UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

SCHOOL OF OCEAN AND EARTH SCIENCE

REPRODUCTIVE ECOLOGY OF A DEEP-WATER SCLERACTINIAN CORAL, *OCULINA VARICOSA* FROM THE SOUTH EAST FLORIDA SHELF

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DOCTOR OF PHILOSOPHY

JULY 2002

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Abstract

Reproductive ecology of a deep-water scleractinian coral, *Oculina varicosa* from the South East Florida Shelf

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Abstract:

The Ivory Tree Coral *Oculina varicosa*, forms extensive bioherms (reefs) of azooxanthellate colonies at depths of 70-100m along the edge of the Florida Hatteras slope. Deepwater *Oculina* reefs support invertebrate and fish communities as diverse as those of tropical coral reefs, and are a critical spawning habitat for a number of commercial fisheries species. A different morphological variant of *O. varicosa* inhabits the near-shore limestone ledges, and these shallow-water colonies were included in the study as an ecological comparison of conspecific populations exploiting different habitats, and as a preliminary reproductive model for the less accessible deep reefs. Colonies from the two populations were confirmed as conspecific using Internal Transcribed Spacer (ITS) sequences of the nuclear ribosomal gene from deep and shallow colonies.

Growth rates (skeletal deposition) of samples from both populations were measured under varying temperature and light regimes. The results showed a significant difference between the two populations under different light conditions. Temperature had no significant effect on growth however. This warrants further investigation, since photosynthetically enhanced calcification did confer an advantage to the shallow zooxanthellate samples, but temperature had no consistent effect on calcium deposition.

Oculina varicosa is a gonochoristic broadcast spawning species, with small eggs (<100μm) and a high fecundity of approximately 4,000-8,000 eggs per cm^{-2} of skeletal surface area for both populations. The gametogenic cycle begins in the early summer and spawning occurs during late summer and fall, with no obvious relationship to lunar or tidal phase. Histological analysis of gonad sections revealed concurrent gametogenic cycles in both populations; however, the deeper populations generally spawned later (September) than their shallow counterparts (July-August).

Embryos developed quickly (<6 hours at 25°C), producing small (160μm), active planulae, which swim to the water surface immediately on hatching. After 24-48 hours the planulae begin to swim throughout the water column, after a week they become demersal and settlement occurred 21 days after spawning.

Planktonic duration was integrated with hydrodynamic information to estimate larval dispersal potential. It appears that larvae not only have the potential to be transported between the deep reef tracts, but may also contribute larvae to near-shore zooxanthellate populations during summer upwelling events.

The deep banks have been under federal protection since 1984, but large areas of this important habitat have been destroyed. The objective of this study was to describe aspects of *O varicosa*, reproductive ecology and provide insight into recovery potential of the damaged reef. The coral produces many highly dispersive larvae, but re-colonisation of damaged areas is extremely slow. Three-dimensional modeling of larval transport, and molecular studies of clonal variability within the systems would provide valuable information on potential natural recruitment rates.

Acknowledgements

A mere thank you is insufficient to express my gratitude to Professors Craig Young and Paul Tyler for the incredible journey though my PhD.

During my tenure with Craig, I have seen and done enough to fill a dozen lifetimes. I have chased urchins across the Bahamian benthos, wrestled mussels from a brine pool in the Gulf of Mexico and ultimately, gazed in awe though a steamy porthole at the wonders of the hydrothermal vents. I shall always be indebted to Craig not only for these memories, but also for teaching me that there really are 36 hours in a day, and you can fill them all if you try hard. To work with Prof. Tyler was a pleasure; his sunny disposition in the face of just about everything, and his visits for a chat and a cup of tea were always greatly appreciated.

I would like to extend a special thank you to Dr. Chris Koenig of Florida State University. Chris has been extremely supportive of my work on the *Oculina* Banks, and it he who initiated this project (for which I now forgive him). Chris has an infectious positive attitude, even under arduous conditions. I am grateful for his help with collections, for involving me in the *Oculina* restoration effort, and for always making me feel that my work was important.

During my travels I have met a great number of bright and talented individuals. I thank them all for their warmth, their humour, and their encouragement during those 'stochastic' events that confound our research efforts. Unfortunately many of these meetings were transient, but the memories will always remain. I would like to say big thank you to my special friends. To Tracy for listening to me grumble, for always helping me, and most of all for making me laugh. To Dan for his help with fieldwork and camaraderie. To Tammy for her compassion and her wonderful ability to put disasters in perspective and especially to Tony who was always willing dive in the lousiest conditions. Thank you for my data, for always making fieldwork fun, but mostly for being my friend. Thanks of course to Steve, my support through the hard times and my partner in the good ones.

This work is dedicated to my Mother, who always believed I could do it, even when I didn't know what or where 'it' was. She has tolerated my familial neglect with grace and this is what I am giving her in return. I hope you think it worthy Mum.

This research was partially supported by MARFIN Grant No. NA87FF0434

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Chapter 1: Introduction

1.1 Distribution of deep-water corals

The term coral 'reef' is usually applied to the high biodiversity hermatypic systems common to tropical waters. For the sake of clarification, the term 'reef' and bioherms will be used synonymously to describe the large structures created by deep-water scleractinian corals. Knowledge of the distribution of most of these deep-water corals is still relatively poor. Geological surveys reveal the presence of structure, but most of the information available on species composition and distribution of these ahermatypic 'reefs' has been gathered from deep-sea trawls (Cairns 1999). The spatial resolution from trawl samples is limited, as collections are integrated over the duration of the trawl, which precludes the discrete sampling protocol necessary for some research projects. There are relatively few species of reef forming deep-water coral species, and the biodiversity of scleractinians within each system is low, unlike shallow water tropical reefs. In many cases, the principal framework builder is a single species such as *Lophelia pertusa, Goniocorella dumosa, Solenosmilia variabilis* and *Oculina varicosa*.

Lophelia pertusa is probably the best known species of deep-water scleractinian. It has a wide distribution, which extends throughout the North Atlantic on both the East and West coastlines, especially in the North East Atlantic around Scotland and Norway. It also occurs extensively in the Eastern Gulf of Mexico and the Caribbean. *Lophelia* has been recorded from depths of 3600m near the Mid-Atlantic Ridge (Bett 1997) to 50m in the Fjords of Norway (Rogers 1999). *Goniocorella dumosa* is widespread in the New Zealand region and occurs most commonly between 300-400m (Cairns 1995). *Solenosmilia variabilis* has a cosmopolitan distribution with the exceptions of continental Antarctica and the North and East Pacific. *Solenosmilia variabilis* is uncommon in the New Zealand region, but is the major framework species of the South Tasmanian seamounts and occurs in large quantities on the Heezen fracture zone in the South Pacific (54°49'S 129°48'W). The depth range for this species is 220-2,165m (Cairns 1982, 1995). *Oculina varicosa* has the most limited distribution of any known deep-water scleractinian, and as it is the subject of this dissertation, it will be dealt with in detail later in this chapter.

Several other species often make a significant contribution to the reef framework; these include *Madrepora oculata, Desmophyllum dianthus, Dendrophyllia cornigera,* and *Enallopsammia* species. *Madrepora oculata* is probably one of the most widespread of the azooxanthellate deep-water corals that contribute to the formation of deep-water reefs. It usually co-occurs with one or more other species, which dominate the reef system. In the North East Atlantic, it often forms the reef framework with *Lophelia pertusa*. In the waters around New Zealand *M. oculata* occurs with *Goniocorella dumosa* (Cairns 1995), where it is most commonly found between 150–500m. *Desmophyllum dianthus* has a worldwide distribution apart from Antarctica and the northern boreal Pacific. In the North Atlantic, *D. dianthus* contributes to the structure of *L. pertusa* reefs, and in the New Zealand region, forms a significant part of *G. dumosa* reefs. Large quantities of *D. dianthus* have also been dredged from off the coast of Chile between 300-800m (Cairns 1982) which may indicate the presence of a deep-water reef formed by this species. In the eastern Atlantic it is found between 80m – 1,694m, depth, in the western Atlantic between 290m – 1,635m, depth, and off New Zealand from 25m – 1,750m, depth (Zibrowius 1980, Cairns 1982). *Dendrophyllia cornigera* forms a component of *Lophelia pertusa* reefs south of 52°N in the North-East Atlantic (Zibrowius 1980). This species is found at depths between 30m – 850m in the Mediterranean, Celtic Sea, Bay of Biscay, Cape Verde Islands, Azores and the Atlantis and Great Meteor Seamounts (Zibrowius 1980; Castric-Fey 1996). The genus *Enallopsammia* is composed of four valid species. *Enallopsammia rostrata* occurs worldwide except Antarctica and the East Pacific, *E. profunda* occurs in the Western Atlantic, *E. pusilla* from the Sulu Sea and *E. marenzelleri,* from the North East Atlantic, Indonesia and New Zealand (Cairns 1995).

Azooxanthellate coral banks or reefs are features that are found on the shelf break and upper bathyal zone on the continental margins around most of the world; they are also frequently found on pinnacles, ridges and seamounts. All of these features interact with the overlying currents to create accelerated flow fields; mean current measurements over peaks of slopes and ridges were twice those of mid slope measurements (Genin et al. 1986). Suspension feeders dominate these high flow habitats, including cnidarians such as gorgonians, antipatharians, actinarians, pennatulids and hydroids, as well as sponges, ascidians and echinoderms (Genin et al. 1986; Boehlert and Genin 1987, Grigg et al. 1987, Kaufmann et al. 1989). Other

research has shown increased numbers of suspension feeders in regions of high current on the vertical walls of a caldera (Tunnicliffe et al. 1985), and along the edges of terraces on seamounts (Moskalev and Galkin 1986). A strong negative correlation has also been noted between coral abundance and sediment cover (Genin et al. 1986, Grigg et al. 1987). The high flow probably delivers food to the benthic organisms, flushes waste products, cleans the substratum and may increase larval supply to the area (Grigg 1974, 1984). The massive Sula Ridge corals rely on a rich supply of zooplankton from the fertile surface waters (Freiwald and Wilson 1998). The need for a planktonic food supply is common to all azooxanthellate corals, so the distribution of deep-water scleractinians may also be influenced by food distributions rather than simply physical flow characteristics.

1.2 Anthropogenic impact on deep-water coral reefs

Since the decline of traditional shelf fisheries, fishermen around the world have begun to target previously unexploited deep-water species of fish (Creasey and Rogers 1999). Many deep-sea fish have life history characteristics that make them particularly vulnerable to overfishing (Rogers 1994; Creasey and Rogers 1999). That such species are being increasingly targeted by fishing fleets is therefore an issue of concern in itself (Aikman 1997; Koslow 1997). It is apparent that some of these commercial deep-sea fishing operations are taking place in areas in which deepwater coral formations exist such as offshore banks, pinnacles and canyons are areas often subject to vigorous hydrodynamic regimes. The upper areas of continental slopes are a habitat particularly favoured by *Lophelia pertusa* (Wilson 1979b), and the occurrence of orange roughy (*Hoplostethus atlanticus*)(Gordon and Hunter 1994) makes them attractive to fishermen, despite the obvious difficulties in trawling such rough ground. Deep-sea fishing is taking place on the continental slope in the North East Atlantic, and high resolution side-scan sonar surveys show evidence of trawl scars on the seafloor (Kenyon et al. 1999). Seabed photographs from the same area show freshly overturned sediments. As *Lophelia pertusa* occurs in these areas, it has probably been impacted by deep-sea fishing (Rogers 1999).

Southern Tasmanian Seamount reefs composed of *Solenosmilia variabilis* have been extensively damaged by trawl fisheries for orange roughy and oreos (*Pseudocyttus maculatus, Allocyttus niger*) (Koslow and Gowlet-Jones 1998). These coral reefs are

similar to other deepwater coral reefs in that they are formed primarily by one or two species of coral, but have a very diverse associated fauna. The reefs on seamounts that have been subject to trawl fishing have been largely destroyed, leaving only finely broken up coral rubble or sand. The substrate of the most heavily fished seamount was >90% bare rock at most depths, and early records from these trawl fisheries recorded a large coral by-catch (Koslow and Gowlett-Jones 1998). The animal communities on impacted seamounts were characteristic of the coral associated community but with a drastically reduced biomass (80-85%) and species richness (60%). It is likely that these communities will take a long time to recover, if they recover at all.

Oil drilling would impact nearby coral reefs with heavy input of silt and sediment, which could smother the fragile polyps. Scouring from suspended sand also damages tissue and oil seepage impacts coral metabolic processes (Rogers 1999). Oil toxicity effects vary from direct mortality, to reduced growth, tissue damage, disruption of cell structure, damage to stimuli response and feeding behaviour and excessive mucus production where chronic contamination has occurred (Loya and Rinkevich, 1980). The effects of oil toxicity can be particularly dramatic on coral reproduction and on the behaviour and survival of coral planulae. Oil contamination can cause coral polyps to prematurely release their brooded planula larvae (Loya and Rinkevich, 1979). Over a longer time, oil contamination can decrease fecundity (Rinkevich and Loya, 1979a,b; Guzmàn and Holst, 1993) or cause complete reproductive failure (Peters et al., 1981). Such effects can last for years after an oil contamination event, especially where the oil is contained within sediments (Guzmàn and Holst, 1993). Planula larvae may be particularly susceptible and chronic oil pollution can prevent planula settlement (Loya and Rinkevich, 1980). As with other forms of exploitation in the deep-sea, the development of deep-sea oil production is taking place without data on many aspects of deep-sea benthic ecology.

1.3 Distribution of the genus Oculina

At the end of 1999, there were 1335 species of scleractinian recognised and 50.3% of them were azooxanthellate species (Cairns 2001). Many of these were deep sea species which have only recently become accessible, but there is a disproportionate amount of research dedicated to the zooxanthellate species, leaving those without symbionts somewhat neglected. The species of interest in this research, *Oculina*

varicose, is facultatively zooxanthellate, but the deepwater populations usually live without symbionts.

Species of the genus *Oculina* have successfully exploited a wide range of different habitats, from the shallow sub-tropical and temperate waters of the Eastern USA (*Oculina varicosa, O. diffusa* and *O. arbuscula*) to the Caribbean (*O diffusa, O. tenella*). Members of the Oculinidae have also been reported from the Galapagos (*O. profunda*), Bermuda (*O. valenciennesi* and *O. diffusa.*) as well as in deep-water (*O variocsa* and *O. vigosa*). One species (*O. patagonica*), has even invaded the Mediterranean Sea from Argentina (Fine and Loya 1994).

Large areas of Bermuda inshore waters are populated by *Oculina* coral communities (*O. diffusa* and *O. valenciennesi*). These corals cover the floors of the inshore waters from approximately 8 to 20 m depth, in turbid sheltered lagoon areas (Meischner et al. 1981). Twenty-one species of scleractinians belonging to 9 genera, including *Oculina* sp. have been found in the last 3 years along the shelf edge and slope off La Parguera, Puerto Rico at 20-50m depths. (Armstrong 1980).

Live colonies of the oculinid genus *Archohelia*, previously known only from the fossil records, were discovered in shallow water off the coast of Central Queensland, Australia. *Archohelia rediviva* was found in 3.5 metres of sheltered, turbid water by the shoreline of a rocky island having no fringing coral reef. The coral is subject to strong tidal currents and large changes in salinity (Wells and Alderslade 1979).

Oculina diffusa and *O. varicosa* also live in turbid, and deep water (*O varicosa*). They have exploited marginal habitats that hermatypic corals would be unable to tolerate. *Oculina varicosa* (Leseur) occurs at depths between 5m and 100m on the Atlantic coast of Florida. It is facultatively zooxanthellate and can tolerate a wide range of environmental conditions including low temperature, high turbidity and low light levels. The shallow populations have been reported as far north as South Carolina, and as far south as the Florida Keys and the Caribbean however the taxonomy of the *Oculinidae* of the Western Atlantic is somewhat confused. (Dr. S. Cairns personal comm.), so the distribution descriptions may be incorrect. These unique deep water *Oculina* reefs (Figure 1.1A) exist only on the shelf edge off eastern Florida, and stretch over 90 nautical miles (167km) from Fort Pierce to Daytona (Macintyre and Milliman 1970; Avent et. al 1977; Reed 1980).

1.4 **Oculina varicosa** ***of the south east Florida shelf***

1.4.1 *Shallow-water* Oculina *habitat*

The shallow near-shore reefs are composed of a series of limestone ledges that run parallel to shore. The first reef line occurs at 3m depth and is dominated by subtidal populations of *Phragmatopoma lapidosa,* or 'wormreef'. The second and third reef lines occur at 4-5m and 6-9m respectively; they support abundant populations of *Oculina varicosa* colonies, which are small (<30cm diameter) and discrete, without the complex tiered structure prevalent in tropical systems (Figure 1.1B). The *Oculina* species that inhabit these ledges are facultatively zooxanthellate. For most of the year, the majority of the colonies are rich dark brown, fading to a golden brown in the summer if the water is warm and clear for extended periods of time. Occasionally, a colony will remain completely or partly bleached, but seem to suffer no adverse consequences of losing the symbionts.

The most common scleractinian species on these near-shore ledges are *O. varicosa* and *O. diffusa*, with occasional occurrence of *Cladocora arbuscula, Siderastrea siderea* and grouped polyps of *Phylangia* sp. and *Astrangia danae*. The visibility on the shallow ledges ranges from 1m to 10m, but during periods of phytoplankton bloom or storm activity, can be reduced to a few centimeters. Particles from the water column are deposited during calm conditions, producing a thick layer of fine sediment on the substratum. Temperature variation over the shallow habitats is quite high (7-29.5°C), partly from seasonal changes, but also because of periodic upwelling events that cause cold deep-ocean water to inundate the near-shore habitats (Smith 1981). These shallow habitats support a large number of fish and invertebrates, which take refuge in the crevices of the limestone matrix. Very little research has been conducted on the community ecology of these habitats, which are under pressure of impact from beach replenishment projects and high nutrient water discharge from the Indian River Lagoon.

1.4.2 *Deep-water* Oculina *bioherms*

In deep water, *O. varicosa* forms massive bushes of fragile colonies, creating continuous tracts of reef on the slopes and tops of pinnacles, similar in structure to deep-water *Lophelia* reefs. The deep *Oculina* bioherms are the only known monospecific coral banks that occur on the North American continental shelf at less

than 200m depth. Similar bioherms of *Lophelia pertusa* and *Enallopsammia profunda* exist in deeper waters at the base of the Florida Hatteras Slope and the Blake Plateau (Reed 2000). All of these deep-water 'reef-building' corals have a similar growth pattern, which is unlike that of typical shallow water species. As the colony increases in diameter, the tangled outer branches block water flow to the core of the colony, and this stagnation causes the inner branches to die. Bioerosional processes weaken the dead coral branches, which eventually break and the colony falls apart. The outer living branches continue to grow, and new recruits may colonize the exposed dead core. As this process continues over thousands of years, these mounds and pinnacles can reach tens of meters in height, with the live coral forming a cover over the unconsolidated dead coral debris below.

The deep shelf-edge *Oculina* reefs form natural spawning grounds for commercially important populations of gag (*Mycteroperca microlepis*) and scamp (*M. phenax*) grouper. They also serve as nursery grounds for juvenile snowy grouper (*Epinephelus niveatus*), and feeding grounds for these and many other commercial fish species including black sea bass (*Centropristis striata*), red grouper (*E. morio*), speckled hind (*E. drummondhayi*), Warsaw grouper (*E. nigritus*), amberjack (*Seriola* sp.), red porgy (*Pagrus pagrus*) and red snapper (*Lutjanus campechanus*) (Gilmore and Jones 1992, Reed 2000). Apart from the valuable fisheries species, the deep water reefs also support very rich communities of invertebrates; faunal diversity on the *Oculina* banks is equivalent to that of many shallow tropical reefs. Over 20,000 individual invertebrates were found living among branches of 42 small *Oculina* colonies, yielding more than 350 different species (Reed 1992, Reed and Hoskin 1987, Reed and Mikkelson 1987, Reed 2000), many of which are important food sources for animals at higher trophic levels.

In 1984, the South Atlantic Fishery Management Council designated 92 square nautical miles of the deep *O. varicosa* banks as a Habitat of Particular Concern (HAPC) because of their importance to the life histories of many commercially valuable species. Mobile fishing gear and anchoring were prohibited to protect the delicate *Oculina* thickets. In 1994, the HAPC was closed to all bottom fishing for 10 years. This area was designated the Experimental *Oculina* Research Reserve or EORR (Figure 1.3). In 2000 the HAPC area was extended to encompass 300 square nautical miles of the shelf edge, which includes most of the known areas of the *Oculina* reefs (Figure 1.2). Parts

Figure 1.1A. Deep (80m) *Oculina varicosa* reef showing azooxanthellate colonies with associated gag grouper (*Mycteroperca microlepis*) and scamp grouper (*M. phenax*). Colonies are >3m in height.

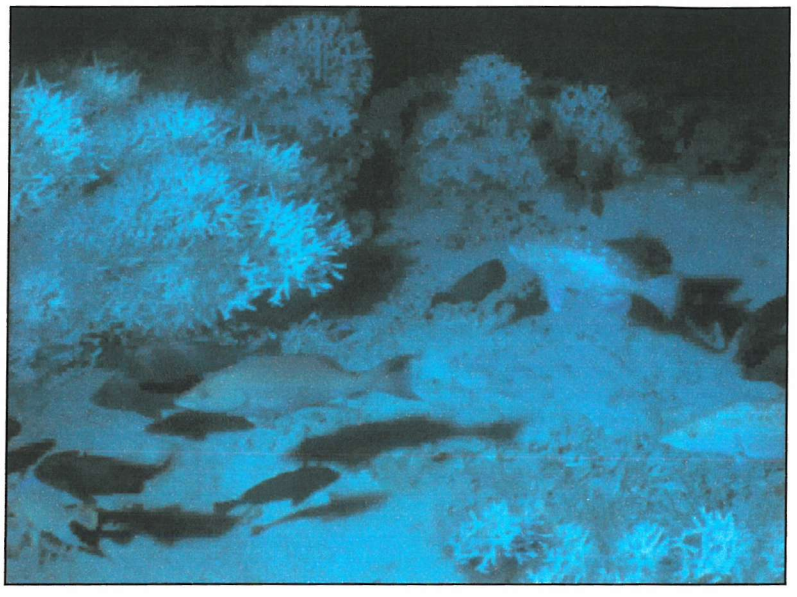


Figure 1.1B: Shallow (6m) zooxanthellate *Oculina varicosa* colonies. Colony diameter is approximately 30cm

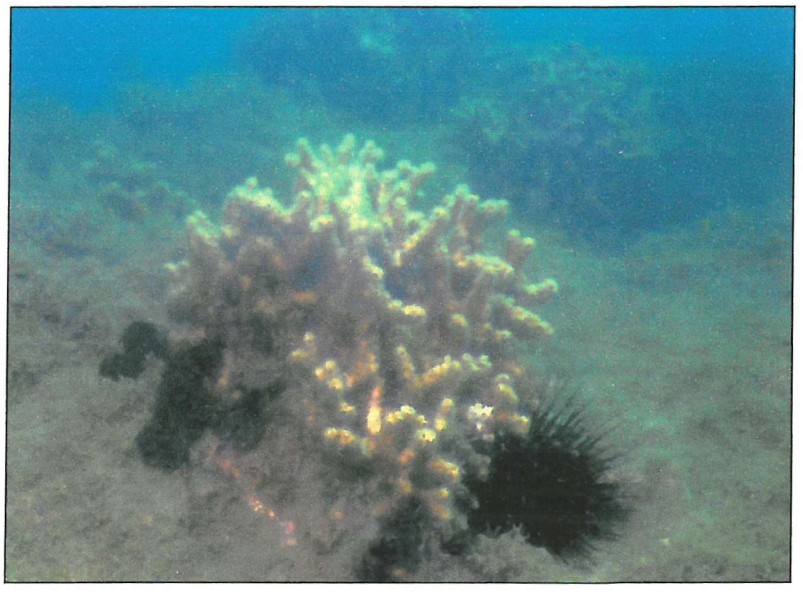


Figure 1.2. Map of the Cental Atlantic Coast of Florida, showing the original Experimental *Oculina* Research Reserve, the previously unprotected area used as a control site for the EORR and the expanded OHAPC boundary.

