ASPECTS OF THE ENERGY BUDGET OF THE CARIBBEAN REEF BUILDING CORAL *Montastrea cavernosa* (Linnaeus): A preliminary *in situ* investigation.

THESIS

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Michael Peter Pearson

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TABLE OF CONTENTS

Appendix-1.2	Circuit diagrams and hermetic housing design for the Mark III automatic respirometer.
Appendix-1.3	Computer programs for the operation of, and decoding data from the automatic respirometer
Appendix-2.1A	Current flow diagram over a coral used for coelenteron sampling experiments at 10 metres.
Appendix-2.1B	Current flow diagram over a patch reef used for coelenteron sampling experiments at 30 metres.

SUMMARY

The purpose of this investigation was to construct a bioenergetic model of energy flow through the Caribbean reef building coral *Montastrea cavernosa* (Linnaeus), and its symbiotic zooxanthellae *Gymnodinium (Symbiodinium) microadriaticum*. All experiments in this investigation were carried out *in situ*.

The energy inputs, the energy expenditure, and the energy retention in the form of tissue growth was determined for corals maintained at two depths (10 metres, and 30 metres), and in two treatment groups (Dark ('bleached') , and 'normal') at each of these depths. The energy losses from the system were calculated by subtraction.

In order to carry out this investigation it was necessary to design and construct an automatic programmable respirometer/data logger for extended deployment on the reef at both study depths. The device constructed was controlled by an ACORN microcomputer. A probe array consisting of 4-Radiometer microcathode oxygen electrodes, 1 linear thermistor, and an internally amplified photocell was used to collect data from corals enclosed in periodically flushing respirometer chambers. Collected data were analyzed in the laboratory using a Commodore 3032 computer system.

A photographic technique was developed to determine the growth rate of colonies maintained at the two depths. It was found that there were marked differences in growth rates between treatment groups at each depth, with those colonies maintained in darkened conditions showing decreased

growth rates relative to colonies maintained in normal conditions. There was no significant difference in tissue cover per unit surface area (mg.dw./cm²) with normal colonies at 10 metres having 18.6281±6.4476 mgdw/cm² and those at 30 meters having 17.9144±3.0803 mgdw/cm². Growth data normalized to the energetic requirements of a standard hypothetical 100 cm² colony showed that a standard colony maintained under normal conditions at 10 metres would require 203.26±132.51 Joules per day, and a similar colony at 30 metres would require 49.17±38.24 joules per day. Colonies maintained in darkened conditions would require 18.85±8.11 joules per day at 10 metres, and 12.49±14.08 Joules per day at 30 metres.

The energetic input due to heterotrophic nutrition on zooplankton was determined using a technique to extract the coelenteron contents from selected colonies for later analysis in the laboratory. It was found that the energy content of a single plankton was 0.00605±0.0025 Joules, and using this value it was found that the energy input to a standard colony having 123 polyps based on an all night collection sampled at 2 hourly intervals was 6.236 Joules per 12 hour nighttime period. It was also found that zooplankton ingestion by a coral colony closely follows zooplankton availability in the water column.

Experiments carried out *in situ* using the automatic respirometer system were used to determine the energy contribution from photosynthetic production by symbiotic zooxanthellae, and the energetic requirements of colony respiration. The decoded results when normalized to the energy equivalents for standard 100 cm² (Joules.100cm^{-2}.12 or 24 hours^{-1}) colonies at both depths showed that Gross Production (Pg) was 17.16% greater for 'normal' colonies at 10 metres than for their counterparts at 30 metres.

Zooxanthellae respiration (Rz) was determined using the density of the symbiont per unit biomass. It was found that (Rz) of 'normal' colonies at 10 metres was 30.71% higher than that of 'normal' colonies at 30 metres. Colony respiration (Rc) was greater for colonies at 10 metres (1124.95 Joules/100cm²/24hrs) than at 30 metres (809.55 Joules/100cm²/24hrs). Animal respiration rates were determined by subtracting the energetic equivalents of zooxanthellae respiration from the calculated equivalent for colony respiration (Rc). It was found that (Ra) in 'normal' colonies at 10 metres was 24.77% higher than (Ra) of 'normal colonies at 30 metres. This increase in animal respiration has been attributed to an increased availability of carbon compounds due to higher levels of productivity in 10 metre colonies.

All the energetic equivalents calculated from *in situ* experiments and converted to the requirements of standard colonies showed that at both 10 metres and 30 metres there was an excess of energy produced and ingested which was not utilized by either member of the symbiosis. It was assumed that all excess energy would be released to the water column in the form of mucus. At 10 metres energy losses to the water column amounted to 110.92 Joules/100cm²/24 hours, or 7.66% of total energy inputs. At 30 metres energy losses amounted to 334.04 Joules/100 cm²/24 hours, or 27.97% of total energy inputs.

It was shown that 56.17% of (P) was translocated to the animal fraction of the symbiosis at 10 metres, and 63.72% of (P) was translocated to the animal fraction at 30 metres.

The results of this investigation show that this species though a voracious feeder on zooplankton does not meet its daily energetic

requirements from heterotrophic nutrition, but that this feeding strategy is necessary for tissue growth. The presence of symbiotic zooxanthellae is vital in that they provide a source of energy which is then translocated to the host in the form of photosynthates to satisfy the energy requirements of the animal fraction of the symbiosis. The results, particularly those from experiments on zooplankton ingestion may suggest that a coral such as *Montastrea cavernosa* with large polyps may be geologically older and less efficient than a small polyped coral such as the *Porites sps.* that proliferates without active feeding on zooplankton.

Montastrea cavernosa(Linnaeus) (2X magnification). Colonies are massive, plocoid, and up to 2-meters in diameter. Growth form can be hemispherical, flat or encrusting. Calices are 5-11mm in diameter with 48 septa per calice, and a trabecular columella swirling clockwise.

COLOURS: Brown, Green, Olive, Red.

BATHYMETRIC RANGE: 0.5 - 95 meters.

(Cairns, 1982)

INTRODUCTION

The success of present day coral reef assemblages is partly due to the development of the association between hermatypic corals and their endosymbiotic zooxanthellae in the Mesozoic (Krasnov,1981). Coral reefs exist as geologically and biologically important shallow water habitats covering an approximate area of 1.9×10^7 km^2 in tropical and subtropical oceanic regions generally recognized as having low levels of productivity (Wells,1957; Goreau,1961). The rates of expansion of coral reefs are primarily dependent on the rate of skeletogenesis of the frame building corals (Goreau,1959; Hein & Risk,1975), and their resistance to physical, chemical, and biological destructive processes (Hein & Risk,1975; MacGeachy & Stearn,1976; MacGeachy,1977; Pomponi,1977; Highsmith,1981).

The anomalous existence of coral reef systems as highly productive areas in an otherwise nutrient poor oceanic environment has prompted much active research. Studies of oceanic waters flowing over reefs have suggested that offshore zooplankton densities, and essential nutrient concentrations (Nitrogen and Phosphorous) are insufficient to meet the energetic requirements of coral reefs on a biomass basis (Sargent & Austin,1949,1954; Johannes *et al*,1969; Tranter & George,1972; Glynn,1973; Johannes, 1974). These investigations though recording downstream depletions in both plankton and nutrients relative to upstream stations, were not specific enough, and suggested that energy flow studies in selected frame building coral species could yield information necessary to understand the mechanisms by which reef systems modify and enrich their oceanic environment.

The energetic requirements for coral growth, maintenance, and reproduction are met either from heterotrophic nutrition from exogenous sources, photosynthetic production by the symbiont followed by translocation of photosynthates to the host, or a combination of both.

Scleractinian corals are generally regarded as suspension feeders on particulate material of animal origin (Yonge,1930; Johannes *etal*,1970; Coles & Strathman,1973; Black & Burris,1983),but they are also capable of active tentacular zooplankton capture (Porter,1974). Other sources of exogenous material also available to coral colonies include dissolved organic material absorbed directly (Johannes *etal*,1970; Goreau,Goreau,& Yonge,1971), and active ingestion of bacteria as described by DiSalvo (1971), Sorokin (1973), and suggested by Moriarity (1979).

Zooplankton present on coral reefs has been shown to originate from two sources, Demersal and Oceanic. It has already been noted that zooplankton depletion studies over water flowing over a reef concluded that the plankton biomass assumed to be extracted by the reef assemblage was insufficient to satisfy the energetic requirements of the system. But those studies did not consider the ability of reefs to retain zooplankton through complex near reef water circulation patterns (Sale,McWilliam, & Anderson, 1976), and the now well established existence of resident (Demersal) reef plankton, and its importance to coral energetic requirements.

Regardless of the source of planktonic material, the importance of zooplankton to coral nutrition is still debated. Vaughn (1912,1919), Mayer (1918), Goreau (1960), Goreau *et al* (1971), Yonge (1973), and others concluded that corals were specialized carnivores due to inherent morphological features, and were able to survive by feeding exclusively on

zooplankton. As proof, Meyers (1918) refers to experiments carried out on *Sideras trea sp.* where sample corals survived in a healthy state when kept in the dark and fed zooplankton. But contemporary experiments by Gravier (1913), Hickson (1924), and Boschma (1925) all suggested that endosymbiotic zooxanthellae had an important role as a supplementary source of nutrition for corals. Yonge and Nicholls (1931) held opposing views, and suggested that zooxanthellae had a purely commensal relationship with the host coral. Yonge and Nicholls's conclusions were based on experiments showing that corals kept in the dark and fed zooplankton survived in good health.

Gohar (1940) repeated the experiments of Yonge and Nicholls (1931) using small polyped and small tentacled corals kept under similar conditions, and reported that none of the test colonies survived. These experiments suggested a relationship between polyp morphology and feeding strategy. Franzisket (1970) examined this relationship and suggested that species with large polyps and low metabolic rates could survive in good health on heterotrophic nutrition from exogenous sources, while those with small polyps and high metabolic rates could not survive without light. These views support those of Kawaguti (1964), and Muscatine and Lenhoff (1967), and are supported by Johannes and Tepley (1974), who found that zooplankton was not a major source of nutritional energy in *Porites lobata* (a small polyped coral). Porter (1974), Muscatine and Porter (1976), and Lewis (1978) all report that zooplankton ingestion was insufficient to meet the daily energetic requirements of *Montastrea cavernosa* and *species* respectively.

Recent work seems to indicate that some hermatypic corals harbouring zooxanthellae are able to satisfy their daily energy requirements by the translocation of photosynthetic products from the symbiont to the host in

shallow water. Davies (1984) working with *Pocillopora eydouxi* in the laboratory reported that the coral was able to meet its daily energy requirements from endogenous sources, and suggested that 48% of the diurnal production could be released to the water column in the form of mucus. McCoskey and Muscatine (1984) similarily reported that colonies of *Stylophora pistillata* maintained at 3-metres in the Red Sea were capable of satisfying their energetic requirements from endogenous sources, but that similar colonies maintained at 35-metres derived only 78% of their respiratory carbon requirements from zooxanthellae photosynthesis (C.Z.A.R.), and the remainder was made up from exogenous sources.

The translocation of photosynthetically reduced carbon compounds from the symbiont to the host was shown by Goreau and Goreau (1960), and Trench (1971), who reported that ^{14}C labelled $Na^{14}CO_3$ fixed by zooxanthellae in the light was later recovered from host tissue. Trench (1971) suggested that the major translocation product was glycerol, but the translocation of fatty acids has also been reported in the coral *Manicina aerolata* (Patton *et al*, 1977). In a recent paper Battey and Patton (1984) suggest two modes of carbon translocation occurring simultaneously. The first being that glycerol is translocated to the host for immediate short term use as the main metabolic substrate, and the second that lipids synthesized by zooxanthellae (triglycerides and wax esters) are translocated to the host in the form of lipid droplets. The latter are then used to generate lipid stores for use during periods of starvation and reproduction. Fitt *et al* (1981) presented data suggesting that the anemone *Anthopleura elegantissima* when starved in the dark shifted from its normal carbohydrate metabolism to a lipid substrate metabolism after 2-days, while starved anemones kept in the light retained a carbohydrate metabolism.

Translocation from the symbiont to the host can occur only if photosynthetic production exceeds zooxanthellae respiration during daylight hours. Attempts have been made to quantify the translocation of fixed carbon to the host (McCloskey *et al*,1981; McCloskey and Muscatine,1984). These workers used complex mathematical equations to determine the percent contribution of photosynthetically fixed carbon compounds available for animal respiration (C.Z.A.R.). Muscatine *et al* (1981) suggest that 40 - 180 % of the daily carbon requirements of *Pocillopora damicornis* and *Fungia scutaria* were met by translocated photosynthates, McCloskey and Muscatine (1984) suggest that colonies of *Stylophora pistillata* at 3-metres on a Red Sea reef were able to meet all their carbon requirements from translocated photosynthates. This technique has been questioned by Davies (1984) who suggests that it would result in an underestimate of translocated products to the host since C-14 labelled products lost from the host tissue to the water column in the form of mucus are not accounted for (Crossland and Barnes, 1980). Davies (1984) assumed that all photosynthetically fixed carbon not required for zooxanthellae growth and respiration was passed to the host tissue. This assumption would yield a better estimate of translocation, and values of 90 % were given for *Pocillopora eydouxi* at 5-metres.

Energetic inputs from endogenous and exogenous sources are partitioned and used by the colony for maintenance metabolism, tissue growth, storage, reproduction, with any excess either being released to the water column in the form of mucus (Crossland *et al*,1983), or recycled from the host to the symbiont (Muscatine and D'Elia,1978).

Growth in coral colonies results from the asexual division of tissue coupled with calcium carbonate ($CaCO_3$) deposition in the form of Aragonite

(Wainwright,1963) results in slow growth rates relative to other non skeletized coelenterates (Barnes,1973). The source of calcium (extracted from seawater), and that of carbonate (from endogenous and exogenous sources) have been described by Muscatine (1971). The conditions necessary for exchange, precipitation, and deposition of these materials by corals have been postulated (Simkiss,1964; Goreau and Bower,1955; Goreau and Goreau,1960; Johnstone, 1981). The translocation of photosynthetically fixed carbon in the form of carbonate (CO_3) to the calcifying sites was proposed by Goreau and Bower (1955), and supported by Pearse and Muscatine (1971). Goreau and Hayes (1977) proposed that calcification in corals occurs in two discrete steps, firstly the process of secreting an organic matrix described by Wainwright (1963) as an intracellular process, and by Johnstone (1976) as a fibrous extracellular component of the skeleton in newly settled polyps of *Pocillopora damicornis*. There is general agreement that the matrix does contain granular material interpreted as seed crystals of $CaCO_3$. And secondly the process of calcification or skeletogenesis, an extracellular process, occurring around the template produced in the first step.

The presence of an organic matrix associated with skeletal growth in corals was first postulated by Silliman (1846), and later by others including Wainwright (1962,1963) who proposed a chitin based matrix in *Pocillopora sp.*. This view is disputed by Yonge (1971) who found chitin only in the genus *Pocillopora*. Mitterer (1978) suggested that the matrix acted as a template that initiated and controlled crystal nucleation and growth either actively by epitaxial overgrowth and the concentration of appropriate ions, or passively by acting as a limiting boundary membrane.

The relationship between growth rate, growth form, and photosynthesis

is well documented. Goreau (1959,1960) reported that corals exhibited increased calcification rates during daytime. This view was later supported by Vandermuelen (1972) who in a series of elegant experiments using the photosynthetic inhibitor D.C.M.U. (3(3,4-dichlorophenyl)-1,8-dimethylurea) reported reduced daytime calcification rates in treated colonies. Calcification rates in corals have been found to be influenced by extrinsic factors such as ambient irradiance levels, water temperature, and the photosynthetic activity of zooxanthellae (Chalker,1975; Barnes and Crossland,1978).

Differential growth rates in corals characterized by skeletal banding have been reported. Highsmith (1979) suggests that skeletal banding is mediated by the combined effects of temperature extremes, and incident light on the photosynthetic activity of zooxanthellae, and the precipitation of $CaCO_3$. He postulates that under conditions of high irradiance, and moderate temperatures (23.7 - 28.5°C) one is likely to observe maximum zooxanthellae activity, rapid secretion of the organic matrix, and increased calcification rate resulting in a low density band. The dense skeletal banding would be formed at temperatures below 23.7°C, when zooxanthellae are less productive, matrix formation is reduced, and passive calcification exceeds the rate of matrix formation. Density banding is now accepted as being an annual phenomenon, and a valuable tool to determine the age of corals (Knutson *et al*,1970; Dodge *et al*,1972; Buddemeir *et al*,1974; Highsmith,1979).

The annual periodicity of the density banding is further confirmed as being related to seasonal fluxes in temperature and incident irradiance by Wellington and Glynn(1983), who suggest that banding is an end product of endogenous processes such as the re-allocation of energy from growth to

reproduction, which in turn may be mediated by exogenous factors such as light and productivity.

A coral species may exhibit several growth forms. These may be genetically controlled, or may be a response to micro or macro environmental conditions (Barnes 1973). Goreau(1963) suggested that corals exhibited a change in morphology with increasing depth. This view was later supported by Dustan (1975) who noted that *Montastrea annularis* showed a strong growth directionality with increasing depth, where deep water colonies were more flattened and had reduced mass compared to more rounded forms found at depths above 15-metres. As well as this tendency towards flattened light optimizing growth forms at depth, there also seems to be a decrease in linear growth correlated with increasing depth (Buddemeier *et al*,1974; Dustan,1975; Baker *et al*,1975; Bak,1976; Chalker and Taylor,1978). Variability in both growth rate and morphology with increasing depth suggests that growth in coral colonies is a light enhanced or limited process. Yet Grigg (1981) reported that incident light alone could not account for differences in rates of calcification, but when considered together with temperature, was highly correlated to growth (r=0.93). This suggests that coral growth may be highly sensitive to other environmental changes. Dodge (1981) and Szmant-Froelich *et al*,(1981) reported that corals existing in areas with a high sediment load,and increased turbidity suffered high mortality rates due to reduced levels of incident light, and inordinate amounts of energy spent on clearing sediment from the feeding surfaces. This view lends some support to Highsmith's (1979) suggestion that energy not spent on maintenance metabolism by both components of the symbiosis may be diverted to growth.

Investigations of the energy inputs from heterotrophic nutrition and

photosynthetic production, and the partitioning of that energy by a coral colony for maintenance metabolism, growth, storage, and reproduction, have for the most part been studied singly. A different approach would be to study each of these processes in terms of a bioenergetic model for a coral colony to assess the contribution and requirements of each of these for both components of a coral zooxanthellae symbiosis. Previous work along these lines has been carried out by Tytler (1982), working in the laboratory on *Anemonia sulcata*, a zooxanthellate littoral anemone common to British shores, and by Davies (1984) using the Pacific coral *Pocillopora eydouxi*, in a laboratory investigation with field extensions for growth determinations at 5-metres. The present investigation seeks to extend the work of Tytler (1982), and Davies (1977, 1984) who carried out their laboratory based investigations, and extrapolated their results to a field situation, by working exclusively in the field and executing all experiments *in situ* at two depths on the fore reef at Discovery Bay.

A bioenergetic model obeying the first law of thermodynamics (that energy in closed system is constant) FIGURE 1-1, was proposed for the Caribbean zooxanthellate reef coral *Montastrea cavernosa*, a ubiquitous frame building coral on Caribbean reefs (Wells,1957; Goodwin *et al*,1976). The model describes energy equations for both the invertebrate and algal fractions of the symbiosis, these being linked by the translocation of photosynthates from the symbiont to the host, and the possible recycling of energy in the reverse direction.

Montastrea cavernosa (Linnaeus) exists as distinct, massive, plocoid colonies showing hemispherical, flat, or encrusting growth forms, between 0.5 - 95 metres (Cairns,1982). Porter (1974) working on this species in Panama showed that the species is highly carnivorous, and suitable for

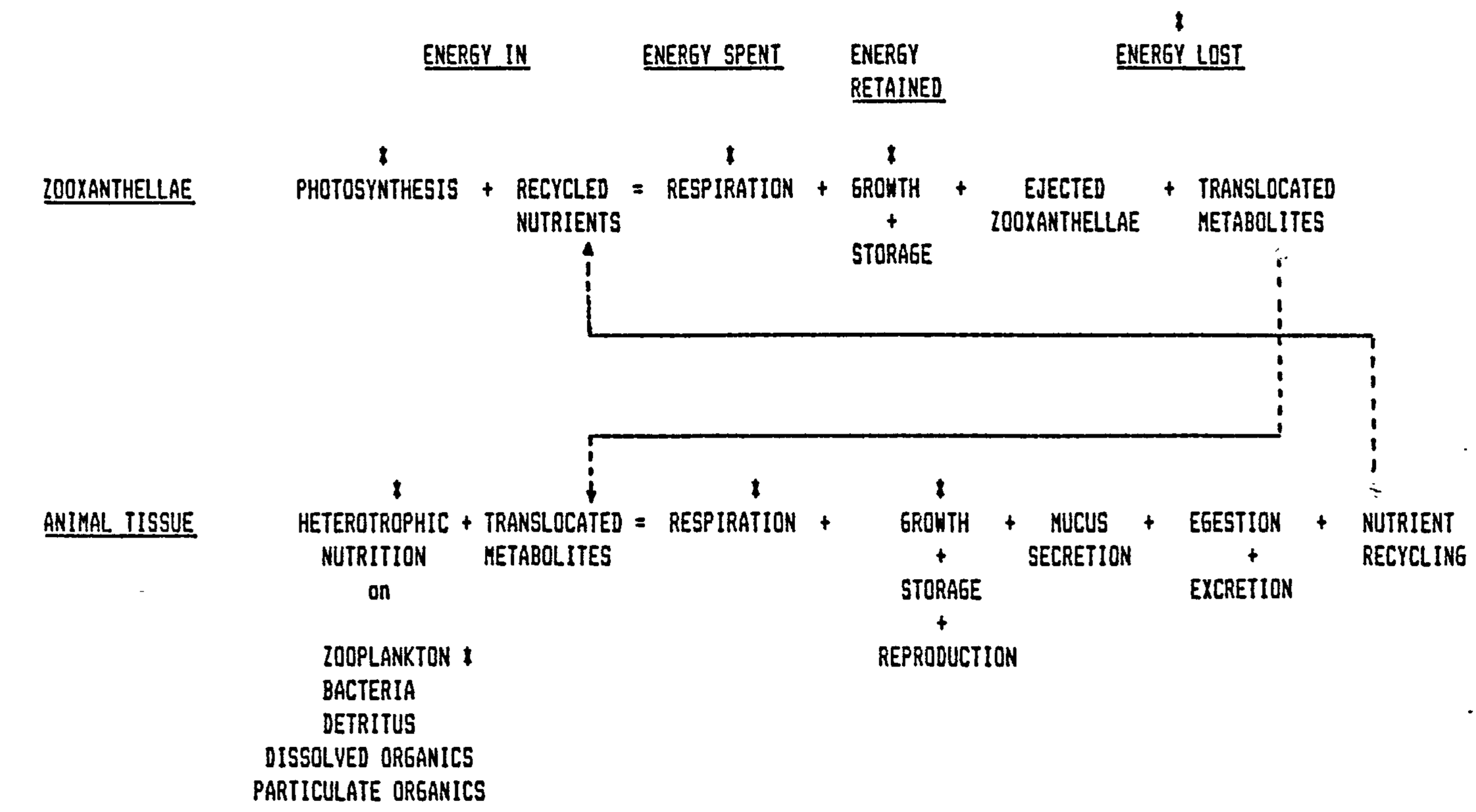


FIGURE 1-1:- A bioenergetic model of the components of an energy budget for a coral animal/zooxanthellae symbiosis. The asterisks indicated those components of the budget measured in this investigation. All values corresponding to energy losses from the system were pooled and determined by subtraction.

direct extraction of ingested planktonic material from the coelenteron. Davies (1977) showed that there was a predicted reduction in algal photosynthesis, and colony respiration with increasing depth. These investigations were the framework on which an *in situ* research programme on this species was planned. The research carried out at two depths, 10-metres, and 30-metres using a purpose built automatic respirometer/data logger, addressed the following questions in addition to constructing an energy budget for a standard 100 cm.^{2}-123 polyp colony;

1)- to determine the degree of dependance on heterotrophic nutrition in this species, and whether there exists a depth dependent factor both in the numbers of plankton ingested by a coral colony, and those available to it for ingestion.

2)- to determine the density of symbiotic zooxanthellae in host tissue over the depth range, and the levels of photosynthetic production at both depths.

3)- to determine whether this species can satisfy its daily energetic requirements from autotrophic nutrition at both depths studied.

4)- to quantify the energetic requirements of tissue growth including algal growth in colonies at both depths, and to assess whether incident irradiance, exogenous nutritional sources, or both are limiting to colony growth.

5)- to compare the results from the *in situ* investigation with those from the laboratory based studies of Davies (1977) on the

same species, and to assess whether laboratory based experiments can be used to complement and groundproof field investigations.

These points in the context of an energy budget for *Montastrea cavernosa* were then used to discuss the role of reef corals in whole reef energetics, and the importance of *in situ* studies using the techniques developed for this research program to broad based coastal resorce management programmes.

CHAPTER - 2

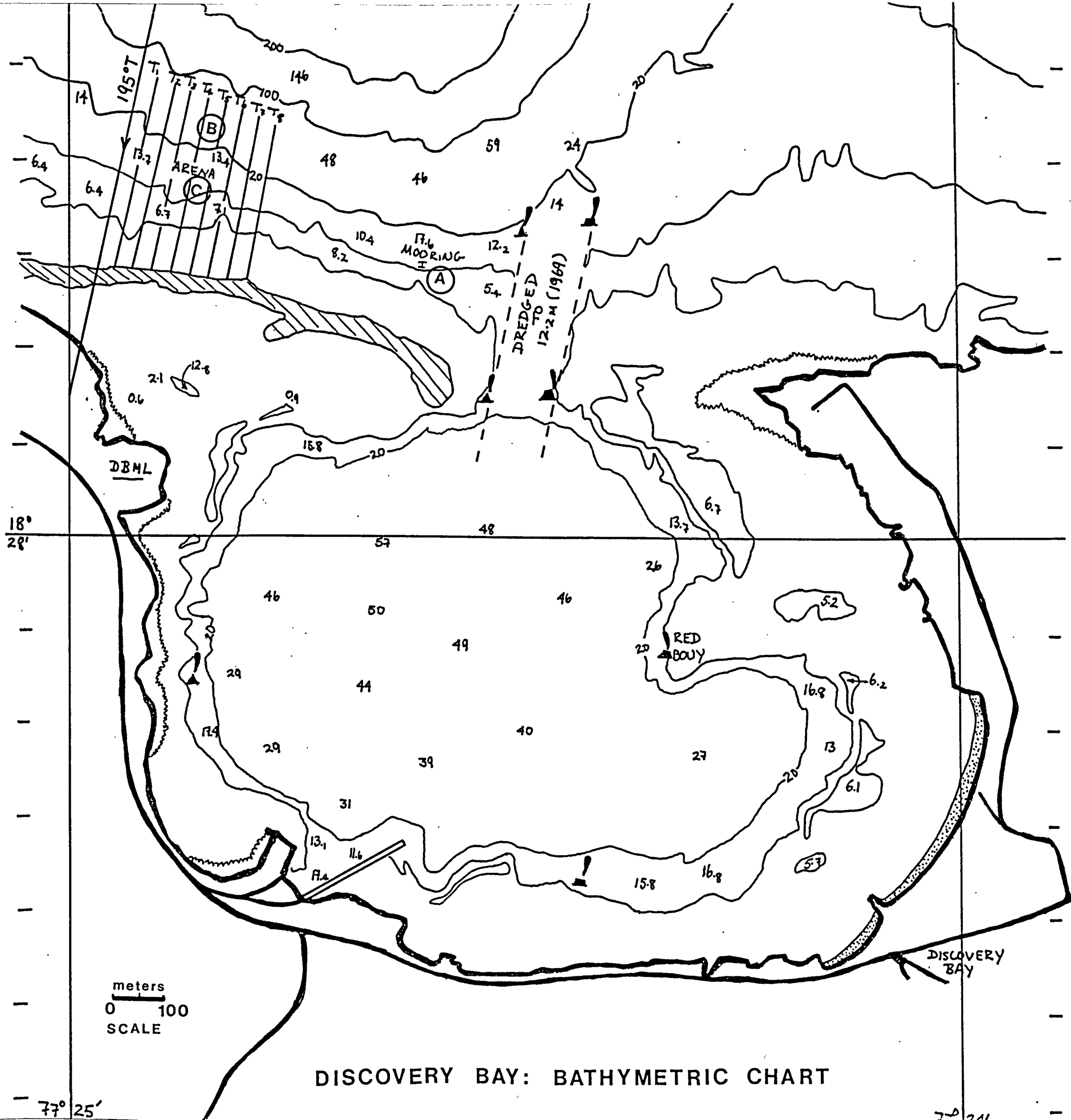
MATERIALS AND METHODS.

SITE DESCRIPTION.

All experiments in this research were carried out *in situ* on the fringing reef at Discovery Bay on the north shore of Jamaica West Indies. Discovery Bay is located 18°27'9" North, and 77°24'30" West, within an area of 1.25 km². It forms a distinctive feature of the Northern coastline, and it has been suggested that the bay represents a drowned river valley excavated by solution of carbonate rock, while being partially cut off from the sea by recent reef formations (Woodley and Robinson,1977). A distinct channel was dredged through the reef to a depth of 12-metres in 1969 to allow passage of ore carrying vessels loading at the Bauxite terminal in the Bay (Figure 2-1). The bay itself is a deep basin reaching depths of 53-metres. Its shape and reduced circulation tends to result in an accumulation of organic and inorganic material of terrigenous origin. These reduce ambient light and result in markedly different coral communities in the bay when compared to those on the seaward fore reef (Reiswig,1973).

The locations selected for this investigation were on the outer fringing reef to the West of the shipping channel (Figure 2-1,positions A,B,C), an area already well described by Goreau and Goreau(1973), Land (1974), Reiswig(1973), Dahl,MacIntyre and Antonius(1974), Goreau and Land(1974), Lang,Hartman and Lang(1975), and more recently Woodley *et al*(1981) following catastrophic destruction by Hurricane Allen.

FIGURE 2-1:
A bathymetric chart of Discovery Bay on the north coast of Jamaica WI. The location of the Marine Laboratory (DBML) is shorewards of the exposed reef crest marked by the hatched area. The study sites on the west fore reef are marked A) for the shallow coelenteron sampling site at 10 meters close to the shipping channel, C) for the main shallow (10 meter) study site-growth and respirometry studies, and B) the deep (30 meter) study site on the fore reef adjacent to the fore reef facies. Lines T_1 to T_8 represent transect lines at 195°True from 45meters to the surface to determine the distribution and numbers of *Montastrea cavernosa* colonies on this section of the fore reef. The fore reef extends seawards to approximately 65 metres after which is the final drop off leading to the Southern slopes of the Cayman trench (Woodley and Robinson 1977).



DISCOVERY BAY: BATHYMETRIC CHART

The fore reef at Discovery Bay has been divided into several zones by those workers mentioned, but only those areas of interest to this work will be described. These are the reef crest mixed zone at 10-metres, and the fore reef slope at 30-metres. These areas were selected since they represented two distinctly different reef environments manifested by differences in spectral irradiance, onshore water movements, surge effects, plankton availability, as well as the availability of suitably sized corals for experimentation. The lower depth limits for this investigation were dictated by safe diving procedure where 30-metres allowed for 20-minutes of no decompression time, the minimum required to perform selected tasks at depth. Study sites were positioned at both 10-metres and 30-metres, the 10-metre site exposed to reef modified water flow patterns (Sale *et al*,1981),and increased irradiance relative to the 30-metre site (Jerlov,1970). The deep study site was also exposed to predominantly onshore oceanic currents.

STUDY SITE DESCRIPTION.

Both study sites (10m-30m) were located in sand channels. The 10-metre site was protected from a prevailing E-N-E current by a patch reef consisting of *Montastrea annularis, Dendrogyra cylindrus, Montastrea cavernosa, Colpophyllia natans, Siderastrea siderea, and Agaricia spp.*. The deep study site was located in the lee of a large coral mound rising to a depth of 21-metres above the sand channel. Sample corals at this site were protected from prevailing water currents in a W-S-W direction (up the sand channel), and from water tumbling off the mound by large colonies of *Montastrea cavernosa* and *Colpophyllia amaranthus*.

Current direction and velocity at both sites was determined by

injecting 5-ml. aliquots of Fluorescein Dye into the water column, and plotting their course for three minutes. Direction was determined using a compass, and velocity by timing the passage of an aliquot of dye over a measured distance.

THE SHALLOW STUDY SITE, 10-METRES.

This region sloped gently to seaward to a depth of approximately 15-meters (Figure 2-1, position C). It had been an area of high coral diversity, but suffered severe damage during and immediately following the onset of Hurricane Allen (1981), resulting in reduced coral fauna , altered community structures, and a marked change in dominant coral species Woodley *et al*,1981).

Corals dominant in this zone are massive colonies of *Montastrea annularis*,abundant field of broken or diseased (Whiteband) *Acropora cervicornis*, colonies of *Diploria strigosa, Agaricia spp., Porites spp., Dendrogyra cylindrus, Colpophyllia natans, Meandrina meandrites, Mycetophyllia spp.*, and isolated colonies of *Montastrea cavernosa.*

THE DEEP STUDY SITE, 30-METRES.

Like the shallow area already described, the deep study site suffered some damage from Hurricane Allen (1981), but here the damage was restricted to previously luxuriant stands of *Acropora cervicornis*, followed later by an enveloping growth of *Lobophora variegata* covering many of the remaining coral colonies. The area is on the seaward boundary of the fore reef *Acropora cevicornis* zone, and the mixed fauna fore reef slope. The area supports a high diversity of coral species with varying growth forms

(massive to platelike) depending on their position on well defined coral mounds, with plating forms being predominant on the steep sides, and massive forms with flaired edges on the tops of the mounds closer to the surface (10-metre depth differential). Specimens of most species of Caribbean reef corals could be found at this depth.

PHYSICAL PARAMETERS INFLUENCING THE FORE REEF AT DISCOVERY BAY.

WINDS. There is considerable seasonal and diurnal variation in both wind direction and intensity. During the winter months there is a North Westerly shift in the Trade Winds resulting in constant winds of 20-40 knots (E-N-E), which create a damaging ground surge over the fore reef at depths above 15-metres. During the summer (May-September) the wind is offshore during the night (Northerly),and in an E-N-E direction 10-20 knots from about 1000- 1700 hours. Turbulent flow over the reef is reduced during the summer months.

TIDES. Tidal range is approximately 0.5 metres with slight variation depending on atmospheric conditions (extreme low pressures, or sustained periods of adverse weather).

CURRENTS. Currrents were predominantly longshore and wind driven in a 260° True heading at 0-1.5 knots. Current direction and velocity was measured directly using the Fluorescein Dye injection method already described.

TEMPERATURES. No extremes in water temperature were recorded on the fore reef at Discovery Bay. Using a thermometer in a protective housing, water temperatures were recorded during each dive. During the Winter months, temperatures of 26 ± 0.3°C were recorded, with summer temperatures rising

1.5°C to 27.5 ± 0.3°C.

SALINITY. Salinity on the fore reef was constant at about 35 ppt. (Woodley and Robinson, 1977).

DISTRIBUTION OF *Montastrea cavernosa* ON THE FORE REEF AT DISCOVERY BAY.

Before corals were collected for the various experiments planned, it was important to gather baseline data on the depth distribution, abundance, and projected surface area (surface area as seen from above in $cm.^2$, determined using a $100cm.^2$ grid) for this species in order that sample size and collection strategy could be determined.

A modified transect method was selected as being the most suitable to cover a large area of reef. Divers starting at a depth of 40 metres swam compass courses (195°True) normal to the reef crest (Figure 2-1), and recorded the presence of *Montastrea cavernosa* colonies in a 1 metre wide band (determined by a 1 metre wide rod carried by the divers). Eight transects were carried out covering an area of 2812 m^2. The results (Figure 2-2) showed that colonies of *Montastrea cavernosa* were clumped at two distinct depths on the fore reef, the first being a band from 5-18 metres, and the second from 34-40 metres. Since colonies of suitable size for respirometry (surface area of $40cm^2$ or less to fit into respirometer chambers) were not found in adequate numbers at the selected study sites, colonies of suitable size were collected from locations further afield but from equivalent depths, and transplanted to their respective study sites.

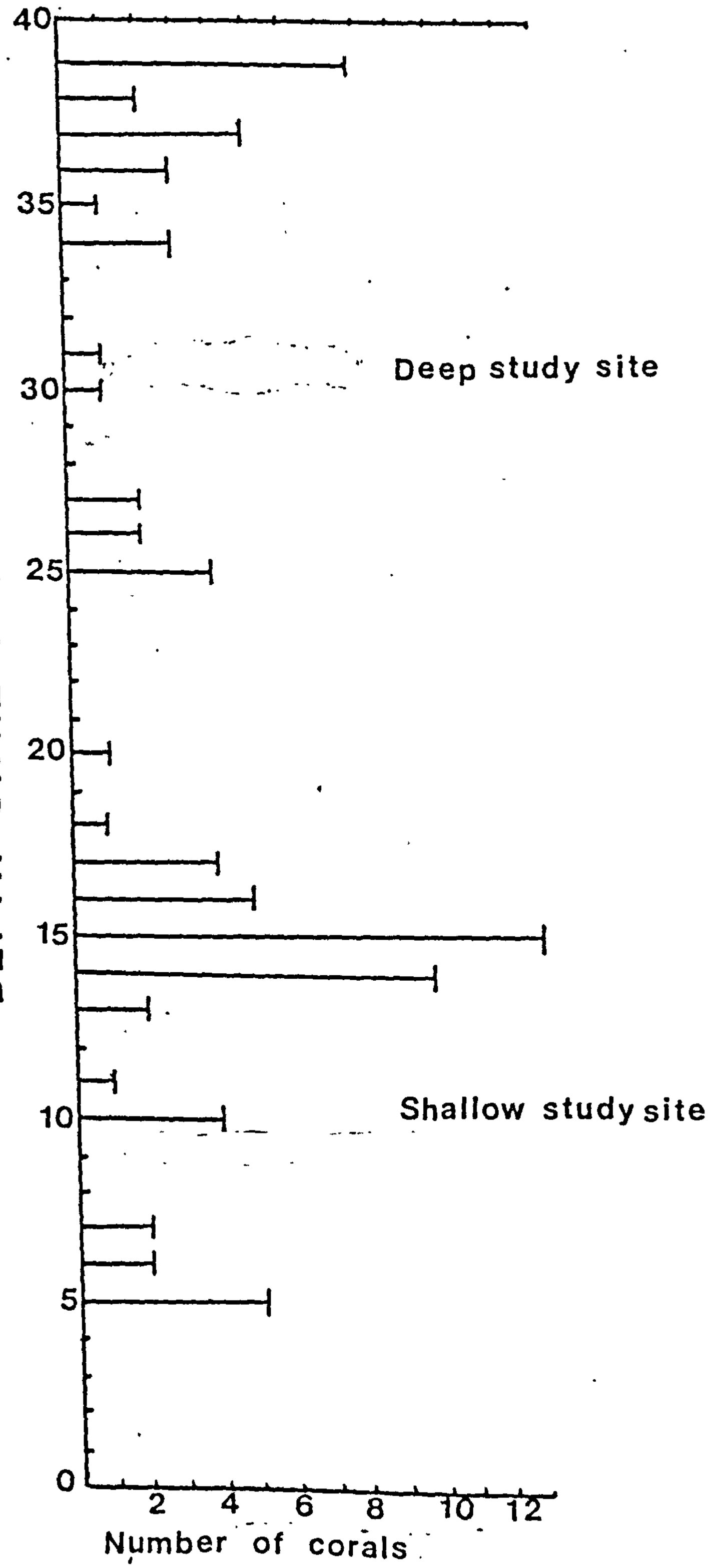
SAMPLE CORAL COLLECTION PROCEDURE

FIGURE 2-2:-

The results from a transect survey of the West fore reef at Discovery Bay to determine the distribution and colony numbers present of *Montastrea cavernosa*. Eight transects were carried out in which 2812m² of the reef surface was surveyed from 40 metres to the surface. The distribution of this species was clumped at two distinct depths, 13 to 18 metres, and 34 to 40 metres. The maximum number of colonies was found at 15 metres with 13 colonies counted.

DISTRIBUTION M. cavernosa

TRANSECT SURVEY (2812 m^2)



Selected colonies were detached from the reef using either a hammer and chisel, or a pneumatic chisel run off a low pressure line from an aqualung. Location, collection depth, and orientation normal to the reef substrate was recorded for each collected coral. Collected corals were then transported to the laboratory in darkened 30-litre fiberglass containers. In the laboratory the corals were placed in running seawater tables (30-cm deep) at 26°C and salinity of 31-35 ppt., the actual value being dependent on precipitation. Since conditions in the laboratory were not ideal (variable physical parameters), collected colonies were quickly cleaned of all visible encrusting organisms, and glued onto numbered perspex plates (7.5cm x 10cm.) using a two part non toxic epoxy compound (Pettit Marine Underwater Patching Compound). Once glued the corals were immediately taken to their respective study sites corresponding to their collection depth, and allowed to recover for a period of 5-14 days. At no time were the corals exposed to either air or direct surface illumination. No visible stress effects were seen (extruded mesenterial filaments, extended periods of polyp contraction). All collected corals survived this treatment and were later used for either growth rate determinations, respirometry, or both.

TREATMENTS OF COLLECTED COLONIES:-

Corals collected and prepared for later experiments were maintained in two treatment groups at both the 10 metre and 30 metre study sites. Thirty colonies were collected and maintained at each of the 10 and 30 metre sites, of those 30 colonies 10 were kept in darkened enclosures at each site with the remaining twenty kept on the reef under normal conditions. Of these thirty colonies not all were of adequate size for use in the respirometer chambers, and consequently were used only for

FIGURE 2-3:-

A photograph showing the automatic respirometer and data logger deployed on the fore reef at 10 meters. The 10 meter study site was in a sand channel protected on three sides by coral growth. Collected corals glued to perspex bases and in the normal treatment group can be seen fixed to asbestos platforms in the fore ground. Grazing by the urchin *Diadema antillarum* controlled algal growth on both the asbestos sheets and the perspex plates. The 30 meter site was also in a sand channel.

photographic growth determinations. the numbers used in growth studies and for respirometry are given in their respective chapters.

THE NORMALLY EXPOSED GROUP:-

Corals fixed to perspex plates were attached to asbestos sheets fixed to the reef by steel shafts. The asbestos sheets were placed in sand channels at both depths (as in Figure 2-3 at 10 metres). This exposed the colonies to somewhat artificial conditions. Firstly there was a reflected light factor from the sand which would otherwise not have been present on normal coral substrates (Brakel, 1981), and secondly the use of sand channels though convenient proved unfortunate since winter storm surges through these channels resulted in some sample loss (n=10 at 10 metres), and on occasion abrasions on the coral tissue surface. Corals damaged in this way were not used for respirometry. The effect of storm surges was pronounced at the 10 metre site but not present at the 30 metre site. Figure (2-4) shows a coral colony from the 10 metre site maintained under normal conditions.

THE DARK TREATMENT GROUP:-

Corals fixed to perspex plates were placed in darkened enclosures constructed from flexible black PVC sheets (100cm x 60cm) with the two longest dimensions fixed by brass screws to weighted wooden bases. The resulting tunnel shaped enclosure was partially sealed at both ends by 4 superimposed layers of coarse black nylon mesh with pores of 5mm x 5mm. The mesh complex allowed water and planktonic material to enter the enclosure but effectively darkened its interior. Colonies maintained in darkened conditions lost all visible pigmentation as shown in the colony in (Figure 2-5) from 10 metres.

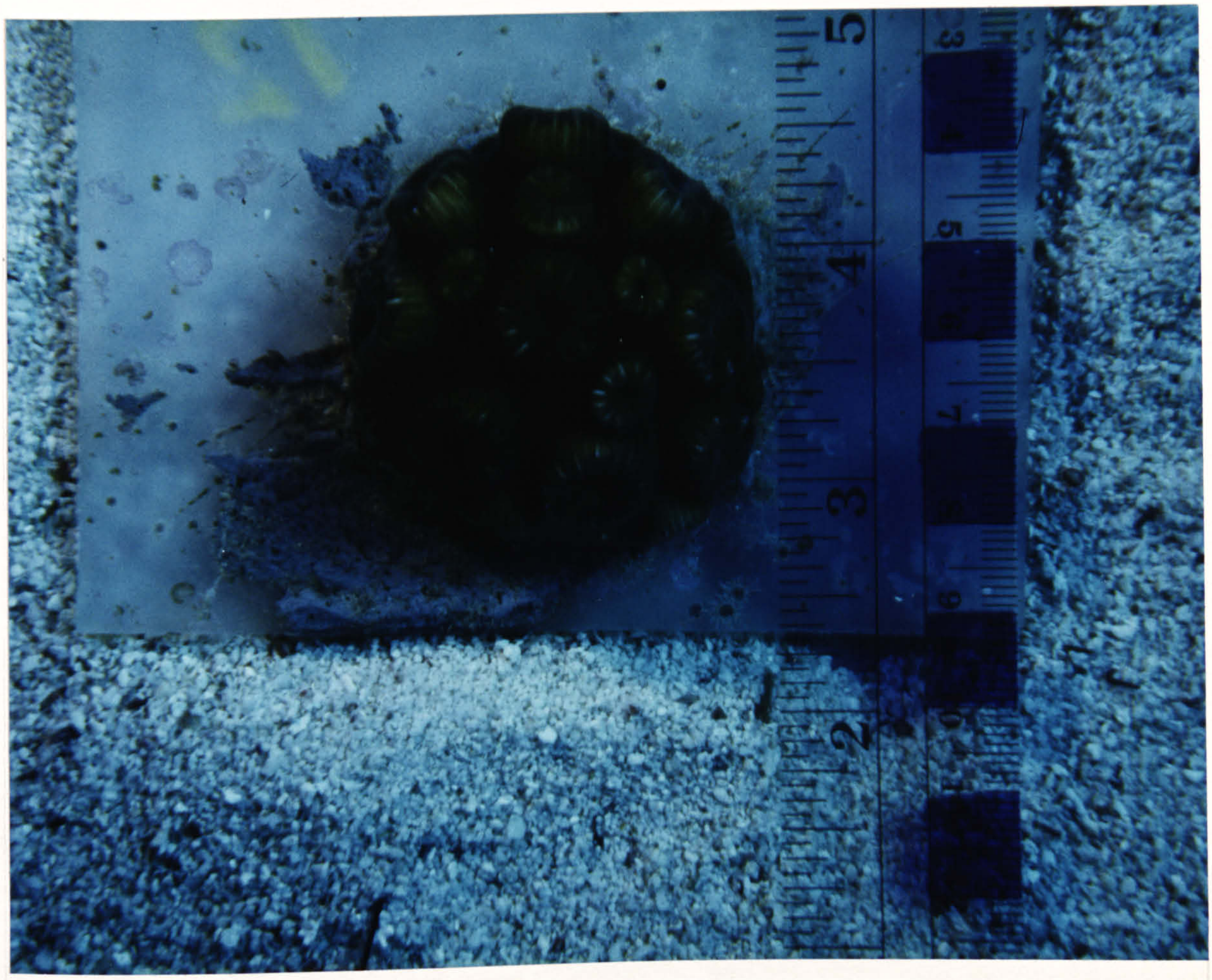
FIGURE 2-4:-

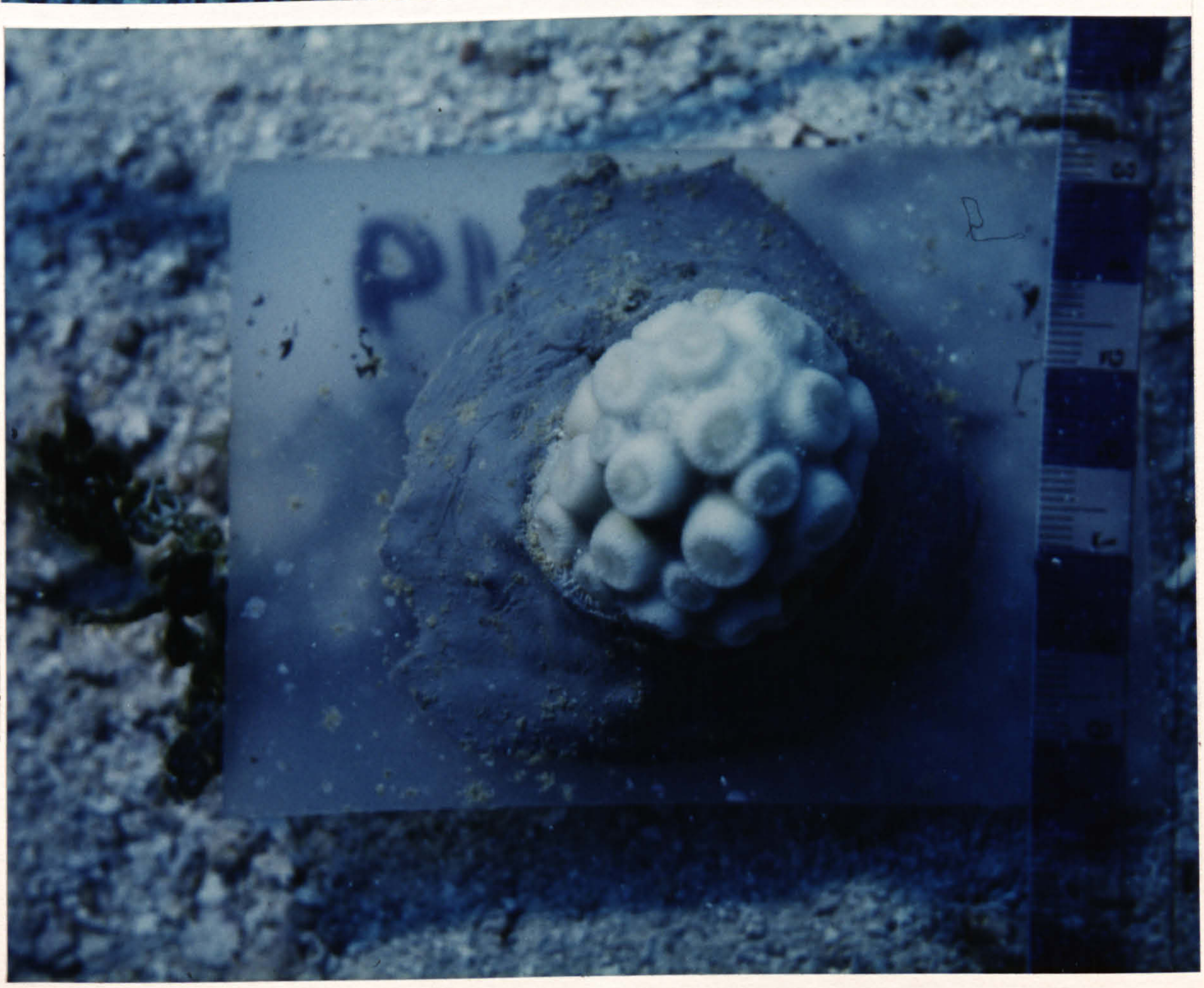
A collected coral colony glued to its perspex base using a two part underwater epoxy patching compound (Pettit Marine). This colony has been kept in normal conditions on the reef and shows a healthy pigmentation. The septa visible through the tissue were the result of damage following a severe storm, the colony recovered fully in 48 hours and no skeletal tissue was visible.

