CORAL PROPAGATION FOR AQUARIUM SPECIMENS

by

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Photograph: I.E. Jordan

Frontispiece: Coral propagation tanks

PREFACE

The work described in this thesis was carried out at the Oceanographic Research Institute, Durban under the supervision of Dr M.H. Schleyer.

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

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ABSTRACT

Coral reefs are being destroyed and degraded by natural and anthropogenic processes. Live corals are becoming increasingly popular as marine aquarium specimens, in both the commercial and private sectors, leading to the degradation of coral reefs. This often has serious economic implications for the fishing, aquarium and tourist industries. It is clear that there is a need for the management and protection of these fragile ecosystems. The artificial propagation of coral is desirable as it will alleviate the demand for wild coral specimens, and will also provide a stock for the rehabilitation of damaged reefs. Although corals are being propagated worldwide by hobbyists, reports on their work are mainly anecdotal and there is little in the scientific literature on the specific requirements for optimal growth rates and survival in suitable coral species. This study thus focused on developing techniques to propagate a range of appropriate coral species and to promote their optimal growth.

The results revealed that different morphological groups of scleractinian corals require specialised techniques of fragmentation and attachment to ensure survival. The corals were broken using a hammer and chisel. Attachment techniques varied from the use of superglue (which is widely used in the United States), to thermoplastic glue. The mean mortality using superglue was 73% (n=120, ±0.167), using epoxy, 62% (n=120, ±0.127) and with thermoplastic glue it was 11% (n=120, ±0.108) Superglue was extremely difficult to work with and proved ineffective, especially when attempting to glue uneven surfaces. Certain species did not survive using this adhesive due to exposure of the coral to air. The most effective method of rapid attachment was the use of thermoplastic glue that set rapidly underwater. The use of electrolysis to promote the attachment of coral nubbins was tested as an alternative to the various adhesives. This method increased the survival of the nubbins and eliminated exposure to air. It has proven suitable for both coral propagation and *in situ* reef rehabilitation.

Growth experiments revealed that the manipulation of current flow, light and the addition of different feeds had different effects on the growth rates of selected candidate species. A suite of optima was thus developed for each species. The majority of species grew best in a bi-directional current flow, with yeast as feed, under actinic blue light. An experiment that combined the optimal current flow, feed and light conditions, revealed that the majority of species grew best under mixed light with yeast as feed.

The trade in corals is sensitive in terms of their handling, transportation and CITES (Convention on International Trade in Endangered Species) status. Having

established the viability of their propagation, consideration was given to appropriate regulatory and marketing procedures to accommodate this sensitivity of the cultivated material.

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CHAPTER 1

INTRODUCTION

1.1 General

The trade in marine organisms has increased greatly in the last eight years (Best, 1997). In 1995, the United States imported 174,140 live corals, and a 49% increase in this figure was recorded in the following year (Curry, 1998). The marine aquarium trade has increased markedly in popularity and volume during the last two decades. The annual, global c.i.f. (cost, insurance and freight) import value in marine ornamental fishes and invertebrates in the 1980s was estimated at US$24-40million (Wood, 1985). By 1990, the global wholesale value in marine ornamental fishes was estimated at US$250million (Barratt & Medley, 1990) with saltwater fishes representing approximately 10-12% of the wholesale value of the ornamental fish trade. In 1992 the global value of the marine ornamental fish retail trade reached US$3,000million, with the marine component estimated at US$360million.

The import value of corals in South Africa (the custom code combines corals and shells) for 1998 was R601598 (64290kg) and the export value was R965354 (307087kg; Stuttaford, 1999). These figures infer that there is a high demand for corals, both for import and export, even though corals are CITES-listed organisms. The propagation of corals would provide a reliable and sustainable supply for this growing trade and the negative impact of collecting them on coral reefs could be reduced. The majority of the work done to date on coral fragmentation has either been *in situ*, focusing on optimal nubbin sizes and suitable reef-building corals for rehabilitation (Franklin *et al.*, 1998; Bruno, 1998), or undertaken by hobbyists from a qualitative perspective. The focus on reef-building corals for rehabilitation has been largely due to the need for rehabilitation of reef systems that have been damaged or over-exploited (Spencer Davies, 1995; Franklin *et al.*, 1998).

Coral reef ecosystems are of commercial, recreational and aesthetic benefit to humans. They are among the most diverse and complex ecosystems (Jameson *et al.* 1995). These ecosystems are under increasing pressure, primarily from anthropogenic disturbance (e.g. dynamite fishing, sample collecting, SCUBA diving, runoff from rivers and pollution; Jameson *et al.*, 1995) and in some cases the problem is further compounded by natural disturbances (e.g. storms; Jameson *et al.*, 1995).

The significant effects of environmental factors on the growth and survival of corals have been studied. Examples of this include light (Meesters *et al.*, 1994), temperature (Yap and Gomez, 1984), sedimentation (Yap and Gomez, 1985; Rice and Hunter, 1992) and water movement (Montebon and Yap, 1995). However, although corals are being propagated in the United States and other countries, there are no data on which species are the most suitable for marine aquaria and what conditions are most favourable for their optimal growth. Work has been done on growing corals in high-nutrient, low-pH seawater (Atkinson *et al.*, 1995), but there have been no studies on the most suitable current regimes, feeding supplements and light regimes for each of the coral species to produce the highest growth. Popular articles such as those of Sykes (1996) merely highlight the need for excellent water quality and high-intensity, full spectrum lighting for the successful propagation of corals.

Although work has been done on the propagation of corals (for a general overview, see Sykes, 1996; Pearse and Muscatine, 1971; Falkowski *et al.*, 1984; Steen, 1986), the manipulation of their physical environment to enhance growth and survival after fragmentation has not been examined. The successful propagation of corals requires the optimisation of the environmental conditions to ensure survival and maximum growth. It has been shown that there are certain factors that, if manipulated correctly, can ensure the survival of the majority of the coral nubbins and even increase their growth. Growth in the wild is influenced by the conditions that surround corals. Different areas on the reef may have different current regimes, food type and availability, and lighting conditions (influenced by turbidity, depth and position on the reef). The same species of coral will often thrive under various environmental conditions and growth may therefore vary in different areas for the same species.

Successful coral propagation for the aquarium trade will require high survival and growth rates of the coral fragments. The aim of this study was to develop techniques to propagate corals in culture systems, the objectives being to determine the:

- Most suitable corals for propagation.
- Most effective fragmentation methods.
- Most effective means of nubbin attachment.
- Environmental conditions that produce the highest growth rates in the subcultures.
- Most suitable permitting and marketing procedures.

1.2 Fragmentation

Many tropical organisms including scleractinian corals, gorgonians, hydrozoans and sponges are known to reproduce asexually through fragmentation (Jackson, 1977; Bothwell, 1981; Highsmith, 1982; Wulff, 1985; Lasker, 1990). Fragments are naturally generated during storms, when waves and objects break branches or lobes off established colonies (Edmunds and Witman, 1991). Corals are relatively slow-growing organisms, and take many years to reach the colony sizes found in the wild. Asexually produced corals have a large initial size and can be produced continuously, whereas sexual offspring are small and are not necessarily produced throughout the year as spawning tends to be seasonal (Williams, 1975). For this reason, asexual reproduction (in this case, fragmentation) offers many advantages over sexual reproduction for the propagation of corals in captivity.

Fragment size is highly variable (Highsmith, 1982; Lasker, 1990) and is mainly determined by morphological characters including branch width and shape, and skeletal density (Highsmith, 1982; Brown *et al.*, 1985). Many species of tropical reef organisms including scleractinian corals, gorgonians, hydrozoans and sponges are known to reproduce asexually through fragmentation (Jackson, 1977; Bothwell, 1981; Lasker, 1990; Lewis, 1991). This provides a primary mode of population growth for those in which sexual reproduction is rare (Tunnicliffe, 1981; Lasker 1984, 1990; Wallace, 1985).

The potential for fragmentation is greater in branching corals as branches grow rapidly, are produced in large numbers, and their breakage often occurs naturally by biological and physical disturbances (Highsmith, 1982). Riegl *et al.* (1995) identified three coral community types according to their damage susceptibility, with the most susceptible types being the branching and tabular scleractinians. The fragmentation of a colony into numerous colonies appears to be the predominant means of reproduction in some corals, and is important in others (Highsmith, 1982). It has been documented that, on some *Pocillopora damicornis* reefs in the tropical eastern Pacific, branches are commonly broken off by wave action, bio-erosion or excavation by foraging fish (Porter, 1972; Glynn, 1974, Wellington, 1981). The fragments colonize adjacent rubble or sandy reef slopes, resulting in lateral reef growth (Porter, 1972; Glynn, 1974; Wellington, 1981). In the wild, three-dimensionally shaped fragments have the highest survival (e.g. *Acropora cervicornis*, in which each branch produces two or three new branches at annual nodes). Thus, regardless of how they come to rest, some part of each fragment will be elevated above the substratum and less likely to be buried or

damaged (Shinn, 1976; Tunnicliffe, 1978, 1980). Although this principle holds true for fragment survival in the wild, it does not necessarily apply to fragments (nubbins) cultured in the laboratory. Nubbins can be removed from all coral morphologies, including encrusting and submassive colonies, that do not naturally reproduce by fragmentation. Such corals may also be propagated in this way with a high survival rate (Sykes, 1996; Highsmith 1982; Lewis, 1991).

1.3 Attachment

The asexual propagation of corals involves the subculture of fragments from the parent colony. The nubbins must be attached to a substratum to ensure their survival. The use of superglue is documented in popular articles (Miller, 1997; GARF, 1999;). While various methods have been outlined and have proven successful for the attachment of coral nubbins, it is unclear whether these methods are in fact the most suitable. Commonly used methods were thus assessed and alternatives sought.

Hilbertz (1981, 1984) adapted the process of electrolysis for the deposition of calcium carbonate to create underwater structures (Bellis, 2002). The technique employs the process of electrolysis based on a galvanic cell. The calcium and magnesium ions in seawater are deposited on a metal template by electrochemical deposition. When connected to a DC power supply, magnesium and calcium minerals precipitate on the cathode while chlorine and oxygen evolve around the anode. The accreted material consists mainly of $CaCO_3$ and its chemical features are similar to that of reef limestone (Meyer & Schuhmacher, 1993). Bellis (2002) notes that Hilbertz suggested that the process could be used to build islands and create underwater homes while simultaneously facilitating the growth of coral and providing a habitat for other reef species. The use of this method was patented for the accretion of large surface structures, building components and elements (Hilbertz, 1981) and for the repair of reinforced concrete structures (Hilbertz, 1984). Van Treeck and Schuhmacher (1999) successfully adapted this technique to attach coral nubbins in the creation of a reef, with no artificial materials, *in situ* near Aqaba in the Red Sea.

The technique was adapted for coral propagation as a means of coral fragment attachment to promote their survival and growth. The previous studies, undertaken by Hilbertz *et al.* (1977) and van Treeck and Schuhmacher (1999), concentrated on the reef-building corals, such as *Acropora, Pocillopora* and *Porites.*

1.4 Current flow

Scleractinian corals, like other colonial organisms, rely on currents and waves to provide some of the physiological necessities of life (Chamberlain *et al.*, 1975). Flow conditions influence the dispersal of waste and reproductive products. Water movement is thus an important factor in the growth and development of coral reefs. Damage, such as the overturning of a branch or breakage of a colony, is also caused by strong currents. This needs to be minimised to ensure survival of corals under culture.

Darwin (1842) noted that coral growth is more vigorous on exposed reefs than in protected areas. Coral morphology is also influenced by turbulence and wave exposure (Hubbard *et al.*, 1991). Current flow governs the hydrodynamics, sedimentation and nutrient distribution on a coral reef. Hydrodynamic conditions influence the shape of the coral colonies, with large encrusted coral heads being the only corals able to survive in turbulent areas (along the reef front). Corals with branched and slender shapes survive better in calmer waters. Sedimentation is also influenced by the current regimes and is important for several reasons: it inhibits the settlement of coral larvae; sediment in suspension increases turbidity and decreases light penetration; and too much sediment stifles the corals. The appropriate water motion is thus essential for the long-term maintenance of corals in aquaria and significantly affects growth, mortality, and the reproductive state of several coral species (Jokiel, 1978).

According to Dennison *et al.* (1988), water motion significantly affects photosynthesis, respiration, and calcification in laboratory cultures of *Acropora formosa*. They conclude that it increases the basic metabolic rates of corals by more than 25%. The movement of coral polyps has been shown to respond to water flow (Hubbard, 1974) and influences the length of the polyps and thickness of the living tissue layer. Photosynthesis and calcification are enhanced in corals found in turbulent habitats and this could account for the higher growth rates in high-energy environments (Jokiel, 1978).

Scleractinian corals are subjected to a wide range of current regimes that, along with colony morphology, can affect the ability of corals to capture zooplankton and other particulate materials (Sebens *et al.*, 1998). It has been shown that zooplankton are an important dietary component in corals and related anthozoans (*Monasteriata cavernosa*, Porter, 1974; various species, Sorokin 1991a; octocorals and *Alcyonium siderium*, Sebens and Koehl, 1984). It has further been established that the

replenishment of nitrogen, phosphorus, and other nutrients that cannot be supplied by symbiotic algae (zooxanthellae) must come from the capture of zooplankton, ingestion of particulate material, or uptake of dissolved compounds (reviewed by Muscatine and Porter, 1977; Sebens, 1987).

1.5 Light

The brilliance in colour of coral reefs is well documented (Takabayashi and Hoegh-Guldberg, 1995; Dove *et al.*, 1995). The intense turquoise or green fluorescence that is often seen in the tissues of hermatypic corals (stimulated by both UV and visible light) is equally spectacular (Catala, 1959; Logan *et al.*, 1990; Schlichter *et al.*, 1994; Mazel, 1995). Early speculations as to the function of fluorescence included its role in photoprotection (Kawaguti, 1944) and/or in the enhancement of the light available for photosynthesis by the zooxanthellae (Kawaguti, 1969).

A noticeable decrease in light intensity, especially with an increase in turbidity, may be harmful to corals. Hermatypic corals are dependent on their symbiotic algae, known as zooxanthellae, for the majority of their nutrition. These zooxanthellae require light for photosynthesis and, to do so effectively, the light must be at the correct intensity and wavelength. Gardiner (1930) showed that the limiting factor for coral growth at depth was illumination and Kawaguti (1969) has shown that the number of species of corals represented on a reef decreases markedly at depths where illumination falls to values of 15-20% of surface illumination. Stoddart (1969a) showed that illumination decreases rapidly below 10 m and that the degree of turbidity of the ambient seawater affects the depth to which light penetrates. Normal growth rates of corals can be reduced by half on a cloudy day (Goreau, 1961) and a substantial increase in the amount of suspended material could reduce growth rates of corals considerably, to the extent that it prevents coral survival in deeper water.

Although light is essential for corals and their symbiotic zooxanthellae, too much light can be detrimental, even on reefs subjected to high solar irradiation (Jokiel, 1980). High solar irradiance may cause photo-inhibition in the algal cells, with subsequent increased concentrations of free oxygen radicals resulting in coral bleaching (Lesser *et al.*, 1990; Shick *et al.*, 1995; Warner et al., 1996; Lesser, 1997). Short wavelength solar irradiation penetrates to considerable depths (Jerlov, 1950) and is not only detrimental to photosynthesis, but can also damage the non-somatic DNA and proteins of marine invertebrates and algae (Shick *et al.*, 1991).

Animals are affected differently by different types of light. The wavelength determines the energy in a particular light band. The shorter the wavelength, the higher the energy but the lower its penetration into water. The longer the wavelength, the lower the energy but the greater its penetration into water. Therefore, blue light yields a greater amount of energy than yellow or red light. It is possible to obtain "equivalent energy loads" by replacing a small amount of short wavelength light with a greater intensity of longer wavelength light (Shick *et al.*, 1991). It must be borne in mind that the energy from a light source is reduced by increasing its distance from the water and the introduction of material between the bulb or light source and the water (shielding, humidity, or dust; Kirk, 1994).

1.6 Feeding

Coelenterates were among the first aquatic animals in which feeding behaviour and nutritional requirements were studied experimentally (Trembley, 1744). Early work on Caribbean species (Vaughan, 1919; Boschma, 1925) and the laboratory observations of researchers during the Great Barrier Reef Expedition (Yonge, 1930a,b) on Indo-Pacific corals, established the effectiveness with which some corals can capture live zooplankton.

Corals are active suspension feeders, utilising two main capture methods: nematocyst adhesion and mucus entrapment (Sebens & Johnson, 1991). Active feeding provides certain nutritional components that the corals are unable to obtain, in the quantities needed, from their zooxanthellae.

Corals are apparently capable of capturing and ingesting *Artemia* for their daily maintenance under laboratory conditions. In a tank study, Coles (1969) showed that three species of Caribbean corals, *Porites*, *Montipora* and *Monastrea*, were able to more than meet their daily energy requirements when fed *Artemia* nauplii *ad libitum* in closed containers. If the assimilation efficiencies of corals approached the high values associated with carnivory in other aquatic animals (Winberg, 1956; Lasker, 1966), then the ingestion rates observed by Coles (1969) would be sufficient to supply their maintenance needs, with enough energy remaining for storage and growth. Most feeding occurred in the first few hours following the introduction of food, after which many *Artemia* died in mucus streams beyond the reach of tentacles and there were insufficient live prey for further feeding (Coles, 1969). Yonge and Nicholls (1931b) showed that corals that had lost their zooxanthellae could live for months if they were

fed concentrations of zooplankton. It is therefore apparent that corals are able to survive on feeds introduced into the system.

Corals are able to use a variety of food sources such as dissolved organic matter (Sorokin, 1973; Al-Moghrabi *et al.*, 1993), particulate organic matter (Lasker, 1981), bacteria (Sorokin,1973; Farrant *et al.*, 1987; Sorokin, 1991a), and zooplankton (Johannes *et al.*, 1970; Sorokin 1991a; Lewis 1992; Sebens *et al.*, 1996). These multiple modes of heterotrophy appear to have evolved for survival in oligotrophic waters (Ferrier-Pagès *et al.*, 2000). There is controversy as to the importance of autotrophic versus heterotrophic nutrition (Goreau *et al.*, 1971; Muscatine, 1973; Edmunds and Davies, 1986; Sebens and Johnson, 1991). Recent observations support the view that zooplankton and detritus may be a major nutritive source of nitrogen and phosphorous for corals (Farrant *et al.*, 1987; Sorokin, 1991a; Lewis, 1992; Ayukai, 1995; Sebens *et al.*, 1996). High concentrations of suspended particulate matter (SPM) in waters over nearshore coral reefs are considered stressful to coral assemblages, mainly by reducing light for photosynthesis and smothering coral tissues (review by Rogers, 1990). However, many reefs with high coral cover are found in relatively turbid conditions (Done, 1982; Veron, 1986). This suggests that turbid conditions are not necessarily detrimental to corals. The SPM on coral reefs constitutes a diverse food source derived from a variety of sources including detrital matter (Marshall, 1965), re-suspended sediment (Larcombe *et al.*, 1995), coral mucus (Coffroth, 1990) and excretory products of other animals (e.g. fish, Meyer and Schultz, 1985). In addition, these particles are subject to colonization by bacteria and micro-algae, which increase the organic value of the food package (Riley, 1963; Wotton, 1988).

Research has also been directed towards autotrophic nutrition (Muscatine, 1990). For many years it was thought that sunlight was the single most important factor for coral growth (Sykes, 1996). Zooxanthellae in the tissues of hermatypic corals, sea anemones and other cnidarians, are dinoflagellate algae that use solar energy in photosynthesis. Pearse and Muscatine (1971) showed a link between algal photosynthesis and coral calcification.

Although hermatypic corals gain the bulk of their carbon requirements from their symbiotic association with unicellular algae (zooxanthellae, e.g. Falkowski *et al.*, 1984; Muscatine, 1990), they are also suspension feeders capable of using a range of food sources (reviewed by Sebens, 1987).

1.7 Current flow, Feeding and Light

Many species of Scleractinia differ morphologically under different environmental conditions (Foster, 1980; Veron, 1995). These variations in morphology, which include colony convexity, shape and pigmentation (Willis, 1985; Gleason, 1992a), corallite structure (Foster, 1979; Willis, 1985; Bruno and Edmunds, 1997), and branch diameter, furcation and spacing (Oliver *et al.*, 1983; Bruno and Edmunds, 1997) have been shown to be phenotypically plastic.

Past studies have demonstrated the significant effects of some environmental factors on the growth and survival of coral transplants *in situ*. Examples of this include light (Meesters *et al.*, 1994), water movement (Montebon and Yap, 1995) and sedimentation (Yap and Gomez, 1985; Rice and Hunter, 1992). However, it is a combination of these factors that most affects the growth of corals. For the purpose of coral propagation, it is essential that the optimal conditions for growth in a tank-based environment be determined. In nature, the factors influencing coral growth (e.g. current flow, feeding and light) are not mutually exclusive. Current flow affects the availability of food, the amount of available light influences the need for active feeding. A logical progression in determining the affect of these factors on coral growth is to determine the combined effect of their optima.

CHAPTER 2

MATERIALS AND METHODS

2.1 Source of experimental colonies

Scleractinian corals were collected at Sodwana Bay, on the north coast of KwaZulu-Natal (Figure 1). The corals were collected on Two-mile Reef, Seven-mile Reef, and Nine-mile Reef, in depths ranging from 16 m to 24 m (Figure 1). Once transported to the Oceanographic Research Institute (ORI) in Durban, the corals were placed in the experimental system (see below) and allowed at least four weeks to acclimatise before experimentation began. The acclimation period was important to ensure that the corals were disease-free, the stress of translocation was reduced and that they would survive in the system.

2.2 Experimental colonies

Thirty-six species of corals were initially collected to provide a variety of morphologies for the selection of the most suitable species for propagation and survival in marine aquaria. The number was reduced after preliminary experiments on their survival (before and after fragmentation) and suitability for quantitative analysis. Twelve species were chosen as candidates for further experimentation on the basis of the following criteria:

- Aesthetic appeal.
- Resilience to change in environmental conditions.
- Survival after fragmentation and attachment.