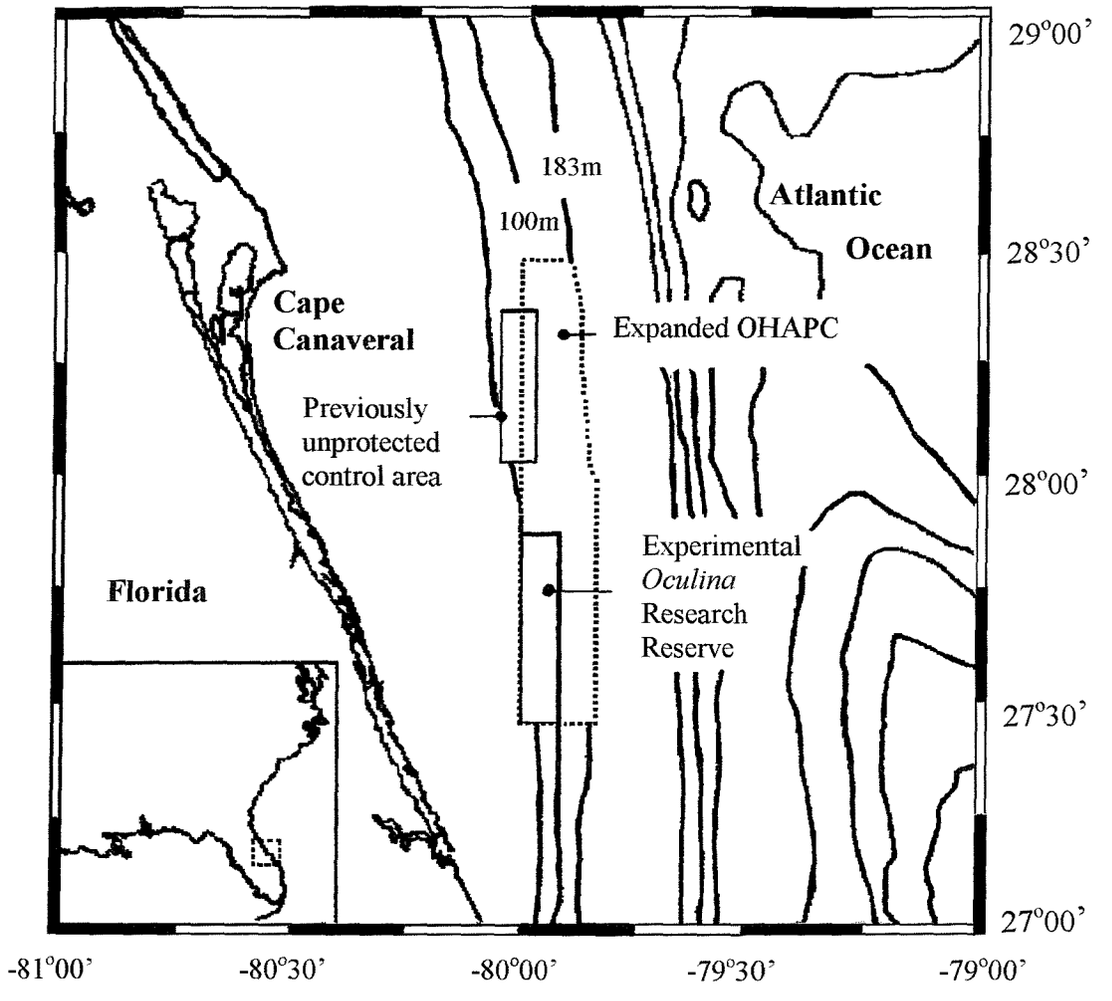
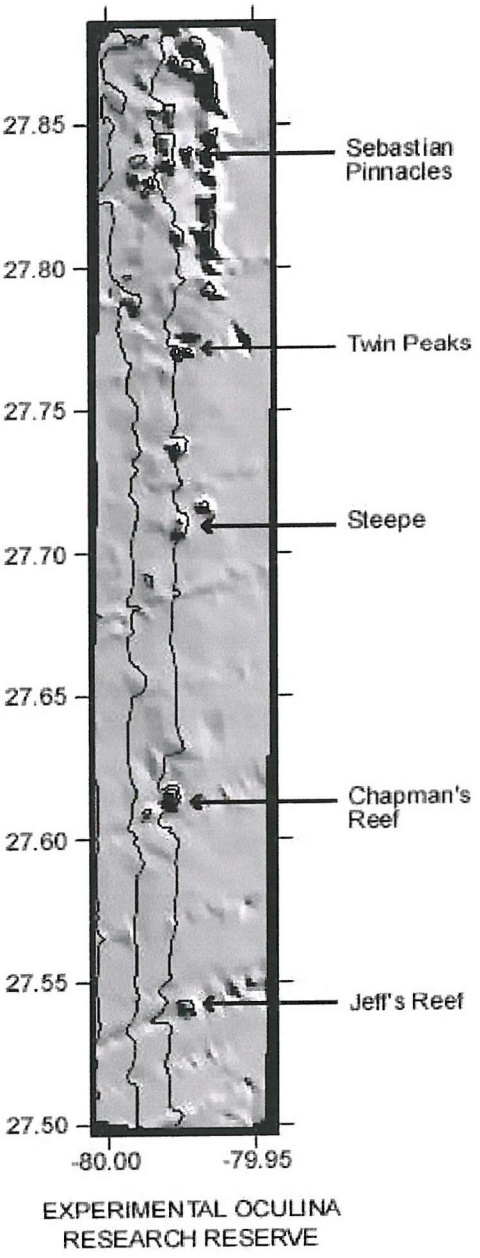


Figure 1.3. Shaded relief map of the EORR showing experimental locations.



of the *Oculina* reefs have been extensively damaged and the previously thick bushes of coral have been reduced to rubble. The exact causes of the damage are open to speculation, but natural processes, disease, unfavorable environmental conditions and destructive fishing practices may all have been contributing factors.

1.5 Coral species boundaries

Scleractinian corals pose problems for species discrimination for several reasons, and it is not surprising that confusion still exists within coral taxonomy. Species may be reproductively isolated, but exhibit much overlap in the relatively few morphological characters used to tell them apart (Lopez and Knowlton 1997). The prevalence of phenotypic plasticity (Willis 1985), slow rates of change in molecular markers (Romano and Palumbi 1996) and long generation times may all contribute to the difficulty in recognizing coral species. Species boundaries may be muddled by extensive and complex hybridization and speciation events, which over time create patterns of reticulate evolution (Veron 1995). The simplicity of coral morphology and development may contribute to successful hybridization that would not be possible in more complex organisms. Conventional taxonomic methods have been augmented in recent years by molecular techniques, which have been used to describe genetic relationships between corals without using morphological features (Stoddardt 1984, Romano and Palumbi 1996, Lopez and Knowlton 1997, Ayre and Hughes 2000, Rogers [in prep]).

1.6 Molecular biology of corals

Although extensive genetic diversity has been suggested for scleractinians, there is only a limited amount of data available on coral genetics. Several studies have been carried out using allozyme electrophoresis. This technique provides insight into coral population structures, but is limited by the relatively small number of testable loci and therefore the limited level of precision. More recently developed techniques based on nucleotide divergence have the potential to further our understanding of the genetic structure of coral populations. Two major difficulties have traditionally been associated with application of DNA based techniques to studies of hermatypic corals. One was the difficulty in preparing high molecular weight DNA as the removal of tissues from polyps shears the DNA strands and renders it useless for digestion and cloning. The

other is the presence of symbiotic dinoflagellates in most coral tissues. The first limitation has been removed with the development of the polymerase chain reaction, which can amplify small amounts of DNA, negating the need for high molecular weight DNA. The second problem may be circumvented by using DNA extracted from symbiont-free gametes for the analyses.

Molecular techniques have been applied extensively in recent years to taxonomic and ecological questions in anthozoan biology. Several studies have used electrophoretic techniques to analyse genetic structure in populations. For example allozyme electrophoresis was used to examine the prevalence of asexual reproduction in a population of deep-sea epizoic anemones *Amphianthus inornata* from 2,200m in the Rockall Trough, North Atlantic Ocean (Bronsdon et al. 1997). Allozymes were used to analyze gene flow among populations of the soft coral *Sinularia flexibilis* on the GBR (Bastidas et al. 2001) and to examine genetic structure of six reef complexes along the South African coastline (Ridgeway et al. 2001). Genetic differences between samples of an intertidal zoanthid, *Zoanthus coppingeri*, from the Great Barrier Reef (Burnett et al. 1995), and clonal structure in the scleractinian coral *Pocillopora damicornis* in Southern Japan (Adjeroud 1999) were also examined using allozyme electrophoresis.

Genetic fingerprinting techniques such as Randomly Amplified Polymorphic DNA markers (RAPD), Random Fragment Length Polymorphism (RFLP), and more recently the PCR-based Amplified Fragment Length Polymorphism (AFLP) have frequently been used to examine genetic makeup of anthozoan genes. RAPD markers were used to assess rates of natural self-fertilization in two common hermaphroditic Caribbean corals from the Florida Keys, *Favia fragum* and *Porites astreoides* (Brazeau et al. 1998), and RAPD analysis, together with comparison of reproductive traits, distinguished a new species of Caribbean gorgonian, *Plexaura kuna* from its congeners (Lasker et al. 1996). Genetic analysis using AFLP was used to determine the role of sexual reproduction for the production of larvae in the hermaphroditic soft coral *Parerythropodium fulvum fulvum* (Barki et al. 2000).

Techniques to analyse ribosomal and mitochondrial DNA and the development of PCR have expanded the possibilities for population genetic analysis. Eukaryotic ribosomal DNA (rDNA) codes for ribosomal RNA, and comprises several hundred tandem repeat copies of the transcription units, interspersed with transcribed and non-transcribed spacer sequences. Many of the rRNA genes are conserved across and

within taxa, but others such as the internal transcribed spacer (ITS) regions are hypervariable in many species (Hillis and Dixon 1991). The application of ITS analysis to coral systematics will be discussed further in chapter 2.

1.7 Patterns of genetic structure in marine populations

Genetic patterns may be generated by historical or recent processes, acting independently or together, at different spatial and temporal scales. An important limitation of genetic approaches that use spatial distribution of allele frequencies for inferring gene flow is the assumption that populations are in evolutionary equilibrium (Knowlton 2000). The pattern of genetic structure at equilibrium depends on many factors such as larval behaviour, circulation patterns, distribution of suitable habitats and geographical scale of sampling (reviewed by Benzie 1999). The ability of larvae to disperse should be the primary determinant of genetic structure (Palumbi 1995). At one extreme, a species with highly dispersive larvae should be panmictic over all except the largest spatial scales. At the other extreme species with non-dispersive larvae should show patterns of isolation at all but the finest scales. Strong relationships have frequently been found between larval dispersal ability and geographic division (Ayre 1990, Palumbi 1994, 1995). However there are cases where dispersal potential only weakly predicted genetic structure (Hellberg 1994, Gomez et al. 2002) These exceptions highlight our limited understanding of how ecological processes generate genetic structure in marine populations.

Interpretation of the genetic structure of populations may be confused by historical events. For example, genetic breaks may occur where no modern barriers exist (Meeus Tde. et al. 1990), genetic continuity may occur where there are no existing dispersal pathways (Weber et al. 2000). Patterns of genetic structure can become very complex after disruptions to gene flow occur repeatedly throughout the species range. Life history strategies with rapid generation times and highly dispersive larvae are conducive to re-establishing equilibrium between migration and gene flow relatively quickly. In those species with non-dispersive larvae and slow generation times, equilibrium recovery may only occur on time scales greater than events that create gene flow disruptions. Hellberg (1995) estimated that the time to reach equilibrium in *Balanophyllia elegans* (a temperate solitary coral with limited larval dispersal) is approximately 40,000 years. Because this is longer than the time between

major climatic changes, this and other similar species, may never reach genetic equilibrium. In conclusion, although there may be a clear relationship between patterns of genetic diversity and geographical distribution at some spatial scales, historical events may contribute to the genetic patterns over different scales, complicating the interpretation of genetic analysis.

1.8 Coral Energetics

1.8.1 Energy resources of tropical corals

Scleractinian corals, like many other marine invertebrates, may contain endosymbiotic zooxanthellae, which fix carbon to support their respiration and growth requirements. Excess carbon is translocated to the host (Muscatine et al. 1983, Davies 1984), principally in the form of glycerol (Muscatine 1967, Trench 1974). The translocated carbon either can be used for host respiration, growth and reproduction, or is released from the animal (Crossland et al. 1980, Muscatine et al. 1981, Edmunds and Davies 1986, Rinkevich 1989, McClosky et al. 1994).

The potential importance of translocated carbon to the host has been well-documented in tropical cnidarian-algal symbiotic relationships (Davies 1984, Muscatine et al. 1984, Steen and Muscatine 1984, Edmunds and Davies 1986, Kremer et al. 1990, Davies 1991, Day 1994, McClosky at al. 1994). The majority of these studies suggest that in shallow water zooxanthellae supply carbon in excess of that required for host respiration. Such autotrophy may be important in oligotrophic waters where energy supplied by heterotrophy may be insufficient to meet host metabolic requirements (Glynn 1973; Porter 1974; Sorokin 1990).

There is evidence in the shallow tropical coral, *Montastrea cavernosa*, that metabolic balance between respiration and photosynthesis, correlates with polyp morphology and behaviour together with zooxanthellae density. (Lasker 1981). The diurnal morph has greater zooxanthellae densities than the nocturnal morph and correspondingly greater rates of gross primary production. Respiration and gross primary productivity (GPP) are both increased by expansion of polyps. Small changes in the size or number of polyps while not affecting the size of the whole colony affect respiration rates, and so colonies with low GPP are characterised by morphologies and behaviour that reduce the maintenance cost. The balance between metabolic demands and energy derived from primary production is further confused by heterotrophic food.

1.8.2 Light enhanced calcification

Various models have been proposed for the mechanism of enhancement of calcification (reviewed by Johnson 1980). The models suggest that photosynthesis by zooxanthellae increases the bicarbonate ion concentration inside the coral tissues, which enhances calcium precipitation. Additionally, the zooxanthellae through he process of nutrient uptake, may remove calcium carbonate crystal inhibitors such as phosphate from calcification sites. Photosynthesis may provide energy for active calcification or promote the synthesis of the organic matrix upon which calcium carbonate is deposited. The uptake of calcium in corals by diffusion has been ruled out for kinetic reasons (Chalker 1976) and is almost certainly a process requiring metabolic energy. Despite the abundance of models, the link between photosynthesis and calcification has yet to be determined (Muller-Parker and D'Elia, 1997). Deep-water scleractinians do not have the benefit of photosynthetically assisted calcium deposition, but can form massive calcified structures. This argues for a system designed to maintain calcium precipitation, with photosynthesis providing a boost rather than playing a vital role in skeleton formation.

1.8.3 Energy resources in non tropical corals

There have been a few attempts to quantify the potential importance of zooxanthellae-fixed carbon to temperate symbiotic cnidarians (Fitt et al. 1982, Schick and Dykens 1984, Tytler and Davies 1986, Stambler and Dubinsky 1987, Verde and McClosky 1996). Temperate symbioses offer an interesting comparison to their tropical counterparts. Bleaching or loss of zooxanthellae in most tropical corals is an indication of extreme physiological stress, but in many temperate Scleractinia, zooxanthellae may be light limited because of seasonally decreased day length and temperature, and high water turbidity. Many temperate corals (including members of the genus *Oculina, Madracis* and *Astrangia*) appear pale or white in poorly lit habitats, as well as darkly coloured with abundant zooxanthellae in shallow well-lit habitats (Miller 1995). In low light conditions, carbon fixation levels may not be able to meet the metabolic demands of both symbiont and host. Davy et al. (1996) modelled carbon budgets for temperate anthozoan-dinoflagellate symbioses, and concluded that under favourable conditions, two of the four anothzoans studied could potentially be autotrophic. However, under annual average field conditions the contribution of zooxanthellae to the animal's

respiratory carbon requirements (CZAR), would be insufficient to meet host metabolic demands, and would need to be supplemented by heterotrophy. Temperate cnidarians may therefore be more dependent on heterotrophy that those living in clear tropical waters. With the exception of the north to mid Atlantic species, *Astrangia danae* (Jaques et al. 1983) the importance of light and feeding to symbiosis and growth in temperate scleractinia is largely unknown.

There also exist, a number of completely azooxanthellate species that live in deep cold, aphotic zones of the world's oceans; these corals derive their energy from heterotrophy alone. Deep water corals are typically ahermatypic, but certain species (such as *Lophelia pertusa, Oculina varicosa, Goniocorella dumosa, Solenosmilia varibilis*) form large 'reef' frameworks, which support extensive invertebrate and vertebrate communities (Zibrowius 1980, Reed 1981, Cairns 1982, Koslow and Gowlett-Jones 1998).

1.8.4 Corals in marginal habitats

Richmond (1987) studied reproduction and growth in different populations of *Pocillopora damicornis*, and discovered that reproductive output is low or non-existent in those populations living in what has been considered marginal habitat. Conspecific colonies in optimal habitat produce larger numbers of propagules than in those from marginal locations; however somatic growth in the former colonies is significantly lower. Ward (1995) found two types of co-existing colonies of *Pocillopora damicornis*, which although they exhibited no morphological differences, had different patterns of energy allocation to growth and reproduction. Those that produced planulae had significantly lower growth rates than those that were sterile or just produced sperm. Ward (1995) also studied the effects of damage on growth and reproduction of *P. damicornis*, with the result that high levels of damage caused the animals to channel energy into growth and repair rather than reproduction. The energy budgets of conspecific populations may therefore be dictated by the environmental conditions under which they exist.

1.9 Asexual Coral Reproduction

Coral polyp division occurs either when one polyp divides into two (intra-tentacular budding) or when a new polyp forms at the colony margin or between two

adjacent polyps (extra-tentacular budding). If the new polyps remain attached to the parent colony, this is simply colony growth; however, if one or more of the polyps detaches and forms a new separate colony it is considered colony reproduction (Richmond 1996). Asexual reproduction in corals occurs through several different kinds of processes, such as fragmentation, polyp expulsion and production of asexual planulae (apomixis).

1.9.1. Fragmentation

Fragmentation is simply the process whereby fragments of existing colonies break off and re-establish themselves elsewhere. Fragmentation is common in branching corals and thin plate corals (Highsmith 1982), where wave action, storm surge, clumsy scuba divers or other forms of physical impact may detach parts of the colony. The fragment may land on a solid surface, attach, and form a new colony or it may tumble around on the benthos and fail to recruit. In coral reef environments that suffer regular physical disturbances, the fragmentation of established colonies is believed to be important in the recruitment (re-colonization) of the local area by branching and free-living corals (Highsmith 1982, Harrison and Wallace 1990). High rates of wave action and sedimentation, for example, would favour fragmentation over the settlement and metamorphosis of sexually produced larvae (Highsmith 1982)

1.9.2 Coral polyp expulsion

This is a mode of asexual reproduction of massive and encrusting corals, which enables them to sustain local populations in disturbed environments. Kramarsky-Winter et al. (1997) observed polyp expulsion in *Favia favus* from the northern Red Sea, and *Oculina patagonica* from the Mediterranean coast of Israel. Individual polyps, including their calices lifted on elongated calcareous stalks before detaching and settling elsewhere. As the new propagule settles, its tissues spread over the stalk and onto the substrate and the polyp forms a new colony. In the laboratory, newly settled propagules of *O. patagonica* and *F. favus* developed and grew into new colonies within two months, and the area of polyp expulsion regenerated within two weeks (Kramarsky-Winter et al. 1997).

This phenomenon differs from the 'polyp bail-out' a process described for several branching pocilloporid species (Sammarco 1982, Richmond 1985). During

polyp bailout dying colonies release whole polyps from their calices. In contrast, polyp expulsion occurs in apparently healthy colonies and the polyp is released with the calix. In both cases, the expelled polyps are larger and developmentally more advanced than sexually produced planulae; this may be a form of size refuge, increasing the polyp's chances of survival in a sub-optimal environment.

1.9.3 Asexual planulae

Some species of coral can produce planulae asexually; for example planulae of *Pocillopora damicornis* were shown to be genetically identical to their brood parents (Stoddart 1983, 1986). Similar results were obtained from electrophoretic studies of *P. damicornis* from the GBR and in the azooxanthellate corals, *Tubastraea coccinea* and *T. diaphina* (Ayre and Resing 1986). Brooded planulae in the anemones *Actinia equina* and *A. tenebrosa* were also produced asexually (Black and Johnson 1979, Carter and Thorpe 1979). There are several theories that suggest mechanisms of sexual planula production. Gametogenic studies have produced conflicting reports however. Production of planulae in *P. damicornis* in Hawaii may develop by a process called apomixis, where larvae develop directly from unfertilized eggs (Martin-Chavez 1986), but studies on *P. damicornis* from Western Australia and the GBR suggest that gametes are not involved in the production of asexual planulae, since the planulae are sometimes produced prior to gamete maturation (Muir 1984, Stoddart and Black 1985). These planulae may develop from differentiation of adult gastrodermis or lipid storage bodies (Muir 1984). Further genetic studies are needed to determine the prevalence of asexual planula production in other corals. Those that produce larvae in the absence of developing embryos, for example *Astroides calycularis*, and those that both brood and broadcast spawn would be likely subjects for further research (Harrison and Wallace 1990).

1.10 Sexual reproduction in corals

1.10.1 Historical perspectives

Sexual reproduction in scleractinians has been studied for over 200 years, and probably the first scientist to document the process was Cavoloni, circa 1790 (Harrison and Wallace 1990), when he observed planulae of *Astroides* in the Mediterranean. Research continued primarily on brooding species, and the few observations of

broadcast spawning in corals (*Caryophyllia smithii*; *Astrangia danae*; *Mancinia areolata*), were dismissed as abnormal or disregarded by researchers (Harrison and Wallace 1990). By the early 1900s brooding had come to be accepted as the normal mode of scleractinian reproduction. Duerden (1902) quoted that "viviparity would appear to be the rule amongst corals" and in 1905 reinforced his opinion with "there is little doubt that all corals are viviparous" (Harrison and Wallace 1990). Throughout the first half of the 20th century, this misconception was supported by studies, which continued to concentrate on planulation of brooding species and did not investigate underlying cycles of gametogenesis. In many species of corals studied, planulae were never found, which led Connell (1973) and Stimson (1978) to suggest that some corals may spawn their gametes for external fertilization, rather than brood planulae. Broadcast spawning of gametes, followed by external fertilization was observed in a number of species in the 1980's (Kojis and Quinn 1981, 1982 a, b, Szmant-Froelich et al. 1980, Bothwell 1981, Fadlallah 1982; Fadlallah and Pearse 1982b; Babcock and Heyward 1986). It is now thought that broadcast spawning is the dominant pattern of sexual reproduction in scleractinian corals, with just 31 species being brooders of 196 species investigated (Harrison and Wallace 1990).