FIGURE 2-5:-

A colony which has been maintained in darkened conditions showing no visible surface pigmentation. Colonies kept in darkened conditions for three weeks lost all pigmentation, but not all their zooxanthellae or the pigmentation of endolythic algae.

A centimeter scale was included in all photographs and was used as a reference point for growth measurements in the laboratory.

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LABORATORY ANALYSIS:-

CALORIMETRY

Two calorimetric techniques were attempted to determine the energy equivalent of samples of coral tissue and zooplankton both previously lyophilized (see relevant chapters for individual treatments). The first technique that of microbomb calorimetry using a Phillipson microbomb calorimeter, and the second that of wet oxidation with potassium dichromate (Johnston, 1949). The microbomb technique was abandoned after repeated trials showed incomplete combustion of the sample, and the need to use large quantities of combustible benzoic acid as a sample carrier. The second technique which required treatment of the samples with a strong oxidant - potassium dichromate ($K_2Cr_2O_7$) was found to be extremely accurate and reproducible, and consequently was used in all calorimetric determinations.

WET OXIDATION WITH DICHROMATE

The method used was based on that of Johnston (1949), and is a colorimetric technique to determine the energy content of sample material.

Samples containing about 2mg of freeze dried material were placed in test tubes previously washed in hot chromic acid to rid them of all organic material. 1.0ml of distilled water, 0.6ml of mercuric sulphate solution, and 0.4ml of 1.0N potassium dichromate solution were added to each tube. The tubes were stoppered with glass marbles and placed in a boiling water bath for 30 minutes. The mixture was then cooled, decanted into 50ml volumetric flasks, and diluted to the mark with distilled water.

Standard solutions containing 66.67umol of potassium dichromate and

blanks reduced with anhydrous sodium sulphite (Na_2SO_3) were included with each batch of determinations.

Absorbances were read at 440nm with a 10mm path length against a reduced blank.

CALCULATION OF CALORIFIC VALUE

In the presence of H_2SO_4, $K_2Cr_2O_7$ can be reduced to chromium (Cr^{3+}) ions in the following reaction (Gotteman and Clymo, 1969).

$$2K_2Cr_2O_7 + 8H_2SO_4 \longrightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 + 8H_2O + 3O_2$$

For each 2mol of $K_2Cr_2O_7$ reduced 3mol of O_2 are released. Therefore the reduction of 66.67umol of $K_2Cr_2O_7$ in the standard solution releases 100umol of O_2 which is equivalent to 45.25 Joules using the general oxy-joule coefficient for a mixture of protein, lipid, and carbohydrate;

1 mg O_2 = 14.14 Joules

1umol O_2 = 452.50 Joules (from Elliot and Davidson, 1975)

Laboratory trials using this technique were carried out on Complan-a high protein convalescent food of known content, and the method found to be highly reliable.

CHAPTER - 3.

THE DESIGN AND DEVELOPMENT OF AN AUTOMATIC RESPIROMETER

INTRODUCTION:-

The decision to investigate the energy budget of the Caribbean reef coral *Montastrea cavernosa - in situ* was a result of previous field work carried out in Grenada W.I., and an extension of the laboratory based studies of Davies (1977) who investigated the respiratory physiology of Caribbean corals including *Montastrea cavernosa* collected from the fore reef at Discovery Bay.

To investigate aspects of the energy budget of *Montastrea cavernosa*, enough components of the energy equation must be known in order that these may then be used to present a bioenergetic model for a standard colony of the species in question. Componets such as heterotrophic nutrition on exogenous sources, and colony growth could be measured directly on test colonies using the techniques of Porter (1974) for the former, and those of Johannes (1969), and Marsh (1970) for the latter. It is more difficult to measure oxygen production by the symbiont, and colony respiration (*in situ*) both being essential to determine the energy inputs to a colony from photosynthetic production and that increment of fixed energy consumed in colony respiration (See chapter 6). In addition to these components of the energy budget, it was also necessary to measure ambient water temperature, and incident irradiance both parameters being important for the analysis of oxygen exchange data.

Three techniques were available to carry out this investigation. Firstly coral colonies collected on the reef could be brought back to the

laboratory at intervals and used in experiments, this procedure was not considered since the combination of *in situ* and laboratory experiments could have added variability to the results. Secondly coral colonies could be maintained on the reef and introduced into respirometers from which water samples could be removed manually for analysis on the surface. And finally an automatic respirometer/data logger capable of long term *in situ* deployment could be designed and constructed. This last technique was the one chosen and this chapter describes the development of the automatic system used in this investigation.

Previous workers (Jaubert, 1977; Mergner and Svoboda, 1977; Svoboda, 1978; Porter, 1980; Muscatine *et al*, 1981) have approached the problem of *in situ* physiological monitoring systems deployable in the sea in a similar manner. For the most part these workers have enclosed commercially available laboratory instrumentation such as YSI oxygen meters, LICOR radiometers, and data recording devices (Porter, 1980) in hermetic housings and deployed these on the reef. These systems lacked programming flexibility, and were unable to control the exeriments in which they were used. Only Liu and Davies, (1982) have constructed a more flexible oceanographic data logger using purpose designed electronics under microprocessor control, but this unit was not suited to biological work. The system described in this section was initially designed in 1979 with field trials and further developments being carried out over the next 3 years.

THE DESIGN AND DEVELOPMENT PROGRAM:-

The process culminating in the design and construction of a working microprocessor controlled automatic respirometer/data logger involved the construction and testing of a series of prototypes constructed and tested over a period of three years. These started with discrete, manually sampled respirometers, through two versions of an automatic system (Mark I, Mark II), and concluded with the last working system (Mark III) used in this research.

THE DESIGN AND CONSTRUCTION OF THE MANUAL RESPIROMETER SYSTEM:-

Respirometers into which test organisms are introduced can be either open flow through systems (having oxygen electrodes monitoring inlet and outlet water), or closed with an oxygen electrode inserted into the chamber and subject to periodic flushing. Flow through chambers are often used when it is important to regulate experimental conditions continously (Niimi, 1978), but such methods have been shown to be affected by lag between the physiological response of the test organism and the detection of that response by the electrodes. The degree to which this lag effect is manifested is directly related to the rate of water flow through a respirometer chamber (Fry, 1971; Niimi, 1978). Lag problems are reduced by using closed respirometer chambers subject to intermittent flushing. Rate of oxygen production and consumption by a test organism are measured by calculating the difference in dissolved oxygen in the respirometer chamber between two consecutive samples.

For this investigation the initial requirements were for a respirometer to be used *in situ* at a depth of 10-metres. The chambers were to be sampled manually at two hourly intervals over a 12-hour daytime

period. Colony respiration was to be measured in a similar manner with the respirometer chambers darkened by a neoprene sleeve to prevent photosynthetic production.

Theoretical chamber volumes were calculated prior to construction following respirometric data from *Montastrea cavernosa* at Discovery Bay (Davies, 1977). A volume of 750 ml was considered adequate for colonies displacing 250-300 ml.

Prototype respirometers were constructed from extruded 0.625 mm (wall thickness) perspex tubes cut to 18cm lengths. The upper section of the tube was sealed using 0.625 mm perspex sheet cut to the diameter of the tube. The lower section was sealed by a removable perspex sheet fitted with four stainless steel snap fasteners, clamping it to the tube against an O-ring fitted into a machined groove on the sheet. An access port (1.0 cm diameter) was cut into a side wall of the tube and sealed with a 2.0 cm self sealing vaccine vial cap. Water samples for oxygen analysis at the surface were extracted through the access port using a 10 cc syringe fitted with a 22-guage hypodermic needle. Water in the chambers was kept in constant motion by a magnetic stirring assembly consisting of a Portescap (Escap-15) 6-volt motor fitted with an Escap E-16/18.6:1 reduction gear head giving 228 RPM at 1.5 volts driving a circular magnet (Figure 3-1, Appendix 1-1). The motor and magnet assembly were fitted into a machined perspex cylinder and sealed by tightening against an O-ring on the upper perspex sheet, and by a screw cap which also acted as an on/off switch tightened onto an O-ring on the top of the machined perspex rod.

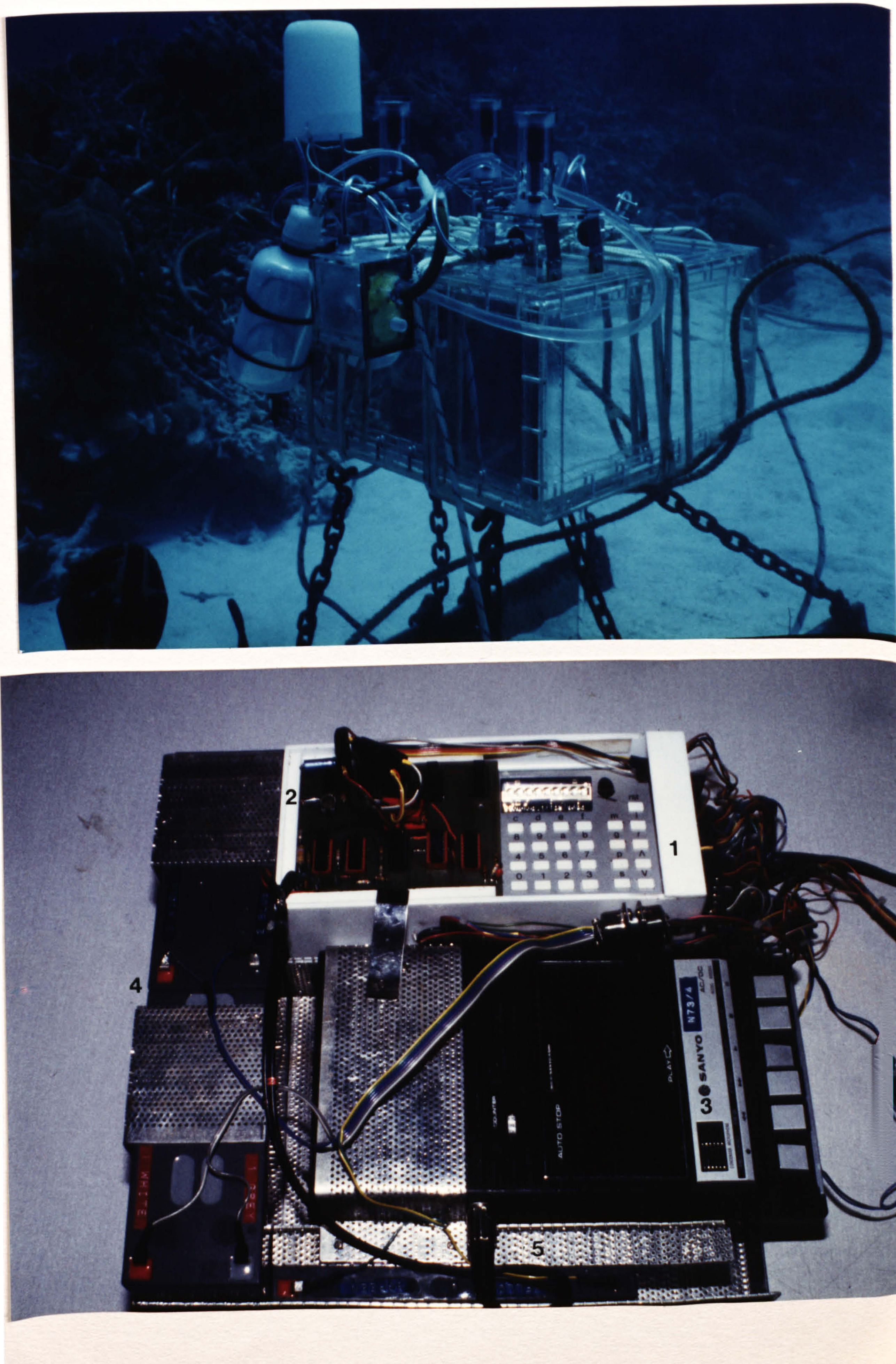
Water samples from 2 respirometers containing coral colonies and a third empty control chamber were extracted at two hourly intervals. At each

sampling interval two 10 ml aliquots were extracted from each chamber, the second following a complete manual flushing of each chamber and serving as the initial sample for the next interval. The water samples were transported to the surface where their oxygen content was determined using a Radiometer type E5046 microcathode oxygen electrode connected to a battery powered oxygen meter. Three oxygen determinations were carried out on each sample.

The results from experiments using this apparatus were reasonably successful, but several points became clear and had to be addressed if the investigation was to continue as an *in situ* exercise. Firstly the volume of the chambers though calculated theoretically was unsuitable to the investigation of an energy budget. The large volume was designed to accomodate corals displacing approximately 300 mls, but it was found that corals of these dimensions harboured large numbers of cryptic organisms which interfered and masked oxygen production and respiration by the coral zooxanthellae symbiosis. The solution to this problem was to redesign the respirometers and reduce their volume to 150 ml to accept smaller colonies thus reducing the risk of heavy infestations of cryptic organisms in the test colonies. The second problem was that the the manual collection of water samples was found to be grossly inefficient. Data resolution was low with data points every two hours, and repeat diving (6-8 dives per day) was dangerous. The only solution to this problem other than transferring the experiments to the laboratory was to design a totally new system capable of collecting data automatically on the reef for long periods of time. This new system would retain the succesful features of the previous one which in this case was the magnetic stirring assembly, and would require dives only to position and retrieve the apparatus.

FIGURE 3-1.
The prototype Mark I automatic respirometer photographed at 10 meters on the fore reef at Discovery Bay. Respirometer chambers were mounted on the upper surface of the outer housing. The entire system was anchored to the bottom using a 100kg iron beam and mooring chains. The respirometer flushing pump is secured to the left with a collapsible 1.5 liter polyethylene bottle to equalize internal pressures with ambient pressures at depth.

FIGURE 3-2.
The electronics package for the Mark I automatic respirometer.
1) ACORN 6502 based microcomputer (keypad and display visible)
2) Cassette recorder interface circuitry
3) Sanyo cassette recorder
4) 2 x 12 volt 5.7 Ah accumulators - main power supply.
5) 1 x 6 volt 1.1 Ah accumulator to drive the tape recorder


1
2
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SANYO
N7374
AUTO STOP
PLAY

THE DESIGN AND DEVELOPMENT OF THE PROTOTYPE AUTOMATIC RESPIROMETER:-

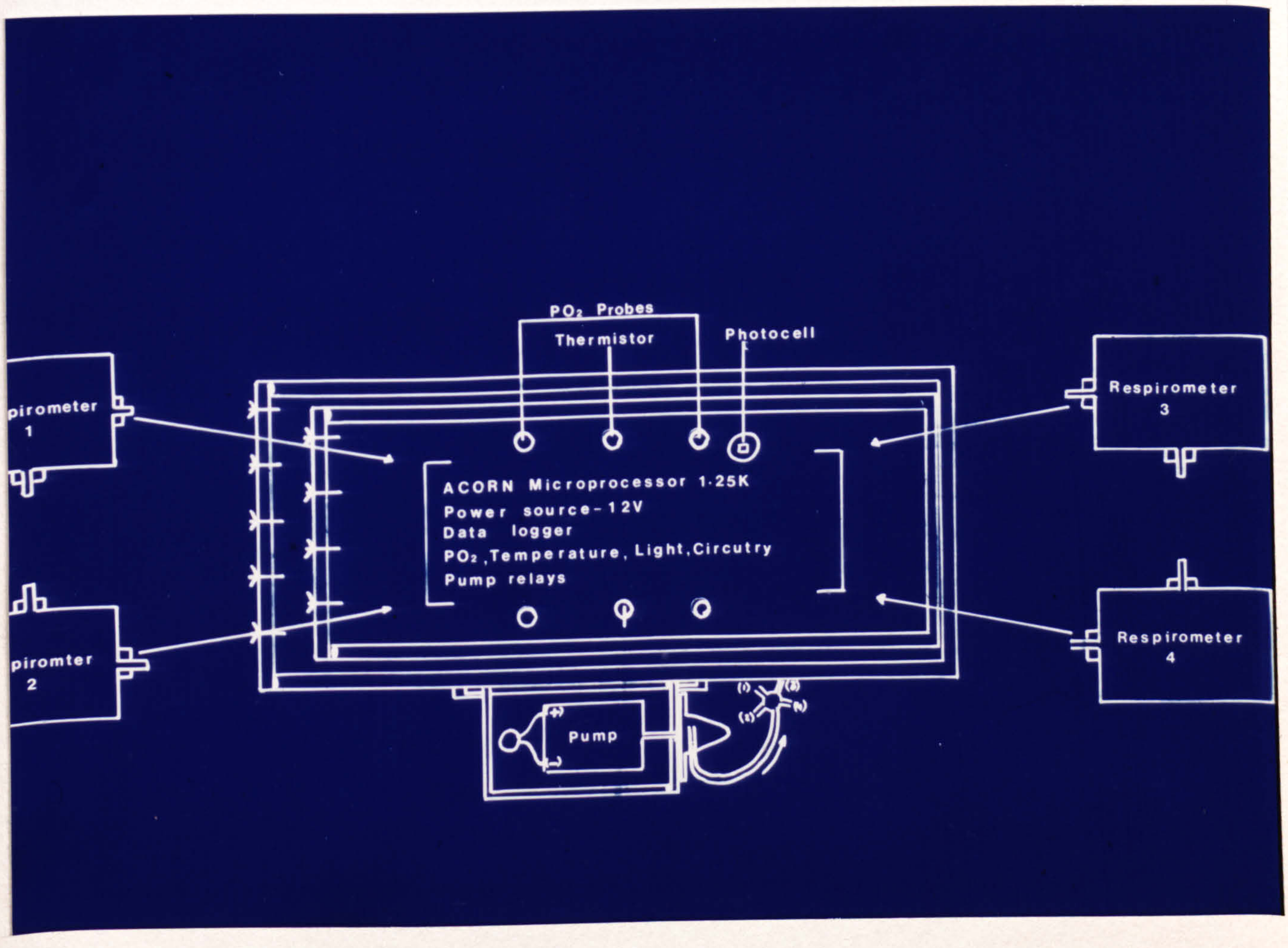
Automatic respirometers and data logging units deployable underwater were not available commercially when the decision was made to adopt this as the solution to *in situ* respirometry. A prototype automatic respirometer consisting of a microprocessor controlled logging unit, probes and their circuitry, a flush pump, a cassette recorder, hermetic housings, and respirometer chambers was constructed (Figure 3-1,3-2). In this section aspects different from those of the second generation (Mark II,III) devices will be described, common points will be refered to Appendix 1-1 a description of that apparatus (Pearson *et al*, 1984).

MICROPROCESSOR CONTROLLED LOGGING UNIT AND PROBE CIRCUITRY - MARK I:-

There were two major differences in design philosophy between the prototype Mark I design and the second generation Marks II and III logging units. Firstly the prototype device used 8-bit accuracy as opposed to 10-bit accuracy used in later models of the apparatus. The data were also stored as 12-bit numbers allowing the detection of parity errors or incomplete data values which were flagged prior to being stored in memory, and rejected when the data were later decoded. Secondly all operations carried out by the prototype (Mark I) unit were done in hardware, these included analogue to digital conversion of the signal from the probes, and the serial transfer of data temporarily stored in computer memory to cassette tape. These operations were controlled by software in the later devices thus providing more flexibility and higher precision more simply and cheaply.

Serious omissions in the prototype unit included no provision for field evaluation of recorded values, and more importantly there was no

FIGURE 3-3.
Exploded diagram depicting the prototype (Mark I) automatic respirometer/data logger and showing the two hermetic perspex housings, the electronics package contained in the inner housing, respirometer flushing pump and its housing, and 4 respirometer chambers anchored to the outer housing.


PO_2 Probes
Thermistor
Photocell
Respirometer 3
ACORN Microprocessor 1·25K
Power source-12V
Data logger
PO_2, Temperature, Light, Circutry
Pump relays
Respirometer 4
Pump

power down facility so that the rapid battery drain resulted in short deployment times.

PROBE ARRAY:-

The probe array used in the prototype automatic respirometer consisted of;

1) four Radiometer microcathode oxygen electrodes (Radiometer E5046).

2) a linear thermistor encased in a steel shaft (Analogue Devices).

3) an internally amplified photocell sensing in the 300 - 1200 nm wavelength range (RS Components).

RESPIROMETER CHAMBER FLUSHING PUMP:-

The respirometer flushing pump was constructed from a 6-volt high speed motor (Marx-Luder M-EM141P) enclosed in a hermetically sealed perspex housing. The pump head was a magnetically coupled Eheim centrifugal pump. The pump assembly was powered by the main computer 12-volt 5.7 amp. ere hour accumulators via a voltage reduction and regulating circuit. The heat generated in the voltage step down was dissipated by means of a large metal heat sink fastened to bulkhead coonectors in the hermetic housing.

DATA LOGGER HOUSING DESIGN:-

The design of a hermetic housing for the prototype device required the selection of a material capable of withstanding hydrostatic pressures up to 4 atmospheres, and a material easily repaired in the field. Following consultations it was decided that the most suitable material would be perspex, and this was used to contruct hermetic housings to contain the data logger (Figures 3-1,3-2,3-3).

electronics, and an outer one through which the probes were led to the respirometers acting as primary protection to the apparatus. Both units were constructed from clear perspex sheet (1.5 cm thick) which was glued and fastened by brass screws at 5 cm intervals. All joins were strengthened further by full length splines cemented internally. These were also meant to improve the sealing of the housings. Access to the interior of each unit was by a removable perspex plate over the open end. These were fixed to the housings by means of .625 cm brass studs and wing nuts spaced at 5-cm intervals, the units were sealed by tightening these, pressing the sealing plates against a neoprene gasket(Figure 3-2).

All power connections and control wiring to the pump, and all probe cables were passed to the exterior using 10 mm Swagelok bulkhead connectors. All wiring from the outer housing to the exterior was passed through clear polyethylene tubing filled and sealed with a two part elastomer potting compund (Sylgard 184).

The two hermetic housings were tested in the laboratory and were cleared for *in situ* deployment at working depths of 10 meters and 30 meters. When subjected to working pressures there was a torsion effect resulting from an improperly constructed outer housing with unequal dimensions at the two ends, the closed face being 3.7 cm larger than the open one. The torsion effect resulted in severe stress cracks on all joins of the outer housing, and substantial flooding when immersed. This problem was eliminated in the field by reinforcing all joins externally with perspex sections glued with hot perspex cement, and constructing an internal framework of aluminium struts to prevent torsion.

head (of plastic construction) resulted in stress cracks in the centrifugal pump back plate. These cracks allowed an aerosol of seawater to be admitted into the dry motor housing. As a temporary solution the pressure differential was eliminated by injecting a volume of air into the motor housing to equalize the internal pressure to ambient pressures. This was done by attaching a collapsible 1.5 liter polyethylene bottle to the pump motor housing which automatically equalized internal pressures with increaing depth (Figure 3-1).

RESPIROMETER CHAMBERS USED IN THE MARK I PROTOTYPE:-

Respirometer chambers were redesigned and their volume reduced to 450mls. The construction of these chambers and a schematic diagram is described in Pearson *et al*, (1984) - Appendix 1-1.

ASSESSMENT OF THE MARK I AUTOMATIC RESPIROMETER:-

There were few redeeming features of the prototype (Mark I) automatic respirometer, and as a result major changes were necessary to improve the apparatus, improve reliability, and ensure data returns during each instrument deployment on the reef.

The hermetic housing used in the Mark I prototype was totally unsuitable. The use of two large perspex housings meant that the inherent bouyancy of the system had to be overcome before it could be submerged, and once submerged required a massive anchoring sytem consisting of a cast iron beam (100 kg) and six lengths of chain to hold the housings on the beam (Figure 3-1). The technique used to seal both housings (brass bolts and wing nuts) was inefficient resulting in uneven pressure of the sealing perspex plate on the sealing gasket. No provision was made to protect the probe array from damage during transit to and from the study site. Only the

respirometer chambers with their magnetic stirring assemblies, and the Swagelok bulkhead connectors were retained from the Mark I version of the apparatus and incorporated into the Marks II and III automatic respirometers. Following criteria adopted from the failures of the Mark I prototype, a new smaller hermetic housing of steel construction was used in the later Marks of the apparatus. The construction of this housing is described in Pearson *et al*, (1984) and included in Appendix 1-2.

A combination of electronic design faults, inadequate programming of the ACORN computer controlling all functions of the system, and power supply problems meant that no useful data was collected from experiments using the Mark I system. But these problems were instrumental in designing the final functional version of the system (Mark III), and must therefore be included for the record in this section.

The first automatic respirometer data logger suffered from a series of small deficiencies that in conjunction produced a device constantly needing field repairs. Each component worked when tested independently, and together when bench tested in the laboratory, but when used *in situ* secondary problems not anticipated in the laboratory corrupted the data stored in RAM (Random Access Memory in the computer) and consequently on magnetic tape. The main cause of data corruption was a sudden voltage drop and power line noise when the respirometer flushing pump was operating. This problem was eliminated from the later versions by running the pump through a clamped voltage regulated switching circuit(Pearson *et al*, 1984 and Appendix 1-2). A second source of data corruption, one not identified until the second device was constructed, was the need to fully isolate the oxygen electrodes from seawater. Failure to do this properly resulted in signal sinking and nonsense data being stored in memory and transmitted to

tape. A full description of the probe isolation procedure is given in Pearson *et al* (1984), and Appendix 1-1.

In the prototype (Mark I) automatic respirometer the ACORN microcomputer was programmed using a main supervisory program that called a series of subroutines to execute such tasks as respirometer chamber flushing, serial transfer of data stored on RAM to tape, probe calibration, and respirometer sampling intervals . All programs were written in assembly language, assembled on a Commodore Pet (3032) and stored on tape. In the field the unit was programmed and calibrated using these program tapes, and placed on standby mode pending transport to the study site. At the conclusion of a logging run the machine was retrieved and returned to the laboratory. In this early device there was no facility for data playback in the field, and therefore no way of identifying malfunctioning probes, flushing cycles, or the end of a run. In addition there was no provision in the program to deal with rapid rates of oxygen production supersaturating the seawater contained in the respirometers, or rates of respiration of sample corals depleting oxygen concentrations in the chambers to dangerously low levels. The problems encountered in the prototype device were corrected in later versions. Programs necessary to operate and control later versions of the system were burned into a 2K EPROM (Erasable Programmable Read Only Memory). Therefore with operating programs in hardware, access and operation of the system was both quicker and less prone to programming errors from misread cassette tapes. A data playback program was written (Appendix 1-3), and a Texas Instruments TI 5010 printing calculator converted to a field printer. Data recorded on magnetic tape were inspected in the laboratory using this apparatus so that anomalies detected in the data identified faults in the hardware which could then be rectified prior to the next deployment.

THE SECOND GENERATION (MARK II,III) AUTOMATIC RESPIROMETERS/DATA LOGGERS

Following *in situ* experiments using the Mark I prototype system a totally new machine incorporating the features already noted was constructed. The new automatic respirometer/data-logger/remote experiment controller (Figures 3-4, 3-5, 3-6) is fully described in Pearson *et al*, (1984), and is included as Appendix B-2 which also includes all the necessary circuit diagrams and programs needed to construct and operate such a device. Only a critical assessment of the Mark II and III versions will be included in this section.

The second generation logger was used over two field seasons and based on that experience a series of modifications or improvements could be incorporated in future versions of this machine.

The dimensions of the steel hermetic housing made the apparatus both extremely portable and stable underwater, but the use of two dissimilar metals (brass and steel) in the construction of the main housing and the pump housing, and the great weight (48 kg) of the system created problems. The use of two dissimilar metals in seawater can result (as in this case) in severe electrolytic reactions that accelerate corrosion in both metals. To reduce corrosion the steel housing was protected and isolated from seawater by two coats of two part marine epoxy paint (International Marine 370), and the brass pump housing by several coats of clear polyurethane. The design of future housings should ensure that all metals exposed to seawater are the same, and if this is not possible a sacrificial zinc anode should be fixed to the housing to reduce the corrosion. The weight of the housing posed a serious threat to divers transporting the system to a study site. To reduce this danger a 50 kg adjustable bouyancy lifting bag was

FIGURE 3-4.
Mark III development of the prototype design photographed at 10 meters on the fore reef at Discovery Bay. The apparatus as seen on the reef consisted of the hermetic sealed housing containing the electronics package, a respirometer flushing pump contained in a brass housing attached to the main housing, 4 respirometer chambers (4 clear or 4 dark or a combination of both)with magnetically coupled stirring assemblies, sensing probes (4 microcathode oxygen electrodes each sensing a separate respirometer chamber, a thermistor inserted in the lower left respirometer, and a photocell in the main housing).

FIGURE 3-5.
A detailed view of the main housing showing 1)-ACORN microcomputer keypad and display, 2)-magnetic reed switch to activate standby mode and trigger the end of run instructions, 3)- respirometer flushing pump housing with the pump inlet manifold seen to the left 4) transit protection plate for the probe array, 5) carrying handle.

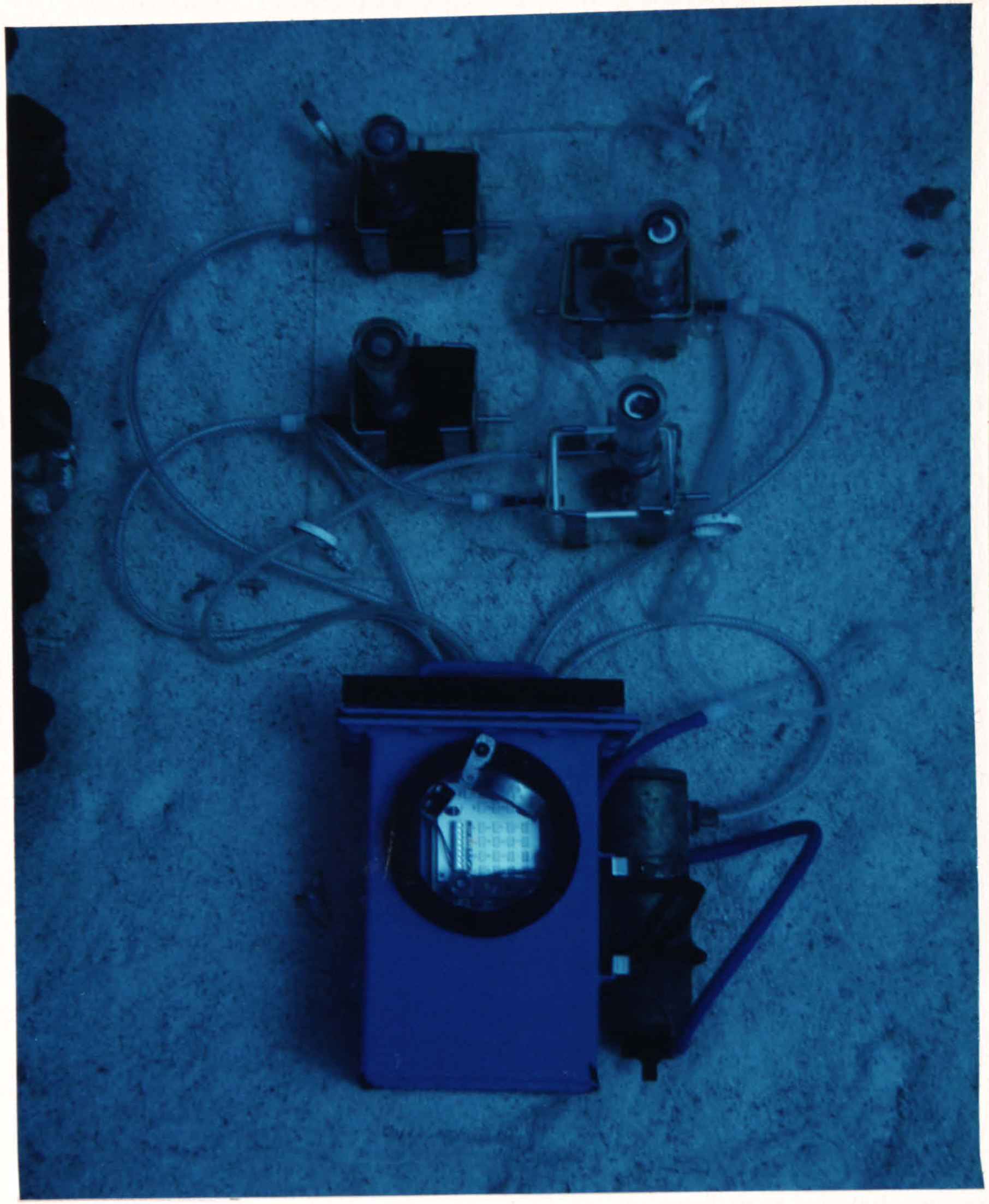


FIGURE 3-6.

Components of the Mark III automatic respirometer/data logger;

1) Hermetic steel housing protected by a two part epoxy paint

2) Perspex viewing port (2.54cm thick) and anchoring bolts.

3) Magnetic reed switch - control for standby mode.

4) 2 x 12 volt 5.7 Ah sealed lead acid accumulators

5) Light emitting diodes (green = standby,red=logging)

6) Photocell.

7) ACORN 6502 based microcomputer with keypad and display.

8) Steel sealing plate carrying the racked electronics.

9) Probe circuitry, one plate per probe.

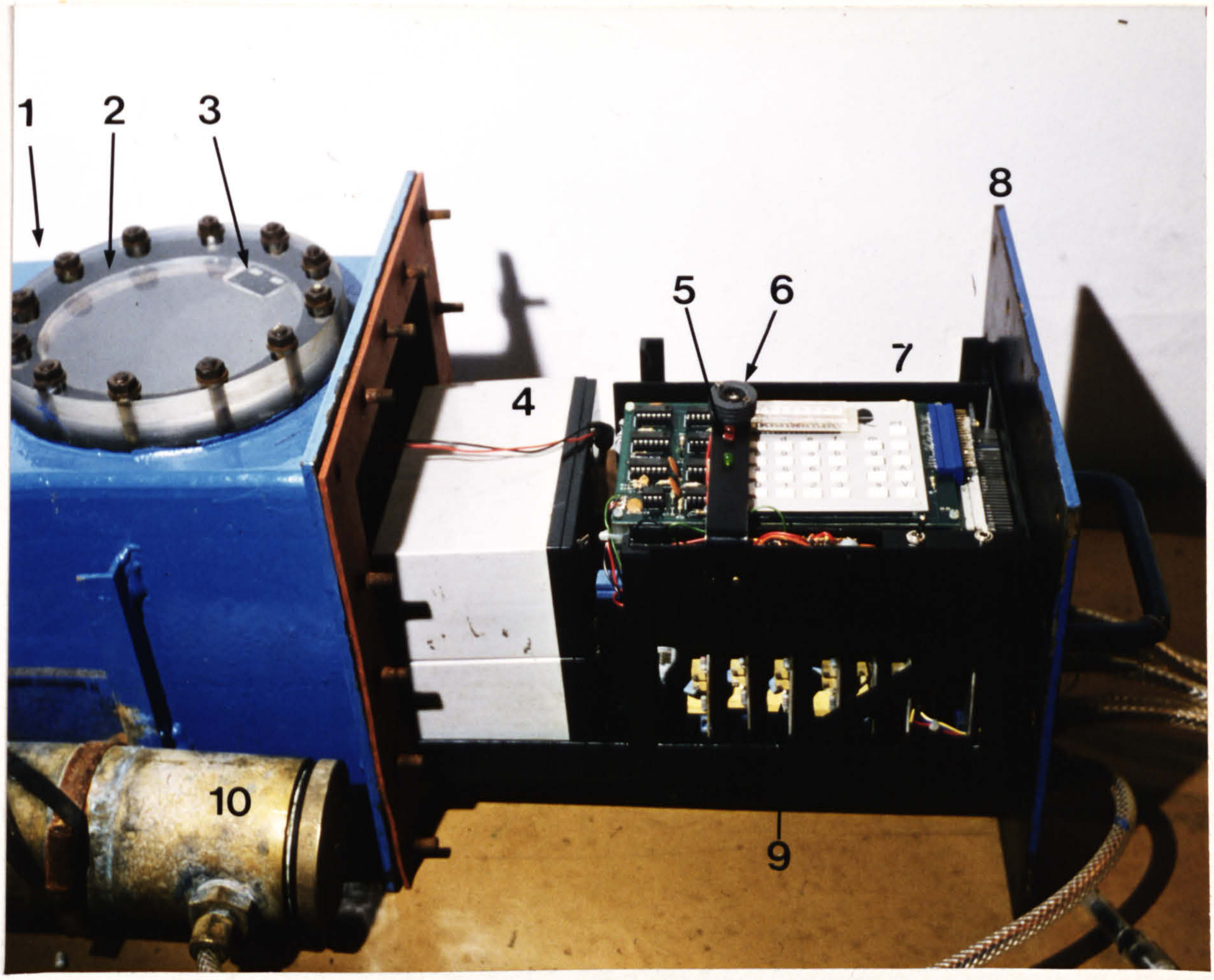
10) Flush pump motor hermetic housing (Machined brass).

11) Probe array in reinforced tubing sealed with Sylgard.

12) Standby switch and magnetic reed switch jack socket.

13) Sealing gasket.

14) Cassette recorder.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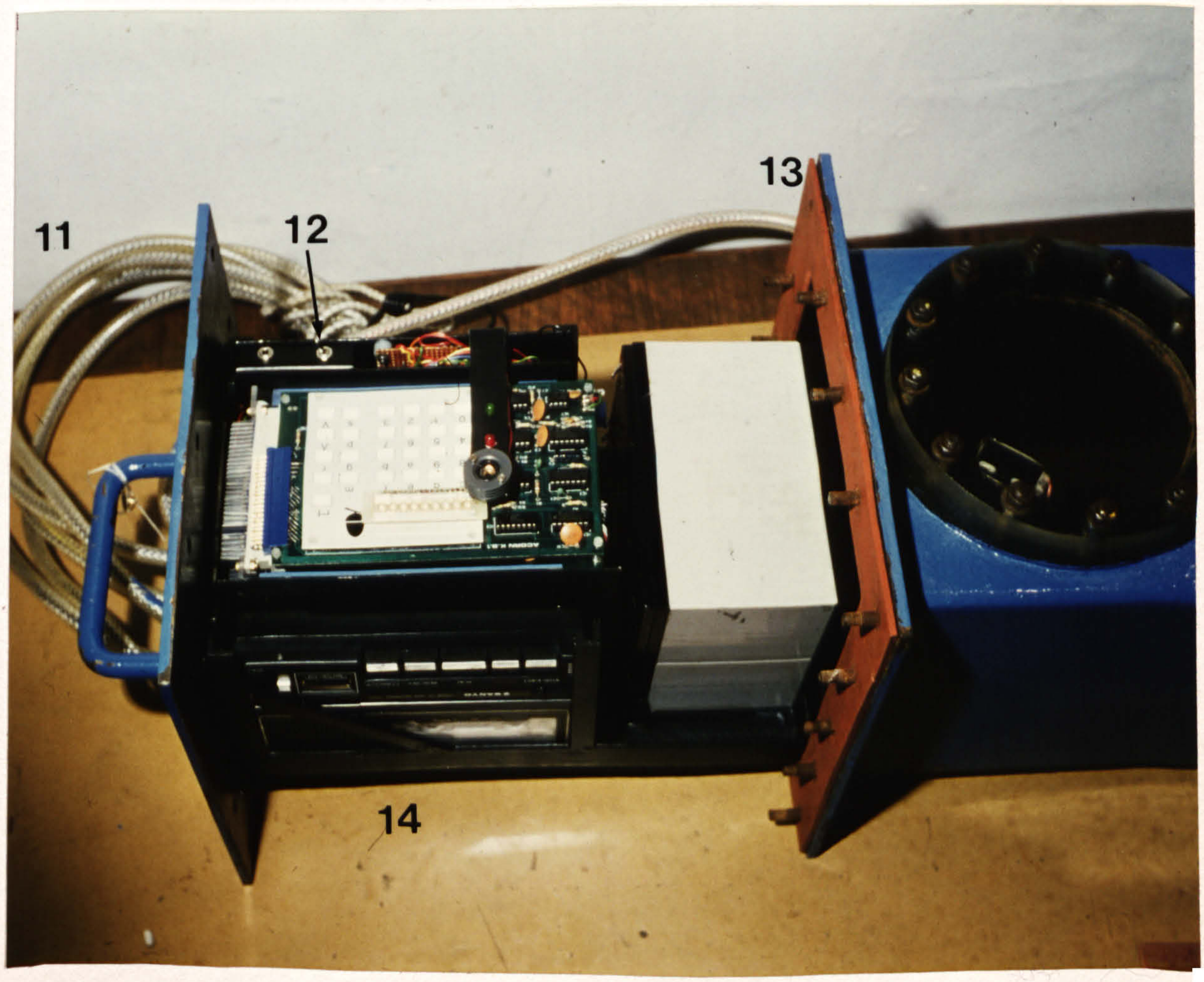
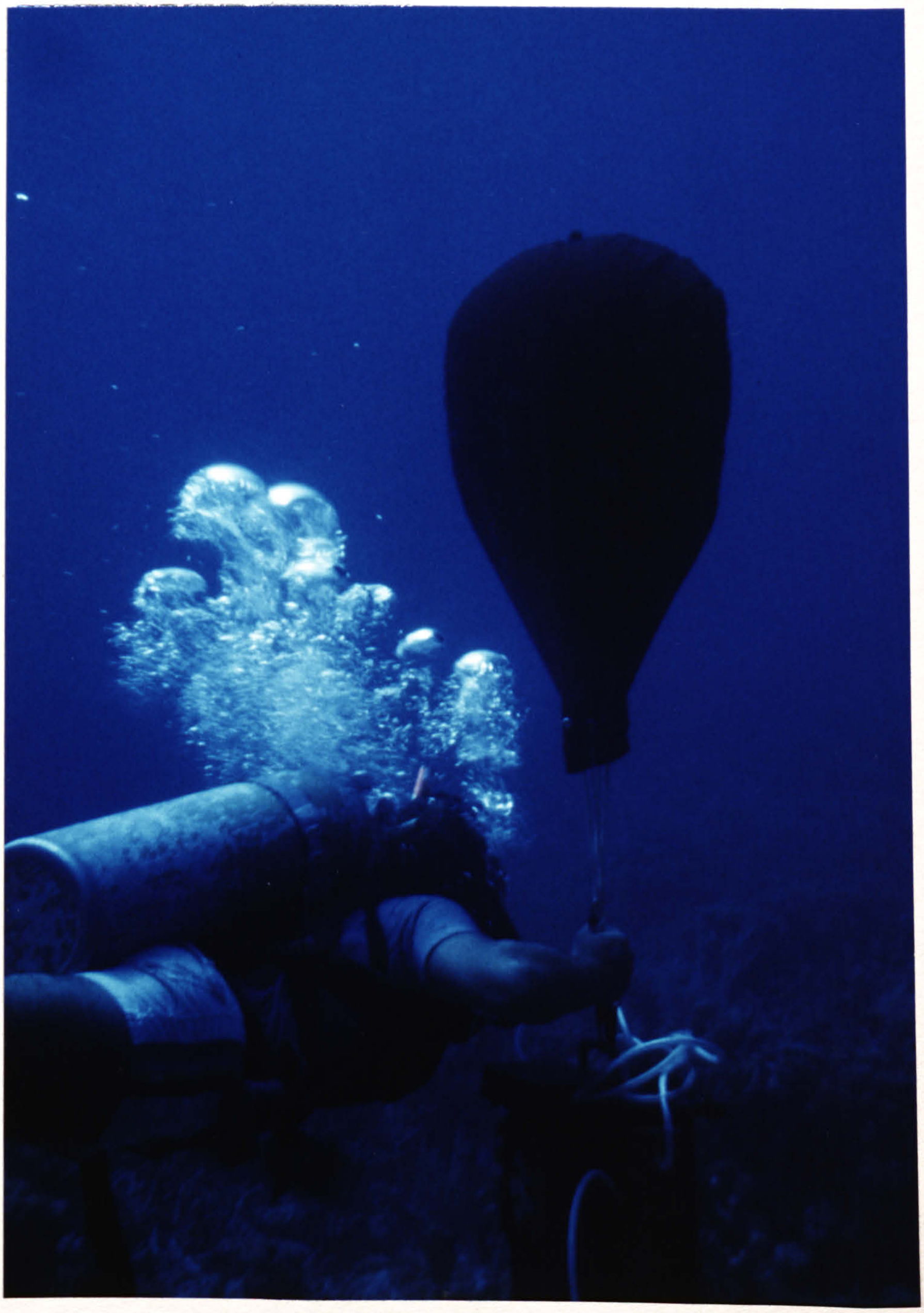

11
12
13
14

FIGURE 3-7.
An adjustable bouyancy lifting bag was required to safely transport the automatic respirometer /data logger (weighing 48 kgs) to the respective study sites.



attached to the main housing to achieve neutral bouyancy underwater (Figure 3-7). Future underwater housings should be constructed from lightweight alloys, aluminium, or synthetics such as honeycombed fiberglass, carbon fibre, or industrial ceramic compounds such as those used in experimental lightweight engines.

Sealing gaskets and bulkhead connectors such as those used in the Marks II and III loggers performed well, but were subject to cracking and leakage problems. Sealing gaskets should be avoided where possible in favour of O-rings in properly machined grooves. Bulkhead connectors for use in underwater instrumentation should be dedicated specialized items and not the less expensive gas fittings used in this investigation.

The programs necessary to operate and control the automatic respirometer (Appendix 1-3) were very effective, but problems did arise that should be addressed in future developments. The main difficulty encountered was that of malfunctioning oxygen electrodes. Faulty electrodes were expected to produce a 1024 value which was recognized by the computer and ignored, but when electrodes were intermittently misreading in a cyclic pattern (as was the case with grounding problems), a lower value was recorded and the respirometer flushing pump was activated. In future developments malfunctioning probes should be made to read zero, and power to that probe should be discontinued for the duration of the experiment. A further addition to the supervisory program controlling the flushing subroutine would be to initiate a flushing cycle if the oxygen concentration in the respirometer chambers fell below 20% of the starting value for a particular hour period. In the present system a flushing cycle was triggered only if the oxygen concentration in the chambers rose 20% above the starting values of an hourly interval.

The power supplies for the system were also a source of difficulties and aggravations. The use of maintenance-free 12 volt 5.7 Ah accumulators would have been a good choice had the charging current at Discovery Bay been constant. The voltage of charged batteries was checked prior to instrument deployment, but the maximum period in which data was recorded was 21 hours during any 24 hour run. For the most part the accumulators only managed to power the system for an average of 9.7 hours for n=14 runs. No electrical faults were found to account for the rapid power rundown, so only the charging system was suspect. Future developments should dispense with rechargeable accumulators and use suitable nickel cadmium batteries which can be charged over a longer period at reduced voltages

The design and development of an automatic respirometer/programmable data logger was a valuable exercise that reduced the constraints of an *in situ* investigation, and was instrumental in the successful completion of this research program. As it stands the device does produce data, this alone justifies the time spent in development, but further development primarily in terms of housing design, data storage, system control programs, and power supplies would make this a very powerful and useful tool in any investigation requiring remote data gathering stations for *in situ* experimentation.

AUTOMATIC RESPIROMETER - FIELD TECHNIQUES AND DATA PROCESSING

This chapter and Pearson *et al* (1984) have described the design and development of the automatic respirometer data logger used in this investigation as well as the programs necessary to operate the system (Appendix 1-3). In this section the procedures required to analyze the data

FIGURE 3-8;

This flow diagram illustrates the steps necessary for the operation of the Mark (III) automatic respirometer/data logger. In order to ensure that the system functioned once on location, each of these steps was carried out during each experimental run.

START

Clean and grease all sealing surfaces and gaskets

Clean all probes, and replace faulty membranes on PO_2 electrodes and isolate them from seawater using silicon grease and teflon paste

Connect freshly charged accumulators after checking the voltages

Check for electrode interaction in a seawater bath

ELECTRODE INTERACTION?

yes

no

Rough calibrate PO_2 electrodes in a stirred seawater bath, check thermistor and photocell, calibrate if required

ALL ELECTRODES IN RANGE ?

no

yes

Load running program, test flush pump, position magnet over reed switch to place equipment in standby mode

Insert new cassette tape into the recorder, press play/record levers, check recorder levels, and insert control jacks.

Seal logger into its steel housing, and transport to the study site on the reef.

Clear all air bubbles from the manifold tubing and respirometer chambers. Insert test corals into the chambers. Start the experimental run by removing the magnet.

Leave *in situ* for 24 hours.

Retrieve apparatus and replace corals in their respective treatment groups. Return the logger to the laboratory.

Open the steel case, remove the logger, rewind the cassette tape, connect the field printer. Print out the data on a paper strip, and check for electrode faults. Remove the accumulator pack and connect to the charger. Remove charged accumulator pack from the charger.

RETURN TO START

collected during an experimental run are described. The section is intended to complement the material presented in Pearson *et al* (1984) and Appendix 1-2. A full description of the steps necessary to prepare and operate the system are shown as a flow chart (Figure 3-8) and are fully explained in the published paper (Appendix 1-1).

DATA RETRIEVAL:-

Data logged during an experimental run and stored serially on magnetic tape had to be retrieved and decoded for final analysis. There were two retrieval requirements, firstly the data collected in the field had to be examined after each run, and problems with the probes or the electronics corrected prior to re-deployment, and secondly data had to be fully analyzed and processed in the laboratory in Glasgow University.

Of the two requirements listed the first was of paramount importance since it would provide a means of visually verifying the data as it came in, and implementing changes in the system to improve data return. In order that preliminary data analyses could be carried out in the field a hard copy of the material stored on tape was essential. Commercial printers compatible with the ACORN microcomputer were found to be too expensive leaving two options, either to load recorded data blocks from cassette tape to computer memory and manually step through and note the memory contents using the keypad, or to construct a simple printer compatible with the ACORN computer by adapting a printing calculator. Of the two choices the second was selected and a Texas Instruments (TI 5010) printing calculator converted to behave as a computer serial printer for decimal numbers.

For the calculator to function as a printer the connections to the button matrix required to display and print a given character (0 - 9), a

paper printing code, and a paper feed code were determined. The internal logic sequence (Figure 3-8) showed that each number character including the printing code and paper feed code required two separate decoding pulses. By constructing a chart showing the desired characters, the ACORN codes necessary to produce them, and the calculator logic (Figure 3-9), it was possible to design external decoding circuitry which would interface the printing calculator with the computer.

The external decoding interface circuitry (Figure 3-10) was a simple 4-line to 12-line decoder where recorded data *wer* accepted as successive digits from the ACORN computer through Port B bits 2 - 5. These data lines were each stabilized by a 47K resistor connected to the ground line preventing spurious pulses from corrùpting the data entering the 4514 (4-bit latch) chip. The 4514 decoded the decimal values entered and activated the corresponding 4066 CMOS switches, so closing contacts in parallel with the buttons in the printer to both display the decoded value from the ACORN memory on the calculator display, and print it on the paper strip.

The data replay program necessary to check the data in the field (Appendix 1-3) also included a data transmission delay subroutine (a 300 msec timing loop). This was necessary to ensure that no values were lost when driving to the printer due to the relatively slow data entry on the calculator, and was determined by the printing rate of the converted calculator. The programmed transmission delay coupled to an internal storage and display of 10 digits was enough to ensure that no recorded values on cassette tape were lost when transmitted to the printer.