These were all hermatypic Scleractinia and were grouped according to their morphology:

2.2.1 Branching corals

***Acropora austera* (Dana, 1846)**

The genus *Acropora* (Family Acroporidae) is the largest scleractinian genus with more than 364 extant species (Veron & Wallace, 1984). African *Acropora* are fairly diverse, and South Africa has 14 species (Riegl *et al.*, 1995). The genus is characterised by its highly organised growth form, which results from a differentiation of polyps into axial and radial corallites (Veron & Wallace, 1984). This genus is variable in growth form as the morphology is affected by environmental conditions (Riegl *et al.*, 1995).

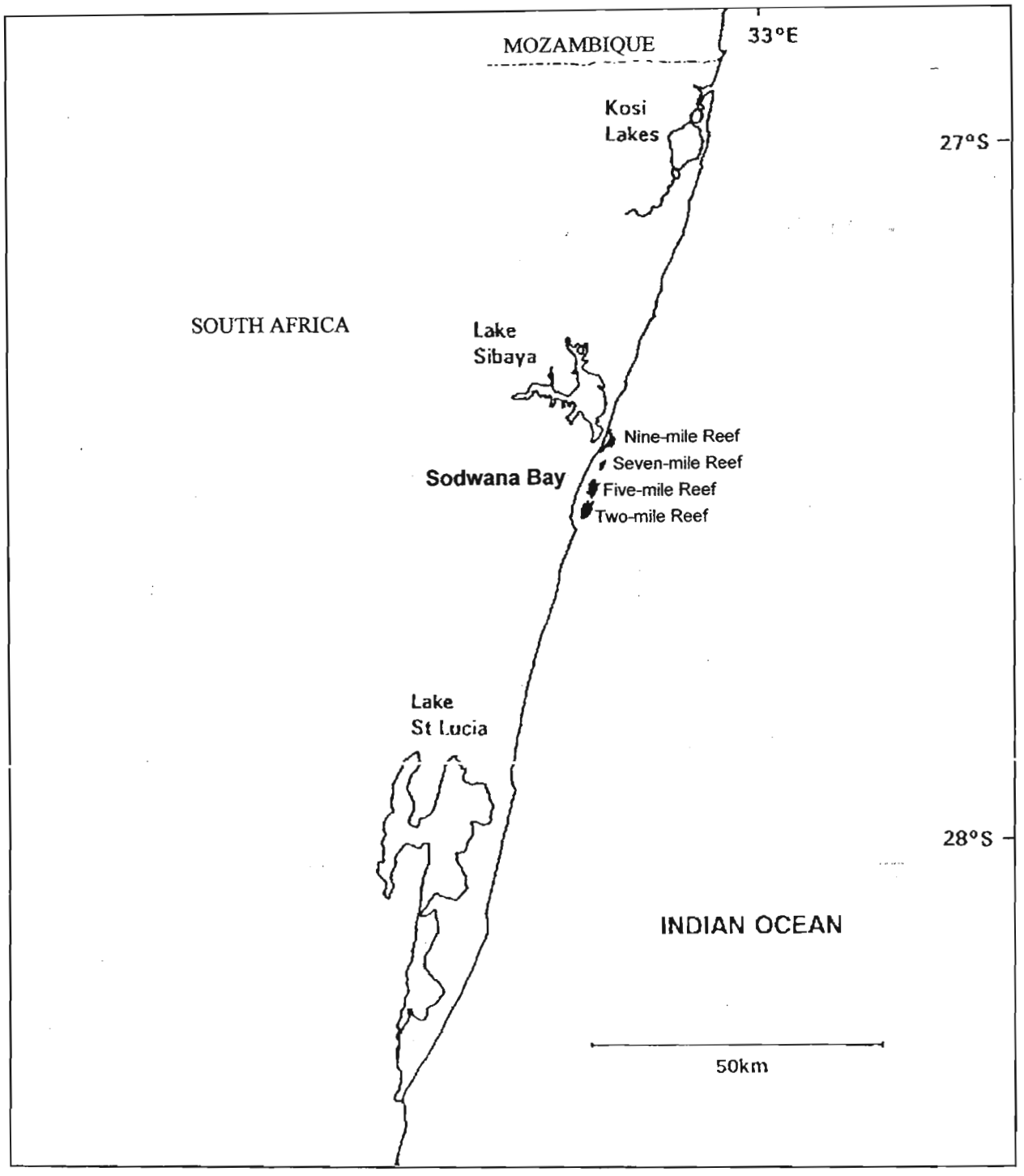

Figure 1: Location of Sodwana Bay reefs where the corals were collected. Adapted from Schleyer (1999)

Acropora austera (Figure 2a) was used in the current flow and feeding experiments as it is the most abundant *Acropora* species in South Africa. It occurs predominantly on reefs deeper than 12 m. The colonies are large and expansive in deeper regions. Living specimens vary in colour from green to a brownish-yellow. *A. austera* has a low density skeleton which allows it to grow rapidly and compete with adjacent colonies (Veron, 1986).

***Pocillopora verrucosa* (Ellis & Solander, 1786)**

The Genus *Pocillopora* is a member of the family Pocilloporidae. The most common genera (*Pocillopora* and *Stylophora*) are found on upper reef slopes exposed to strong wave action, as well as in deep water and lagoons (Veron, 1986). The genus is easily identified by wart-like growths, called *verrucae*, which cover the colonies. They are highly polymorphic with a variety of growth forms developing in response to wave action and light availability (Veron, 1986). All species of *Pocillopora* are relatively stunted when exposed to heavy wave action. In deeper, calmer water, the branches are thinner and more wide-spread. *Pocillopora* is hardy, widely distributed and common in the Indo-Pacific region. *P. verrucosa* (Figure 2b) was used in this study and is brown in colour.

***Stylophora pistillata* (Esper, 1797)**

Another member of the Pocilloporidae (Veron, 1986), *Stylophora pistillata* (Figure 2c), is an extremely hardy coral. This species has a wide distribution as colonies, attached to flotsam, may be transported hundreds of kilometres and can produce more larvae *en route*. *S. pistillata* colonies have blunt-ended branches that may be thick and sub-massive, and the polyps are extended only at night (Veron, 1986). The colour ranges from uniform green or blue to brown. This species is common in most shallow-water reef areas exposed to strong wave action and may be a dominant species on exposed reef fronts (Veron, 1986).

2.2.2 Exsert corallite corals

***Galaxea fascicularis* (Linnaeus, 1767)**

This genus is one of the two that constitute the family Oculinidae (Veron, 1986). The polyps of these corals are amongst the most beautiful in all the corals. Each corallite has a circle of delicate sabre-like septa surrounded by an outer circle of softly coloured translucent tentacles, which usually possess white tips. The colonies have groups of long, branching, tubular corallites linked by layers of tiny plates, the outermost layer of which is covered by living tissue (Veron, 1986).

Galaxea is often dominant on inshore fringing reefs, sometimes to the exclusion of all other corals. The colonies may be enormous in size and vary in shape according to local conditions. The species used in this study, *Galaxea fascicularis* (Figure 2d), has small, cushion-shaped, domed or irregular colonies. A small number of long sweeper tentacles are used to sting other colonies and may be used for capturing actively swimming zooplankton (Veron, 1986). The polyps are usually a mixture of green, red and brown. The polyps are frequently extended during the day.

***Blastomussa merleti* (Wells, 1961)**

The genus *Blastomussa* belongs to the family Mussidae (Veron, 1986). Mussids are easily recognised by their heavily constructed skeletons with large teeth on the septa. The polyps are usually thick, fleshy and colourful. Polyps of the different genera are generally similar with large numbers of sturdy tentacles that are well suited for capturing actively swimming zooplankton. *Blastomussa* is the only coral in which two distinct colour morphs are found: one red and the other brown or greenish-brown. As the colonies grow, the individual corallites often lose their organic interconnections. The colony, by definition, then ceases to be a colony and becomes a clone of solitary individuals that presumably compete with each other for food and space (Veron, 1986). The species propagated in this study, *Blastomussa merleti* (Figure 2e), has corallites less than 7 mm in diameter. The polyp colour is either dark red or greenish-brown and the oral disc may be green, blue or yellow.

2.2.3 Submassive corals

***Alveopora spongiosa* (Dana, 1846)**

The genus *Alveopora*, of the family Poritidae, was once considered to be in a family or subfamily of its own (Veron, 1986). More recently a taxonomist has claimed that *Alveopora* and *Goniopora* (of the same family) are "hardly separable" (Veron, 1986). The skeletons of the two genera are distinct, but have much in common. Likewise, the polyps can be distinguished readily by the number of tentacles present (12 in *Alveopora* and 24 in *Goniopora*), but are otherwise similar in appearance and behaviour (Veron, 1986). No species of *Alveopora* is common, and their occurrence on reefs is unpredictable with the different species tending to occupy different habitats. The colonies are massive or branching, often with irregular shapes (Veron, 1986). The skeletal structure is very light, consisting of interconnecting rods and spines. The polyps are large and fleshy and are normally extended day and night. *Alveopora spongiosa* (Figure 2f), which was used in this study, is composed of short,

irregularly dividing knob-like branches. The polyps are long when extended and are usually brown with a white centre in each oral disc.

***Goniopora djiboutiensis* (Vaughan, 1907)**

Members of the genus *Goniopora* are most commonly found in turbid water protected from strong wave action (Veron, 1986). Although they do not possess sweeper tentacles they are generally aggressive as the polyps are sometimes very extended (40cm) and attack any other coral within reach. The skeletal structures in *Goniopora* are better developed than *Alveopora*. *Goniopora djiboutiensis* (Figure 3a) was used in this study, its colour normally being a light brown or beige with a white centre in each oral disc.

2.2.4 Encrusting corals

***Montipora monasteriata* (Forskål, 1775)**

This is the second-largest coral genus in terms of species having 211 nominal species (Veron, 1986). It belongs to the family Acroporidae. Colonies may be submassive, laminar, foliaceous, encrusting or branching, with very small corallites and polyps that are usually extended only at night (Veron, 1986). *Montipora monasteriata* (Figure 3b) colonies are submassive or plate-like and their surface is uniformly covered with tuberculae. The corallites are immersed between the tuberculae. The colour may be blue or brown, uniform or mottled.

***Hydnophora exesa* (Pallas, 1766)**

This is a common genus easily distinguished by the beautifully sculpted hydnophores that cover the colony surface (Veron, 1986). The genus *Hydnophora* belongs to the family Merulinidae and consists of approximately 22 nominal species but probably only five true species (Veron, 1986). The colonies are massive, encrusting or arborescent. The hydnophores that characterise the genus are formed where the common walls between corallites intersect and develop into conical mounds (Veron, 1986). The polyps are usually extended only at night. Short tentacles surround the base of each hydnophore, with one tentacle between each pair of septa. A green colour morph of *Hydnophora exesa* (Figure 3c) was used in this study.

***Gardinoseris planulata* (Dana, 1846)**

Gardinoseris species (family Agariciidae; Veron, 1986) are found on protected reef slopes and are common in lagoons, but not on reef flats or slopes exposed to

wave action. They have very fine tentacles that are rarely extended and only during the night. *Gardinoseris* forms an encrustation with laminar margins and the corallites are immersed with poorly defined walls but are separated by acute ridges formed by thickening of the septo-costae (Veron, 1986). The septa seldom fuse and are continuous between adjacent corallite centres. The margins are smooth or finely serrated and are closely packed (Veron, 1986). The colour is brownish with green margins. The species used in this study was *Gardinoseris planulata* (Figure 3d). This species usually lives in clear water (Veron, 1986).

***Pachyseris speciosa* (Dana, 1846)**

Pachyseris is also a member of the family Agariciidae (Veron, 1986). *Pachyseris speciosa* colonies (Figure 3e), used in this study, are unifacial laminae with characteristic parallel ridges, usually forming horizontal tiers along reef edges (Veron, 1986). More than one row of corallites may occur between the ridges. Columellae are absent. The colour is pale brown. These corals are extremely fragile and are easily broken along naturally occurring "fault lines" in the skeleton, a characteristic that is advantageous in their culture.

2.2.5 Encrusting corals with exsert corallites

***Echinopora lamellosa* (Esper, 1795)**

The Faviidae constitute one of the most important coral families (Veron, 1986). It is the largest in terms of the number of genera, and ranks next to the Acroporidae in the number of species and overall abundance in most reef habitats throughout the Indo-Pacific region (Veron, 1986). *Echinopora* is of varying growth form. Some colonies form encrusting plates which may overlap, forming tiers of plates on a reef edge (Veron, 1986). Other colonies consist only of plates or branches, but it is unclear what regulates their growth form as they occur in a wide range of environments (Veron, 1986). *Echinopora lamellosa* was used in this study and is dark purple or green in colour (Figure 3f).

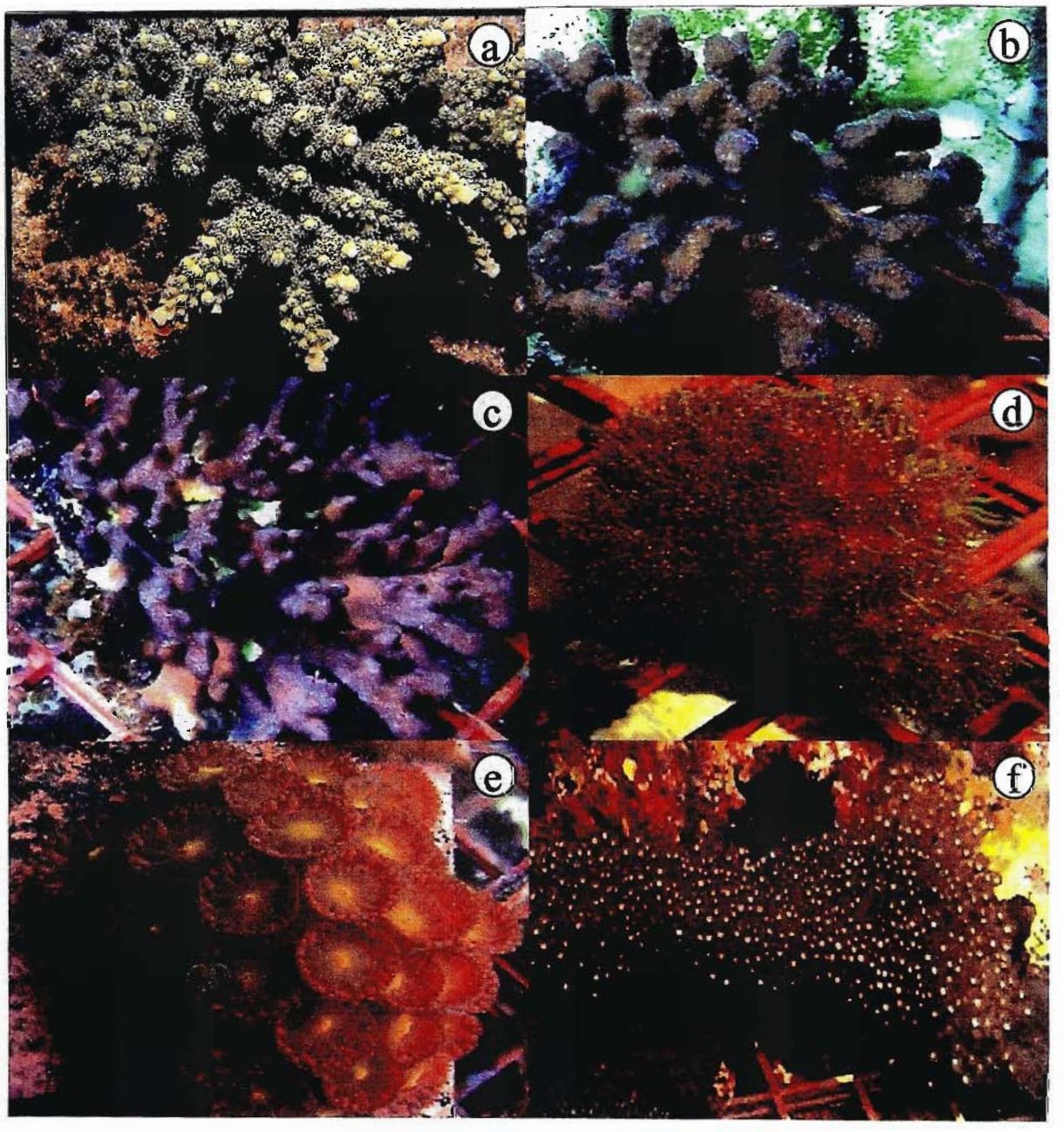

Figure 2: Candidate coral species used in the propagation study:
(a) *A. austera*, (b) *P. verrucosa*, (c) *S. pistillata*, (d) *G. fascicularis*,
(e) *B. merleti*, (f) *A. spongiosa*.

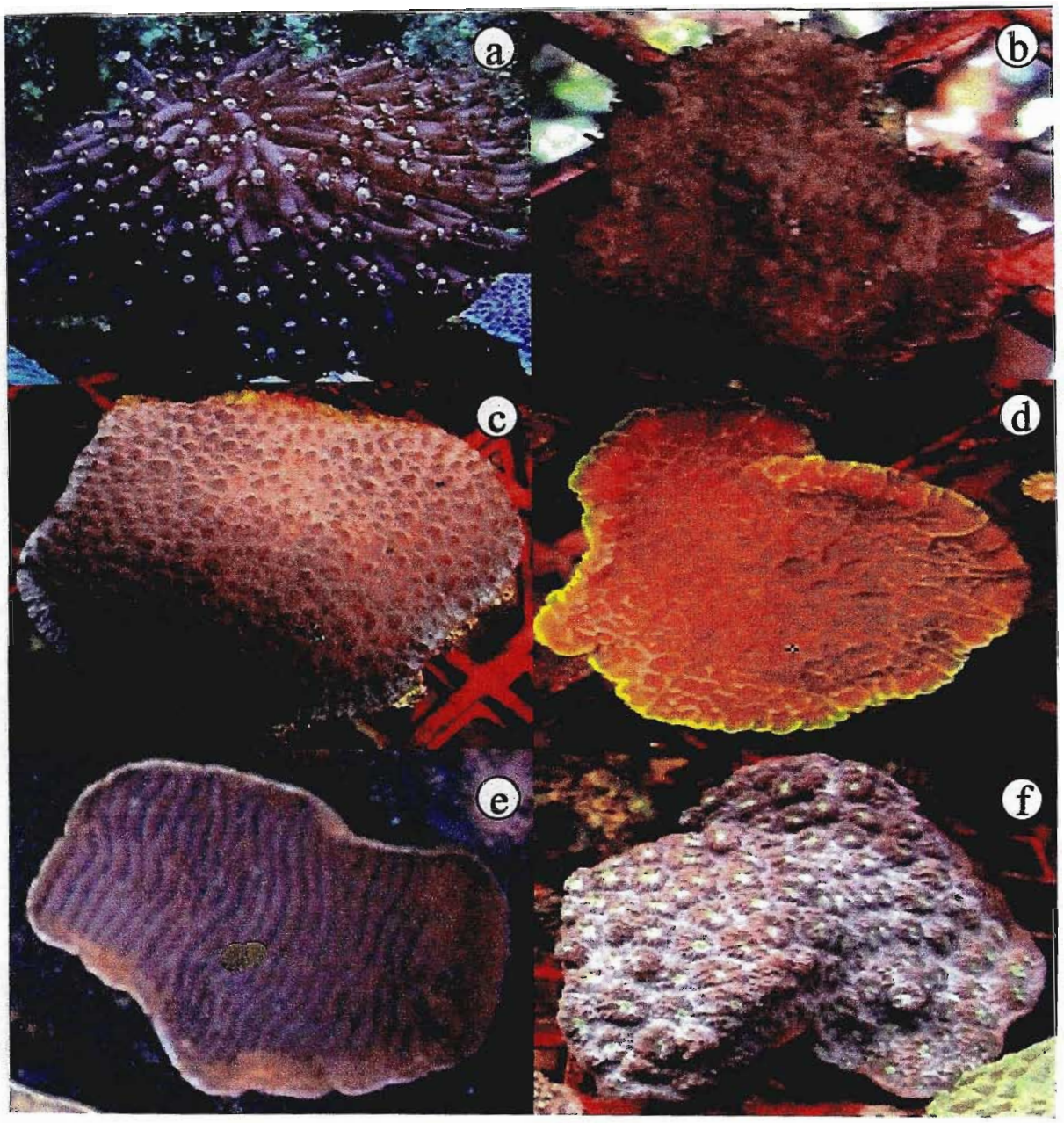

Figure 3: Candidate coral species used in the propagation study:
(a) *G. djiboutiensis*, (b) *M. monasteriata*, (c) *H. exesa*, (d) *G. planulata*,
(e) *P. speciosa*, (f) *E. lamellosa*.

2.3 Grouping

The candidate species were grouped into five morphological categories (Table 1), according to similarities in their response to the experimental propagation conditions. The species names that appear in the graphs (Chapter 3) were shortened according to the international codes (English et *al.*, 1994) in Table 1.

Table 1: Grouping of species according to similarity in morphology and response to experimental propagation conditions.

Morphological Category	Species	Code
Branching	*Acropora austera*	ACR AUST
	Pocillopora verrucosa	POC VERR
	Stylophora pistillata	STY PIST
Exsert corallite	*Blastomussa merleti*	BLA MERL
	Galaxea fascicularis	GAL FASC
Submassive	*Alveopora spongiosa*	ALV SPON
	Goniopora djiboutiensis	GON DJIB
Encrusting	*Gardinoseris planulata*	GAR PLAN
	Hydnophora exesa	HYD EXES
	Montipora monasteriata	MON MONA
	Pachyseris speciosa	PAC SPEC
Encrusting with exsert corallites	*Echinopora lamellosa*	ECH LAME

2.4 System design and management

The coral propagation system consisted of ten 800 l free-standing, white, semi-translucent, plastic tanks (Figure 4). An open-flow system was used, with each tank having a separate inflow and outflow. Each tank was isolated in terms of environmental control to prevent cross-contamination. A well-point seawater supply fed the system with a continuous inflow of sand-filtered seawater. White, translucent tanks were used as they provided incident, indirect solar radiation to all regions in the tanks. The tanks were shaded with two layers of 80% shade cloth, to reduce the intensity of direct light, as they were located out-of-doors and were exposed to natural daylight.