1.10.2 Patterns of sexual reproduction

Scleractinian reproductive patterns generally fall into one of four categories: gonochorism, protandrous or protogynous hermaphroditism and simultaneous hermaphroditism (Fadlallah 1983b). Hermaphroditism may occur within each polyp or at the colony level with male and some female polyps in the same colony. In addition, species may be broadcast spawners or larval brooders. The former tends to occur in large colonies with short annual spawning periods, whereas brooding often occurs in small colonies that occupy unstable habitats and have multiple planulating cycles per year, (Szmant 1986). There are also those that have mixed developmental mode, or that produce planulae asexually (Stimson 1978, Stoddart 1983, Schlesinger and Loya 1985, Willis et al. 1985). Hermaphroditic species have colonies or solitary polyps, which produce both male and female gametes during their lifetime. A species may be a simultaneous or a sequential hermaphrodite. The former produce testes and ovaries at the same time within an individual polyp or colony, and the latter develop ova and sperm at different times. Sequential hermaphroditism can be subdivided into three categories: *alternating*,

changing sex more than once; *protandrous*, maturing first as a male then becoming female and *protogynous*, maturing first as a female then becoming male. (Ghiselin 1974). When male and female gametes develop sequentially during a single reproductive season, the term *sequential cosexual* should be applied (Policansky 1982). Hermaphroditic broadcast spawners seem to dominate species studied to date, with gonochoric broadcast spawning being the next most common strategy. True sequential hermaphroditism has not yet been demonstrated in scleractinian corals (Policansky 1982, Fautin 1990, Harrison and Wallace 1990) although in some species, the production of sperm and eggs occur at different times within a colony to avoid self-fertilisation. Incomplete temporal sampling could mislead investigators to conclude that a species is gonochoric, whereas in fact it may be hermaphroditic.

Much of the work on sexual reproduction in scleractinians has been concentrated on stony corals from the Great Barrier Reef in Australia, with information also available from species in the Caribbean, Florida, the Bahamas, the Red Sea, Japan and Hawaii. There are still some parts of the world where scleractinian fauna is abundant, but little research has been done, for example, Indo-Pacific Oceanic Islands, Southeast Asia and African reefs (Harrison and Wallace 1990). Information on reproduction of either temperate or deep-water corals is also sparse, and in the few species that have been studied, there is no discernible trend in reproductive strategy. The solitary temperate coral *Caryophyillia smithii* is found in both deep and shallow waters off the southern coast of England. This is a broadcast spawning species with external fertilization and embryonic development, producing a planktotrophic planula larva (Hiscock and Howlett 1977, Tranter et al. 1982). A similar reproductive strategy was found in another solitary temperate coral *Paracyathus stearnsii* from central California, which spawns in early spring (Fadlallah and Pearse 1982a). Other cold-water solitary corals, such as *Balanophyllia elegans* (Fadlallah and Pearse 1982b), and *Balanophyllia regia* (Fadlallah 1983a, Kinchington 1981) have internal fertilization. They release fully developed planulae, which may either swim or crawl for short periods as larvae before settlement and metamorphosis. Understanding of reproductive mode provides a fundamental basis for any work on larval retention, dispersal potential and genetic exchange between populations.

1.10.3 Gonad Development

Although scleractinians do not have true organs, the sites of development for testes (spermaries) and ovaries are generally referred to as gonads. In most corals the gametes develop within the mesenteries, surrounded by mesoglea and gastrodermis. There are some species (e.g. *Stylophora pistillata, Pocillopora damicornis, P. verrucosa*) in which the gonads develop on stalks attached to the mesentery, enveloped by mesogloea and gastrodermis (Fadlallah 1983b).

Gonad development begins when primordial germ cells, develop in the mesenterial gastrodermis (Szmant-Froelich et al. 1980, 1985, Delvoye 1982, Wyers 1985). Primary oogonia (stage I) are recognised by the relatively large nucleus and small amount of cytoplasm. These cells proliferate by mitotic division and migrate from the gastrodermis into the mesogloea of the mesenteries. They undergo meiosis to form primary oocytes (stage II) which begin to undergo vitellogenesis. The nucleus enlarges to form a large germinal vesicle with a conspicuous nucleolus (Delvoye 1982). As oocyte development proceeds, the cytoplasmic volume becomes proportionately larger than the nucleus, and the eggs become irregularly shaped as they conform to mesenterial morphology (stage III). A vitelline membrane forms beneath the plasma membrane, and the germinal vesicle migrates towards the periphery of the cytoplasm at the animal pole (Harrison and Wallace 1990). The cytoplasm may become pigmented (e.g., red, pink, orange, yellow, purple) some weeks prior to spawning (Harrison et al. 1984, Babcock 1986). In the final stages of oocyte maturation (stage IV), the nuclear chromatin condenses (Szmant–Froelich et al. 1980), then the germinal vesicle disappears prior to fertilization (Rinkevich and Loya 1979a). In a number of scleractinian species some oocytes are resorbed or undergo fusion during oogenesis (Delvoye 1982; Wyers 1985), and may serve a nutritional function for the remaining developing eggs. Unspawned or unfertilized oocytes are also resorbed in some species (Szmant-Froelich et al. 1980, Rinkevich and Loya 1979a). However, the phenomenon of oocyte resorption is poorly understood. Factors such as influence of exogenous factors on oocyte degeneration, which eggs are sacrificed, and how resorption occurs, are still unknown (Harrison and Wallace 1990). Unfertilized scleractinian eggs typically have an outer membrane with microvilli, a cortical layer composed of various vesicles of varying structure, mitochondria, crystalline inclusions and yolk granules (Schmidt and Shafer 1980, Szmant-Froelich et al. 1980, Harrison 1988a). The yolk granules

occupy most of the ooplasm and probably consist mainly of lipids, since the caloric value of the eggs is similar to that of pure lipid. (Harrison 1988a, Rinkevich and Loya 1979a).

Male germ cells originate in the same way as females; they are first observed as groups of very small cells, resembling enlarged interstitial cells, gathered in the gastrodermis (Delvoye 1982, Szmant-Froelich et al. 1985). These cells then enter the mesogloea and the early testes are enlarged by proliferation of spermatogonia by mitotic division (Szmant-Froelich et al. 1980, 1985, Delvoye 1982). The spermatogonia then differentiate into primary spermatocytes, which undergo meiosis to form secondary spermatocytes then spermatids (Delvoye 1982). As spermatogenesis proceeds, the cytoplasm and nuclear chromatin condenses, the mid-piece organelles are organised and the flagellum is formed (Harrison 1988b). These final stages of sperm development can take place very rapidly (Harrison et al. 1984).

Two types of sperm have been observed in scleractinian corals (Harrison 1985, 1988b). In one form the head and mid-piece are pear shaped or ovoid. Adjacent to the nucleus lie small vesicles that correspond in size and position to the "pro-acrosomal" vesicles of other anthozoans (Miller 1983); however a defined acrosome appears to be absent. These types are found in the hermaphroditic coral species of several families and may be characteristic of hermaphroditic corals in general (Harrison 1985, 1988b). A different sperm type is seen in other corals, with a pointed conical head and mid-piece, and in some species a large anterior process of unknown function extends from the apex of the nucleus. The structure might have an acrosomal function (Fadlallah 1983b), although it is an extension of the nucleus rather than a separate acrosomal structure (Harrison 1988b). This type of sperm has been found among gonochoric members of various coral families, but it is unknown whether this sperm structure is characteristic of gonochorism. The different types may be divided by sexual pattern but they also occur across different families. If defined by sexual pattern, there may be a functional basis for the difference in sperm morphology. Alternatively, sperm structure may have a phylogenetic basis, suggesting substantial divergence in the scleractinia or polyphyletic origins (Harrison 1985, Harrison and Wallace 1990).

1.10.4 Spawning and planula release

Coral species have a variety of reproductive schedules; some are seasonal, some periodic and non-seasonal, and some are continuous. Studies on coral reproductive cycles suggest that broadcast spawners have synchronous annual or seasonal gametogenesis and spawning (Hiscock and Howlett 1977, Bothwell 1981, Fadlallah 1981, Kojis and Quinn 1982 a, b). The majority of brooding colonial corals however have asynchronous continuous, gametogenesis (Fadlallah 1983b). Developmental cycles may culminate in short periods (number of days) of spawning or larval release (Kojis and Quinn 1981, Willis et al. 1985, Minchin 1993) or protracted release over a number of weeks (Fadlallah 1981, 1982). Many broadcast spawners release their larvae simultaneously (often correlated to some part of the lunar or tidal phase), as demonstrated by the mass spawning events documented for corals of the Great Barrier reef (Babcock et al. 1986, 1994). Brooders tend to have prolonged periods of planula release (Stimson 1978, van Moorsel 1980, 1981, Kitchington 1981), up to six months in the case of *Stylophora pistillata* (Rinckevich and Loya 1979b). In those species with continuous reproductive cycles, release occurs either continuously, or according to monthly lunar schedules (Fadlallah 1983b).

In corals, environmental factors, such as temperature, tides and lunar cycles have been correlated with reproductive activity but the precise mechanisms are not described. Temperature has been suggested as an important factor in the timing of reproduction, with different species spawning in response to a specific temperature range. For example, spawning in *Astrangia danae* (Szmant-Froelich et al. 1980) and planular release in *Rhizopsammia minuta* (Fadlallah 1983b) are correlated with maximum seawater temperature. Gametogenesis and gamete spawning in *Caryophyllai smithii*, are possibly controlled in the field by a gradual drop followed by a rise in seawater temperature, respectively (Tranter et al. 1982). In *Agaricia agaricites*, rising seawater temperature initiates planular release and leads to increase in planular production (Fadlallah 1983b).

Lunar schedules of spawning and planular release are well documented (see Fadlallah 1983b, Harriot 1983, Babcock et al. 1994). Planular release by *Pocillopora damicornis* was enhanced by solar UV radiation, and inhibited by its absence (Jokiel and York 1982) therefore reproductive cycles may have a solar component.

Stimson (1978) proposed that planular release by shallow-water reef corals during low tides facilitate retention of the planulae in the same habitat. Kojis and Quinn (1981) also suggest that the negative buoyancy of egg clumps of *Goniastrea australensis*, and the timing of their release in shallow reef areas facilitate their retention near parent colonies. Buoyant eggs produced by *Favites abdita* and *Leptoria phrygia* are not likely to remain near parent colonies but, since spawning by these two species takes place on neap tide, embryos are probably retained within the area (Kojis and Quinn 1982 b).

There is evidence that spawning in corals can be stimulated by gonadal material of the opposite sex; placing small pieces of male gonadal tissue in female polyps, and vice versa, induced spawning in *Astrangia danae* (Szmant-Froelich et al. 1980). Tranter et al. (1982) suggested that sperm released by the males of the broadcasting coral *C. smithii* can stimulate release of ripe eggs by the female (although the initial cue for sperm release was not defined). Chemical receptor cells in the oral epidermis of females are probably responsible for detecting sperm, which causes contractions of the oral disc and consequent expulsion of ova. Spawning coordination by males is probably not crucial for brooding species, but may be so for species of gonochoristic broadcasting spawners that must spawn simultaneously to achieve egg fertilization.

1.10.5 Embryonic development

Details of the early stages of development in brooding species are still poorly understood. When large amounts of yolk are present in the oocytes, cleavage may be superficial or epiblastic (Campbell 1974) and this yolk also makes histological examination difficult. The product of superficial cleavage is normally a stereoblastula, and instead of having a blastocoel, the embryo is filled with yolky vacuoles, surrounded by a single layer of epithelium. Gastrulation seems to occur by means of multipolar delamination (splitting of cells to form another layer) (Campbell 1974). Advanced embryos have a uniform continuous layer of epithelial cells in the ectoderm (Szmant-Froelich, et al. 1980, Fadlallah and Pearse 1982a). A middle lamella or mesogloea lies below the ectoderm and beneath the mesogloea, next to the coelenteron, is the endoderm. Endoderm cells are less regular that the outer epithelial layer. Further embryonic development occurs through differentiation of endoderm and ectoderm cells (Vandermeulen 1974, 1975). Some of these cells are strictly embryonic, but most of the

adult cell types (except the calicoblasts) are also present in the fully developed embryos. In the larva, further development occurs when a blastopore at the oral pole invaginates to form the oral pore and the stomodeum (gut lining near the oral pore, derived from ectodermal epithelium). The septa or mesenteries are derived from outfolding of the endoderm and mesogloea from the inner body wall into the coelenteric cavity. Mergner (1971) describes these later stages secondary larvae.

Corals such as *Astrangia danae, A. lajollaensis, Caryophyllia smithii*, and *Paracyathis stearnsii* release small eggs without large quantities of yolk, so early cleavage patterns can be readily observed. Szmant-Froelich et al. (1980) observed early development *of A. danae* in the laboratory, and noticed that the first divisions were radial but, subsequently blastomeres rearranged into a pseudospiral arrangement. A blastocoel was formed and the embryo became ciliated at the 128 cell stage (6-8 hours after fertilisation), after which they commenced swimming and became positively phototactic. Gastrulation took place when the larva elongated and produced an apical tuft; a similar structure was observed in *C. smithii* (Tranter et al. 1982) and several other anthozoan planulae (Martin and Koss 2001). The stomadeum was formed 24 hours post fertilization and the resultant larva is the smallest known (70μm) for a scleractinian larva.

Development in broadcasting lecithotrophic eggs may be obscured by large amounts of yolk, creating incomplete cleavage planes. Any differences in early development between broadcasting and brooding species are probably a consequence the differences in quantity of yolk in the egg, rather than a phylogenetic constraint.

1.10.6 Larval development and behaviour

The larval biology of scleractinins has been studied most intensively in warm tropical shallow species (Atoda 1951 a, b, Stimson 1978, Rinkevich and Loya 1979a, b, Bothwell 1981, Kojis and Quinn 1981 a,b, Fadlallah 1983 b). In contrast, there have been few studies of temperate species (Van Moorsel 1980, 1981, Fadlallah 1981, Tranter et al. 1982, Fadlallah and Pearse 1982b). Apart from the work on *Oculina varicosa* larval biology (Brooke and Young in submission), there has been nothing published to date on the larval biology of deepwater scleractinians.

Scleractinian planulae are elongate ciliated larvae with ectodermal and endodermal layers separated by a mesogloea and surrounding a central coelenteron. All scleractinia produce one form or another of this general planula; however the term planula may cover a wide range of developmental stages. For example, the ciliated stages of the externally developing embryo as well as advanced larvae with mouth, stomodeum and mesenteries (such as edwardsia and halcampoides) are all referred to as larvae. The definition seems to pertain to all free-living motile stages prior to settlement. A variety of adjectives have been used to describe scleractinian larvae; elongate, hemispherical, piriform (pear shaped), vermiform (worm shaped), barrel shaped, bullet shaped and rod like. These descriptions may apply to the larvae of any stony coral, since planulae are capable of changing their shapes. In general, a planula is elongate along the aboral axis, the aboral end being narrower than the oral end during locomotion. The larva swims backwards (i.e. aboral end anterior). The ectoderm of the aboral end is thicker and some workers have proposed that these cells may have chemically mediated sensory or adhesive functions (Lyons 1973, Vandermeulen 1974, Rinkevich and Loya 1979a, Kinchington 1981). Planulae generally have a ciliated ectoderm, but in larvae of some broadcasting species, an apical tuft has been observed at the aboral pole. This tuft was present in *C. smithii* for the first 2/3 weeks of larval life, then was replaced by a blister like swelling just before settlement (Tranter et al. 1982). Blisters or bumps at the aboral pole have also been described for *S. pistillata*, (Rinkevich and Loya 1979a), and they may have some role in the settlement process.

In brooding species the planulae are generally large (1-2mm) and energy rich when they emerge from the parent polyp. As well as the advantage of an internal food supply, large size may reduce the impact of predation by small planktivores. Some broadcasting species however produce small larvae (75-200μm) with little or no energy supplies. In *C. smithii*, larvae feed by trapping food particles on trailing mucous strings which they subsequently ingest (Tranter et al. 1982). Other circumstantial evidence for feeding comes from larvae of *Porites porites*, which became threadlike and incompetent when deprived of particulate organic material (Goreau et al. 1981). Development of an oral pore and stomodeum is documented in planulae of many species. Some planulae have been seen to extrude mucus and debris from the mouth, indicating that the coelenteron is open to the exterior via the mouth. The oral pole is also extensively

ciliated, therefore the apparatus for feeding appears to be present; however particulate feeding by planulae is still mostly unexplored. Another form of nutrition available to some planulae is translocation of photosynthates produced by symbiotic zooxanthellae. This has been documented by Richmond (1981) for larvae of *Pocillopora damicornis*. Hayes and Goreau (1977) observed uptake of material through the ectoderm of *Porites porites* via pinocytosis and, since adult corals have been seen to take up DOM, it is quite possible that planulae can do so also.

Scleractinian larvae may be defined by habitat; they are either planktonic or benthic. The planktonic planula is far more common, and although all coral larvae become demersal prior to settlement, benthic planulae are non swimming crawlers. So far only *Balanophyllia elegans* (Gerodette 1981) is known to produce strictly benthic larvae, although larvae of some other corals *(B. regia, Isophyllia dipsacaea, Astroides calycularis*) are suspected to behave similarly (Kinchington 1981, Fadlallah and Pearse 1982a).

Some common patterns exist in the swimming behaviour of coral planulae. Most are observed to swim in a rotary clockwise or anti-clockwise fashion around the anterior-posterior axis. They can attach to surfaces temporarily before final settlement and metamorphosis. Initial phases of swimming in many planulae are characterised by positive phototactic and negative geotactic responses (although most of the reports of phototaxis in the literature have not been rigorously tested as such, but are assumed from larval behaviour). Later larval stages are reported as being photonegative since they prefer shaded areas of the benthos, but all benthic larvae have to be either photonegative or geopositive prior to settlement in order to reach the substrate.

Although small planktonic larvae are unable to swim against the force of prevailing tides and currents, they can position themselves vertically in the water column in response to environmental factors or endogenous rhythms (Chia and Bickell 1978). Response to cues such as light, gravity and temperature are used to position the larvae in the water column. In the laboratory, brooded planulae exhibited a tendency to swim near the water surface initially, then moved throughout the water column or became demersal (Harrigan 1972, Lewis 1974, Szmant-Froelich et al. 1985). This has been attributed to changes in phototactic or geotactic response, although in most cases the tactic responses have not been tested. Kawaguti (1941) studied planular tactic responses in several species, and found evidence of phototaxis, geotaxis, thigmotaxis

(response to touch) and rheotaxis in slow currents. Responses differed between species and correlated with the quantity of zooxanthellae in the larva. Kawaguti (1941) concluded that the tactic responses of planulae play an important role in settlement and distribution of corals on reefs.

Most larvae of brooding corals hatch from large lipid-rich eggs and possess yolk in the coelenteron, which provides sufficient food to last through settlement and metamorphosis (Fadllallah 1983b). A small number of species (*Pocillopora damicornis*, *Seriatopora hystrix*, *Stylopora pistillata*, and *Favia fragrum*) release zooxanthellate larvae (Richmond 1981). Translocation of photosynthetic products has been documented for *P. damicornis* planulae, which allows the larva to remain planktonic for extended periods (Richmond 1981, Fadllallah 1983b). Larvae of *Fungia scutaria* begin to feed shortly after they are released, and take up zooxanthellae through the mouth as well as food particles (Schwartz et al. 1999). The symbionts appear in the endoderm a few hours later and begin photosynthesis. Another source of planula nutrition may be uptake of dissolved organic material, which is a strong possibility since adult corals have been shown to take up DOM (Chia 1972, Stevens and Schinske 1975). If a larva is able to exploit external energy, the duration of larval life and therefore dispersal potential may be increased.

1.10.7 Settlement and metamorphosis

Chemoreception and mechanoreception are probably the primary mechanisms that cue cnidarian planulae to initiate settlement (Chia and Bickell 1978). Swimming planulae exhibit interim phases of settlement behaviour, during which they settle temporarily on the substratum, then resume swimming. A combination of sufficient developmental progress and appropriate conditions are probably required for permanent settlement to occur. The aboral ectoderm of *P. damicornis* contains several different cell types, including three types of secretory cell, four types of cnidoblast cells and a series of flagellated cells (Vandermeulen 1974, 1975).

Scleractinian planulae can presumably discriminate between different substrata, however there are conflicting reports regarding the specificity and conditions required for settlement. Biological conditioning (bacterial and algal films) of the substratum is needed for settlement to occur in some corals (Harrigan 1972, Loya 1976 a, b). Other studies indicate that larvae will settle quite readily on unconditioned substratum (Lewis 1974).

Larval swimming and settling behaviours accounted for much of the adult distribution of the leaf coral *Agaricia humilis* in Bonaire (Raimondi and Morse 2000). Swimming behaviour placed larvae in shallow water, orientation caused preferential settlement on the undersides of surfaces, and chemosensory recognition of molecules associated with a specific crustose red alga, induced settlement and metamorphosis. The consequences of atypical larval behavior were severe and included decreased survivorship, growth, and ability to reproduce sexually. The tumor-promoting phorbol ester TPA induced metamorphosis in planulae of the Red Sea soft coral species *Heteroxenia fuscescens, Xenia umbellata, Dendronephthya hemprichii, Litophyton arboreum* and *Parerythropodium fulvum fulvum*, and the stony coral *Stylophora pistillata* (Henning et al. 1996). Extracts or presence of crustose algae have also been successfully used to stimulate metamorphosis in other species of scleractinian. For example, larvae of *Acropora millepora* were induced to metamorphose by six species of crustose or coralline algae (Heyward and Negri 1999). Metamorphosis also occurred when larvae were exposed to skeleton of the coral *Goniastrea retiformis* and to calcified reef rubble, demonstrating that metamorphosis was possible in the absence of encrusting algae. Goreau (1981) demonstrated spatial aggregation and preference for horizontal substratum by larvae of *P. porites.* Gregarious settlement occurs in response to stimuli of adults and juveniles of conspecifics (Doyle 1975).

1.11 Project objectives

The *Oculina* reefs are commercially valuable as a critical fisheries habitat, and ecologically important as a highly diverse and unusual ecosystem. Despite being placed under protection, the coral has declined so severely that the reefs are in danger of extinction. The overall objective of this research was to provide information on ecologically relevant aspects of *O. varicosa* biology and ecology that may be useful in efforts to restore the damaged areas and implement management strategies for the *Oculina* banks.

The first objective was to determine whether the deep and shallow populations were con-specific. If so, then the more easily accessible shallow water populations could be used to determine basic reproductive strategy of the species.