Final data processing including conversion to appropriate units,

CHARACTER	ACORN CODES PORT-B 2 3 4 5	HEXADECIMAL BEFORE INCREMENTING
0	*	0
1	*	1
2	* *	2
3	*	3
4	* *	4
5	* *	5
6	* * *	6
7	*	7
8	* *	8
9	* *	9
print	* * *	A
paper feed	* *	B

FIGURE 3-9:- ACORN computer codes necessary to produce characters on the printer calculator

NUMBER	CONTACT ON CALCULATOR EDGE CONNECTOR TO PRODUCE THE NUMBER 1 2 3 4 5 6 7 8 9 10 11 12 13 -- pin numbers
0	* * 6 and 11
1	* * 6 and 10
2	* * 6 and 9
3	* * 6 and 8
4	* * 6 and 7
5	* * 7 and 13
6	* * 7 and 12
7	* * 7 and 11
8	* * 7 and 10
9	* * 7 and 9
print	* * 8 and 10
paper feed	* * 3 and 4

FIGURE 3-9:- Pin connections on the printing calculator edge connector necessary to produce characters 0 to 9 on paper tape, to print the value, and to advance the paper strip in preparation for the next number to be printed. There was a 300 msec delay required in the transmission of data from the computer to the printer in order that data did not overflow the memory buffer in the calculator (10 numbers).

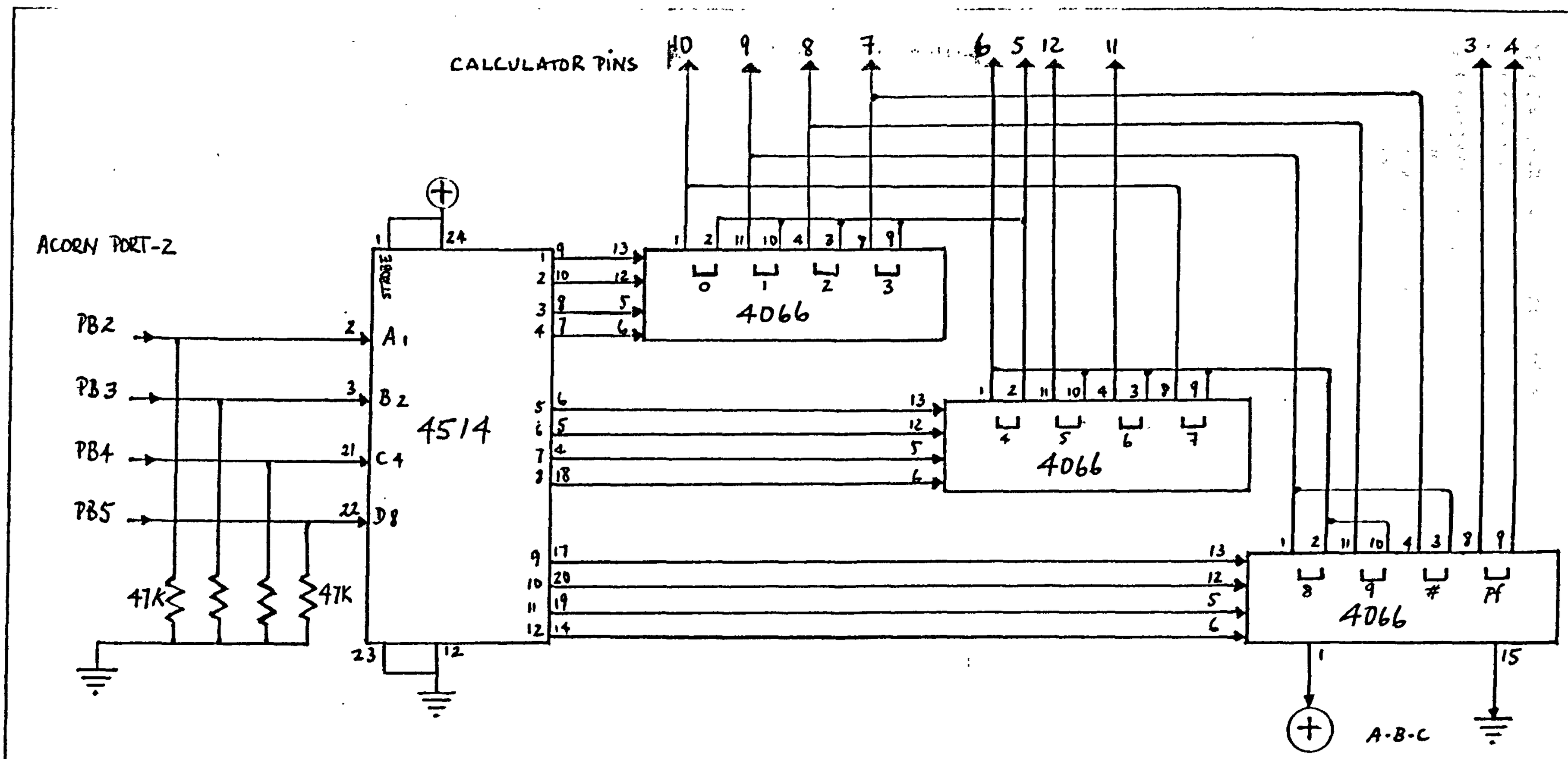


FIGURE 3-10.

Circuitry necessary to decode recorded data on tape using a TI-5010 printing calculator. This simple circuit was a 4-line to 12-line decoder where data was accepted as successive digits from the ACORN, and eventually printed on a paper strip.

preliminary statistical analysis, and plotting of the converted data points was carried out at Glasgow University using a CBM- PET (3032) 6502 based computer driving a Digiplot X-Y Plotter.

Data stored on cassette tape in the field were replayed through decoding circuitry in blocks of 252 bytes transmitted serially to the PET computer. Data were displayed on the VDU as they were transmitted. Where necessary the values were edited on the screen (ie)-if the range of three values recorded at a given 5-minute interval for a given channel was greater or less than 5 units. When this condition arose the program halted and the computer questioned the validity of the recorded values, and requested the desired average value from the operator. Once a block of 252 bytes was transmitted from magnetic tape and edited in the computer, a hard copy was printed on an Epson MX-80 dot matrix printer and simultaneously recorded on a floppy disk . Data recorded on disk could then be recalled at any time for final analysis. The program required to do this is listed in (Appendix 1-3).

CONVERSION OF RECORDED DATA TO ACCEPTED UNITS FOR FINAL ANALYSIS:-

Before data recorded on disks could be analyzed the values (in ACORN computer units) had to be converted to real values. The formulae required were programmed into the PET computer (Appendix 1-3), and are listed below.

1) Dissolved Oxygen Concentration in Respirometer Chambers:-

Variables:-

a) Starting oxygen value from winkler titration : input D.
b) ACORN units corresponding to starting value a) : input E.
c) Respirometer volume - sample displacement : input V.
d) Average three values taken after a 5-min.period : labelled AV.
e) Oxygen value conversion coefficient : labelled QT.
f) Oxygen value : labelled OV.
g) Oxygen value from previous 5-minute period : labelled OZ.

FORMULAE;

i) Oxygen conversion coefficient (QT) = $\frac{D}{1000} \times \frac{V}{E}$

where 1000 = 1000 mls.

ii) Oxygen value after a given 5-minute period (OV) in ulO^2

$$(OV) = \frac{AV \times QT}{(1 + 0.03(TE - 26.5°C)}$$

where the average of the three recorded values after any 5-minute period (AV) is multiplied by the oxygen conversion coefficient (QT), and the resulting value is corrected for temperature drift (± 3% per °C Severinghaus and Bradley, 1971) . The value is then converted to an hourly rate using the oxygen value from the previous 5-minute interval that had been stored as OZ using the formula

$$DR = (OV - OZ) \times 12$$

The value DR of O_2 in ulO_2/hour was then plotted using the digiplot X-Y plotter.

Conversion of Temperature Recordings from Respirometer Chambers:-

The linear temperature probe sensed changes in seawater temperature inside the respirometer chambers, and the recorded values were used both as a record of temperature change over an experimental run, and to correct the oxygen probes for temperature drift.

VARIABLES:-

a) Calibrated temperature (low value, 0°C) : input L
b) ACORN units corresponding to (a) : input A
c) Starting temperature *in situ* (26.5°C) : input H
d) ACORN units corresponding to (c) : input B
e) Temperature conversion coefficient : labelled TS
f) Temperature value at end of a 5-min. interval : labelled TE
g) Average of 3-values recorded every 5-minutes : labelled AV

FORMULAE:-

i) Temperature conversion coefficient (TS) = $\frac{(H - L)}{(B - A)}$

ii) Temperature value (TE) = AV x TS since the probe was linear

Conversion of Incident Light Recordings:-

The sensor measuring incident irradiance at the study sites was positioned inside the perspex window of the Marks II and III automatic respirometers.

VARIABLES:-

a) Incident light at working depth 5039±.05uE/m²/min : input G
b) ACORN units corresponding to (a) : input H
c) Light conversion coefficient : label-LQ
d) Incident light value : label-LI
e) Average of 3 values recorded after a 5-min. period : label-AV

FORMULAE:-

i) Incident light conversion coefficient (LQ) = $\frac{G}{H}$

ii) Incident light value for any 5-minute period (LI) = AV x LQ

CHAPTER - 4.

THE ENERGETIC REQUIREMENTS OF TISSUE GROWTH IN *Montastrea cavernosa*.

INTRODUCTION:-

The growth of reef corals has been studied extensively since approximately 1846 (Silliman), with workers investigating coral growth under different environmental conditions (Roos, 1964,1967,1971; Wijsman-Best, 1972; Graus *et al*, 1974; Chamberlain and Graus, 1975; Houck *et al*, 1977; Jokiel, 1978; Rogers, 1979), and growth form as an environmental indicator (Shinn, 1966; Graus, 1975). Recent work has concentrated on the calcification process, and those factors both physical and physiological affecting it. Studies investigating the energetic requirements of growth in hermatypic corals have been carried out by Davies (1984) who used a combination of *in situ* incubations of collected specimens of *Pocillopora eydouxi* at 5-metres followed by buoyant weighing in the laboratory to determine that component of the energy budget which was allocated to tissue growth. In the present investigation colony growth was measured *in situ* to determine the rate of skeletal extension in colonies of *Montastrea cavernosa* at two depths.

The relationship between growth form, growth rate, and zooxanthellar photosynthesis is well documented. Enhanced calcification rates in hermatypic corals in daytime were described by Goreau (1959,1960), and later supported by Vandermuelen (1972) who used DCMU (A photosynthetic inhibitor) to show that daytime calcification rates in treated colonies were reduced relative to untreated colonies. Goreau (1963) and Dustan (1979) have noted that corals such as *Montastrea annularis* showed a strong growth directionality with increasing depth, with deeper colonies having

reduced mass and flattened morphologies optimizing the irradiated photosynthetic surface relative to their shallow water counterparts having more massive and hemispherical morphologies. In addition to these changing morphological patterns with increasing depth there also seems to be a decrease in linear growth with increasing depth suggesting that growth in coral colonies harbouring symbiotic zooxanthellae is a light enhanced or limited process (Buddemeier *et al*, 1974; Dustan, 1975; Baker *et al*, 1975; Bak, 1976; Chalker and Taylor,1978).

Bak (1976) noted that the rate of calcification in corals is related to surface area. He suggested that tissue growth results in an enlarged calicoblastic layer which in turn forms a greater surface for skeletogenesis, work showed that the growth rate of small and large colonies of *Montastrea cavernosa* did not differ significantly, and suggested that the accumulation of calcium carbonate per unit of living tissue was the same for small and large colonies. This view formed the rationale for all growth determinations in this investigation. Tissue growth was considered essential for skeleton extension. Therefore the energy allocated to growth was calculated from predictions of the amount of tissue laid down during a given time interval, and the energy equivalent of a unit biomass of that tissue. It was assumed that tissue growth was a linear function of surface area.

MATERIALS AND METHODS

Several techniques are currently used to determine growth rates of scleractinian corals. These are for the most part destructive methods, measuring the increase in skeletal mass or the linear extension of colonies. The most widely used techniques are listed below;

1) the use of skeletal stains- *in situ* marking with Alizarin Red-S (Barnes, 1973; Lamberts, 1978).

2) laboratory weighing of corals in seawater - Buoyant weighing technique (Davies, 1984).

3) surface area changes measured using the aluminium foil method (Marsh, 1970).

4) weighing of coral colonies *in situ* (Bak, 1976). A difficult and costly technique affected by physical factors (temperature, water density, and water movements).

5) direct measurement of branch extension rates (Chalker and Taylor, 1978)

6) growth rate determinations from X-radiographs of seasonal density bands (Buddemeir *et al*, 1974; Wellington and Glynn, 1983).

For the current investigation these growth measuring techniques were not suitable. The X-radiographic method was rejected since it would require marking the corals at the start of the experiment with a metal compound which could later be recognized during analysis, this technique had no precedent in the literature. Alizarin staining was rejected because in preliminary tests it was found that not all test colonies showed dye incorporation in the skeleton. All mass weighing techniques were eliminated since it was found that colonies of *Montastrea cavernosa* were unpredictably infested by boring cryptic organisms. The presence of boring organisms and the documented removal of skeletal material by these (Goreau and Hartman, 1963; [illegible]; Hein and Risk, 1975; Bak, 1976; MacGeachy, 1977; Pomponi, 1981) would have made these methods unreliable in a long term experiment. The aluminium foil method (Marsh, 1970) was retained but used in conjuction with a photographic method developed for this investigation.

PHOTOGRAPHIC METHOD FOR GROWTH DETERMINATIONS IN SCLERACTINIAN CORALS

Corals were photographed in a vertical plane at intervals to determine linear growth rate. Photography was carried out *in situ* using a Nikonos IV-A underwater camera equipped with a 35-mm lens, a Nikonos close up kit with a 35-mm framer, and a Toshiba TM-II synchronized strobe. All photographs were taken on Ektachrome 400 (Kodak) diapositive film.

Corals at 10-metres were photographed at monthly intervals from 31/8/81 to 31/10/81, and again on 6/6/82. Colonies at 30-metres were not photographed on 31/9/81. Since the 10-metre site was subject to severe ground surge conditions during storms, 10 colonies from the normal treatment group were removed on 31/10/81 for colony growth and tissue biomass determinations. Three of these colonies were used in tissue removal trials and were later discarded. The remaining 7 colonies were used and are included in Table 4-1. These 10 colonies were collected as protection from winter storms to avoid the risk of specimen damage and loss. This precaution was warranted since only two of the remaining 14 colonies in the normal treatment group on the reef remained on site following the winter storms. No colonies were taken from those corals kept in darkened chambers at 10 metres, or from either treatment group at the 30-metre site. In these corals growth was measured over the full 10-month period of the experiment.

All photographs were developed and projected onto a blank sheet of paper. The outline of the projected images including the centimeter scale photographed with each colony (Figure 2-4,2-5) were traced. The area of the traces were determined using a digitizing pad (Watanabe) connected to a Commodore Pet (CBM-3032) microcomputer loaded with the necessary programs

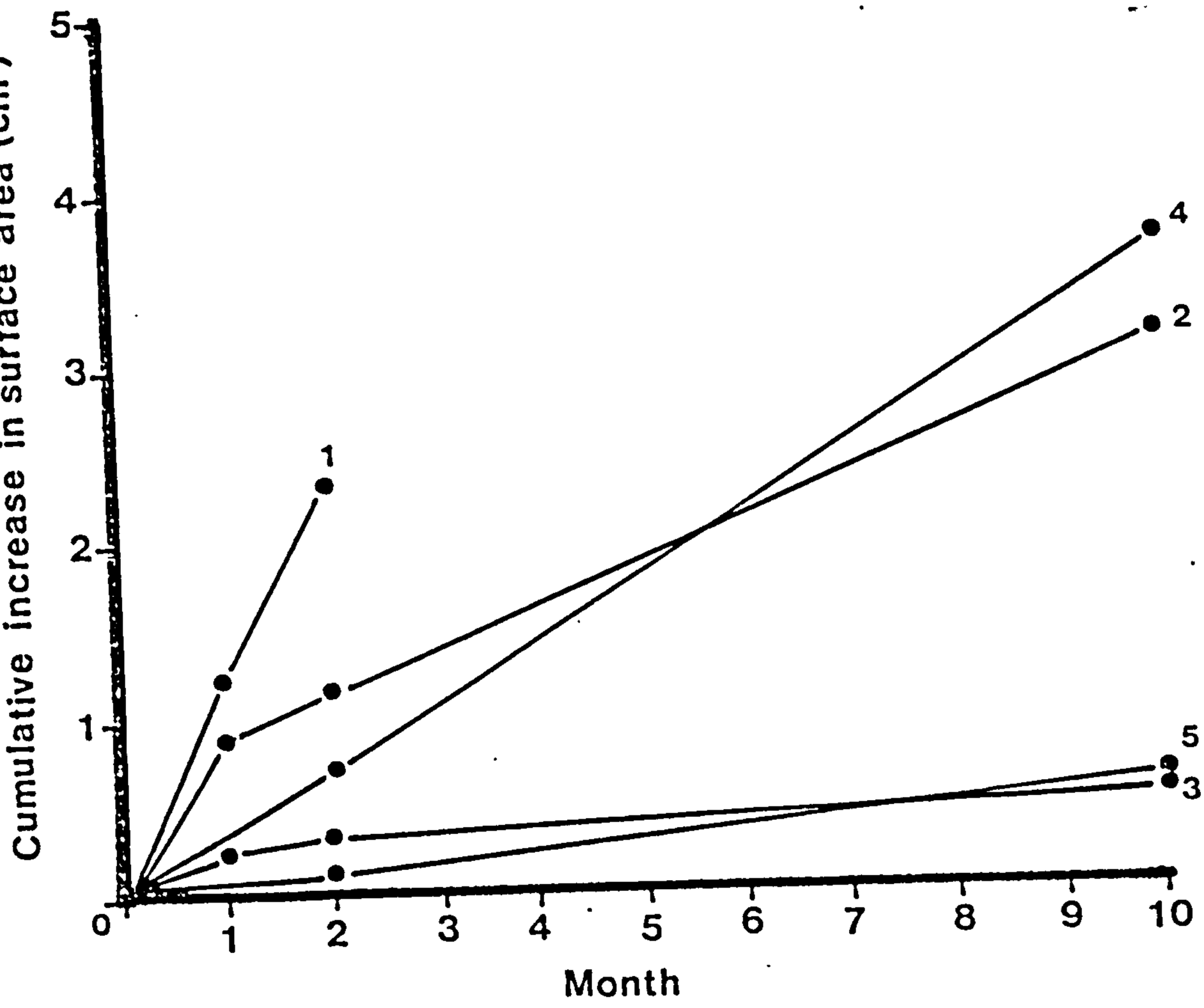


FIGURE 4-1:-
The cumulative increase in surface area (cm²) for coral colonies in all treatment groups at 10 meters, and 30 meters.
Trace (1) is for n=9 'normal' colonies at 10 meters.- 2 months
Trace (2) is for n=2 'normal' colonies at 10 meters -10 months
Trace (3) is for n=6 'bleached' colonies at 10 meters -10 months
Trace (4) is for n=8 'normal' colonies at 30 meters -10 months
Trace (5) is for n=5 'bleached' colonies at 30 meters -10 months

Each trace was outlined on the digitizing pad three times, and a statistical mean calculated. The mean value was taken as the photographic area for that colony. If the spread of values for a given trace was $\pm$ 0.5cm^2 then all three outlines for that trace were repeated.

Since growth was essentially linear with time (Figure 4-1) the area from the first photograph taken of each colony was subtracted from the last photographic area to give an increment of linear extension. This increment was divided by the time interval between photographs to give a unit of colony growth per day.

When the experiments on the reef were completed the colonies were placed in nylon jars and covered with Millipore filtered seawater, and transported to Glasgow University frozen at -10°C. In the laboratory corals were thawed and the tissue removed using the Water Pik technique (Johannes and Weibe, 1970). It was found that tissue from these previously frozen colonies separated completely (microscopic examinaton of blasted skeletons showed no residual tissue) from the skeleton using small volumes of Millipore filtered seawater (50ml.). The tissue water solution was placed in 50ml nylon centrifuge tubes and spun for 5-minutes at 1500 RPM. The supernatant was discarded and the pellet lyophilized to 10^{-3} Torr. The dried tissue was then weighed to determine tissue biomass for each colony. The energy equivalent of a unit tissue biomass (mg.d.w.) was determined directly by wet oxidation with potassium dichromate. 3-5mg subsamples of lyophilized tissue were processed following the method described in Chapter 2.

After tissue removal the surface area of each colony used was determined using the foil method of Marsh (1970). The foil area was used to calculate

a conversion coefficient for all photographic areas. This was necessary since the photographic areas were one di mensional and could underestimate actual colony growth in two dimensions. The conversion coefficient was then used to convert all increases in surface areas determined from photographs into true surface area values. The ratio of the surface area to tissue biomass determined for each coral was used to convert growth expressed in surface area units into units of biomass. The relationship between biomass and energy content determined for each coral was then used to convert growth into units of Joules per day. Finally the data were normalized and expressed on the basis of Joules per day for a 'standard' 100cm² coral.

RESULTS

Mean increases in surface area in all treatment groups were linear (Figure 4-1) with mean skeletal extension rates decreasing over the depth range. This was not the case for traces 2 and 4 in Figure (4-1) where 'normal' colonies at 30 metres showed a growth rate greater than their 10 metre counterparts, but the validity of this result is suspect since only 2 corals survived the winter storms at the 10 metre site for the full 10 month period.

There were marked differences in colony growth between treatments at each depth with those colonies maintained in darkened conditions showing decreased growth rates relative to normal colonies. An analysis of growth rates at both 10 metres and 30 metres was carried out comparing the growth rates of 'normal' and 'bleached' colonies after 2 and 10 months. When comparing the growth rates at the 10 metre site after 2 months for n=9 normal and n=6 bleached colonies (Table 4-3), the growth of bleached colonies was 16.24% that of normal colonies. After 10 months for n=2 normal

and n=6 bleached colonies there was little change with growth rates of bleached colonies being 16.71% that of normal colonies. A similar analysis at the 30 metre site showed that after 2 months (N=8 normal and n=5 bleached colonies) the growth rate of bleached colonies was 3.32% that of normal colonies, while after 10 months the difference increases to 14.58% that of normal colonies (Table 4-3)

Figure (4-1) is misleading since the plot for normal colonies maintained at the shallow site for 10 months (n=2) is similar to that of normal colonies at the 30 metre site (n=8) over the same interval. But when the linear trend of all 10 month plots is applied to the 2 month plot (n=9) at 10 metres, then the extrapolated line would yield a higher increase in surface area in the order of 11.6910cm^2 over the 10 month period. This value would be purely hypothetical since severe winter storms resulting in increased turbidity, and reduced light intensities, could in effect reduce growth rates (Wellington and Glynn, 1983).

Corals collected from the shallow site (n=15), of which eight colonies (n=2 normal and n=6 bleached) had survived the winter storms, and from the deep site (n=13) were processed The resultant data are presented in Table 4-1 for the former and Table 4-2 for the latter. Means and standard deviations of all biomass and energy relationships were calculated for corals maintained under normal conditions (n=9 over 2 months, and n=2 over 10 months at 10 metres, and n=8 at 30 metres over 10 months), and those kept in darkened chambers (n=6 shallow, and n=5 deep, both over 10 months).

Mean daily growth increments ($cm^2.day^{-1}$) for corals maintained normally at 10 metres were 0.0434 $\pm$ 0.0134 cm^2 per day (Table 4-1), while

CORAL #	DRY WT. (grams)	FOIL AREA (cm^2)	PHOTO AREA (cm^2)	PHOTO AREA/ FOIL AREA CONV COEFF	GROWTH FROM PHOTO cm^2/day	GROWTH IN FOIL AREA cm^2/day	DRY WT/ cm^2FOIL mgdw/day	MG.DW./DAY (FOIL)	J/MG.DW.	J/CORAL/ DAY	J/100cm/DAY
					NORMAL TREATMENT GROUP						
1	.1353	10.6430	9.0020	.8458	.0037	.0398	12.7126	.5060	36.12	18.28	171.76
2	.0593	8.2156	6.6209	.8059	.0294	.0365	7.2180	.2634	21.63	5.69	69.35
3	.1692	6.5505	5.2781	.8057	.0284	.0352	25.8301	.9092	35.57	32.34	498.55
4	.2486	13.9504	11.4699	.8222	.0564	.0686	17.8203	1.2225	36.53	44.66	320.12
5	.6772	26.7396	25.1213	.9357	.0599	.0640	25.5326	1.1341	38.43	62.79	234.85
6 *	.3683	18.8245	16.4564	.8742	.0327	.0374	19.5644	.7317	32.92	24.09	127.96
7**	.3921	15.5390	15.2174	.9793	.0280	.0294	25.2333	.7418	28.67	21.27	136.87
8	.4863	24.6297	23.3991	.9500	.0388	.0408	19.7444	.8056	37.64	30.32	123.11
9	.1634	11.6740	11.2583	.9644	.0379	.0393	13.9969	.5501	31.15	17.13	146.78
$\bar{x}$		15.1962				.0434	18.6281	.7627	33.18	28.51	203.26
±S.D		7.0151				.0134	6.4476	.3030	5.37	16.88	132.51
					DARK TREATMENT GROUP						
10	.3610	23.5693	22.9015	.9717	.0096	.0099	15.2783	.1512	21.63	3.27	13.88
11	.3729	16.3071	17.5061	1.0735	.0027	.0025	22.8673	.0572	24.14	1.38	8.48
12	.3970	16.8130	15.7598	.9374	.0077	.0082	23.6126	.1936	22.25	4.31	25.62
13	.2977	12.7028	13.5076	1.0633	.0065	.0061	23.4357	.1429	27.13	3.88	30.52
14	.3601	23.5693	24.0209	1.0192	.0096	.0094	15.2783	.1436	24.73	3.55	15.07
15	.2873	15.5799	16.3017	1.0463	.0049	.0047	18.4404	.0867	35.13	3.04	19.55
$\bar{X}$		18.0902				.0068	19.8187	.1292	25.84	3.24	18.85
±SD		4.4761				.0028	3.9975	.0490	4.95	1.01	8.11

TABLE 4-1:- Colony growth and tissue biomass relationships for corals from the normal treatment groups at the 10 meter study site. Growth in normal corals is calculated for a 2-month period. Means and standard deviations are given in the appropriately labelled rows, and are used in later calculations. Corals marked with an asterisk in the 'normal' group were kept over a 10 month period.

CORAL #	DRY WT. (grams)	FOIL AREA (cm^2)	PHOTO AREA (cm^2)	PHOTO AREA/ FOIL AREA CONV COEFF	GROWTH FROM PHOTO cm^2/day	GROWTH IN FOIL AREA cm^2/day	DRY WT/ cm^2FOIL mgdw/day	MG.DW./DAY (FOIL)	J/MG.DW.	J/CORAL/ DAY	J/100cm/DAY
					NORMAL TREATMENT GROUP						
1	.2369	15.0381	16.3449	1.0869	.0124	.0114	15.7533	.1796	36.51	6.56	43.60
2	.0833	5.3479	5.8126	1.0869	.0127	.0117	15.5762	.1822	39.24	7.16	133.83
3	.3475	22.3168	26.1377	1.1712	.0070	.0060	15.5712	.0934	33.96	3.17	14.21
4	.2787	12.4603	11.8406	.9503	.0101	.0106	22.3670	.2371	35.69	8.46	67.91
5	.1481	11.4007	21.4515	1.8816	.0092	.0049	13.0255	.0638	47.13	3.01	26.37
6	.2965	18.1582	34.3225	1.8902	.0136	.0072	16.3287	.1176	31.89	3.75	20.65
7	.7246	34.8734	23.6792	.6790	.0110	.0162	22.5337	.3650	35.87	13.09	36.54
8	.4913	22.1709	26.5869	1.1992	.0162	.0135	22.1597	.2991	36.52	10.92	49.27
$\bar{x}$		17.2078			.0102	17.9144	.1922	37.10	7.01		49.17
+S.D		8.9700			.0038	3.8028	.1038	4.57	3.70		38.24
					DARK TREATMENT GROUP						
9	.7148	13.5978	14.7251	1.0869	.0027	.0025	52.5673	.1314	29.82	3.92	28.82
10	.3564	29.5651	32.1344	1.0869	.0012	.0011	12.0547	.0133	23.03	0.31	1.04
11	.2492	17.8347	27.3232	1.5320	.0012	.0008	13.9728	.0112	24.96	0.28	1.57
12	.2415	12.0631	9.3231	.7729	.0009	.0012	20.0197	.0240	20.92	0.50	4.16
13	.3903	10.7401	9.2761	.8637	.0027	.0031	36.3404	.1126	25.64	2.89	26.88
$\bar{X}$		16.7602			.0017	26.9909	.0586	24.87	1.58		12.49
±SD		7.3913			.0010	17.1954	.0587	3.32	1.17		14.08

TABLE 4-2:- Colony growth and tissue biomass relationships for corals from the normal treatment groups at the 30 metre study site. Means and standard deviations are given in the appropriately labelled rows, and are used in later calculations.

	MONTH-0	MONTH-1	MONTH-2	MONTH-10
'NORMAL' COLONIES (10 metres)				
Mean surface area (photo)in cm^2				
n=9	11.2713 ± 6.4018	12.4591 ± 7.0442	13.5481 ± 6.7492	----------
n=2	12.9377 ± 0.7116	13.7457 ± 0.7101	14.4357 ± 0.7475	15.8369 ± 0.8961
Mean increase in surface area(cm2,				
n=9	0	1.1878 ± 0.9024	1.1504 ± 0.4494	----------
n=2	0	0.8080 ± 0.0015	0.9360 ± 0.3954	1.4012 ± 0.1285
Cumulative increase given in cm^2				
n=9	0	1.1878 ± 0.9024	2.3382 ± 0.7551	----------
n=2	0	0.8080 ± 0.0015	1.1744 ± 0.3838	3.1452 ± 0.5124
'BLEACHED' COLONIES(10 metres (n=6)				
Mean surface area (photo) in cm^2	17.8072 ± 4.1206	18.0110 ± 4.2231	18.1872 ± 4.2431	18.3329 ± 4.6889
Mean increase in surface area(cm^2)	0	0.2037 ± 0.1521	0.1762 ± 0.2093	0.1457 ± 0.2140
Cumulative increase given in cm^2	0	0.2037 ± 0.1521	0.3799 ± 0.3599	0.5256 ± 0.6017
'NORMAL' COLONIES (30 metres) (n=8)				
Mean surface area (photo) in cm^2	17.2689 ± 9.0364	----------	17.8563 ± 9.0593	20.7727 ± 9.0877
Mean increase in surface area(cm^2)	0	----------	0.7138 ± 0.2337	2.9164 ± 0.9995
Cumulative increase given in cm^2	0	----------	0.7138 ± 0.2337	3.6297 ± 1.2096
'BLEACHED' COLONIES (30 metres) (n=5)				
Mean surface area (photo) in cm^2	18.0271 ± 10.6996	----------	18.0508 ± 10.6868	18.5564 ± 10.5744
Mean increase in surface area(cm^2)	0	----------	0.0237 ± 0.0418	0.5055 ± 0.2417
Cumulative increase given in cm^2	0	----------	0.0237 ± 0.0418	0.5293 ± 0.2692

TABLE 4-3:- Mean and cumulative increase in surface area determined photographically from (n=as shown above) colonies maintained *in situ* in two treatments groups (normal and darkened) at two depths (10 metres and 30 metres) on the fore reef at Discovery Bay. Photographic areas were determined using a Watanabe Digitizer, and a Commodore 3032 computer.

at 30 metres daily skeletal extension rates for normal colonies were lower at 0.0102 ± 0.0039 cm^2 per day (Table 4-2). Tissue growth necessary to produce that increase in surface area at 10 metres was 0.7627±0.3030 mg.dw per day (Table 4-1). At 30 metres mean tissue growth rates required to produce the recorded increase in surface area were lower at 0.1922 ± 0.1038 mg.dw. (Table 4-2).

Similar relationships calculated for corals maintained in dark enclosures showed that at 10 metres skeletal extension rates for (n=6) colonies were 0.0068 ± 0.0028 cm^2 per day requiring tissue growth rates of 0.1292 ± 0.0490 mg.dw. per day (Table 4-1). At 30 metres skeletal extension rates of 0.0017±0.0010 cm^2 per day requiring tissue growth rates of 0.0586 ± 0.0587 mg.dw. per day (Table 4-2).

A one way analysis of variance was used on growth data relationships within and among the treatment groups at both study sites.

The results from Table(4-4)suggest that tissue cover (mg.dw./cm^2 (foil)) was similar in all treatment groups within and between the two depths with $P<.05$ in all cases. Mean tissue cover in normal colonies at 10 metres was 18.6281±6.4476 mg.dw./cm^2 (n=9) Table(4-1), and 17.9144±3.0803 mg.dw./cm^2 (n=8) at 30 metres (Table 4-2). These values compare favourably with those of Lewis (1981) who reports tissue cover (dry weight) per cm^2 of 21.0±4.7 mg/cm^2 for *Montastrea sp.* at Barbados.

Significant differences were found in the energy equivalence of a unit biomass between normal and bleached colonies at both depths (Table 4-5), with tissue from normal corals having a greater energy equivalent relative to bleached colonies (Tables 4-1,4-2). There were no significant

STUDY SITE	NORMAL CORALS		BLEACHED CORALS	
	X	±S.D.	X	±S.D.
SHALLOW	18.6281 (n=9)	6.4476	19.8188 (n=6)	3.9975
DEEP	17.9144 (n=8)	3.8028	26.9909 (n=5)	17.1954

ONE WAY ANALYSIS OF VARIANCE:-

TREATMENT	df_1	df_2	F_s	$F_{.05}$	PROBABILITY
BETWEEN DEPTHS					
NORMAL	1	15	.13	4.54	P<.05 NOT SIGNIFICANT
BLEACHED	1	9	1.00	5.12	P<.05 NOT SIGNIFICANT
WITHIN DEPTHS					
SHALLOW	1	13	.10	4.61	P<<.05 NOT SIGNIFICANT
DEEP	1	11	2.17	4.84	P<.05 NOT SIGNIFICANT

TABLE 4-4:- Tissue cover per unit area (mg.dw./cm^2) for colonies from 10 metres and 30 metres in both treatment groups.

SF 1

STUDY SITE	NORMAL CORALS		BLEACHED CORALS	
	X	± S.D.	X	± S.D.
SHALLOW	33.18 (n=9)	5.37	25.84 (n=6)	4.95
DEEP	37.10 (n=8)	4.57	24.87 (n=5)	3.32

ONE WAY ANALYSIS OF VARIANCE:-

TREATMENT	df_1	df_2	F_s	$F_{.05}$	PROBABILITY
BETWEEN DEPTHS					
NORMAL	1	15	0.28	4.54	P<.05 NOT SIGNIFICANT
BLEACHED	1	9	0.14	5.12	P<.05 NOT SIGNIFICANT
WITHIN DEPTHS					
SHALLOW	1	13	8.20	4.61	P>.05 SIGNIFICANT
DEEP	1	11	2.17	4.84	P>.05 VERY SIGNIFICANT

TABLE 4-5:- Energy equivalentsin Joules per mg.dw. from colonies in two treatment groups at 10 metres and 30 metres in both treatnment groups.

STUDY SITE	NORMAL CORALS		BLEACHED CORALS	
	X	±S.D.	X	±S.D.
SHALLOW	.0434 (n=9)	0.0134	0.0068 (n=6)	0.0039
DEEP	0.0102 (n=8)	0.0039	0.0017 (n=5)	0.0010

ONE WAY ANALYSIS OF VARIANCE:-

TREATMENT	df_1	df_2	$F_=$	$F_{.05}$	PROBABILITY
BETWEEN DEPTHS					
NORMAL	1	15	45.38	4.54	P>.05 SIGNIFICANT
BLEACHED	1	9	13.74	5.12	P>>.05 SIGNIFICANT
WITHIN DEPTHS					
SHALLOW	1	13	42.33	4.61	P>>.05 SIGNIFICANT
DEEP	1	11	2.17	4.84	P>>.05 SIGNIFICANT

TABLE 4-6:- colony growth in cm^2 per day (foil) for corals at both depths and from both treatment groups.

differences in either normal or bleached colonies over the depth range with ($P<<.05$) in both cases (Table 4-5). This observation lends support to the observations of Fitt *et al* (1981), Patton *et al* (1983), Battey and Patton (1984) who suggest that colonies kept under darkened conditions tend to shift their main metabolic substrate from lipid (of photosynthetic origin and translocated as droplets to the host from the zooxanthellae) to carbohydrates. Fitt and Pardy (1981) noted that the shift to carbohydrate substrates occurred after a period of 48 hours in the dark, after the stored lipid had been depleted.

Significant differences ($P>>.05$) were found in an analysis of colony growth (cm^2/day) within and between all treatment groups at both depths (this analysis was based on the findings of Bak (1976)). Normal corals in all cases had growth rates in excess of bleached colonies with means of 0.0434 ± 0.0134 cm^2/day versus 0.0068 ± 0.0039 cm^2/day at 10 metres, and 0.0102 ± 0.0039 cm^2/day versus 0.0017 ± 0.0010 cm^2/day at 30 metres (Table 4-6). An analysis of the daily tissue growth increments (mg.dw/colony/day) of colonies in both treatment groups over the depth range (Table 4-7) showed that there was no significant difference ($P<.05$) in tissue growth between shallow and deep bleached specimens. Differences between all other treatment groups were highly significant ($P>>.05$). Similarily no significant differences were found in the daily energetic requirements for growth in bleached colonies normalized to 100 cm^2 standard colonies between the depth ranges (Table 4-8). Normal colonies showed significant differences between 10 metres and 30 metres, with those at 10 metres retaining more energy in the form of tissue growth (302.26 ± 132.51 Joules/$100cm^2$/day at 10 metres against 49.17 ± 38.24 Joules/$100cm^2$/day at 30 metres). No significant differences were found in the energy retained as tissue growth between normal and bleached colonies at 30 metres. At 10

STUDY SITE	NORMAL CORALS		BLEACHED CORALS	
	X	± S.D.	X	±S.D.
SHALLOW	.7627 (n=9)	0.3030	0.1292 (n=6)	0.0490
DEEP	0.1922 (n=8)	0.1038	0.0586 (n=5)	0.0587

ONE WAY ANALYSIS OF VARIANCE:-

TREATMENT	df_1	df_2	F_s	$F_{.05}$	PROBABILITY
BETWEEN DEPTHS					
NORMAL	1	15	24.02	4.54	P<.05 NOT SIGNIFICANT
BLEACHED	1	9	4.74	5.12	P<.05 NOT SIGNIFICANT
WITHIN DEPTHS					
SHALLOW	1	13	25.16	4.61	P>>.05 SIGNIFICANT
DEEP	1	11	6.77	4.84	P>>.05 SIGNIFICANT

TABLE 4-7:- Daily tissue growth increment per coral colony per day (mg.dw./colony/day) for corals in both treatment groups at both depths[2].

STUDY SITE	NORMAL CORALS		BLEACHED CORALS	
	X	±S.D.	X	±S.D.
SHALLOW	.0434 (n=9)	0.0134	0.0068 (n=6)	0.0039
DEEP	0.0102 (n=8)	0.0039	0.0017 (n=5)	0.0010

ONE WAY ANALYSIS OF VARIANCE:-

TREATMENT	df_1	df_2	F_s	$F_{.05}$	PROBABILITY
BETWEEN DEPTHS					
NORMAL	1	15	45.38	4.54	P>.05 SIGNIFICANT
BLEACHED	1	9	13.74	5.12	P>>.05 SIGNIFICANT
WITHIN DEPTHS					
SHALLOW	1	13	42.33	4.61	P>>.05 SIGNIFICANT
DEEP	1	11	2.17	4.84	P>>.05 SIGNIFICANT

TABLE 4-6:- colony growth in cm^2 per day (foil) for corals at both depths and from both treatment groups.

STUDY SITE	NORMAL CORALS		BLEACHED CORALS	
	X	± S.D.	X	±S.D.
SHALLOW	.7627 (n=9)	0.3030	0.1292 (n=6)	0.0490
DEEP	0.1922 (n=8)	0.1038	0.0586 (n=5)	0.0587

ONE WAY ANALYSIS OF VARIANCE:-

TREATMENT	df_1	df_2	$F_=$	$F_{.05}$	PROBABILITY
BETWEEN DEPTHS					
NORMAL	1	15	24.02	4.54	P<.05 NOT SIGNIFICANT
BLEACHED	1	9	4.74	5.12	P<.05 NOT SIGNIFICANT
WITHIN DEPTHS					
SHALLOW	1	13	25.16	4.61	P>>.05 SIGNIFICANT
DEEP	1	11	6.77	4.84	P>>.05 SIGNIFICANT

TABLE 4-7:- Daily tissue growth increment per coral colony per day (mg.dw./colony/day) for corals in both treatment groups at both depths[2].

STUDY SITE	NORMAL CORALS		BLEACHED CORALS	
	X	± S.D.	X	± S.D.
SHALLOW	203.26 (n=9)	132.51	18.85 (n=6)	8.11
DEEP	49.17 (n=8)	38.24	12.49 (n=5)	14.08

ONE WAY ANALYSIS OF VARIANCE:-

TREATMENT	df_1	df_2	F_s	$F_{.05}$	PROBABILITY
BETWEEN DEPTHS					
NORMAL	1	15	10.80	4.54	P>.05 SIGNIFICANT
BLEACHED	1	9	.87	5.12	P<<.05 NOT SIGNIFICANT
WITHIN DEPTHS					
SHALLOW	1	13	12.17	4.61	P>.05 SIGNIFICANT
DEEP	1	11	4.12	4.84	P<.05 SIGNIFICANT

TABLE 4-8:- Mean daily energy requirements (in Joules) for growth in standard 100cm² colonies from two treatment groups at two depths.

metres the opposite was true with normal colonies retaining or requiring 75.8% more energy as tissue growth than bleached colonies.

The differences encountered in the analysis of bleached colonies at 10 metres and 30 metres are of great interest. No significant differences were found in tissue cover per unit surface area, or in the energy equivalent of a unit biomass from colonies at the two depths. Significant differences were found in the analysis of colony growth per day (cm^2/day) with shallow bleached colonies having mean skeletal extension rates of 0.0068 ± 0.0039 cm^2/day, and those at 30 metres having mean extension rates of 0.0017 ± 0.0010 cm^2/day (Table 4-6).

In this investigation an assumption has been made that skeletal extension requires tissue growth and its associated calicoblastic layer necessary for skeletogenesis (Bak,1976; Mitterer, 1978). From Table (4-7) it can be seen that there was no significant difference ($P<.05$) in daily tissue growth increments of bleached colonies at both depths. Although variance analysis does not detect a significant difference in the tissue growth rates of the shallow and deep bleached colonies, nevertheless the shallow colonies do show a 54.64% higher growth rate than the deep colonies. This is in accord with the observed differences in the rate of surface area increase in these two groups.

Data have been normalized to the requirements of a standard hypothetical 100 cm^2 colony. These data will be used in later sections to produce an energy budget for this species. A 100cm^2 colony maintained under normal conditions will retain or require 203.26 ± 132.51 Joules per day at 10 meters, and 49.17 ± 38.24 Joules per day at thirty metres. Colonies maintained in darkened conditions will require 18.85 ± 8.11 Joules per day at

10 metres, and 12.49±14.08 Joules per day at 30 metres for tissue growth.

DISCUSSION

The mean increase in surface area for colonies at the 10 metre site was 1.4012±0.1285 cm^2 per 10 month period which can be extrapolated to 1.6814±0.1542 cm^2 per year for normal corals (n=2), and 0.1457±0.2140 cm^2 per 10 month period which can be extrapolated to .1748±0.2568 cm^2 per year for bleached colonies (n=6). At 30 metres the mean increase in surface area was 2.9164±.09995 cm^2 per 10 month period which can be extrapolated to 3.4997±1.1994 cm^2 per year for normal colonies (n=8), and 0.5055±0.2417 cm^2 per 10 month period which can be extrapolated to 0.6066±0.2900 cm^2 per year for (n=5) bleached colonies. These results though showing decreased growth rates with increasing depth cannot be compared with values reported in the literature since these have measured linear growth in coral colonies (Webber and White, 1977; Highsmith *et al*, 1983; Wellington and Glynn, 1983), and not changes in surface area over a given time interval which were measured in this investigation.

Reported growth rates of *Montastrea cavernosa* measured using X-radiography showed mean linear extensions of 6.55mm/year (Weber and White, 1977) and 4.36mm/year (Highsmith *et al*, 1983). Linear extension rates of Caribbean massive colonies measured using X radiography fall between 3.0mm/year for colonies of *Porites astreoides* (Gladfelter, 1978), and 6.55mm/year for colonies of *Montastrea cavernosa* (Weber and White, 1977). In massive coral colonies from Panama and Eniwetak, mean linear growth rates were higher than in Caribbean species with most colonies falling between 5.7mm/year (*Favia pallida*, Highsmith 1977 at Eniwetak) and 1.32± 0.061cm/year for colonies of *Pavona clavus* collected from 5-7 meters

in the Gulf of Panama (Wellington and Glynn, 1983).

Future research using the photographic technique developed in this investigation should consider collecting data from photographs in such a way as to allow direct comparison with published material in the literature. In order that these comparisons can be made, rates of linear extension must be determined either by geometric approximations (From the sample colony's principal dimensions) or by X-radiography determining linear extension from density banding.

CHAPTER - 5

HETEROTROPHIC NUTRITION ON ZOOPLANKTON AND THE CONTRIBUTION TO THE ENERGY BUDGET OF *Montastrea cavernosa*

INTRODUCTION.

Montastrea cavernosa was selected for this investigation partly because of its importance as a frame building coral on Caribbean reefs(Goodwin *et al*, 1976), but also because direct *in situ* determinations of zooplankton ingestion by individual polyps (Porter, 1974) could be carried out to calculate the contribution of heterotrophic nutrition on zooplankton to the daily energy requirements of a standard 100 cm.^{2} colony.

Zooplankton on coral reefs has been shown to originate from two sources, oceanic (Odum and Odum, 1955) and demersal (Porter, 1974). Investigations of the nutritional importance of planktonic material to coral reef energetics were initially carried out using flow depletion studies (Sargent and Austin, 1949,1954; Odum and Odum, 1955; Tranter and George, 1972). These investigations measuring changes in zooplankton biomass upstream and downstream of a coral reef, agree that depletions in the order of 20-60% were insufficient to meet the energetic requirements of coral reefs. These early studies did not consider the existence of resident reef plankton (demersal plankton) as described by Bakus (1964), Emery (1968), Porter (1974), Alldredge and King (1977,1980), Grimm and Clayshulte (1981), Ohlhorst (1982). The abundance of demersal plankton has been shown to be highly variable with maximum densities in the water column recorded approximately 2-hours after sunset (Ohlhost, 1982). Vertical migration of demersal plankton has been linked to mucus production by corals (Hamner and

Carleton, 1979; Walter and Pasamonte, 1981), and likewise feeding patterns of reef organisms including corals has been linked to these vertical migrations (Macurda, 1976).

In this investigation an attempt has been made to quantify the energy input from zooplankton ingestion to the daily energy requirements of standard colonies of *Montastrea cavernosa*, and to assess the effects of *in situ* plankton densities and species diversity on this contribution to the energy budget.

MATERIALS AND METHODS.

The investigation of zooplankton ingestion by *Montastrea cavernosa* was approached both ecologically investigating the feeding efficiencies of sample colonies relative to plankton density in the water column, and energetically quantifying the energy input to the colony from heterotrophic nutrition on zooplankton. It was of particular interest to determine whether this species could satisfy its daily energy requirements from heterotrophic nutrition alone as suggested by Yonge (1971).

5-1. SELECTION OF CORALS.

Coral colonies other than those collected for growth and respirometry were used for sampling ingested plankton. 5 hemispherical colonies approximately 1 metre in diameter were selected at two locations on the fore reef (Figure 2-1, Positions A and B). Position A at 10 metres close to the shipping channel, and the second Position B adjacent to the deep study site at 30 metres on an easily accessible patch reef on which *Montastrea cavernosa* was the dominant coral (Appendix 2). Of the colonies selected,

only one (sampled by myself) was used in the analysis, the others sampled by field assistants showed too many sampling inconsistencies (corallite fragments, sand, and high levels of coral tissue) to be used in the analysis. All results shown were for a coral at the 10 metre study site at position (A).

Preliminary dives prior to plankton collection dives were used to map current patterns over selected coral heads, and to divide those colonies into four sampling quadrats normal to current flow patterns over each specimen (Figure 5-1). Flow patterns were determined by repeatedly injecting aliquots of fluorescein dye upstream of each colony, and mapping the movement and direction of the dye over the coral head. Diagrams depicting these flow patterns are presented in Appendix 2-1.

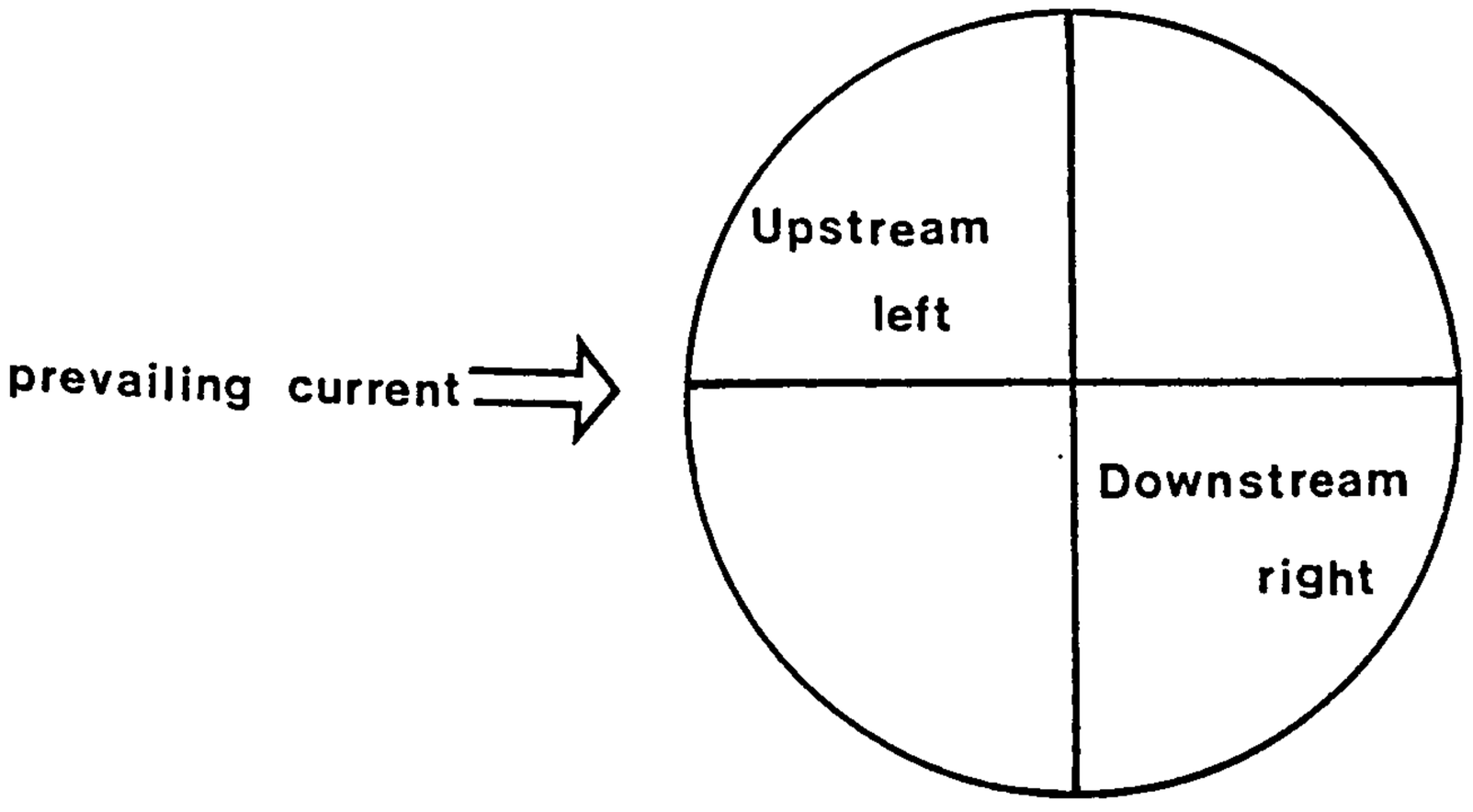


FIGURE 5-1. Sampling quadrat division normal to the prevailing current. Each coral colony was divided into two upstream and two downstream quadrats.