Trickle filters consisting of PVC pipes filled with coiled plastic shavings and limestone were attached to the inflow pipes to reduce the incidence of air-bubbles and nitrogen supersaturation in the water. These bubbles can cause mortality in corals as they collect under the surface tissues, irritate the coral polyps and promote mucus production. This causes a metabolic drain on the corals and can lead to mortality.

The tanks were heated using thermostatically controlled 100 W immersion heaters to maintain temperatures above the permissible minimum of 19°C (Veron, 1986) during the winter months. Heaters were not used during the summer months. Each tank was equipped with two powerhead pumps that provided water circulation in the tanks. The pumps were positioned at opposite corners, on either side of the inflow pipes. The powerheads were set on a 4 min switching cycle with only one under power during each cycle. The interval was chosen to allow sufficient time for alternating currents to be established in the tanks. The alternation between powerhead functioning created a bi-directional current flow in the tanks, except in the current flow experiment (see below). The corals grew under natural light and photoperiod except in the artificial lighting experiments (see below). The nubbins were placed on plastic trays (bread trays) in the tanks. These trays allowed for water movement in all directions around the nubbins. The nubbins were attached to glass slides rather than to the trays as they were weighed weekly. These, in turn, were attached to the trays to prevent them from being dislodged by water movement.

2.5 Transportation and Acclimation

The corals were carefully removed with very little handling of the living tissue. They were collected in depths between 15-25 m and placed in temporary holding pools approximately 60 cm in depth. Screening from excess light is important for the survival of shallow water hermatypic corals (Salih *et al.*, 1997). Shade cloth was thus placed over the pools to reduce the light intensity. Frequent water changes were made to ensure that the water quality was maintained. Each coral colony was placed in its own plastic bag for transportation to the experimental tanks on completion of the field collection. The corners of the bags were cut off and holes made in the bags to allow for water circulation and eliminate dead spaces in the bags during transportation. The bags were suspended from an overhead support in a transport tank filled with seawater.

After transportation it was necessary to allow the corals to acclimatise before experimental work could commence. The corals were placed in holding tanks and left for approximately four weeks. They required this period of adjustment to the new environmental conditions in the tanks, due to the stressful nature of removal, handling and transportation. During the acclimation period the corals were monitored for necrosis, damage and any tissue mortality. Necrosis was common on the peripheral edges of fractured corals, and on the associated "live rock". Sponges on the live rock

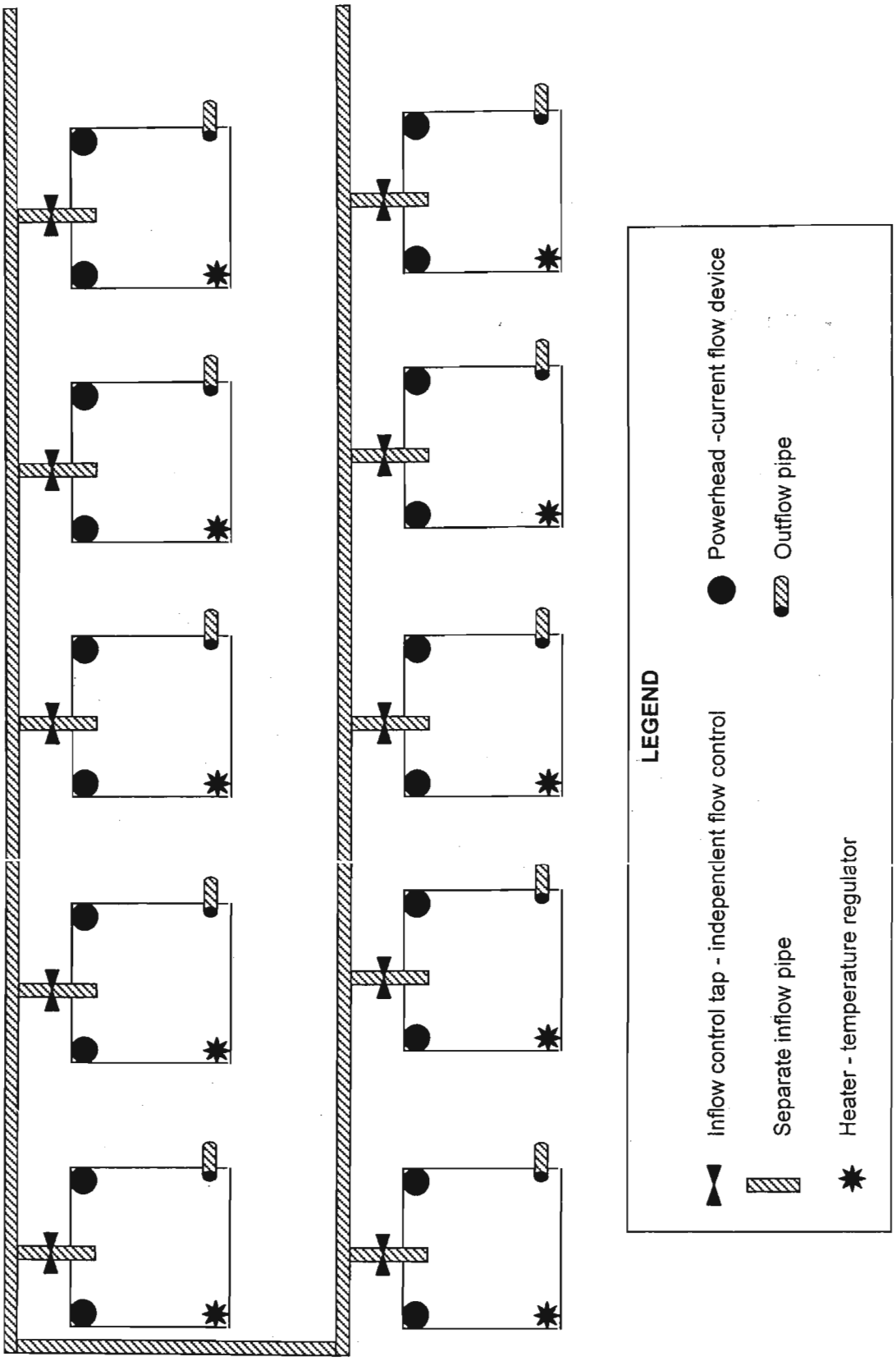

Figure 4: Outdoor coral propagation system consisting of ten 800 l tanks.

were particularly susceptible to mortality and their tissues became necrotic. This was monitored and removed as it caused disease if left unattended.

2.6 Fragmentation

The morphological variety of the experimental corals required a range of fragmentation techniques. It was important to minimise the stress involved and the exposure of the peripheral areas. The appropriate nubbin size was an important consideration. Previous studies on fragment size, for *in situ* transplantation, have indicated that it has a significant effect on coral survival (Hughes and Jackson, 1985; Harriott and Fisk, 1988; Bowden-Kerby, 1996). This relationship has, however, been shown to be significant for some species and not for others (Clark and Edwards, 1995).

The hard exoskeletons of corals are relatively easy to fragment with a hammer and chisel. The most effective means of fragmenting the encrusting, submassive and corals with exsert corallites, was along a thinning in the skeleton. Breakage along a "fault line" made fragmentation easier and less traumatic for the tissue. The branching corals were fragmented using side-cutters or a hammer and chisel. Small branches or fragments, between 2 and 4 cm, were removed from the colonies in all cases (Table 2). All the corals were fragmented whilst submerged to reduce physical contact and avoid exposure to air.

Table 2: The dimensions (cm) of the morphological categories of corals used in the propagation experiments (only the height is given for branching corals).

Morphological category	**Mean size (cm)**	**Mean mass (g)**	**Number of polyps**
Branching	2 - 4	8	> 100
Exsert corallite	2 x 4	3	3 - 5
Submassive	3 x 3	3	50 - 100
Encrusting	3 x 3	4	> 100
Encrusting with exsert corallites	3 x 4	5	> 100

The nubbin size was a matter of convenience rather than selection, but a greater number of smaller sized nubbins were fragmented from a parent colony than large nubbins. Once fragmented, the nubbins were placed in fresh seawater and rinsed well to remove excess mucus and damaged tissue. They were replaced into the experimental tanks as soon as possible.

2.7 Attachment

Popular articles outline the most effective methods for coral nubbin attachment (GARF, 1997); however, these are trial-and-error methods that may not be the most effective for the corals. Various adhesives were thus tested, including superglue, epoxy and thermoplastic glue. Glass microscope slides were used as the substratum for attachment of the nubbins. The slides were lightweight, non-toxic and transparent and were chosen for ease of attachment to the holding trays and their inconspicuous nature once placed in a tank. Algae and other epiphytes soon overgrow the slides and they can be broken to fit on any surface or in any crevice upon transplantation to their final location in the tank or on the reef. The slides were bead-blasted to provide a rough surface for the adhesives. The coral fragments were removed from the water to apply the adhesives. The surfaces to be glued were dried using soft tissue paper for this purpose.

Superglue was applied to the base of four replicate nubbins of each candidate species, and the slides to which they were attached. Pressure was applied to the nubbins for one minute after attachment. The nubbins were then placed in a bowl of fresh seawater for 10 min to remove excess mucus before returning them to the grow-out tank.

Marine epoxy was used to attach four replicates of each species to slides. An excess of epoxy was placed on the slide to provide support for the nubbin. The nubbin was pushed into the epoxy and the nubbin was immediately placed in a bowl of fresh seawater for 10 min before returning it to the grow-out tank.

Thermoplastic glue was applied to four replicates per species. A glue-gun was used to heat the glue. Glue was placed on the slide in excess and allowed to cool for 10 sec. The nubbin was then pushed into the glue and immediately placed in a bowl of fresh seawater for 10 min before returning it to the grow-out tank.

2.8 Electrolytic Attachment

Electrolytic attachment was tested as an alternative method of attachment. The principle described by Hilbertz *et al.* (1977), refined by Hilbertz (1992) and further developed by Schuhmacher and Schillak (1994) was used and is based on that of a galvanic cell. Folded sheets of non-galvanized steel (chicken-mesh) were used as the cathode and a titanium mesh as the anode. The sheets were folded to reduce predator impact. The system was energized for 18 h per day with a current of approximately 2.8

$A.m^{-2}$ (van Treeck & Schuhmacher, 1999). Van Treeck and Schuhmacher (1999) only tested six species (*Stylophora pistillata*, *Pocillopora damicornis*, *Acropora variabilis*, *Acropora squamosa*, *Montipora danae* and *Pavona varians*) that were known for their ability to form new colonies from fragments (Highsmith, 1982; Harrison & Wallace, 1990; Richmond & Hunter, 1990).

The method was adapted for the purpose of this study to determine whether this attachment technique, developed by van Treeck and Schuhmacher (1997), could be applied in a tank-based propagation system. A preliminary experiment was conducted with A 4 A battery charger at 3 $A.m^{-2}$ on a 12 h:12 h on/off cycle. The preliminary experiment was intended merely to test the success of the technique in fragment attachment and therefore only *Pocillopora verrucosa* nubbins (n=20) were used. The controls were a system with wire mesh and no charge and a system with no wire mesh or charge.

The experiment was expanded to include all of the twelve candidate species. Three scenarios were tested namely; a 12 h:12 h cycle, a 24 h continuous charge, and a 12 h/24 h system (an initial 12 h:12 h on/off cycle for the first two weeks which was then increased to a 24 h continuous charge). The mesh was not folded in these experiments. The nubbins were checked daily to determine the attachment rate ($n=4.sp.^{-1}.experiment^{-1}$) by manipulating them gently to establish whether they had cemented to the mesh.

2.9 Environmental response monitoring

Growth rate was considered the most effective means of measuring nubbin response to varying conditions. The buoyant weight technique of determining weight changes (Franzisket, 1964; Maragos, 1972) was employed in the study. This technique of measuring coral growth involves weighing live coral cuttings while suspended in a buoyant medium of seawater.

The method is the most suitable for the following reasons:

- It is a direct physical measurement of aragonite ($CaCO_3$) deposition, and is unaffected by factors such as the amount of water retained in the porous skeleton, the accumulation of tissue and mucus, and the biomass of epiphytes and non-calcified commensal organisms on and in the skeleton.
- Corals are morphologically asymmetrical, and it is therefore difficult to use dimensions as an accurate measure of growth.

- Corals are extremely sensitive to any exposure to air that could retard the growth of the corals and even cause mortality. This technique does not require removal of the corals from the water for their measurement and repeated growth determinations can be conducted on the same nubbin.

The technique, derived from Archimedes' Principle, states that the weight of an object in air is equal to the object's weight in a liquid plus the weight of the liquid displaced by the object. This means that the actual mass of an object can be determined even while submerged.

The following terms were used in the relationship between aragonite skeleton buoyant weight and dry weight (Jokiel *et al.*, 1978):

D_w = density of fluid used in weighing (seawater)

D_a = density of skeletal material (aragonite)

W_a = total dry weight of skeleton (aragonite)

W_w = measured buoyant weight of specimen

V_a = volume of skeletal material (aragonite) in specimen

= volume of liquid (seawater) displaced by aragonite

According to Archimedes' Principle:

$$W_a = W_w + (V_a.D_w) \quad \text{.....(1)}$$

where $V_a.D_w$ = weight of liquid (seawater) displaced.

Since $V_a = W_a.D_{a-1}$, it can be substituted for V_a in equation (1):

$$W_a = W_w + (W_a.D_{a-1}.D_w) \quad \text{.....(2)}$$

or

$$W_a = \frac{W_w}{1 - (D_w.D_{a-1})} \quad \text{.....(3)}$$

The density of aragonite (D_a = 2.93g/cc) and the approximate density of sea water (D_w = 1.03) can then be substituted in equation (3):

$$W_w = W_a (1 - 1.03/2.93)$$

$$= 0.649W_a$$

therefore:

$$\mathbf{W_a = 1.54W_w} \quad \text{.....(4)}$$

Coral cuttings were weighed on a weekly basis and growth increments were determined using formula (4).

2.10 Lag prior to growth

It was found that a lag period occurred in the growth experiments before the deposition of calcium carbonate began. During this lag period, the corals were monitored and several qualitative stages of development were recorded. These stages provided an indication of the progress in nubbin condition before quantitative measurements were detectable.

2.11 Current Flow

Three 800 l tanks were used for this experiment, with each tank being exposed to a different current flow regime (Table 3). Aquaclear 301 powerheads (220V, 8W, 60Hz, 0.05AMP) were used to create the currents. Four replicates of each of the twelve candidate species were placed in each tank. Buoyant weight was measured on a weekly basis to monitor growth and descriptive observations were recorded.

Table 3: Current flow regimes implemented in coral nubbin growth experiments.

Current Flow Regime	Description
Bi-directional	Two powerheads, powered alternately every 4 min, generating an oscillating current.
Intermittent unidirectional	One powerhead was used and was powered on a 4 min on/off cycle.
None (control)	No powerheads were used.

2.12 Light

A system of four 800 l tanks was constructed for the lighting experiment. With the exception of the control, each tank was isolated from exposure to light from adjacent tanks and from natural daylight, being separated by black corrugated plastic and black screening material. This prevented any light from entering the tanks except from the light sources above each tank. Five different lighting regimes were tested (Table 4). The four artificially lit tanks were set on a 12 h:12 h light:dark cycle while the tank in natural light conformed to the diurnal pattern. Four replicates of the twelve candidate species were placed on racks within each tank. Buoyant weight was measured on a weekly basis to monitor growth and descriptive observations of nubbin condition were recorded.

Table 4: Types of lighting used in the coral nubbin growth experiments, with specifications.

Light Regime	Designation	Specifications
Standard white fluorescent	White	4x 400 LUX; 40W tubes; white light.
Sunglo	Yellow	4x 300LUX; 40W tubes; predominantly yellow spectrum.
Powerglo	Blue	4x 180LUX; 40W tubes; predominantly blue with a red spectrum peak.
Marineglo	Red	4 x 105LUX; 40W tubes; predominantly red spectrum.
Natural (control)	Daylight	Varying light intensities; sunlight.

2.13 Feeding

Five 800 l tanks were used for this experiment. Each tank was subjected to a different feeding regime (Table 5). Four replicates of each of the twelve candidate species were placed in each tank. Buoyant weight was measured on a weekly basis to monitor growth and descriptive observations of nubbin condition were recorded. Daily feeds were introduced into the tanks in excess at dusk and the retention time of the food was approximately four hours, allowing sufficient time for the corals to feed.

Table 5: Feeding regimes and food preparation methods used in the coral nubbin growth experiments.

Feed Type	Preparation method
Yeast	25g Dry yeast dissolved in approximately 1 l of luke-warm fresh water.
Soya Flour	25g Dry soya flour in 1 l of fresh water strained through netting (mesh size = 1mm) to remove large particles.
Artemia	Live *Artemia* harvested and introduced in approximately 1 l of seawater.
Green Water	Approximately 1 l of a mixed culture, predominantly of a marine-adapted *Chlorella*, harvested from a Sea World culture.
None (control)	Approximately 1 l of fresh water.

2.14 Current flow, feeding and light

Six 800 l tanks were used. The current flow, feeding and lighting regimes that produced the optimal growth in the other experiments were combined in this experiment (Table 6). The bi-directional current flow was employed as it was found to be optimal for the majority of corals. The feeding and light optima were varied to determine the best combination. Four replicates of each of the twelve candidate species were placed in each experimental tank. The nubbins were weighed weekly according to the buoyant weight technique.

Table 6:Combinations of optimal current, feeding and light optima in coral nubbin growth experiments. (*Combination of all the feeds from the feeding experiment,**Combination of Powerglo and Marineglo lights).

Tank	Current Flow	Feeding	Light
1	Bi-directional	Yeast	Powerglo
2	Bi-directional	Yeast	Marineglo
3	Bi-directional	Yeast	Combination**
4	Bi-directional	Combination*	Combination**
5 (control)	Bi-directional	Yeast	Natural
6 (control)	Bi-directional	Combination*	Natural

2.15 Analyses

The mass measurements, obtained from the buoyant weight technique, were used to calculate mean percentage mass increments for each species under each treatment. This was done using cumulative percentage mass changes rather than instantaneous mass measurements to reduce the standard deviation and normalise the data.

The following equation was used to determine the percentage mass increments:

$$\frac{\text{final mass} - \text{initial mass}}{\text{initial mass}} * 100$$

Growth rates were expressed as mean weekly mass increases ($g.week^{-1}$). A normalized growth rate (Ferrier-Pagès *et al.*, 2000) was used to determine growth rate as follows:

$G\ (g.week^{-1}) = (M_{t+1} - M_t)/(M_t.(T_{t+1}-T_t))$,

where M_t and M_{t+1} are the coral mass at the start (Tt) and end (T_{t+1}) of each growth interval.

Significant differences between the treatments, for each species, were determined using ANOVA (Sokal & Rohlf, 1995).

CHAPTER 3

RESULTS

3.1 Adhesives (Figure 3.1)

The mortality of nubbins after a one week period of post-attachment varied between 40% and 90% using superglue, between 40% and 80% using epoxy and between 0% and 40% using thermoplastic glue among the twelve candidate species. The thermoplastic glue yielded the most successful results with three species showing 100% survival and the lowest survival being 60%. *B. merleti* and *G. fascicularis* yielded relatively high survival rates using the superglue and epoxy.

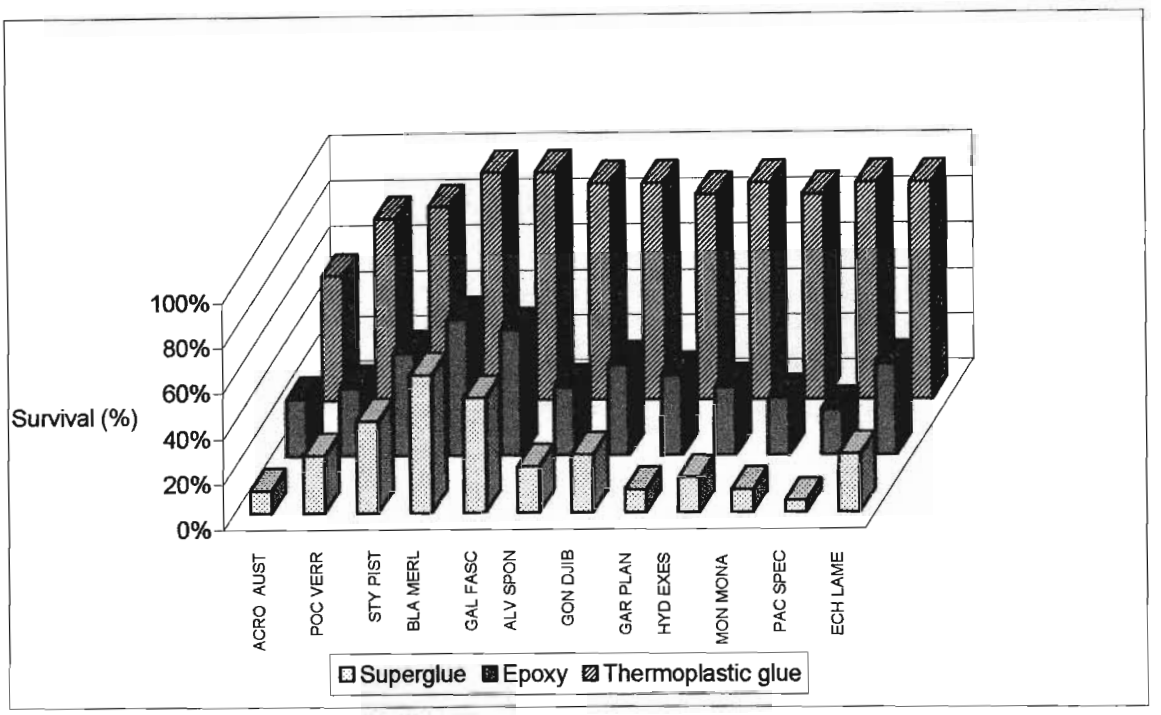

Figure 3.1: Survival (%) of coral nubbins one week after attachment.