The next objective was to compare the growth rate and energetic status of the two populations. The deep reefs are azooxanthellate, therefore skeletal growth occurs

without photosynthetically enhanced calcification, and reduced growth rates could affect recovery from physical disturbance. The deep habitat would generally be considered 'marginal' for coral growth. Therefore it is possible that the corals are under physiological stress, which would make them more vulnerable to anthropogenic disturbance.

The final series of objectives concentrated on the reproductive ecology of the species. Information on reproductive strategy, gametogenic cycles, fecundity, larval biology and dispersal potential is vital to understanding how the ecosystem functions and how quickly it may recover from disturbance.

Chapter 2: Genetic analysis of *Oculina varicosa* populations

2.1 Introduction

2.1.1 The problem of coral species

High levels of intra-specific phenotypic plasticity in scleractinians are well-documented (Willis 1985, Willis and Ayre 1985). Inaccurately defined species boundaries, based traditionally on skeletal characteristics alone, may be partly responsible for this phenomenon (Miller 1994). Recent studies have challenged morphologically defined species boundaries with evidence from molecular genetics, reproductive biology or physiology of some coral species. These include *Stylophora* sp. (Gattuso et al. 1991), *Platygyra* sp. (Miller 1994), *Montipora digitata* (Stobart and Benzie 1994), and *Montastrea annularis* (Lopez and Knowlton 1997). Despite research efforts, it remains a challenge to set consistent and operationally meaningful species boundaries for scleractinian corals based on agreement of evidence from many biological characteristics (Miller 1994, Veron 1995, Harrison 1998). Temporal and spatial environmental heterogeneity increases genetic variability through differential selection pressures (Hedgecock 1994), and periodic disturbances such as storms and freshets may increase intra-specific variation through disruptive selection (Potts 1984). Although hermatypic corals occur mostly within 20m of the water surface, the three-dimensional marine environment over their range can vary greatly in temperature, light and current speed (Veron 1995). Species may be reproductively well isolated but exhibit much overlap in the characters used to tell them apart (Romano and Palumbi 1996). Phenotypic plasticity, slow rates of change in molecular characters, relatively recent origins and long generation times may all contribute to difficulty in recognising coral species. Species boundaries in corals may also be muddled because of extensive and complex patterns of hybridisation (Veron 1995). Many corals participate in mass spawning events that potentially provide numerous opportunities for inter-specific fertilization events. Progeny of such crosses often survive, perhaps because the relatively simple morphology of corals imposes few constraints.

Disruptive intermittent gene flow in coral populations may also have led to the process of reticulate evolution in scleractinian taxa. This process, comprising a network of diverging and converging species via alternating processes of speciation and hybridisation, may be facilitated by mixing of gametes by surface circulation, combined

with hybridisation of scleractinian "species". The expansion of species boundaries through reticulate evolution may have resulted in quasi-species or "metaspecies" with greater morphological and genetic variability than that typically seen in other species (Veron 1995). The reticulate patterns of evolution within and among scleractinian species are supported by morphological and genetic data of the genus *Acropora* (Odorico and Miller 1997, Diekmann et al. 2001). Shallow water colonies of *Stylophora sp*, previously described as *S. pistillata* (Esper 1797) in the Gulf of Aqaba (Red Sea) more closely resembled *S. mordax* morphometrically (Gatuso et al 1991). It is possible however, that the shallow and deep-water colonies of *S. pistillata* at this location were representatives of two ends of the morphological spectrum. Deep and shallow populations of *Oculina varicosa* from the East Coast of Florida differ greatly in their morphology, but have been accepted as con-specific from morphological identification.

2.1.2 Genetic analysis of deep-water anthozoans

Genetic structure and species delineation of shallow water corals is still confused, and virtually nothing is known about deep-water species. Deep-water research poses various logistical and economic constraints, since field sites are often remote habitats on the high seas, which are expensive and difficult to access. Consequently, less expertise is available for deep-water corals as the species are less well known and rarely studied. Traditional field techniques often cannot be applied to these systems, but ecological questions may be answered indirectly by using molecular techniques. For example, allozyme electrophoresis was used to examine genetic consequences of asexual reproduction in a North Atlantic population of *Amphianthus inornata* (Bronsdon et al. 1997); also, microsatellites and RAPD's (Randomly amplified polymorphic DNA) have been used to assess the clonal structure of *Lophelia pertusa* reefs (Rogers in prep). Genetic structure can provide useful indirect information about larval dispersal potential, relative contribution of sexual and asexual reproduction to reef structure, and intra-specific variability within the population, but results should be interpreted with caution taking historical events into account.

2.1.3 Application of ITS analysis to coral systematics

The 5.8S and internal transcribed spacer (ITS) regions of the rRNA transcription unit were used to study systematic relationships between tropical corallimorpharians (Chen 1996). AFLP and ITS sequence comparisons were also used to determine that the morphologically defined single species of Caribbean *Montastrea annularis* was actually a species complex (Lopez and Knowlton 1997). Intra-specific variability was described for the Pacific scleractinians *Pocillopora damicornis* and *Stylophora pistillata* using internal transcribed sequences of the ribosomal RNA gene (Takabayashi 2000). Phylogenetic relationships between the five most commonly recognized species of the scleractinian *Madracis* were investigated using the ITS1-5.8S-ITS2 region of the ribosomal DNA (Diekmann et al 2001). The evolution of hermaphroditism was investigated in 14 European and North American species of *Alcyonium*, using the ITS regions of the nuclear ribosomal gene to construct a molecular phylogeny (McFadden et al 2001). Smith et al. (1997) developed coral-specific PCR primers to amplify the ribosomal inter-genic spacer (IGS) region, which varies substantially within and between populations for a number of animal and plant species. Sequence variation of ITS-1 was used to study variation in ahermatypic corals from california (Beauchamp and Powers 1996). They found a wide range of sequence variation between species, very little intra-specific variation in *Paracyathus stearnsii*, but quite high numbers (14) of variable nucleotide sites in *Balanophylia elgans*.

Deep and shallow populations of *Oculina varicosa* display extreme morphological differences, which are probably the result of environmentally mediated phenotypic plasticity. Alternatively, the different colony types may represent two or more distinct species or sub species. The objective of this study was to use molecular techniques to determine whether the deep and shallow populations of *Oculina varicosa* are truly conspecific, which required a genetic technique with sufficient resolution to distinguish between inter and intra-specific genetic variation. Sequence analysis of the internal transcribed spacer regions (ITS-1 and 2) of nuclear ribosomal DNA was used in this study to compare *O. varicosa* populations from deep and shallow water habitats.

2.2 Objective

Determine whether the deep and shallow populations of *Oculina varicosa* are truly conspecific, using variation of the internal transcribed spacer (ITS) genes of nuclear ribosomal DNA (rDNA).

2.3 Materials and methods

2.3.1 Field collections

Two study sites with abundant populations of *O. varicosa* were selected from depths of 6m and 80m. A small branch (<5g) of *O. varicosa* was collected from each of 10 distinct colonies at each of the study sites. The shallow samples were collected from near-shore ledges approximately 500m offshore from Pepper Park (27°32.54'N, 79°58.73'W) in Fort Pierce, Florida. Corals were collected by hand using scuba, transported to the laboratory alive, and immediately transferred to 95% ethanol. Samples were taken from colonies 30-40m apart to avoid collection of fragmented clones. At the deep site, (approximately 27km north-east of the shallow site) known locally as Jeff's Reef (27°32.8'N, 79°58.8'W), collections were made using the manipulator claw of the Johnson Sea Link II research submersible. These deep samples were taken from colonies at least 30m apart, placed in individual containers, and preserved in 95% ethanol on board the ship.

2.3.2 Sample preparation

Tissue from five adjacent polyps was removed from each sample using a clean scalpel and placed in 2ml plastic tubes with 600µl of TNE buffer (see Appendix I for details of solutions). The tissue was ground with a disposable pestle and left for 5 minutes. The TNE was then replaced with a digestion solution consisting of 600µl of TNE, 10 µl of 20mg ml^{-1} proteinase K, and 24µl of 20% SDS. The sample was left to incubate for 3 hours at 55°C, inverting every 30 minutes to help dissolve the tissue. After incubation, 300µl of 6M NaCl was added to the sample. This was then mixed for 20 minutes by hand to precipitate proteins and other cellular debris. The solution was then centrifuged at 11,000 rpm. The supernatant was transferred to a new 2ml tube, 600µl of chloroform/isoamyl alcohol was added and the resultant solution mixed by hand for 1 minute. The sample was centrifuged at 11,000 rpm for 1 minute and the

upper aqueous phase was carefully removed under a fume hood, then transferred to another labeled tube. The lower phase was discarded. To precipitate the DNA, 750 µl of ice cold isopropanol, was added and mixed by hand for 2-3 minutes. The solution was then centrifuged at 11,000 rpm (4°C) for 25 minutes, the supernatant removed and the DNA pellet washed in ice cold 70% ethanol. The sample was centrifuged for another 5 minutes at 11,000 rpm (4°C). This last step was then repeated and the DNA pellet was air-dried, then re-suspended in 50µl of TE buffer.

Aliquots (2µl) of DNA extract were loaded into a 1% agarose electrophoresis mini-gel with ethidium bromide and allowed to run under 40mA current for approximately 1.5 hours. The gel was then photographed under UV to visualise the DNA. DNA was quantified using UviDoc system in conjunction with UviSoft DNA quantification software.

2.3.3 Polymerase Chain Reaction Amplification

Amplification of ITS-1 and part of the 5.8s region was carried out using the IS and A4 primers from Chen et al. (1996):

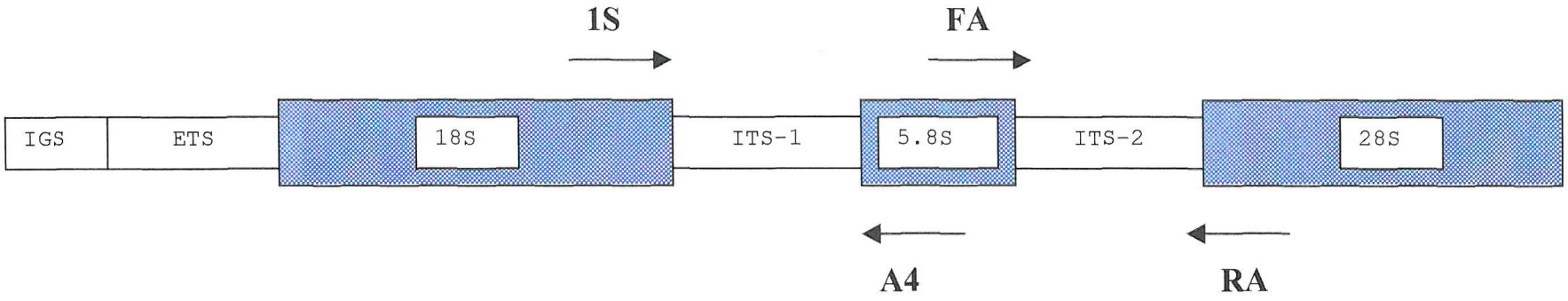
IS (5'-GGTACCCTTTGTACACACCGCCCGTCGCT-3')
A4 (5'-ACACTCAGACAGACATG-3')

Amplification of the ITS-2 region was carried out using primers ITS-2FA and ITS-2RA. These were modified by Rogers (in prep.) from primers A7 and 2SS from Chen et al (1996) for use on the ITS-2 region of *Lophelia pertusa* (Scleractinia, Caryophyllidae)

ITS2FA (5'-CGAATCTTTGAACGCAAATG-3')
ITS2RA (5'-ACAGACGGGGTTGTCACC-3')

The transcribed rDNA subunits (18S, 5.8S and 28S), the ITS-1 and 2 regions, and position of each primer are represented as a schematic in Figure 2.1. Each PCR (100µl reaction volume) contained 2ng (1µl) of DNA template, 50mM KCl, 10mM Tris HCl, pH 8.3, 3mM $MgCl_2$, 0.2mM of each dNTP, 50pMol of each primer and 1unit of Taq polymerase.

Figure 2.1: A schematic diagram of one repeat of eukaryotic ribosomal RNA. Relative locations of the PCR primers used in this study are shown. Key: IGS: intergenic (untranscribed) spacer; ETS: external transcribed spacer; 18S, 5.8S and 28S: ribosomal RNA subunit genes; ITS-1 and 2: internal transcribed spacers 1 and 2.



An initial denaturation step of 94°C for 2 minutes was carried out prior to addition of TAQ polymerase. Amplification of ITS-1 was then carried out using the protocol of Chen et al. (1996):

1 cycle at 95°C (3 minutes), 50°C (1 minute), 72°C (2 minutes).
4 cycles of 94°C (30s), 50°C (1 minute), 72°C (2 minutes).
25 cycles of 94°C (30s), 57°C (1 minute), 72°C (2 minutes).
1 cycle at 75°C (10 minutes).

Negative controls without template DNA were included, and samples of *Oculina diffusa* and *O. patagonica,* were included to compare variability between related species. *Lophelia pertusa* and *Madrepora oculata* are species of deepwater ahermatypic scleractinia and were included as outgroups in the study (sequences from A. D. Rogers, British Antarctic Survey, Cambridge).

The PCR products were purified using QIA-quick PCR purification kit (Qiagen) following procedures recommended by the manufacturers. The purified samples were then quantified for the cycle sequencing step using agarose gels with Ethidium bromide as before. Small quantities of DNA (2-5ng) were used with a Qiagen an Applied Biosystems Big-Dye terminator cycle sequencing kit, following protocols recommended by the manufacturers. This process amplifies a large number of fragments of the sequence of interest of different sizes. The terminal nucleotide of each fragment is fluorescently labeled with a fluorophore that emits a specific wavelength under laser excitation depending on the type of nucleotide (A,T,G,C). The DNA fragments were separated and then analysed on an Applied Biosystems Model 377 automated DNA sequencer (Perkin Elmer). The sequence fluorograms were observed using the programme 'Chromas' and analysed as described below.

2.3.4 Sequence analysis

The raw sequences were aligned using the programme Clustal W (www.ebi.ac.uk/clustalw) (Thompson et al. 1994) and BLAST searches for ITS-1 and 2 were performed using the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov). This program matched the experimental sequences to those in the database to verify that they represented the appropriate scleractinian genes, and to

determine the approximate location of the ribosomal and ITS sequences. Reverse sequences were complemented using WebAngis (www.angis.org.au) and checked against the original sequences in Chromas for accuracy. The edited sequences were aligned using the programme Clustal W on default settings. The aligned sequences were bootstrapped (1000 repetitions) using WebAngis and the genetic divergence between all sequence pairs was calculated using the Kimura 2-parameter model (Kimura 1980). Phylogenetic trees of each bootstrapped combination were created using the neighbour joining model (Saitou and Nei 1987), with the deep-water coral *Lophelia pertusa* as the outgroup root. Final consensus phylogenetic trees for both ITS-1 and ITS-2 were determined separately using WebAngis.

2.4 Results

2.4.1 ITS-1 sequences

PCR amplification of part of 18S, 5.8S and ITS-1 using Is/A4 primers resulted in DNA sequences of 744-1072 base pairs (bp). The ITS-1 region itself was approximately 230bp in length, but parts of the flanking ribosomal genes (18S and 5.8S) were also sequenced (Figure 2.2A). A Blast search on an ITS-1 sequence from a shallow *O. varicosa* sample produced the closest association (89% sequence similarity) with sequences from *Heliofungia actiniforms* (Scleractinia, Fungiina, Fungiidae). The *H. actiniforms* sequence included part of the 18S ribosomal gene, all of the ITS-1 region and part of the 5.8S ribosomal gene. A deep sample of *O. varicosa* sample produced a 91% identity with this *H. actiniforms* sequence (Genbank Accession Number: AF038906). Alignment of the ITS-1 sequences of *O. varicosa* samples (Figure 2.3A) shows heterogeneous regions between base pairs 19-26, 113-116, and 153-156. Within these regions, 10 base pair locations show insertion/deletion mutations, five transitions and three transversions. There are also isolated incidents of insertion/deletion (12), transitions (4) and transversions (8) elsewhere in the sequences. Percent genetic divergence was low within deep colonies (mean=2.19, SD=2.43), within shallow colonies (mean=1.93, SD=1.27), and between deep and shallow colonies (mean=2.66, SD=2.18). The consensus tree from the manipulated data separated outgroups *Lophelia pertusa* and *Madrepora oculata*, from the other sequences. The remaining samples, including the congeneric *Oculina diffusa* and *O. patagonica*, show no species grouping

(Figure 2.4A). The numbers on the forks of the branches represent the number of times that arrangement occurred within the 1000 different trees generated.

2.4.2 ITS-2 sequences

PCR amplification of part of 5.8S, 28S and ITS-2 with FA/RA primers resulted in DNA sequences of 757-1198 bp. The ITS-2 region alone was approximately 200bp in length, but parts of the flanking ribosomal genes (5.8S and 28S) were also sequenced (Figure 2.2B). Few of the ITS-2 samples produced clean DNA sequences; many contained a large number of nucleotide positions that could not be identified and the samples were discarded. A blast search against a shallow *O. varicosa* ITS-2 sample produced an 86% similarity with Haplotype MB6 of *Plesiastrea versipora* (Scleractinia, Faviina, Faviidae). The *P. versipora* sequence included partial ITS-1, full ribosomal 5.8S and partial ITS-2 regions. A deep-water sample produced 84% similarity with haplotype B37 of *P. versipora* (Genbank Accession numbers: AF483789 and AF483796 respectively). Alignment of the ITS-2 sequences (Figure 2.3B) shows a higher level of heterogeneity than for the ITS-1 region, and is particularly prevalent in regions between base pairs 26-36, 69-78, 122-131, 169-179 and 216-221. There are a total of 18 deletion/insertion locations, 6 transitions and 11 transversions. Percent genetic divergence was low within shallow colonies (mean=5.3, SD=0.01), but slightly higher within deep colonies (mean=15.7, SD=0.05), and between deep and shallow colonies (mean=12.3, SD=0.08). The consensus tree created from the manipulated data (Figure 2.4B) placed *L. pertusa* on a separate branch from the remainder of the samples, which show no clear grouping by population. The congeneric *O. diffusa* was placed with the *O. varicosa* samples.

2.5 Discussion

2.5.1 Genetic variability

Recent studies have suggested that the extent of variability in ITS regions differ greatly between species of scleractinian corals. Sequence variation of up to 29% has been reported for *Acropora valida* across the ITS-1 region (Odorica and Miller 1997). At the other end of the spectrum, variability of only 4% was found in *Paracyathus stearnsii* (Beauchamp and Powers 1996). Analysis of four species of Pacific coral showed that variation in genetic divergence of the ITS-1 region was species specific:

Figure 2.2: Sections of PCR amplification sequences from deep *O. varicosa* sample. A) Part of 18S, ITS-1 and part of 5S (primers 1S/A4) B) Part of 5.8S, ITS-2 and part of 28S (primers ITS2 FA/RA).

A

```
GCGCCGGATG CCGGAAAGTT GGTCAAACTT GATCATTTAG AGGAAGTAAA      50
                                                18S • ITS-1
AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT CATTACCGAT      100

GCAATAATCC AAAACAAAAA CGACTCCGTC GTTTTGTATA CCGTGTGAAC      150

TGTACCCAAC CATTTAGGGG TCGGCTGGTC CGTTAAATGG CGGTCGCGCG      200

TGACTCGATC GCGCGCGCTC CGCCAGCCCC GCCTGTGTTT TTTTTTATCA      250

ACATACGTTT ATACGATCTG ACAAACTGTG GCCGCTCTTC GGAGCGTGTC      300
                     ITS-1 • 5.8S
GCGAATTGAG CAAAAAACGA GATACAACTT TTGACGGTGG ATCTCTTGGC      350

TCACGCATCG ATGAAGAACG CAGCCAGCTG CGATAAGTAG TGTGAATTGC      400
```

B

```
                                  5.8S • ITS-2
CTCCCAGGAG CATGTCTGTC TGAGTGTCGG ATATCAATGC ATCGCACTTG      50

GCTGGCTGCC AAACCAAAGA GTGCGGCGTT GAGGCGTCAC GGCCAGCTTT      100

GCTTTGGCCG TGTCCCTTGA AGGTCAGTGA GACTCGCCGT GGCATGGGTC      150

ATCTACCTGC ACACTCGCGG AGGCTAAAAA CTGTTTTGTC CTTCGCGTGC      200
                                                ITS-2 •
.TCGGAGCGGC CTGCCCGGCG GGACGCAGAG GCGATAAAAA AGACAACACT     250
28S
TTAATTATCT TGACCTCAGA TCAGGCAAGG CTACCCGCTG AATTTAAGCA      300
```

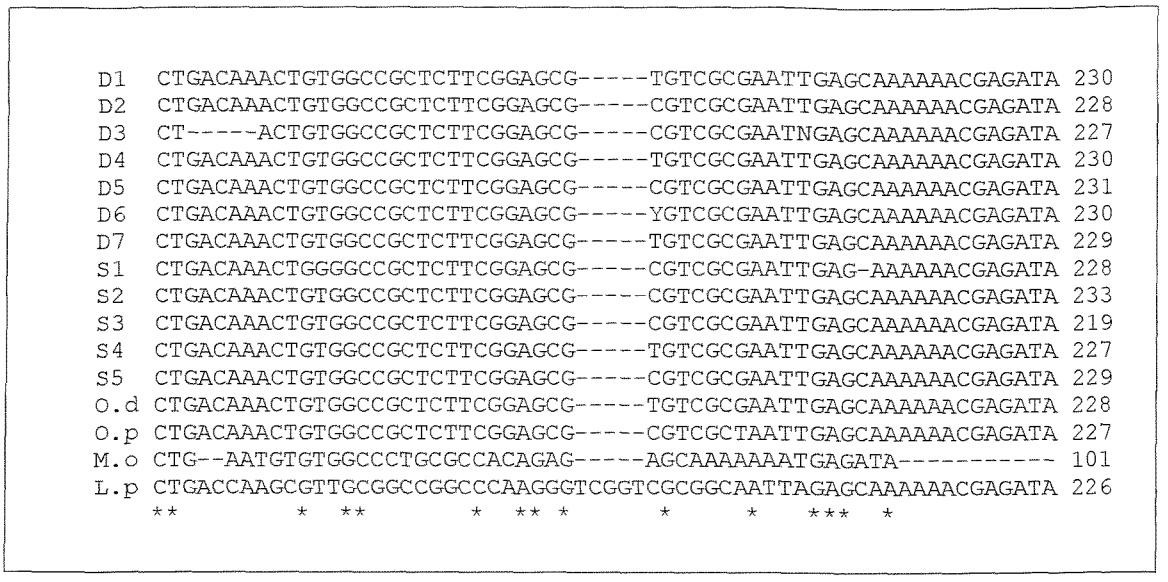
Figure 2.3. Alignment of A) ITS-1 and B) ITS-2 regions of coral samples using ClustalW. Species Key: M.o: *Madrepora oculata*; L.p: *Lophelia pertusa*; O.d: *O. diffusa*; O.p: *O. patagonica*; D1-D7: O. *varicosa* deep samples; S1-S5: *O. varicosa* shallow samples. Sequence codes: R=A/G; Y=C/T; M=A/C; K=G/T; S=G/C; W=A/T.