The rate of water flow over each coral head was determined by repeatedly timing the passage of an aliquot of fluorescein dye over a marked 1-metre trajectory. The volume of water from which zooplankton could be extracted by a coral surface could then be determined from the tissue surface area exposed to a water current.

5-2. SAMPLING OF COELENTERON CONTENTS.

In order to determine the energetic input to a coral colony from heterotrophic nutrition on zooplankton, the coelenteron contents (gut contents) of 24 individual polyps were extracted, the ingested plankton identified, and the energy equivalent of that ingested plankton determined at a later date by Wet Oxidation with potassium dichromate.

The techniques used for *in situ* extractions of gut contents from individual polyps of *Montastrea cavernosa* have been described by Porter (1974,1978). Porter (1974) used a hypodermic syringe with a large syringe needle (2mm. diameter) to extract gut contents from *Montastrea cavernosa* colonies in Panama. The collected sample was then placed into a 5 ml. Vacutainer tube prepared with 5% formalin, and two stains, Alcian Blue for mucus, and Eosin-Y Red for animal tissue, and counted in the laboratory. In this investigation the extraction process remained unchanged from Porter's except for the use of the specified syringe needle which was found to cause unacceptable damage to both the tissue and the corallite. The syringe needle was discarded, and extractions carried out using the hypodermic syringe only. This change reduced both tissue and skeletal damage yet retained the same extraction capabilities.

Twenty four polyps were sampled from each selected coral colony during a collection dive, six polyps from each quadrat. Three corals were sampled during each dive yielding a total of 72 gut content extractions.

The gut contents were fixed in a buffered formalin solution and stained following the method specified by Porter (1974,1978).

5-2-1. COELENTERON SAMPLING INTERVALS.

Sampling intervals were designed to determine whether there was a relationship between plankton density in the water column and the number of plankton ingested by a coral colony.

In order to ensure optimum data return per collection dive at the 10 metre collection site, the following sampling regimes were selected;

1)- Sampling at 2-hourly intervals (1800-0600) during a single night,

2)- Spot collections (1800-0500). Single collection dives during different lunar phases to cover the effect of lunar cycles, and vertical migration patterns of zooplankton.

Since plankton in the water column were found to peak at 20:00 hours (approximately 2-hours after sunset) at the 10 metre site (see Figure 5-4), an observation subsequently verified by Ohlhorst (1982), sampling at the 30 metre site was carried out only at this time. Gut content extractions were performed on three separate occasions at 30 metres.

5-3. COELENTERON CONTENT EXTRACTIONS *in situ* AT 10 METRES.

5-3-1. THE ALL NIGHT SAMPLE

Gut contents were extracted from 24 polyps of a single colony at 2-hourly intervals starting at 18:00 hours. Due to repeat diving limitations the 04:00hr. collection was not carried out, and the values given are a mean calculated from the 02:00 and the 06:00 samples.

Zooplankton density in the water column was determined using a light trap (See Figure 5-2), and the total number of plankton including species

composition determined. The total number of collected zooplankton was plotted against time to determine vertical migration patterns.

Energy input from zooplankton ingestion was calculated in the following manner;

1) Number of z'plankton ingested per polyp = $\frac{\text{Total of z'plankton ingested}}{\text{24 polyps sampled}}$

2) Energy input per polyp = Result (1) x Energy equivalent (J) of 1 plankton

3) Normalizing to a standard colony 100cm² with 123 polyps = Result (2) x123.

The number of polyps per 100 cm² standard colony was determined as shown in section 5-4.

5-3-2. SPOT SAMPLES AT 10 METERS.

Gut samples collected at a specific time on different nights yielded very little useful information, and are presented here as a discussion point for zooplankton ingestion by coral colonies relative to zooplankton availability in the water column, and the species composition of those plankton. The same assumptions hold for the spot samples as for the all night sample

5-3-3. AN ESTIMATE OF ZOOPLANKTON CAPTURE EFFICIENCIES BY SAMPLE CORALS.

For the purpose of data interpretation a rough estimate of capture efficiencies was required to assess the effect that changes in plankton density, and species composition had on plankton capture by a coral colony. This calculation could also be used to assess the effect of environmental influences such as storm surge on plankton ingestion. Capture efficiency

was calculated using the following formula;

$$\text{CAPTURE EFFICIENCY} = \frac{\text{TOTAL NUMBER OF ZOOPLANKTON INGESTED}}{\text{DENSITY OF ZOOPLANKTON .LITRE}^{-1}}$$

5-3-4. ADDITIONAL ZOOPLANKTON COLLECTIONS.

5-3-4.1. PLANKTON TOWS

Plankton tows were carried out following each gut sampling dive at a depth of 8 metres when corals at the 10 metre site were sampled, and at 25 metres when corals at the 30 metre site were sampled.

An 80u net with a 50cm. mouth and 1.5 metres in length was used in all tows. The tow depth was calculated from the amount of line paid out, the line angle determined by a protractor and plumb line arrangement, for 5-minutes at a speed of approximately 1-knot. Boat speed was calculated by timing the passage of a dropped floating object past a marked 2-meter length of the boat. Towed volumes were calculated using the formula given in Tranter (1968).

Collected samples were decanted into a 1500ml. stoppered erlenmeyer flask prepared with 30 ml. of 10% buffered formalin, and transported to the laboratory.

5-3-4.2. TRAPPED PLANKTON SAMPLES

Zooplankton was also collected *in situ* adjacent to colonies selected during each gut sample using a light trap. The light trap was constructed from a darkened 1.5 liter wide mouth, screw top, polyethylene flask. The base of the flask was removed and replaced by a suitable rubber stopper. A section of 80u plankton netting was fixed to the neck of the bottle using a

silicon aquarium sealant. The screw top was retained (Figure 5-2).

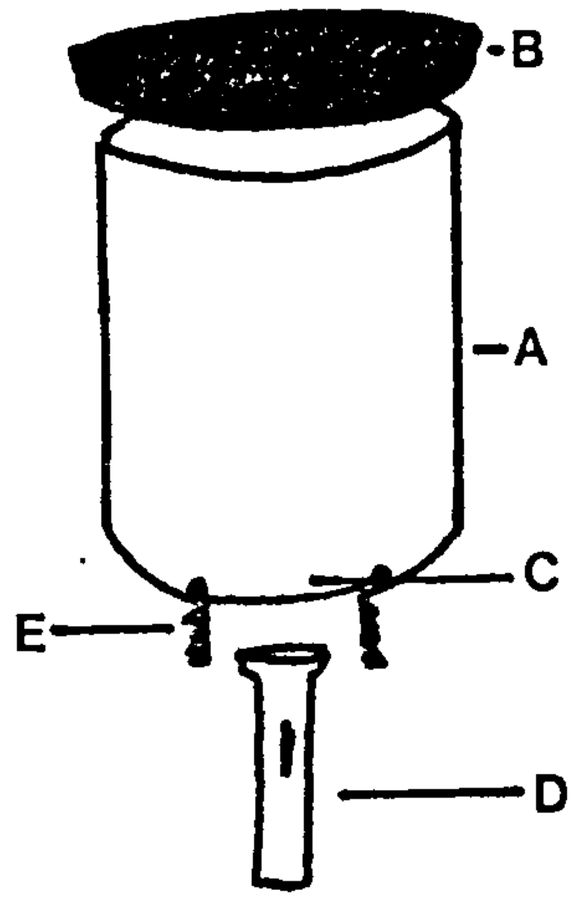


FIGURE 5-2: Cross sectional view of a zooplankton light trap, showing (A) darkened polyethylene flask, (B) rubber stopper, (C) 80u plankton netting, (D) underwater light, (E) screw top.

To collect a sample a diver removed both the stopper and the screw top. The flask was then inverted in a neck down position, and a powerful underwater light (Q-Lite) was played upwards through the flask. After a sampling period of 5-minutes both the rubber stopper and screw cap were replaced, and the trapped zooplankton carried to the surface. The sample was then decanted into a 1500ml. stoppered Erlenmeyer flask prepared with 30 ml. 10% buffered formalin.

This technique was found to collect a representative number of zooplankton. A comparison of trap collected plankton against tow collected plankton showed that species diversity, size range, and relative numbers collected were similar, therefore the possibility of having collected purely phototactic forms in the light trap was eliminated.

5-4. PROCESSING OF EXTRACTED AND COLLECTED ZOOPLANKTON.

5-4-1. COELENTERON (GUT) CONTENTS

Each Vacutainer containing the fixed gut contents of a single polyp was examined independently. The total number of zooplankton, and the species composition of each tube was recorded. The contents of all 24 tubes corresponding to extractions from a single colony were pooled following examination, and decanted into a single scintillation vial. The vial was then stored at -10°C pending transport to Glasgow University for further analysis.

5-4-2. ADDITIONAL ZOOPLANKTON COLLECTIONS

Plankton collections both towed and trapped were decanted into 1.5 litre graduated cylinders, sealed with Parafilm, and the contents allowed to settle for 3-hours. The settled volume was recorded, and the supernatant discarded. The settled sample was fractioned into a counting and identification fraction (A), and a storage fraction (B) to be used for later analysis. Samples in fraction (A) were counted in the field using a Bausch and Lomb binocular dissecting microscope (x 10 magnification) using a rafter cell. Total zooplankton numbers present in each collection were determined using the total settled volume. In addition the following were also recorded;

5-4-2.1. Taxonomic Composition:- The taxonomic composition of collected samples was determined by examining and counting four .5 ml aliquots extracted from a mixed sample of settled plankton. Different taxa were identified and the number in each taxa recorded.

5-4-2.2. Size Distribution:- Plankton identified in the previous section were measured using an ocular micrometer (.1mm divisions) and their lengths recorded. They were then placed in one of three groups ; <.5mm,

>.5mm<1.0mm, >1.0mm. The results from all collections were then averaged to determine the mean size distribution (n=12) for zooplankton populations on the fore reef at Discovery Bay.

Fraction (B) retained for later biochemical analysis were all treated in the following manner; 3-5ml aliquots of settled zooplankton were placed in 10ml nylon scintillation vials, and the volume made up with Millipore filtered seawater. The vials were then stored at -10°C pending transport to Glasgow.

5-5. ENERGY EQUIVALENTS

The energy equivalent of a single average plankton was determined by wet oxidation of 3-5mg of lyophilyzed material previously stored at -10°C at Discovery Bay, where the number of settled plankton per ml had been determined. The energy equivalent of a single plankton was calculated by dividing the energy equivalent in Joules of a mg of plankton by the total number of plankton contained in that unit weight. The number of zooplankton per mg dry weight was determined by counting 1.0 ml aliquot subsamples of settled material, and drying these at 60 °C to constant weight, the number contained in a 1 mg of dried material was then determined. Since the material had been stored in formalin prior to storage at -10°C the possibility existed that the energy equivalent determined by wet oxidation would not be an accurate measure of the actual energy equivalence, and hence would underestimate the contribution from zooplankton ingestion to the energy requirements of a coral colony. Comparisons with previously published data (Ostapenya and Shikina, 1974; Johannes and Tepley,1974) showed that the results from this investigation were acceptable.

The energy equivalent of a single plankton was used to determine the

energy contribution from zooplankton ingestion to the requirements of a standard colony of 100cm² having 123 ± 32 polyps.

5-6. NUMBER OF POLYPS PER COLONY:-

Morphometric measurements of n=70 colonies had been carried out during the first field season in Grenada W.I. The results showed that colonies had a mean polyp diameter of 0.88 ± 0.26cm and an average of 1.23 ± 0.32 polyps.cm^{-2}. From these results a 100cm² colony would have 123 ± 32 polyps. This value was used in all normalizing calculations.

5-7. RESULTS:-

5-7-1. ENERGY EQUIVALENTS OF COLLECTED PLANKTON:-

The energy content of a single average plankton (Table 5-1) determined using wet oxidation yielded mean values of 23.5436 ± 3.7491 Joules.mg^{-1} or 5.6937 ± 0.9067 calories.mg^{-1}, with the energy equivalent of a single plankton calculated as 0.00605 ± 0.0025 Joules or 0.00146 ± 0.00059 calories per plankton. These values compare favourably with those of Ostapenya and Shikina (1973) who report 5.56 calories.mg^{-1} of tropical plankton, and that of Johannes and Tepley (1974) of 0.0016 calories per plankton. These values are all lower than those of Porter (1974) who calculated the energy equivalent of a single plankton as 0.2012 calories (0.8319 Joules).

All subsequent calculations were done using the energy equivalent of 1 plankton as being 0.00605 Joules.

5-7-2. ZOOPLANKTON SPECIES COMPOSITION AND SIZE DISTRIBUTION:-

TOW#	# PLANKTON/ml SETTLED VOLUME	PLANKTON(#) PER MG.DW.	JOULES/ml	CALORIES/ml	JOULES/ mg.dw.	CALORIES/ mg.dw	JOULES/ PLANKTON	CALORIES/ PLANKTON
T- 1	3370	6607	9.776	2.364	19.1616	4.6340	0.00290	0.00070
T- 2	1530	2997	12.784	3.092	25.0574	6.0598	0.00836	0.00202
T- 3	3375	6617	15.227	3.682	29.8450	7.2177	0.00451	0.00109
T- 4	3045	5962	15.545	3.759	30.4676	7.3682	0.00511	0.00124
T- 5	3575	6996	11.280	2.728	22.1081	5.3466	0.00316	0.00076
T- 6	2780	5448	10.896	2.635	21.3562	5.1647	0.00392	0.00095
T- 7	1045	2047	9.693	2.344	18.9977	4.5944	0.00928	0.00224
T- 8	1725	3379	11.310	2.735	22.1676	5.3610	0.00656	0.00159
T- 9	1720	3371	12.350	2.987	24.2060	5.8539	0.00718	0.00174
T-10	2195	4299	11.887	2.875	23.2985	5.6345	0.00542	0.00131
T-11	1285	----	------	-----	------	-----	------	------
T-12	1120	2194	11.385	2.753	22.3146	5.3965	0.01017	0.00246
$\bar{X}$ ±S.D.	2235 (±945) (n=12)	4538 (±1853) (n=11)	12.0121 (±1.9128) (n=11)	2.9049 (±0.4625) (n=11)	23.5436 (±3.7491) (n=11)	5.6937 (±0.9067) (n=11)	0.00605 (±0.0025) (n=11)	0.00146 ±0.00059 (N=11)

TABLE 5-1:

Energy equivalents for settled plankton collected from plankton tows at 8 meters from the fore reef at Discovery Bay. The energy equivalents are given in both Joules and Calories, the values in Joules were determined using wet oxidation with Potassium dichromate.

DATE OF TOW	11/9/81	12/9/81	13/9/81	31/8/8	14/9/81	28/9/80	18/9/80	13/10/80	24/9/80	26/9/80	16/9/80	30/9/80
TIME OF TOW	20:00	20:30	20:30	20:30	20:30	20:00	01:00	20:00	24:00	05:00	18:00	20:00
TOW NUMBER	1	2	3	4	5	6	7	8	9	10	11	12
SPECIES	NUMBER OF EACH SPECIES IN A TOW											
CNIDARIA LARVAE	2		1	1								
MEDUSAE			3	4					3			
PLATYHELMINTHES LARVAE			1									
POLYCHAETE LARVAE			2	1				2			1	
POLYCHAETE ADULT	1			1		14			3			
SIPUNCULID LARVAE				1							1	
GASTROPOD LARVAE			2	1	4	2			8	6		
PELECYPOD LARVAE					7	4				25		8
PTEROPODA	13	65	16	16	64	22	32	3	12	16	27	24
CLADOCERA				4	4						1	
OSTRACODA	3	9	2	6	4	4			5	9		11
COPEPODA	275	143	455	242	420	312	81	252	208	196	189	145
NAUPLII	3	4	3	6	7	12					1	
CIRRIPED LARVAE	1	2	1	2	2	4				5		
MYSIDACEA	8	5	8	4	5	2	5			11		4
CUMACEA	2				1						1	
AMPHIPODA	2			4	1							
SHRIMP LARVAE	1	10	18	16	15	5	1	4		13		4
ANOMURA LARVAE	6	3	2	1		3						
BRACHYURA LARVAE	5			2	4	2		2	7			2
ECHINODERM LARVAE	1		3	2	3					4		
ISOPODA	2		2	1	2							
LARVACEA	12											
CHAETOGNATHA	29	16	12	12	69	31	24	17		32	1	
FISH OVA	1	6	13	26	12	12		10	9	18	3	9
FISH LARVAE	4	8	2	14	4	3	3					4
FORAMINIFERA	10	10	7	9	26	15	12	4		22	16	10
RADIOLARIAN	1				1	1	2				1	3
DINOFLAGELLATE-PERIDINIUM	243	17	69	185	51	87	31	39	34	17	6	
DIATOMS	39	7	52	47	9	21	28	12	56	65	9	
SALPA		1	2	1								
NUMBER OF PLANKTON/ml (from TABLE 5-1)	3370	1530	3375	3045	3575	2780	1045	1725	1720	2195	1285	1120

TABLE 5-2:-

Plankton species identification counted from 5 replicates of 0.2ml taken from a thoroughly mixed sample that had been previously settled in a graduated cylinder. The counts from the 5 replicates were pooled and the total recorded in the table.

Species composition and size distribution of the free and coelenteron samples of zooplankton were compared to determine whether there was a preferred size range, and species of plankton ingested.

Identification, total numbers collected, and size distribution studies were carried out on all plankton tows. The results are given in Table 5-2 showing the relative abundance of each species encountered. The collections show a clear predominance of copepods, these comprising 37-74% of any given sample. Most plankton identified from towed collections were also found in gut samples, the only exceptions being Medusae, Cumacea, Brachyura larvae, and Salps. That representatives of most plankton species collected were found in gut samples is not surprising judging from the size distribution and sample percentage histogram (Figure 5-3). For n=12 tows, 93.57 $\pm$ 3.47% of all plankton collected had a total length less than or equal to 0.5mm, while 4.21 $\pm$ 2.01% were greater than 0.5mm but less than 1.0cm, and only 2.2 $\pm$ 1.67% were greater than 1.0cm. All plankton species identified from towed collections were represented in the < 0.5mm group, and it was plankton from this group that was predominant in gut samples.

VERTICAL MIGRATION PATTERNS OF COLLECTED ZOOPLANKTON.

From Table 5-2 it can be seen that species composition was similar in all tows, but the density of zooplankton in the water column was highly variable ranging from 1045 to 3575 plankters per ml settled volume. This observation supports Ohlhorst (1982) who showed a similar variation in density at Discovery Bay. Such ranges in zooplankton density can be attributed to different sampling times reflecting vertical migration patterns, and to environmental conditions such a storm surge that tended to reduce the density of zooplankton in the water column.

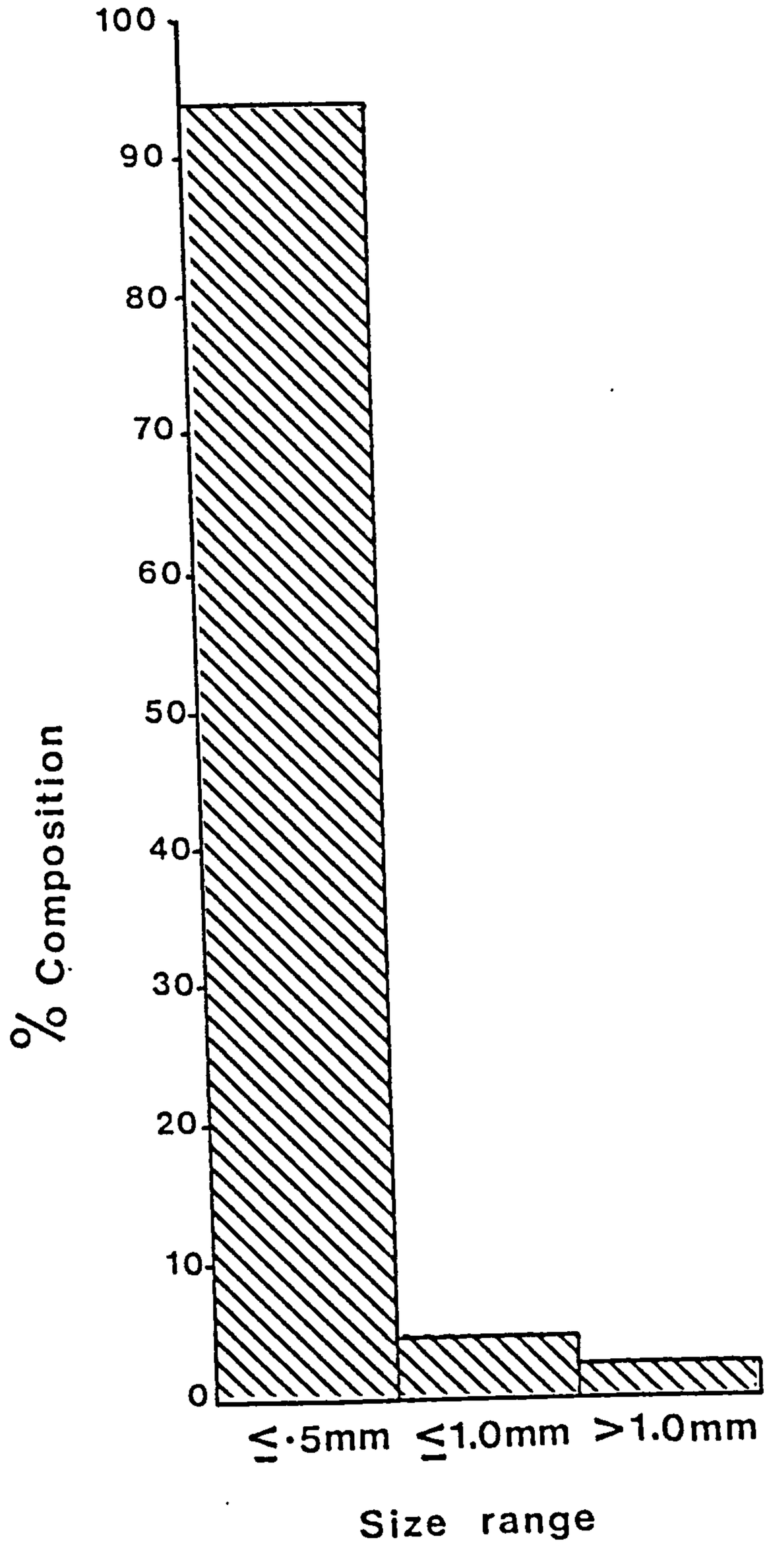


FIGURE 5-3:- Zooplankton size distibution from n=12 plankton tows. The catch was counted, measured using an ocular micrometer, and placed in one of three size classes, less than or equal to 0.5mm, less than or equal to 1.0mm, and greater than 1.0mm. The mean catch percentage from the 12 tows was subdivided as follows; < 0.5mm (93.57±3.48%), < 1.0mm (4.21±2.01%), > 1.0mm (2.20±1.68%).

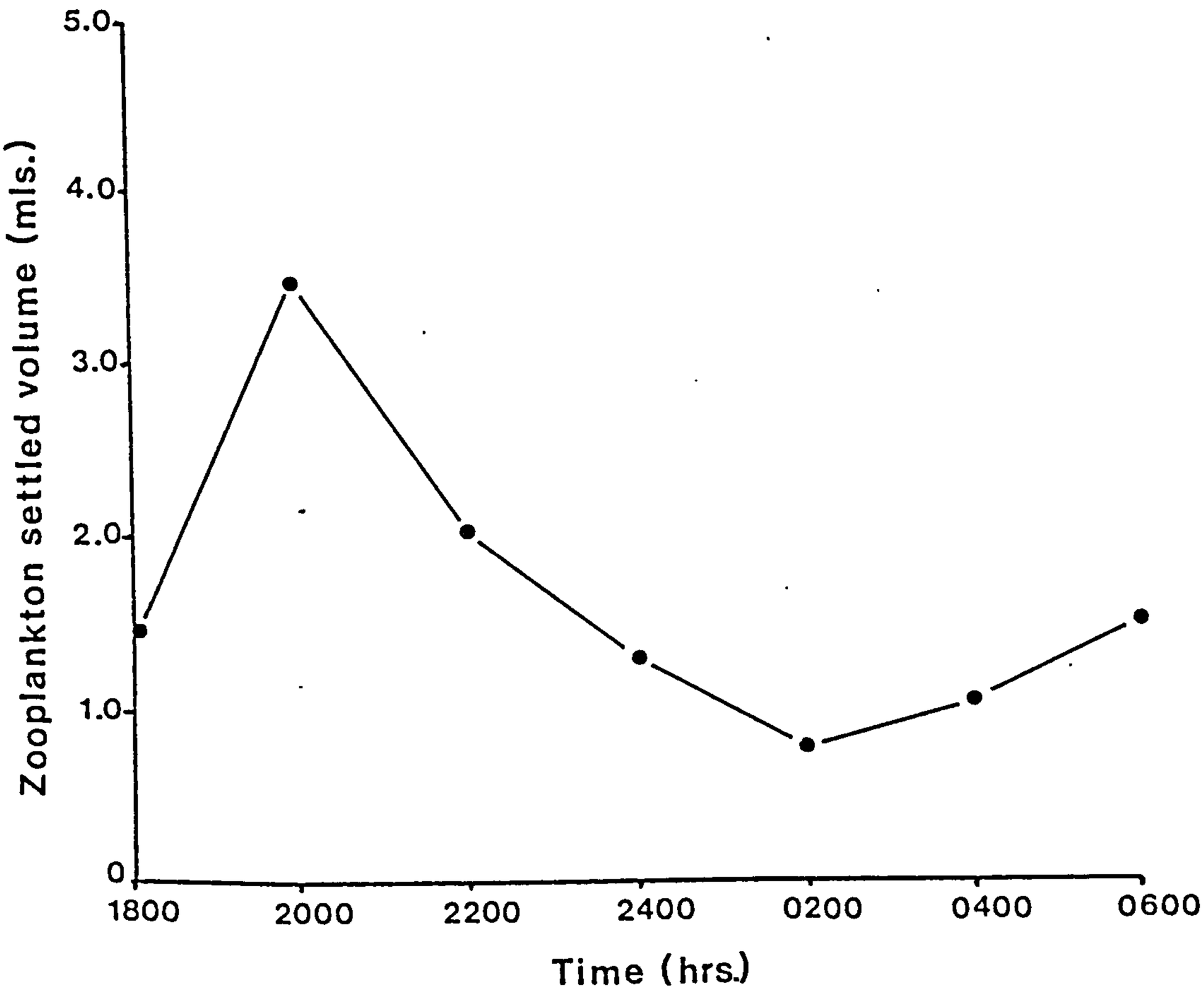


FIGURE 5-4:-
Zooplankton present in the water column the 1st of October, 1980. The collections were made using a plankton tow at 8 metres for 5-minutes at a speed of 1-knot. The tows were made immediately following a gut sampling dive. Settled volume is directly related to zooplankton density in the water column.

The vertical migration of planktonic forms was continuous throughout the night time period (18:00-06:00) with two characteristic peaks, the first at 20:00 hours, and the second at 06:00 hours (Figure 5-4). This vertical migration pattern characteristic of most oceanic environments and similar to those reported by Ohlhorst (1982) at Discovery Bay, was assumed to remain constant throughout the period of this investigation.

5-7-3. EXTRACTION OF COELENTERON CONTENTS:-

All ingested plankton was assumed to have been ingested in the two hours prior to sample extraction following zooplankton digestibility studies on the same species carried out by Porter (1974).

5-7-3.1. GUT CONTENT EXTRACTIONS AT 2-HOURLY INTERVALS - SINGLE NIGHT:-

The density of zooplankton in the water column during the all night sample (Table 5-3) was low relative to other nights with a maximum of 4133 zooplankton per litre at 20:00 hours, and a minimum of 1786 zooplankton per litre at 02:00 hours. Zooplankton ingestion in the upstream and downstream quadrats of sample colonies (Figure 5-5) showed that plankton ingestion closely followed the changes in zooplankton density in the water column (Figure 5-4). A linear relationship between zooplankton ingestion by a coral colony and zooplankton density in the water column is suggested, with a maximum ingestion of 68 plankton/24 polyps occurring at 20:00 hours, and minimum ingestion of 13 plankters/24 polyps occurring at 02:00 hours. This linearity has also been observed in tentaculate and lobate ctenophores (Reeve *et al*,1978) where ingestion rates increased directly with increased food availability. These results clearly suggest that *Montastrea cavernosa* is an obligate feeder ingesting proportionally greater or lesser numbers of zooplankton depending on zooplankton density and in some cases species

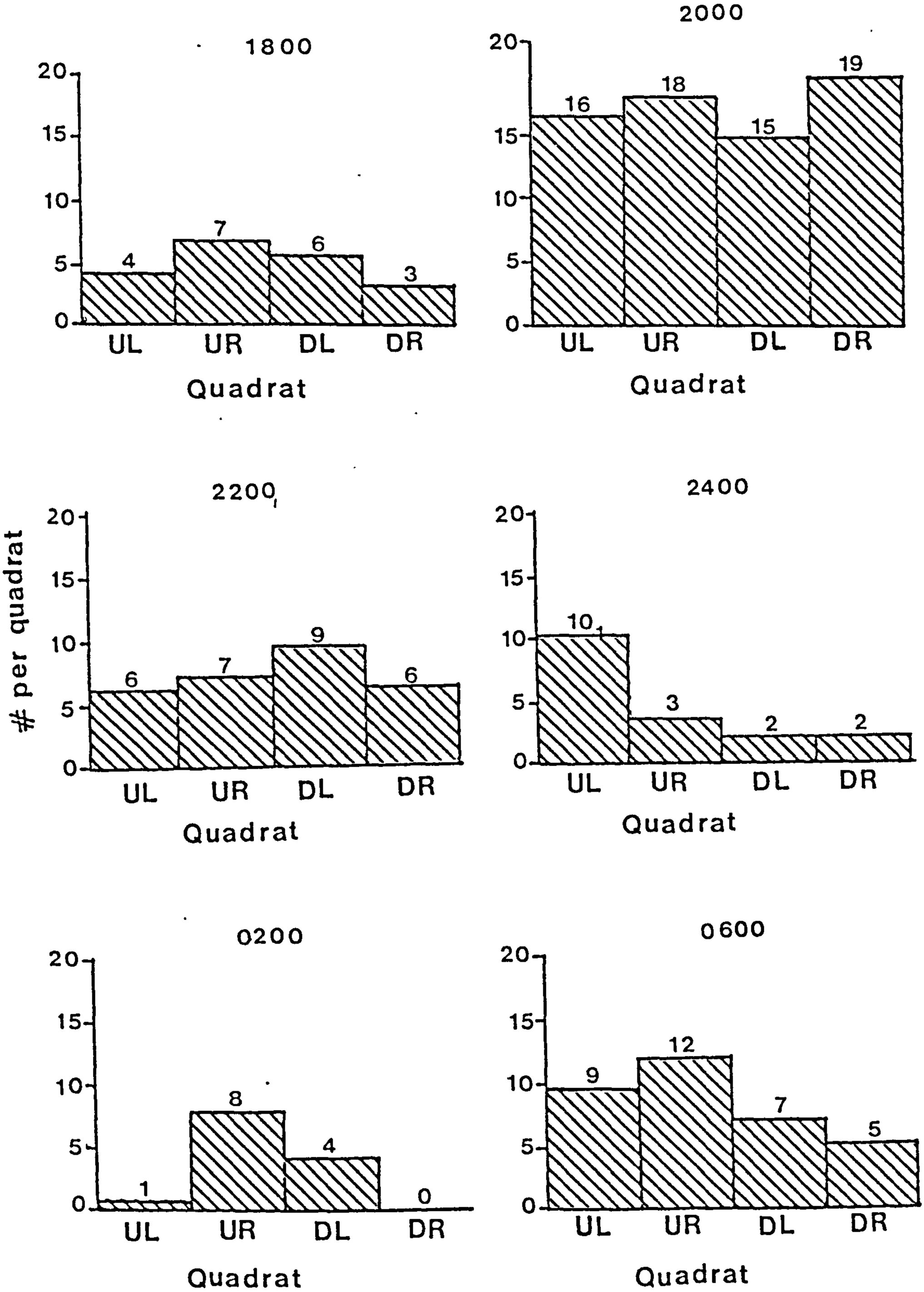


FIGURE 5-5:-
Histograms showing pooled quadrat ingestion numbers for a coral the 1st of October, 1980. Gut sampling times are shown above each histogram. The number of ingested zooplankton closely follows zooplankton availability in the water column (Figure 5-4), and is reflected in the energetic input to the colony during each sampling interval (Table 5-4)

TIME	SETTLED VOLUME ml	# PLANKTON PER VOLUME	%	#PLANKTON/ POLYP	JOULES/ POLYP	CALORIES/ POLYP
18:00	1.45	2147	41	0.816	0.0049	0.0012
20:00	3.50	4133	100	2.830	0.0171	0.0041
22:00	2.00	2973	57	1.166	0.0070	0.0017
24:00	1.35	2537	39	0.708	0.0043	0.0010
02:00	0.80	1786	23	0.542	0.0033	0.0079
04:00	1.05	2279	30	0.946	0.0057	0.0014 **
06:00	1.50	3670	43	1.375	0.0083	0.0020

TABLE 5-3: Energetic inputs from gut sample collections during an all night sample on 1st October, 1980. The summed input during the 12 hour period sampled (with the 04:00 hr sample extrapolated as the mean between the samples at 02:00 and 06:00 hours) yielded 0.0507 Joules, this being equivalent to 6.236 Joules in a standard colony having 123 polyps ($100cm^2$).

composition in the water column.

The energetic contribution from heterotrophic nutrition on zooplankton during the 12-hour period sampled (including the extrapolated value at 04:00 hrs) was 0.0507 Joules.polyp^{-1} (Table 5-3). Normalizing to a standard colony (100cm^2 with 123 polyps), the energy input from zooplankton ingestion would be 6.236 Joules.12-hours^{-1}.

A rough estimate of capture efficiency was also calculated for each sample interval. As expected the highest capture efficiency (1.64%) was recorded when plankton density was highest at 20:00 hours,while the lowest capture efficiency (0.67%) was recorded at 24:00 hours, not at the expected 02:00 hours when density was also at its lowest and capture efficiency was (0.728%). This anomaly remains difficult to explain since other than the total number of zooplankton present, the species composition, size distribution, and oceanographic conditions remained unchanged. It is possible that in this instance the colony took longer to expand and actively feed following the collection at 22:00 hours resulting in fewer plankton being caught during this interval.

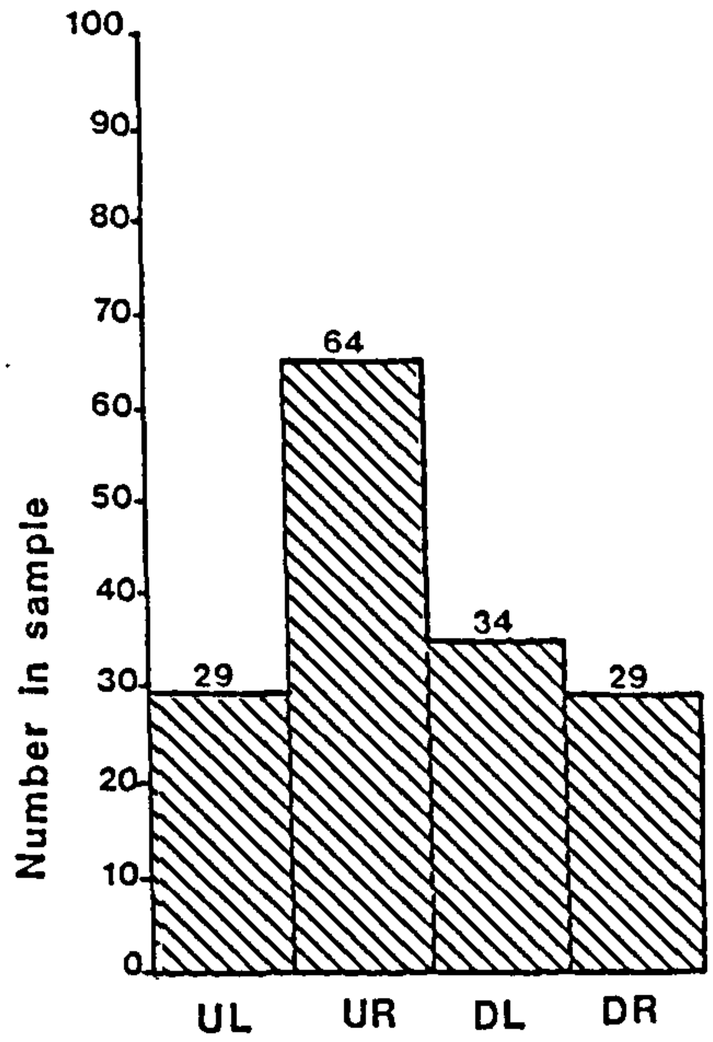
5-7-3.2. SPOT SAMPLES AT DIFFERENT TIMES DURING DIFFERENT NIGHTS 10 METERS

i) it was assumed that all zooplankton extracted from the coeleteron of the sample colony had been ingested during the two hours prior to each collection (Porter, 1974)

ii) the conversion of ingested plankton to its energy equivalent assumes full digestion of all ingested plankton.

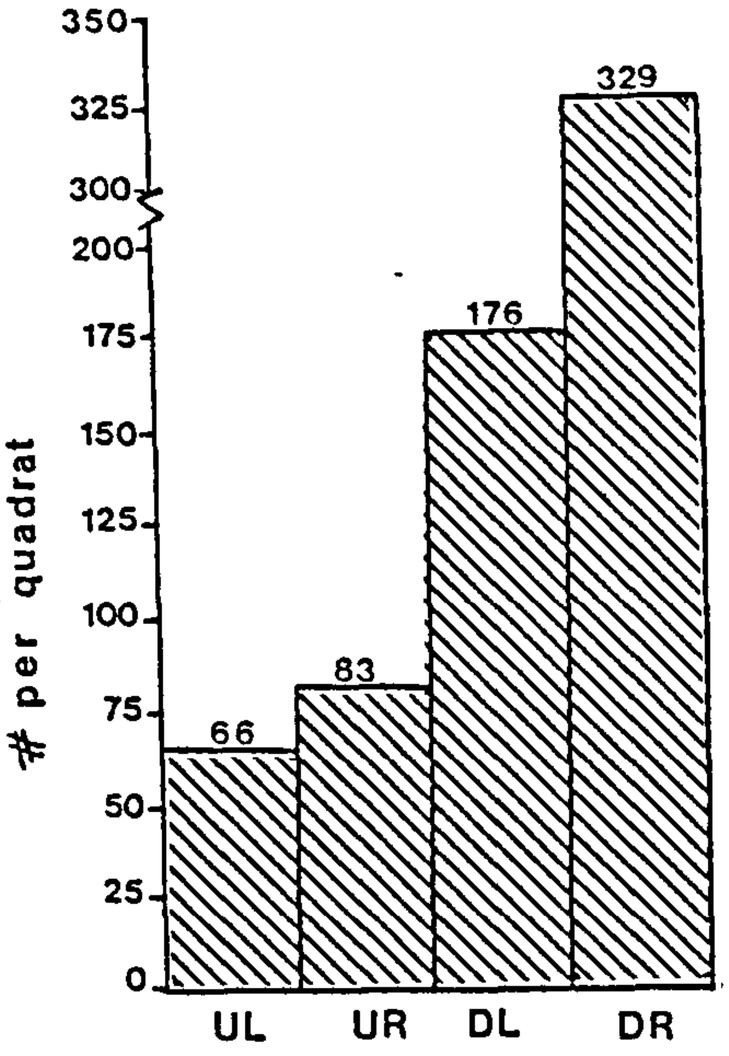
SAMPLE #	LUNAR PHASE	DATE	TIME COLLECTED	SETTLED VOLUME(ml)	# PLANKTON PER VOLUME	TOTAL # INGESTED	% CAPTURE EFFICIENCY	# INGESTED PER POLYP	JOULES/ POLYP	JOULES PER STANDARD 123 POLYP 100cm² COLONY
A	NEW MOON	16/9/80	18:00	2.7	5873	156	2.65	6.5	0.0393	4.8370
B	WANING MOON	28/9/80	20:00	5.4	11763	654	5.56	27.25	0.1649	20.2827
C	WANING MOON	30/9/81	20:00	6.8	14745	147	0.99	6.12	0.0370	4.5510
D	FULL MOON	11/8/80	20:00	1.6	3464	17	0.49	0.71	0.0043	0.5289
E	FULL MOON	12/8/81	20:30	6.6	14324	205	1.43	8.54	0.0517	6.3591
F	FULL MOON+1	13/8/81	20:30	6.7	14732	253	1.72	10.54	0.0638	7.8474
G	FULL MOON+2	14/8/81	20:30	7.8	16921	214	1.26	8.91	0.0539	6.6297
H	FULL MOON-1	24/9/81	24:00	1.6	3405	132	3.87	5.50	0.0333	4.0959
I	FULL MOON-3	18/9/80	01:00	3.1	6706	86	1.28	3.58	0.0216	1.5498
J	FULL MOON	26/9/80	05:00	2.4	5230	108	2.06	4.50	0.0272	3.3456

TABLE 5-4, and FIGURE 5-6 (facing page): Data collected from spot gut sampling collections at the 10 metre site. The data from each collection are presented in the form of a histogram with the number ingested by each sampling quadrat of 6 given at the top of each column. Energy equivalents were calculated as per the text, and normalized to the energetic inputs of a standard 100cm² 123 polyp colony.



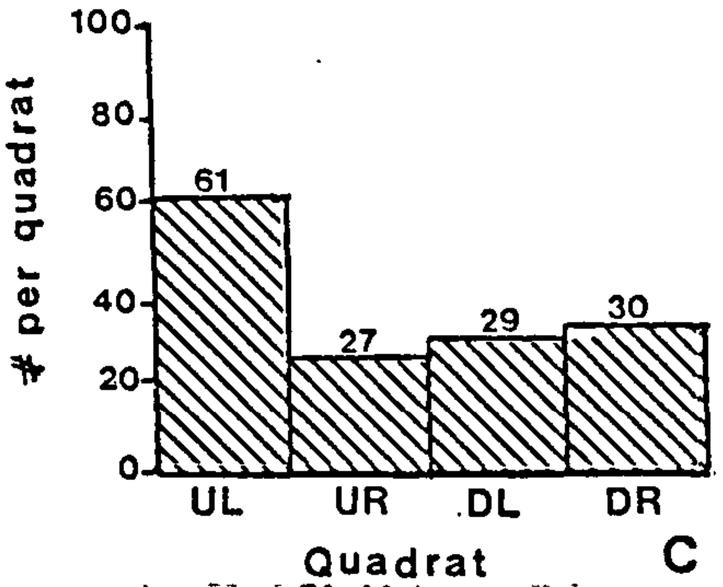
A

Quadrat
18:00 hours
16th of September, 1980



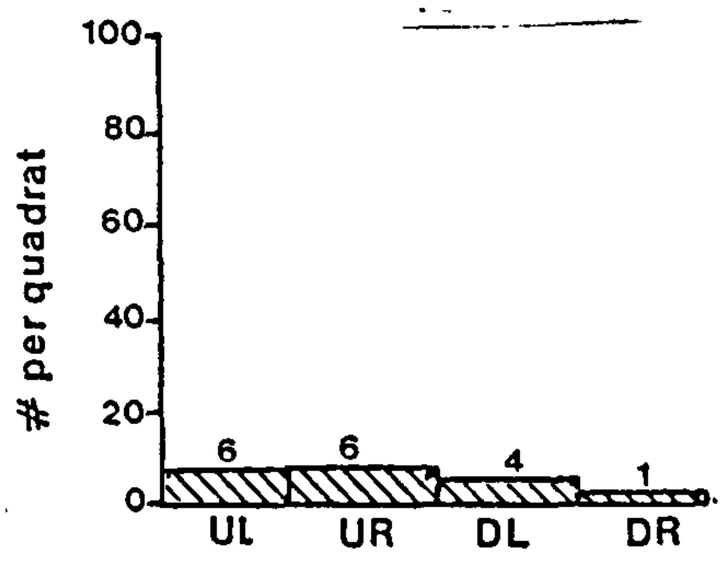
B

Quadrat
20:00 hours
28th of September, 1980



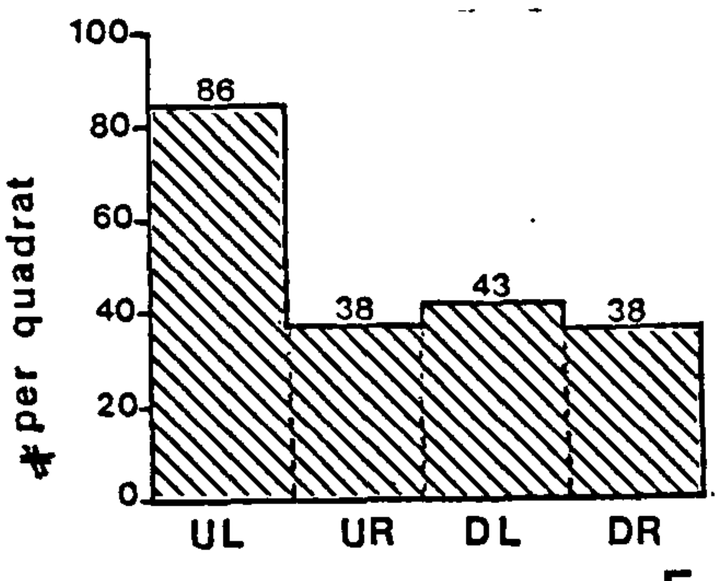
C

Quadrat
20:00 hours
30th of September 1981



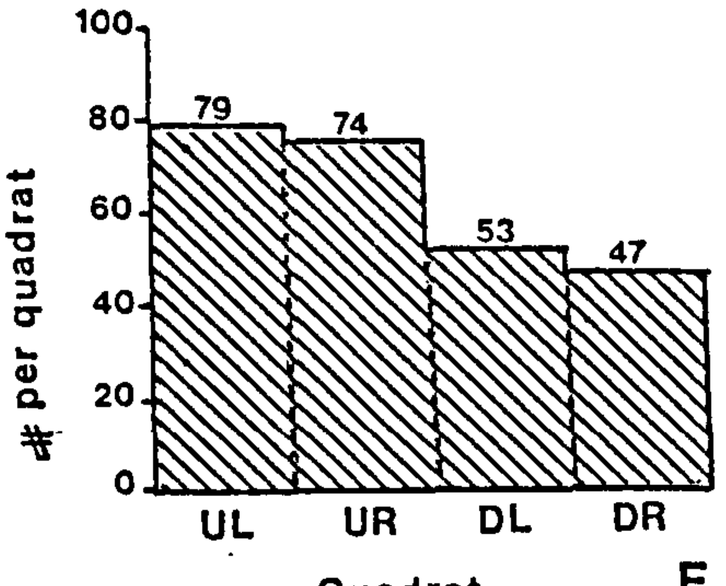
D

Quadrat
20:00 hours
11th of August 1980



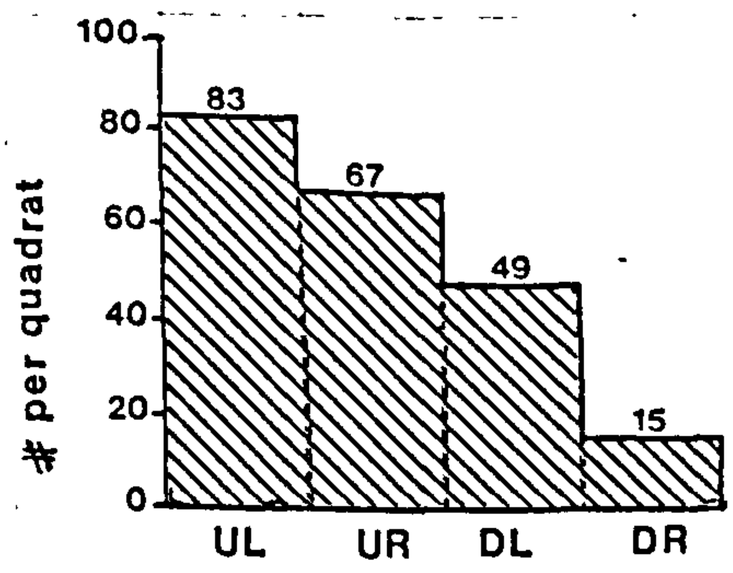
E

Quadrat
20:30 hours
12th of August, 1981



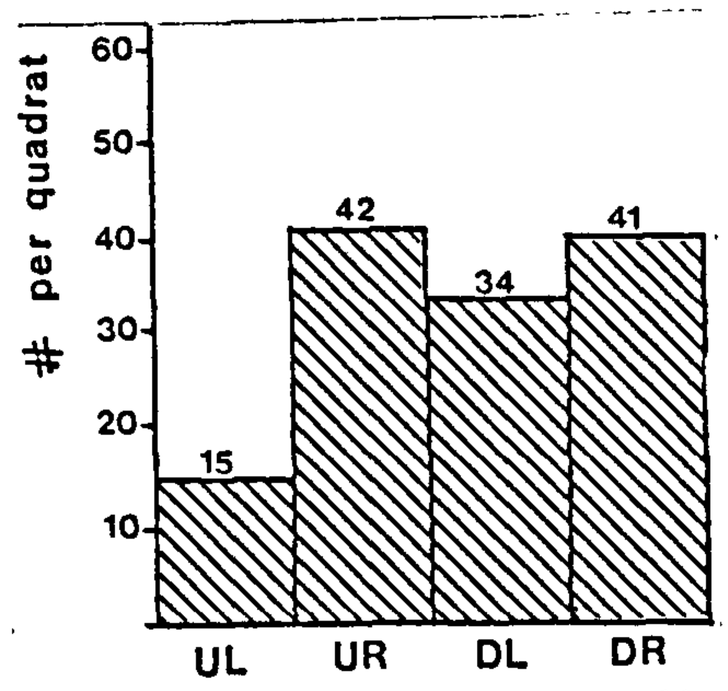
F

Quadrat
20:30 hours
13th of August, 1981



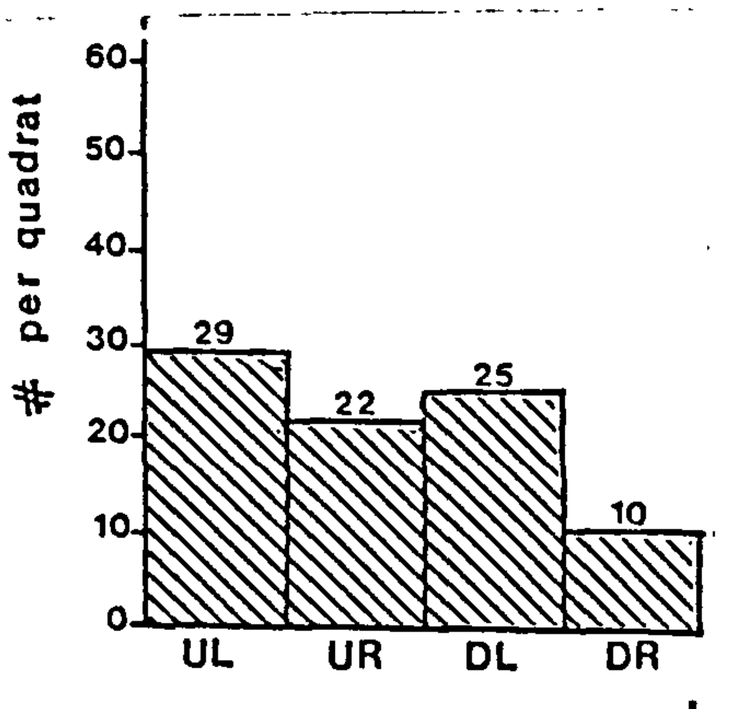
G

Quadrat
20:30 hours
14th of August, 1981



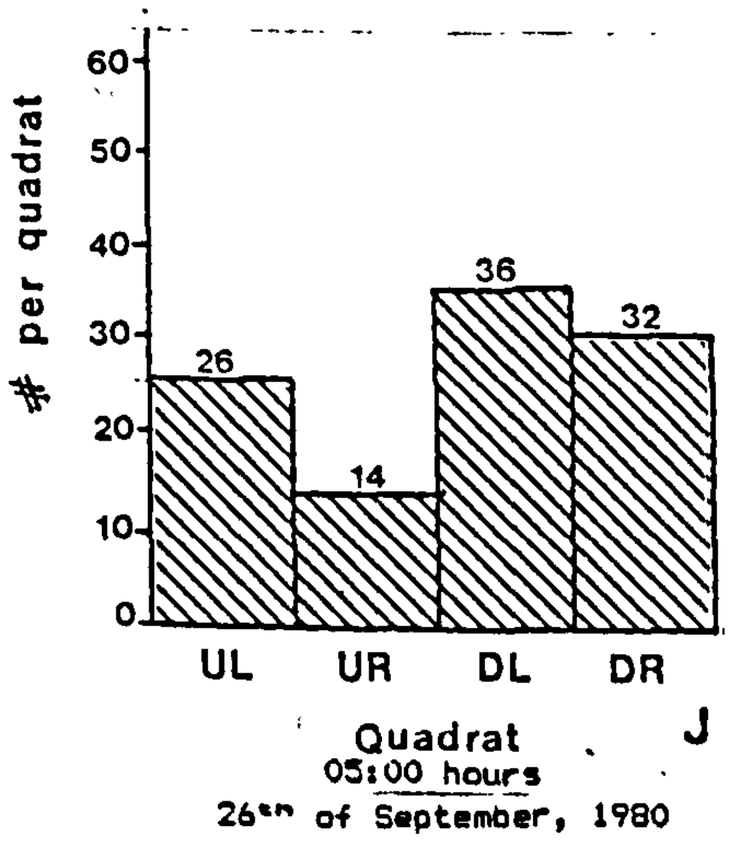
H

Quadrat
24:00 hours
24th of September, 1981



I

Quadrat
01:00 hours
18th of September, 1980



J

Quadrat
05:00 hours
26th of September, 1980

SPOT SAMPLE AT 18:00 HOURS:-

Figure 5-6(A) and Table(5-4) represent extrapolations from a spot gut content collection at 18:00 hours during a waxing (new) moon. Plankton density was high at 5873 zooplankton per liter collected using the light trap. Capture efficiency was high at 2.65% reflecting this high plankton density.