3.2 Electrolytic Attachment (Figure 3.2; Table 7)

The preliminary 12h:12h experiment yielded a low mortality of 10% (n=20) at the end of attachment. Calcium carbonate deposition began evenly over the mesh after four days and the *P. verrucosa* were firmly attached within three weeks (n=20, sd=±0.50). The subsequent experiments were also successful, with a low mortality of 4% (n=48). *P. verrucosa, A. austera, S. pistillata, M. monasteriata*, *G. planulata* and *H. exesa* (n=4.sp.treatment^{-1}) nubbins were attached within one and a half weeks (n=72, sd=±0.75) in the 12h cycle, the 24h continuous charge and the 12h:24h regimes; *G. fascicularis, E. lamellosa* and *P. speciosa* (n=4.sp.treatment^{-1}) after 2 weeks (n=36,

sd=±0.60); and the *G. djibouteinsis* and *A. spongiosa* (n=4.sp.treatment^{-1}) within three weeks (n=24, sd=±0.45).

The greatest success of attachment was gained with *A. austera, M. monasteriata, G. planulata* and *P. speciosa*, with all fragments attaching regardless of the experimental regime (12h, 12h:24h, 24h). *B. merleti* was the only species that did not become attached in any of the treatments. The 24h and 12h:24h treatments

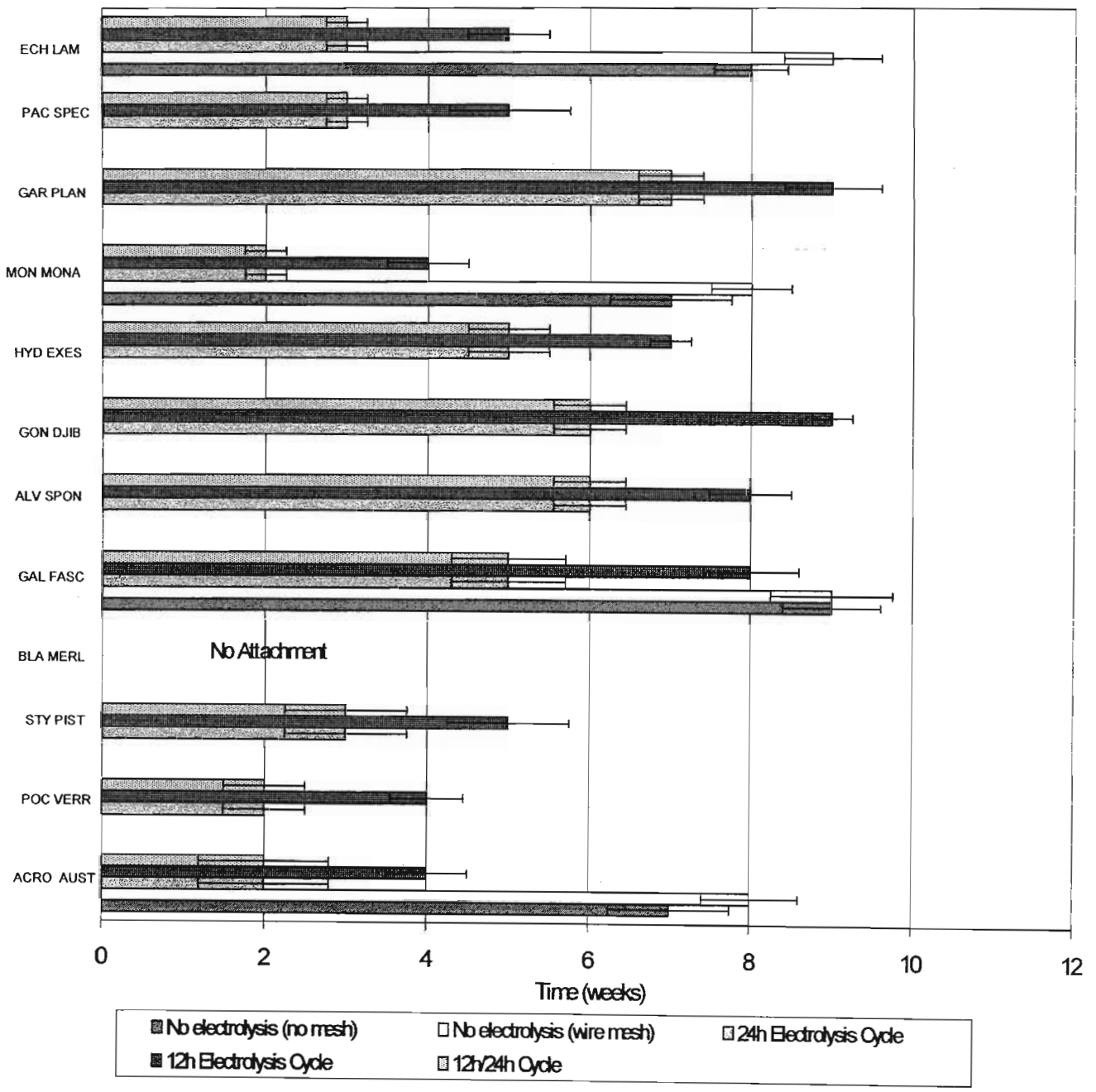

Figure 3.2: Time taken for nubbins to attach with varying combinations of electrolysis. Bars = standard deviation.

produced the fastest attachment in all of the corals with which electrolysis was successful.

Signs of tissue deterioration were observed on the fragments placed under continuous charge in the 24h cycle. The stressed fragments began to lose colour after a few days of exposure, but their colour was regained after approximately one and a half weeks. The encrusting corals bleached quicker than the branching corals.

The presence of the galvanic charge reduced algal growth on the exposed edges of the nubbins. There was a high degree of peripheral algal overgrowth in the systems with no electrolysis. No bleaching occurred in the 12h:24h regime.

There was a significant difference in attachment rates between the 24h regime and the controls ($p<0.05$), and between the 12h and 24h cycles. The 12h cycle and the controls were not significantly different.

Table 7: ANOVA comparison of coral nubbin attachment rate using different electrolytic scenarios. Bold figures denote significant difference.

Charge Type Comparison	N	d.f.	F	P
24h vs12h	60	59	3.56	**0.023**
12h vs no charge with mesh	60	59	1.23	0.380
No charge with mesh vs no charge, no mesh	60	59	0.68	0.260
24h vs no charge with mesh	60	59	4.28	**0.010**
24h vs no charge, no mesh	60	59	2.9	**0.050**
12h vs no charge, no mesh	60	59	0.81	0.400

3.3 Lag prior to growth (Table 8)

The corals that were grouped together (Table 1) underwent similar lag periods during the current flow experiment while the lag periods in the other three experiments (feeding, light and combination experiments) were variable. The lag periods for all the corals were reduced by the bi-directional flow except in the encrustations in which the lag time did not vary. The lag period for all of the corals, except *G. djibouteinsis,* was reduced in the yeast regime. The lag period of the latter was lower with the *Artemia* feed and combinations that included *Artemia*. Lag periods were reduced in general by a bi-directional current, yeast-feed, blue and red spectrum lighting and combinations of artificial lighting and supplementary feeding.

Nubbin development was observed during the lag period and varied for each species in each regime. All the nubbins underwent skeletal exposure at the start of propagation where some skeleton was exposed on fragmentation depending on the degree of colony calcification. This encouraged algal fouling on some of the coral species. Peripheral algal overgrowth did not occur on *A. austera*, *P. verrucosa*, and *G. fascicularis* and tissue regeneration occurred immediately, followed by peripheral tissue growth, polyp formation and aragonite deposition before noticeable changes in buoyant

weight were recorded. The remaining species, *S. pistillata, B. merleti, G. djiboutiensis, A. spongiosa, M. monasteriata, G. planulata, P. speciosa*, *H. exesa* and *E. lamellosa*, all encountered high degrees of algal overgrowth. In the lighting experiments, it was observed that the lights with strong blue and red spectra discouraged algal overgrowth and the lag period was reduced.

Table 8: Mean lag periods (weeks) in coral nubbins under different propagation conditions. Individual lag periods are given in parenthesis for species that did not conform to their assigned grouping.

	Branching	**Exsert Corallite**	**Submassive**	**Encrusting**
			GON DJIB/ALV SPON	
CURRENT				
Bi-directional	2	2	3/3	3
Unidirectional	3	4	5/5	3
None	3	4	5/5	3
FEEDING				
Yeast	2	1	3/2	3 (2^{Mont})
Soya Flour	3	3	4/4	4(3^{Hyd}/3^{Pachy})
Artemia	3	2	2/3	5
Chlorella	3	4	4/4	4 (3^{Gard})
None	3	4	5/5	4 (3^{Echi})
LIGHT				
Fluorescent	3	3	5/5	5
Sunglo	3	4	5/5	6
Powergio	1	1	2/2	1
Marineglo	2	1	2/2	2
Natural	2	2	3/3	3
COMBINATION				
Yeast/Powerglo	1	1	3/2	2
Yeast/Marineglo	1	1	3/2	2 (1^{Echi})
Yeast/Combination light	1	1	3/2	3
Combination feed & light	3	2	2/3	1 (3^{Echi})
Yeast/Natural daylight	2	2	3/2	4
Combimation feed/ Natural daylight	3	4	2/3	4

3.4 Current Flow (Figure 3.3; Table 9)

The majority of candidate corals grew best in the bi-directional current flow. One submassive (*G. djiboutiensis*) and one encrusting (*B. merleti*) coral grew faster in a unidirectional current. Significant differences in growth rates between the current flow regimes were found in all but one species (*H. exesa*, Table 38). There was no significant difference in the growth of *P. speciosa* in the unidirectional current and the

control (Table 44), but its growth was faster under these conditions than in the bi-directional current. Three species (*H. exesa*, *M. monasteriata* and *G. planulata*) yielded faster growth in the currentless regime than in the unidirectional current.

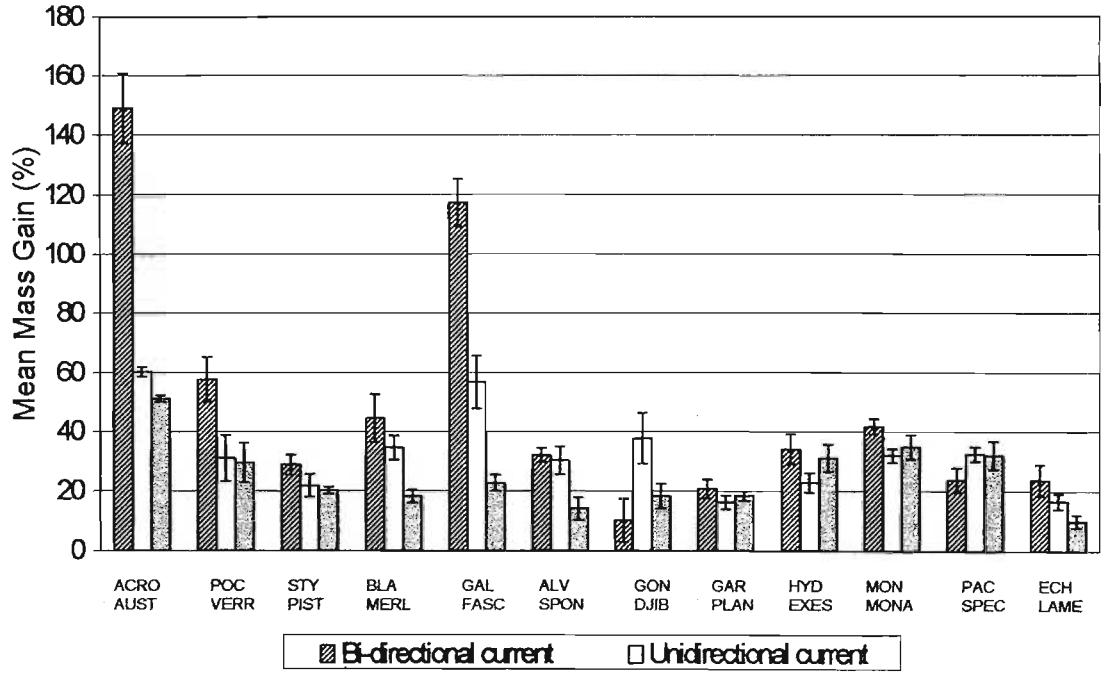

Figure 3.3: Cumulative mean mass gain (%) of the twelve candidate coral species under different current flow regimes over a three month period.

Table 9: Mean weekly growth rates of the twelve candidate coral species under different current flow regimes. Bold figures = highest growth rates.

Species	Bi-directional Current ($g.week^{-1}$)	Unidirectional Current ($g.week^{-1}$)	Control ($g.week^{-1}$)
Acropora austera	**0.038**	0.017	0.011
Pocillopora verrucosa	**0.007**	0.005	0.005
Stylophora pistillata	**0.012**	0.008	0.007
Blastomussa merleti	**0.011**	**0.011**	0.005
Galaxea fascicularis	**0.013**	0.011	0.000
Alveopora spongiosa	**0.01**	0.009	0.003
Goniopora djiboutiensis	0.009	**0.010**	0.005
Echinopora lamellosa	**0.009**	0.006	0.007
Gardinoseris planulata	0.003	**0.004**	**0.004**
Hydnophora exesa	0.006	0.004	**0.009**
Montipora monasteriata	0.005	**0.006**	**0.006**
Pachyseris speciosa	**0.004**	0.002	0.002

3.5 Light (Figure 3.4; Table 10)

The majority of corals grew the fastest in the artificial lighting rich in the blue and red spectra, with the greatest increments under the blue lighting. High nubbin mortality (50%; n=48) occurred under the standard white fluorescent and yellow-rich lights. The mortality was accounted for by six of the candidates, namely *A. spongiosa*, *E. lamellosa*, *G. planulata*, *H. exesa*, *M. monasteriata* and *P. speciosa* that underwent bleaching with 100% mortality. There was no significant difference in growth of *B. merleti* in the blue and red spectrum lighting regimes (Table 22). There was a significant difference between the blue and red spectrum lighting and the other regimes (Table 22). The polyps also appeared healthier with larger, more vivid oral discs under blue and red lighting. No mortality occurred amongst the candidates in natural light (the control) but the growth was slower. Those species that did not bleach under the fluorescent and yellow lighting attained higher growth than under natural light (Figure 3.4).

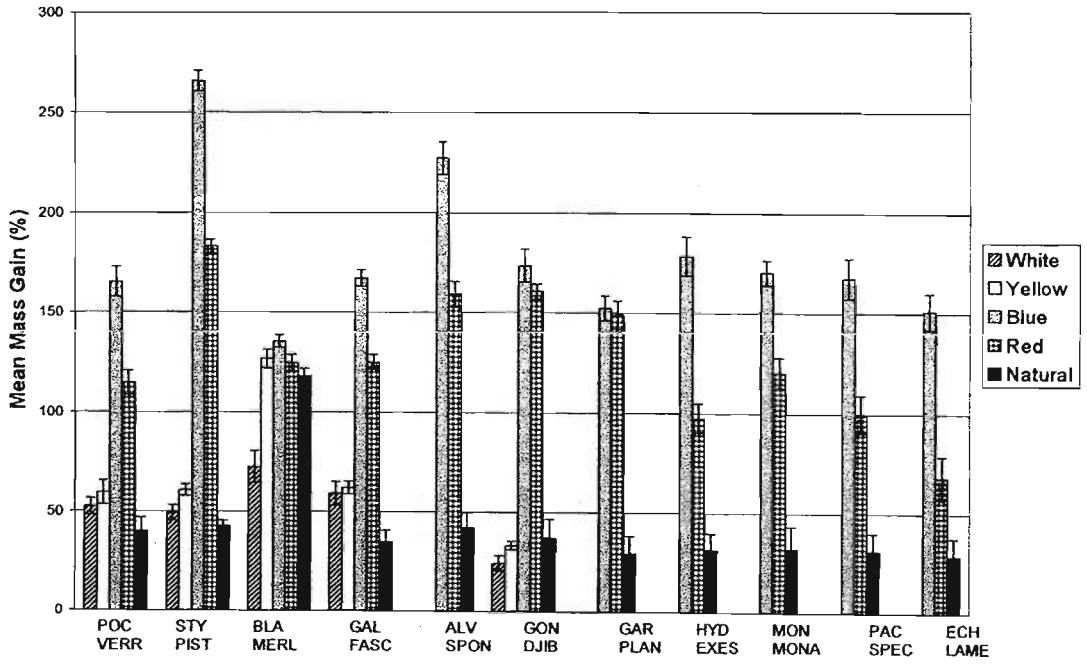

Figure 3.4: Cumulative mean mass gain (%) of the twelve candidate coral species under different lighting regimes over a three month period.

The mean growth rates of all the candidate species, except *M. monasteriata*, were greatest in the Powerglo (blue light) regime. The growth rate of *M. monasteriata* was highest under the Marineglo (red light) lights. *A. spongiosa, M. monasteriata, H. exesa* and *E. lamellosa* underwent negative growth rates in the standard white fluorescent and Sunglo (yellow light) regimes, while *Gardinoseris* underwent negative growth under the Sunglo (yellow) light (Table 10).

Table 10: Mean weekly growth rates of the twelve candidate coral species under different types of light. Bold figures = highest growth rates.

Species	Fluorescent ($g.week^{-1}$)	Sunglo ($g.week^{-1}$)	Powerglo ($g.week^{-1}$)	Marineglo ($g.week^{-1}$)	Control (daylight) ($g.week^{-1}$)
Pocillopora verrucosa	0.056	0.081	**0.104**	0.082	0.03
Stylophora pistillata	0.041	0.124	**0.201**	0.141	0.032
Blastomussa merleti	0.065	0.089	**0.104**	0.082	0.036
Galaxea fascicularis	0.042	0.047	**0.129**	0.095	0.026
Alveopora spongiosa	-0.024	-0.022	**0.083**	0.073	0.011
Goniopora djiboutiensis	0.034	0.047	**0.131**	0.122	0.028
Echinopora lamellosa	-0.009	-0.009	0.027	**0.033**	0.004
Gardinoseris planulata	-0.008	-0.011	**0.03**	0.015	0.005
Hydnophora exesa	0.01	-0.006	0.022	**0.012**	0.003
Montipora monasteriata	0.007	0.01	**0.099**	0.08	0.037
Pachyseris speciosa	-0.011	-0.014	**0.114**	0.051	0.022

3.6 Feeding (Figure 3.5; Table 11)

Nubbin growth varied in the five feeding regimes. Most of the corals grew fastest when yeast was added to the system, especially the branching corals and *M. monasteriata*. Two of the encrusting corals grew faster in the soya flour. One submassive coral grew best with *Artemia* as food, one encrusting coral with a *Chlorella* culture and the encrustation with exsert corallites grew best under control conditions (no feed). *A. spongiosa* and *G. djiboutiensis* underwent very high percentage mass increases (>400%) with *Artemia* as food, as did *B. merleti* and *G. fascicularis* (> 300%).

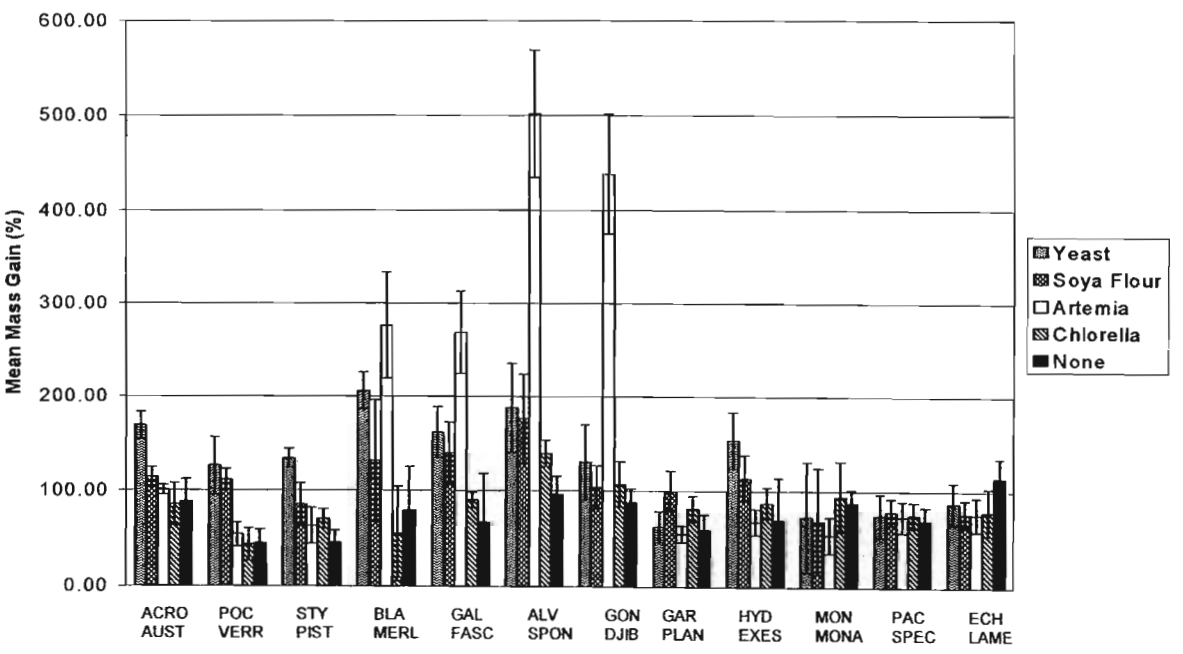

Figure 3.5: Cumulative mean mass gain (%) of the twelve candidate coral species under different feeding regimes over a three month period.