A

```
D1    ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
D2    ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTAGACCGTGTGAACTG 58
D3    ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
D4    ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
D5    ACCGATGCAATA-ATCCAAAAWCAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 59
D6    ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
D7    ACCGATGCAATA-ATCCAAAAACAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 59
S1    ACCGATGCAATA-ATCCAAAAACAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 59
S2    ACCGATGCAATA-ATCCAAAA-MCAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
S3    ACCGATG---TA-ATCCAAAA----AAACGACTCCGTCGTT---TGTACCGTGTGAACTG 49
S4    ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
S5    ACCGATGCAATA-ATCCAAAAAACAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 59
O.d   ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
O.p   ACCGATGCAATA-ATCCAAAA-CAAAAACGACTCCGTCGTTTTGTATACCGTGTGAACTG 58
M.o   ACCGATAAAAGT-ATCCAACA------ACTACCCAGTTGT-------ACTGTGTGAACTG 46
L.p   ACCGATGATATTCGTCCAACA------ACTACACAGTTGT-------ACCTTGTGAACCG 47
      ******        ***** *      ** ** * ** **        **  ******* *

D1    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGATC-- 116
D2    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 116
D3    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGATCGC 118
D4    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGATC-- 116
D5    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGATC-- 117
D6    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGWTC-- 116
D7    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 117
S1    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 117
S2    TACCCAACCATTTAGGGGTCGGTTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGATCTC 118
S3    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 107
S4    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 116
S5    TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 117
O.d   TACCCAACCATTNAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 116
O.p   TACCCAACCATTTAGGGGTCGGCTGGTCCGTTAAATGGCGGTCGCGCGTGACTCGTTC-- 116
M.o   TACCAAACTATT-AGGGGTCGGCTAGTCCGTTAAATGGTGGTCGGACGCGAGA-GATC-- 102
L.p   TACCAAACTATTCAGGGGTCGGCTTGTCCGTTAAATGGTCGACGGACGCGAGATAATC-- 105
      **** *** *** ********* * *************  * **  ** **     **

D1    GCGCGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT-TTATCAACATACGTTTATACGAT 173
D2    GCGTGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT---ATCATCATACGTTTATACGAT 171
D3    GCGCGCGCTCCNANAGCCCCGCGTGTGTGTT--TTA-TCATCATCATACGATCTGACAAA 175
D4    GCGCGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT-TTATCAACATACGTTTATACGAT 173
D5    GCGCGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTW-TWATCATCATACGTTTATACGAT 174
D6    GCGCGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTW-TYATCATCATACGTTTATACGAT 173
D7    GCGCGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT---ATCATCATACGTTTATACGAT 172
S1    GCGTGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT---ATCATCATACGTTTATACGAT 172
S2    GCGTGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTAATCATCAACATACGTTTATACGAT 176
S3    GCGTGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT---ATCATCATACGTTTATACGAT 162
S4    GCGTGCGCTCCGCCAGCCCCGCCTGTGTTTT--TT----ATCATCATACGTTTATACGAT 170
S5    GCGTGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT---ATCATCATACGTTTATACGAT 172
O.d   GCGCGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTT---ATCAACATACGTTTATACGAT 171
O.p   GCGTGCGCTCCGCCAGCCCCGCCTGTGTTTT--TTA----TCWTCATACGTTTATACGAT 170
M.o   GCGGTCGCTCCGCCAGCCCCGCTTGTGTTTT--TTA----CAATCATAAGTGT-CAAAGT 155
L.p   GCGGCCGCTCGACCGGCCCCGCACGCGTTTTGTTTTTTTATAAACATATGTGTAC-CTGT 164
      ***  *****     *******  * ** **  **          **** *
```



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D1   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----TGTCGCGAATTGAGCAAAAAACGAGATA 230
D2   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----CGTCGCGAATTGAGCAAAAAACGAGATA 228
D3   CT-----ACTGTGGCCGCTCTTCGGAGCG-----CGTCGCGAATNGAGCAAAAAACGAGATA 227
D4   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----TGTCGCGAATTGAGCAAAAAACGAGATA 230
D5   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----CGTCGCGAATTGAGCAAAAAACGAGATA 231
D6   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----YGTCGCGAATTGAGCAAAAAACGAGATA 230
D7   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----TGTCGCGAATTGAGCAAAAAACGAGATA 229
S1   CTGACAAACTGGGGCCGCTCTTCGGAGCG-----CGTCGCGAATTGAG-AAAAAACGAGATA 228
S2   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----CGTCGCGAATTGAGCAAAAAACGAGATA 233
S3   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----CGTCGCGAATTGAGCAAAAAACGAGATA 219
S4   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----TGTCGCGAATTGAGCAAAAAACGAGATA 227
S5   CTGACAAACTGTGGCCGCTCTTCGGAGCG-----CGTCGCGAATTGAGCAAAAAACGAGATA 229
O.d CTGACAAACTGTGGCCGCTCTTCGGAGCG-----TGTCGCGAATTGAGCAAAAAACGAGATA 228
O.p CTGACAAACTGTGGCCGCTCTTCGGAGCG-----CGTCGCTAATTGAGCAAAAAACGAGATA 227
M.o CTG--AATGTGTGGCCCTGCGCCACAGAG-----AGCAAAAAAATGAGATA----------- 101
L.p CTGACCAAGCGTTGCGGCCGGCCCAAGGGTCGGTCGCGGCAATTAGAGCAAAAAACGAGATA 226
    **        *  **       *  ** *      *     *   ***  *
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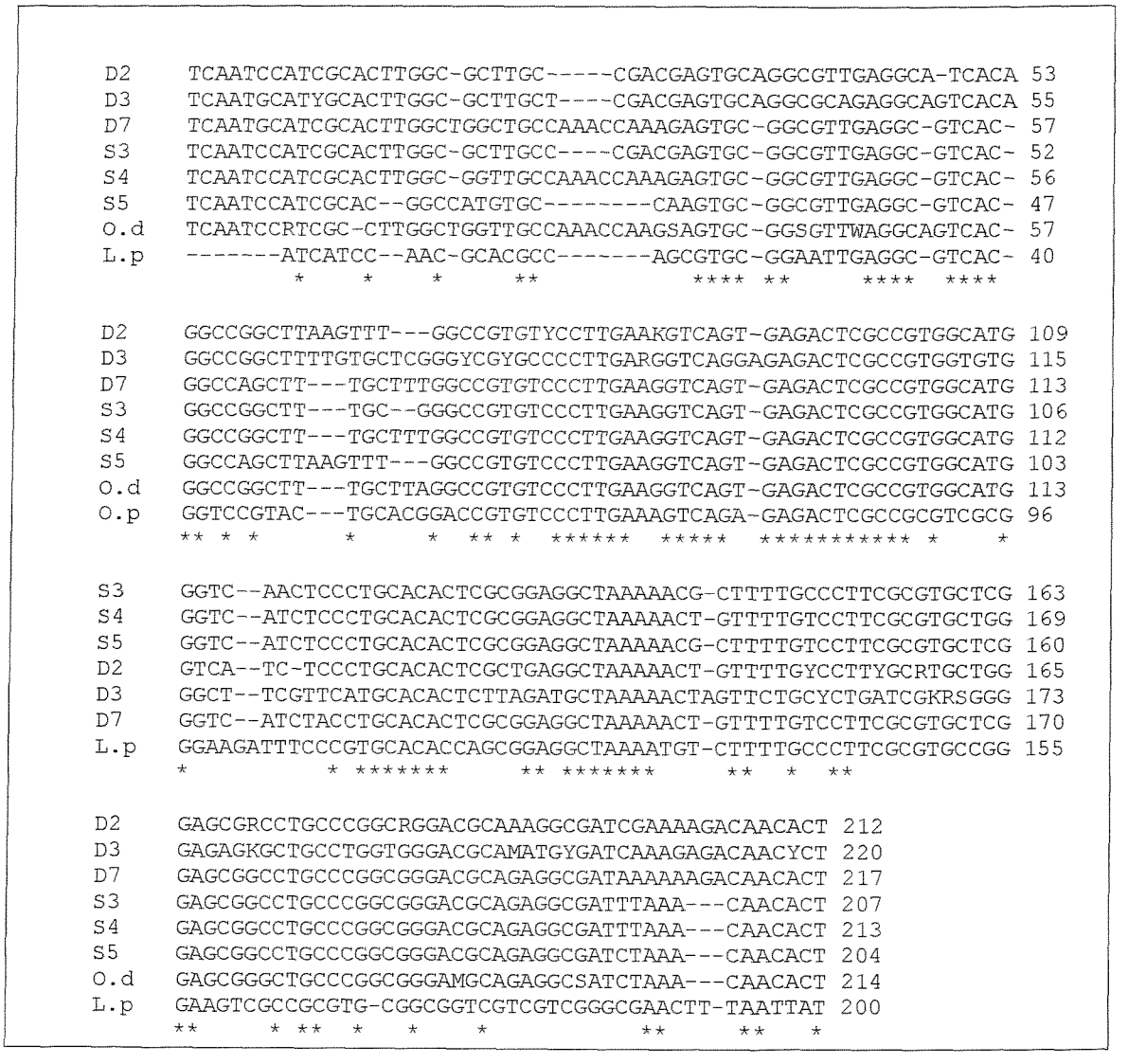
B



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D2    TCAATCCATCGCACTTGGC-GCTTGC-----CGACGAGTGCAGGCGTTGAGGCA-TCACA 53
D3    TCAATGCATYGCACTTGGC-GCTTGCT----CGACGAGTGCAGGCGCAGAGGCAGTCACA 55
D7    TCAATGCATCGCACTTGGCTGGCTGCCAAACCAAAGAGTGC-GGCGTTGAGGC-GTCAC- 57
S3    TCAATCCATCGCACTTGGC-GCTTGCC----CGACGAGTGC-GGCGTTGAGGC-GTCAC- 52
S4    TCAATCCATCGCACTTGGC-GGTTGCCAAACCAAAGAGTGC-GGCGTTGAGGC-GTCAC- 56
S5    TCAATCCATCGCAC--GGCCATGTGC--------CAAGTGC-GGCGTTGAGGC-GTCAC- 47
O.d   TCAATCCRTCGC-CTTGGCTGGTTGCCAAACCAAGSAGTGC-GGSGTTWAGGCAGTCAC- 57
L.p   -------ATCATCC--AAC-GCACGCC-------AGCGTGC-GGAATTGAGGC-GTCAC- 40
                *    *    *     **           **** **     ****  ****

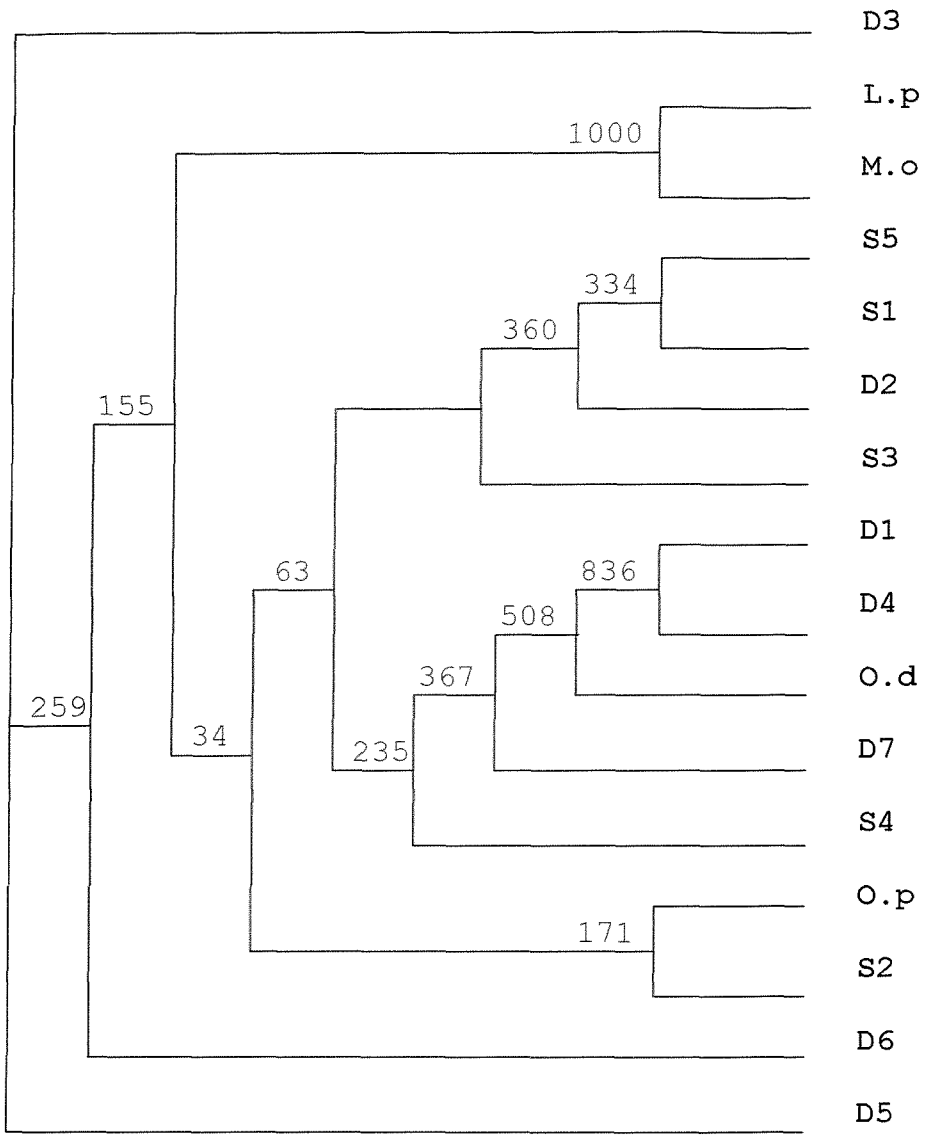
D2    GGCCGGCTTAAGTTT---GGCCGTGTYCCTTGAAKGTCAGT-GAGACTCGCCGTGGCATG 109
D3    GGCCGGCTTTTGTGCTCGGGYCGYGCCCCTTGARGGTCAGGAGAGACTCGCCGTGGTGTG 115
D7    GGCCAGCTT---TGCTTTGGCCGTGTCCCTTGAAGGTCAGT-GAGACTCGCCGTGGCATG 113
S3    GGCCGGCTT---TGC--GGGCCGTGTCCCTTGAAGGTCAGT-GAGACTCGCCGTGGCATG 106
S4    GGCCGGCTT---TGCTTTGGCCGTGTCCCTTGAAGGTCAGT-GAGACTCGCCGTGGCATG 112
S5    GGCCAGCTTAAGTTT---GGCCGTGTCCCTTGAAGGTCAGT-GAGACTCGCCGTGGCATG 103
O.d   GGCCGGCTT---TGCTTAGGCCGTGTCCCTTGAAGGTCAGT-GAGACTCGCCGTGGCATG 113
O.p   GGTCCGTAC---TGCACGGACCGTGTCCCTTGAAAGTCAGA-GAGACTCGCCGCGTCGCG 96
      ** * *      *     *  ** *  ******  *****  ********** *    *

S3    GGTC--AACTCCCTGCACACTCGCGGAGGCTAAAAACG-CTTTTGCCCTTCGCGTGCTCG 163
S4    GGTC--ATCTCCCTGCACACTCGCGGAGGCTAAAAACT-GTTTTGTCCTTCGCGTGCTGG 169
S5    GGTC--ATCTCCCTGCACACTCGCGGAGGCTAAAAACG-CTTTTGTCCTTCGCGTGCTCG 160
D2    GTCA--TC-TCCCTGCACACTCGCTGAGGCTAAAAACT-GTTTTGYCCTTYGCRTGCTGG 165
D3    GGCT--TCGTTCATGCACACTCTTAGATGCTAAAAACTAGTTCTGCYCTGATCGKRSGGG 173
D7    GGTC--ATCTACCTGCACACTCGCGGAGGCTAAAAACT-GTTTTGTCCTTCGCGTGCTCG 170
L.p   GGAAGATTTCCCGTGCACACCAGCGGAGGCTAAAATGT-CTTTTGCCCTTCGCGTGCCGG 155
      *          * *******     ** *******     **  *  **