With this density the extrapolated energetic input during a 12-hour nightime period would be 0.0393 Joules/polyp, or 4.8370 Joules per standard 100 cm^2 colony having 123 polyps (Table 5-4).

SPOT SAMPLES COLLECTED AT 20:00 HOURS:-

Figures (5-6(B), 5-6(C), 5-6(D)) and Tables (5-4) represent extrapolations of gut sample extractions taken at 20:00 hours during periods of peak plankton density.

Table (5-4) and Figure (5-6(B)) are for a sample collected on the 28th September, 1980 during a waning moon. The plankton density was 11,763 plankton per liter, collected using the light trap, the majority of these being gravid polychaetes (epitokes) seen to be swarming on this particular night. Because of the highly unpredictable movement of these plankters in the water column , capture efficiency was high at 5.56% most of these being the result of collisions between the polychaete and the coral. The high capture efficiency is reflected in the energy input per polyp which was calculated to be 0.1649 Joules, or 20.2827 Joules per 100cm^2 standard colony. The contribution from zooplankton in this instance was expected to be higher since individual polyps were observed to consume epitokes repeatedly at short intervals. Gut samples were replete of egg masses, but these were not included in in the counts, only identifiable polychaetes and

other plankton were counted. Burst polychaetes devoid of egg masses were seen to be ejected by individual polyps suggesting that plankton capture continued despite the coelenteron being filled to capacity. This latter point reinforces the suggestion that this species is an obligate feeder.

Table 5-4 and Figure 5-6(C) represent a sample extracted and collected at 20:00 hours the 30th of September 1980 during a full moon. Plankton density was high at 14,745 plankters per litre but capture efficiency was low at 0.996% reflecting bottom surge conditions. In conditions where bottom surge intermittently agitates the substrate suspending particulate material, many of the polyps remained contracted. Under these conditions the energy input per polyp was 0.0370 Joules per polyp which yielded 4.5510 Joules /12 when normalized to the equivalent inputs for a standard colony.

Table 5-4 and Figure 5-6(D) are for a sample extracted at 20:00 hours on the 11th of August 1981 during a full moon. Plankton density was very low at 3464 plankters per litre with a high proportion of these being highly motile chaetognaths. The composition of this sample and the ability of chaetognaths to escape tentacular capture even when in direct contact with the tentacle surface was reflected in low capture efficiencies of 0.4908%. The extrapolated energy input was 0.0043 Joules per polyp yielding an energetic input of 0.5289 Joules for a standard 123 polyp colony. This sample suggests that during periods of reduced plankton density the energetic input to a coral colony could be further reduced if one of the dominant planktonic forms present are able to successfully evade capture by the coral.

SPOT SAMPLES COLLECTED AT 20:30 HOURS

Figures (5-6(E), 5-6(F), 5-6(G)) and Table (5-4) represent spot gut samples extracted from polyps at 10 meters at 20:30 hours on three consecutive days starting on the 12th to the 14th of August 1981, with the full moon being on the 12th of August.

Plankton density was at its lowest during the full moon with 14,324 plankton/litre, the density rose to a maximum two days into waning when 16,921 plankton/litre were recorded. Capture efficiencies were similar for all three days 1.43%, 1.72%, 1.26% respectively. The lowest capture efficiency corresponding to conditions of maximum plankton density but under storm surge conditions. Pooled quadrat ingestion values for these collections (Figures 5-6(E) to 5-6(G)) suggest that there was signifcantly more plankton caught by the upstream quadrats than the downstream quadrats ($P>.001$), adding some support to current aided plankton capture. This was not the case in all collections where there was no significant difference between plankton capture by upstream and downstream quadrats.

The energetic contribution from zooplankton ingestion was similar for all three days 0.0517, 0.0638, 0.0539 Joules per polyp for the 12th to the 14th respectively. When normalized to the energy input of a standard 123 polyp colony these would be equivalent to 6.3591 Joules on the 12th, 7.8474 Joules on the 13th, and 6.6297 Joules on the 14th.

SPOT SAMPLES COLLECTED AT 24:00 HOURS AND 01:00 HOURS:-

Extrapolations for spot collections at 24:00 hours (Figure 5-6(H), Table 5-4), and at 01:00 hours (Figure 5-6(I), Table 5-4) show that for the former capture efficiency was high at 3.87% with plankton density being moderate at 3405 plankton/liter these being mainly small copepods (2-6mm). Capture efficiency for the latter was lower at 1.28% with plankton density

higher at 6706 plankton/liter, the lower capture efficiency reflecting the presence of larger and more motile forms in the water column.

The energy input per polyp was 0.0333 Joules at 24:00 hours equivalent to an energetic input of 4.0959 Joules in a standard colony (Table 5-4), and 0.0126 Joules at 01:00 hours equivalent to 1.5498 Joules for a standard 123 polyp coral (Table 5-4).

SPOT SAMPLE COLLECTED AT 05:00 HOURS:-

The plankton density in the water column during a gut sample collection at 05:00 hours on the 26th of September 1980 during a full moon was 5230 plankton/litre (Table 5-4, Figure 5-6(J)). The collection was carried out in strong surge conditions with a marked reflected offshore ground wave. Capture efficiency was high at 2.065% as was the energetic contribution from zooplankton extrapolated at 0.0272 Joules per polyp equivalent to 3.3456 Joules per 123 polyp standard colony.

It was of interest to note that on this particular collection with the prevailing oceanographic conditions, the downstream and onshore quadrats of the sampled colony ingested more plankton relative to the upstream colonies(Figure 5-15). This observation may in part be due to the strong offshore ground swell cancelling the effects of the onshore swell at periodic intervals thus creating periodic intervals of slack water. This would tend to influence and assist zooplankton capture by downstream polyps who normally exist in slight plankton shadows in hemispherical colonies (Porter, 1974).

5-7-3.3. GUT SAMPLE EXTRACTIONS FROM COLONIES AT 30 METERS AT 20:00 HOURS:-

No zooplankton was extracted from sampled polyps of colonies at 30 metres on the fore reef at Discovery Bay. Three excursions to that depth at 20:00 hours in which 72 separate polyps were sampled using the quadrat technique used at the shallow site showed no identifiable plankton.

Since no zooplankton was recovered the energy input from zooplankton ingestion at 30 metres must be taken to be zero for the purpose of the energy equation (Chapter-7), this does not preclude the possibility that *Montastrea cavenosa* colonies at this depth do feed on zooplankton and other exogenous nutritional sources. Future research should consider the possibility of alternate nutritional strategies with increasing depth.

5-8. DISCUSSION.

Differing views regarding the dependance of hermatypic corals on heterotrophic nutrition have persisted since Vaughn (1912, 1919) and Gravier (1913). Presently with improved laboratory and field techniques the question still remains; do hermatypic corals need to feed heterotrophically to survive, and to what extent are they dependant on external nutritional sources. This investigation attempted to answer these question within the context of an energy budget for *Montastrea cavernosa*.

The results from the spot sampling experiments and the all night two hourly sampling regime showed that the energy contribution to a coral colony from heterotrophic nutrition on zooplankton was highly variable, ranging from 0.5289 to 20.2827 Joules/standard colony. The contribution from the all night sample contributing 6.24 Joules to a standard coral colony. This result was lower than expected and accounted for only 0.77% of the daily energy requirements of the animal fraction of a standard 100cm²

colony at 10 metres (See Chapter 7, Figure 7-2). The percent contribution is lower than expected considering observations of continuous voracious feeding by colonies of *Montastrea cavernosa*, but agrees with Porter (1974) who reported mean percent contribution rates of 3.4% (range 0.1-11.6) for the same species in Panama, and Johannes and Tepley (1974) who working with *Porites lobata* (a small polyped coral) reported percent contributions from zooplankton ingestion of 10-20% of the energy requirements of test colonies.

In this research the energy contributions from zooplankton ingestion were based on a series of assumptions based on the work of Porter (1974), Ohlhorst (1982). Following the results of Porter (1974), and Nicholls (cited Yonge, 1930) who report complete digestion of ingested zooplankton after 2-hours in the coelenteron, all intact and recognizable plankton in the gut sample were assumed to have been ingested in the two hours prior to the sample. This assumption validates the two hourly sampling regime of the all night sample. The results presented would tend to underestimate the contribution of heterotrophic nutrition to the energy equation since they do not include possible daytime feeding (Lasker, 1978), or the contributions from bacteria (DiSalvo, 1974; Sorokin, 1974; Moriarity, 1979), from dissolved organics (Muscatine and Porter, 1977), and organic aggregates (Johannes, 1967; Coles and Strathman, 1973)

The results also suggest that *Montastrea cavernosa* must be primarily an autotrophic organism but also an obligate feeder on zooplankton this will be shown in later sections. Zooplankton ingestion occurred throughout the 12-hour nighttime period with ingestion rates dependent on plankton density and species composition in the water column. Reeve *et al* (1978) noted that ingestion rates in lobate and tentaculate ctenophores were

linearly related to food concentration, The results from this investigation support those results. Ingeston rates by individual polyps were affected by extrinsic factors such as bottom surge conditions which prevented polyps from fully extending their tentacles (visual observation), by zooplankton species composition where large or extremely motile forms such as *Sagitta sp.* were ingested with difficulty, and finally by the orientation of the colony and its position relative to the reef profile, in which case exposure of feeding surfaces to altered or deflected water flow as well as position on a reef facies with little suitable substrate for demersal plankton would tend to reduce zooplankton ingestion, as shown from current flow studies over a large patch reef at 30 metres (Appendix 2-1).

Sampling of coral gut contents and plankton in the water column at the 30-metre site yielded what could be the most revealing data. In three collection dives to this depth no ingested plankton was recovered from 72 polyps sampled. Likewise few plankters (15-200/litre for n=6 collections) were caught at this depth using tows and the light trap. These results suggest that corals located on offshore reef slopes at Discovery Bay may exist in conditions of extremely low plankton densities relative to colonies at 10-metres. The proximity of the 30 metre sampling site to the fore reef slope and deep offshore water make this site markedly different both ecologically and oceanographically than the 10-metre site. The deep collection site would be bathed by predominatly onshore currents (Woodley and Robinson, 1977) low in plankton densities (Sargent and Austin,1949, 1954; Tranter and George,1972). This area will also tend to retain fewer demersal zooplankton due to reduced available and suitable substrates (Sand, coral rubble, etc.) associated with a vertical reef facies. Those plankton present will tend to drift shorewards during vertical migrations since near reef circulation patterns normally retaining demersal plankton

(Sale, McWilliam and Anderson, 1984) would not be present on the fore reef facies. The overall effect of a shorewards drift of demersal plankton, and a partial replacement by oceanic plankton would be a sustained plankton paucity and reduced ingestion rates directly related to lower plankton densities in coral colonies on the deep fore reef. Reduced zooplankton ingestion rates would also tend to decrease the supply of essential amino acids necessary for tissue growth, but these may be supplied by alternate exogenous sources such as bacteria, dissolved and particulate organics.Depth related trophic plasticity as alluded to by the results of this investigation could form the basis of a new research programme.

It will be shown in Chapter 7 that since the contribution from zooplankton ingestion to the daily energy requirements of this species is extremely low (<1%, from the all night sample), then it can be assumed that zooplankton ingestion by *Montastrea cavernosa* is not energetically important, but that it satisfies a secondary role such as tissue growth.

Growth studies on 'bleached' and zooxanthellate corals at 10 metres and 30 metres showed that colonies kept at 10 metres grew significantly faster ($P>.05$) than colonies at 30 metres. If the results from 'bleached' colonies only are considered, thus eliminating the effect of light enhanced calcification in zooxanthellate corals (Goreau,1959), then all tissue growth can be attributed to heterotrophic nutrition, and passive calcification (Gladfelter,1982). Therefore since more plankton is available in shallow areas (Sale, McWilliam and Anderson, 1976; Birkeland and Smalley, 1981; Walter and Pasamonte, 1981), then ingestion rates and colony growth rates will tend to be higher in colonies located in areas where water currents and near reef circulation patterns result in increased plankton densities.

CHAPTER - 6.

PHOTOSYNTHETIC OXYGEN PRODUCTION AND COLONY RESPIRATION.

INTRODUCTION

Investigations of the physiological and ecological characteristics of the coral zooxanthellae symbiosis have concentrated on photosynthetic and respiratory measurements (both of the colony and isolated zooxanthellae) to determine the ability of autotrophic nutrition to supply the carbon requirements of the symbiosis (Beyers 1966; Kanwisher and Wainwright 1967; Roffman 1968; Franzisket 1969; Pillai and Nair 1972; Wethey and Porter 1976; Muscatine and Porter 1976), but only Tytler (1982) and Davies (1984) have approached the problem in terms of the energetic requirements of the symbiosis. These investigations have all been carried out in the laboratory, or as in the case of Roffman (1968), and Davies (1984), in the laboratory using animals collected and maintained on the reef prior to use. More recently laboratory investigations have been giving way to field research programs where the test organisms are maintained and experimented on *in situ* (Svoboda 1978; Porter 1981; McCloskey and Muscatine 1984; Chalker 1984) The shift of research programs from the laboratory to the reef has been made possible by the rapid advances in technologies enabling the deployment of remote sensing devices capable of recording physiological data *in situ*.

In this investigation an attempt has been made to determine the energy contribution from photosynthetic production by symbiotic zooxanthellae in coral tissue to the energy requirements of whole *Montastrea cavernosa* colonies. An *in situ* automatic respirometer was used in a series of experiments at two depths (10 and 30 metres) to record

oxygen flux in sample corals placed in periodically flushing closed respirometers. Rates of change of oxygen concentrations in the respirometer chambers were used to calculate all energy equivalents. The data were analyzed on a unit biomass (mg.dw. coral tissue), and unit surface area (cm^2) basis, and the results normalized to a standard 100cm^2 colony.

MATERIALS AND METHODS

The data presented in this section were recorded *in situ* using the prototype automatic respirometer/data logger described in Chapter-3, and as such must be treated as preliminary results from a prototype device. The data were found to be highly comparable to published results.

FIELD TECHNIQUES

Animal collection and maintenance on the reef have already been described in Chapter-2, while instrument preparation prior to deployment on the reef, recovery of the apparatus following termination of a run, and field data control have been covered in Chapter-3

ADDITIONAL TECHNIQUES

1) DEPTH ATTENUATION OF INCIDENT IRRADIANCE

Measurements of the attenuation of incident irradiance in the working depths (0 to 40 metres) of this investigation were carried out on the fore reef at Discovery Bay using a quantum light meter Licor-185, and a Licor-192S quantum sensor . Readings were taken at 10ft. (3 metre) intervals from 40 metres to the surface at 12:00 hours. The results were used to post-calibrate light readings recorded by the *in situ* data logging apparatus.

2) ZOOXANTHELLAE : DETERMINATION OF POPULATION SIZE

In order to express respiration of the zooxanthellae (Rz) in terms of the number of zooxanthellae present in host tissues it was necessary to measure the density of zooxanthellae per unit surface area, and per unit biomass in n=50 corals collected throughout the investigation over the depth range studied. The corals used include those used in respirometry.

Two separate techniques were used to determine zooxanthellae density, the first a field procedure on freshly collected corals, and the second a laboratory technique used on corals returned to Glasgow University for final analysis;

1) Tissue was removed from the skeleton of collected coral colonies in the field using the Water Pik technique of Johannes and Weibe (1970). The blastate was homogenized for 3-minutes in a Waring blender and then decanted into 150ml centrifuge tubes through 3 layers of gauze to remove skeletal fragments. The blastate was centrifuged at 3000 rpm for 5-minutes, the supernatant decanted off and the pellet washed with 10ml Millipore filtered seawater and whirlymixed into suspension. The suspension was then transferred to a glass Potter homogenizer using a Pasteur pipette and homogenized for a further two minutes. Six subsamples of thoroughly mixed suspension were counted using a haemocytometer. The surface area of coral colonies processed in the field were determined using the aluminium foil method of Marsh (1970), and zooxanthellae density expressed in terms of a unit surface area (cm^2).

2) Coral colonies used in growth and respiration studies at Discovery Bay and later transported frozen to Glasgow for final analysis were also used for zooxanthellae counts. Tissue removal techniques from thawed

colonies, and the freeze drying method to determine tissue biomass have already been described (Chapter-4). Following tissue biomass determinations of each colony, 10mg subsamples of lyophilized material were rehydrated in 10ml of Millipore filtered seawater and homogenized in a glass Potter homogenizer for 3-minutes. Six subsamples of this solution were then counted using a haemocytometer. Surface areas of corals processed in Glasgow were determined using the aluminium foil method of Marsh (1970).

Zooxanthellae counts were expressed on a unit biomass (mg.dw.) and unit surface area (cm^2) basis in the laboratory, and unit surface area only in field processed colonies. An analysis of variance between zooxanthellae counts carried out in the field and those carried out in the laboratory showed that there was no significant difference in the results ($P<.001$ for n=372 counts). Based on this analysis, only laboratory counts which included an accurate measure of tissue biomass were used in this section.

MEASUREMENT OF PHOTOSYNTHETIC PRODUCTION AND COLONY RESPIRATION

Of the n=60 colonies collected for growth studies at both study sites, n=30 colonies at 10 metres and n=30 colonies at 30 metres were used to determine rates of photosynthetic production and dark respiration. These colonies were treated similarly to those used in growth studies, but were placed into respirometer chambers at intervals for periods of 24-hours during the final field session (16/4/82 to 10/6/82).

Corals selected for experiments using the automatic respirometer were systematically placed in respirometer chambers to measure photosynthetic oxygen evolution and colony respiration which was then recorded on magnetic tape in 5-minute intervals for the duration of an experimental run. Three colonies were measured simultaneously during each run.

Since actual logging time for each instrument deployment was unpredictable due to accumulator charging problems, each coral colony was used several times in order that a complete 24-hour recording could be made for each colony used. The apparatus was deployed on three separate times for each series of three corals used (0700-0700; 1200-1200; 1800-1800), but the actual instrument deployment on the reef could be 2 hours before or after these ideal times depending on field conditions, and repeat diving limitations. These variations from the ideal times were taken into consideration in later runs to make up the 24 hour cycle. Since this experimental strategy had to be adopted much of the data presented are for colonies from the 10 meter site. Difficulties with the instrument and remaining operative field time prevented extensive deployment at the 30 metre site.

Field analysis of data recorded *in situ* showed that much of this information was invalid due either to electrode grounding and electrolyte leakage problems, or to power supply problems since the accumulators were often unable to retain their charge and as a result the electrode calibration drifted. The latter problem limited running times by both reducing the period in which the device was actually recording *in situ*, and by rendering those data [illegible] invalid once the electrode calibration started to drift. Electrode drift due to voltage problems was identified by a linear decline in recorded data points which continued unchanged through a flushing cycle. Voltage problems were eventually eliminated by re-wiring the -5 volt regulating circuitry, and replacing the voltage regulator. All power lines were replaced and all printed circuit boards cleaned of possible salt corrosion and sealed with a polyurethane spray.

Data presented in this section are for experimental runs in which no corruption due to electrical or electrode faults could be detected. The dates on which the recordings were made are as follows; the 12/5/1982 at 12:00 hours for the traces in Figures 6-2A, and 6-2B, and on the 25/5/82 at 17:00 hours for the three corals from which data were recorded and presented in Figure 6-4..

RETRIEVAL OF CORAL COLONIES

All corals used in P/R experiments and still remaining on the reef at the end of the investigation were collected from both the 10 metre and the 30 metre study sites and transported to the laboratory.

Before processing each colony prior to their eventual transport to Glasgow University, each coral used in respirometry was placed in a respirometer chamber filled with seawater to determine the volume displaced by each colony. The seawater contained in the respirometer chamber was then decanted into a 500ml graduated cylinder. The volume of the chamber was then determined by subtracting the displaced volume from the total chamber volume. These volumes were then used in all subsequent P/R calculations to determine dissolved oxygen concentrations in the chambers.

The coral colonies were then placed in numbered, nylon screw top jars filled with Millipore filtered seawater, and kept frozen at -10°C pending transport to Glasgow University for further analysis

LABORATORY TECHNIQUES.

1) ANIMAL TREATMENTS

Tissue biomass and surface areas of coral colonies used in

respirometry were determined as in Chapter-4

2) ENERGY EQUIVALENTS: TISSUE

The energy content of six replicate subsamples (3-5mg) of lyophilized tissue from each colony used in respirometry was determined using wet oxidation (Chapter-2)

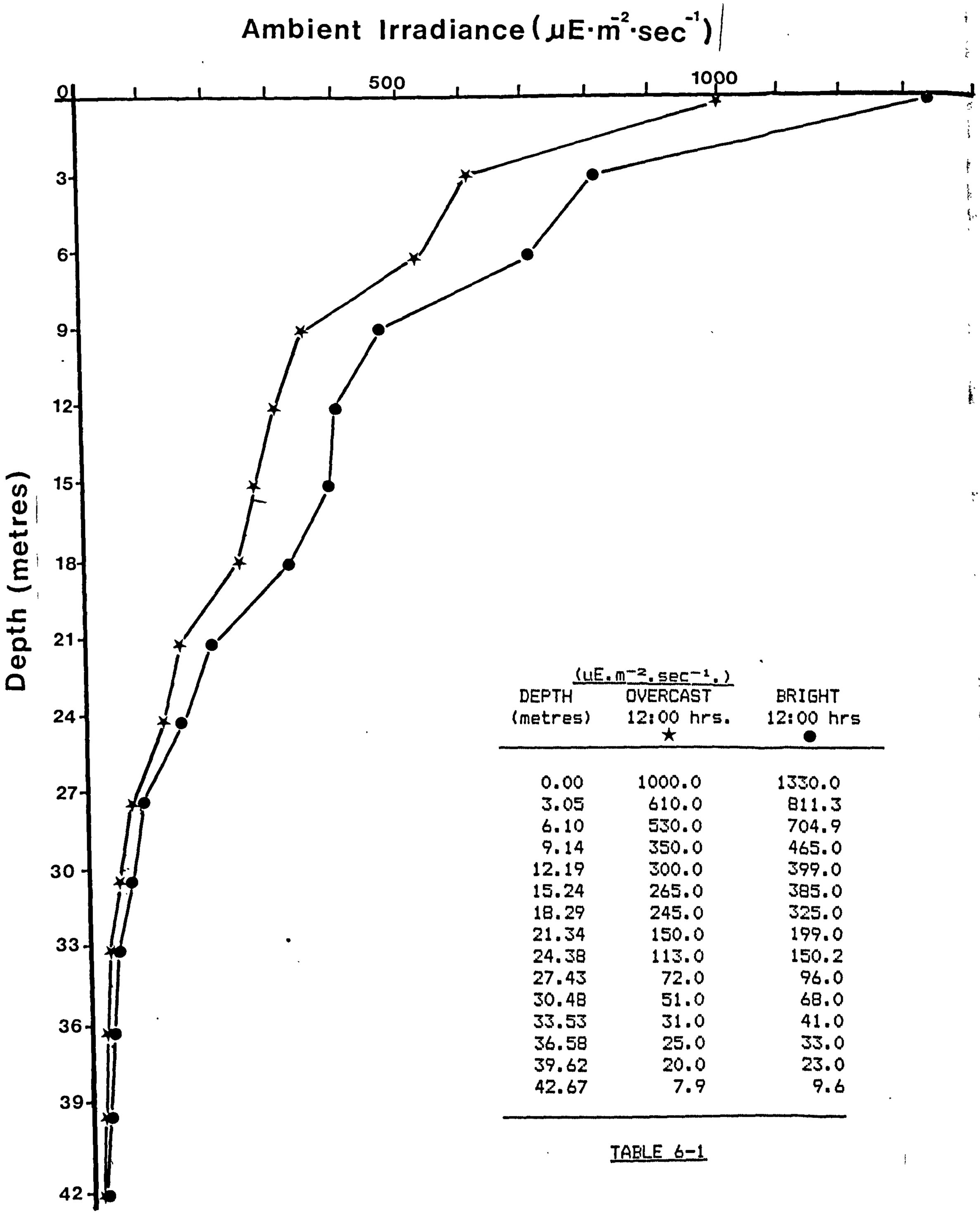
3) PHOTOSYNTHESIS AND RESPIRATION DATA

Data stored on magnetic tape were decoded and plotted using a Commodore 3032 microcomputer and a Digiplot X-Y plotter (Chapter-3). Decoded P/R data were presented on an hourly rate basis (Net oxygen production or colony respiration ($\mu lO_2.hour^{-1}$), with temperature and incident light given in °C and $\mu Einsteins.m^{-2}.sec^{-1}$, respectively.

Production and respiration values were converted to rates per unit biomass (mg.dw.), and unit surface area (cm^2), by dividing by the total dry tissue weight and the surface area respectively for each colony used.

Oxygen consumption values were then converted to energy equivalents by assuming that the primary metabolic substrate was lipid (Patton *et al*, 1977; Crossland *et al*, 1980), and using the lipid oxy-joule coefficient of 19.63 (Elliot and Davison, 1975). The energy input due to photosynthetic production was determined by assuming that 6 mol of oxygen resulted in the formation of 1 mol of glucose with an energy equivalent of 2817 KJoules (Lehninger 1973; see also Tytler 1982; Davies 1984). Energy equivalents for photosynthetic oxygen production were calculated for gross photosynthetic oxygen production rates determined by summing hourly dark respiration rates (colony) to hourly net photosynthetic production rates (colony). The assumption that daytime respiration was the same as nightime (dark)

FIGURE 6-1, and TABLE 6-1:- Ambient photosynthetic photon flux density measured on the fore reef at Discovery Bay under 100% overcast conditions, and 100% clear conditions. The measurements were made from the surface to 40 meters at 10 foot (3.3 meter) intervals using a Licor 185 meter with a Licor 192S underwated quantum sensor.



DEPTH (metres)	OVERCAST 12:00 hrs. ★	BRIGHT 12:00 hrs ●
	(uE.m⁻².sec⁻¹.)	
0.00	1000.0	1330.0
3.05	610.0	811.3
6.10	530.0	704.9
9.14	350.0	465.0
12.19	300.0	399.0
15.24	265.0	385.0
18.29	245.0	325.0
21.34	150.0	199.0
24.38	113.0	150.2
27.43	72.0	96.0
30.48	51.0	68.0
33.53	31.0	41.0
36.58	25.0	33.0
39.62	20.0	23.0
42.67	7.9	9.6

TABLE 6-1

respiration was made.

Dark respiration rates of zooxanthellae freshly isolated from *Montastrea cavernosa* colonies were determined *in vitro* at Discovery Bay by Davies 1982, and presented in Davies (1984).

RESULTS AND DISCUSSION:-

DEPTH ATTENUATION OF INCIDENT IRRADIANCE

Photosynthetic oxygen production in reef corals is a function of incident irradiance. On coral reefs a correlation exists between the lower depth distributions of hermatypic corals and the lower limit of the photic zone (Wells 1957).

Measurements of incident irradiance levels ($\mu E.m^{-2}.sec^{-1}$) from the surface to 40 metres on the fore reef at Discovery Bay (Figure 6-1, Table 6-1) show that 30.19% of surface irradiance was available to colonies at 10 metres, while only 1.86% of that irradiance reached deep water colonies at 30 metres. Incident light at the 10 metre site comprises most of the elements of the visible spectrum, while only Blue-Green light was present and available to deep water forms (Jerlov 1970). Comparisons between the two depths showed that 5.9% of that irradiance available to 10 metre colonies was available to deep water colonies at 30 metres (Table 6-1). This result compares favourably with those of McCloskey and Muscatine (1984) who report an 8% (of IRRADIANCE AT 3M WAS AVAILABLE AT 35M.) in available irradiance between 3 metres and 35 metres in the Gulf of Aqaba. But the results are lower than those of Porter (1980) who recorded maximum irradiance levels at 12:00 hours of approximately 6.0 $\mu E.m^{-2}.min^{-1} \times 10^4$ at 10 metres at Discovery Bay.

PHOTOSYNTHETIC PRODUCTION AND RESPIRATION IN *Montastrea cavernosa* COLONIES:

Decoded data from *in situ* experiments at the 10 metre site presented in Figure (6-2A, 6-2B), and summarized in Figure (6-3), show that during a given experimental run at 10 metres where both colonies used were exposed to the same conditions (irradiance, flushing intervals, temperature rises), net production rates of the coral colonies were not significantly different ($P<.01$). Mean production values (hourly production values divided by the number of hours sampled) recorded were $1.9355 \pm .4513\ \mu l O_2.mgdw^{-1}.hr^{-1}$, and $1.67 \pm .6296\ \mu l O_2.mgdw^{-1}.hr^{-1}$ for colonies (A) and (B) respectively (Table 6-3). The results are in the range of those of Davies (1977) who working in the laboratory recorded production values of $2.215 \pm .649\ \mu l O_2.mg^{-1}.hr^{-1}$ on the same species collected at 13 metres from the fore reef at Discovery Bay.

Mean colony respiration rates (Figure 6-4) showed no sig nificant difference ($P<.05$ from 1 way ANOVA) between all the colonies used at 10 metres, or when comparisons of mean colony respiration rates per unit biomass were carried out over the depth range (Table 6-3). Mean rates of $1.2818 \pm .5561\ \mu l O_2.mg^{-1}.hr^{-1}$ for n=5 colonies were recorded at 10 metres, and $.9592 \pm .3988\ \mu l O_2.mg^{-1}.hr^{-1}$ for n=2 colonies at 30 metres. Respiration rates of 30 metre colonies were approximately 74.8% those of the 10 metre colonies. The recorded values though substantially higher than the $.7836 \pm .2607\ \mu l O_2.mg^{-1}.hr^{-1}$ for colonies collected at 13 metres and $.613 \pm .104\ ul O_2.mg^{-1}.hr^{-1}$ for colonies collected at 40 metres recorded by Davies (1977), show comparable decreased respiration rates with increasing depth with Davies (1977) showing that the respiration rates of 40 metre *Montastrea cavernosa* colonies were 78.5% those of 13 metre colonies in laboratory based experiments at Discovery Bay.

Since net photosynthetic production and colony respiration rates for

FIGURE 6-2A:-

Traces from decoded data points from experiments at the 10 metres study site. All data was recorded from 3 channels at 5-minute intervals during an experimental run from 12:00 to 19:15 hours on th 12.th of May, 1982.

Data points for coral A day 1 were recorded at 5-minute intervals, and are expressed as $\mu l O_2 . mgdw^{-1} . hour^{-1}$. The bold line represents the mean hourly production or respiration rate for a 1 hour interval.

Included in every five minute recording were data for incident irradiance expressed here in $\mu E . m^{-2} . sec^{-1}$, and data for water temperature (°C) in the respirometer chambers. the temperature was measured in a single chamber, but it was assumed that the temperature rise would be the same for all the chambers in use during a particular run.

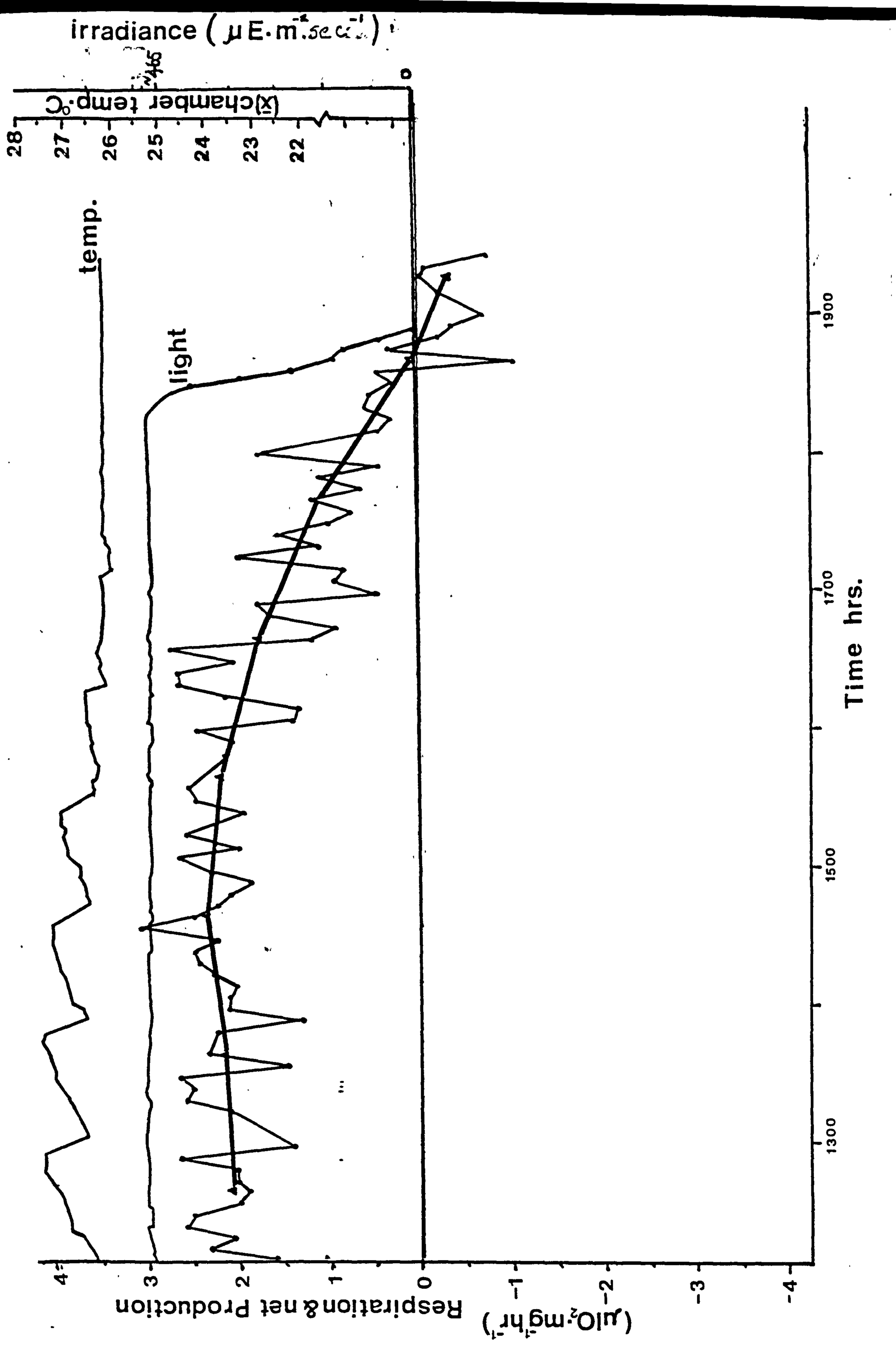

irradiance (μE·m⁻²·sec⁻¹)
0
(x̄)chamber temp.°C
28
27
26
25
24
23
22
temp.
light
1900
1700
1500
1300
Time hrs.
Respiration & net Production
(μlO₂·mg⁻¹hr⁻¹)
4
3
2
1
0
-1
-2
-3
-4

FIGURE 6-2B:-

Traces from decoded data points from experiments at the 10 metre study site. All data was recorded from three channels at the end of each 5-minute period from 12:00 to 19:15 on the 12th of May, 1982. This trace corresponds to coral B day-1. The traces for chamber temperature (°C), And incident irradiance are identical to those of Figure 6-2A.

Both sets of data points (6-2A, and 6-2B) and subsequent analysis showed that there was no significant difference in (P net) per mg.dw. between corals A and B.

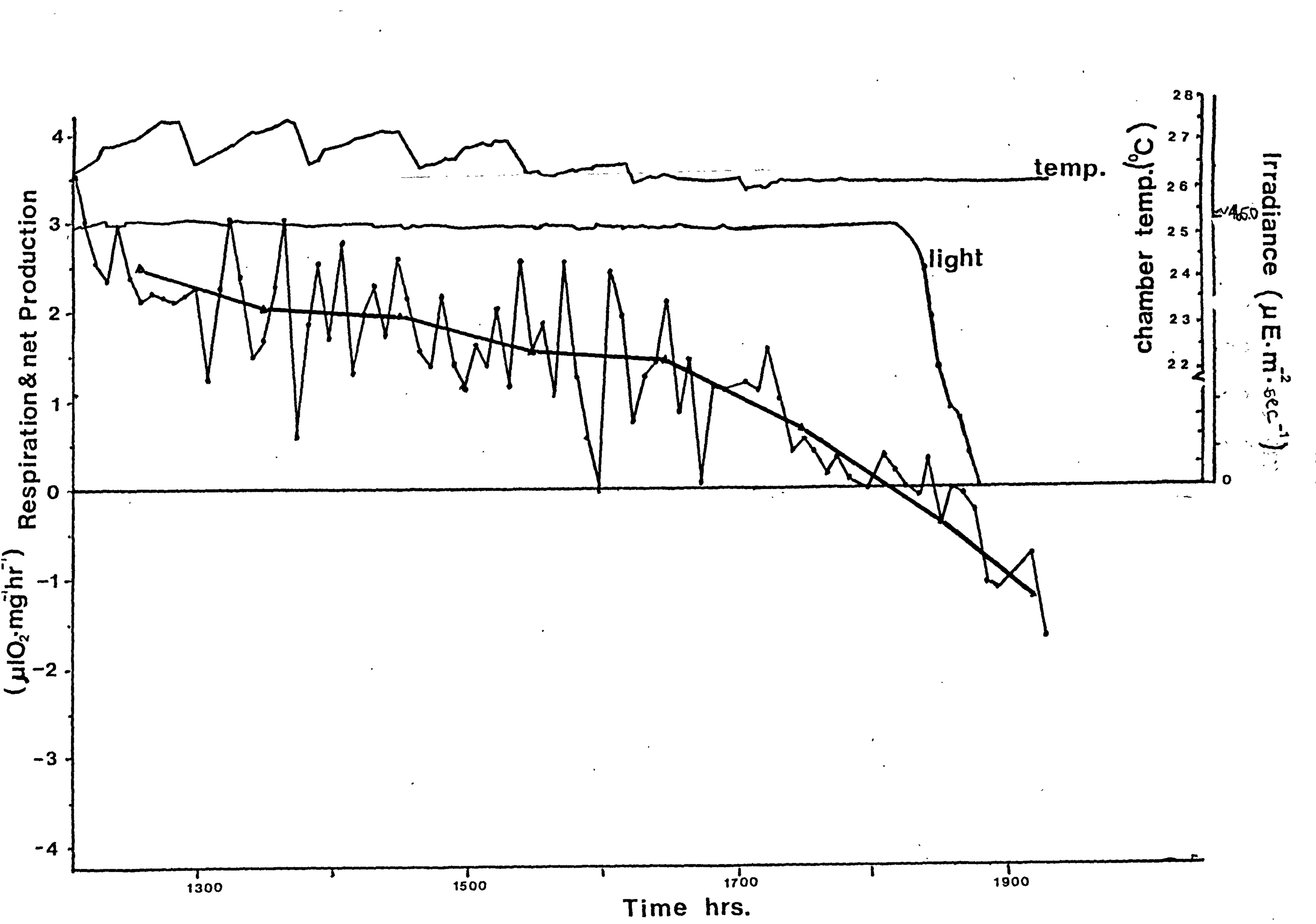
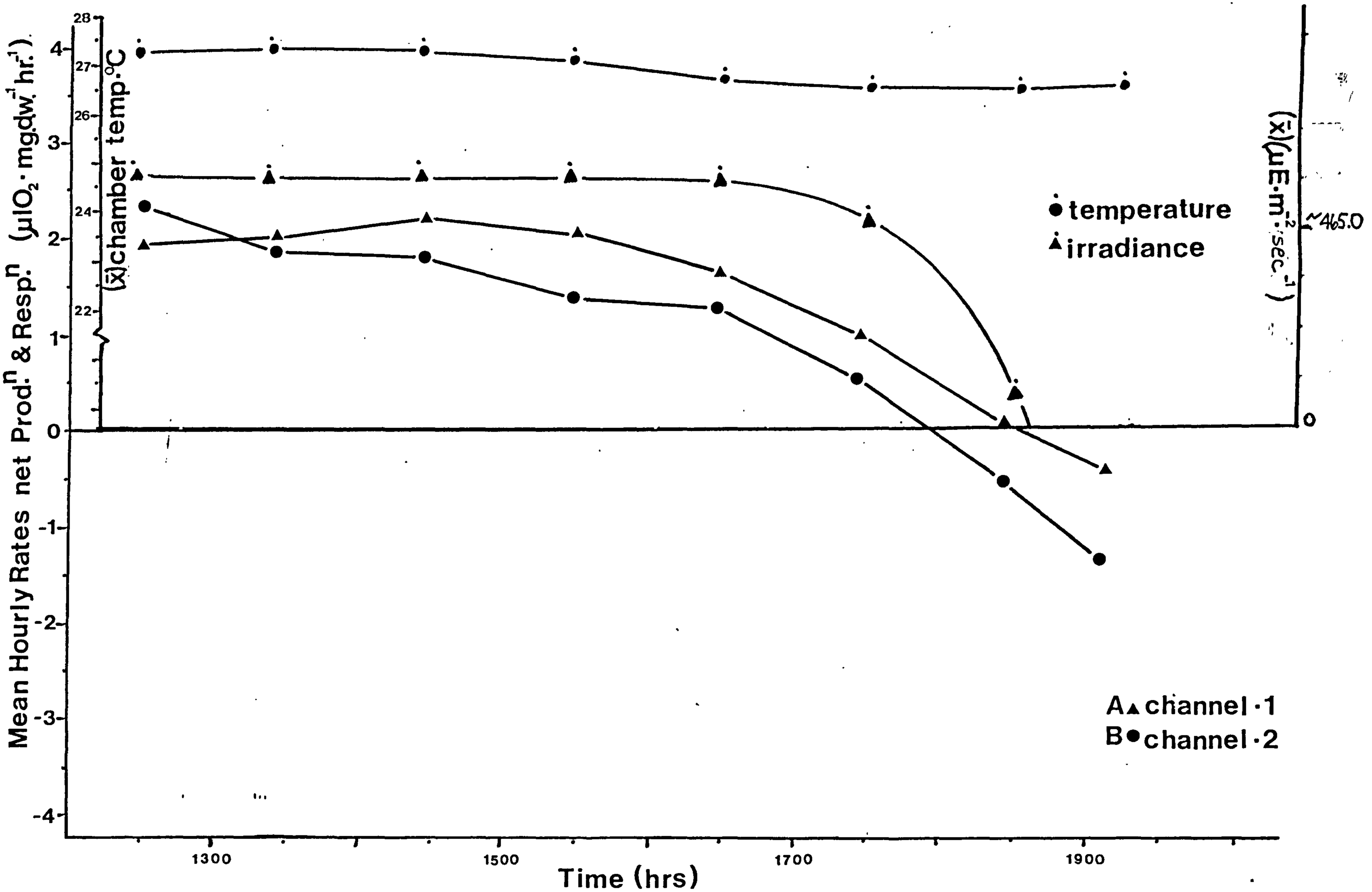

temp.
light
chamber temp.(°C)
Irradiance (μE.m⁻².sec⁻¹)
Respiration & net Production
(μlO₂.mg⁻¹hr⁻¹)
28
27
26
25
24
23
22
4
3
2
1
0
-1
-2
-3
-4
460
1300
1500
1700
1900
Time hrs.

FIGURE 6-3:-

Mean hourly net production rates determined for corals A and B in ($\mu l O_2 . mg.dw.^{-1} . hour^{-1}$). Mean hourly rates were also calculated for chamber temperature (°C) and incident irradiance ($\mu E . m^{-2} . sec^{-1}$).

The data presented in this figure was extracted from those data presented in Figures 6-2A, and 6-2B, and are shown in those figures as the bold line contained within the data points for each 5-minute period for the duration of the run.


Mean Hourly Rates net Prod.n & Resp.n (µlO2·mg.dw.-1 hr.-1)
(x̄)chamber temp.°C
(x̄)(µE·m-2·sec-1)
~465.0
temperature
irradiance
A▲ channel·1
B● channel·2
Time (hrs)
1300
1500
1700
1900
28
27
26
24
22
4
3
2
1
0
-1
-2
-3
-4

separate coral colonies during any given run were not significantly different (P<.01) when expressed on a unit biomass (mgdw), or a unit surface area (cm^2), it was possible to extrapolate a 24-hour plot from those data presented in Figure (6-3,6-4) to produce Figure (6-5). It was assumed that the mean hourly production rates ($\mu l O_2.mgdw^{-1}.hr^{-1}$ from sunrise to 12:00 hrs would be the mirror image of those recorded from 12:00 to sunset. This assumption was based on data presented by Svoboda (1978) and (Porter 1980) showing similar plots for *Stylophora pistillata* and *Montastrea annularis* respectively.

Maximum photosynthesis to respiration ratios (Pmax/R) exceeded 1.0 at both depths investigated, 2.8042±.5077 and 2.9008±1.5364 for corals at 10 meters and 30 metres respectively. The results agree with Davies (1977) who recorded Pmax to R ratios of 2.879±.529 at 13 metres and 2.764±.61 at 40 metres for the same species, and Wells (1973) who notes Pmax to R ratios of 2.9 for *Montastrea cavernosa*. P(net) to R ratios for n=4 colonies at 10 metres of 1.2411±.3196 were calculated, and similarily a mean P/R ratio of 1.411±.9710 calculated for colonies at 30 metres (Table 6-3).

From Table (6-3) a P/R ratio of .9814 was calculated for coral-B at 10 metres on day 2. This result suggests that colony respiration exceeded photosynthetic production, but this result may be due to altered electrode sensitivity since P(net) rates are 42.5% lower than when the same colony was previously measured (Coral-B, day 1). P(net) measurements for coral (A) show only a 4.7% difference over both days. Means and standard deviations given in Table (6-3) include these values.

Since data from laboratory experiments (Davies 1977) on the same species appear to be compatible and comparable with the *in situ*

FIGURE 6-4:- Mean hourly respiration rates ($\mu l O_2.mgdw^{-1}.hour^{-1}$) for three separate colonies (C, D, E,) at 10 metres. Data points were recorded from three channels simultaneously at the end of each 5-minute period from 17:00 to 02:15. these data were recorded on the 25th of May 1982.