The mean growth rates of *A. austera, P. verrucosa, S. pistillata, B. merleti, M. monasteriata* and *E. lamellosa* were increased with the addition of yeast. The mean growth rate of *P. speciosa* was highest in the soya flour and *Chlorella* regimes. The *Artemia* regime elicited the highest growth rates in *A. spongiosa, G. djiboutiensis* and *G. fascicularis*, while the growth rates of *H. exesa* and *G. planulata* were greatest with the *Chlorella* feed.

Table 11: Mean weekly growth rates of the twelve candidate species using different feeds. Bold figures = fastest growth rates.

Species	Yeast ($g.week^{-1}$)	Soya Flour ($g.week^{-1}$)	*Artemia* ($g.week^{-1}$)	*Chlorella* ($g.week^{-1}$)	Control ($g.week^{-1}$)
Acropora austera	**0.032**	0.018	0.022	0.015	0.014
Pocillopora verrucosa	**0.025**	0.017	0.01	0.015	0.006
Stylophora pistillata	**0.032**	0.022	0.011	0.01	0.009
Blastomussa merleti	0.051	0.034	**0.073**	0.016	0.023
Galaxea fascicularis	0.03	0.026	**0.058**	0.016	0.014
Alveopora spongiosa	0.005	0.048	**0.118**	0.03	0.002
Goniopora djiboutiensis	0.042	0.032	**0.115**	0.032	0.018
Echinopora lamellosa	**0.027**	0.017	0.017	0.016	0.013
Gardinoseris planulata	0.013	0.02	0.012	**0.021**	0.012
Hydnophora exesa	0.018	0.016	0.014	**0.021**	0.015
Montipora monasteriata	0.016	**0.019**	0.017	**0.019**	0.016
Pachyseris speciosa	**0.019**	0.014	0.016	0.015	0.016

3.7 Current Flow, Feeding and Light (Figure 3.6)

The propagation candidates grew faster in the combined treatments than in the individual manipulations. Some of the species that did not increase greatly in mass in the individual treatments, increased significantly under the combined optima. The branching corals and *A. spongiosa* grew best in the yeast and blue light regime, *E. lamellosa* in the yeast and red light, *G. fascicularis* in the yeast and red/blue light, and *G. djiboutiensis* and the encrusting corals grew best with the combined feeds and the red/blue light.

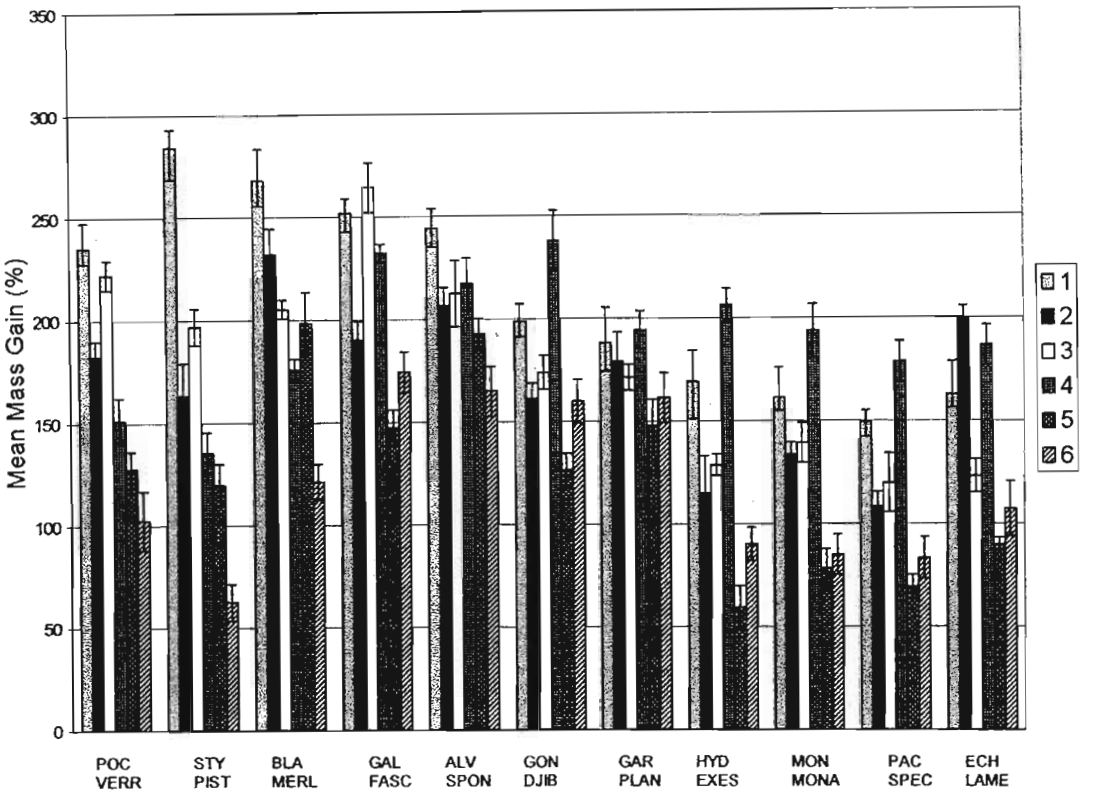

Figure 3.6: Cumulative mean mass gain (%) of candidate coralspecies in different combinations of the selected optima. 1=yeast & blue light, 2=yeast & red light, 3= yeast & blue/red light, 4= combined feed & blue/red light, 5= yeast & natural light, 6=combined feed & natural light.

3.8 Individual species results

3.8.1 Acropora austera (Figure 3.7)

This species proved extremely sensitive to environmental changes, exposure to air and handling. Care was needed when collecting and transporting. Once the parent colonies had acclimated successfully, fragmentation was simple. The skeleton was easily snapped and the branching morphology lent itself to this process.

Nubbin mortality was 66.7% (n=30) after fragmentation and attachment. The growth experiments yielded high growth with a bi-directional current and yeast feed; however, mortality was high in both the current flow (75%, n=20) and feeding (65%, n=20) experiments. The mass increase with yeast as a feed was19.8% greater than with the bi-directional current. This species was excluded from further experimentation due to its high mortality.

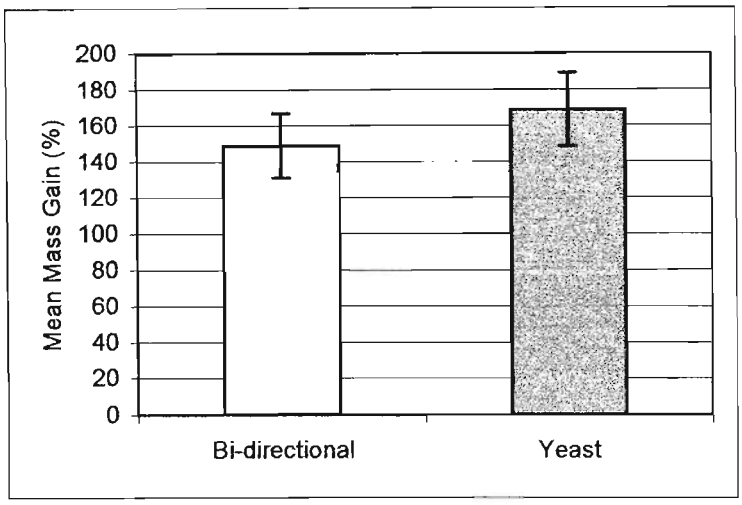

Figure 3.7: The mean mass gain of *A. austera* under the optimal current and feeding regimes.

Table 12: Results of ANOVA between growth in the bi-directional, unidirectional and the control regimes for *A. austera* nubbins. Bold figures denote significant difference.

Source of Variation	Df	SS	F	P-value
Bi-directional/Unidirectional	7	10.747	53.143	**<0.001**
Bi-directional/control	7	15.468	9.594	**0.004**
Unidirectional/control	7	3.566	1.221	0.568

Table 13: Results of ANOVA between *A. austera* nubbin growth in the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes. Bold figures denote significant difference.

Source of Variation	Df	SS	F	P-value
Yeast/Soya flour	7	10.747	53.143	**<0.001**
Yeast/*Artemia*	7	15.468	9.594	**0.004**
Yeast/*Chlorella*	7	55.894	13.645	**<0.001**
Yeast/control	7	32.154	16.554	**<0.001**
Soya flour/*Artemia*	7	2.531	1.052	0.325
Soya flour/*Chlorella*	7	5.710	2.101	0.447
Soya flour/control	7	3.219	1.027	0.362
Artemia/*Chlorella*	7	0.249	0.145	0.713
Artemia/control	7	1.189	0.366	0.568
Chlorella/control	7	1.985	0.523	0.445

3.8.2 Pocillopora verrucosa (Figure 3.8)

This species was easily broken into fragments between branches, but care was required for nubbin attachment. Although it is considered an easy coral to work with and propagate in the literature, this did not prove the case in this study. Parent colonies should be submerged during fragmentation to reduce exposure to air. The living tissue layer is extremely thin and therefore vulnerable to damage during handling and fragmentation. Nubbin mortality after fragmentation and attachment was 53.3% (n=30). The mass of the nubbins did not increase much in the current flow experiment, but the highest growth occurred in the bi-directional flow. Successively higher growths were obtained in the bi-directional current (28.8%), yeast feed (133.8%), blue spectrum light (165.4%), and the combination of bi-directional flow, yeast and blue light (235.2%; Figure 8). The combination of the three optima resulted in a significantly higher growth ($p<0.05$) of the nubbins relative to the light experiment (increase of 69.8%).

ANOVA showed that there was a significant difference between the bi-directional and unidirectional current flows ($p<0.001$, Table 14) and between the bi-directional and control current regimes ($p<0.001$, Table 14). There was no significant difference between the unidirectional and control current regimes ($p=0.786$, Table 14).

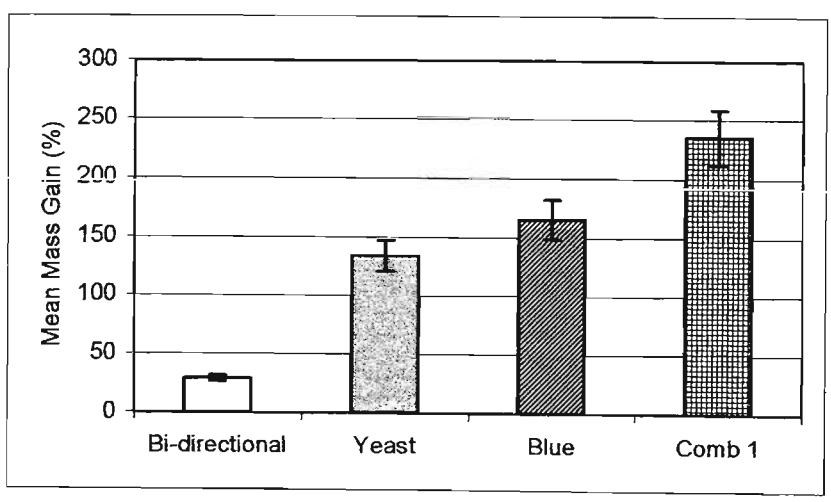

Figure 3.8: The mean mass gain of *P. verrucosa* under the optimal regimes. Comb 1=Tank 1 in Table 4.

Table 14: Results of ANOVA between growth in the bi-directional, unidirectional and control current regimes for *P. verrucosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	2.602	44.179	**<0.001**
Bi-directional/control	7	64.021	19.325	**<0.001**
Unidirectional/control	7	0.0264	0.566	0.786

Table 15: Results of ANOVA between *P. verrucosa* nubbin growth in the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	10.747	53.143	**<0.001**
Yeast/*Artemia*	7	16.532	10.125	**0.005**
Yeast/*Chlorella*	7	33.217	13.487	**<0.001**
Yeast/control	7	55.354	12.668	**<0.001**
Soya flour/*Artemia*	7	5.647	2.124	0.412
Soya flour/*Chlorella*	7	3.713	1.549	0.388
Soya flour/control	7	3.855	1.031	0.350
Artemia/*Chlorella*	7	0.463	0.218	0.625
Artemia/control	7	2.458	1.974	0.432
Chlorella/control	7	2.056	0.416	0.395

Table 16: Results of the ANOVA test between the standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *P. verrucosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent/Sunglo	7	1.350	3.488	0.074
Standard white fluorescent /Powerglo	7	39.073	18.287	**<0.001**
Standard white fluorescent /Marineglo	7	12.670	11.142	**0.003**
Standard white fluorescent /control	7	0.002	0.100	0.922
Sunglo/Powerglo	7	23.465	11.283	**0.002**
Sunglo/Marineglo	7	5.748	4.624	**0.040**
Sunglo/control	7	1.249	3.547	0.072
Powerglo/Marineglo	7	5.986	2.115	0.158
Powerglo/control	7	35.486	18.332	**<0.001**
Marineglo/control	7	12.323	11.210	**0.003**

3.8.3 Stylophora pistillata (Figure 3.9)

This is a resilient species that is easily broken into fragments. The parent colonies and nubbins retained their colour throughout experimentation. The mortality of nubbins was 40% (n=30) after fragmentation and attachment. The optima from all the regimes yielded an increase of 50% in growth. Light and the combination of light, current and feeding produced the highest growth rates (265.6% and 284.3% respectively). The growth in the light and the combination experiments was not significantly different ($p>0.05$). There was an 18.7% increase in growth in the combined feeding and light experiments relative to the light treatment alone (Figure 3.9).

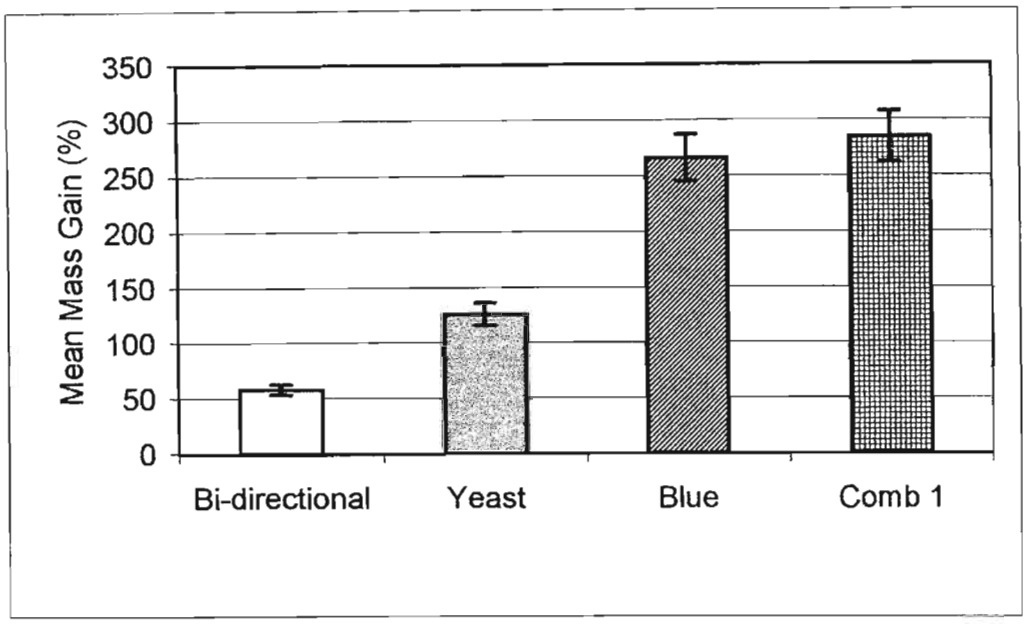

Figure 3.9: The mean mass gain of *S. pistillata* under the optimal regimes. Comb 1=Tank 1 in Table 4.

Table 17: Results of ANOVA between growth in the bi-directional, unidirectional and control current regimes for *S. pistillata* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	6.587	25.066	**<0.001**
Bi-directional/control	7	30.219	16.027	**<0.001**
Unidirectional/control	7	15.031	8.673	0.609

Table 18: Results of ANOVA between *S. pistillata* nubbin growth in the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	0.003	0.048	0.828
Yeast/*Artemia*	7	14.227	10.559	**0.002**
Yeast/*Chlorella*	7	29.769	11.331	**<0.001**
Yeast/control	7	23.247	15.815	**<0.001**
Soya flour/*Artemia*	7	4.563	3.261	0.598
Soya flour/*Chlorella*	7	2.664	1.678	0.454
Soya flour/control	7	3.061	1.992	0.381
Artemia/*Chlorella*	7	2.374	1.085	0.290
Artemia/control	7	6.329	2.379	0.486
Chlorella/control	7	0.038	0.042	0.737

Table 19: Results of ANOVA between the Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *S. pistillata* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent/Sunglo	7	1.868	5.910	**0.023**
Standard white fluorescent /Powerglo	7	62.001	18.299	**<0.001**
Standard white fluorescent /Marineglo	7	39.495	24.360	**<0.001**
Standard white fluorescent /control	7	0.509	3.741	0.075
Sunglo/Powerglo	7	42.394	11.672	**<0.001**
Sunglo/Marineglo	7	24.135	12.923	**0.002**
Sunglo/control	7	0.432	1.144	0.295
Powerglo/Marineglo	7	2.665	0.517	0.479
Powerglo/control	7	51.381	14.888	**<0.001**
Marineglo/control	7	31.022	18.405	**<0.001**

3.8.4 Blastomussa merleti (Figure 3.10)

This species lent itself to propagation. Although the skeleton is thick and dense, it is easily fragmented using a hammer and chisel. The mortality after fragmentation and attachment was low (26.6%, n=30), making *B. merleti* an ideal propagation candidate. The polyps are resilient and tissue regeneration is rapid. This species proved to be the hardiest species and no mortalities occurred during the growth experiments. The fragments survived well in all environmental conditions, but grew best in the yeast (205.8%) and combination (284.3%) regimes. Although this species did not grow as well under the blue light regime, the combination of blue light and yeast feed promoted growth and there was a significant difference between this growth rate and that in the yeast treatment ($p<0.05$). The combination treatment yielded a 61.9% greater growth than in the yeast experiment (Figure 3.10).

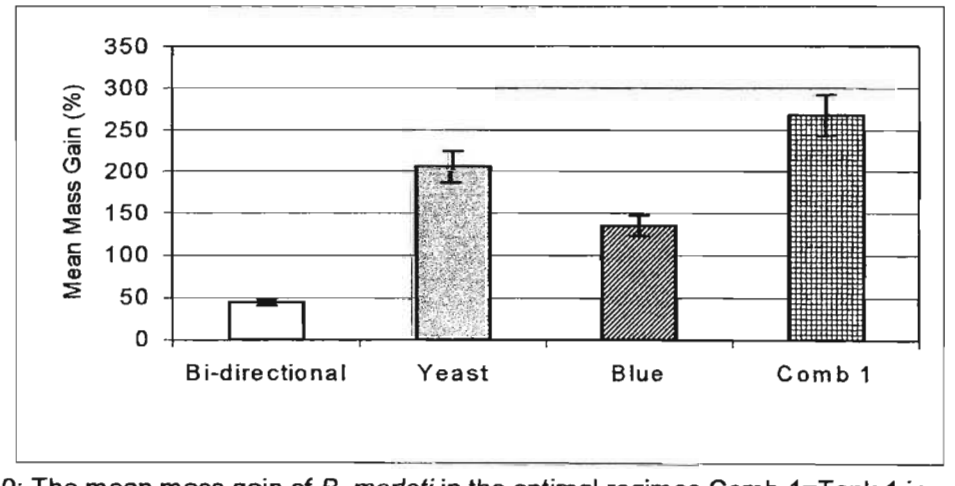

Figure 3.10: The mean mass gain of *B. merleti* in the optimal regimes Comb 1=Tank 1 in Table 4.

Table 20: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *B. merleti* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	5.685	2.519	0.423
Bi-directional/control	7	1.722	11.607	**0.003**
Unidirectional/control	7	2.351	12.723	**0.002**

Table 21: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control Feed regimes for *B. merleti* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	4.516	4.298	0.070
Yeast/*Artemia*	7	0.668	5.765	**0.023**
Yeast/*Chlorella*	7	5.626	5.971	**0.040**
Yeast/control	7	3.744	6.126	**0.030**
Soya flour/*Artemia*	7	1.986	13.370	**0.002**
Soya flour/*Chlorella*	7	11.951	9.734	**0.009**
Soya flour/control	7	2.145	12.226	**0.003**
Artemia/Chlorella	7	2.369	14.221	**0.001**
Artemia/control	7	3.775	18.991	**0.001**
Chlorella/control	7	4.516	4.298	**0.043**

Table 22: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *B. merleti* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent /Sunglo	7	4.100	2.564	0.112
Standard white fluorescent /Powerglo	7	18.955	4.683	**0.011**
Standard white fluorescent /Marineglo	7	3.638	2.231	0.148
Standard white fluorescent /control	7	0.981	1.247	0.275
Sunglo/Powerglo	7	5.424	1.938	0.177
Sunglo/Marineglo	7	0.014	0.007	0.934
Sunglo/control	7	9.092	8.133	**0.009**
Powerglo/Marineglo	7	5.860	2.115	0.159
Powerglo/control	7	28.560	14.383	**0.001**
Marineglo/control	7	8.396	7.306	**0.012**

3.8.5 Galaxea fascicularis (Figure 3.11)

This species is easily fragmented as the corallites are exsert, but nubbin size is important. If the nubbin is too small, the corallites may separate as they are loosely held together by a thin layer of calcium carbonate and living tissue. This species proved highly suitable for coral propagation. The mortality after fragmentation and attachment was 30% (n=30) and there was no mortality throughout the growth experiments. *G. fascicularis* grew best (264.4%) in the combination treatment of a bi-directional current, yeast feed and combination of blue and red spectra lighting (Figure 3.11).