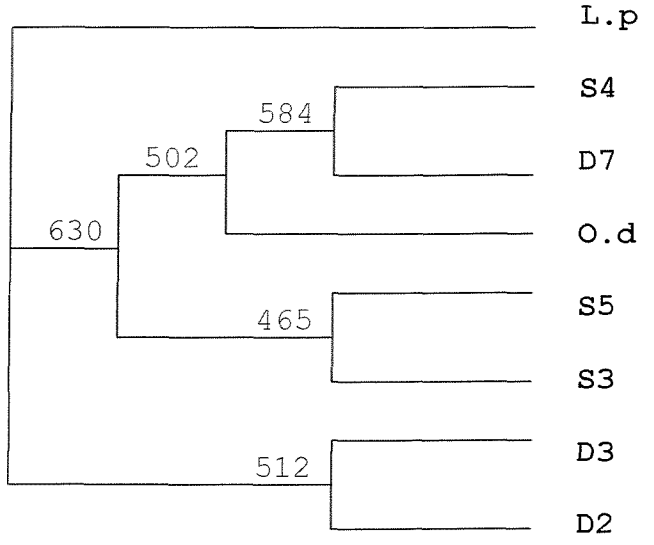
D2    GAGCGRCCTGCCCGGCRGGACGCAAAGGCGATCGAAAAGACAACACT 212
D3    GAGAGKGCTGCCTGGTGGGACGCAMATGYGATCAAAGAGACAACYCT 220
D7    GAGCGGCCTGCCCGGCGGGACGCAGAGGCGATAAAAAAGACAACACT 217
S3    GAGCGGCCTGCCCGGCGGGACGCAGAGGCGATTTAAA---CAACACT 207
S4    GAGCGGCCTGCCCGGCGGGACGCAGAGGCGATTTAAA---CAACACT 213
S5    GAGCGGCCTGCCCGGCGGGACGCAGAGGCGATCTAAA---CAACACT 204
O.d   GAGCGGGCTGCCCGGCGGGAMGCAGAGGCSATCTAAA---CAACACT 214
L.p   GAAGTCGCCGCGTG-CGGCGGTCGTCGTCGGGCGAACTT-TAATTAT 200
      **     * **  *   *   *          **     **   *
```

Figure 2.4. Consensus trees for A) ITS-1 and B) ITS-2 (n=1000 trees). Species key: M.o: *Madrepora oculata*; L.p: *Lophelia pertusa*; O.d: *O. diffusa*; O.p: *O. patagonica*; D1-D7: O. *varicosa* deep samples; S1-S5: *O. varicosa* shallow samples.

A



B



15% in *Goniopora tenuidens*, 2% in *Heliofungia actiniformis*, 11% in *Acropora longicyathus* and 10% in *Stylophora pistillata* (Takabayashi, 2000). A very low variability (1%) was found amongst closely related species of *Montastrea annularis*, *M. franksi*, and *M. faveolata* (Lopez and Knowlton 1997), which is similar to the genetic divergence found within deep and shallow populations in this study. The genetic divergence in the ITS-2 sequences was higher than in the ITS-1 sequences. The opposite trend was found in *Acropora nasuta* (Takabayashi 2000), and was explained by differential mutation and/or homogenisation rates in the two regions.

The amplification of ITS-1 was more successful than for ITS-2 sequences, which showed high levels of polymorphism and possible presence of microsatellite regions. Re-designing the primers may improve the sequence quality, but tandem repeats of these small sequences cause problems with primer slippage. Allele polymorphism within colonies creates double peaks in genetic sequences, making it impossible to distinguish between nucleotides after the microsatellite region. Alternatively, it has been shown for several species of invertebrates that rRNA encoding genes may show intragenomic variation (Takabayashi 2000). This means that instead of all copies of the genes in the multi-gene family being identical (through concerted evolution) they show variation. The number of acceptable ITS-2 sequences was therefore small and the value of the data limited to corroboration of the ITS-1 data. One way of analyzing the variable repeat regions within the ITS-2 region would be to analyse the size of amplicons for this region as with standard microsatellite analysis. This may confirm whether the region in question was simply a repeat with Mendelian inheritance, or whether the ITS region showed intragenomic variation. The region may be useful as a genetic population marker.

2.5.2 Consensus trees

The consensus tree for ITS-1 placed *Lophelia pertusa* and *Madrepora oculata* as separate groups, but all of the other samples, including the congeneric *O. diffusa* and *O. patagonica*, were placed on interspersed branches of the tree. The consensus tree for ITS-2 supports the evidence from the ITS-1 tree, that not only are the deep and shallow populations of *O. varicosa* conspecific, but that they are also closely related to *O. diffusa*. A consensus tree represents the most frequent combination of sequence branches, but some junctions on both trees are assigned a low number, which implies

uncertainty in the branch positioning. It is clear however, that these data show no genetic distinction between samples from the different populations of *O. varicosa*. Indeed there was some evidence based on ITS-1 data that *O. diffusa* and *O. patagonica* may indeed be conspecific. This is extremely interesting as *O. varicosa* has one of the most restricted geographic distributions known amongst cold-water corals (Rogers, 1999). If it is the case that these three *Oculina sp.* are conspecific then its recognized distribution would be greatly increased and more in line with species such as *Lophelia pertusa* and *Madrepora oculata*. It would also change the emphasis on the conservation status of Florida *O. varicosa* to one of maintaining intraspecific genetic variability and range rather than maintaining species survival. However, as previously discussed, the ITS region in *Oculina* shows a low level of variation compared to many species of Scleractinia. Clearly further investigations are required to clarify the genetic structure of the Oculinidae.

2.5.3 Reproduction and genetic homogeneity

Scleractinians exhibit a number of different reproductive traits; corals may be gonochoristic or hermaphroditic and may broadcast gametes or brood planulae. Broadcast spawning is thought to facilitate larval dispersal over larger distances than brooded larvae, which often spend short periods of time in the plankton (Veron, 1995). A low level of genetic variation in the ITS-1 regions of *O. varicosa*, a broadcast spawning species, supports the premise that high dispersal potential is often associated with low genetic diversity because of the homogenising effect of gene flow. There are, however, exceptions to this rule (Palumbi 1994), and previous studies with scleractinians on the Great Barrier Reef have shown a lack of correlation between reproductive strategy and genetic diversity. Brooding species such as *Acropora cuneata* and *Pocillopora damicornis* exhibited as much gene flow as the broadcast spawning species, *Acropora hyacynthus* and *Acropora valida*. In contrast, other brooders such as *Stylophora pistillata* and *Seriatopa hystrix* had weak genetic connections over the same geographic range (Ayre and Hughes, 2000). In a different study of four Australian species, *Goniastrea temuidens*, a gonochoric spawner, *Acropora longicyathus*, a hermaphroditic spawner and *Stylophora pistillata*, a brooder, all exhibited similar intermediate levels of sequence variability (Takabayashi 2000). The lowest variation was shown by the fourth species, *Heliofungia actiniformes*, which has been shown to

exhibit both broadcast spawning (Willis et al 1985) and brooding (Abe 1937) reproductive modes. It is impossible to support generalisations on the influence of reproductive mode on genetic variation when data on both of these aspects of coral biology are still limited. In addition, genetic variation is dramatically influenced by other factors including the history of the study populations and the effective size of the populations. Historical bottlenecks are well known to reduce the genetic diversity of wild populations, for example striped river bass (Waldman et al. 1998) and Northern elephant seal (Weber et al. 2000).

The continental shelf on the Atlantic coast of Florida has a very complex hydrodynamic regime (see page 125), influenced strongly by the proximity of the Florida Current. Deep and shallow-water *O. varicosa* samples were collected from sites at least 27km apart. However, strong upwelling events during the summer spawning season of *O. varicosa* provide a hydrodynamic mechanism to facilitate gene flow between the deep and shallow water populations, thus preventing speciation.

2.5.4 Implications for conservation

Since the two *Oculina* populations are the same species there is (or has been in the recent past), genetic exchange either between the two habitats, or from one habitat to another. The former scenario would be advantageous to the recovery of the deep banks for two reasons. Firstly, the shallow habitats could potentially supply larvae to the shelf edge habitat, thus increasing the number of recruits into damaged areas. Secondly, larval influx into damaged areas from both deep and shallow colonies would increase genetic diversity of recruits, preventing 'founder effects' and genetic bottlenecks. If the shallow populations are a significant source of recruits for the deep reefs, then the species should be managed as a unit, conferring protection on the currently neglected shallow reefs. Alternatively, if the shallow ledges are a sink for deepwater larvae, then the damaged areas can only be re-colonised by the few remaining deep-water colonies.

2.5.5 Future research

Information on natural recruitment rates and gene flow between the populations is needed to assess recovery potential of the *Oculina* banks; however, conventional ecological techniques are not easily applicable to the deep shelf habitat. Molecular

techniques can provide insight into ecological processes. For example, high clonal variability within a reef area would imply influx of genetic material from outside the system, whereas a low variability indicates that the system was created primarily from asexual growth and colony fragmentation. Microsatellite analysis has been used to determine clonal variability of *Lophelia pertusa* reefs (Rogers In prep). Microsatellites are small tandem repeat sequences of a few base pairs in length. They are non-coding sequences and are selectively neutral. They also vary at the level of the individual and therefore have a high resolution in determining clonal variation. Microsatellite analysis could be used equally well to determine clonal variation of the *Oculina* reefs, and to study the metapopulation dynamics of the *Oculina* populations. This information could be a useful for management of the reefs; a high recruitment rate and strong genetic exchange implies that the provision of appropriate substrate and removal of damaging impact would be sufficient to facilitate reef recovery. If recruitment rates and gene flow are low, transplants may be needed to seed the damaged areas with new colonies. Transplants traditionally have met with limited success, and low fragment survival often does not justify the expense of the operation or the sacrifice of the transplants.

2.6 Conclusions

Results from initial *O. varicosa* ITS-1 sequencing supports the generally accepted morphological conclusion that deep-water populations on the edge of the Florida shelf are conspecific with those occurring as smaller isolated colonies in the shallow near-shore ledges. The limited ITS-2 sequence analysis also supports the ITS-1 data. This study creates a foundation for further studies of the population structure of *O. varicosa*, as well as species definitions for the *Oculinidae* of the south eastern US Coast. The deep *O. varicosa* populations have been destroyed to such an extent that future re-colonisation may exhibit founder effects or genetic bottlenecks, especially if larval recruitment rates are low. Genetic population studies (AFLP or microsatellite techniques) are the next step in understanding relative contribution of sexual and asexual reproduction of the deep reefs and estimating re-colonisation potential of damaged areas.

Chapter 3: Growth and Respiration

3.1 Introduction

3.1.1 Oculina *growth rates*

Oculina varicosa is a facultatively zooxanthellate species, retaining algal symbionts when light levels are sufficiently high, but surviving without them under low light conditions. The presence of algal symbionts should enhance energy supply and increase growth. However, *in situ* growth studies (Reed 1982) indicate that linear extension rates are higher in the deep azooxanthellate *O. varicosa* than in the shallow zooxanthellate populations. A congeneric species, *Oculina arbuscula* inhabits inshore and near-shore areas of South and North Carolina. Unlike *O. varicosa* it does not form deep reef structures, but can attain colony sizes of up to 0.5m diameter, in both light and dark habitats. Miller (1995) measured growth rates in terms of percentage change in wet mass over defined times, under different temperature, light and feeding regimes, both in the laboratory and in the field. The results of the study showed that growth rates were highest under conditions of high light and warm temperatures, but colony abundance was greater in the cooler dark environments, where one would expect growth potential to be lower. Miller concluded that biotic rather than physical factors were probably influencing *O. arbuscula* distribution and abundance.

3.1.2 Energy allocation in marginal habitats

Reproductive output of *Pocillopora damicornis* was low or non-existent in populations living in what has been considered marginal habitat on the Pacific coast of Panama (Richmond 1987). Conspecific colonies in optimal habitat produced larger numbers of propagules than those from marginal locations; however somatic growth in these colonies was significantly lower. Ward (1995) found two types of co-existing colonies of *Pocillopora damicornis* which, although they exhibited no morphological differences, had different patterns of energy allocation to growth and reproduction. Those that produced planulae had significantly lower growth rates than those that were sterile or just produced sperm (Ward 1995). High levels of damage also caused *P. damicornis* colonies to channel energy into growth and repair rather than reproduction. The energy budgets of conspecific populations may therefore be dictated by the environmental conditions under which they exist. The two populations of *O. varicosa* live in different environmental conditions, under different energetic constraints. It is

possible therefore that they may display differences in growth rates, energy stores and reproductive output.

3.1.3 Importance of temperate symbiosis

Many marine cnidarians contain endosymbiotic zooxanthellae, which fix atmospheric carbon, often in excess of their own metabolic demands. A portion of this excess photosynthate is translocated to the host (Muscatine et al. 1983; Davies 1984). The translocated carbon is potentially available for host respiration, growth and reproduction, or it may be lost (Crossland et al. 1980a,b, Muscatine et al. 1981, Edmunds and Davies 1986, Rinkevich 1989). The potential importance of translocated carbon to the host has been investigated in various tropical marine cnidarian-algal symbioses (Davies, 1984, Muscatine et al.1984, Edmunds and Davies 1986, Davies 1991, Day 1994, McClosky et al. 1994). The majority of these studies suggest that in clear shallow water, the symbionts produce sufficient excess carbon to fulfill the metabolic demands of the host. Such autotrophy may be vital in oligotrophic systems where there may not be enough POC for the coral to survive by heterotrophy alone (Glynn 1973, Sorokin 1990). In contrast, there have been few attempts to quantify the potential importance of zooxanthellae-fixed carbon to temperate symbiotic cnidarians (Fitt et al. 1982, Schick and Dykens 1984, Tytler and Davies 1986, Farrant et al. 1987, Stambler and Dubinsky 1987, Verde and McClosky 1996). Temperate symbioses offer an interesting comparison to their tropical counterparts. Bleaching or loss of zooxanthellae in most tropical corals is an indication of extreme physiological stress, but in many temperate Scleractinia, zooxanthellae may be light-limited because of seasonally-decreased day length and temperature, and high water turbidity. Many temperate corals (including members of the genera *Oculina, Madracis* and *Astrangia*) appear pale or white in poorly-lit habitats, as well as darkly coloured with abundant zooxanthellae in shallow well lit habitats (Miller 1995, Brooke pers obs.). In low light conditions, carbon fixation levels may not be able to meet the metabolic demands of both symbiont and host. Carbon budgets for temperate anthozoan-dinoflagellate symbioses, showed that under favourable conditions, two of the four anthozoans studied could potentially be autotrophic (Davy et al. 1996). However, under annual average field conditions the contribution of zooxanthellae to the respiratory carbon requirements (CZAR) of the host, would be insufficient to meet host metabolic demands, and would need to be supplemented by heterotrophy. Temperate cnidarians

may therefore be more dependent on heterotrophy than those living in clear tropical waters. With the exception of the North to mid-Atlantic species, *Astrangia danae* (Jaques et al. 1983) the importance of light and feeding to symbiosis and growth in temperate scleractinians is largely unknown.

3.2 Objectives

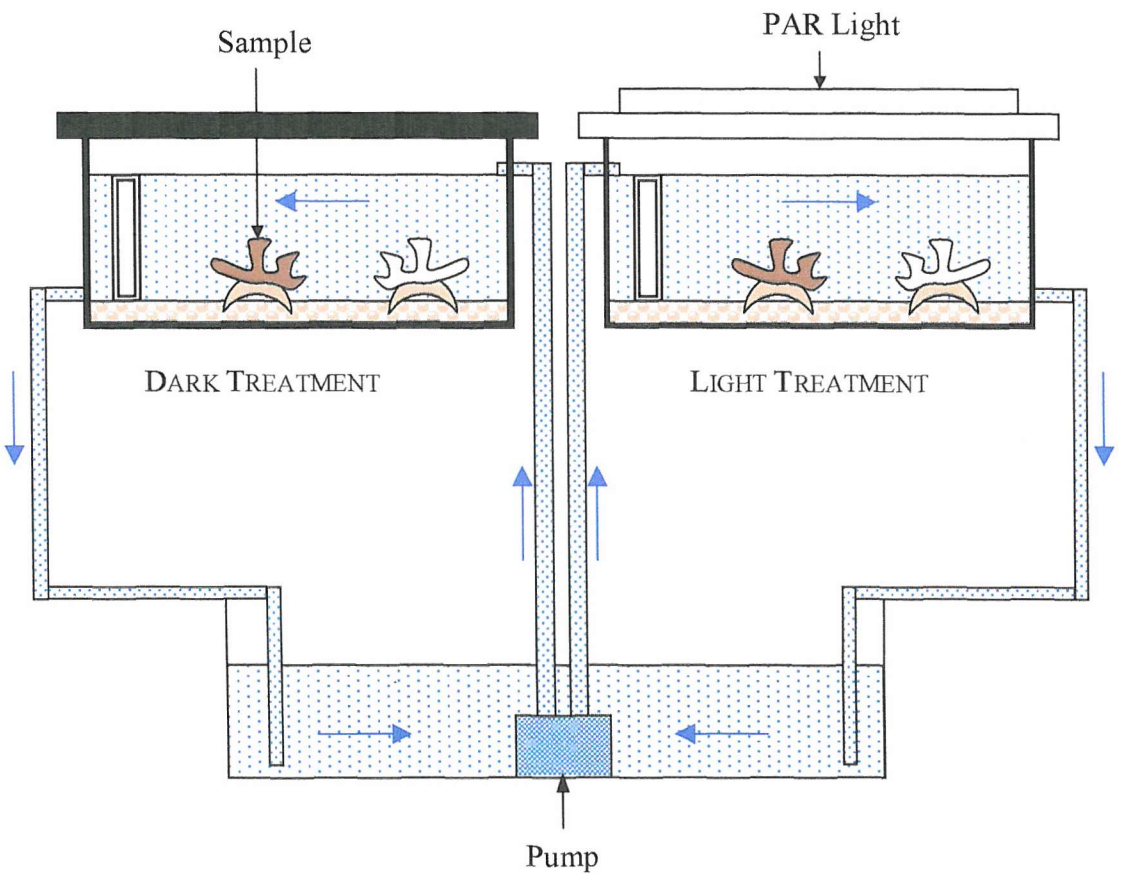
1) Compare growth rates of deep and shallow-water coral samples under different experimental light and temperature regimes.
2) Compare *in situ* growth rates of deep and shallow-water samples, using reciprocal transplants from deep and shallow populations.
3) Measure polyp dry weight and lipid content seasonally to assess nutritional status of the tissues.
4) Measure dark respiration rate and oxygen production under different light levels to determine primary productivity of zooxanthellate *O. varicosa* samples.
5) Measure *in situ* temperature at the deep and shallow sites using temperature data loggers, and present experimental results on the light of environmental conditions.

3.3 Materials and Methods

3.3.1 Laboratory Growth Rate Experiment

Coral samples from deep and shallow populations were kept under different temperature and light regimes in a re-circulating aquarium system (figure 3.1). One set of tanks was maintained at 25°C with thermostatically controlled aquarium heaters, and the second set was kept at 16°C, using a re-circulating chiller system (Universal Marine Industries, model TC-200). All of the tanks had a 2cm-deep layer of calcareous gravel substratum to maintain calcium levels in the system. Half of one set were illuminated on a 12h:12h light:dark cycle, using PAR lamps for aquarium use. The irradience level for the light treatment was set at 9×10^{20} photons m^{-2} s^{-1}; a similar light level was recorded at 6m depth on a sunny late morning in April at the shallow study site. The shallow colonies were too small to provide samples for all replicates, so all of the samples from both deep and shallow sites were selected from different colonies. The experiment had an orthogonal 3 factor design (Table 3.1) with five samples of coral from each population in each of the two replicates per treatment. Seawater for the experiment was raw seawater pumped from ¼ of a mile offshore. The water was filtered through a 20μm bag-filter but was otherwise untreated.

Figure 3.1. Schematic of the re-circulating aquarium system used in the growth rate study. The warm treatments (25°C) had aquarium heaters inside the sump and the cold treatments (16°C) were circulated through a chiller to maintain required temperature.



Every month the tanks were cleaned and the water replaced, but a 50% water exchange was done every week to keep the system clean. Every second day the corals were fed newly hatched brine shrimp. The nauplii were not quantified, but the total volume of shrimp was divided equally between the tanks and each individual sample was fed by releasing a stream of shrimp over the sample with a glass pipette. The flow through the tanks was shut off prior to feeding and resumed the following day. Any unconsumed shrimp were then flushed from the tanks into a bag filter, which was then cleaned to remove the debris.

The buoyant weight of the samples was measured by using a modified Mettler Balance (model AE163) (Figure 3.2), and the equivalent weight in air was subsequently calculated using formulae described by Davies (1989). This technique is preferable to measuring weight in air of organisms such as corals, where incremental weight gain is very small, and the weight of water associated with the sample could mask slight increases. The downfall of this method is that only the increase resulting from skeletal deposition is recorded, as coral tissue has a similar density to water.

Coral fragments have a natural tendency to try to attach to a substratum, so the samples were each fastened with a small cable tie to a clam shell to avoid overgrowth onto the gravel in the tanks, which would render the sample useless. The coral-shell unit was then re-weighed in water and was recorded as the initial buoyant weight. Five clam shells without coral pieces were also weighed and placed in each of the tanks to account for any changes in the weight of the shells over time. The coral-shell units and empty control shells were re-weighed monthly to monitor growth rate.

3.3.2 Field growth rate experiment

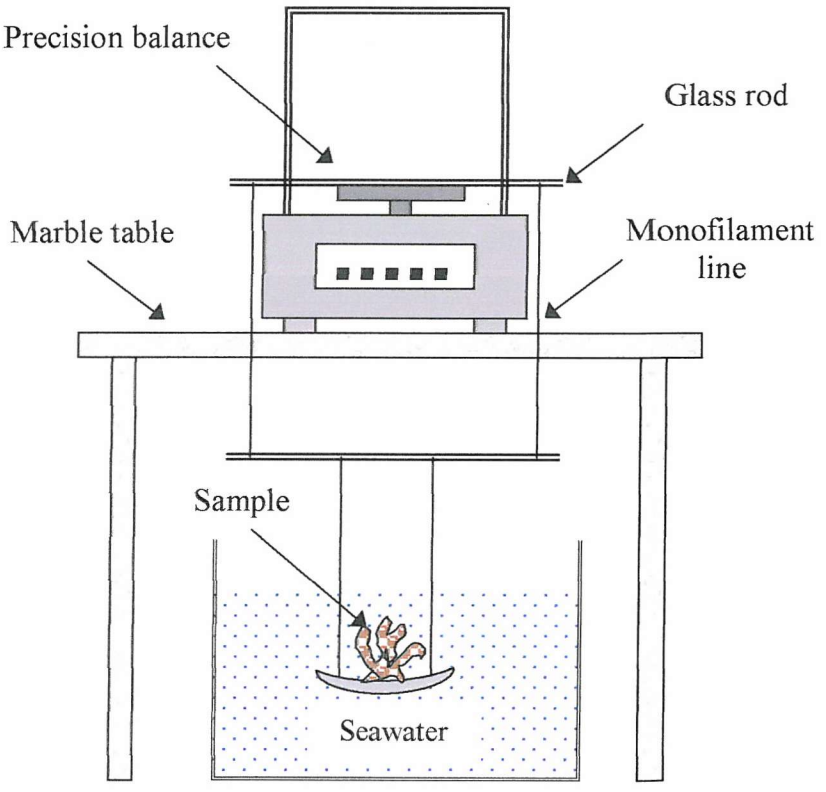
For this experiment, small (<20g) pieces of coral were removed from 6 different healthy colonies at both deep and shallow study sites. The buoyant weight of each sample was measured as previously described; it was then attached to a Plexiglas plate with a cable tie and re-weighed.

For field deployment the coral plates were attached to a square concrete paving-slab (20cm x 20cm x 8cm) using ¼" wedge anchors. Control blocks were made using pre-weighed Plexiglas plates without coral pieces. These blanks served to control for growth of fouling organisms, and to act as potential recruitment substratum for planulae. A marker float was constructed from yellow polypropylene line and attached to each block to facilitate recovery for re-weighing.

Table 3.1. Summary of experimental design for growth rate experiment, showing levels of treatment factors: Light, Temperature and Population.

LIGHT REGIME	TEMPERATURE	POPULATION
Diurnal	25°C	Shallow
Diurnal	25°C	Deep
Dark	25°C	Shallow
Dark	25°C	Deep
Diurnal	16°C	Shallow
Diurnal	16°C	Deep
Dark	16°C	Shallow
Dark	16°C	Deep

Figure 3.2. Schematic of the apparatus used to take buoyant weight measurements, showing a modifed precision balance and samples immersed in seawater.



Blocks were deployed in six replicate groups of three at both deep and shallow study sites. Each group consisted of one control block, one block with a deep sample, and one with a shallow sample. One set of blocks was deployed using the Johnson Sealink Research Submersible on June 23, 1999 at a depth of 83m on Jeff's Reef (27°32.54 N, 79°58.73 W). Another set were deployed using scuba on July 25, 1999 at a depth of 6m at Pepper Park (27°29.478N, 80°17.286W). The shallow experiment was visited weekly, or as conditions permitted, and the deep site was re-visited in April 2000.

3.3.3 Measurement of lipid content and polyp dry weight