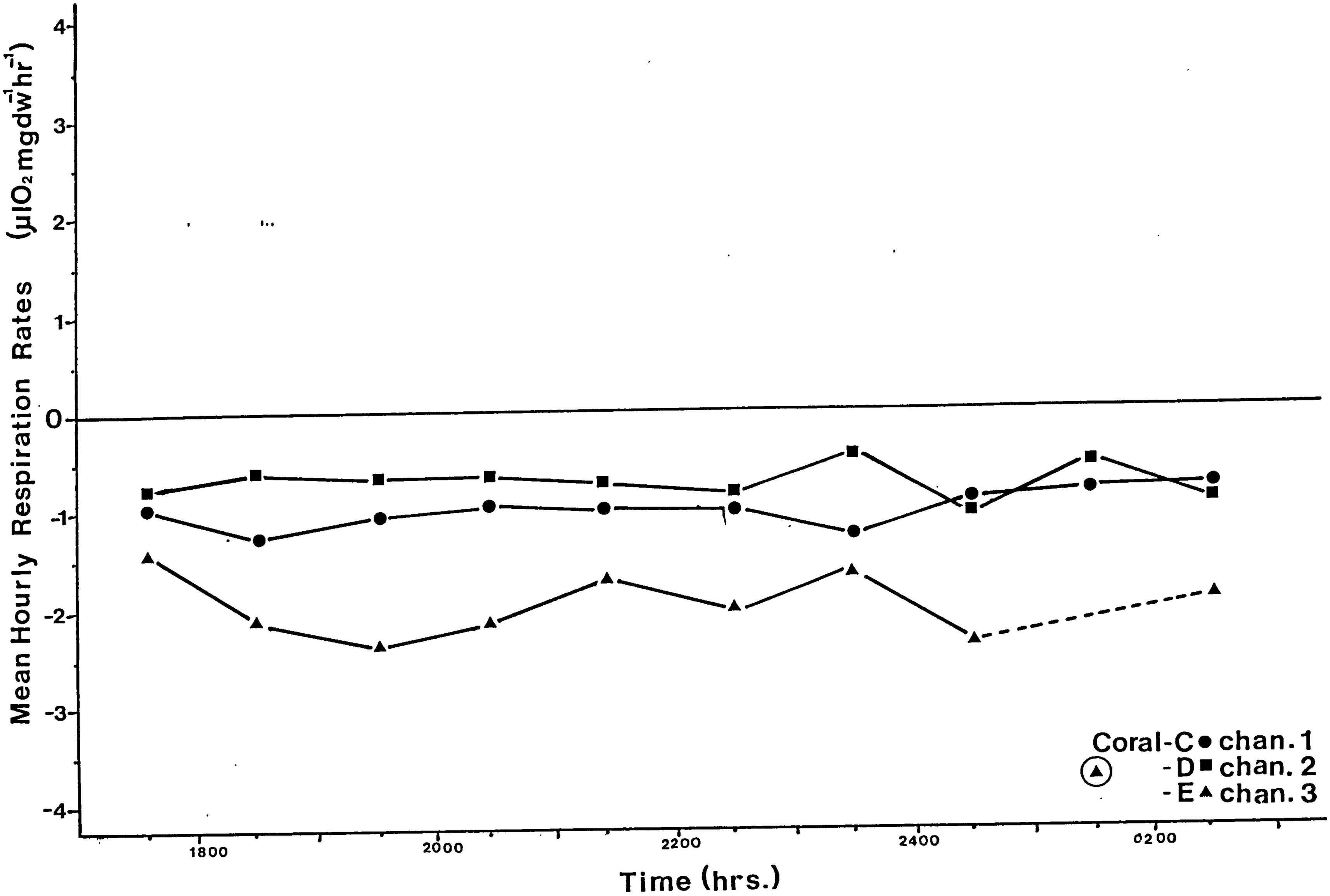

Mean Hourly Respiration Rates (µlO2 mgdw-1 hr-1)
4
3
2
1
0
-1
-2
-3
-4
1800
2000
2200
2400
0200
Time (hrs.)
Coral-C ● chan. 1
-D ■ chan. 2
-E ▲ chan. 3

FIGURE 6-5:- 24-hour plot from data presented in Figures 6-2, and 6-3. The assumptions made for plotting extrapolated points is given in the text.

(------) extrapolated results.
(_____) calculated mean hourly values from decoded data.
(▲) coral (A) at 10 meters.
(●) coral (B) at 10 meters.
(■) incident irradiance data at 10 meters.
(◘) respirometer chamber temperature.

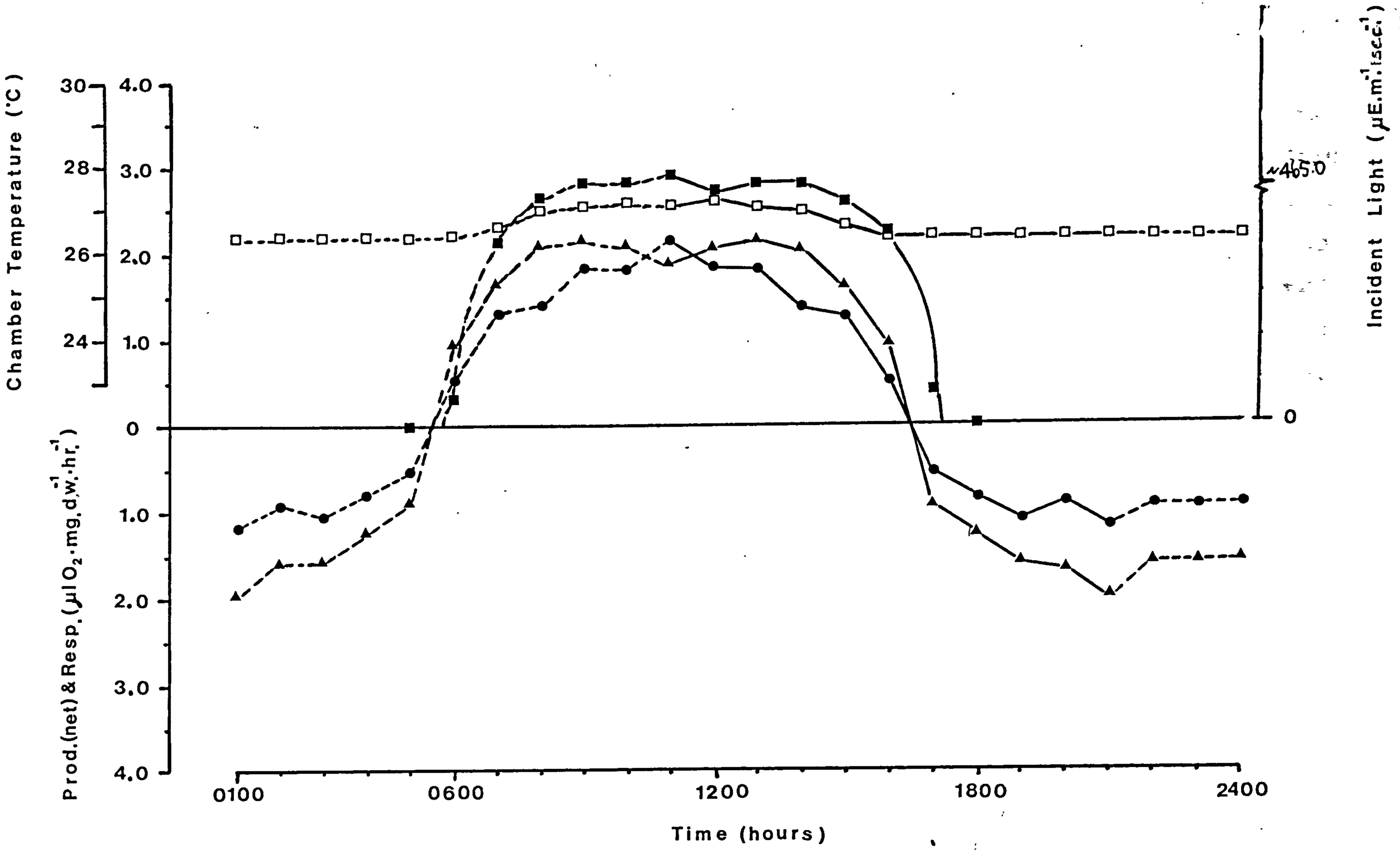

Chamber Temperature (°C)
30
28
26
24
Prod.(net) & Resp. (µl O2·mg.d.w.-1·hr.-1)
4.0
3.0
2.0
1.0
0
1.0
2.0
3.0
4.0
Incident Light (µE.m-1.sec-1)
~465.0
0
0100
0600
1200
1800
2400
Time (hours)

Fig 1

experiments of this investigation, the importance of laboratory based experiments to ground proof and complement *in situ* research becomes essential to field based research programs. A program combining baseline ecological recordings (dissolved oxygen, temperature, light, conductivity, and pH) and laboratory based biochemical and physiological experiments could provide a powerful tool for coastal resource management programs.

DENSITY OF ZOOXANTHELLAE IN HOST TISSUES:-

Since photosynthetic production in reef corals is directly related to zooxanthellar abundance in host tissues, it was necessary to determine the density of zooxanthellae in *Montastrea cavernosa* colonies collected from 10 metres and 30 metres on the fore reef at Discovery Bay to investigate whether algal density per unit biomass (mg.dw.), and unit surface area (cm^2), remained constant or changed over the depth range studied.

The density of zooxanthellae remained virtually unchanged over the depth range studied (Table 6-2). No significant difference ($P<.01$) was found in zooxanthellae density normalized to unit biomass, or unit surface area. Similar results have been reported by Drew (1972), but they do not agree with those reported by Falkowski and Dubinsky (1981) who found that algal numbers increased with increasing depth in *Stylophora pistillata*, or the results of Titlyanov (1980) and Muscatine *et al* (1984) who both report decreasing zooxanthellae numbers with increasing depth in the corals *Stylophora mordax* and *Stylophora pistillata* respectively.

Comparisons between 'bleached' colonies kept at 10 metres and 30 metres showed no significant difference ($P<.01$) in zooxanthellae numbers per unit biomass or unit surface area. That 'bleached' colonies should contain zooxanthellae was surprising, but this suggests that in the absence

TREATMENT GROUP	#ZOOXANTHELLAE/ MG.DW.	#ZOOXANTHELLAE/ CM^2	#ZOOXANTHELLAE/ MG.DW.	#ZOOXANTHELLAE/ CM^2	1-WAY ANOVA (P .01)
	10 metres		30 metres		
NORMAL	(1)	(2)	(3)	(4)	1 v/s 3 not significant
$\bar{x}$	2.72×10^5	4.36×10^4	1.96×10^5	3.99×10^4	
±S.D.	1.43×10^5 (n=18)	1.05×10^4 (n=18)	9.37×10^4 (n=15)	1.97×10^4 (n=15)	2 v/s 4 not significant
'BLEACHED'	(5)	(6)	(7)	(8)	5 v/s 7 not significant
$\bar{x}$	7.09×10^4	1.33×10^4	4.37×10^4	5.84×10^5	
±S.D.	3.84×10^4 (n=6)	7.16×10^5 (n=6)	2.75×10^4 (n=7)	4.82×10^5 (n=7)	6 v/s 8 not significant

TABLE 6-2:- Mean zooxanthellae densities from *Montastrea cavernosa* colonies in two treatment groups (normal and 'bleached') collected from 10 metres and 30 metres. 1 way ANOVA showed that there was no significant difference between depths within each treatment group. The zooxanthellae counts presented are for the corals used in the respirometer chambers at both depths. There was no significant difference ($P<.01$) between the 'normal colonies used in experiments and those from n=50 colonies collected for zooxanthellae density analysis.

of light a seed population of zooxanthellae remains in the endoderm of zooxanthellate corals. This result could go some distance in explaining the re-pigmentation of 'bleached' colonies returned to a photic environment after an extended dark incubation period.

Zooxanthellae numbers per mg.dw. of 'bleached'coral tissue were $7.09\pm3.84 \times 10^4 \pm 3.84 \times 10^4$ for n=6 colonies, or 26.1% that of normal colonies at 10 metres, and $4.37\pm2.75 \times 10^4 \pm 2.75 \times 10^4$ for n=7 colonies, or 22.3% that of normal colonies at 30 metres. The lower number of zooxanthellae in 'bleached' colonies at 30 metres could be explained by the lower zooxanthellae densities of 30 metre normal colonies relative to their 10 metre counterparts, even though this difference was not significant. Since colonies were bleached in mesh fronted, darkened enclosures, depth related downward irradiances can be eliminated as the causative agent for increased algal numbers in 10 metre colonies. Increased levels of scattered, and reflected light, from the substrate and suspended particulate material (Jerlov, 1970; Brakel, 1979) could account for the increase in zooxanthellae density in the tissue of 'bleached' colonies.

As seen in the previous section, mean gross production (Table 6-3) in $\mu l O_2.mgdw^{-1}.hr^{-1}$, and $\mu l O^2.cm^{-2}.hr^{-1}$, remains virtually unchanged with normal colonies at 30 metres having production levels 86% those of their 10 metre counterparts. This result suggests that there must be a degree of zooxanthellae photoadaptation since production in the deep water forms is maintained at 86% with only 5.9% of the irradiance available to 10 metre colonies. Photoadaptation may be the result of;

1) increased chlorophyll a concentrations per algal cell (Prezelin and Sweeney, 1979; Falkowski *et al*, 1981; Dustan, 1982).

2) the possible existence of two distinct depth limited

CORAL	SURFACE AREA (cm^2)	DRY WEIGHT (mgdw)	$\bar{X}$ PHOTOSYNTHETIC PRODUCTION-net $\mu lO_2/mg/hr$	$\mu lO_2/cm^2/hr$	$\bar{X}$ PHOTOSYNTHETIC PRODUCTION gross $\mu lO_2/mg/hr$	$\mu lO_2/cm^2/hr$	MEAN COLONY RESPIRATION $\mu lO_2/mg/hr$	$\mu lO_2/cm^2/hr$	P(max.)-gross $\mu lO_2/mg/hr$	P(net) R	P(max) R
					10 METRES						
A day-1	12.2528	182.46	1.9355± .4513	28.8274± 6.7259	3.5954± 0.7507	59.5390± 7.6532	----------	----------	4.0077	1.1660	2.4144
A day-2	12.2528	182.46	1.8438±1.3640	34.1070±25.2212	3.5037± 1.6634	64.8186±27.8102	1.6599±.2994	30.7116±5.4468	4.4682	1.1108	2.6918
B day-1	18.8245	368.31	1.6730± .6296	34.3013±12.2954	2.2635± 0.9712	54.6584±12.6481	----------	----------	3.4774	1.7063	3.5466
B day-2	18.8245	368.31	.9623± .8080	19.8273±17.1869	1.9428± 1.6865	40.1844±36.5248	.9805±.1878	20.3571±3.8111	2.5141	.9814	2.5641
C day-1	26.7396	677.21	----------	----------	----------	----------	.9864±.1512	24.4140±4.2118	------	------	------
D day-1	15.5390	392.13	----------	----------	----------	----------	.7233±.1753	18.6379±4.5658	------	------	------
E day-1	11.2727	162.93	----------	----------	----------	----------	2.0589±.3957	29.9297±5.7457	------	------	------
					30 METRES						
F day-1	22.3168	347.50	0.4906±0.0861	9.0095± 2.6582	1.1678± 0.2523	22.4735± 4.8363	.6772±.1662	13.4640±3.3300	1.2287	.7245	1.8144
G day-1	15.0381	236.90	2.6037±1.0482	40.7914±16.5239	3.8449± 2.2410	60.3443±26.7539	1.2412±1.646	19.5529±25.937	4.9489	2.0977	3.9872

TABLE 6-3:- Decoded data from *in situ* experiments using the automatic respirometer/data logger. The recordings were made at two depths 10 meters (n=5), and 30 meters (n=2). The values given are means of hourly recordings at 5 minute intervals, and all recordings for each 5 minute period have been decoded and expressed on an hourly rate basis. Colony respiration data for corals (A and B) on day 1 are not included since the the battery supply failed soon after sunset. Similarily data for photosynthetic production in corals (C, D, and E) are not present since the instrument was deployed at 17:00 hrs during that run.

zooxanthellae strains, one shallow (0 - 15m), and one deep (>15m) as suggested by Dustan (1979).

3) depth related morphological characters changing from hemispherical in the shallows to platelike at depth to maximize light capturing surfaces (Kawaguti, 1937; Goreau, 1959; Barnes, 1973)

OTHER PARAMETERS MEASURED:-

Temperature was measured using a linear thermistor sensing changes in water temperature inside a respirometer chamber. Temperature recordings were used to correct electrode drift due to temperature fluctuations (Chapter-3). Water temperature rose approximately 1.1°C between respirometer flushings during peak daylight hours (Figures 6-2A, 6-2B, and summarized in Figure 6-3), while ambient water temperature, measured by divers using a thermometer, remained constant at 26.5±.03°C. The recorded temperature rise was probably due to a greenhouse effect in the closed perspex respirometer chambers, as suggested by a rapid drop in chamber temperature to the 26.5°C level following a flushing cycle.

Incident irradiance levels at the study sites were also recorded during each experiment, but as can be seen from the plotted data (Figures 6-2A and B, 6-3), maximum irradiances of 465.0 $\mu E.m^{-2}.sec^{-1}$ (TABLE 6-1) were reached over a forty minute interval (the reverse of the time taken to go from sensor saturation to zero at sunset). This short interval to or from sensor saturation suggests that the photocell was saturating at lower intensities than had been planned, even though the sensing surface was masked by a neutral grating allowing 50 % of available light to reach it.

The photocell was post calibrated using data from Figure 6-1 and

Table 6-1. Since most of the incident irradiance data were lost, much of the interesting data regarding changing irradiance levels and their corresponding rates of gross production cannot be included. The plotted irradiance data have been included as a reference point (sunrise and sunset) for the photosynthesis and respiration data.

ENERGY EQUIVALENTS:-

Photosynthetic oxygen production and colony respiration rates recorded *in situ* were decoded and converted to energy equivalents (Joules). From Table (6-4) it can be seen that for those 10 metre colonies analyzed, the energy derived from photosynthetic oxygen production (P(gross).12hrs^{-1}) exceeded the requirements of the colony as determined by colony respiration (Rc). Calculated P/R ratios were 1.16 and 1.28 for corals A and B respectively. In both these corals respiration by the zooxanthellar fraction of the symbiosis exceeded respiration of the animal fraction, with mean zooxanthellae respiration comprising 59.9±13.6% of colony respiration. This increased percentage of zooxanthellae respiration relative to animal respiration may be the result of either slightly higher mean zooxanthellae densities (2.72 x 10^5±1.43 x10^5 zooxanthellae/mgdw (n=18)) in 10 metre colonies relative to 30 metre colonies with 1.96 x 10^5±9.37x10^4 zooxanthellae/mgdw for (n=15) colonies at 30 metres, or to discrepancies in respiration rates of isolated zooxanthellae measured *in vivo*, since isolated zooxanthellae have been shown to have increased respiration rates relative to zooxanthellae in host tissues.

Results from deep water colonies at 30 metres show unacceptable variability and must therefore be treated lightly. P to R ratios calculated from gross photosynthetic production (Pg) and colony respiration (Rc),

CORAL	Σ PROD'n(net) μlO_2/mgdw/12h	Σ PROD'n(gross) μlO_2/mg/12hr	RESP'n animal(Σ) μlO_2/mg/24hr	%Rc	Σ RESP'n zooxant μlO_2/mgdw/24hr	%Rc	Σ RESP'n colony μlO_2/mgdw/24hr	# ZOOXANTHELLAE mg.dw.	Pg(JOULES) Rc(JOULES)
A Day 2	19.9430	41.5752	19.0829	49.7	19.2931	50.3	30.3760	3.08×10^5	1.16
(Joules)		(0.8714)	(0.3746)		(0.3787)		(0.7533)		
B Day 2	18.7904	20.2529	7.1830	30.5	16.3490	69.5	23.5320	2.61×10^5	1.28
(Joules)		(0.5922)	(0.1410)		(0.3209)		(0.4619)		
F Day 1	5.8872	14.0136	8.1096	49.6	8.1432	50.4	16.2528	1.03×10^5	0.92
(Joules)		(0.2937)	(0.1592)		(0.3190)				
6 Day 1	31.2440	46.1384	18.6478	72.3	7.1410	27.7	25.7888	1.14×10^5	1.91
(Joules)		(0.9670)	(0.3660)		(0.1402)		(0.5062)		

TABLE 6-4: Production and respiration results from decoded data. The values were summed over the recorded period and were then converted to P(net), P(gross), and R(colony) rates for corals at both 10 metres and 30 metres. The highest net production values (coral G) at 30 metres is probably due to the short recording times using the automatic respirometer at that depth. The values for respiration of zooxanthellae were derived from the total zooxanthellae numbers, and respiration data from freshly extracted zooxanthellae and determined *in vitro* by Davies (1982, and published 1984). The energetic equivalent for the production and respiration data presented is given in the rows labelled (Joules).

suggest that coral (F) with a P/R ratio of 0.92 was not able to satisfy its daily energy requirements from translocated photosynthetic material, and would have to make up the energetic shortfall from heterotrophic sources. Conversely coral (G) with a P/R ratio of 1.91 should be able to satisfy its energetic requirements from photosynthetic production and the translocation of photosynthates to the host. Clearly a discrepancy exists in these results since both colonies were exposed to the same conditions on the reef, both were treated similarly during experiments, and both had similar zooxanthellae numbers per unit biomass 1.30×10^{5} (coral F) and 1.14×10^{5} (coral G). It seems reasonable to expect these colonies to show less variability in P/R ratios as was seen in colonies at the 10 metre site, but this was not the case. One must therefore suspect either instrument performance at the greater depth, or that variation was induced by limited data returns at 30 metres and is therefore an artefact easily avoided by improved instrument performance.

Energy equivalents calculated from mean results from both the 10 metre and 30 metre sites (Table 6-5) show that gross production rates (Pg) of zooxanthellae (Joules.mgdw^{-1}.12hrs^{-1}) were .7739 Joules at 10 metres, and .6666 Joules at 30 metres. These results show that there was a 13.86% decrease in (Pg) at 30 metres relative to colonies maintained at the 10 metre site. This decrease suggests that there is a reduction in the photosynthetic rate of zooxanthellae with decreased irradiance at the greater depth.

Colony respiration rates expressed in Joules/mg.dw./24hours were higher for 10 metre colonies (.6039 Joules), than for 30 metre colonies (.4519$\pm$.1879 Joules), a decrease of 25.16% at 30 metres (Table 6-5). This decrease in colony respiration rates with increasing depth has been

CORAL	PROD'n (net) $\mu l O_2/mgdw/12h$	PROD'n (gross) $\mu l O_2/mg/12hr$	RESP'n animal $\mu l O_2/mg/24hr$	%Rc	RESP'n zooxant $\mu l O_2/mgdw/24hr$	%Rc	RESP'n colony $\mu l O_2/mgdw/24hr$	ZOOXANTHELLAE mg.dw.	Pg (JOULES) Rc (JOULES)
10 metres	19.3667±5.2944	34.9140± 9.4202	13.7256	44.6	17.0376	55.4	30.7632±13.3464	2.72×10^{5}±1.46	1.13
(Joules)		.7739±.1985	.2694		.3345		.6039±.2620	(n=18)	
30 metres	18.5656+17.9304	30.0760±22.7160	10.7424	46.7	12.2784	53.3	23.0208±9.5712	1.96×10^{5}±0.937	1.31
(Joules)		.6666±.4761	.2109		.2410		.4519±.1879	(n=15)	

TABLE 6-5:- Mean values calculated from the summed data in Table 6-4. The values for zooxanthellae respiration were determined using the results of Davies (1984) for 10^6 zooxanthellae determined *in vivo* from algae freshly extracted from *Montastrea cavernosa* at Discovery Bay. Respiration for the animal fraction of the symbiosis was determined by subtraction (Rc-Rz). The enery equivalent for the values given are presented in the rows labelled (Joules), and the units are in Joules $.mg^{-1}.$time interval^{-1} (12 or 24 hours).

reported previously (Spencer-Davies, 1980; McCloskey and Muscatine, 1984), and may be due to ;

A) decreased numbers (density) of zooxanthellae, though not a significant difference, in colonies from the 30 metre depth (Table 6-5). Zooxanthellae respiration expressed as a percentage of colony respiration does decrease with increasing depth, 55.4% and 53.3% for colonies at 10 metres and 30 metres respectively.

B) increased respiration rates in corals at the 10 metre site could be expected since there was a slight increase in zooxanthellar productivity with decreasing depth. Higher levels of productivity would increase the availability of carbon compounds to the colony, which would be reflected in increased respiration rates with decreasing depth, or the reverse with increasing depth (Svoboda, 1978; Fitt *et al*, 1982; McCloskey and Muscatine, 1984).

Respiration rates (Joules.mgdw^{-1}.24hrs^{-1}) of the animal fraction of the symbiosis do reflect the latter statement with .2694 J.mg^{-1}.24hrs^{-1}being respired by the animal fraction at 10 metres, and .2109 J.mg^{-1}.24hrs^{-1} at 30 metres, a reduction in the rate of respiration of the animal fraction of 21.7% at 30 metres.

Zooxanthellar respiration rates (Rz) expressed in Joules/number of zooxanthellae per mg.dw/24 hours were consistently higher than respiration rates for the animal fraction (Ra). At the 10 metre depth (Rz) was .3345 J.mg^{-1}.24hrs^{-1}, or 55.4% of colony respiration (Rc), while at the 30 metre site (Rz) was .2410 J.mg^{-1}.24hrs^{-1}, or 53.3% of (Rc) (Table 6-5). Similar results have been reported by McCloskey and Muscatine (1984) who suggest that zooxanthellae accounted for 48% of colony respiration at 3 metres, and

85% of colony respiration at 35 metres in the coral *Stylophora pistillata* in the Gulf of Aqaba.

Despite the limitations imposed by the few adequate data recordings, the values presented in this section are useful in constructing a preliminary energy budget for *Montastrea cavernosa* colonies normalized to a standard 100cm² surface area.

THE RESULTS NORMALIZED TO A STANDARD *Montastrea cavernosa* COLONY:-

Using the data presented in Table 6-5 it was possible to normalize the results given per unit biomass to those of a standard 100cm² colony.

Tissue cover per unit surface area (mg.dw./cm²) for this species was 18.6281±6.4476 mg.cm^{-2} at 10 metres, and 17.9144±3.8028 mg.cm^{-2} at 30 metres (Tables 4-1,4-2). Tissue cover in standard 100cm² colonies will then be 1862.81±644.76 mg at 10 metres, and 1791.44±380.28 mg at 30 metres.

Using tissue biomass values given above, and the results presented in Table 6-5, given in Joules per unit biomass, it is then possible to calculate the following energy equivalents, all normalized to a standard 100 cm² colony;

	SHALLOW (10m)	DEEP (30m)
Gross Production (Pg) (J/100cm²/12hours)	1441.63	1194.17
Zooxanth.Respiration(Rz) (J/100cm²/24hours)	623.11	431.74
+	+	+
Animal Respiration (Ra) (J/100cm²/24hours)	501.84	377.81

Colony Respiration (Rc) (J/100cm²/24hours)	1124.95	809.55
Excess energy (Pg -Rc) (J/100cm²/24hours)	316.68	384.62

TABLE 6-6:- The energetic equivalents for photosynthetic production, and colony respiration data collected *in situ* using the automatic respirometer system and given in Table (6-5). The energetic equivalents for zooxanthellae respiration were calculated using the data of Spencer-Davies (1984). The values given for Animal respiration were determined by subtraction (Rc - Rz).

The excess energy from photosynthetic production (Pg) could be expended in tissue growth, laid down as storage compounds (Patton 1977), and some could be lost to the water column in the form of mucus (Coles and Strathman 1973; Crossland *et al* 1980; Spencer-Davies 1984). The values in Table 6-6 do not include energetic inputs from heterotrophic sources, or the energetic cost of tissue growth, these will be dealt with in Chapter-7 where the values from Table 6-6 will be recalled, and will be used to construct an energy budget for *Montastrea cavernosa*.

CHAPTER 7.

THE ENERGY BUDGET OF *Montastrea cavernosa*

All the experiments to determine the daily energy requirements of *Montastrea cavernosa* were carried out *in situ* at two depths (10 metres and 30 metres) on the fore reef at Discovery Bay Jamaica. In the previous chapters the energetic equivalents of all measured components of the budget were presented and discussed. In this chapter those results are used to describe a preliminary bioenergetic model for this species.

All parameters in the bioenergetic model (Figure 7-1) have been dealt with in the literature, but only Tytler (1982) working with the symbiotic anemone *Anemonia sulcata* in the laboratory, and Davies (1984) working with the Pacific reef building coral *Pocillopora eydouxi* have attempted to present the daily energy requirements of these species in terms of an energy budget.

Using the method of Tytler (1982), and Davies (1984), the symbiosis was separated into its animal and zooxanthellae fractions, and an energy budget determined for each. The preliminary values presented suggest that;

1) *Montastrea cavernosa* is able to meet its daily energy requirements from photosynthetic production over the depth range studied.

2) Heterotrophic nutrition on exogenous sources does not seem to be energetically important to this species.

3) Heterotrophic nutrition on exogenous sources seems to be necessary for tissue growth.

4) Depth related variations in the energy budget of colonies seem to exist, and these may have important ecological implications.

5) Corals with large polyps and low surface to volume ratios (Porter 1976) previously assumed to be highly dependent on heterotrophic nutrition, may in fact be on a lower evolutionary scale and geologically older than corals with small polyps and high surface to volume ratios.

MATERIALS AND METHODS

Not all the componets of a 24-hour energy budget for a coral animal - zooxanthellae symbiosis as shown in Figure 7-1, were measured. Only those components marked with an asterisk were measured or calculated, and were included in the bioenergetic model (Table 7-2).

The methods for determining the energy equivalents of photosynthetic production, colony respiration, zooxanthellae respiration, and colony growth have already been discussed in previous chapters. Only those techniques not previously described will be discussed in this section.

All measured and calculated values of the energy budget were expressed per unit biomass (mg. dried weight), and normalized to the requirements of a standard colony having 100 cm^2 and 123 polyps. A standard colony of this surface area would be covered by 1862.81 $\pm$ 644.76 mg.d.w. of tissue at 10 metres, and 1791.44 $\pm$ 380.28 mg.d.w. at 30 metres (Tables 4-1,4-2). To convert values expressed per unit biomass to those of standard

FIGURE 7-1:- A bioenergetic model of the components of an energy budget for a coral animal/zooxanthellae symbiosis. The asterisks indicated those components of the budget measured in this investigation. All values corresponding to energy losses from the system were pooled and determined by subtraction.

colonies at 10 and 30 metres, these were multiplied by the respective total biomass for colonies at either depth.

RESPIRATION OF ZOOXANTHELLAE

Respiration rates of freshly isolated zooxanthellae were determined by Davies (1982, published 1984). The mean respiration rate at 28°C was 2.61 ± .41 $\mu l O_2$ for 10^6 zooxanthellae^{-1}.hour^{-1}. This value was converted to its energetic equivalent assuming the metabolic substrate was lipid, and using the oxy-joule coefficient of 19.63 (Elliot and Davidson, 1975).

The mean number of zooxanthellae per mg.d.w. was $2.72 \times 10^5 \pm 1.43 \times 10^5$ for n=18 colonies at 10 metres, and $1.96 \times 10^5 \pm 9.37 \times 10^4$ for n=15 colonies at 30 metres (Table 6-2). Using the respiration rate determined by Davies (1984), mean zooxanthellae respiration rates (converted to Joules) per mg.d.w. were .3345 Joules/24 hours at 10 metres, and .2410 Joules/24 hours at 30 metres (Table 7-1).

RESPIRATION: ANIMAL TISSUE FRACTION.

Respiration rates of the animal tissue fraction of the symbiosis, and their energy equivalents were determined by subtraction. The energy equivalent of zooxanthellae respiration (Joules.24hrs.$^{-1}$) was subtracted from the energy equivalent of colony respiration (Rc) in (Joules.24hrs.$^{-1}$), with the result being that proportion of available energy in Joules.24hrs.$^{-1}$ required by the animal fraction for maintenance metabolism over a 24 hour period. This resulted in 501.84 Joules.24hrs.$^{-1}$ at 10 metres, and 377.81 Joules.24hrs.$^{-1}$ at 30 metres (Table 7-1).

GROWTH OF ZOOXANTHELLAE.

Mean zooxanthellae densities per mg.d.w. for 10 metre and 30 metre colonies are given in Table 6-2. Zooxanthellae growth rate was determined using daily tissue growth rates normalized to a standard 100 cm.^{2} colony using data given in (Tables 4-1,4-2), and zooxanthellae numbers per unit biomass (Table 6-2). At 10 metres the mean tissue growth rate of 'normal' colonies normalized to rates of a standard 100 cm.^{2} colony was 5.0190 $\pm$ 1.9939 mg.24hrs.$^{-1}$ requiring 1.36 x 10^6 'new' zooxanthellae. At 30 metres the mean tissue growth rate of 'normal' colonies was 1.1169 $\pm$ .6032 mg.24hrs.$^{-1}$ requiring 2.19 x 10^5 'new' zooxanthellae.

Using the energy equivalent of 6.43 Joules per 10^6 zooxanthellae determined by Tytler (1982) for zooxanthellae isolated from *Anemonia sulcata*, the energy equivalent represented by the 'new' zoxanthellae at 10 metres was 8.74 Joules/24 hours, 1.41 Joules/24 hours for colonies at 30 metres.

Determinations of the energy equivalent of zooxanthellae isolated from *Montastrea cavernosa* were carried out, but the results (0.73 $\pm$ 1.96 Joules.10^6zooxanthellae) were very much lower than those of Tytler (1982) for zooxanthellae extracted from *Anemonia sulcata*. The disparity in the results, and the high standard deviation makes the present results suspect, consequently these results were not used in the analysis in favour of those determined by Tytler (1982)

LOSSES FROM ZOOXANTHELLAE.

No data are available regarding the rate of removal of senescent

zooxanthellae from host tissue. This phenomenon has been reported (Odum & Odum,1955; Steele,1976), but as yet no hard data are available. Energy losses due to zooxanthellae ejection were lumped into the total losses from the zooxanthellae fraction of the energy budget. Losses from the zooxanthellae budget were calculated by subtraction, where;

ENERGY IN - (ENERGY SPENT + ENERGY RETAINED) = ENERGY LOST

(photosynthesis) - (respiration + growth)

For this bioenergetic model the energy losses from zooxanthellae were assumed to be translocated *in toto* to the animal tissue fraction.

LOSSES FROM THE ANIMAL TISSUE FRACTION.

Losses from the animal tissue fraction of the symbiosis, or that energy not used in maintenance metabolism, or retained as tissue growth or storage compounds, was assumed to be released to the water column in the form of mucus, egested, or excreted material. For this budget all losses were lumped, and were calculated by subtraction.

A proportion of the material considered lost from the animal tissue fraction to the water column has been shown to be recycled to the symbiont (Muscatine and D'Elia, 1978; Rynkevich and Loya, 1984).

COMPONENTS OF THE ENERGY BUDGET	SHALLOW (10 metres)		DEEP (30 metres)	
	Joules/mg.dw./24hrs.	Joules/100cm²coral	Joules/mg.dw./24hrs	Joules/100cm²coral
GROSS PRODUCTION (Pg)	0.7739 (12-hours)	1441.63 (12-hours)	0.6666 (12-hours)	1194.17 (12-hours)
COLONY RESPIRATION (Rc)	0.6039	1124.95	0.4519	809.55
ANIMAL RESPIRATION (Ra)	0.2694	501.84	0.2109	377.81
ZOOXANTHELLAE RESPIRATION (Rz)	0.3345	623.11	0.2410	431.74
MEAN TISSUE GROWTH				
'NORMAL' COLONIES	0.1091	203.26	0.0274	49.17
'BLEACHED' COLONIES	0.0101	18.85	0.0070	12.49
HETEROTROPHIC NUTRITION				
ZOOPLANKTON	0.0507 J/polyp/12hrs	6.24 J/123Polyps/ 12 hours	0.0000	0.00
MEAN GROWTH ZOOXANTHELLAE	NUMBER ZOOX./24-HRS.	JOULES/24.HOURS	NUMBER ZOOX./24-HRS.	JOULES/24-HOURS
'NORMAL' COLONIES	1.36×10^4	8.74	2.19×10^4	1.41
'BLEACHED' COLONIES				

TABLE 7-1:- Energy equivalents of the different components of the energy budget given per unit biomass, and normalized to a standard 100cm² coral colony. The values given are in Joules per 24 hours unless otherwise stated. The energetic equivalent for tissue growth was calculated by subtracting the equivalent for zooxanthellae growth from the equivalent for total tissue growth.

		ENERGY IN				=ENERGY SPENT		ENERGY RETAINED		ENERGY LOST
		Photosynthesis	+ Zooplankton	+ Translocation	=	Respiration	+	Growth	+	Losses
A	ZOOXANTHELLAE	1441.63	+ -------	+ ---------	=	623.11	+	8.74	+	809.78
	ANIMAL	-------	+ 6.24	+ 809.78	=	501.84	+	203.26	+	110.92
B	ZOOXANTHELLAE	1194.17	+ -------	+ ---------	=	431.74	+	1.41	+	761.02
	ANIMAL	-------	+ -------	+ 761.02	=	377.81	+	49.17	+	334.04

TABLE 7-2:- The energy budget for standard 100cm² *Montastrea cavernosa* colonies at 10 metres (A), and 30 metres (B). The equations are calculated using total tissue biomass figures of 1862.81 mg.dw. at 10 metres, and 1791.94 mg.dw. at 30 metres. Values for zooxanthellae respiration were determined using data presented by Davies (1984).

RESULTS AND DISCUSSION.

Energy budgets for both 10 metre and 30 metre colonies are discussed in terms of that increment of energy (Joules) derived from or required by each component of the equation. The results are analyzed within the framework of the budget, and compared to previously published research.

PHOTOSYNTHETIC PRODUCTION BY SYMBIOTIC ZOOXANTHELLAE.

Hermatypic (zooxanthellate) *Montastrea cavernosa* colonies were able to satisfy their daily energy requirements from the translocation of photosynthates from the symbiont to the host. Gross photosynthetic production (Pg) assuming a 12-hour period of photosynthetically active radiation (PAR) was 0.7739 Joules.mg.d.w.$^{-1}$.12hrs.$^{-1}$, or 1441.63 Joules.12hrs.$^{-1}$, and 0.6666 Joules per mg.dw.$^{-1}$, or 1128.61 Joules.12hrs.$^{-1}$ normalized to standard 100 cm^2 colonies at both 10 metres and 30 metres respectively (Table 7-1). At 30 metres Pg was reduced by 13.86% relative to Pg at 10 metres.

Photosynthetic production rates were reduced at 30 metres, but this decrease of 13.86% in Pg relative to 10 metre colonies was small when compared to reductions in Pg rates of 76% recorded for colonies of *Styl'ophora pistillata* at 35 metres relative to colonies at 3 metres (McCloskey and Muscatine, 1984). This discrepancy may be attributed to incomplete experimental runs at 30 metres, where only limited photsynthetic production data were recorded using the automatic respirometer/data logger.

The energy equivalent resulting from photosynthetic production (Pg) at 10 metres was significantly lower than that recorded by Davies (1984), who notes an equivalent of 3.54 Joules per mg. per 12 hours for *Pocillopora eydouxi* colonies at 5 metres. This difference may be due to the different techniques used to determine tissue biomass, with Davies (1984) removing tissue fixed in 4% formalin by skeletal solution in 10% HCL. This technique may underestimate total biomass since some tissue may be lost to either the formalin or the acid. Likewise the Water Pik technique used in this investigation may overestimate total biomass since salts will tend to be lyophilized along with the blastate.

For both 10 metre and 30 metre colonies, the P/R ratio in Joules was greater than 1.0, 1.28 for the former, and 1.47 for the latter (Table 7-1). A P/R ratio greater than 1.0 is indicative of autotrophy. These ratios compare favourably with those of Svoboda and Poorman (1980) who report ratios of 1.24 $\pm$ .2 for n=29 zooxanthellate Red Sea corals.

Energy inputs to the zooxanthellae budget may also originate from nutrient recycling from host to symbiont (Lewis, 1973; Taylor, 1973; Franzisket, 1974; D'Elia, 1977; Patton *et al*,1977; D'Elia, 1978). The recycling mechanism - enhanced by light - may serve to reduce the need for external particulate energy supplies in zooxanthellate corals. No energy equivalents were determined for this process, and all losses from the animal fraction of the budget were lumped and assumed lost to the water column.

ZOOXANTHELLAE RESPIRATION.

Respiration rates of freshly isolated zooxanthellae (Rz) measured *in*

vitro were used to calculate the energy equivalent required for respiration (maintenance metabolism) of zooxanthellae contained per unit biomass. The results were used to extrapolate the energy requirements of (Rz) for standard 100 cm.^{2} colonies at both depths.

The energy requirements of Rz, 683.11 Joules per 24 hours for a standard 100 cm.^{2} colony at 10 metres constituted 55.39% of the maintenance requirements of the colony (Rc), while at 30 metres Rz was 431.74 Joules per 24 hours for a standard colony or 53.33% of Rc (Table 7-1). These results compare favourably with those of McCloskey and Muscatine (1984) who show that Rz in *Styl ophora pistillata* comprised 48% of Rc at 3 metres, and 85% of Rc at 35 metres. But these results are not compatible with those of Davies (1984) who reports that Rz was 19.19% of Rc in *Pocillopora eydouxi* in the laboratory.

Zooxanthellae respiration rates determined *in vitro* have been shown to be eight times higher than algal respiration rates *in situ* (McCloskey and Muscatine, 1984). These workers also report a 30% decrease in Rz in 35 metre colonies of *Styl ophora pistillata* relative to 3 metre colonies. Since in this investigation the same value for Rz was used for both 10 metre and 30 metre colonies, the possibility exists that there could be an eightfold overestimate in Rz at 10 metres and an even greater overestimate at 30 metres. Decreased *in situ* Rz requirements would result in an even greater proportion of that energy fixed by photosynthesis being translocated to the host.

GROWTH OF ZOOXANTHELLAE.

Zooxanthellae growth rates, or rates of mitotic division of

zooxanthellae necessary to populate the endoderm of 'new' tissue growth were 1.36 x 10^{6} zooxanthellae in standard 100 cm.^{2} colonies at 10 metres, and 2.19 x 10^{5} zoōxanthellae in standard colonies at 30 metres. The energy equivalent required for this growth was 8.74 Joules.24hrs.$^{-1}$ at 10 metres, and 1.41 Joules.24hrs.$^{-1}$ at 30 metres (Table 7-1).

The energetic requirements of zooxanthellae growth at 30 metres was 16.13% that of colonies at 10 metres. This result suggests reduced algal growth rates at 30 metres. This result was expected since skeletal extension rates and therefore tissue growth rates were also reduced at 30 metres relative to colonies at 10 metres (Tables 4-1,4-2).

At 10 metres the energy retained in the form of 'new' zooxanthellae Gz was 0.66% of Pg. This figure is higher than that obtained by Davies (1984) who recorded Gz rates requiring 0.087% of Pg in *Pocillopora eydouxi* at 5 metres. In the present investigation Gz in colonies at 30 metres was 0.12% of Pg. These results are lower than those of McCloskey and Muscatine (1984) who report carbon specific growth rates of 5.4 and 2.6 $\mu gC.cm.^{-2}.day^{-1}$ for zooxanthellae from colonies of *Stylophora pistillata* at 3 metres and 35 metres respectively. As a proportion of Pg these would be 1.99% at 3 metres, and 4.02% at 35 metres. That a greater proportion of available energy is utilized by zooxanthellae for growth at 35 metres was unexpected, since both tissue growth and algal respiration were reduced relative to those of 3 metre colonies. This phenomenon could be explained by an increased algal turnover rate as suggested by (Odum and Odum, 1955) in 35 metre colonies, but this possibility was not considered by McCloskey and Muscatine, 1984 .

ENERGY LOSSES FROM ZOOXANTHELLAE.

Since no data are available regarding the culling and rate of ejection of senescent zooxanthellae from host tissue (Steele, 1976), it was assumed that all the energy fixed by photosynthesis which was not used in algal maintenance, metabolism and growth would be translocated *in toto* to the host tissue. The concept of translocation of photosynthates from the symbiont to the host is now well established (Von Holt and Von Holt, 1968; Muscatine and Cernichiari, 1969; Trench, 1970,1971; Chalker and Taylor, 1971; Blanquet *et al*, 1979; Tytler, 1982; Davies, 1984; McCloskey and Muscatine, 1984).

In this investigation the energy equivalent of translocated material normalized to standard 100 $cm.^{2}$ colonies at 10 metres and 30 metres was 809.78 Joules.24hrs.$^{-1}$, and 761.02 Joules.24hrs.$^{-1}$ respectively (Table 7-2). As a percentage of the energy available from photosynthetic oxygen production, it was calculated that at 10 metres 56.17% of Pg would be translocated to the host, and at 30 metres this would increase to 63.73% of Pg. These rates of translocation compare favourably with published values; Von holt and Von holt (1968) reported the translocation of 40 - 50% of photosynthetically fixed carbon to the host, Taylor (1969) reports 58% translocation in *Anemonia sulcata*, Tytler (1982) reported higher values of 68.6 - 76.8% for the same species. Muscatine *et al*, (1981) showed that 63 -68% of the carbon requirements of *Pocillopora damicornis* were met by photosynthetic production, and finally Davies (1984) reported the translocation of 90% of photosynthetically fixed energy to the host in *Pocillopora eydouxi*.

In the present investigation translocation of photosynthates to the host was higher in 30 metre colonies than in colonies at 10 metres,

suggesting that zooxanthellae were photoadapted to the reduced irradiances at 30 metres (Dustan, 1979) and contributed a greater proportion of fixed energy to the requirements of the colony. Wethey and Porter (1976) suggested that the translocation of photosynthates from the algae to the host should increase in shade adapted corals. The present results seem to support this view. Increased translocation may also be the result of decreased algal growth requirements at 30 metres (Table 7-2), and may be caused by an artificially high Pg in 30 metre colonies.

Since no zooplankton were extracted from the coelenteron of 30 metre colonies, then the increased translocation rates from zooxanthellae to the host may be necessary to satisfy the energetic and nutritional requirements of colonies at that depth.

ENERGY INPUTS: THE ANIMAL TISSUE FRACTION.

Only energy inputs from heterotrophic nutrition, and that translocated to the host from its symbiont were considered. Since other exogenous energetic material (bacteria, detritus, particulate and dissolved organic material) were presumably available to the coral colonies at both depths, the values presented are an underestimate of that energetic input readily available to the colonies from heterotrophic nutrition.

No actual energy figures from exogenous inputs have been included in previous published material (Davies, 1984; McCloskey and Muscatine, 1984). Muscatine and Porter (1977) approximated the energetic input from zooplanktivory using gut content examinations collected 2-hours after sunset from colonies of *Montastrea cavernosa* in Panama by Porter (1974). In this investigation repeated gut sampling experiments (n=11) including an

all night sample at 2-hourly intervals yielded interesting results.

The energetic benefits from zooplankton ingestion (the values used in the budget being those from the all night sample) were minimal contributing approximately 6.24 Joules.12hrs^{-1} (Tables 7-1,7-2) or 0.77% of the total energy input to the animal fraction of standard colonies being derived from zooplankton ingestion at 10 metres. No zooplankton was found in the guts of 30 metre colonies after 3 collection dives in which 72 polyps were sampled, therefore the contribution from heterotrophic nutrition was taken to be zero. Since zooplankton ingestion was shown to be highly variable from the spot samples (Figure 5-6, Table 5-4), the contribution to the energy budget for this species could be higher than the value given above. As an example, the spot sample taken at 20:00 hours the 28/9/80 would contribute 20.2827 Joules to a standard 100 cm^2 colony at that time alone. This input would be equivalent to 2.5% of the requirements of a colony at 10 metres, but this percentage contribution would be expected to be higher if the colony continued to feed throughout the nighttime period.

Muscatine and Porter (1977) suggested that up to 20% of the daily energy requirements of *Montastrea cavernosa* were met by zooplankton ingestion, but this was based on the assumption that the colony fed only at dusk and dawn. The results from this investigation show that this species seems to be an obligate feeder ingesting a small proportion of available zooplankton throughout the night, with capture efficiencies being dependent on zooplankton density and species composition in the water column (Chapter 5). Since zooplankton density in the water column is variable throughout the nighttime period, depending on vertical migration patterns, then capture efficiencies will also be highly variable. The energetic inputs from heterotrophic nutrition on zooplankton will be small and often

intermittent when near reef water movement is severe and prevents active feeding by coral colonies.

This species does feed actively on zooplankton, but the benefits derived from this activity are minimal. Since this species is ubiquitous on Caribbean reefs (Wells, 1957; Goodwin *et al*, 1976), then its success may be related to its highly carnivorous feeding strategy necessary to provide an exogenous source of amino acids and essential nutrients present only in growth limiting concentrations in the water column (D'Elia, 1977).

The energetic costs of feeding, tentacular extension and retraction, and Specific Dynamic Action (SDA) as measured by Jobling (1978) in the plaice *Pleuronectes platessa*, and Tytler (1982) in *Anemonia sulcata*, were not measured in this investigation. Tytler (1982) reported that SDA - the increase in maintenance metabolism immediately following a meal - required 6.7% of that energy available to the animal fraction of the symbiosis. An increase in energy requirements of this magnitude throughout the nighttime feeding period in *Montastrea cavernosa* would reduce that proportion of energy lost from the symbiosis in the form of mucus.

Lasker (1980) reported tentacular extension during daylight periods in *Montastrea cavernosa* colonies from Panama. This was not seen at Discovery Bay, and hence it was assumed that the corals were not actively feeding on zooplankton during daylight hours.

RESPIRATION: ANIMAL TISSUE FRACTION.

The energy equivalents required for maintenance metabolism of the animal tissue fraction of the symbiosis (Ra) were determined by sutraction.

The energetic requirements of Ra in 'normal' colonies at 10 metres was 0.2694 Joules.mg.d.w.$^{-1}$.24hrs^{-1} (Table 7-1), or 34.8% of colony respiration at that depth. These values when normalized to the respiratory requirement of the animal tissue fraction of a standard 100 cm.^{2} colony would be equivalent to 501.84 Joules per 24 hours (Table 7-2). At 30 metres Ra was reduced at 0.2109 Joules.24hrs^{-1} (Table 7-1), or 31.64% of colony respiration (Rc). These values when normalized to the requirements of standard 100 cm.$^{-2}$ colonies at 30 metres, yielded an energy requirement of 377.81 Joules per 24 hours (Table 7-2). In terms of the total energy available to the animal tissue fraction, 61.97% was partitioned for Ra at 10 metres, while at 30 metres only 49.64% was spent on Ra. This reduction suggests decreased Ra at 30 metres, and confirms the results of Wethey and Porter (1976), Davies (1980), McCloskey and Muscatine (1984) who similarily showed that respiration rates decreased with increasing depth. Decreased Ra of 30 metre colonies relative to 10 metre colonies may be due to slightly lower rates of photosynthetic production at 30 metres, and consequently reduced inputs of metabolic substrates (carbohydrates) to the animal fraction of the symbiosis. Fitt *et al*, (1982) showed that Ra increased in response to carbon availability in *Anthopluera elegantissima*. Similar results have been described by Muscatine *et al*, (1981) and McCloskey and Muscatine (1984) who suggest that carbon available for animal respiration of zooxanthellae origin (C.Z.A.R.) decreased with increasing depth. Comparable C.Z.A.R. value for *Montastrea cavernosa* could not be calculated following the method of Muscatine *et al*, (1981), since zooxanthellae biomass per unit tissue biomass was not known.

GROWTH OF ANIMAL TISSUE.

There was 75.45% less energy required for animal tissue growth at 30

metres than at 10 metres. Colonies maintained at 30 metres exhibited mean growth rates of 0.1922 ± 0.1038 mg.d.w.24 hrs^{-1}, or 49.17±38.24 Joules.24hrs.$^{-1}$ for standard 100 cm^2 colonies at that depth. At 10 metres colonies had mean growth rates of 0.7627 ± 0.3030 mg.d.w.24hrs^{-1}, or 203.26 Joules per 24 hours when normalized : to a standard colony at 10 metres (Table 7-2).

Only a small percentage of the total energy input to the animal fraction was expended on tissue growth, 24.49% at 10 metres, and 6.46% at 30 metres. These values show that growth rates of shallow colonies were 73.62% higher than those at 30 metres. The results support the findings of Bak (1976) who reported decreased colony growth rates with increasing depth in the Caribbean coral *Meandrina meandrites*, and those of Buddemeier *et al*, (1974), Dustan (1976) who noted a decrease in linear growth rates with increasing depth.