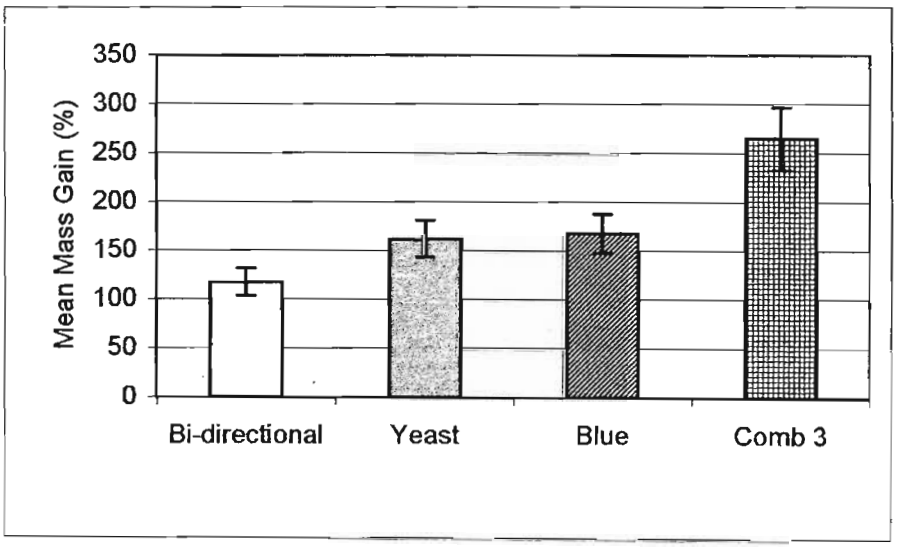

Figure 3.11: The mean mass gain of *G. fascicularis* in the optimal regimes. Comb 3=Tank 3 in Table 4.

Table 23: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *G. fascicularis* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	15.217	9.602	0.578
Bi-directional/control	7	16.672	12.426	**0.003**
Unidirectional/control	7	14.326	12.733	**0.005**

Table 24: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *G. fascicularis* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	4.613	7.821	**0.032**
Yeast/*Artemia*	7	13.618	9.513	**0.019**
Yeast/*Chlorella*	7	4.871	5.925	**0.028**
Yeast/control	7	5.432	5.851	**0.042**
Soya flour/*Artemia*	7	3.164	14.554	**0.003**
Soya flour/*Chlorella*	7	11.537	8.991	**0.008**
Soya flour/control	7	4.129	5.822	**0.011**
Artemia/*Chlorella*	7	2.012	13.291	**0.002**
Artemia/control	7	2.938	11.612	**0.004**
Chlorella/control	7	5.932	4.014	0.091

Table 25: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *G. fascicularis* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent /Sunglo	7	0.0175	0.048	0.828
Standard white fluorescent /Powerglo	7	13.703	8.662	**0.007**
Standard white fluorescent /Marineglo	7	4.739	4.628	**0.042**
Standard white fluorescent /control	7	0.5195	1.936	0.177
Sunglo/Powerglo	7	12.74	8.033	**0.009**
Sunglo/Marineglo	7	4.180	4.066	0.055
Sunglo/control	7	0.728	2.672	0.115
Powerglo/Marineglo	7	2.325	1.035	0.319
Powerglo/control	7	19.558	13.109	**0.001**
Marineglo/control	7	8.396	8.989	**0.006**

3.8.6 Alveopora spongiosa (Figure 3.12)

This species was easily fragmented as the skeleton is very porous and relatively weak. It was important to separate a large part of the skeleton with living tissue as *A. spongiosa* has long polyps that retract when disturbed. The polyps had a tendency to bleach during the first few weeks of experimentation. The colour was soon regained and no polyp mortality occurred. Nubbin mortality was relatively high after fragmentation and attachment (53.3%, n=30). *A. spongiosa* did not show a high growth in the current flow experiment, with the bi-directional current only yielding a 32.9% growth increase (Figure 3.12). This indicated that current flow was not the major factor influencing growth. The highest growths were obtained in the yeast (187.9%), blue light

(227.2%) and combination of bi-directional current, yeast and blue light (244.1%) treatments. There was no significant difference between the blue light and combination regimes ($p<0.05$), however the combination treatment yielded the highest growth (Figure 3.12).

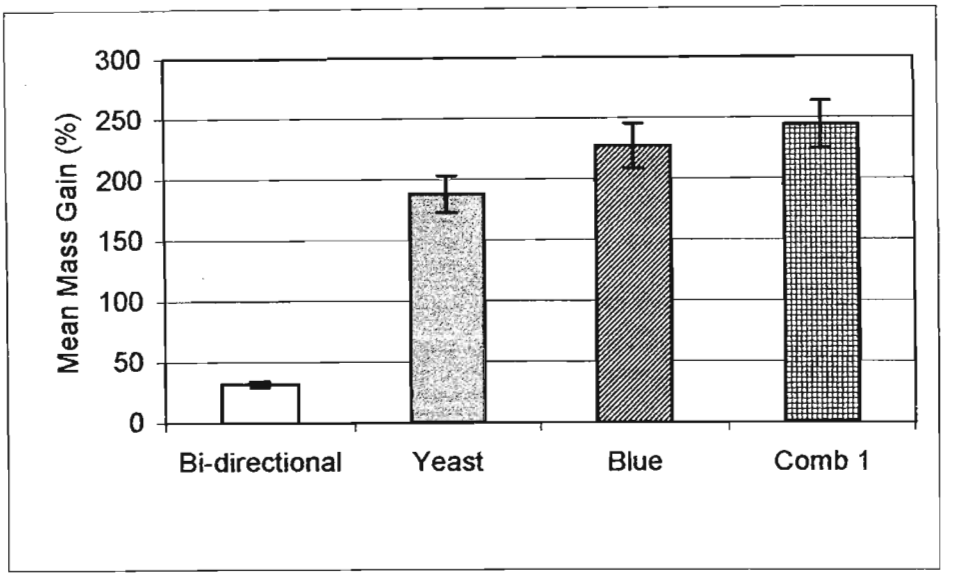

Figure 3.12: The mean mass gain of *A. spongiosa* in the optimal regimes. Comb 3=Tank 3 in Table 4.

Table 26: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *A. spongiosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	2.297	1.109	0.339
Bi-directional/control	7	1.235	11.888	**0.003**
Unidirectional/control	7	10.931	8.722	**0.001**

Table 27: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *A. spongiosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	0.269	0.254	0.824
Yeast/*Artemia*	7	182.348	62.311	**<0.001**
Yeast/*Chlorella*	7	1.988	0.624	0.453
Yeast/control	7	0.003	0.048	0.828
Soya flour/*Artemia*	7	173.622	47.182	**<0.001**
Soya flour/*Chlorella*	7	0.1504	0.721	0.403
Soya flour/control	7	0.024	0.039	0.716
Artemia/*Chlorella*	7	165.975	39.265	**<0.001**
Artemia/control	7	185.487	60.771	**<0.001**
Chlorella/control	7	15.694	9.658	0.591

Table 28: Results of ANOVA between Standard whitefluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *A. spongiosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent /Sunglo	7	0.2501	0.136	0.716
Standard white fluorescent /Powerglo	7	189.135	57.212	**<0.001**
Standard white fluorescent /Marineglo	7	150.842	67.722	**<0.001**
Standard white fluorescent /control	7	58.275	61.800	**<0.001**
Sunglo/Powerglo	7	175.630	50.747	**<0.001**
Sunglo/Marineglo	7	138.808	58.264	**<0.001**
Sunglo/control	7	50.890	46.349	**<0.001**
Powerglo/Marineglo	7	2.134	0.562	0.461
Powerglo/control	7	37.440	14.604	**<0.001**
Marineglo/control	7	21.603	14.546	**<0.001**

3.8.7 Goniopora djiboutiensis (Figure 3.13)

This species was very similar to *A. spongiosa* in terms of fragmentation and attahcment. The nubbin mortality after fragmentation and attachment was 46.7% (n=30). This species responded well to the *Artemia* feed (154.9% growth), unlike the majority of the other species (Figure 3.13). The nubbins grew best in the blue light (173.6%) and the combination of a bi-directional current, mixed of feeds and combined lighting (237.9%). There was no significant difference between the *Artemia* and blue light treatments ($p<0.05$). The combination treatment yielded 64.3% more growth than the blue light regime (Figure 3.13).

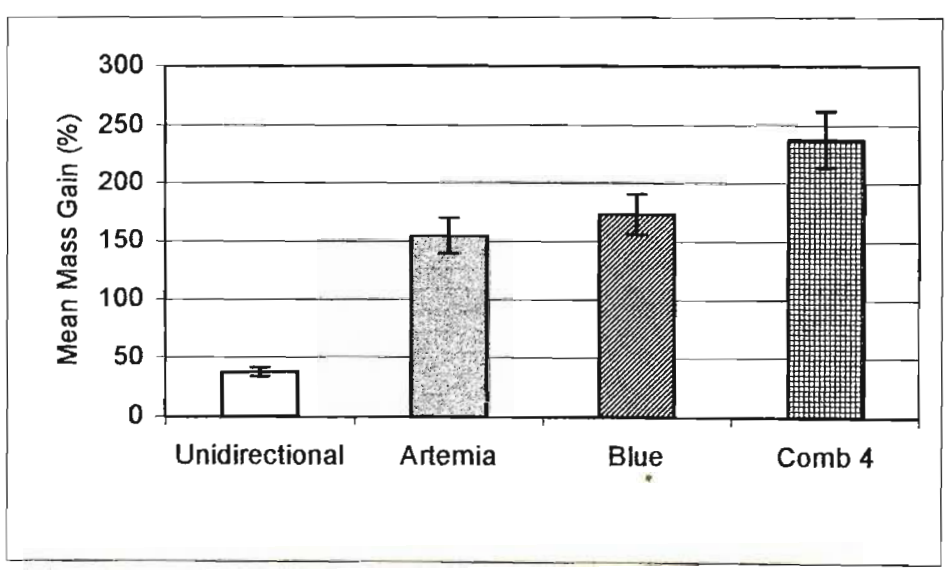

Figure 3.13: The mean mass gain of *G. djiboutiensis* in the optimal regimes. Comb 4=Tank 4 in Table 4.

Table 29: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *G. djiboutiensis* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	9.874	1.231	0.329
Bi-directional/control	7	1.235	11.888	**0.003**
Unidirectional/control	7	9.854	7.841	**0.002**

Table 30: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *G. djiboutiensis* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	14.874	9.216	0.587
Yeast/*Artemia*	7	9.657	48.574	**<0.001**
Yeast/*Chlorella*	7	5.148	9.652	0.416
Yeast/control	7	3.510	1.348	0.397
Soya flour/*Artemia*	7	46.872	11.247	**<0.001**
Soya flour/*Chlorella*	7	0.037	0.021	0.771
Soya flour/control	7	12.321	8.873	0.645
Artemia/Chlorella	7	2.548	9.574	**0.008**
Artemia/control	7	33.187	17.569	**<0.001**
Chlorella/control	7	6.254	4.741	0.093

Table 31: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *G. fascicularis* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent /Sunglo	7	1.358	1.262	0.273
Standard white fluorescent /Powerglo	7	41.673	14.279	**<0.001**
Standard white fluorescent /Marineglo	7	36.882	14.189	**<0.001**
Standard white fluorescent /control	7	0.160	0.295	0.592
Sunglo/Powerglo	7	27.987	8.946	**0.006**
Sunglo/Marineglo	7	24.087	8.574	**0.007**
Sunglo/control	7	0.585	0.777	0.387
Powerglo/Marineglo	7	0.146	0.314	0.861
Powerglo/control	7	36.66	14.128	**0.010**
Marineglo/control	7	32.179	14.141	**0.010**

3.8.8 Echinopora lamellosa (Figure 3.14)

This species, although an encrustation, responded differently in the different experiments. For this reason it was grouped alone as an encrusting coral with exsert corallites. Nubbin mortality was 46.5% (n=30). It responded similarly to the other corals

in the current flow and lighting experiments (growing best in the bi-directional current and blue lighting), but it grew fastest in the control feed (114.1%) and the combination of bi-directional current, yeast and red lighting (198.9%). Growth in the combined treatment was 47.9% higher than in the blue light regime (Figure 3.14).

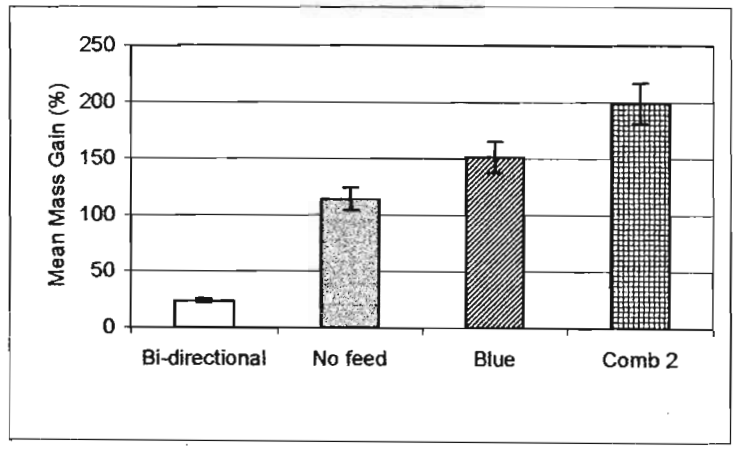

Figure 3.14: The mean mass gain of *E. lamellosa* in the optimal regimes. Comb 2=Tank 2 in Table 4.

Table 32: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *E. lamellosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	1.055	45.214	**<0.001**
Bi-directional/control	7	10.213	50.612	**<0.001**
Unidirectional/control	7	14.678	9.612	0.621

Table 33: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *E. lamellosa* nubbins.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	15.235	9.324	0.564
Yeast/*Artemia*	7	4.891	7.989	0.033
Yeast/*Chlorella*	7	2.948	1.543	0.237
Yeast/control	7	0.019	0.058	0.699
Soya flour/*Artemia*	7	5.946	2.237	0.478
Soya flour/*Chlorella*	7	6.659	5.324	0.129
Soya flour/control	7	5.997	3.983	0.099
Artemia/*Chlorella*	7	0.361	0.048	0.076
Artemia/control	7	7.014	2.249	0.196
Chlorella/control	7	13.590	10.287	0.557

Table 34: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *E. lamellosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent /Sunglo	7	0.358	0.095	0.761
Standard white fluorescent /Powerglo	7	376.391	92.939	**<0.001**
Standard white fluorescent /Marineglo	7	187.118	89.062	**<0.001**
Standard white fluorescent /control	7	140.198	85.289	**<0.001**
Sunglo/Powerglo	7	353.539	75.089	**<0.001**
Sunglo/Marineglo	7	171.111	62.011	**<0.001**
Sunglo/control	7	126.391	54.901	**<0.001**
Powerglo/Marineglo	7	32.738	10.786	**0.003**
Powerglo/control	7	57.158	22.171	**<0.001**
Marineglo/control	7	3.380	5.373	**0.030**

3.8.9 Gardinoseris planulata (Figure 3.15)

This species was extremely hardy and retained its colour in all the experiments. The mortality of nubbins after fragmentation and attachment was 50% (n=30). This was attributed to the very delicate and fragile nature of these nubbins and to the fact that the live tissue layer is very thin. There was no significant difference between the growth in the blue light and combination treatments ($p<0.05$). The combination treatment of bi-directional current, combination feeds and combined lighting (193.6%; Figure 3.15) produced the highest growth. In the feeding experiment, the *Chlorella* culture produced the highest growth (94.5%).

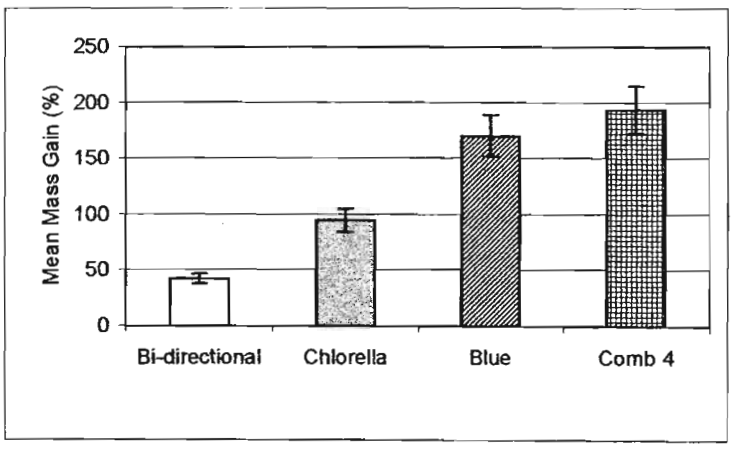

Figure 3.15: The mean mass gain of *G. planulata* in the optimal regimes. Comb 4=Tank 4 in Table 4.

Table 35: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *G. planulata* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	2.687	1.092	0.335
Bi-directional/control	7	0.152	7.000	**0.015**
Unidirectional/control	7	0.6676	5.766	**0.023**

Table 36: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *G. planulata* nubbins.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	4.987	2.165	0.597
Yeast/*Artemia*	7	3.361	1.464	0.264
Yeast/*Chlorella*	7	6.214	4.579	0.091
Yeast/control	7	0.457	3.682	0.069
Soya flour/*Artemia*	7	15.231	9.532	0.564
Soya flour/*Chlorella*	7	2.361	1.841	0.239
Soya flour/control	7	0.456	0.057	0.071
Artemia/*Chlorella*	7	17.642	12.010	0.548
Artemia/control	7	0.196	0.864	0.436
Chlorella/control	7	2.961	1.554	0.345

Table 37: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *G. planulata* nubbins. Bold figures denote significant difference.

Source of Variation	Df	SS	F	P-value
Standard white fluorescent /Sunglo	7	0.286	0.102	0.752
Standard white fluorescent /Powerglo	7	279.358	83.478	**<0.001**
Standard white fluorescent/Marineglo	7	198.969	84.786	**<0.001**
Standard white fluorescent /control	7	102.704	76.272	**<0.001**
Sunglo/Powerglo	7	261.779	71.495	**<0.001**
Sunglo/Marineglo	7	184.179	69.195	**<0.001**
Sunglo/control	7	92.158	55.465	**<0.001**
Powerglo/Marineglo	7	6.804	2.113	0.159
Powerglo/control	7	43.292	19.502	**<0.001**
Marineglo/control	7	15.770	12.926	**0.001**

3.8.10 Hydnophora exesa (Figure 3.16)

This species was a successful candidate although mortality after fragmentation was high (56.67%, n=30). The polyps retained their green colour in all the experiments except under the standard white fluorescent and yellow light experiments where there was100% mortality. This species was vulnerable to high intensity light and bleaching occurred with all the nubbins. The bi-directional current was the best flow regime but only produced a 20.73% (Figure 3.16) mass increase. The highest mass increase was observed in the combination of a bi-directional current, combination feed and combination light regime (206.29% mass increase). This was however not significantly different to the blue light regime ($p<0.05$). In the feeding experiment, *H. exesa* grew best in the soya flour treatment (100.02% mass increase; Figure 3.16).

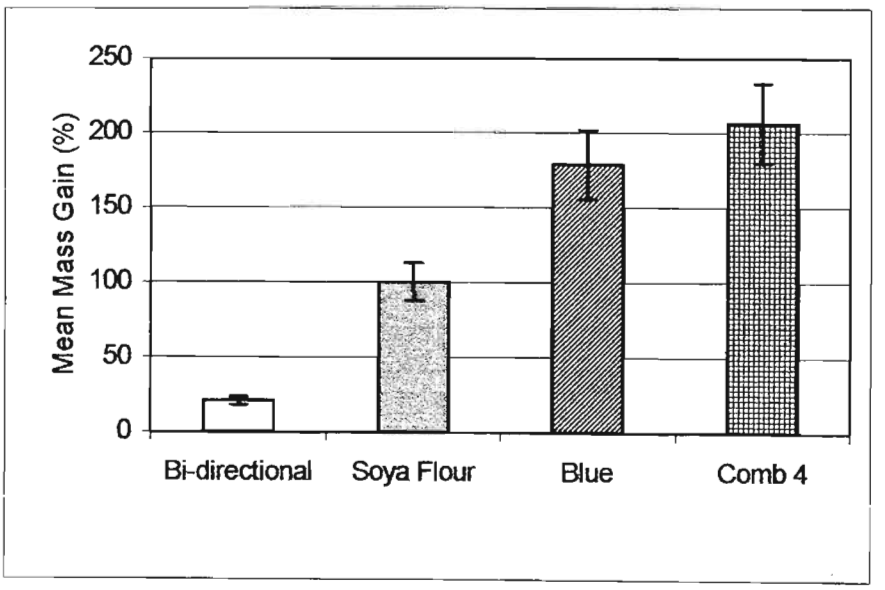

Figure 3.16: The mean mass gain of *H. exesa* in the optimal regimes. Comb 4=Tank 4 in Table 4.

Table 38: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *H. exesa* nubbins.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	0.047	1.75	0.228
Bi-directional/control	7	0.032	1.89	0.232
Unidirectional/control	7	0.056	2.67	0.317

Table 39: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *H. exesa* nubbins.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	16.325	8.547	0.651
Yeast/*Artemia*	7	14.331	10.214	0.521
Yeast/*Chlorella*	7	2.984	1.487	0.398
Yeast/control	7	0.056	1.847	0.214
Soya flour/*Artemia*	7	6.354	4.515	0.091
Soya flour/*Chlorella*	7	1.984	1.247	0.084
Soya flour/control	7	0.042	1.94	0.236
Artemia/*Chlorella*	7	13.283	9.561	0.556
Artemia/control	7	2.313	1.682	0.087
Chlorella/control	7	15.662	9.338	0.621

Table 40: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *H. exesa* nubbins. Bold figures denote significant difference.