Coral samples were taken monthly, or as frequently as weather permitted, from the shallow site at Pepper Park. Samples from the deep site were collected during cruises to Jeff's Reef. Collections were made using a National Marine Fisheries Service (NMFS) 'Phantom' ROV (February 1999), the Johnson Sea Link research submersible (June 1999), a 'Rescue ROV' from Harbor Branch Oceanographic Institution (April and July 2000), or the 'Clelia' research submersible (March 2001, September 2001). Two collections were made (March 1998, September 2000) outside the *Oculina* Habitat of Particular Concern (HAPC) at a site off Cape Canaveral (28°29.82′ N, 80°01.24′ W), using a small (1.5m swath) trawling device.

Samples were initially fixed in 10% formalin in seawater. After 24 hours (or as soon as possible, they were decalcified overnight with 10% hydrochloric acid. The number of polyps in each sample was counted before being transferred to an oven (56°C) to dry for 24 hours. The samples were weighed (μg) using a fine precision balance, and placed in a solution of methanol/chloroform (1:2 v/v), which removes lipid from the tissues as it dissolves in the chloroform fraction.

After 48 hours of extraction, the samples were placed back in the oven for 12 hours to evaporate the solvent. The samples were then re-weighed and the difference between the initial and post extraction weights represents the quantity of lipid that has been removed from the tissue. The data from each population were analysed for seasonal differences in polyp dry weight and lipid content using a one way analysis of variance. Dry weight and lipid content of the populations were then compared using a one-tailed paired t-test, with an a *priori* assumption that the shallow-water samples could have higher values because of the warmer temperatures in the shallow habitat and the zooxanthellae in the corals.

3.3.4 Respiration and primary productivity of zooxanthellate O. varicosa

3.3.4.1 Dark respiration measurements

Coral samples were kept without feeding in the dark for 24 hours to stabilise respiration rates, and avoid post-prandial respiratory responses. The coral sample was rinsed with 0.22μm-filtered seawater (to remove particulate material) and its volume was measured by displacement. The sample was then placed in a 50ml respiration chamber, filled with 0.22μm-filtered seawater. The chamber was sealed with a lid, into which an oxygen electrode was inserted. The water in the respiration chamber was mixed continuously, using a magnetic stirring disc and plate. The disc was placed in the chamber, a screen platform placed over it and the coral sample positioned on top of the screen.

Prior to each coral measurement a blank was run using just 0.22μm-filtered seawater, to determine the oxygen consumption of the electrode, and to ensure the electrode reading was stable. The chamber was darkened with light-impermeable black plastic so that oxygen production from photosynthesis would not affect the respiration measurement. After an initial measurement of oxygen content, the coral was left to incubate for 5 minutes, or until the oxygen levels approach 70% of oxygen saturation. Below this level, the corals begin compensatory respiration. Readings from the YSI oxygen electrodes were logged directly onto a laptop computer, which were subsequently saved as spreadsheet files. Oxygen flux was measured and standardised to cm^{-2} of tissue area for replicated shallow samples (n=2), and oxygen consumption rates were calculated for both the coral and electrode. Respiration rates were converted to carbon equivalents (Muscatine et al. 1983, 1984) by multiplying by the carbon conversion factor of 0.375 (Muscatine et al. 1984) and an assumed respiratory quotient of 0.8 (Muscatine 1990)

3.3.4.2 Determination of potential primary production.

After measuring dark respiration of the samples, the light-impermeable cover was removed from the apparatus and a photosynthetically active radiation (PAR) lamp was used to illuminate the chamber. Photon flux was measured with a LICOR light meter. A range of light intensity was used, beginning with the weakest illumination, and increasing in increments of 50 μE (μmol photons m^{-2} s^{-1}) until photosynthetic saturation point was reached. As with the respiration experiment, the samples were incubated at a particular light level for 5 minutes, or until the water in the chamber

became oxygen-saturated. After incubation the coral sample was covered to allow oxygen levels to fall to 75%, before the next illuminated incubation began.

Gross and net photosynthetic rates were converted to carbon equivalents by multiplying oxygen production by 0.375 and dividing by the photosynthetic quotient of 1.1 for zooxanthellae (Muscatine 1984). The relationship between light intensity and photosynthesis is an indicator of photosynthetic efficiency and photosynthetic capacity of the alga (Muscatine 1984). Several photosynthetic characteristics were determined. 1) P_{max}: the photosynthetic rate at light saturation. 2) I_c: the compensation intensity at which net coral photosynthesis=0. 3) α: Light use efficiency at sub-saturating light intensities, which is the initial slope of the hyperbolic photosynthesis-irradience curve. 4) I_k: the minimum light intensity that produces maximum photosynthesis, which is the intersection point between the initial photosynthetic slope and P_{max}.

3.3.5 Measurement of bottom-temperature at the deep and shallow habitats.

A temperature data logger was deployed at each of the habitats to monitor fluctuations in bottom temperature over time. A small, inexpensive 'Hobotemp' data logger was deployed at the shallow site in August 2000. The battery power expired in February 2001, but poor diving conditions precluded recovery until May 2001. A second 'Hobotemp' was deployed from May 2000 until April 2002. Temperatures on the deep reef were recorded using a Falmouth Scientific 2D-ACM coastal current meter, with an incorporated temperature data-logger. This was deployed at the same time as the *in situ* growth experiment in April 2000, and recovered one year later.

3.4 Results

3.4.1 Growth rates from laboratory experiments

The shallow water zooxanthellate coral samples had higher average annual growth rates in the light treatments than their azooxanthellate deep-water counterparts, and the dark cold treatments produced the lowest growth rates in the experiment (Figure 3.3). The raw growth rate data was represented as a percentage so a square-root arcsine transform was applied to the data prior to analysis. Results of a two way ANOVA using population and light as factors, showed a significant effect of population ($F=9.56$, $p=0.004$), light ($F=5.85$, $p=0.020$), and the interaction between factors ($F=7.53$, $p=0.009$). The same analysis was applied to the data for population and temperature, yielding similar results for population ($F=7.50$, $p=0.009$), however

there was no significant effect of temperature (F=0.003, p=0.959) on growth rate and the interaction between population and temperature was not significant (F=2.05, p=0.160). Light and temperature were compared independently of population, and a significant effect of light on growth was shown (F=4.90, p=0.032). The effect of temperature was not significant (F=0.122, p=0.728), neither was the interaction of light and temperature (F=1.351, p=0.252). Table 3.2 shows a summary of ANOVA results.

3.4.2 In situ *growth rates*

The shallow blocks were monitored for transplant health every week until 22 August. At that time, one of the deep samples had been invaded by boring polychaete worms, probably *Eunice* sp., and although sedimentation was high, the other coral transplants (both deep and shallow) appeared healthy. A series of hurricanes and bad weather subsequently prevented diving on the site until the following 24 June. On revisiting the site, all of the deep-water and two of the shallow-water transplants had died. The remaining shallow samples were partially alive, but had not attached to the plates. One of the transplant blocks was covered in barnacle recruits, but no new coral colonies were observed on any of the blocks. The transplants at the deep-water reef were re-visited in April 2000, and only 4 of the 6 sets of blocks were found. The samples were partially alive, but again, had not attached to the substratum. There was a thick layer of sediment on the block itself, but the live coral samples appeared clean and sediment-free. The experiments at both sites were discarded because of the high level of mortality encountered.

3.4.3 Seasonal changes in polyp dry weight and lipid content

One way ANOVA shows a significant difference between sample months in polyp dry weight for samples of deep (F=3.58, p=0.01) and shallow (F=3.79, p=0.003) populations (Figure 3.4A). An analysis of % lipid content showed similar trends for deep (F=20.80, p<0.001) and shallow (F=9.51, p<0.001) samples (Figure 3.4B). Since the shallow corals are zooxanthellate and the near-shore habitat has a higher annual mean temperature than the deep reefs an *a priori* assumption was made that the shallow water habitat would confer an energetic advantage over the deep habitat. A one-tailed paired t-test was used to compare data from the two populations, and showed no significant difference for either polyp dry weight (t=0.74, p=0.26) or % lipid content (t=0.145, p=0.45).

Figure 3.3. Growth rates of coral samples from deep and shallow *O. varicosa* populations, maintained under different light and temperature conditions for 13 months. Error bars represent standard deviation from mean % monthly increase in skeletal deposition

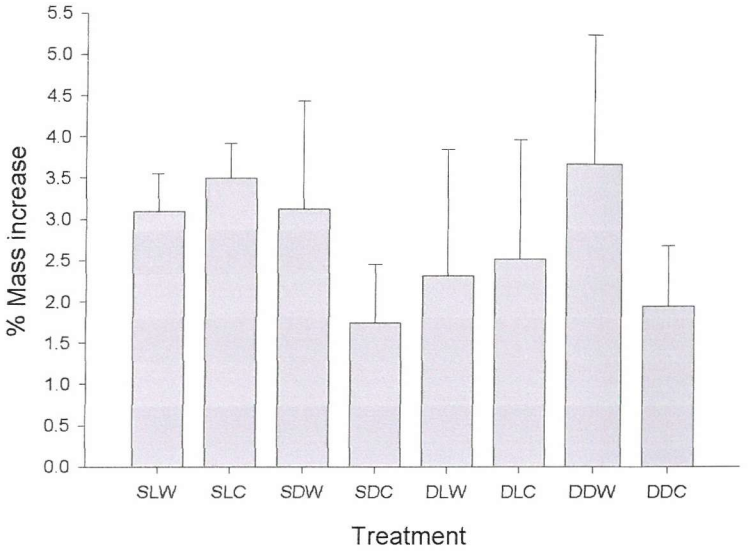
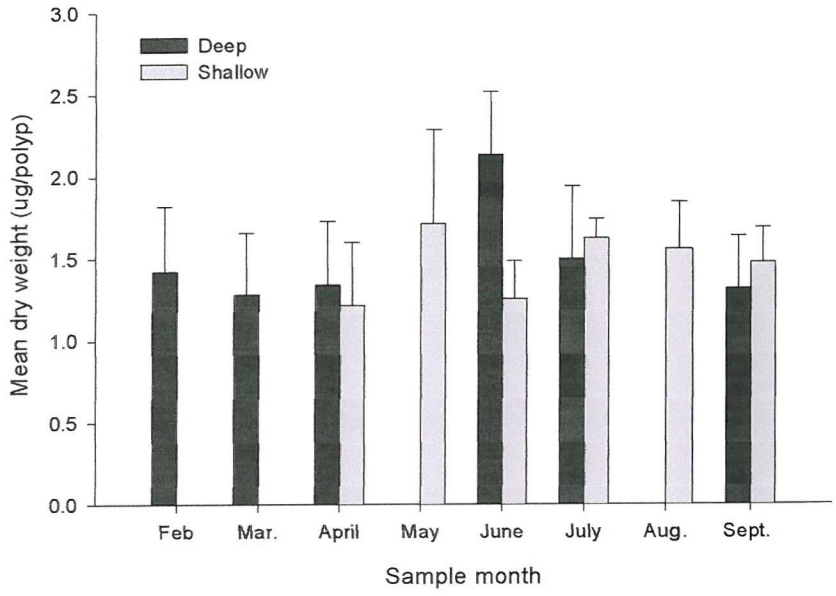


Table 3.2. Summary of results of two-way analysis of variance using factors: Population (deep and shallow), Light (dark and diurnal light) and Temperature (warm and cold) on arcsin-transformed % mass increase data. The F statistic and p values are shown for the factors and their interactions. Significant effect is designated by an asterisk.

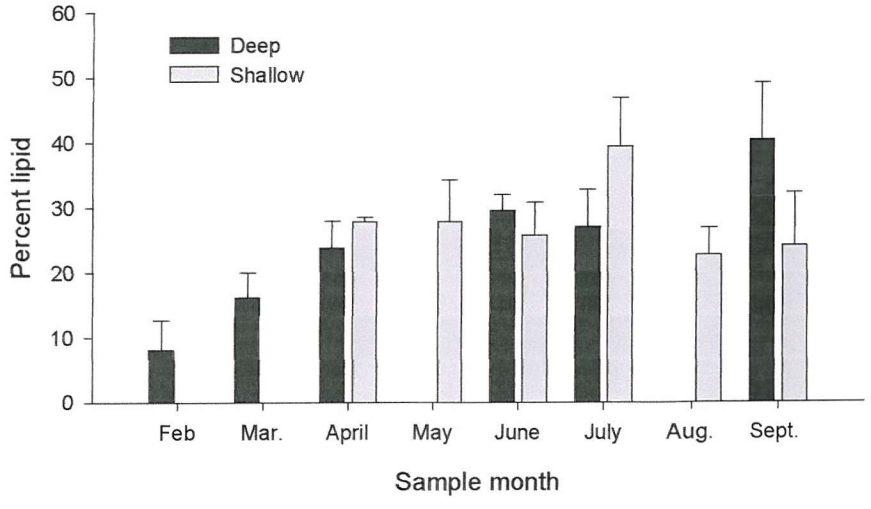
Source of Variance	**F**	**P**
Population	9.56	0.004*
Light	5.85	0.020*
Population x Light	7.53	0.009*
Population	7.504	0.009*
Temperature	0.003	0.959
Population x Temperature	2.050	0.160
Light	4.903	0.032*
Temperature	0.122	0.728
Light x Temperature	1.351	0.252

Figure 3.4. Seasonal changes in A) polyp dry weight (μg) B) % lipid content per polyp of *O. varicosa* samples from deep and shallow populations. Error bars are standard deviations from the mean of 6-10 samples.

A



B



3.4.4 Respiration and primary productivity

Coral respiration (oxygen consumption) rate at 25°C was 8.59 μg O_2 hr^{-1} cm^{-2} tissue. After the dark respiration runs, the sample was exposed to a range of increasing light intensities, and photosynthetic oxygen production was measured (Figure 3.5). The values for net and gross photosynthesis, plus their carbon equivalents are summarised in Table 3.3. The light compensation point (I_c) occurred at 60.5μE. The photosynthetic saturation point (P_{max}) was approximately 16.5μg O_2 hr^{-1} cm^{-2} and occurred between 200-250μE. Photo-inhibition was observed beyond 300μE. The initial slope of the curve (α) was 0.16 and the onset of light saturation (I_k) occurred at approximately 150μE.

3.4.5 Temperature data from deep and shallow sites

Simultaneous temperature data were only available for both sites between August 2000 and February 2001 (Figure3.6), and during that time period there was no significant difference in temperature between the two habitats ($t=-0.47$, $p=0.64$).

During the gametogenic months, average monthly temperatures recorded at Jeff's Reef in February (16.9°C), March (17.5°C), and April (20.7°C) of 2001 were significantly lower (paired t-test: $t=6.17$, $p<0.001$) than recorded the following February (20.2°C), March (21.9°C) and April (24.1°C) at Pepper Park. It is noteworthy however, that the temperature increase from February to April was 4°C at both sites, and 5.5°C at Pepper Park between February and May 2001.

During the spawning season, at Jeff's Reef the monthly average temperatures during July, August and September of 2000 were 19.25°C, 20.09°C, and 21.82°C respectively. These temperatures were several degrees lower than those recorded at Pepper Park in September 2000 (28.13°C), and during July (25.79°C), August (26.93°C), and September (27.16°C) of 2001.

3.5 Discussion

3.5.1 Laboratory growth rates

The high level of variance may have masked some of the treatment effects for the monthly mass increase data; however, the annual skeletal deposition data show clear response to treatment conditions. As expected, the shallow zooxanthellate samples grew faster under light conditions than did the azooxanthellate deep-water amples. The effect of light-induced calcium deposition reported for

Table 3.3. Summary of net and gross photosynthetic rates of *O. varicosa* shallow-water samples (μg O_2 cm^{-2} tissue hr^{-1}), and their carbon equivalents ($P_{Net}C$ and $P_{Gross}C$) for the experimental light intensities 0-500μE (μmol PAR m^{-2} s^{-1}).

LI μE	P_{Net}	P_{Gross}	P_{Net} C	P_{Gross} C
0	-8.59	0	-2.58	0
15	-5.76	2.83	-1.96	0.97
51	-1.76	6.84	-0.60	2.33
75	2.33	10.93	0.80	3.72
105	8.67	17.26	2.96	5.89
150	14.02	22.62	4.78	7.71
200	16.46	25.06	5.61	8.54
300	16.67	25.26	5.68	8.61
500	13.62	22.21	4.64	7.57

Figure 3.5. Effect of light intensity on oxygen flux for zooxanthellate *O. varicosa* sample. Each data point was generated from regression analysis of oxygen flux over time for a specific experimental light intensity. Error bars represent 95% confidence interval for the regression. P_{max}: maximum photosynthetic rate Ic: compensation point of respiration and photosynthesis. Ik: the minimum light intensity that produces maximum photosynthesis.

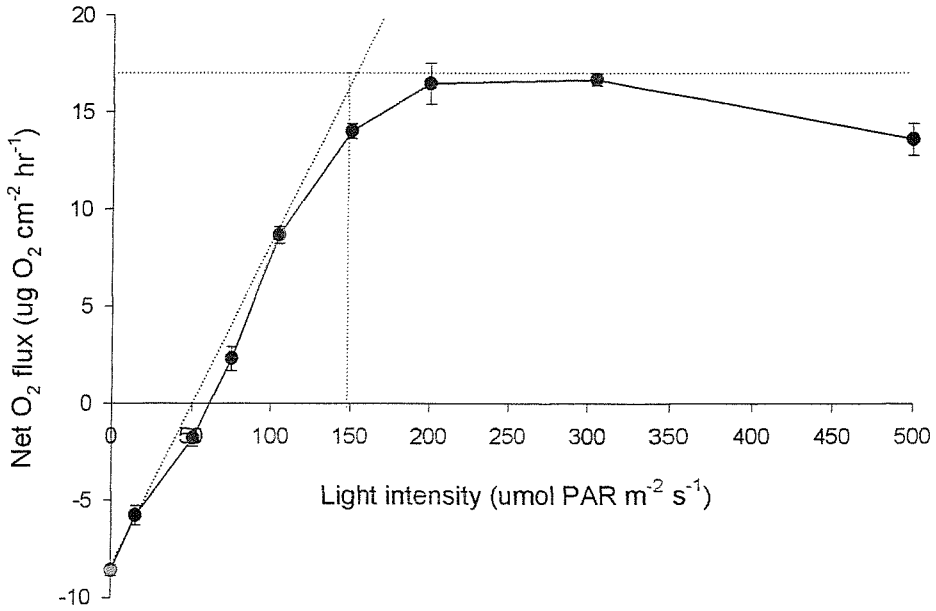
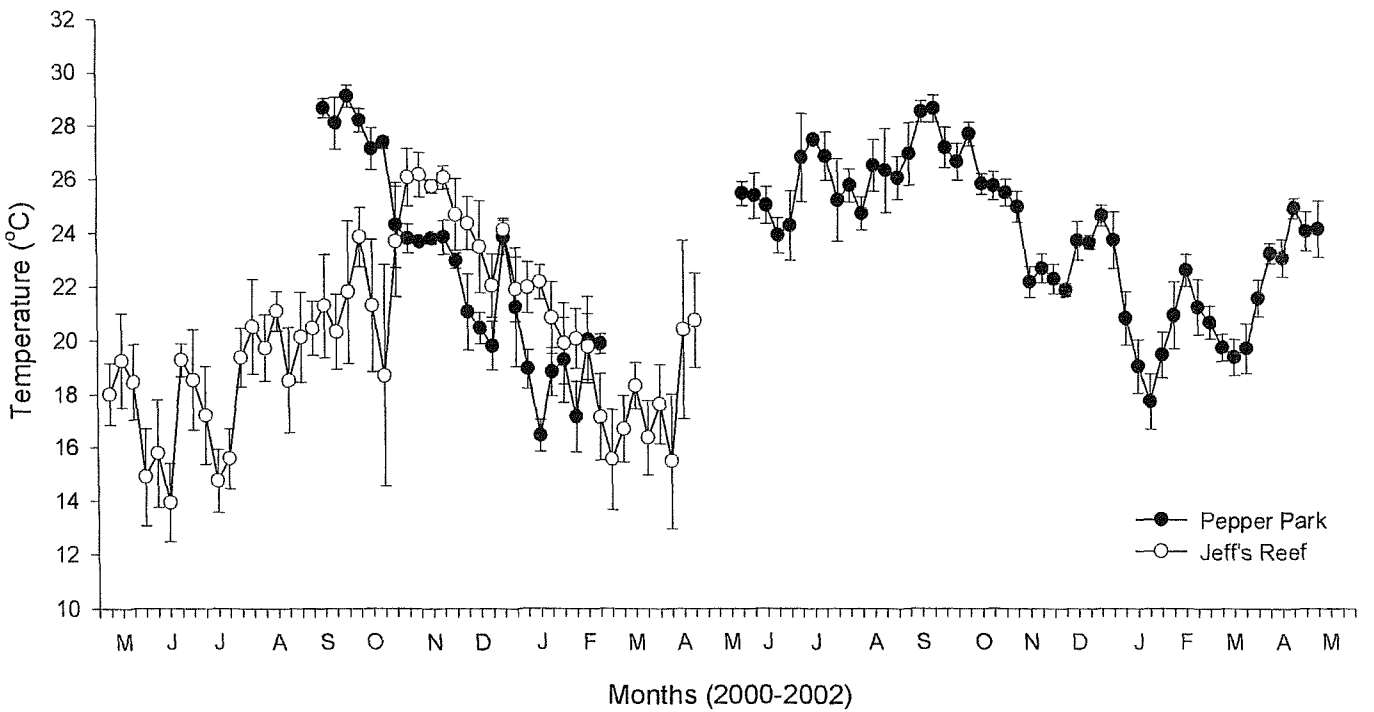


Figure 3.6. Seasonal changes in bottom water temperature (°C) at the deep (80m) and shallow (6m) study sites, Jeff's Reff and Pepper Park, respectively.



zooxanthellate corals (Chalker 1981, Smith and Douglas 1987) had a positive effect on growth rates but the dark warm samples did as well in both populations as those with light. A study by Marshall (1996) showed equal calcification rates in zooxanthellate (*Galaxea fascicularis*) and azooxanthellate (*Tubastrea fulkneri*) tropical corals. Jaques (1983) studied energetic parameters of the temperate coral *A. danae* and found that although light enhances calcification under some circumstances, the relationship between light intensity and calcium deposition is not simple.

Although *O. varicosa* can thrive in the azooxanthellate form, the presence of algal symbionts seems to confer some advantage to the coral. Also as expected, the higher experimental temperature had a positive effect on growth in samples from both populations. Increasing temperature increases metabolic rate, and would promote growth assuming that nutritional status was sufficient.

Presentation of growth rate in terms of calcium deposition rather than linear extension has possibly explained the apparent paradoxical results of Reed (1981), who found a greater linear growth rate in deep (cold-water) populations of *O. varicosa*. Thick wave-resistant branches, characteristic of shallow-water colonies require greater skeletal deposition per unit linear extension than the thin fragile branches of the deep colonies. Calcium deposition was slower at lower temperatures, but branches of the deep reefs may be sufficiently fine that their linear growth is faster than the shallow colonies, despite lower average temperatures, which did not have a significant impact on growth in this experiment.

Variation between the samples was high. These data were presented in terms of percentage, which is a function of the sample size since an absolute quantity of aragonite deposit (presented in figure 3.3) will be a greater percentage of a small sample than a large one. The coral growth form varied between samples; some attached readily to the clamshell base and polyps rapidly over the surface of the shell. These began to deposit skeleton, producing a high growth increment. Others of the samples however, never attached to the shells and growth was via extension of existing branches, which seems to be a slower growth mechanism.

The deep-water samples in the light treatments were more susceptible to algal overgrowth than shallow-water samples. Although this observation was not tested experimentally, the shallow samples may be more adapted to withstand algal overgrowth since they live in a high nutrient, insolated environment where macro-algae can thrive. The deep-water samples do not need such an adaptation since they

live in an environment that does not support observable levels of zooxanthellae, or other plant life, for most of the year. Deep-water samples did however, produce mucus much more readily and copiously than the shallow samples. This may be an adaptation to the extremely high sediment levels that can occur on the shelf-edge reefs (personal observation), allowing the deep-water corals to shed particles rapidly before the polyps suffocate.

3.5.2 In situ *growth rate experiment*

This experiment was not successful in either habitat. In the shallows the corals were damaged and broken by late summer tropical storms, and at the deep site the corals, although still alive, did not appear healthy so growth was not measured.

In both habitats, the transplant blocks were placed in areas where *O. varicosa* colonies were abundant, but differences between the microhabitat of the natural colonies and the transplanted corals may account for much of their mortality. Small natural colonies are attached to the benthos (or, on the deep reefs, to exposed surfaces of other coral colonies), where they are protected from high currents and turbulence by the rugosity of the substratum. The experimental samples were tied to plates and attached to the upper surface of the blocks, and were therefore exposed to currents and turbulence. Indeed, the elevated nature of the block would have magnified ambient current speed as it passed over the coral transplant.

In retrospect, the attachment of the coral to the plate should have been more secure. In a laboratory setting with static water, a cable tie is sufficient to hold the coral immobile until it can attach to the substratum, but in the ocean, the abrasion of the sample against both the cable tie and the plate probably contributed to breakage and tissue death. In the natural environment, especially the turbulent near-shore ledge epoxy cement would have created a more stable attachment. The blocks should also have been placed in more sheltered areas, or the plates should have been attached directly to the natural substratum rather than placed on an elevated surface.

3.5.3. Respiration and primary productivity

The respiratory and photosynthetic parameters measured for *O. varicosa* are similar to those recorded for shade-adapted *Stylophora pistillata* in the Sinai peninsula reef flats (Porter et al. 1984), although maximum net photosynthesis was higher for *S.*

pistillata (P_{net}:24$\mu gO_2cm^{-2}hr^{-1}$) than for *O. varicosa* (P_{net}:16 $\mu gO_2cm^{-2}hr^{-1}$). This could be the result of differences in density or clade designation of the zooxanthellae.

Comparison of shade adapted and light adapted colonies of *S. pistillata*, indicated a significantly higher light-use efficiency at low light level in shade-adapted colonies. The minimum light intensity for maximum photosynthesis (I_k) and the compensation irradiance (I_c) were both significantly lower in shade-adapted specimens, which means that photosynthetic rate increases from darkness to saturation irradiance faster than in light adapted corals. The values of I_c and I_k for *O. varicosa* (60.5μE and 150μE) were comparable to shade adapted *S. pistillata* (38μE and 161μE). Other studies have shown that maximum net photosynthetic rates are greater in light adapted corals than shade-adapted corals (Falkowski and Dubinski 1981), and that chlorophyll a levels were lower in corals (Porter et al. 1984) and zooxanthellae (Zvalinskii 1980, Dustan 1982) from deep than from shallow water. Unlike most tropical corals, *Oculina varicosa* lives for most of the time under light conditions that can vary rapidly in both deep and shallow habitats, depending on tidal cycle, storm activity, upwelling events and algal blooms. The speed of photo-adaption in *O. varicosa* is unknown but would be an ecologically important avenue to show how quickly these corals can respond rapid changes in their environment.

Contribution of translocated carbon from zooxanthellae to animal respiration (CZAR) was studied in light and shade adapted *S. pistillata* colonies (Muscatine et al. 1984). Translocation supplied more than the total carbon needed for respiration in light-adapted colonies, which derived only 6% of their carbon requirements from heterotrophy. However, nearly 50% of the carbon requirements of shade-adapted colonies came from particulate matter and DOM. Carbon budgets of the temperate anthozoans: *Cereus pedunculatus*, *Anthopleura ballii* and *Anemonia viridis* (sea anemones), plus the zoanthid *Isozoanthus sulcatus* were studied under irradiance regimes comparable to those encountered in the field (Davy et al. 1996). Results from the study showed that *C. pedunculatus* and *A. ballii* require a heterotrophic food source to survive, whereas *A. viridis* and *I. sulcatus* are potentially autotrophic under favourable conditions. Considering annual variations in field conditions, the authors suggest a general need for heterotrophically-derived carbon in temperate anthozoans.

Oculina varicosa lives in nutrient-rich habitats where POC is abundant, and this species can survive apparently indefinitely, without symbionts. A study of carbon

budgets under different light and temperature conditions would provide insight into the energetic significance of photosynthetically-derived carbon to this species.

3.5.4 Seasonal changes in polyp dry weight and lipid content