The growth rates of animal tissue in 'bleached' corals (semi-aposymbiotic) were lower than those of normal corals at both depths. At 10 metres mean tissue growth rates were 0.1292 ± 0.0490 mg.d.w. .24hrs^{-1} which was equivalent to 18.85±8.11 Joules per 24 hours in a standard colony (Table 7-1), colonies at 30 metres had mean growth rates of 0.0586 + 0.0587 mg.d.w. .24hrs^{-1}, or 12.49±14.08 Joules per 24 hours in a standard colony (Table 7-1) . These growth rates were 9.27% of those of normal corals at 10 metres, and 25.40% those of normal colonies at 30 metres.

Tissue growth of 'bleached' colonies was probably largely due to exogenous nutritional sources including zooplankton. Reeve *et al* (1978) showed that the Ctenophore *Pleurobranchia mnemopis* retained 3-11% of ingested material as tissue growth and storage material, while Tytler

(1982) showed that 33.5% of ingested squid mantle was retained by the anemone *Anemonia sulcata*. These 'bleached' colonies were not wholly aposymbiotic, since from Table (6-2) it can be seen that bleached colonies at 10 metres retained 9.27 x 10^3 ± 59 zooxanthellae per mg.d.w., and those at 30 metres retained 2.58 x 10^3 ± 16 zooxanthellae per mg.d.w.. If these algae were photosynthetically active, then they could have contributed to calcification, and translocated photsynthetically reduced carbon compounds for tissue growth. For this discussion it was assumed that all growth in bleached colonies was due exclusively to exogenous energy inputs. If this assumption is correct, one can subtract the tissue growth of bleached colonies from those of normal colonies with the remainder being that fraction of animal tissue growth resulting from translocated photosynthates. From this assumption it could be said that translocation from the symbiont to the host contributed 77.25% of the energetic requirements for tissue growth at 10 metres, and 95.18% of the requirements at 30 metres. This suggests that in this species an exogenous energy supply is necessary for growth, and that tissue growth would be limited by a reduced or absent supply.

At 30 metres the difference in growth rate between normal and bleached colonies was less than at the 10 metre site. Since growth rate was reduced at 30 metres, and no ingested plankton was recovered from the guts of corals at that depth, then one can assume that alternate sources of exogenous nutritional material such as bacteria (Sorokin, 1973; Moriarity,1981), particulate organic material (D'Elia, 1977; Muscatine and D'Elia, 1978), detritus (McCloskey and Chesher, 1971), and dissolved organic material (Johannes *et al*, 1970) were ingested. If most ingested exogenous material was in this form, then the increased growth rate of bleached colonies relative to normal colonies at 10 metres could be

explained. The exogenous materials described would be available to corals in both treatment groups in equal quantities, but colonies on the fore reef slope close to the fore reef facies, were exposed to upwelling currents up the fore reef facies, and a general onshore flow of oceanic water. This onshore flow would have a slightly higher concentration of inorganic phosphates and nitrates (Sverdrup *et al*, 1942) necessary and limiting to animal tissue growth in tropical marine ecosystems (Kawaguti, 1953; Franzisket, 1970; D'Elia, 1977). Therefore in the absence of suitable quantities of zooplankton, it would be expected that the difference between growth rates in bleached and normal colonies at this depth should be less than between their counterparts at 10 metres.

Bleached colonies at both depths were shown to harbour what have been termed 'seed' populations of zooxanthellae in this investigation. These may exist to enable the colony to actively take up phosphates, nitrates, and ammonia from solution as suggested by D'Elia (1977), and Muscatine and D'Elia (1978), this phenomenon occurring in the presence of light. This process may have taken place in corals maintained in darkened chambers, since some light must have impinged on the colonies to enable endolythic algae to retain their pigmentation, and spread inwards into the colony. The workers mentioned previously noted that ahermatypic corals such as *Tubastrea sp.* were unable to actively take up nutrients from solution in the presence of light.

Some of the energy partitioned for growth would also be retained in the form of lipid storage compounds. Fitt *et al* (1981), investigating the metabolism of the anemone *Anthopleura elegantissima* noted that after two days starvation in the dark the metabolism shifted from the normal carbohydrate substrate metabolism to a lipid substrate metabolism. These

results supported those of Blanquet *et al*, (1979), who suggested that lipid stores in corals were of photosynthetic origin. In this investigation storage compounds and their energetic equivalents were not investigated, therefore those values presented as that fraction of energy retained as growth (Table 7-2) must be an underestimate since they do not include the equivalents for storage compounds.

ENERGY LOSSES FROM THE ANIMAL TISSUE FRACTION

It is assumed in this work that all excess energy both of exogenous and endogenous origin was released to the water column in the form of mucus. Mucus secretions by corals have been reported by Crossland *et al*,(1980), who reported that 40% of photosynthetically fixed carbon was lost as mucus secretions from the coral *Acropora acuminata*, by Tytler (1982) who recorded energy losses in the form of mucus of 14.3% and 45.3% in *Anemonia sulcata* maintained at 70 $\mu E.m^{-2}.sec^{-1}$ and 140 $\mu E.m^{-2}.sec^{-1}$ respectively, and finally by Davies (1984) who reported losses of 48% in the coral *Pocillopora eydouxi* at 5 metres. These values are all higher than those calculated for corals maintained at 10 metres, where energetic losses to the water column amounted to 110.92 Joules per standard colony, or 13.59% of the total energy input to the animal tissue fraction of the symbiosis (Table 7-2). But the values are within the range of those calculated for colonies maintained at 30 metres, where energetic losses amounted to 334.04 Joules per standard colony or 43.89% of the energy inputs (Table 7-2).

The values representing the energetic losses from the bioenergetic model are purely arbitrary since the extent of nutrient recycling from the host to the symbiont has not been quantified in this research. Recycling

mechanisms between host and symbiont may be an effective means of reducing the excretion of essential nutrients (Lewis, 1973; Taylor, 1973; Pomeroy *et al*, 1974; Muscatine and Porter, 1976). In a recent paper Rynkevich and Loya (1983) reported that there were no significant losses of labelled photosynthetic products from *Stylophora pistillata* after 48 hours, but that 80% of these were lost after 1 month presumably in the form of mucus, larval release, tissue growth, and maintenance metabolism. These results suggest an efficient recycling mechanism in that species. Similar experiments on *Acropora formosa* (Crossland *et al*, 1980) suggest that 50 - 60% of labelled photosynthetic products are lost 40 hours post incubation. Such disparity between species may be indicative of successful colonization of a reef by a particular species. Those with well developed recycling mechanisms that are able to derive their energetic requirements from autotrophy and light enhanced uptake of nutrient material in solution (high surface to volume corals Porter 1976) may be more successful than low surface to volume corals requiring active heterotrophic feeding to complement their nutritional input. Results from the energy budget equations may provide information regarding the successful colonization of a reef by a particular species. Accurate data regarding its dependance on different nutritional strategies, and the partitioning of that energy input to the different components of the budget, may indicate how that species will tolerate changes in the reef environment. Environmental changes may force a coral to alter its budgetary requirements to partition extra energy for specific tasks such as sediment clearing from feeding surfaces during periods of excessive sediment load on estuarine reefs, increased active feeding on exogenous material during periods of high turbidity not conducive to normal rates of photosynthetic production. Corals able to exhibit flexibility in their budgetary requirements, those with the greatest trophic plasticity should be the more successful colonizers on a

CHAPTER - 8

Investigation of the Energy Budget of *Montastrea cavernōsa*: present trends and future developments.

Montastrea cavernosa is a ubiquitous frame building scleractinian coral on Western Atlantic reefs (Wells,1957; Goodwin *et al*,1976). An investigation was carried out to determine how energy fixed by endosymbiotic zooxanthellae via photosynthetic production, and ingested from exogenous sources, was partitioned by the colony for maintenance metabolism, growth, storage, and finally what proportion was lost to the water column, or translocated as excess production to the host.

A series of experiments using a purpose built automatic respirometer /data logger suggests that at the two depths studied, 10 metres and 30 metres, photosynthetic production exceeded colony respiration, and was able to fully satisfy the daily energetic requirements of the colony.

The energetic benefits derived from heterotrophic nutrition on zooplankton were minimal, and accounted for only < 1 % of the total energy input of colonies at 10 metres. No such value could be calculated for colonies at 30 metres, since no zooplankton was extracted from the coelenterons of sample colonies, but this does not preclude zooplankton ingestion, or heterotrophic nutrition on other exogenous material. Active feeding on zooplankton was seen to occur throughout the night at the 10 metre site, but ingestion was shown to be dependent on zooplankton density, zooplankton species, and water movement. Capture of zooplankton by a coral colony was reduced during periods of low plankton density in the water column, periods of severe ground surge, and when highly motile forms such

as chaetognaths formed the bulk of available plankton.

Heterotrophic nutrition on zooplankton, though not energetically important, was essential for tissue growth. This was shown by the continued but reduced growth rates of 'bleached' colonies at both depths. 'Bleached' colonies at 30 metres exhibited reduced growth rates relative to 10 metre colonies. Since no zooplankton was recovered from the guts of 30 metre colonies, these must have satisfied their biochemical requirements for tissue growth from other exogenous material such as Bacteria (DiSalvo,1971; Sorokin,1973; Moriarity,1979), Dissolved and particulate organics (Johannes *et al*,1970; Black and Burris,1983), detritus (Lewis,1973), and possibly by light enhanced nutrient uptake as suggested by D'Elia (1977) by the remaining zooxanthellae. Bleached colonies maintained in darkened conditions for eight months still had endodermal zooxanthellae, suggesting that a remnant 'seed' population of zooxanthellae remains in the host tissue, and are able to reinfect the host when returned to a normally irradiated environment. Repigmentation was always seen in colonies returned to a normal photic environment.

Nutrient recycling mechanisms from the host to the symbiont have been reported (D'Elia,1977; Muscatine and D'Elia,1978). This mechanism, coupled with an active uptake of growth limiting nutrients by the colony in the presence of light (D'Elia,1977), provides corals with the ability to exist in and modify what would otherwise be a reduced fauna environment. The symbiosis enables a coral colony to exhibit a remarkable degree of trophic plasticity, a benefit in an environment noted for inconsistent supplies of exogenous nutritional material.

This research project though technically difficult and physically

demanding, largely achieved the intended goals. The expected data return from the prototype automatic respirometer/data logger was well below that originally projected, but in hindsight this is not surprising. Improvements both in technology and experimental design would streamline the method, improve data reliability, and reduce data processing time.

TECHNICAL IMPROVEMENTS

1)- The use of a sensitive microcathode oxygen electrode created difficulties. It would have been preferable to use a robust medium area cathode electrode such as the YSI model 5311. Oxygen depletion at the sensing head can be eliminated by increasing the RPM of the stirring assembly and the effect of greater consumption is cancelled by frequent microcomputer-controlled flushing.

2)- The probe array should be increased to include pH and Conductivity electrodes, and it may be desirable to add a controlled zooplankton sampling camera (Benthos Undersea Systems Technology).

3)- Many of the electrical faults encountered, including blown microchips, could be avoided by enclosing sensitive circuitry in a hermetic elastomer compound. Backup circuits with gold plated edge connectors should be available to replace failed circuitry.

4)- A microprocessor programmable in BASIC would be preferable to the ACORN minicomputer programmed in machine language used in this investigation.

5)- On board data processing with only fully decoded data being stored on magnetic tape, or preferably in removable battery backed static RAM packs

would improve the system. Data retrieval would then simply involve serial transfer to a shore based microcomputer where the data would be permanently stored on disk.

6)- An improved hermetic housing constructed of lighter metal alloys and sealed by hydrostatic pressures would improve system portability and allow long term deployment in the field without the corrosion problems of the steel housing used for the prototype device.

7)- The use of dedicated underwater connectors with gold plated terminals for all external connections would reduce data corruption due to increased resistance from corrosive processes on external electrical leads.

8)- The use of a low power C-MOS microcomputer in future developments would reduce power consumption in the system, and increase underwater deployment time.

IMPROVEMENTS IN THE EXPERIMENTAL METHOD

This investigation could have been improved by combining *in situ* experiments with laboratory based experiments to both support and complement the field experiments. Support experiments were not carried out since it was suspected that the combination of laboratory based experimental results and those from *in situ* work could inject variation, and make the analysis difficult. The results from this research show that there is strong correlation between *in situ* results, and the results from laboratory based research on the same species (Davies,1977), and therefore future experiments can be designed to combine these two in the same research programme. Such a programme could provide a useful diagnostic tool for

environmental monitoring, and coastal resource management.

The following laboratory based experiments could have added valuable information to this research:

a)- Chlorophyll-a concentrations of extracted zooxanthellae from 'bleached' colonies at both 10 meters and 30 meters, to determine whether 'seed' zooxanthellae were active or dormant in the host tissue.

b)- Thermal tolerance experiments to assess the degree of survivable stress in field populations. This information could be invaluable to a management programme

c)- To quantify energy expenditure for sediment clearing from feeding surfaces, to assess whether increased sediment load would severely alter the energy budget as suggested by Szmant-Froelich *et al* (1981)

d)- To investigate the effect of short term stress on growth rate, to assess the possibility of using growth in coral colonies as an indicator of deleterious environmental change.

e)- Losses and uptake of dissolved organic material and specific nutrients by the colony to determine the extent of nutrient recyling from the host to the symbiont. These results would be used to calculate an energetic equivalent for recycled material.

f)- To quantify short term variations in lipid concentrations of

coral colonies in an effort to determine whether lipid stores are depleted during periods of plankton paucity, or during periods of rough weather when colonies are unable to feed normally.

An investigation such as the one presented in this thesis has an obvious application to environmental monitoring, and resource management programs. Using the *in situ* logging aparatus, it would be possible to characterise the physiological response of a selected ubiquitous benthic organism under different environmental conditions (inshore lagoon, offshore reef front, estuarine, prone to frequent effluent discharges, thermal plumes, etc.). The data could then be used to construct a cause/effect predictive model based on the information for the species selected, and apply the results to create guidelines for coastal development strategies.

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APPENDIX 1.1.

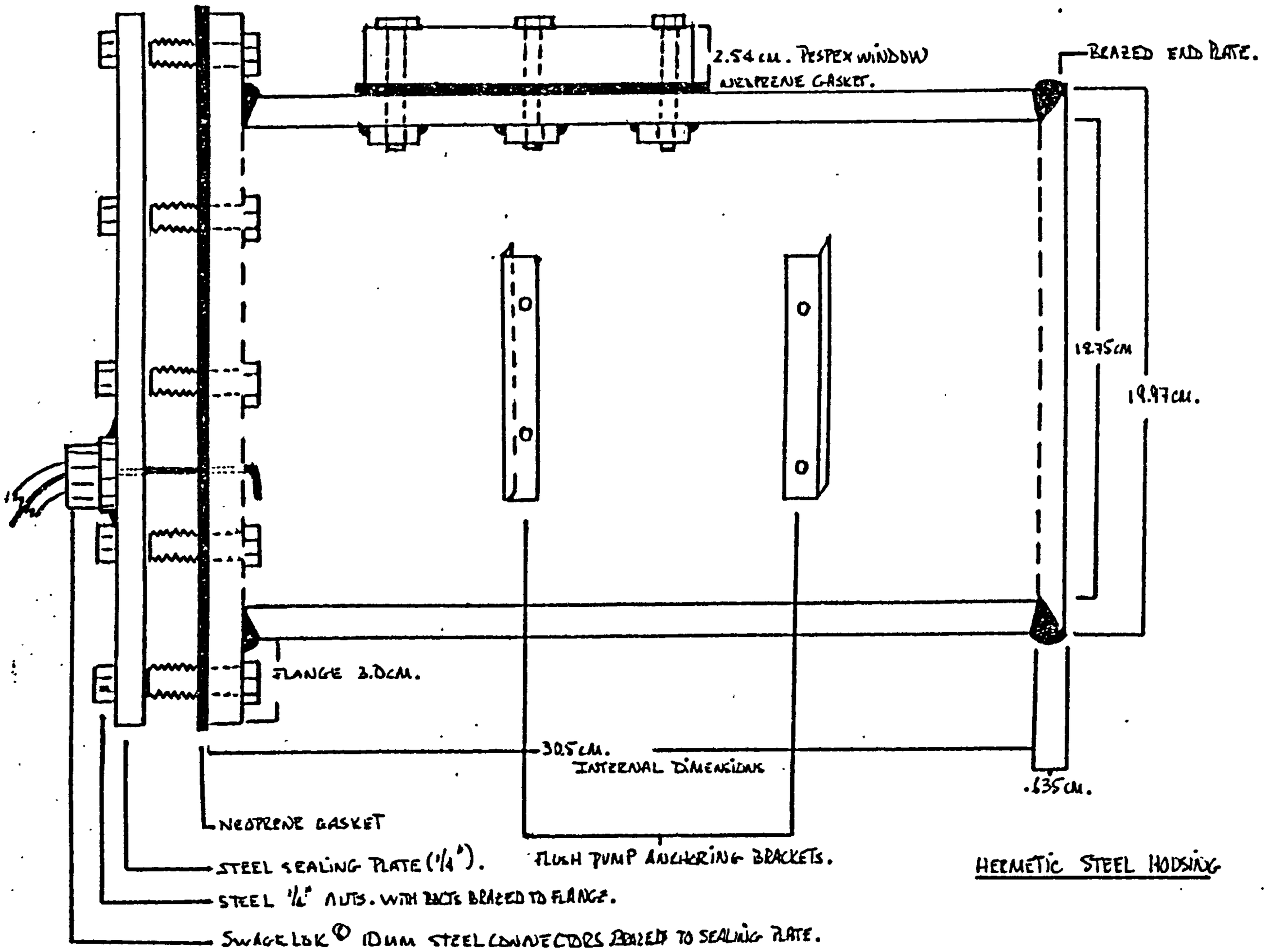
This paper published in Journal of Experimental Marine Biology and Ecology Volume 74, pages 231-239, is included to complement the material in Chapter-3 concerning the development of the Mark III automatic respirometer /data logger.

APPENDIX 1.2.

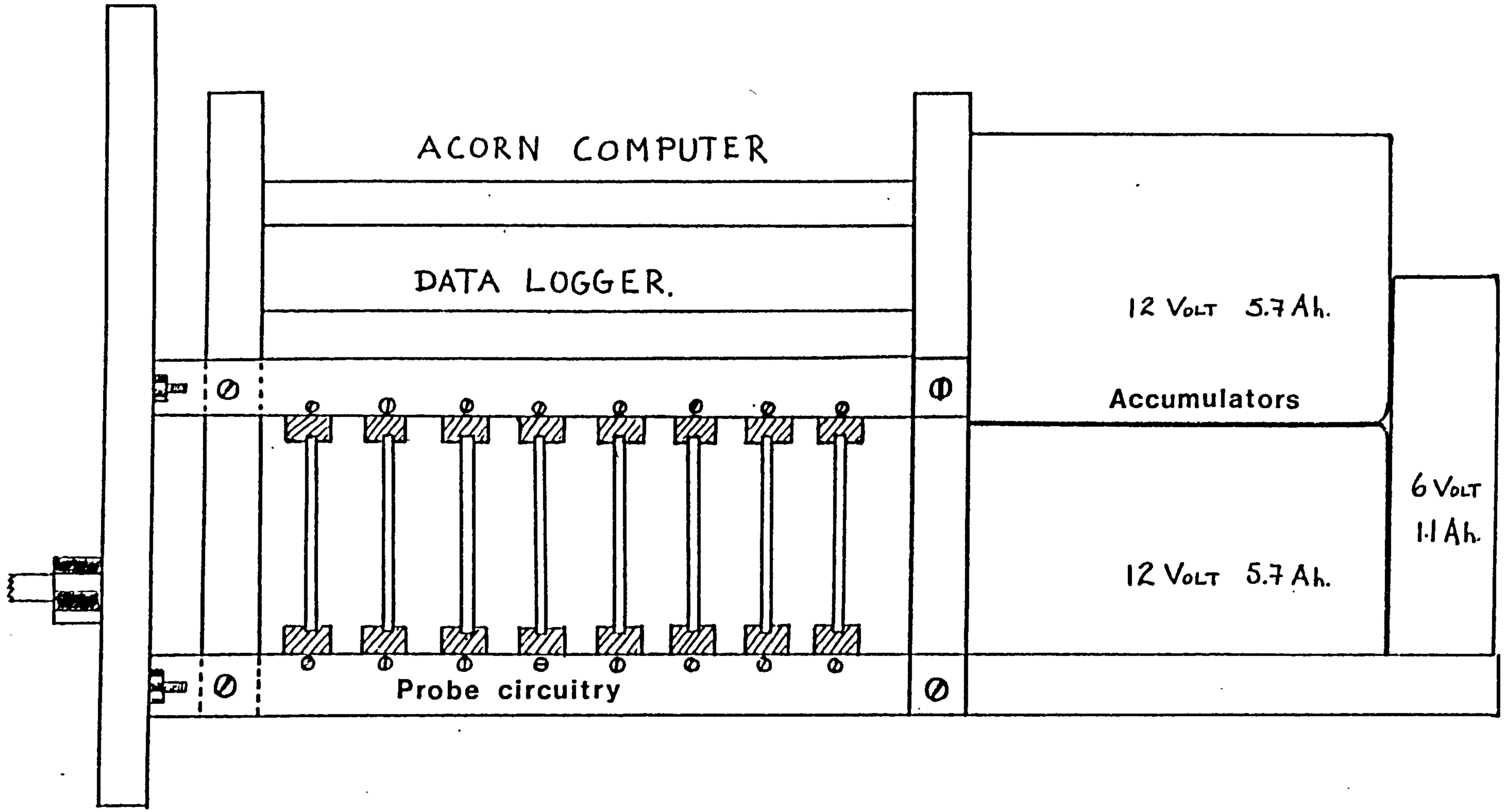
Components of the Mark III automatic respirometer/data logger;

1) Hermetic steel housing, dimensions and construction.
2) Internal arrangement of the electronic components and accumulators mounted on an aluminium rack fixed to the interior of the steel sealing plate.
3) Data logging circuitry. This circuit being composed of three main units, a timing circuit, circuitry and relays for the flushing pump, and the digital to analogue conversion circuitry.
4) Oxygen electrode circuit
5) Photocell circuitry.
6) Thermistor circuitry.
7) Power supply circuitry and connections.
8) Battery charger circuit.

MARK III LOGGER HERMETIC HOUSING



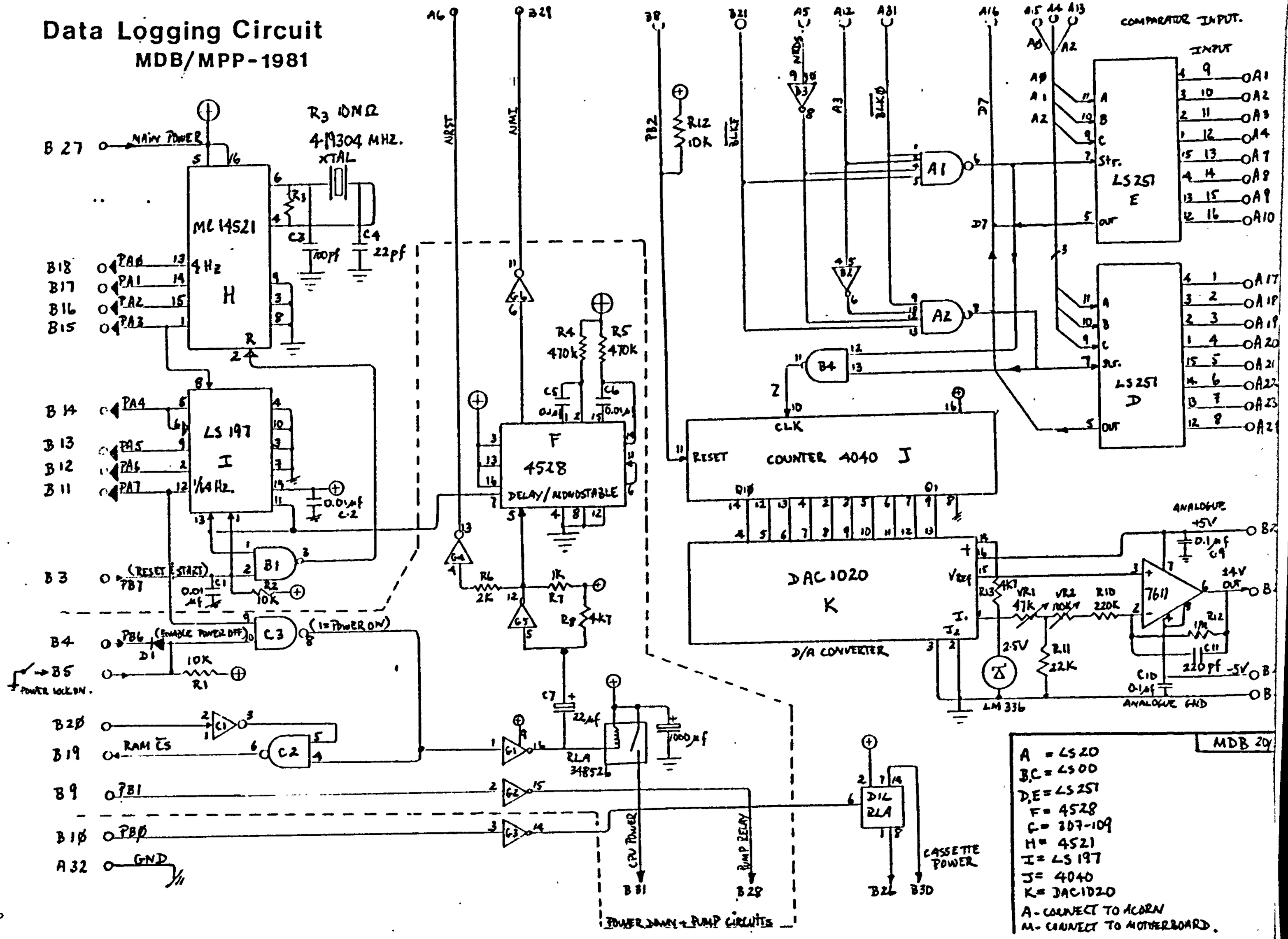
HERMETIC STEEL HOUSING



HOUSING : INTERNAL RACK

Data Logging Circuit

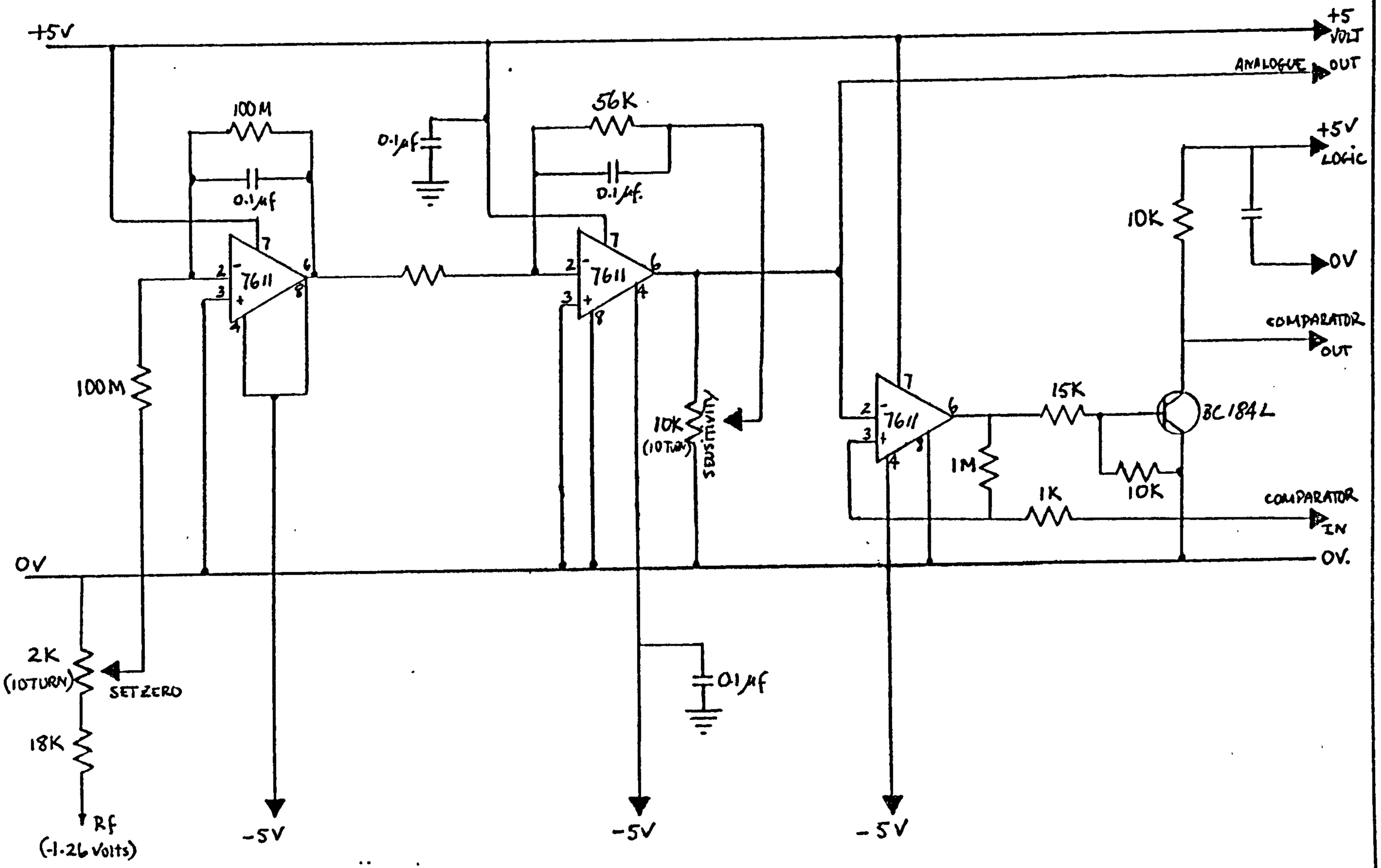
MDB/MPP-1981



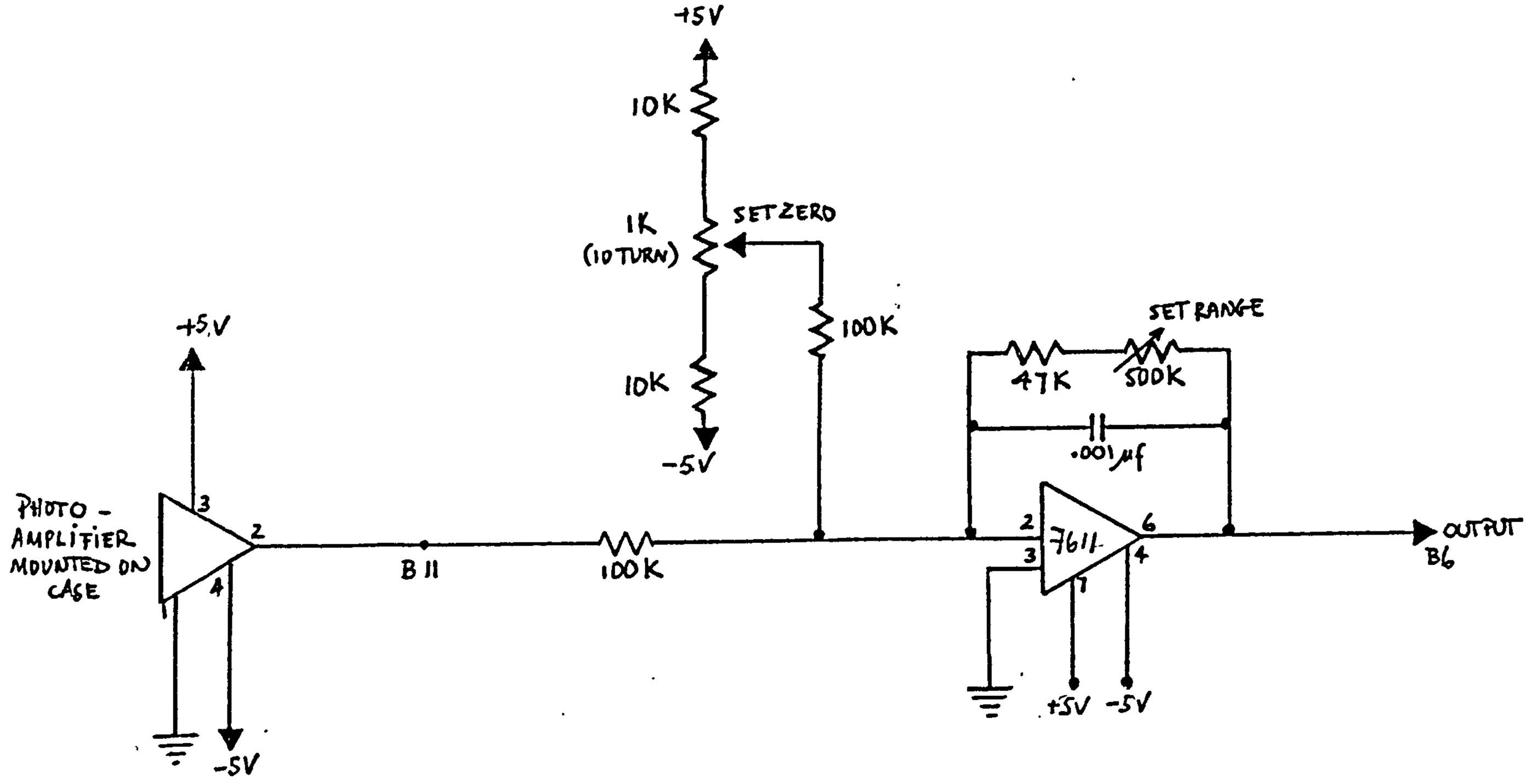
MDB 20

A = LS20
B,C = LS00
D,E = LS251
F = 4528
G = 307-109
H = 4521
I = LS 197
J = 4040
K = DAC1020
A - CONNECT TO ACORN
M - CONNECT TO MOTHERBOARD.

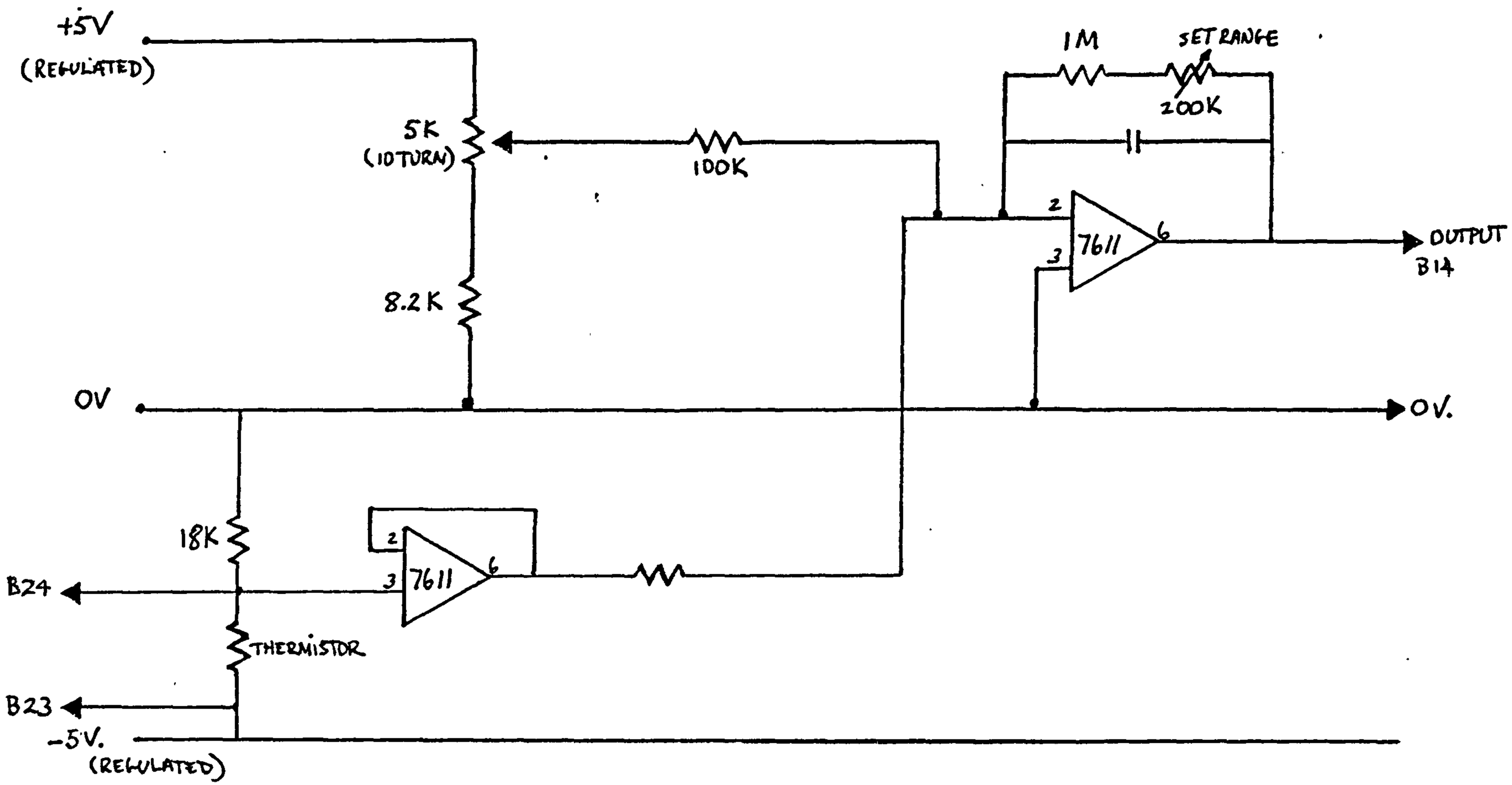
OXYGEN ELECTRODE CIRCUIT



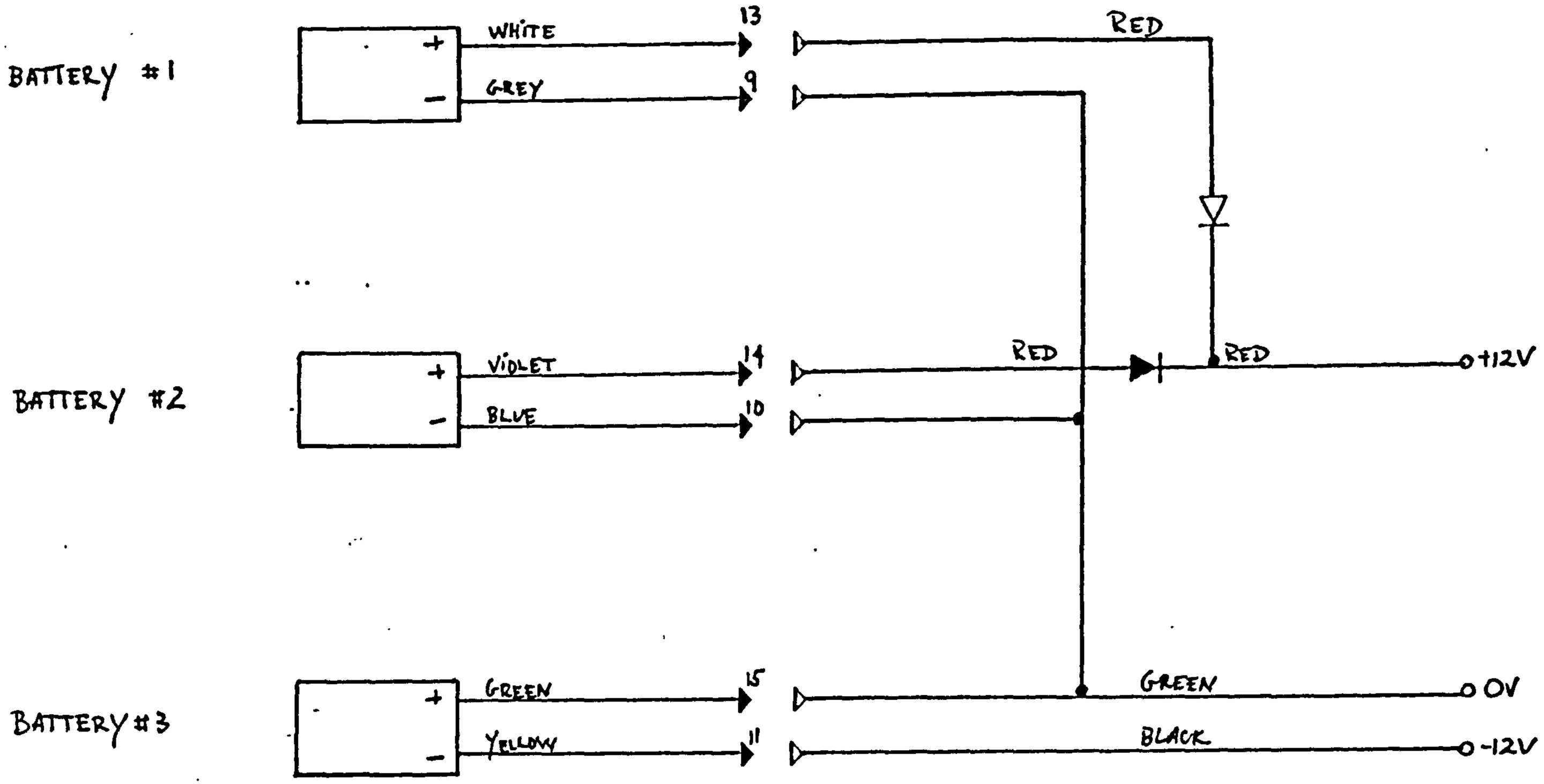
PHOTOCELL CIRCUIT



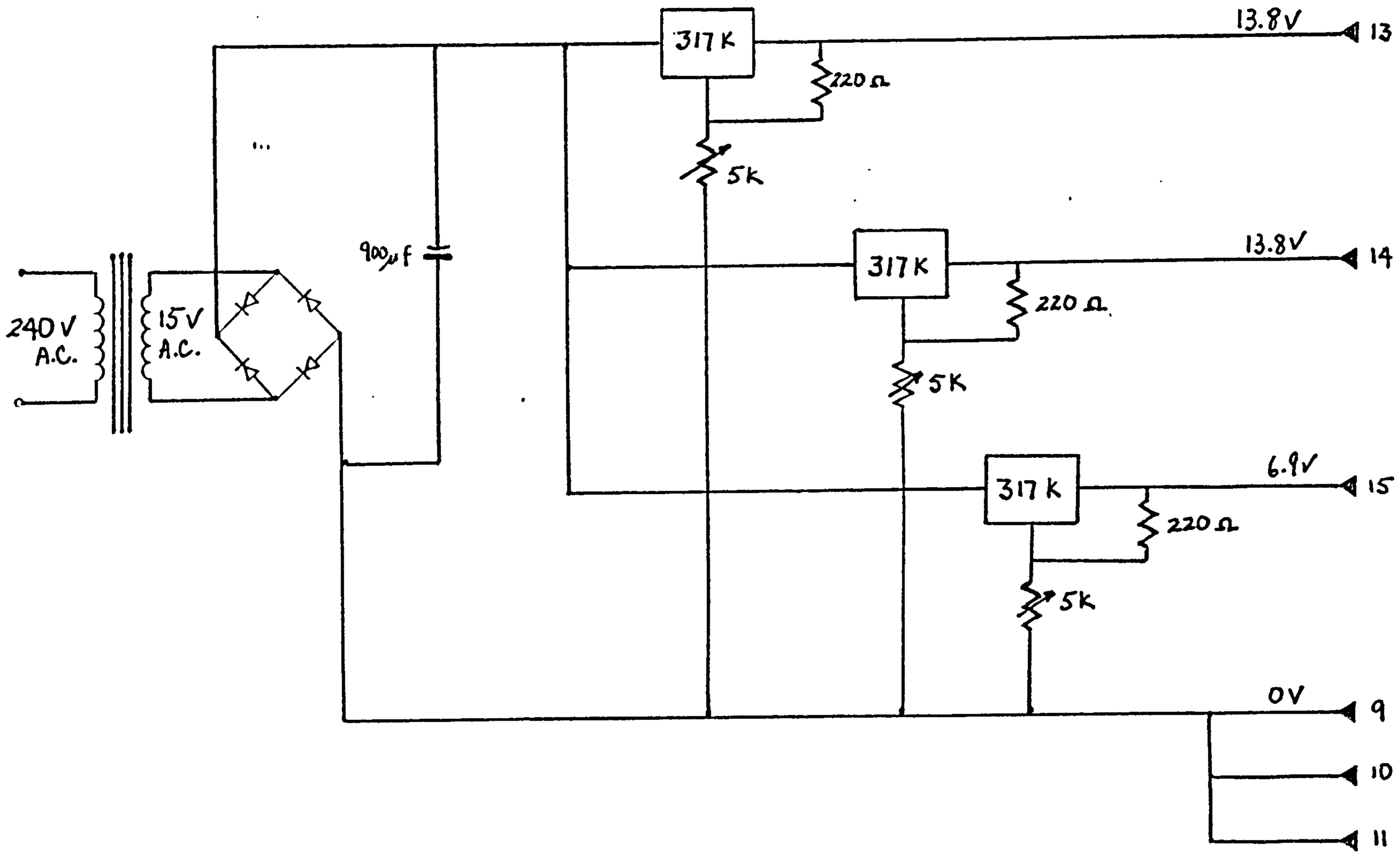
THERMISTOR CIRCUIT



POWER SUPPLY CIRCUITRY



BATTERY CHARGER CIRCUIT



APPENDIX 1.3.