Source of Variation	Df	SS	F	P-value
Standard white fluorescent /Sunglo	7	0.6077	0.098	0.758
Standard white fluorescent /Powerglo	7	416.000	57.891	**<0.001**
Standard white fluorescent /Marineglo	7	251.734	53.736	**<0.001**
Standard white fluorescent /control	7	137.425	39.126	**<0.001**
Sunglo/Powerglo	7	384.808	58.084	**<0.001**
Sunglo/Marineglo	7	227.594	55.194	**<0.001**
Sunglo/control	7	119.756	40.575	**<0.001**
Powerglo/Marineglo	7	20.523	4.043	0.056
Powerglo/control	7	75.225	19.268	**<0.001**
Marineglo/control	7	17.164	12.326	**0.002**

3.8.11 Montipora monasteriata (Figure 3.17)

M. monasteriata is easy to fragment but care must be taken not to damage the tissue layer as it is extremely thin. The nubbin mortality after fragmentation and attachment was 60% (n=30) and some initial bleaching occurred in all of the experiments. The nubbins used in the growth experiments recovered within two weeks of commencement. The standard white fluorescent and yellow light experiments resulted in 100% mortality. The combination of a bi-directional current, combination feed and combined light treatment produced the highest growth (194.4%). There was no significant difference between the yeast and blue light regimes ($p<0.05$). The bi-

directional current was the best flow regime but yielded a low increase in growth (34%) in comparison to the feeding, light and combination experiments (Figure 3.17).

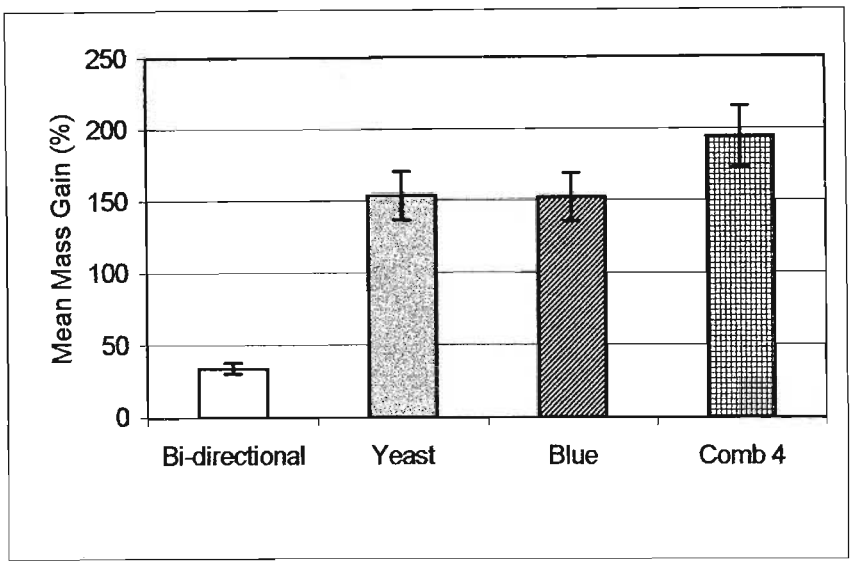

Figure 3.17: The mean mass gain of *M. monasteriata* in the optimal regimes. Comb 4=Tank 4 in Table 4.

Table 41: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *M. monasteriata* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	0.452	11.451	**0.003**
Bi-directional/control	7	0.784	12.947	**0.001**
Unidirectional/control	7	14.525	10.211	0.524

Table 42: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *M. monasteriata* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	0.784	5.891	**0.020**
Yeast/*Artemia*	7	10.599	10.772	**0.008**
Yeast/*Chlorella*	7	2.321	12.013	**0.003**
Yeast/control	7	2.458	14.999	**0.001**
Soya flour/*Artemia*	7	18.250	12.551	0.564
Soya flour/*Chlorella*	7	3.856	2.337	0.123
Soya flour/control	7	10.540	8.325	0.655
Artemia/*Chlorella*	7	2.956	1.845	0.334
Artemia/control	7	0.452	4.369	0.062
Chlorella/control	7	0.008	0.092	0.084

Table 43: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *M. monasteriata* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Standard white fluorescent /Sunglo	7	1.206	0.235	0.632
Standard white fluorescent /Powerglo	7	294.135	60.010	**<0.001**
Standard white fluorescent /Marineglo	7	310.155	64.864	**<0.001**
Standard white fluorescent /control	7	98.572	37.515	**<0.001**
Sunglo/Powerglo	7	257.670	52.162	**<0.001**
Sunglo/Marineglo	7	272.679	56.573	**<0.001**
Sunglo/control	7	77.971	29.247	**<0.001**
Powerglo/Marineglo	7	0.212	0.046	0.832
Powerglo/control	7	52.157	21.346	**<0.001**
Marineglo/control	7	59.026	25.402	**<0.001**

3.8.12 Pachyseris speciosa (Figure 3.18)

This species is easily fragmented along naturally occurring "fault lines". It is, however, a fragile coral as the skeleton is very thin, as is the tissue layer. The mortality after fragmentation and attachment was 46.7% (n=30) and there was 100% mortality in the standard white fluorescent and yellow light experiments. In the current flow experiment, this species grew best in the unidirectional current (37.7% mass increase; Figure 3.18). The highest growth occurred in the combination of bi-directional current, combination feed and combined light (178.5%). This was not significantly different to the blue light regime ($p<0.05$). The soya flour treatment provided the highest growth (78.7%) in the feeding experiment (Figure 3.18).

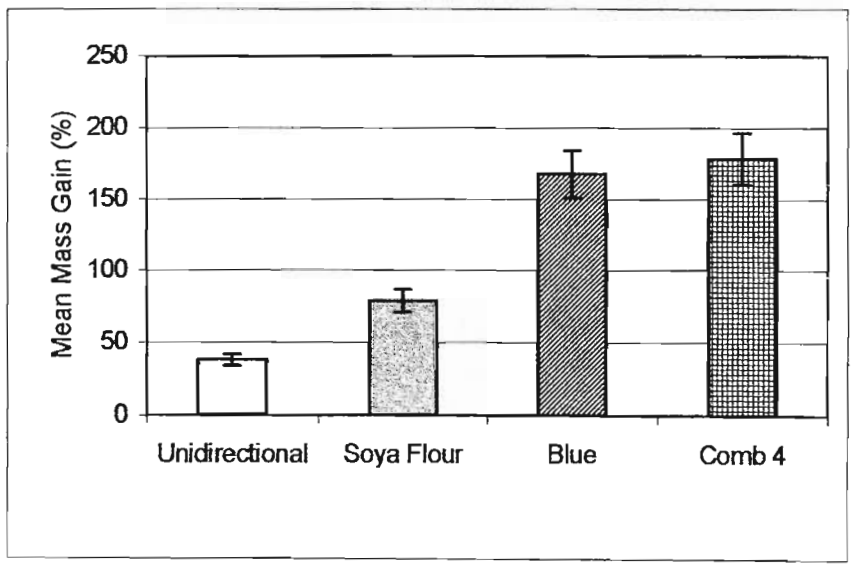

Figure 18: The mean mass gain of *P. speciosa* in the optimal regimes. Comb 4=Tank 4 in Table 4.

Table 44: Results of ANOVA between the bi-directional, unidirectional and control current regimes for *P. speciosa* nubbins. Bold figures denote significant difference.

Source of Variation	df	SS	F	P-value
Bi-directional/Unidirectional	7	0.240	9.000	**0.007**
Bi-directional/control	7	2.581	13.636	**0.001**
Unidirectional/control	7	14.257	10.239	0.661

Table 45: Results of ANOVA between the yeast, soya flour, *Artemia*, *Chlorella* and control feed regimes for *P. speciosa* nubbins.

Source of Variation	df	SS	F	P-value
Yeast/Soya flour	7	15.012	9.235	0.598
Yeast/*Artemia*	7	15.135	9.984	0.532
Yeast/*Chlorella*	7	14.365	10.258	0.574
Yeast/control	7	16.982	12.344	0.632
Soya flour/*Artemia*	7	2.987	0.851	0.491
Soya flour/*Chlorelia*	7	9.314	8.662	0.422
Soya flour/control	7	4.293	2.038	0.399
Artemia/Chlorella	7	2.125	0.694	0.458
Artemia/control	7	3.845	1.540	0.511
Chlorella/control	7	2.368	0.867	0.387

Table 46: Results of ANOVA between Standard white fluorescent, Sunglo, Marineglo, Powerglo and control light regimes for *P. speciosa* nubbins. Bold figures denote significant difference.

Source of Variation	Df	SS	F	P-value
Standard white fluorescent/Sunglo	7	0.002	0.098	0.756
Standard white fluorescent /Powerglo	7	46.914	27.363	**<0.001**
Standard white fluorescent /Marineglo	7	13.128	19.794	**<0.001**
Standard white fluorescent /control	7	1.792	21.603	**<0.001**
Sunglo/Powerglo	7	46.311	26.979	**<0.001**
Sunglo/Marineglo	7	12.810	19.256	**<0.001**
Sunglo/control	7	1.675	19.713	**<0.001**
Powerglo/Marineglo	7	10.408	4.410	**0.046**
Powerglo/control	7	30.370	17.064	**<0.001**
Marineglo/control	7	5.220	7.166	**0.013**

3.9 Linear regression equations

Linear regression equations were determined for the optimal regimes in each experiment for each species (Tables 47-50). Linear regressions provided the best fit to describe the growth of the coral nubbins in the given time-frame.

Table 47: Linear regression equations of growth of the twelve candidate coral species under their optimal current regime: y=cumulative mass increase (g), x=time (weeks).

Species	**Optimal regime**	**Regression equation**	r^2
Acropora austera	Bi-directional	y=0.1303x-0.3503	0.9939
Pocillopora verrucosa	Bi-directional	y=0.0851x-0.2216	0.9958
Stylophora pistillata	Bi-directional	y=0.1049x-0.4065	0.9807
Blastomussa merleti	Bi-directional	y=0.0573x-0.1865	0.9935
Galaxea fascicularis	Bi-directional	y=0.1274x-0.7816	0.9359
Alveopora spongiosa	Bi-directional	y=0.0474x-0.1000	0.9937
Goniopora djiboutiensis	Bi-directional	y=0.0541x-0.1800	0.9920
Echinopora lamellosa	Bi-directional	y=0.0439x-0.2273	0.9666
Gardinoseris planulata	Bi-directional	y=0.0490x-0.2468	0.9723
Hydnophora exesa	Bi-directional	y=0.0298x-0.1589	0.9590
Montipora monasteriata	Bi-directional	y=0.0526x-0.1279	0.9938
Pachyseris speciosa	Bi-directional	y=0.0410x-0.1923	0.9804

Table 48: Linear regression equations of growth of the twelve candidate coral species under their optimal light regime: y=cumulative mass increase (g), x=time (weeks).

Species	**Optimal regime**	**Regression equation**	r^2
Pocillopora verrucosa	Blue	y=0.4277x-0.7019	0.9891
Stylophora pistillata	Blue	y=0.4738x-0.5356	0.9979
Blastomussa merleti	Blue	y=0.4913x-0.5183	0.9987
Galaxea fascicularis	Blue	y=0.5011x-0.3615	0.9976
Alveopora spongiosa	Blue	y=0.5699x-0.9663	0.9893
Goniopora djiboutiensis	Blue	y=0.5702x-0.9644	0.9920
Echinopora lamellosa	Blue	y=0.5573x-0.5298	0.9985
Gardinoseris planulata	Blue	y=0.6651x-1.2577	0.9898
Hydnophora exesa	Blue	y=0.7063x-0.825	0.9985
Montipora monasteriata	Blue	y=0.4913x-0.5183	0.9987
Pachyseris speciosa	Blue	y=0.5273x-0.4683	0.9995

Table 49: Linear regression equations of growth of the twelve candidate coral species under their optimal feeding regime: y=cumulative mass increase (g), x=time (weeks).

Species	Optimal regime	Regression equation	r^2
Acropora austera	Yeast	y=0.1942x-2.5408	0.9944
Pocillopora verrucosa	Yeast	y=0.1554x-4.6310	0.7989
Stylophora pistillata	Yeast	y=0.0957x-3.2157	0.2773
Blastomussa merleti	Yeast	y=0.3288x-3.8932	0.8225
Galaxea fascicularis	Yeast	y=0.1380x-4.0893	0.1778
Alveopora spongiosa	Yeast	y=0.1320x-2.4207	0.4048
Goniopora djiboutiensis	*Artemia*	y=0.2742x-2.3327	0.8030
Echinopora lamellosa	No feed	y=0.0613x-3.5173	0.1003
Gardinoseris planulata	*Chlorella*	y=0.0815x-4.1260	0.0656
Hydnophora exesa	Soya Flour	y=0.0935x-4.3010	0.8673
Montipora monasteriata	Yeast	y=0.1142x-3.9950	0.0650
Pachyseris speciosa	Soya Flour	y=0.0933x-3.0756	0.5135

Table 50: Linear regression equations of growth of the twelve candidate coral species under their optimal combination regime: y=cumulative mass increase (g), x=time (weeks).

Species	Optimal regime	Regression equation	r^2
Pocillopora verrucosa	Yeast/Blue light	y=0.1487x-3.3540	0.9889
Stylophora pistillata	Yeast/Blue light	y=0.9364x-2.4692	0.9763
Blastomussa merleti	Yeast/Blue light	y=0.0734x-5.2273	0.8924
Galaxea fascicularis	Yeast/Blue&Red light	y=0.1624x-7.3022	0.9541
Alveopora spongiosa	Yeast/Blue light	y=0.0935x-5.6683	0.8961
Goniopora djiboutiensis	Combination Feed/Light	y=0.1588x-4.9877	0.9335
Echinopora lamellosa	Yeast/Red light	y=0.9609x-3.4501	0.9652
Gardinoseris planulata	Combination Feed/Light	y=0.5371x-2.3155	0.9423
Hydnophora exesa	Combination Feed/Light	y=0.9267x-2.5837	0.9976
Montipora monasteriata	Combination Feed/Light	y=0.2491x-3.2400	0.9830
Pachyseris speciosa	Combination Feed/Light	y=0.3511x-4.5714	0.9848

CHAPTER 4

DISCUSSION

4.1 General

The results of this study proved useful in determining the criteria required to produce maximum growth in the propagation of coral species. Twelve candidates were chosen as the most suitable for propagation. After the first two experiments (Current Flow and Feeding) it was realised that, although *A. austera* appeared to be a likely candidate, it was extremely difficult to work with and was very sensitive to changes in environmental conditions. It was therefore abandoned as a candidate for further propagation and growth experiments.

This highlighted the need for the careful choice of corals for propagation according to the following criteria:

- Adaptation to a change in environmental conditions (e.g. corals in South Africa must be able to survive translocation from depths of 16-22 m to less than 2 m.
- Survival under propagation.
- Aesthetic appeal (generally brightly coloured corals, or corals with extended polyps or branches are favoured by aquarists).
- Growth rate (faster growing corals are preferable as they take less time to reach marketable size).
- Variety in morphology (attempts were made to include species of different morphology to provide variation).

There are some obvious reasons for attempting to increase the growth rates of coral nubbins for a propagation operation. The faster the nubbins grow, the less time they remain in the system and the quicker they become available for sale or transplantation. There were also other reasons for attempting to determine what factors influence the growth and survival of each of the coral species, namely to establish:

- The ability of colonies or nubbins to adjust to a new environment.
- The response of each species to new environmental conditions.
- The tolerance of each species to the different propagation conditions.

It became evident that the additional feeding and artificial lighting increased the growth of the nubbins quite considerably and a combination of all the factors further increased their growth. For the purpose of this study the cumulative and incremental percentage mass increases were compared and analysed. These were determined using the mean cumulative mass changes of the nubbins. It has been suggested that initial size influences nubbin growth (Sykes, 1996); however, if the change in mass was calculated on a weekly basis it can be shown that growth was relative to size. Therefore, relative growth rate was constant in nubbins of the same species under the same conditions, regardless of size.

It was possible to optimise the conditions to produce the fastest growth in the majority of species. In a commercial venture, it would be advisable to house each species in separate tanks, thereby providing the specific requirements for maximum growth in each species.

4.2 Acclimation and Lag prior to growth

The corals were adapted to the natural conditions they were collected in and a period of acclimation was important for the parent colonies to recover from stress and to acclimitise under new environmental conditions.

The lag periods of all the candidate coral species were reduced when the corals were exposed to their optimal conditions (Table 8). This suggests that when the corals are not compromised, they are better able to direct energy into repair and growth. The introduction of artificial lighting and food supplementation further improved the situation, resulting in a reduction in lag period. This was most reduced when the corals were exposed to combinations of optimal feeding and light (Table 8).

4.3 Fragmentation

Highsmith (1982) suggested that there are intra- and inter-specific trade-offs in corals between producing small fragments with relatively low survival and large fragments with high survival. Highsmith's model was based on the survival of hurricane-generated fragments of *Acropora palmata* in which survival was size-dependent (Highsmith *et al.*, 1980) and also on the fact that whole-colony survival is size-dependent in many coral species (e.g. Connell, 1973; Hughes and Jackson, 1985; Hughes and Connell, 1987). Although the general predictions of Highsmith's model are widely cited (Denny *et al.*, 1985; Lewis, 1991), there are some experimental studies that do not support them (e.g. Bruno, 1998). Coral fragmentation is often discussed as

if it is a process optimised by natural selection (e.g. Highsmith, 1982; Lewis, 1991), but in some cases it may be an unavoidable and potentially detrimental consequence of a branching morphology in a high-energy environment (Bruno, 1998). In the latter case, most fragments die regardless of their size.

The survival of fragmented corals is not necessarily size-dependent (Bruno, 1998) as fragmentation is a random occurrence. Fragment survival in the wild is usually affected by sedimentation and dispersal distance from the reef (Bruno, 1998). However, Riegl (1995) studied the degree to which corals tolerate sedimentation and concluded that it is rarely the decisive lethal factor under normal water movement on the reef. Bruno (1998) showed that, in *Madracis mirabilis,* there was no significant relationship between fragment size and survival (Bruno, 1998). Survival was equated with position on the reef and other factors rather than size.

Other factors may affect fragment survival, such as intra-species variation in phenotypic plasticity, tissue regeneration, susceptibility to disease (Bak and Criens, 1981), damage susceptibility (Riegl and Cook, 1995) corallite morphology (Highsmith, 1982) and predator preferences (Rylaarsdam, 1983; Knowlton *et al.*, 1990). Evidence suggests that fragment survival may be highly habitat-and species-specific (Bruno, 1998; Rogers *et al.*, 1982; Wahle, 1983; Heyward and Collins, 1985).

The present study eliminated the influences of predators, position on reef and sedimentation as the experimentation was tank-based. It is evident that fragment size is not an issue in coral propagation as fragments as small as two polyps survived (exsert corallite corals, Table 2). Corals with either exsert corallites or large polyps (*B. merleti* and *G. fascicularis*), fragmented this small, showed high survival. These corals have a thicker tissue layer and are thus more resilient to tissue damage, injury and air exposure. The branching (*A. austera, P. verrucosa* and *S. pistillata*) and encrusting (*M. monasteriata, H. exesa, G. planulata, P. speciosa* and *E. lamellosa*) corals have very thin tissue layers, making them more susceptible to tissue damage and air exposure. These corals must therefore be fragmented into larger nubbins. The submassive corals (*G. djiboutiensis* and *A. spongiosa*) have very porous and soft skeletons with long extended polyps. When either exposed to air or disturbed, the polyps retract relatively deep into the skeleton. The fragments must therefore include the majority of the skeleton below the polyps and must thus be quite big in comparison to the exsert corallite corals.

Fragment survival in a tank-based operation is more dependent on exposure and stress to the parent colonies (prior to fragmentation) and fragments. Therefore,

corals should be kept submerged during the fragmentation process and the fragments should be well rinsed in clean seawater to flush away mucus that is produced during fragmentation.

4.4 Attachment

4.4.1 Adhesives

Coral propagation is dependent on a combination of factors for success, one of these being the initial attachment of fragments to the substratum. Attempts at coral propagation are futile if the nubbins do not survive this procedure. Great care was taken to determine the most effective technique of coral fragment attachment. In the short-term, it was easier and quicker to attach fragments to a substratum (such as a glass slide) by means of an adhesive.

The use of superglue required that the nubbins be exposed for too long in air and the toxicity of the glue caused peripheral tissue necrosis and mortality. The superglue was also difficult to work with as the adhesive had to be applied to both the base of the fragment and the substratum (glass slide). The bases of the fragments were not always flat and the adhesive surface was therefore not even, making attachment more difficult. The use of superglue required the application of a certain amount of pressure to the fragments to ensure that the adhesive bonded. This pressure may have been the cause of some of the nubbin mortality experienced in species such as *P. verrucosa*. This species has an extremely thin tissue layer covering the hard skeleton and, therefore, pressure damaged the coral polyps and may have caused mortality. This, coupled with the longer exposure to air, could have caused the high mortality using this adhesive.

The use of epoxy required only a few seconds of air exposure, and was simple to use as a small "holdfast" could be formed on the glass slide to hold the fragment in place. However, the epoxy did not adhere very well to the base of the fragments and the bases had to be well-dried before placing epoxy on them. Once the fragments had been secured, they could be replaced in the tank immediately as the epoxy was designed for marine application and set under water. The nubbins detached from the epoxy in some cases.

The use of thermoplastic glue also required only a few seconds of exposure of the nubbins to air. Although this adhesive was relatively hot, it caused no peripheral tissue damage. This was attributed to the fact that the glue cooled rapidly when the nubbins were re-submerged in water. The thermoplastic glue proved the most

successful (Figure 3.1) as a "holdfast" could be formed on the glass slide and further glue could be manipulated around the fragment. The glue set rapidly under water and no pressure was required to ensure adhesion.

4.4.2 Electrolysis

The use of electrolysis proved slower (Figure 3.2) as a means of attachment but caused no peripheral tissue damage, while promoting a more natural form of attachment and growth. Electrolysis also reduced algal invasion of the lesion at the breakage site as $CaCO_3$ was deposited around the exposed areas. This appeared to reduce the stress on the corals and more energy could be channeled into growth and the formation of new polyps.

The initial rate of calcium $CaCO_3$ deposition was observed to be greater around the living nubbins than on the rest of the mesh. This finding concurred with that of van Treeck and Schuhmacher (1997). However, these authors provided no explanation for this phenomenon. It appears that $CaCO_3$ deposition occurs more readily around the bases of the nubbin skeletons and is probably enhanced by biological deposition. After one week, accretion began to cement *A. austera*, *P. verrucosa*, *S. pistillata*, *M. monasteriata*, *P. speciosa* and *E. lamellosa* nubbins to the mesh in the 12 h system. All the fragments were well-attached to the mesh within a maximum of nine weeks, except for *B. merleti* (Figure 3.2). The maximum attachment period was further reduced to seven weeks in the 24 h system. Polyp proliferation appeared to take place at the attachment sites.

M. monasteriata, H. exesa and *E. lamellosa* nubbins underwent a degree of bleaching in the initial few days. No mortality occurred and the polyps regained colour within two weeks. This was possibly caused by the continuous charge in the system that causes localised changes in pH; the values close to the cathodic surface may increase to as high as 11 (Schuhmacher and Schillak, 1994). Van Treeck and Schuhmacher (1997) observed no damage to the coral tissue in the vicinity of the cathode and the pH increase was of no concern to them as the *in situ* nature of their experimental system allowed for continual water replacement around the cathode. It was a cause for concern in the present study as it may have been the cause of bleaching in the 24 h system. Although the water through-flow was continuous in the 800 l tank, it would have been insufficient to prevent some stagnation in the vicinity of the cathode. The two powerhead pumps that generated a bi-directional current would

have helped prevent dead spaces in the tank; however, a pH shift sufficient to cause bleaching may have occurred.

Nubbin mortality was extremely low in this experiment. Van Treeck and Schuhmacher (1997) attributed the losses in their experiments to factors such as wave action, predation and death caused by intrinsic factors such as the health of the nubbins, survival after fragmentation (physical stress), disease and bleaching. Mortality in this study was attributable to such intrinsic factors as none of the other factors applied in the laboratory. It has been reported by Yap *et al.* (1992) that high mortality rates in *Pocillopora* sp. nubbins could be due to fragmentation and transplantation stress. The high mortalities of *P. damicornis* in van Treeck and Schuhmacher's (1997) study and of *P. verrucosa* in this study support this finding. It was further observed in this study that *P. verrucosa* fragments did not tolerate exposure to air and a great deal of handling. Van Treeck and Schuhmacher (1997) proposed that the fragmentation process rather than the electrolytic process was the cause of their high nubbin mortality.

B. merleti was the only species that did not attach in any of the electrolysis systems (Figure 3.2). This was attributed to inclusions of unconsolidated sediment in fragments (i.e. the surfaces in contact with the mesh were not necessarily composed of $CaCO_3$). The deposit on the mesh would therefore have to accumulate until it fused with the $CaCO_3$ of the *B. merleti* nubbins.

There were no signs of peripheral algal overgrowth in the electrolysis systems. The electrolytic process may have created an environment that was unfavourable for algae to grow and epiphytes to survive. As mentioned above, the electrolytic process causes an increase in the pH around the cathode (Schuhmacher and Schillak, 1994). It is possible that this increase in pH created unsuitable conditions for algae. There was also no evidence of decaying tissue at the fragmentation sites or peripheral tissue damage in electrolytically attached nubbins, unlike the methods involving adhesives.

Electrolytic attachment has great potential for coral propagation and reef rehabilitation. The nubbins are easily attached and growth is promoted. It involves very little handling and exposure to air is unnecessary. No toxic materials are involved in attachment.

The design of van Treeck and Schuhmacher (1997) could be modified to yield greater nubbin survival in reef rehabilitation applications. Instead of using an *in situ* electrolytic system, nubbins could be attached to smaller sections of mesh in the laboratory (as was done in this study). Once the nubbins attain suitable size and are

firmly attached, the meshes could be transported for placement on the reef. This would reduce initial predation and the consequences of wave action that may disrupt nubbin attachment and would, furthermore, be advantageous on reefs where it would be impossible to install an electrolytic system.

4.5 Current Flow

Current flow had a profound effect on the growth of some corals. The branching corals were more dependent on the presence of a current and in particular a bi-directional current. This is due to the fact that "dead" areas develop between the branches within the colony in the absence of a current. An accumulation of mucus and sediment deposition occurs within these "dead" areas. A current is therefore required to flush the waste out. The current also serves to distribute nutrients and particles for uptake by the polyps. Encrusting corals, on the other hand, are more dependent on maximising their exposure to solar radiation for photosynthesis (Chamberlain *et al.*, 1975). They lack branches and are not as dependent on currents for survival.

Chamberlain *et al.* (1975) tested the effect of water flow on corals using cleaned museum specimens. The main factor that affects water flow in corals is colony porosity. This pertains to the openness of the coral framework. A branched colony is defined, for this purpose, as a framework of solid branches enclosing a system of interconnected passageways. Water movement through the colony must, therefore, depend to a great extent on the porosity of the framework. Colony porosity affects both the nature and interior velocity of water flow. When branches are widely spaced and the porosity is correspondingly high, as in *Acropora palmata* and *A. cervicornis*, incoming water encounters little resistance. Momentum loss occurs slowly and water can penetrate further into the colony. In tightly branched, low porosity colonies, momentum loss occurs quickly and the flow rapidly stagnates in the interior of the colony. For this reason, branching corals such as *A. austera*, *P. verrucosa* and *S. pistillata* require continuous, bi-directional currents for the removal of waste and mucus.

Another factor investigated by Chamberlain *et al.* (1975) was the effect of branching pattern on the internal flow in coral colonies. He compared the flow properties of randomly and regularly branched colonies. In a regularly branched colony, water follows the path of least resistance and moves through the widest inter-branch channels. A major benefit of a regular branch pattern would be to create channels that allow the penetration of nutrient-laden water deep into the colony interior, and to facilitate mucus and waste water removal (Chamberlain *et al.*, 1975). In colonies with

randomly spaced branches, water flows through irregular channels that are perpendicular to the external current direction. Stagnation becomes excessive behind clusters of closely spaced branches in a unidirectional current. The implications of this are that corals such as *A. austera*, *P. verrucosa* and S. *pistillata* require a continuous bi-directional current.

Water flow on a reef is seldom unidirectional. A colony may be subjected to surges and currents as well as flow deflected from other colonies on the reef. Chamberlain *et al.* (1975) examined the effects of variation in colony orientation, relative to the direction of water flow, using two orientations of a colony. Inter-branch channels were aligned parallel to the external current direction in the first, while the channels were inclined at 45° to the external current direction in the second. Both orientations resulted in the same flow characteristics with water following the paths of least resistance, but the paths of water movement were different in the two orientations. Such intra-colony flow variations may significantly affect functions such as mucus and sediment removal, and factors such as nutrient distribution and larval dispersal, especially where strong currents prevail. Encrusting corals again do not require the same amount of water movement within the colonies and, in these experiments, a unidirectional or currentless regime proved more beneficial.

4.6 Light

Light is essential both for corals and their symbiotic algae, but too much light can be a problem even in high light reef habitats (Jokiel, 1980). The corals used in these experiments were collected at depths where the light intensity was not as great as that provided in the experimental tanks. Although the corals went through a period of acclimation after collection, it is thought that a light regime most similar to their natural environment would have more suitable. Symbiotic algal photosynthesis is affected by high solar irradiances, which may cause photo-inhibition resulting in increases in the concentration of reactive oxygen and even coral bleaching (Lesser *et al.*, 1990; Shick *et al.*, 1995; Warner *et al.*, 1996). This theory is supported by the observation of initial bleaching in some of the encrusting coral fragments when exposed to higher levels of solar irradiance, in water less than one meter deep, in the lighting experiment (Figure 3.5). This greater photo-sensitivity is again related to their greater dependence on zooxanthellar photosynthesis as encrusting corals are endowed with greater concentrations of zooxanthellae (Shick *et al.*, 1995).

Short wavelength solar radiation that penetrates to considerable depths (Jerlov, 1950) can be detrimental to photosynthesis and may damage the DNA and proteins of marine invertebrates and algae (Shick *et al.*, 1991). Although this type of light is harmful to corals (Salih *et al.*, 1998), the colonies in this experiment grew best under the short wavelength blue light. This may have been due to natural photo-protection (Shick *et al.*, 1991) in the corals. Some corals, such as the acroporids (e.g. *Acropora hyacinthus, A. palifera* and *A. nobilis*) were found to have both intensely fluorescent morphs as well as non-fluorescent morphs, which are almost indistinguishable under visible light (*Salih et al.*, 1998). Fluorescent pigments have been found in the tissues of most corals, in colonies from shallow intertidal habitats, to those from lower light, shaded or deeper water habitats (Salih *et al.*, 1998). Salih *et al.* (1998) proposed that polyp fluorescence could have a function in screening out excess light in corals growing in shallow reef habitats.

According to Salih *et al.* (1998), many corals are either high-light (HL) or low-light (LL) adapted. The results of this study corresponded with the classification of Salih *et al.* (1998) with the South African HL adapted corals being *G. fascicularis* and *H. exesa*, and the LL adapted corals being *A. spongiosa* and *M. monasteriata.* The balance listed by Salih *et al.*, 1998) were both HL and LL adapted, representatives of this group being *S. pistillata, P. verrucosa, G. djiboutiensis,* and *E. lamellosa*. These grew under all lighting conditions (Figure 3.5). The species that were only LL adapted were encrusting corals that grew best under the blue and red spectrum lighting and suffered bleaching and mortality under the standard white fluorescent and yellow lighting (Figure 3.5). The significance of this relative to their greater dependence on photosynthesis has already been mentioned.

4.7 Feeding

Most studies conducted on coral nutrition have focused on autotrophy, focusing on their associated symbiotic zooxanthellae and nutrient uptake (Atkinson *et al.*, 1995; Ferrier-Pagès et al., 2000). Other works have focused on the effects of water flow on heterotrophic feeding (Johnson & Sebens, 1993). The feeding experiment in this study was aimed at assessing the effect of supplementary feeding on coral growth.

Tsuchida and Potts (1994) conducted an experiment on the effects of food on the sea anemone *Anthopleura elegantissima*. This anemone possesses zooxanthellar symbionts and thus has similarities with hermatypic corals in terms of its nutrition. Despite considerable evidence for autotrophic feeding on algal photosynthate, host

cnidarians almost always retain morphological and behavioural attributes for holozoic feeding (Yonge, 1930a,b; Sebens, 1981a, 1982a,b; Zamar, 1986). Tsuchida and Potts (1994) found that anemones given food increased in size, while unfed anemones decreased in size even when exposed to light. This provided evidence that these organisms rely on active feeding rather than autotrophy.

Corals rely more on the symbiotic autotrophy (Ferrier-Pagès *et al.*, 1998) than the above example, but they do retain the ability to feed actively. This suggests that they use this mode of feeding to some extent. The morphological differences between corals (ranging, in terms of polyp size, from large to almost inconspicuous) suggests that their reliance on active feeding is variable. Corals with large (*B. merleti* and *G. fascicularis*) or extended (*A. spongiosa* and *G. djiboutiensis*) polyps make use of active feeding more than the encrusting corals (e.g. *M. monasteriata* and *G. planulata*). This was supported by the results in this experiment as the exsert corallite and submassive corals increased in growth significantly with the addition of feeds (Fig 3.4, Table 10 and Section 3.8). *G. fascicularis* polyps actively removed *Artemia* from the surrounding water while the encrusting corals (e.g. *M. monasteriata, G. planulata* and *P. speciosa*) demonstrated no visible responses to the addition of feeds (pers. obs.). *B. merleti*, *A. spongiosa* and *G. djiboutiensis* were observed taking particles of yeast and soya flour from the water (pers. obs.).

On coral reefs, SPM constitutes a diverse food source derived from a variety of origins including detrital matter (Marshall, 1965), re-suspended sediment (Larcombe *et al.*, 1995), coral mucus (Coffroth, 1990) and excretory products from other animals (e.g. from fish; Meyer & Schultz, 1985). In addition, such particles are subject to colonization by bacteria and microalgae, which increases the organic value of the food (Riley, 1963; Wotton, 1988). It was therefore assumed that the corals in this study were ingesting the feeds to some extent, but this was difficult to determine or quantify. The aim of the experiment, however, was to determine whether or not the addition of certain feeds had an affect on growth. It was therefore possible to deduce that the addition of certain feeds did increase the growth rates of the nubbins. The nubbins in the feeding experiment were presented with a two-fold advantage in that they were exposed to light during the day and feed supplementation in the evening. Predation on micro-organisms and particulate matter could be an important trophic link providing a positive energy budget for the symbiotic association of zooxanthellae in corals (Ferrier-Pagès *et al.*, 1998).

Porter (1976) proposed a model in which corals with small polyps and a large colony surface:light exposure ratio (encrusting corals) rely mainly on zooxanthellar photosynthesis for their energy while corals with large polyps and a relatively low surface:light exposure (branching, submassive and exsert corallite colonies) rely heavily on zooplankton capture. This study supported this theory in that the branching corals, and those with large polyps grew faster in the feed supplemented regimes (Figure 3.4).

It was possible to increase the growth of the corals with the addition of feeds, without the corals being compromised in terms of light reduction as they were fed at dusk. Heterotrophic nutrition is known to occur even if the colonies can satisfy their carbon metabolism via photosynthesis and zooplankton feeding seems to supplement autotrophic nutrition (Ferrier-Pagès *et al.*, 1998).

4.8 Current Flow, Feeding and Light

Scleractinia are exposed to a wide range of current flow regimes in nature that, together with morphology, could affect the ability of corals to capture zooplankton and other particulate materials (Sebens *et al.*, 1998). It was possible to increase the growth of the corals by manipulating a single factor (such as feeding), but by combining the optima of all the factors, it was possible to increase the growth rate even further.

Polymorphism is found in corals and was originally explained by simple cause and effect relationships. For example, the flattening of colonies in deeper water to form plate-like morphologies (Goreau, 1959; Dustan, 1975) was attributed to photosynthetic acclimation through the enhancement of light capture by symbiotic zooxanthellae (Graus and Macintyre, 1976). Further studies indicated that skeleton morphology could influence particle capture (Helmuth and Sebens, 1993; Johnson and Sebens, 1993), mass transfer of nutrients (Patterson, 1992a,b; Shashar *et al.*, 1993; Lesser *et al.*, 1994; Helmuth *et al.*, 1997a,b) and tissue attachment (Brown *et al.*, 1983). No polymorphism was observed in this study.

When presented with the optimal current flow, feeding and lighting conditions, all the corals grew faster. The presence of a bi-directional current facilitated the distribution of the feeds, making the particles more accessible to the corals. Short-term turbidity only occurred after the introduction of feeds at dusk and did not affect the peak photosynthetic period. The optimal lighting promoted enhanced photosynthetic activity. The corals thus benefited from the enhanced combination of the optimal feeding and lighting regimes. The relatively small differences in the response of the corals to their

environmental conditions was reflected in their morphology, leading to their separation into the morphological groups used in this study.

4.9 Implications of coral propagation

4.9.1 Structure of a commercial operation

The initiation of a commercial venture requires a sustainable supply of corals to meet the demands of the market. The time taken from fragmentation to sale should also be considered in order to make the venture viable.

As no minimum size proved necessary for coral nubbin survival (Section 4.2), the initial size of fragments could be based purely on economic criteria. In other words, the smaller the fragments, the greater the number of propagules that would be obtained. It would, however, take longer for the fragments to reach marketable size. The latter may also be variable and would be largely dependent on the market.

The conditions required to enhance the growth of coral nubbins and calculate the time-frame and quantities of fragments required to ensure a sustainable and viable business can be determined. The results from the growth experiments can be used to determine which regime would be most cost-effective. For example, the combination regime may produce the highest growth rates but at a higher cost (given the need for lights, feed and pumps), whereas the addition of feed alone may produce a relatively high growth at a reduced cost. Regression equations of growth were calculated using the mean cumulative mass changes of the corals over time (Tables 47-50). These equations can be used to determine how long a nubbin of a particular species, in a given regime, will take to reach the size required.

For example: If an *H. exesa* nubbin initially weighed 5g and a nubbin of 50g was required for sale, it would be possible to calculate the time required for it to attain this mass:

The percentage mass increase is 900%

Mass gain (g) = 45g

These values are then substituted into the regression equations to determine the time required to reach the required size, where the mass gain (g) = y and time (weeks) = x:

In the bi-directional current flow regime:

y = 0.0298x – 0.1589.....(Table 47)

x =1515 weeks

In the soya flour feeding regime:

y = 0.0935x - 4.301.....(Table 48)

x = 527 weeks

In the blue light regime:

y = 0.7063x – 0.825.....(Table 49)

x = 65 weeks

In the combination regime:

y = 0.9267x – 2.583.....(Table 50)

x= 51 weeks

The time for the nubbin to reach marketable was reduced by a factor of thirty in the combination regime (lag period included).

The design of a commercial venture would depend mainly on the scale of the intended operation. Holding tanks with stable environmental parameters and a history of successful and preferably rapid coral growth would be essential. The best system would be to have holding tanks for the parent colonies and then separate species-specific grow-out tanks for the nubbins, allowing the flexibility to provide optimal lighting, water movement and feeding requirements for each species. The system used in this study would be ideal for the purpose of propagation, with only a few modifications. It is suggested that, to optimise a grow-out system, a series of long, shallow, backlit tanks stacked above each other (on shelving) would be the best design. This would provide considerable holding space for the corals, with sufficient accessibility. It would also reduce the floor-space required, which would reduce the cost of the venture considerably.

The manpower required would be minimal as, once the nubbins are fragmented and placed in the tanks, very little maintenance is required. The nubbins need only to be monitored for signs of necrosis, disease and polyp quality and the system monitored for water quality and to ensure that water is flowing continuously, the powerhead

pumps are working and the lights are operational. In the case of feed being required for a particular species, this would be added daily at dusk. The system would best be constructed indoors for the control of temperature and lighting. It is difficult to outline a specific structure for a commercial venture as a great deal would depend on the scale of the operation.

4.9.2 Reef rehabilitation

While coral propagation in South Africa would primarily be aimed at providing the aquarium market with indigenous coral specimens, reef rehabilitation would be a secondary consideration for the future. The recovery of damaged reefs can be accelerated by rehabilitation techniques (Westmacott *et al.*, 2000). Innovations in coral propagation could enhance these efforts. In doing so, it is essential that the scale involved in reef rehabilitation be considered. In the eventuality of reef rehabilitation being required, the application of coral propagation techniques would vary from country to country. South Africa's coral reefs are unique in that they are relatively deep and are subjected to strong currents that make working conditions difficult (Schleyer, 1999). In places such as the Philippines, the reefs are shallow and have many sheltered areas where coral propagation can be done *in situ* (Heeger *et al.*, 1999). A South African situation would thus entail tank-based coral propagation, requiring the most effective and efficient propagation techniques.

Reef restoration is generally expensive and not always successful (Westmacott *et al.*, 2000). Managers must therefore assess the situation carefully before initiating such a programme. This can only be done with sufficient knowledge of the processes and techniques involved. A number of factors must be considered:

- The objectives of the rehabilitation project must be established. Are the reefs being restored for biodiversity conservation, tourism, fishing, protection from coastal erosion or purely for research?
- The scale of the project. The extent of the degraded area must be determined. Is it a specific location (i.e. anchor scar or boat grounding), a section of the reef, or an entire reef complex? If the degraded area is large, careful thought must be given as to where the rehabilitation efforts would best be directed in terms of current patterns, exposure to wave action, sources of pollution and turbidity.
- The cost of the project must be evaluated.

- The viability of the proposed method must be considered: Which method will be most cost-effective at the site? The method selected must not cause additional damage to the reef.
- The project should continue long enough to ensure its success and to monitor the results.

The propagation of corals could also aid in maintaining the biodiversity of corals. The deterioration of coral reefs, due to both anthropogenic and natural disturbances, coupled with the slow growth rate of corals, makes these habitats vulnerable. This could result in a loss of biodiversity. The propagation of corals could assist in maintaining the gene pool and thus biodiversity.

Coral bleaching has been mild in South Africa (Schleyer, 1999) but is increasing and is a problem elsewhere in the world (Schleyer & Griffiths, submitted). The protection of coral reefs from such threats as global warming will require research and management strategies to ensure their survival. The study of genetic variability and resilience in corals to the stresses that cause coral bleaching will provide information as to the availability and suitability of resistant clones and whether propagation could ensure a sustainable supply for this purpose. This can best be accomplished in tank cultures and management strategies for reef rehabilitation.

4.10 Regulation

The existing threats to the biodiversity, structure and function of coral reef ecosystems are compounded by the unsustainable extraction of corals for aquarium and ornamental specimens, jewellery, and construction materials (U.S. Department of State, 1998). Coral extraction is driven by international demand, with most collecting occurring in the Indo-Pacific region. For over three decades, the Philippines was the major supplier of ornamental coral for the international market. A ban on coral exports from the Philippines caused a change in the market, with Indonesia becoming the largest supplier in the 1990's (U.S. Department of State, 1998). Since 1995, coral exports from Fiji, Mozambique, Taiwan and Tonga have increased. Concern about over-exploitation led to the listing of most coral species in CITES Appendix II, which requires that exporters file trade records including information on the destination, taxa, and quantities exported.

Corals are protected in South Africa. This is mainly due to the fact the coral reefs in this country are relatively deep and under conservation. South Africa does not, currently, have a problem with the harvesting or poaching of corals *per se*, but if the

market for corals increases a problem could arise. The propagation of corals provides an alternative supply for this market. However, certain management actions would need to be implemented to ensure that local corals are not poached and sold as propagated corals. The export of live corals is not as much of an issue as the sale of indigenous corals in the country of origin. It is more difficult to prove that an indigenous specimen was not collected illegally. A permit system for the sale of propagated corals should thus be structured in a similar fashion to the CITES export permits, requiring information on the buyer, seller, taxa, sizes (weights), colour and quantity.

The onus, however, should rest neither on the retailer nor the buyer to regulate the movement of corals. This must remain a governmental responsibility. If an effective permit system is established whereby the mass of the coral, the species and a reference code are provided, there is nothing further the retailer could do to ensure that the trade in corals remains legal. Problems in this regard should be surmountable and the advantages of opening a propagation market outweigh the likelihood of an increase in poaching if this alternative is not encouraged.

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