Programs necessary to calibrate, operate, decode the data recorded on cassette tape and plot that data using an X-Y plotter. The programs are each identified by a short description contained in the asterisked section.

```
                        ;**********************************
                        ;*
                        ;* CALIBRATION ROUTINE. NEEDS
                        ;* CONVRT AND DDEC
                        ;*................20/07/81
                        ;**********************************
                        ;
                        ;
                      BDDR2=$923
                      DISP=$FE0C
                      CONVRT=$F38E
                      DDEC=$F1E0
                      CHNO=$80
                      PIB2=$921
                      HEXTD=$FE7A
                        ;
                        ;
                        *=$F100
F100 A9C7       CAL     LDA  #$C7
F102 8D2309             STA  BDDR2          !SET ODB
F105 D8                 CLD                 SET BINARY
F106 A900               LDA  #0
F108 8D2109             STA  PIB2           !DISABLE POWER OFF
F10B AA                 TAX
F10C A008               LDY  #8
F10E 960F       CLEAR   STX  $F,Y           !CLEAR DISP
F110 88                 DEY
F111 D0FB               BNE  CLEAR
F113 A97F               LDA  #$7F
F115 850E               STA  $E
F117 A480       MEASR   LDY  CHNO           !GET CHANNEL #
F119 208EF3             JSR  CONVRT         !GET VALUE
F11C 20E0F1             JSR  DDEC           !CONVERT TO DECIMAL
F11F A232               LDX  #50
F121 200CFE     SHOW    JSR  DISP           !DISPLAY
F124 9005               BCC  KEY            !REPEAT IF NO KEY
F126 CA                 DEX
F127 D0F8               BNE  SHOW           !10 DISPLAYS
F129 F0EC               BEQ  MEASR          (ALWAYS)
F12B 8480       KEY     STA  CHNO           !STORE KEY
F12D A000               LDY  #0
F12F 207AFE             JSR  HEXTD          !SET CHANNEL # DISPLA
F132 10E3               BPL  MEASR          !ALWAYS
F134                    END
```

```
 2  033a                ;**************************************
 4  033a                ;** running program
 6  033a                ;** ------------------
 8  033a                ;** to collect data using the
10  033a                ;** acorn in situ logger system
12  033a                ;** ..............18/07/81
14  033a                ;**************************************
16  033a                ;
18  0923                bddr2=$923
20  0037                offl=$37
22  0038                ovrscl=$38
24  0039                cycles=$39
26  f13a                timer=$f13a
28  f400                flush=$f400
30  f160                pdwn=$f160
32  f390                scan=$f390
34  fe0c                disp=$fe0c
36  f0d0                recblk=$f0d0
38  0200                storel=$200
40  0300                storeh=$300
42  0025                savy=$25
44  003e                ovrl=$3e
46  003d                ovrh=$3d
48  002a                check=$2a
50  033a                ;
52  033a                *=$f000
54  f000  a9c7    start       lda  £$c7
56  f002  8d2309              sta  bddr2          set odb
58  f005  d8                 cld                 set binary
60  f006  a90c                lda  £12
62  f008  852a                sta  check
64  f00a  a900                lda  £0
```

```
:       66  f00c  8d0909          sta  $909          pump off
        68  f00f  8d0809          sta  $908          tape off
        70  f012  8d0e09          sta  $90e          clear pb-6
        72  f015  850e            sta  $e            single display scan
        74  f017  8538            sta  ovrscl        clr.overscale flag
        76  f019  a200            ldx  £0
        78  f01b  a008            ldy  £8
        80  f01d  960f    clear   stx  $f,y
        82  f01f  88              dey
        84  f020  d0fb            bne  clear
        86  f022  a9ff            lda  £$ff
        88  f024  8537            sta  offl          set off flag
        90  f026  a2e4            ldx  £228
        92  f028  a974            lda  £$74
        94  f02a  851c            sta  $1c
        96  f02c  a9f1            lda  £$f1
        98  f02e  851d            sta  $1d           set nmi pointer high
       100  f030  a901            lda  £01
       102  f032  8539            sta  cycles        set cycles =1
       104  f034  2060f1          jsr  pdwn          wait for reed switch
       106  f037  a900            lda  £0
       108  f039  8537            sta  offl          clear offl
       110  f03b  2000f4          jsr  flush
       112  f03e  203af1  cont    jsr  timer         power down for resid.
                                                     residual
       114  f041  2000f7          jsr  scan
       116  f044  c62a            dec  check
       118  f046  f00a            beq  c5
       120  f048  a538            lda  ovrscl
       122  f04a  f012            beq  c2
       124  f04c  2000f4          jsr  flush
       126  f04f  4c5ef0          jmp  c2
       128  f052  a90c    c5      lda  £12
       130  f054  852a            sta  check
```

```
132  f056  2000f4          jsr  flush
134  f059  a90f            lda  £$f
136  f05b  9d0003          sta  storeh,x       end of hour marker
138  f05e  8a        c2    txa                 :
140  f05f  f018            beq  c3             if x=0,end block
142  f061  a9ed            lda  £$ed
144  f063  8510            sta  $10            s to display
146  f065  a005            ldy  £5
148  f067  8425            sty  savy
150  f069  8d1809          sta  $918           tape on
152  f06c  200cfe    c4    jsr  disp
154  f06f  c625            dec  savy
156  f071  d0f9            bne  c4             delay to inch tape
158  f073  8d0809          sta  $908           tape off
160  f076  4c3ef0          jmp  cont           next power down
162  f079                ;
164  f079                ;***** end of block******
166  f079  20d0f0    c3    jsr  recblk         record data block
168  f07c  4c3ef0          jmp  cont
170  f07f                ;
172  f07f                ;***** finish off when reed closed***
174  f07f  ca        dend  dex
176  f080  a900      e1    lda  £0
178  f082  9d0002          sta  storel,x
180  f085  a90b            lda  £$b
182  f087  9d0003          sta  storeh,x
184  f08a  ca              dex
186  f08b  d0f3            bne  e1
188  f08d  20d0f0          jsr  recblk         record last block
190  f090  4c00f0          jmp  start
192  f093                ;
194  f093                ;
196  f093                ;**note--data storage down from $03e3 (h) & $02e3 (l)
```

```
  1  033A                ;**************************************
  3  033A                ;**TIMER SUBROUTINE
  5  033A                ;**-----------------
  7  033A                ;**POWER DOWN FOR THE REST OF TIME TO
  9  033A                ;**GO (TTG) AND THEN RESETS TTG=5MINS.
 11  033A                ;**.................21/7/81
 13  033A                ;**************************************
 15  033A                ;
 17  0041              - TTGL=$41
 19  0042              - TTGH=$42
 21  0040              - T2=$40
 23  0045              - RMNDR=$45
 25  0039              - CYCLES=$39
 27  F3E0               - CLOCK=$F3E0
 29  0046               - T3=$46
 31  090B               - REED=$90B
 33  0921               - PIB2=$921
 35  0923               - DDR2=$923
 37  0037               - OFFL=$37
 39  FE7A               - HEXTD=$FE7A
 41  F05C               - DEND=$F05C
 43  FE0C               - DISP=$FE0C
 45  0024               - SAVX=$24
 47  0025               - SAVY=$25
 49  0026               - PCL=$26
 51  0027               - PCH=$27
 53  033A                ;
 55  033A                *=$F13A
 57  F13A  20E0F3  TIMER       JSR  CLOCK
 59  F13D  38                  SEC
 61  F13E  A541                LDA  TTGL
 63  F140  E540                SBC  T2
 65  F142  8545                STA  RMNDR
 67  F144  A542                LDA  TTGH
 69  F146  E900                SBC  #0
 71  F148  8539                STA  CYCLES          TTG-T2 (CYCLES=H,RMNDR=L)
 73  F14A  18                  CLC
 75  F14B  2645                ROL  RMNDR
 77  F14D  2639                ROL  CYCLES          DIVIDE BY 128
 79  F14F  18                  CLC
 81  F150  6645                ROR  RMNDR           REMAINDER
 83  F152  18                  CLC
 85  F153  A545                LDA  RMNDR
 87  F155  6540                ADC  T2
 89  F157  8546                STA  T3
 91  F159  20E0F3  LOOP        JSR  CLOCK
 93  F15C  C546                CMP  T3              COMPARE TIME TO GO
 95  F15E  D0F9                BNE  LOOP
 97  F160  8624    PDWN        STX  SAVX            SAVE X
 99  F162  68                  PLA
101  F163  8526                STA  PCL
103  F165  68                  PLA
105  F166  8527                STA  PCH             SAVE STACK POINTER
107  F168  8D0F09  PDWN2       STA  $90F            CLEAR PB-7
109  F16B  8D1F09              STA  $91F            SET PB7
111  F16E  8D1E09              STA  $91E            SET PB6
113  F171  4C71F1  HOLD        JMP  HOLD            ALWAYS WAIT
115  F174                ;
117  F174                ;**** POWER UP ****
```

```
119  F174                ;
121  F174  A9C7    POWER       LDA  #$C7
123  F176  8D2309              STA  BDDR2        CONFIG.PORT
125  F179  D8                 CLD               SET BINARY
127  F17A  A900                LDA  #0
129  F17C  8D2109              STA  PIB2         P,T OFF,B6 LOW B7 LOW
131  F17F  8D1F09              STA  $91F         SET PB-7
133  F182  850E                STA  $E           SINGLE DISP SCAN
135  F184  A200                LDX  #0
137  F186  8640                STX  T2
139  F188  A000                LDY  #0
141  F18A  960F    CLEAR       STX  $F,Y
143  F18C  88                  DEY
145  F18D  D0FB                BNE  CLEAR        CLEAR DISP
147  F18F  A624                LDX  SAVX
149  F191  2C0B03              BIT  KEED
151  F194  1007                BPL  P2
153  F196  C639                DCC  CYCLES       REED OPEN
155  F198  D01B                BNE  CYDISP       NEXT CYCLE
157  F19A  4CA6F1              JMP  FINISH
159  F19D  A537    P2          LDA  OFFL
161  F19F  D0C7                BNE  PDWN2        OFF FLAG SET SO STAY OFF
163  F1A1  C637                DEC  OFFL
165  F1A3  4C7FF0              JMP  DCND         QUIT
167  F1A6  A904    FINISH      LDA  #4
169  F1A8  8542                STA  TTGH
171  F1AA  A9A0                LDA  #176
173  F1AC  8541                STA  TTGL
175  F1AE  A527                LDA  PCH
177  F1B0  48                  PHA
179  F1B1  A526                LDA  PCL
181  F1B3  48                  PHA               RESTORE STACK POINTER
183  F1B4  60                  RTS               SET TTG TO 1200
185  F1B5                ;
187  F1B5  A958    CYDISP      LDA  #$58
189  F1B7  8510                STA  $10          SYMBOL=C
191  F1B9  A539                LDA  CYCLES
193  F1BB  A007                LDY  #7
195  F1BD  207AFE              JSR  HEXTD
197  F1C0  A014                LDY  #20
199  F1C2  8425                STY  SAVY
201  F1C4  200CFE  SHOW        JSR  DISP
203  F1C7  C625                DEC  SAVY
205  F1C9  D0F9                BNE  SHOW         DISPLAY CYCLES
207  F1CB  4C60F1              JMP  PDWN2        POWER DOWN FOR NEXT ~~CYCL~~ CYCLE
```

```
0   033a                ;**********************************
2   033a                ;** scan subroutine
4   033a                ;** ---------------
6   033a                ;** scans all 6 analogue channels,
8   033a                ;** checks for overscale on 1-4,
10  033a                ;** and stores end of scan marker.
12  033a                ;**...........01/04/82
14  033a                ;**********************************
16  033a                ;
18  003b                scount=$3b
20  f38e                convrt=$f38e
22  0021                ach=$21
24  0020                acl=$20
26  0038                ovrscl=$38
28  f23e                parity=$f23e
30  0200                storel=$200
32  003e                ovrl=$3e
34  0300                storeh=$300
36  003d                ovrh=$3d
38  003f                flsind=$3f
40  033a                ;
42  033a                *=$f700
44  f700  a903    scan        lda  £03
46  f702  853b              sta  scount       count=3 for three counts
48  f704  a006    p3        ldy  £6           set 6 channels
50  f706  208ef3  p2        jsr  convrt
52  f709  c005              cpy  £5
54  f70b  f02e              beq  p1           if>4
56  f70d  b02c              bcs  p1
58  f70f  a521              lda  ach
60  f711  c903              cmp  £3
62  f713  9006              bcc  p4
64  f715  a520              lda  acl
66  f717  c9fd              cmp  £8fd  branch if>1020
```

```
68  f719  b020                bcs  p1
70  f71b  a521      p4         lda  ach
72  f71d  c53d                 cmp  ovrh          load high comp to overs.
74  f71f  901a                 bcc  p1            branch if <high byte
76  f721  a520                 lda  acl
78  f723  c53e                 cmp  ovrl
80  f725  9014                 bcc  p1            branch if < low byte
82  f727  a9ff                 lda  £$ff
84  f729  8538                 sta  ovrscl        set overscl flag
86  f72b  a53f                 lda  flsind
88  f72d            ;this section only during flush scan
90  f72d  f00c                 beq  p1            not a flush scan
92  f72f  a900                 lda  £0
 94  f731  8538                sta  ovrscl
 96  f733  a521                lda  ach
 98  f735  853d                sta  ovrh          load h-byte store in ove
le location
100  f737  a520                lda  acl
102  f739  853e                sta  ovrl
104  f73b            ;from here on for all scans
106  f73b  203ef2    p1         jsr  parity
108  f73e  ca                   dex
110  f73f  a520                 lda  acl
112  f741  9d0002               sta  storel,x
114  f744  a521                 lda  ach
116  f746  9d0003               sta  storeh,x      store value in memory
118  f749  88                   dey
120  f74a  d0ba                 bne  p2
122  f74c  c63b                 dec  scount
124  f74e  d0b4                 bne  p3
126  f750  ca                   dex
128  f751  a900                 lda  £0
130  f753  9d0002               sta  storel,x
132  f756  a90c                 lda  £$c
134  f758  9d0003               sta  storeh,x      end of scan marker
```

```
  0  033a                ;**********************************
 20  033a                ;** flushing subroutine of the
 40  033a                ;** running program
 60  033a                ;** ------------------
 80  033a                ;** ..............02/4/82
100  033a                ;**********************************
120  033a                ;
140  f700                scan=$f700
160  0200                storel=$200
180  0300                storeh=$300
200  0044                flstim=$44
220  0050                waitm=$50
240  003f                flsind=$3f
260  fe0c                disp=$fe0c
280  003d                ovrh=$3d
300  003e                ovrl=$3e
320  0038                ovrscl=$38
340  f3e0                clock=$f3e0
360  033a                ;
380  033a                *=$f400
400  f400  a949    flush       lda  £73        pump display character
420  f402  8510                sta  $10
440  f404  a900                lda  £0
460  f406  853d                sta  ovrh
480  f408  853e                sta  ovrl       reset ovrscl levels
500  f40a  8d1909              sta  $919       pump on
520  f40d  20e0f3  p9          jsr  clock
540  f410  c544                cmp  flstim     comp. t2 to flush time
560  f412  f008                beq  p8
580  f414  b006                bcs  p8
600  f416  200cfe              jsr  disp
620  f419  4c0df4              jmp  p9
```

```
640  f41c  8d0909    p8      sta  $909          pump off
641  f41f  20e0f3            jsr  clock
642  f422  18                clc
643  f423  6544              adc  flstim
644  f425  8550              sta  waitm         calculate wait
645  f427  20e0f3    p1      jsr  clock
646  f42a  c550              cmp  waitm         comp. t2 to waitm
647  f42c  f008              beq  p2
648  f42e  ea ea
649  f430  200cfe            jsr  disp
650  f433  4c27f4            jmp  p1            wait for pump pots to di
660  f436  a9ff      p2      lda  #$ff
680  f438  853f              sta  flsind        set flush indicator
700  f43a  2000f7            jsr  scan          do flush scan
720  f43d  a900              lda  #0
740  f43f  853f              sta  flsind        clear flush indicator
760  f441  18                clc
780  f442  a53e              lda  ovrl
800  f444  6964              adc  #100
820  f446  853e              sta  ovrl
840  f448  a53d              lda  ovrh
860  f44a  6900              adc  #0
880  f44c  853d              sta  ovrh          add 100 to the overscale
900  f44e  a9ff              lda  #$ff
920  f450  9d0002            sta  storel,x      set flush marker
940  f453  60                rts
```

```
                ;**********************************
                ;*
                ;* DOUBLE PRECISION BINARY TO
                ;* DECIMAL CONVERTER. CALL
                ;* WITH #IN FAC (20,21),RETURN
                ;* WITH X,Y UNCHANGED:RESULT
                ;* IN (30-33)AND DISPLAY.
                ;* MAXIMUM DISP=1023
                ;*.................18/7/81
                ;**********************************
                ;
                ;
ACL=$20
ACH=$21
SAVX=$24
SAVY=$25
RESULT=$30-4
TEMP=$26
HEXTD=$FE7A
                ;
                ;
                *=$F1E0
F1E0  8624      DDEC    STX  SAVX
F1E2  8425              STY  SAVY       !SAVE X,Y
F1E4  A004              LDY  #4         !FIRST DIGIT POINTER
F1E6  A903              LDA  #3
F1E8  2521              AND  ACH
F1EA  8521              STA  ACH        !MASK TO 10 BITS
F1EC  A200      LOOP    LDX  #0
F1EE  A520      L1      LDA  ACL
F1F0  38                SEC
F1F1  F913F2            SBC  TABLE1+1,Y !SUB. POWER OF 10(LOW)
F1F4  8526              STA  TEMP
F1F6  A521              LDA  ACH
F1F8  F917F2            SBC  TABLE3,Y   !SUB. POWER OF 10(HIGH
F1FB  900A              BCC  DONE       !JUMP OUT IF CARRY CLEF
F1FD  8521              STA  ACH                        CLEAR
F1FF  A526              LDA  TEMP
F201  8520              STA  ACL        !SAVE VALUE
F203  E8                INX
F204  4CEEF1            JMP  L1
F207  962C      DONE    STX  RESULT,Y   !STORE DEC.DIGIT
F209  8A                TXA
F20A  207AFE            JSR  HEXTD      !SET UP DISPLAY DIGIT
F20D  C3                INY
F20E  C008              CPY  #8         !DONE 4PRINT
F210  D0DA              BNE  LOOP       !NO,DO NEXT
F212  A624      TABLE1  LDX  SAVX
F214  A425              LDY  SAVY
F216  60                RTS
F217                    ;
F217  E8640A    TABLE3  BYT  232,100,10
F21A  01                BYT  1
F21B  030000    TABLE4  BYT  3,0,0
F21E  00                BYT  0
```

```
  2  033A                    ;**********************************
  4  033A                    ;*
  6  033A                    ;*SUBROUTINE TO MAKE AD CONVER
  8  033A                    ;*(AVERAGE OF FOUR READINGS)
 10  033A                    ;*CALL WITH CHANNEL NO.1-16 IN
 12  033A                    ;*Y. RETURNS WITH VALUE IN
 14  033A                    ;*PSEUDO AC(20,21)(L,H)AND X
 16  033A                    ;*UNCHANGED. BDDR2 MUST BE SET
 18  033A                    ;*CONVERSION TIME 250MS FULLSC
 20  033A                    ;* .............17/07/81
 22  033A                    ;**********************************
 24  033A                    ;
 26  0020                   ACL=$20
 28  0021                   ACH=$21
 30  0022                   COUNTL=$22
 32  0023                   COUNTH=$23
 34  0024                   SAVX=$24
 36  0025                   SAVY=$25
 38  C000                   COMP=$C000
 40  091A                   DAZS=$91A
 42  090A                   DAZC=$90A
 44  FED0                   HWAIT=$FED0
 46  FED7                   WAIT2=$FED7
 48  001A                    TY=$1A
 50  033A                    ;
 52  033A                    *=$F38E
 54  F38E  8624      CONVRT      STX  SAVX          !SAVE X
 56  F390  A900                  LDA  #0
 58  F392  8520                  STA  ACL
 60  F394  8521                  STA  ACH           !ZERO VALUE
 62  F396  A204                  LDX  #4
 64  F398  80                    DEY
 66  F399  A900      START       LDA  #0
 68  F39B  8522                  STA  COUNTL
 70  F39D  A904                  LDA  #4
 72  F39F  8523                  STA  COUNTH        !SET FS LIMIT
 74  F3A1  8D1A09                STA  DAZS
 76  F3A4  8D0A09                STA  DAZC          !ZERO D/A
 78  F3A7  20D0FE                JSR  HWAIT         !1.6 MSEC DELAY
 80  F3AA  B900C0                LDA  COMP,Y        !D/A=1
 82  F3AD  B900C0    READ        LDA  COMP,Y        !INC.D-A & READ
 84  F3B0  1018                  BPL  NXTCYC        !JMP IF COMP=0(BIT 7
 86  F3B2  C622                  DEC  COUNTL
 88  F3B4  D004                  BNE  R1
 90  F3B6  C623                  DEC  COUNTH
 92  F3B8  F010                  BEQ  NXTCYC        !JMP OUT IF FSV
 94  F3BA  E620      R1          INC  ACL
 96  F3BC  D002                  BNE  R2
 98  F3BE  E621                  INC  ACH           !INCREMENT VALUE
100  F3C0  841A      R2          STY  TY
102  F3C2  A003                  LDY  #3
104  F3C4  20D7FE                JSR  WAIT2         !768 MSEC DELAY
106  F3C7  4CADF3                JMP  READ          !REPEAT ALWAYS
108  F3CA  CA        NXTCYC      DEX
110  F3CB  D0CC                  BNE  START         !NEXT CYCLE
112  F3CD  A202                  LDX  #2
114  F3CF  4621      DIVIDE      LSR  ACH           !DIVIDE BY FOUR
116  F3D1  6620                  ROR  ACL
118  F3D3  CA                    DEX
```

```
 1   033A                ;**********************************
 3   033A                ;* *
 5   033A                ;* SUROUTINE TO GENERATE PARI-*
 7   033A                ;*TY BIT IN PSEUDO AC($20,$21)*
 9   033A                ;*(L,H). X,Y UNCHANGED *
11   033A                ;* DATA FORMAT: *
13   033A                ;*P,FLAG,9,8//7,6,5,4,3,2,1,0 *
15   033A                ;* *
17   033A                ;**********************************
19   033A                ;
21   0020                ACL=$20
23   0021                ACH=$21
25   0022                DAT1=$22
27   0024                SAVX=$24
28   033A                ;
29   033A                *=$F23E
30   F23E                ;
31   F23E  8624    PARITY      STX  SAVX        !SAVE X
33   F240  A208                LDX  #8          !SET FIRST EIGHT BITS
35   F242  A520                LDA  ACL         !LOW BYTE
37   F244  8522                STA  DAT1
39   F246  A900                LDA  #0          !ZERO PARITY (AC)
41   F248  205CF2              JSR  P2          !ZERO PARITY (AC)
43   F24B  A621                LDX  ACH
45   F24D  8622                STX  DAT1        !HIGH BYTE
47   F24F  A203                LDX  #3          !SET LAST THREE BITS
49   F251  205CF2              JSR  P2          !CALCULATE PARITY
51   F254  18                  CLC
53   F255  6521                ADC  ACH
55   F257  8521                STA  ACH         !ADD PARITY TO PSEUDO (AC)

57   F259  A624                LDX  SAVX
59   F25B  60                  RTS
60   F25C                ;
61   F25C  6622    P2          ROR  DAT1        !BIT TO C
63   F25E  9002                BCC  P3          !BIT NOT= 0
65   F260  4908                EOR  #8          !N.SWITCH PARITY
67   F262  CA      P3          DEX
69   F263  D0F7                BNE  P2          !NOT LAST BIT CONTINUE
71   F265  60                  RTS
73   F266                      END
```

```
                        ;**********************************
                        ;*
                        ;*SUBROUTINE TO MAKE AD CONVER
                        ;*(AVERAGE OF FOUR READINGS)
                        ;*CALL WITH CHANNEL NO.1-16 IN
                        ;*Y. RETURNS WITH VALUE IN
                        ;*PSEUDO AC(20,21)(L,H)AND X
                        ;*UNCHANGED. BDDR2 MUST BE SET
                        ;*CONVERSION TIME 250MS FULLSC
                        ;* .............17/07/81
                        ;**********************************
                        ;
                        ACL=$20
                        ACH=$21
                        COUNTL=$22
                        COUNTH=$23
                        SAVX=$24
                        SAVY=$25
                        COMP=$C000
                        DAZS=$91A
                        DAZC=$90A
                        HWAIT=$FED0
                        WAIT2=$FED7
                        TY=$1A
                        ;
                        *=$F38E
F38E 8624     CONVRT    STX  SAVX          !SAVE X
F390 A900               LDA  #0
F392 8520               STA  ACL
F394 8521               STA  ACH           !ZERO VALUE
F396 A204               LDX  #4
F398 88                 DEY
F399 A900     START     LDA  #0
F39B 8522               STA  COUNTL
F39D A904               LDA  #4
F39F 8523               STA  COUNTH        !SET FS LIMIT
F3A1 8D1A09             STA  DAZS
F3A4 8D0A09             STA  DAZC          !ZERO D/A
F3A7 20D0FE             JSR  HWAIT         !1.6 MSEC DELAY
F3AA B900C0             LDA  COMP,Y        !D/A=1
F3AD B900C0   READ      LDA  COMP,Y        !INC.D-A & READ
F3B0 1018               BPL  NXTCYC        !JMP IF COMP=0(BIT 7
F3B2 C622               DEC  COUNTL
F3B4 D004               BNE  R1
F3B6 C623               DEC  COUNTH
F3B8 F010               BEQ  NXTCYC        !JMP OUT IF FSV
F3BA E620     R1        INC  ACL
F3BC D002               BNE  R2
F3BE E621               INC  ACH           !INCREMENT VALUE
F3C0 841A     R2        STY  TY
F3C2 A003               LDY  #3
F3C4 20D7FE             JSR  WAIT2         !768 MSEC DELAY
F3C7 4CADF3             JMP  READ          !REPEAT ALWAYS
F3CA CA       NXTCYC    DEX
F3CB D0CC               BNE  START         !NEXT CYCLE
F3CD A202               LDX  #2
F3CF 4621     DIVIDE    LSR  ACH           !DIVIDE BY FOUR
F3D1 6620               ROR  ACL
F3D3 CA                 DEX                                  RIGHT
F3D4 D0F9               BNE  DIVIDE        !SHIFT VALUE TWO ~~RIGHT~~
F3D6 A624               LDX  SAVX          !RESTORE X
F3D8 C8                 INY                RESTORE Y VALUE (CH#)
F3D9 60                 RTS
```

```
                                ;*****************************************
                                ;** SUBROUTINE TO RECORD A BLOCK
                                ;** OF DATA DURING THE RUNNING
                                ;** PROGRAM (TAKES 37 SECS)
                                ;** ............18/7/81
                                ;*****************************************
                                ;
                                ADDR1=$E22
                                STORCH=$300
                                STOREL=$200
                                DISP=$FE0C
                                PUTWRD=$F266
                                ;
                                *=$F0D0
F0D0  8D1809    RECBLK      STA  $918        TAPE ON
F0D3  A946                  LDA  #$46
F0D5  8510                  STA  $10         TO DISPLAY
F0D7  A2FF                  LDX  #$FF
F0D9  200CFE    P5          JSR  DISP        5-SECOND DELAY
F0DC  CA                    DEX
F0DD  D0FA                  BNE  P5
F0DF  A940                  LDA  #$40
F0E1  8D220E                STA  ADDR1       CONFIGURE PORT 1
F0E4  8D160E                STA  $E16        SET 1-TONE
F0E7  A2E3                  LDX  #227.       SET 227 WORDS
F0E9  2066F2    P6          BNE  P6...
F0EC  CA                    DEX
F0ED  D0FA                  JSR  PUTWORD.
F0EF  8D0809                STA  $908        TAPE OFF
F0F2  A2E4                  LDX  #228
F0F4  60                    RTS
```

```
                        ;**********************************
                        ;*
                        ;* SUBROUTINE TO TRANSMIT 12
                        ;* BIT WORDS TO TAPE//DATA
                        ;* STORED IN TWO LOCATIONS
                        ;* POINTED TO BY:
                        ;* 200+X(LS BYTE)
                        ;* 300+X(MS BYTE)
                        ;* SET X TO FF FIRST
                        ;*
                        ;* MDD 17/7/81
                        ;**********************************
                        ;
                        ;
                        PIA1=$C20
                        WAIT=$FECD
                        STOREH=$300
                        STOREL=$200
                        ;
                        ;
                        *=$F266
F266  A00C      PUTWRD  LDY  #12            !SET 12 BITS
F268  8C200E            STY  PIA1
F26B  7E0003    AGAIN   ROR  STOREH,X
F26E  7E0002            ROR  STOREL,X
F271  6A                ROR  A              !THREE BYTE ROTATE
F272  20CDFE            JSR  WAIT
F275  6A                ROR  A              !DATA BIT TO A-6
F276  8D200E            STA  PIA1           !SEND BIT
F279  88                DEY
F27A  D0EF              BNE  AGAIN          !KEEP GOING
F27C  20CDFE            JSR  WAIT           !WAIT FOR LAST BIT TO
F27F  88                DEY
F280  8C200E            STY  PIA1           !SEND STOP BIT (Y=FF)
F283  A01D              LDY  #29
F285  20CDFE    GAP     JSR  WAIT
F288  88                DEY
F289  D0FA              BNE  GAP            !TWO WORD WAIT
F28B  60                RTS
F28C                    END
                        ;**********************************************
                        ;**CLOCK SUBROUTINE
                        ;**----------------
                        ;**MUST BE CALLED EVERY 32 SECONDS
                        ;**OF POWER UP TO KEEP TTG(TIME TO GO)
                        ;**UPDATED. RETURNS WITH TIME READ
                        ;**IN THE ACCUMULATOR.
                        ;**.....................22/07/81
                        ;**********************************
                        ;
                        T2=$40
                        TTGL=$41
                        TTGH=$42
                        PIA2=$920
                        ;
                        *=$F3E0
F3E0  AD2009    CLOCK   LDA  PIA2           READ COUNTER
F3E3  18                CLC
F3E4  6980              ADC  #$80           REVERSE MSB
F3E6  48                PHA
F3E7  38                SEC
F3E8  E540              SBC  T2             SUBTRACT T2
F3EA  1002              BPL  CLK2
F3EC  C642              DEC  TTGH           SUBTRACT FF FROM T TO GO
F3EE  68        CLK2    PLA
```

```
 2  033A                ;**********************************
 4  033A                ;*
 6  033A                ;*PROGRAM TO READ TAPE INTO MEMORY
 8  033A                ;*AND THEN TRANSMIT TO PRINTER AND
10  033A                ;*DISPLAY. ENTER CONTROL DIGITS:
12  033A                ;*ABCD>0. A=DISPLAY NEXT # ONLY
14  033A                ;*
16  033A                ;*BE PRINTED TURN ON TAPE AND
18  033A                ;*PRESS CONTROL KEY TO START
20  033A                ;*..............29/07/81--4
22  033A                ;**********************************
24  033A                ;
26  033A                ;
28  0923                BDDR2=$923
30  0020                ACL=$20
32  0021                ACH=$21
34  FE38                QDATFE=$FE38
36  0E20                PIA1=$0E20
38  0921                PIB2=$921
40  FE0C                DISP=$FE0C
42  FEF3                RESET=$FEF3
44  F560                GETWRD=$F560
46  0200                STOREL=$200
48  0300                STOREH=$300
50  0920                PIA2=$920
52  0027                CONMSK=$27
54  0028                CONWD=$28
56  033A                ;
58  033A                ;
60  033A                *=$F480
62  F480  A900              LDA  #0
64  F482  8520              STA  ACL
66  F484  8521              STA  ACH            !ZERO PROMPT
68  F486  A9FD              LDA  #$FD
70  F488  8D2309            STA  BDDR2          !SET PORT-B
72  F48B  A220              LDX  #$20
74  F48D  2038FE            JSR  QDATFE         !KEY IN CONTROL DATA
76  F490  A200              LDX  #0
78  F492  D8                CLD                 SET BINARY
80  F493  A008              LDY  #8
82  F495  960F          BLANK   STX  $F,Y
84  F497  88                DEY
86  F498  D0FD              BNE  BLANK          BLANK DISPLAY
88  F49A  A521              LDA  ACH
90  F49C  29F0              AND  #$F0
92  F49E  F002              BEQ  S1
94  F4A0  A980              LDA  #$80
96  F4A2  48            S1  PHA                 !SET KEY CONTROL FLAG
98  F4A3  A521              LDA  ACH
100 F4A5  290F              AND  #$F
102 F4A7  F004              BEQ  S2
104 F4A9  68                PLA
106 F4AA  0904              ORA  #4
108 F4AC  48                PHA                 !SET FIRST # FLAG
110 F4AD  A520          S2  LDA  ACL
112 F4AF  29F0              AND  #$F0
114 F4B1  F004              BEQ  S3
116 F4D3  68                PLA
118 F4B4  0902              ORA  #2
```

```
120  F4B6  48                PHA                 !SET SECOND FLAG
122  F4B7  A520      S3 :    LDA  ACL
124  F4B9  290F              AND  #$F
126  F4BB  F004              BEQ  S4
128  F4BD  68                PLA
130  F4BE  8901              ORA  #1
132  F4C0  48                PHA                 !SET THIRD # FLAG
134  F4C1  68        S4      PLA
136  F4C2  8528              STA  CONWD          !STORE CONTROL WORD
138  F4C4  A900      START   LDA  #0
140  F4C6  850C              STA  $0E            NON REPETITIVE SCAN TO DISPLAY

142  F4C8  8D2103            STA  PIB2
144  F4CB  A981              LDA  #$81
146  F4CD  8D2103            STA  PIB2           !RESET TIMER AND START TAPE

148  F4D0  AD2003    WAIT1   LDA  PIA2           !READ TIMER
150  F4D3  C98C              CMP  #$8C
152  F4D5  D0F9              BNE  WAIT1          !WAIT 3 SEC
154  F4D7  2C200E    WAIT2   BIT  PIA1
156  F4DA  10FB              BPL  WAIT2          !WAIT FOR 1 TONE
158  F4DC                    ,
160  F4DC                    ;*** READ IN ONE BLOCK ***
162  F4DC  A2E4                      LDX  #228
164  F4DE  20 00 F6  READ            JSR  GETWRD         !GET WORD
166  F4E1  CA                        DEX
168  F4E2  D0FA                      BNE  READ           !GET NEXT WORD
170  F4E4  4CE7F4                    JMP  SCAN           JUMP
172  F4E7  A900      SCAN            LDA  #0
174  F4E9  8D2103                    STA  PIB2           !STOP TAPE
176  F4EC                    ,******* PRINT DOWN BLOCK******
178  F4EC  A2E4                      LDX  #228           !START BLOCK
180  F4EE  A004      C3              LDY  #4
182  F4F0  8427                      STY  CONMSK         !SET CONTROL MASK
184  F4F2  A006      C0              LDY  #6             Y=5
186  F4F4  A526      C1              LDA  CONWD
188  F4F6  2527                      AND  CONMSK
190  F4F8  F018                      BEQ  C2             (0=NON PRINT)
192  F4FA  BD0002                    LDA  STOREL,X
194  F4FD  8520                      STA  ACL
196  F4FF  BD0003                    LDA  STOREH,X
198  F502  8521                      STA  ACH
200  F504  2041F5                    JSR  PRINT
202  F507  A980                      LDA  #$80
204  F509  2526                      AND  CONWD          !WAIT FOR KEY
206  F50B  F005                      BEQ  C2
208  F50D  200CFC    SHOW            JSR  DISP           !DISPLAY #
210  F510  90FB                      BCC  SHOW           !WAIT FOR KEY
212  F512  CA        C2              DEX
214  F513  F022                      BEQ  ERROR          !>255
216  F515  88                        DEY
218  F516  D0DC                      BNE  C1             !NEXT #
220  F518  18                        CLC
222  F519  6627                      ROR  CONMSK         NEXT CONTROL MASK
224  F51B  D0D5                      BNE  C0
226  F51D  A90B                      LDA  #$B
228  F51F  2074F5    JSR'ED          JSR  WAIT           !END OF SCAN PRINT PAPE
ED
230  F522  EA                        NOP
232  F523  EA                        NOP
234  F524  BD0002                    LDA  STOREL,X
236  F527  8520                      STA  ACL
238  F529  BD0003                    LDA  STOREH,X
```

```
240  F32C  8521                    STA  ACH
242  F32E  2041F5                  JSR  PRINT
244  F331  CA                      DEX                    SKIP END OF SCAN MARKER
246  F332  D0DA                    BNE  C3
248  F334  4CC4F4                  JMP  START             END OF BLOCK GET NEXT
250  F337  A979        ERROR       LDA  #$79
252  F339  8510                    STA  $10
254  F33B  200CFE      ERR2        JSR  DISP
256  F33E  4C3BF3                  JMP  ERR2
258  F341                ;
260  F341                ;
262  F341                ;*********************************
264  F341                ;*
266  F341                ;*PRINT ONE 4-DIGIT # FROM
268  F341                ;*DOUBLE PRECISION HEX. CALL
270  F341                ;*WITH # IN PAC(20,21).X,Y SAME
272  F341                ;*
274  F341                ;*********************************
276  F341                ;
278  0024                SAVX=$24
280  0025                SAVY=$25
282  0026                NZF=$26
284  F1E0                DDEC=$F1E0
286  F341  8624          PRINT       STX  SAVX
288  F343  8425                      STY  SAVY
290  F345  20E0F1                    JSR  DDEC              !CONVERT TO DECIMAL IN 30
;
292  F348  A000                      LDY  #0
294  F34A  8426                      STY  NZF               !CLEAR LEADING ZERO FLAG
296  F34C  A204                      LDX  #4
298  F34E  B9 00 00      P1          LDA  $F30,Y            !GET DIG.(CORR.ADD.=$30)
300  F351  D003                      BNE  P2
302  F353  C526                      CMP  NZF
304  F355  D004                      BNE  P2
306  F357  A9FF                      LDA  #$FF              !NON PRINTING CHARACTER I
LEADING ZERO
308  F359  D002                      BNE  P3
310  F35B  C626          P2          INC  NZF               ;SET NON ZERO FLAG
312  F35D  2074F3        P3          JSR  XMIT
314  F360  C8                        INY
316  F361  C003                      CPY  #3
318  F363  D002                      BNE  P4
320  F365  C626                      INC  NZF               LEAVE LAST 0
322  F367  CA            P4          DEX
324  F368  D0E4                      BNE  P1                !NEXT DIGIT
326  F36A  A90A                      LDA  #1A
328  F36C  2074F3                    JSR  XMIT  ←JSR PTD    !PRINT #
330  F36F  A624                      LDX  SAVX
332  F371  A425                      LDY  SAVY
334  F373  60                        RTS
336  F374                ;
338  F374                ;**TRANSMIT ONE CHARACTER TO PRINTER.CHARACTER IN ACCU
**
340  F374                ;
342  0029                SAVY2=$29
344  F374  8429          XMIT        STY  SAVY2
346  F376  18                        CLC
348  F377  6901                      ADC  #1
350  F379  0A                        ASL  A
352  F37A  0A                        ASL  A
354  F37B  8D2100                    STA  PIB2              !TRANSMIT SHIFTED CH TO
T
356  F37E  200CFE                    JSR  DISP
359  F581  200CFE                    JSR  DISP              !46 MS DELAY
```

```
360   F584   8D2100      STA   PIB2          !TRANSMIT 0
362   F587   200CFE      JSR   DISP
364   F58A   200CFE      JSR   DISP
366   F58D   200CFE      JSR   DISP          :
368   F590   200CFE      JSR   DISP
370   F593   200CFE      JSR   DISP          115 MS DELAY .
372   F596   A429        LDY   SAVY2
374   F598   60          RTS
376   F599               END
```

377
378 JSR XMIT
379 STY SAVY2 PFD
380 JSR DISP
381 LDY SAVY2
RTS

```
  1   033A               ;*********************************
  3   033A               ;* SUBROUTINE TO GET 12 BIT
  5   033A               ;* WORD FROM TAPE INTO 300,X
  7   033A               ;* 200,X RETURNS WITH MS
  9   033A               ;* BYTE IN AC. (300,X)=MS
 11   033A               ;* BYTE (200,X)=LS BYTE.
 13   033A               ;*.............24/07/81--1
 15   033A               ;*********************************
 17   033A               ;
 19   0E20               PIA1=$E20
 21   0E21               PIB1=$E21
 23   FEDD               ;WAIT=$FEDD
 25   FECD               WAIT=$FECD
 27   0300               STOREH=$300
 29   0200               STOREL=$200
 31   033A               ;
 33   033A               *=$F600
 35   F600   A00C        GETWRD      LDY   #12
 37   F602   2C200E      START       BIT   PIA1          !WAIT FOR START BIT
 39   F605   30FB                    BMI   START
 41   F607   20D0FE                  JSR   HWAIT         !HALF BIT WAIT
 43   F60A   20CDFE      INPUT       JSR   WAIT          !1-BIT WAIT
 45   F60D   0E200E                  ASL   PIA1          !BIT TO CARRY
 47   F610   7E0003                  ROR   STOREH,X
 49   F613   7E0002                  ROR   STOREL,X      2 BYTE ROTATE
 51   F616   80                      DEY
 53   F617   D0F1                    BNE   INPUT
 55   F619   A00F                    LDY   #15
 57   F61B   20CDFE      GAP2        JSR   WAIT          !BIT TO CARRY
 59   F61E   88                      DEY
 61   F61F   D0FA                    BNE   GAP2          !ONE WORD GAP
 63   F621   A004                    LDY   #4
 65   F623   5E0003      SHIFT       LSR   STOREH,X
 67   F626   7E0002                  ROR   STOREL,X      RIGHT JUSTIFY
 69   F629   88                      DEY
 71   F62A   D0F7                    BNE   SHIFT
 73   F62C   BD0003                  LDA   STOREH,X      MSBYTE TO AC
 75   F62F   8D210E                  STA   PIB1          AND DISPLAY
 77   F632   60                      RTS
 79   F633               END
```

```
10 rem***program to read data
20 rem***from disk and to convert
30 rem***data into real corrected
40 rem***values-program corrects
50 rem***po-2 readings for temperature
60 rem***drift,and chamber volume.
70 rem***mdb/mpp. 21/10/82
80 open 4,4
90 dim x(18),e(18),fl(200) :
100 poke59468,14
110 gosub 1650
120 input "disk drive #";dd$
130 input "filename";ff$
140 cr$=chr$(13)
150 open 15,8,15:open8,8,8,dd$+":"+ff$+",s,r
160 input#15,en$,em$:if en$="00"goto190
170 print"disk error";em$;"
180 close 8:close15:goto120
190 ev=5:rem absolute difference const  190rem*************
200 rem*****************************
210 print"enter flush  points"
220 for j=0 to 300
230 input fl(j)
240 if fl(j)=0 goto 260
250 next j
260 dc=0:kk=1
270 rem**temperature conversion***
280 print"temperature conversion"
290 print "----------------------"
300 print"enter low temperature"
310 input l
320 print "enter acorn value":input a
330 print "enter high temperature"
340 input h
350 print"enter acorn value":input b
360 ts=(h-l)/(b-a)
370 rem***po-2 conversion****
380 print"po-2 conversion"
390 print"enter start value"
400 input d
410 print "enter acorn value":input e
420 print "enter chamber volume"
430 input v
440 qt=(d/1000)*v/e
450 rem***light conversion***
460 print "light conversion"
470 print"-----------------"
480 print "enter high value"
490 input g
500 print "enter acorn value":input h
510 lq=g/h
520 print "enter channel #"
530 input cn
```

```
540 gosub 1130
550 l=xm/s
560 c=1
570 if c=fl(dc) then dc=dc+1
580 for j=1 to 18
590 input#8,x(j),e(j)
600 next j
610 if st=64 then print"end of file":end
620 print""
630 for jj=1 to18 step 6
640 print
650 for k=0 to 5
660 print  x(jj+k);"  ";
670 next k
680 print
690 next jj
700 if cn<>5 goto 770
710 cm=cn
720 gosub 960
730 li=av*lq
740 py=li
750 gosub 1440
760 gosub 1190
770 cm=6
780 gosub 960
790 te=av*ts
800 if cn<>cm goto 840
810 py=te
820 gosub 1440
830 gosub1190
840 cm=cn
850 gosub 960
860 ov=av*qt/(1+0.03*(te-26.5))
870 dr=(ov-oz)*12
880 py=dr-yn
890 print#4,dr
900 gosub 1440
910 oz=ov
920 if c<>1thenc=c+1:goto570
930 gosub 1500
940 goto 550
950 rem***subroutine to check values***
960 j=7-cm
970 et=abs(x(j)-x(j+6))+abs(x(j+12)-x(j+6))
980 av=(x(j)+x(j+6)+x(j+12))/3
990 if et>ev then print "channel #";cm:input "correct value";av
1000 return
1010 rem*****************************
1020 rem***
1030 rem*** program to plot data
1040 rem*** from disk to digiplot
1050 rem***
1060 rem*** variable to be plotted may be
1070 rem*** selected from the 7 stored
1080 rem***
1090 rem*** mdb/act 20/05/82
1100 rem***
1110 rem*****************************
1120 print"*************************"
1130 print"program to plot acorn data
1140 print"*************************"
1150 print "enter max. value of y": input ym
1160 print "enter min. value of y":input yn
1170 rem*****************************
1180 rem***  draw axes   ***
1190 b=int((ym-yn)/0.1)
1200 a=int(1500/b)
```

```
1230 print "H";:sys28160
1240 print "M200,200";:sys28160
1250 print "X0,";a;",";b;",";:sys28160
1260 print"M60,";pn;:sys28160
1270 print"P";ym;:sys28160
1280 print"M60,200";:sys28160
1290 print"P";yn;:sys28160
1300 rem***  plot x axis
1310 print "M200,200";:sys28160
1320 print "X1,100,25";:sys28160
1330 print"M2600,100";:sys28160
1340 print"P5.0";:sys28160
1350 py=int(-yn/(ym-yn)*1500+200)
1360 print"M200,";py;:sys28160
1370 print"L1";:sys28160
1380 print"B50";:sys28160
1390 print"D2600,";py;:sys28160
1400 s=5:rem scan interval
1410 xm=300:rem time scale
1420 return
1430 rem*** plot point **
1440 x=s/xm*2500
1450 py=int(py/(ym-yn)*1500+200)
1460 px=int(x*(c-dc))+200
1470 print "M";px;",";py;:sys 28160
1480 print"N4";: sys28160:print"R0,0";:sys28160
1490 return
1500 print "H";:sys28160
1510 print "plot complete"
1520 print "press c to continue"
1530 print "or press e to end"
1540 get e$: if e$="" goto 1540
1550 if e$="e" goto 1610
1560 print"insert new sheet of paper"
1570 print"press space when ready to continue"
1580 get c$: if c$="" goto 1580
1590 if c$=" " goto 1600
1600 return
1610 print"H";:sys28160
1620 print"end of plot"
1630 close 8
1640 end
1650 forn=28160to28328:reada:pokeN,a:next:poke59459,255
1660 poke1,0:poke2,110:return
1670 data 169,128,133,255,133,16,169
1680 data 0,133,254,133,15,166,216,240
1690 data 12,24,105,40,144,2,230,16,202
1700 data 240,2,208,244,24,101,198,144
1710 data 2,230,16,133,15,160,0,169,255
1720 data 205,18,232,208,108,173,77,232
1730 data 41,2,240,242,140,65,232,165
1740 data 255,197,16,208,6,165,254,197
1750 data 15,240,42,177,254,41,127,201
1760 data 96,208,5,169,32,76,96,110,201
1770 data 27,16,5,9,96,76,96,110,201
1780 data 32,16,2,9,64,9,128,141,65,232
1790 data 230,254,208,190,230,255,76
1800 data 39,110,169,138,141,65,232,169
1810 data 128,133,255,132,254,232,169
1820 data 32,145,254,200,192,40,208,249
1830 data 202,240,14,24,165,254,105,40
1840 data 133,254,144,237,230,255,76
1850 data 124,110,169,19,32,216,227,96
1860 data 32,115,110,169,31,133,18,169
1870 data 64,133,17,56,76,176,199
1880 print"error":stop
ready.
```

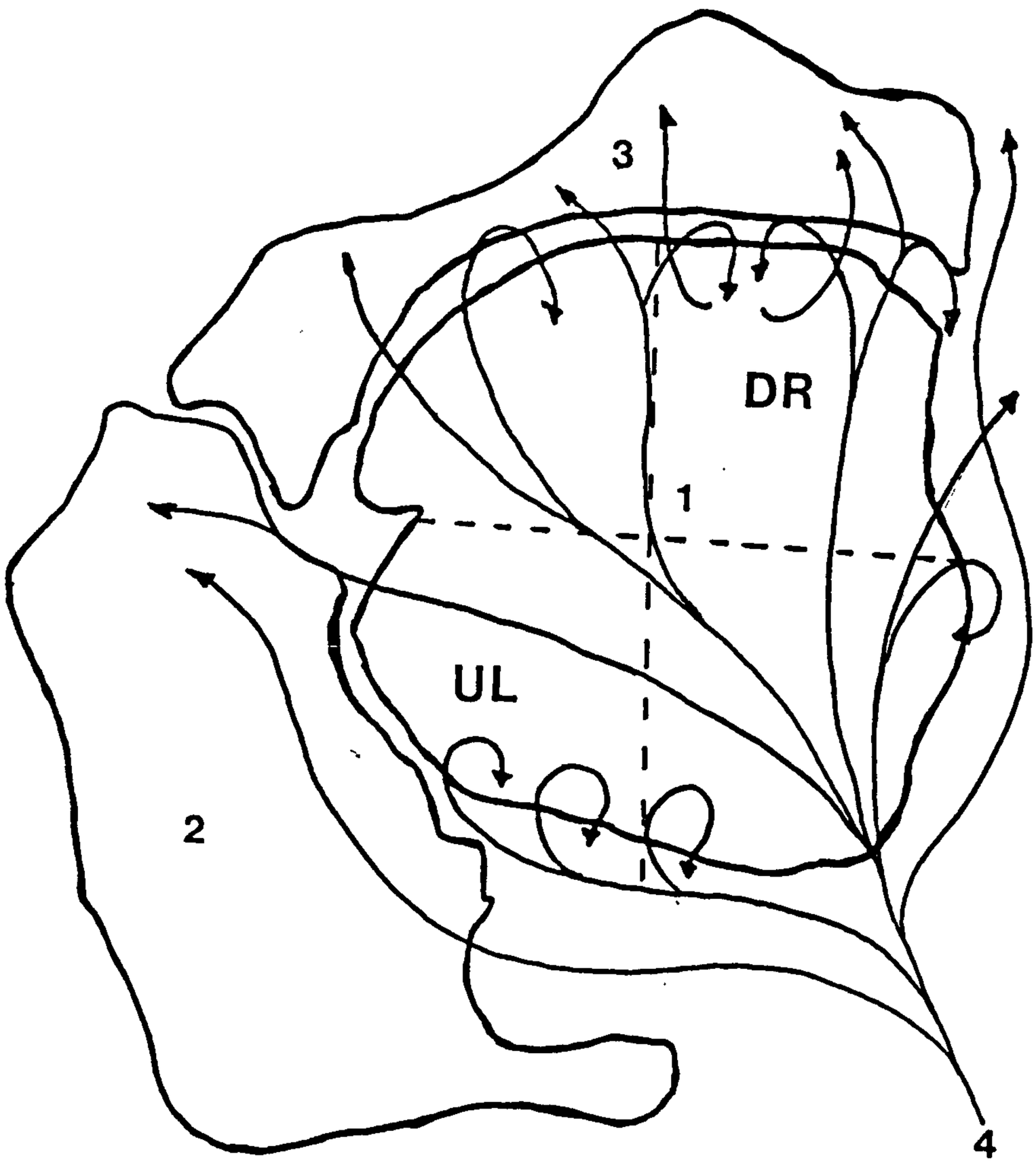
```
10 rem*** basic program to read and
20 rem*** data tapes from acorn loggers
30 rem***
40 rem*** mdb/act/mp16/09/82
50 rem*** new version with new address  56 rem*** star
60 rem*** end address $6e8f(28303)
70 rem***version modified to convert
80 rem***temperature and po2 readings
90 rem***
100 rem*** data may be stored on disk
110 rem***
120 rem**************************
130 print"     **********************"
140 print"   program to print acorn data"
150 print"     **********************"
160 poke53,110
170 gosub1260
180 poke 59459,2:g=0
190 poke 59471,0:rem tape off
200 dim x(256),m%(256),e%(256)
210 print"date?":input d$
220 print"experiment no.?": input e$
230 open 4,4
240 print#4,"Date",d$;"          Experiment No.",e$
250 print#4
260 close 4
270 goto 560
280 rem**************************
290 rem***temperature conversion
300 print""temperature conversion"
310 print"-----------------------"
320 print"enter low temperature"
330 input l
340 print"enter acorn value": input a
350 print"enter high temperature"
360 input h
370 print"enter acorn value":input b
380 t=h-l:c=b-a:s=t/c
390 rem**************************
400 rem**
410 rem***po2 conversion**********
420 print""po2 conversion"
430 print"---------------"
440 print "enter acorn value for po2=0"
450 input v
460 print"enter acorn value for po2=130"
470 input w
480 p=w-v:r=130/p
490 print""press a" for auto print"
500 print"or m" for manual print"
510 get au$: if au$="" then 510
520 print""press d" to store data on disk"
530 print" otherwise press space""
540 get dz$: ifdz$="" goto 540
550 if dz$<> "d" goto 660
560 input "disk drive No.";dd$
570 input "filename";ff$
580 cr$=chr$(13)
590 open 15,8,15:open8,8,8,dd$+":"+ff$+",s,w"
600 input#15,en$,em$: if en$="00" goto 630
610 print"disk error ";em$;"    "
620 close8: close 15: goto 560
630 if dz$="d" then print#8,ff$;cr$;
640 rem**************************
650 rem*** read a block from tape **
660 poke 59471,2: rem--turn on tape
670 print""reading data""
680 n=0: nn=0
```

```
700 if peek(59471)and128>0 then n=n+1:goto 770
710 n=0: nn=nn+1
720 if nn>500 then goto 730
730 poke 59471,0
740 print""out of data"
750 close8: close15
760 end
770 if n<50 goto 700: rem--wait for pilot
780 for j=1 to 228
790 x(j)=usr(0):m%(j)=peek(15):e%(j)=peek(16)
800 printj;
810 if j=228 goto 850
820 next j
830 **********************************f
840 rem*** print down block **
850 poke 59471,0
860 g=g+1
870 print"block";g
880 print"block";g:print
890 print
900 print"                value                marker    error"
910 for k=1 to j
920 printk,x(k),m%(k),e%(k)
930 next k
940 open4,4
950 print#4,"block";g:print#4
960 print#4,"    temp.        light   po2-4    po2-3";
970 print#4,"    po2-2    po2-1"
980 print#4,"    ------------------------------------
990 print#4,"--------"
1000 for k=1 to j step 19
1010 for l= k to k+17 step6
1020 a$="":b$="      #####"
1030 n=0
1040 for z=1 to l+5
1050 n=n+1
1060 y(n)=x(z)
1070 a$=a$+b$
1080 if e%(z)>0 then a$=a$+"*":ye(n)=-1 :go to 1100
1090 a$=a$+" ":ye(n)=0
1100 next z
1110 print using#4,a$,y(1),y(2),y(3),y(4),y(5),y(6)
1120 rem ######
1130 for jj=1 to 6
1140 print#8,y(jj);",";ye(jj);cr$;
1150 next jj
1160 next l
1170 print#4
1180 next k
1190 print#4,chr$(12)
1200 close 4
1210 if au$="a" goto 650
1220 print"    press space" for next block"
1230 print"    or press e" to end"
1240 get z$: if z$="" goto 1240
1250 if z$=" " goto 650
1260 if z$="e" goto 740
```

```
1270 forn=28160 to 28303:reada:poken,a:next
1280 poke1,0:poke2,110:return
1290 data 120,169,0,133,16,160,12,169
1300 data 255,205,18,232,208,123,44,79
1310 data 232,48,244,173,65,232,32,109
1320 data 110,44,79,232,48,233,32,115
1330 data 110,14,79,232,144,6,169,128
1340 data 69,16,133,16,110,142,110,110
1350 data 143,110,136,208,233,32,115
1360 data 110,44,79,232,48,1,200,32,115
1370 data 110,44,79,232,48,1,200,152
1380 data 5,16,133,16,24,173,142,110
1390 data 42,41,128,133,15,160,4,78,142
1400 data 110,110,143,110,136,208,247
1410 data 173,142,110,41,3,172,143,110
1420 data 32,109,210,88,96,162,8,32,121
1430 data 110,96,162,16,32,121,110,96
1440 data 173,77,232,41,2,240,249,173
1450 data 65,232,202,208,243,169,0,96
1460 data 169,255,133,16,96,234,234,170
ready.
```

APPENDIX 2-1A:

Current flow over a *Montastrea cavernosa* (coral-1) colony used for coelenteron sampling extractions at 10 metres on the fore reef at Discovery Bay (Position A, Figure 2-1). Sampling quadrats were divided as shown in the diagram, two upstream quadrats and two downstream quadrats. Coral-2 was a large colony of *Montastrea annularis*, and coral-3 a colony of *Diploria labrinthiformis*.


3
DR
1
UL
2
4