The samples were composed of both male and female corals. The presence of males would decrease the absolute value of lipid per polyp and increase the variance of the sample; however, sex ratios from samples taken for oocyte data were approximately equal so the meaning of the data was not compromised. Seasonal changes were measured in both polyp dry weight and lipid content, both being greater during the summer when the corals are reproductive. Lipid content was highest in July for the shallow corals and September for the deep corals (although no deep-water August samples were available for comparison). The presence of large numbers of eggs in the females could account for the elevated lipid levels during these periods of peak spawning activity. The levels of lipid are very similar for both populations, which implies equality between the habitats in terms of energy allocation to reproduction. Growth rate, and fecundity data from chapter 4, support the suggestion that this species reproduces in each habitat type with equal efficiency.

Environmental differences between the habitats had no apparent impact on the polyp size or the lipid content, since there was no difference between the data sets (figure 3.4B). The growth rate data support the idea that corals from both habitats are sufficiently resilient to fluctuations in environmental conditions, that the habitat specific differences have little impact on the energetics of the corals. The former measurement was prompted by the observation that tissue of shallow samples consistently appeared thicker and more robust than tissue of the deepwater polyps. The data do not support this observation, which means that either the shallow water tissues retain more fluid, which creates the illusion of bigger size, or the weighing scale was not sufficiently accurate with such tiny quantities.

3.5.5 Temperature data from the deep and shallow habitats

During the winter the corals are non-reproductive, and unfortunately the sampling dates of the temperature data from the different sites do not coincide during the gametogenic months (April-July) or the spawning months (July-September), when comparative temperatures would be of more ecological relevance. A general rise in temperature during the spring appears to be common to both sites and although the

absolute values are different, the spring temperature increase may be the trigger for *Oculina* gametogenesis. Seasonal temperature changes have been correlated with timing of reproductive cycles in other species of scleractinians (Harrison and Wallace 1990).

Temperature differences between the deep and shallow habitats during the spawning season could conceivably cause differences in gamete maturation rates, and ultimately in the timing of spawning. The ecological impacts of variation in spawning depend on effects of temperature on embryos and larvae and on time-specific larval transport mechanisms.

3.6 Conclusions

The growth rate data indicate that, zooxanthellae may confer an energetic advantage on the corals, as skeletal deposition rate was significantly higher in the presence of symbionts. Temperature did not have a significant effect on skeletal deposition rates, which is surprising since metabolic rate is directly affected by temperature. The presentation of growth rate in terms of calcium deposition rather than linear extension has explained the apparent paradoxical results of Reed (1981), who found a greater linear growth rate in deep (cold-water) populations of *O. varicosa*. The results are still not fully explained since the highest deposition occurred in the dark with deep samples (ie no help from symbionts).

Lipid levels of both populations were elevated during the spawning season, probably because of the presence of lipid rich eggs in the mesenteries. The lipid data also imply that the energetic allocation to reproduction is similar for deep and shallow populations. This subject will be addressed in chapter 5.

The respiration and photosynthetic parameters of zooxanthellate *O. varicosa*, are comparable to those of shade-adapted tropical species which experience similar light intensities, but more extensive studies are needed before ecologically relevant conclusions may be drawn regarding the energetic significance of photosynthetically derived carbon in this species.

Temperature data showed considerable seasonal variation within the different habitats with occasional rapid temperature fluctuations. The organisms that exploit these habitats are able to tolerate the environmental conditions, but studies of temperature specific respiration will determine whether temperature extremes cause physiological stress to the corals. The very limited amount of temperature data (current

and historical) from the *Oculina* habitats, indicates that the spring increase in water temperature coincides with the onset of gametogenesis in *O. varicosa*. These annual temperature cycles are highly variable; therefore much more data are required to establish the role of temperature in regulation of reproductive cycles.

Chapter 4: Gametogenesis, fecundity and spawning in deep and shallow populations of *Oculina varicosa*

4.1 Introduction

4.1.1 Reproduction of cold-water scleractinians

Most of the research published on coral reproductive ecology has focused on tropical reef-dwelling corals, and those living in temperate, cold-water and deep-water habitats have been somewhat neglected. These colder habitats usually preclude survival of the hermatypic corals prevalent in tropical oligotrophic ecosystems, and are dominated by branching or solitary species.

Deepwater corals thrive on topographical features such as seamounts, shelf edges and other hard bottom areas with high current speeds. Information on deepwater scleractinians is very limited and much of the information comes from a study on the deep-sea coral bioherms in the New Zealand region. These bioherms contain a scleractinian assemblage of at least five colonial species, while ahermatypic coral reefs in other ocean basins are usually dominated by only one or two colonial species. The following information was obtained via personal communication with the scientist (S. Burgess) and had not yet been published.

Coral species studied included four colonial species, *Enallopsammia rostrata, Goniocorella dumosa, Madrepora oculata* and *Solenosmilia variabilis,* plus one solitary species *Desmophyllum dianthus. Solenosmilia variabilis* was found to be gonochoric, a trait that was also suggested for *E. rostrata, G. dumosa* and *M. oculata,* although colonies of only one sex were collected for these species. Broadcast spawning was the probable mode of reproduction, with fertilisation occurring in late April or May. There was a high level of synchrony between species in the seamount localities studied. High fecundities were estimated for *E. rostrata* (>144 oocytes per polyp), *G. dumosa* (>480 oocytes per polyp) and *S. variabilis* (>290 oocytes per polyp), with a negative correlation between oocyte size and number observed for all three species.

Reproductive biology and seasonality have been studied in the deep-water solitary coral *Fungiacyathus marenzelleri* from 2200m depth in the NE Atlantic by Waller et al. (in press). This species was a quasi-continuous reproducer with overlapping gametogenesis for both spermatocysts and oocytes. All individuals were gonochoric, and produced high numbers (~2900) of large eggs (~750μm), suggesting

lecithotrophic development. Broadcasting of gametes was inferred, possibly during June/July.

4.1.2 Effect of depth on coral reproduction

There is evidence in the literature that depth may affect reproductive output in cnidarians. For example, two Jamaican species of *Zoanthus* showed reduced reproductive activity with increasing depth (Karlson 1981), and fecundity of surface colonies of *Acropora palifera* was approximately twice that of colonies from depths greater than 12m (Kojis and Quinn 1983). Shallow colonies of *Stylophora pistillata* also released much greater numbers of planulae and had a larger number of reproductive colonies than deep colonies (Rinkevich and Loya 1987). These species were all from oligotrophic systems where algal sybionts commonly supplement anthozoan nutrition. When low light level compromises the symbionts, the nutrition derived from them also decreases and reproductive effort appears to suffer. Non-tropical species are frequently azooxanthellate; without the benefit of energy from algal symbionts, they rely on heterotrophy for their energy intake. The presence of zooxanthellae also stimulates skeletal deposition and increases growth of zooxanthellate species. *Oculina varicosa* lives in a sub-tropical high-nutrient environment and can survive without algal symbionts, although the contribution of the zooxanthellae to the coral nutrition is unknown.

4.1.3 Spawning cues

Cycles of spawning and gametogenesis of many species of coral have been shown to correlate with factors such as temperature (Goreau et al. 1981; Tranter et al. 1982), season (Szmant-Froelich et al. 1980; Fadlallah and Pearse 1982 a, b), and lunar phase (Lewis1974; Stimson 1978). Non-tropical shallow habitats are often highly variable and predictable cues such as changes in day-length, rather than more variable factors such as seasonal temperature changes, may control reproductive cycles. The deep-sea is less variable but shallow water cues such as day-length or lunar phase may attenuate in the deep ocean. Rigorous tests of the cues controlling biological rhythms require experiments that are beyond the scope of this project; however, the schedule of observed spawning events will be discussed in the light of possible environmental influence. This chapter will compare reproductive periodicity, fecundity and spawning of deep and shallow populations of *Oculina varicosa*.

4.2 Objectives

1. Describe the reproductive strategy and gametogenic cycles of *O. varicosa* colonies in both deep and shallow water populations.
2. Determine whether gametogenesis is synchronous within each population.
3. Determine whether gametogenesis varies inter-annually within populations.
4. Compare the gametogenic cycles of deep and shallow populations.
5. Determine whether fecundity varies intra-annually (both populations) and inter-annually (deep population).
6. Compare the fecundity of deep and shallow populations.
7. Correlate spawning events with tidal and lunar phase, and time of day.

4.3 Materials and Methods:

4.3.1 Sampling schedule for deep populations

Samples of deep-water populations were collected on eight separate cruises between March 1998 and September 2001. Twelve sets of samples were taken in total, from between 80 and 100 meters depth. Six collections were made at 'Jeff's Reef' (27°32.54′ N; 79°58.73′ W), using either a National Marine Fisheries Service (NMFS) 'Phantom' ROV (February 1999), the Johnson Sea Link research submersible (June 1999), a 'Rescue ROV' from Harbor Branch Oceanographic Institution (April and July 2000), or the 'Clelia' research submersible (March 2001, September 2001). Two collections were made (March 1998, September 2000) outside the *Oculina* Habitat of Particular Concern. (OHAPC) at a site off Cape Canaveral (28°29.82′ N; 80°01.24′ W), using a small (1.5m swath) trawling device. One set of samples was collected from a site called the 'Steeple' (27°43.60′ N; 79°58.75′ W), in February 1999, using the NMFS 'Phantom' ROV. A set of samples was also collected from Eau Gallie (28°08.6′ N; 79°59.5′ W), Sebastian Pinnacles (27°50.974′ N; 79°57.698′ W) and Chapman's Reef (27°36.4′ N; 79°58.4′ W) in September 2001 using the 'Clelia' research submersible. During each collection, samples were taken from several (5-12) different healthy colonies. Small pieces of each branch were preserved immediately for genetic analysis (95% ETOH), histology (10% buffered formalin) and lipid analysis (frozen at -60°C).

4.3.2 Sampling schedule for shallow populations

Samples of shallow-water *O. varicosa* colonies were collected monthly (weather permitting) using scuba during 1998 and 1999. In 2000 and 2001, samples

were collected only during the reproductive season in order to examine inter-annual variability in gametogenic cycle, and to obtain gametes for larval culture. Small pieces of coral (<5cm branch length) were removed with hammer and chisel from 10 different colonies per sampling date. Shallow water *Oculina* colonies are too small to permit repeated sampling of the same colony, therefore colonies were haphazardly selected during each sampling event, but were at least 20m apart to avoid sampling of fragmented clones.

4.3.3 Determination of reproductive season and gametogenic cycle

For histological examination, polyps at the base of the sample branches were used, as the small polyps at the branch tips were potentially non-reproductive (Rinkevich and Loya, 1987). After decalcification in 10% hydrochloric acid for 6-10 hours, polyps were dehydrated through a series of ethanol concentrations, embedded in paraffin wax, cut into 8μm sections, and stained using Mayer's Haematoxylin/Eosin B staining techniques. Images of oocytes were taken using an Optronics digital camera attached to an Olympus BX50 compound microscope. The area of each oocyte was measured and recorded using Image Tool (UTHSCSA) image analysis software. Oocyte 'feret' diameter was calculated, which estimates the diameter of a hypothetical circle with the same area as the object measured.

$$\text{Feret diameter} = \sqrt{\frac{4 \times \text{area}}{\pi}}$$

Information on oocyte feret diameter and size frequency distribution was used to infer the timing of the gametogenic cycle. The percentage of oocytes with a diameter of 60μm or above (which represents lower limit of mature oocytes) was also calculated from the histological data. Oocyte size-frequency distributions were generated using the oocyte diameter data. For the male colonies, the histological sections were examined and the percentage of gonads containing mature sperm was calculated for 20 mesenteries per colony at each sampling date. Analysis of variance was used to test for significant ($p<0.05$) intra and inter-month differences, as well as inter-annual variation in both male and female data sets (Grant and Tyler 1983a, 1983b). Samples from both deep and shallow populations were processed in the same manner.

4.3.4 Fecundity

Samples collected during the spawning season (late July to September) in 2001 from the shallow site, and 2000 and 2001 from the deep sites, were used for fecundity analysis. Samples were fixed in 10% seawater-buffered formalin solution for 24 hours and then transferred to 30% ethanol for dissection using fine forceps and needles under a dissecting microscope. The polyp length and total number of eggs per polyp were recorded for 3 polyps from each female colony. Regression analysis was used to determine whether fecundity increases as a function of increasing polyp length. For samples from both deep and shallow populations, the intra and inter-colony variation was tested using analysis of variance ($p<0.05$), and for the deep samples, inter-annual variation was also calculated. Some of the female colonies had spawned prior to collection, and therefore could not be used for fecundity estimates.

To facilitate comparison of fecundity between populations, the number of polyps cm^{-2} of skeletal surface area was estimated from samples of both deep and shallow skeletons. A piece of aluminum foil was cut to a known size (either $25cm^2$ or $12.5cm^2$), and wrapped around the branches of a sample of *Oculina* skeleton. The number of polyps covered by the foil was counted, and repeated for 12 skeletons of both deep and shallow populations. The average number of polyps cm^{-2} of surface skeletal area was then calculated.

4.3.5 Spawning

Small samples of coral from 8-13 different colonies were collected from the field during periods of greatest egg diameter. In 1998, the samples were maintained separately in 2-liter glass bowls containing 20μm-filtered seawater at ambient temperature (25-27°C), with a view to obtaining separate samples of eggs and sperm for experiments. In subsequent years the coral samples were combined in a small aquarium containing filtered seawater and exposed to natural light cycles at ambient temperature. Information on time of day, lunar phase and tidal cycle were taken during or after gamete release in an effort to correlate these parameters with natural spawning events.

4.4 Results

4.4.1 Reproductive strategy and gametogenic cycles

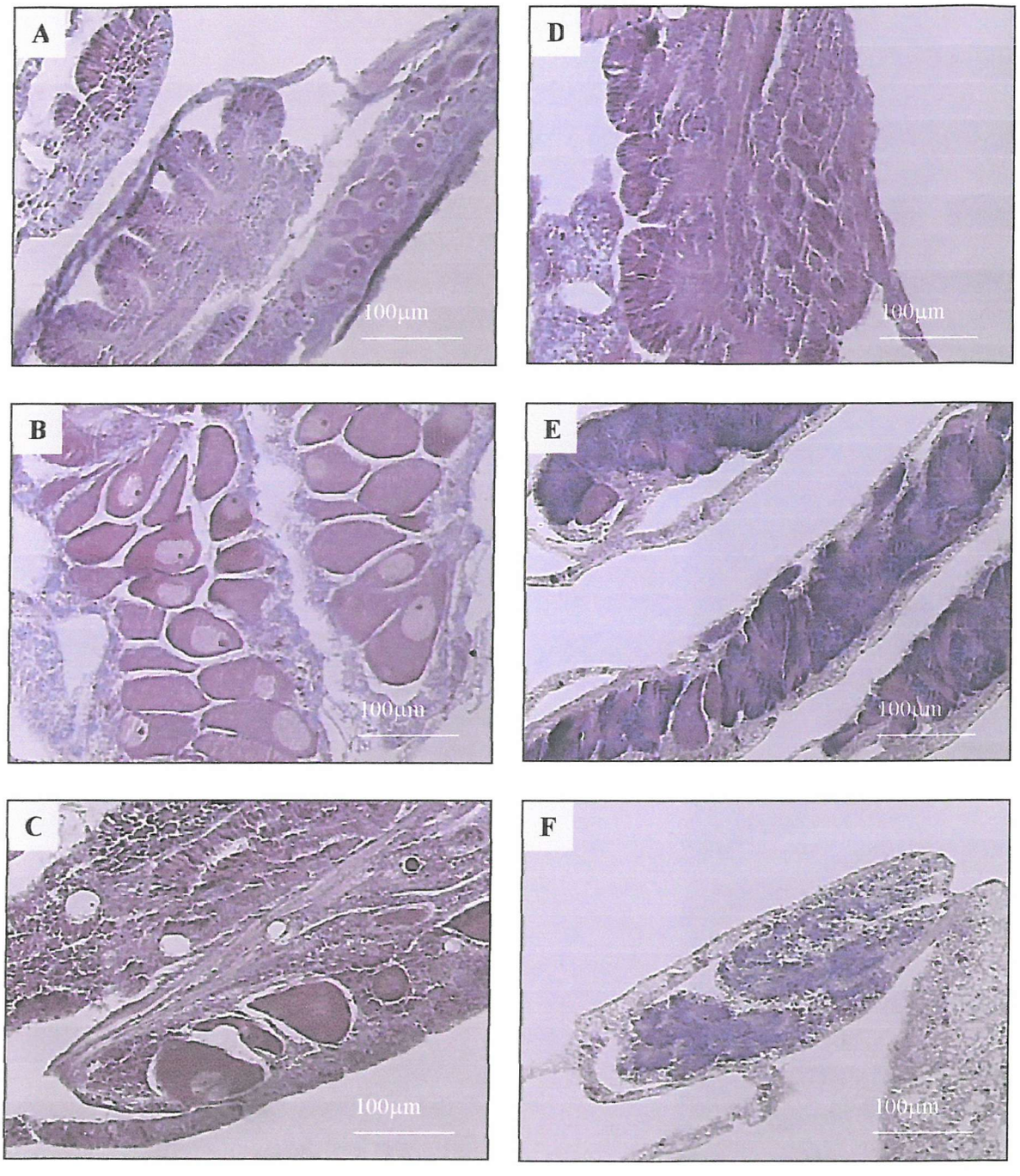
The reproductive tissues of scleractinians are not contained within a true organ, but for ease of description, they will henceforth be referred to as gonads. In *Oculina varicosa* the gonads are situated inside the mesenteries, closely associated with the mesenterial filament. *Oculina varicosa* has 12 pairs of mesenteries, with one member of each pair usually larger than the other. There appears to be just one large area of reproductive tissue per mesentery, which expands as the reproductive season proceeds and distends the mesenteries. The oocytes are compressed irregularly-shaped discs in the mesentery, but become spherical just prior to expulsion, or shortly thereafter. The testes have many lobes, giving the impression of multiple gonads in histological sections of males. Small genital pores are visible in the mesentery wall of ripe adults, which may allow passage of gametes into the gastrovascular cavity prior to spawning; however this has yet to be established.

Time series samples from both deep and shallow populations indicate that *Oculina varicosa* is a gonochoristic broadcast spawning species, with an annual, seasonal gametogenic cycle. Figures 4.1A, B, C show the progression of gametogenesis in females and Figures 4.1D, E and F show development of male gonads. Histological information shows that gametogenesis begins in April, and culminates in a protracted spawning season that occurs from late July to early September. Some remnant eggs are present in October and November, but from December to April, no reproductive material was observed in either deep or shallow populations.

4.4.1.1 Gametogenesis in deep-water populations

Histological sections of polyps collected in March 1998 and February 1999 show no indication of reproductive activity. In June 1999, small oocytes (mean 41.83μm, SD=7.21) in the early stages of vitellogenesis were observed. Larger oocytes were observed in July 1999 (56.56μm, SD= 0) and 2000 (46.37μm, SD=4.69), and a small spawning event occurred in the laboratory on August 9, 2000. Samples collected in September 2000 and 2001 contained the largest oocytes (69.41μm, SD=7.85 and 61.91μm, SD=7.85 respectively)(Figure 4.2A). A large spawning event was observed by samples collected from the deep reef on the night of September 9,

Figure 4.1A-F. Images showing the progression of gametogenesis in *O. varicosa* polyps: A) early female gametogenesis B) late gametogenesis showing vitellogenic oocytes C) post spawning female showing remnants of late oocytes D) early male gametogenesis E) late stage testes F) post spawning male. Scale bars = 100μm.



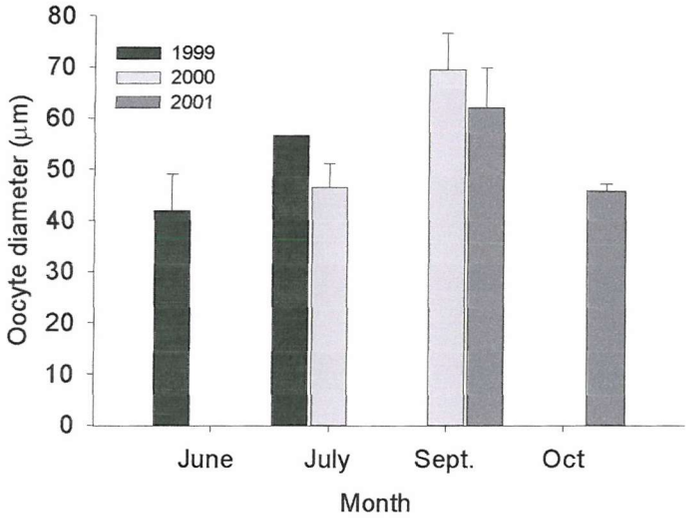
2000 and samples collected from the cruise to the deep reef in September 2001 spawned on the night of September 7, but fewer than 1000 eggs were released. The data show a decrease in mean oocyte diameter between September and October 2001. A small number of late stage oocytes and some small oocytes (45.49μm, SD=1.39) were present in October. These gonads did not appear to be undergoing a second gametogenic cycle as the eggs were few and poorly organized within the gonad area; rather they appeared to be remnant oocytes from the gametogenic cycle. The percentage of mature male gonads was low in late June 1999 (6.57%), but increased rapidly in July 1999 to 44% (Figure 4.2B). In 2000, development was slower, but by September of both 2000 and 2001, 74% of the male mesenteries contained ripe testes. The percentage of mature testes present in the mesenteries fell considerably in October to less than 1%. The percentage of mature gametes increased synchronously in males and females as the reproductive cycles progressed (Figure 4.2B).

4.4.1.2 Gametogenesis in shallow-water populations

Oocyte diameter increased throughout the gametogenic cycle, from April through to September, for 1998, 1999 and 2000 (Figure 4.3A). Samples collected in December January February and March show no indication of gametogenic activity. Oogonia were first observed in samples from April 1999 (3.75μm, SD=0.22). Oocyte size increased in May 1998 (26.75μm, SD=5.28) and 1999 (20.93μm, SD=4.46), with oocytes still of uniform size. Oocyte diameter increased as the gametogenic cycles progressed, through June (38.69μm, SD=10.79; 41.01μm, SD=6.42; 49.62μm, SD=13.56) and July (65.76μm, SD=8.13; 60.09μm, SD=6.48; 67.44μm, SD=7.42). Peak oocyte size occurred in August (68.45μm, SD=2.51; 80.95μm, SD=2.41) and September (76.74μm, SD=8.34; 64.43μm, SD=9.41) of 1998 and 1999. Spawning occurred in the laboratory by samples collected from the field during the late summer months. Samples collected in November 1998 contained a small number of large oocytes (94.4μm, SD=13.49). Since the eggs were large and rather diffuse, they are thought to be the remnants of the gametogenic cycle. As in the deepwater samples, the percentage of late stage gametes increased synchronously in males and females as the reproductive cycles progressed (Figure 4.3B).

Figure 4.2. A) Oocyte development over time for deep-water *Oculina* colonies. Fifty oocytes were measured from three different colonies per sample date, except July 1999 when only one female colony was available. B) Percentage of gametogenic material in the late stages of development i.e. male gonads with heavily stained sperm and females with oocytes >60μm in diameter. Twenty mesenteries were scored for gonad maturity from each of 3 different male and female colonies per month

A



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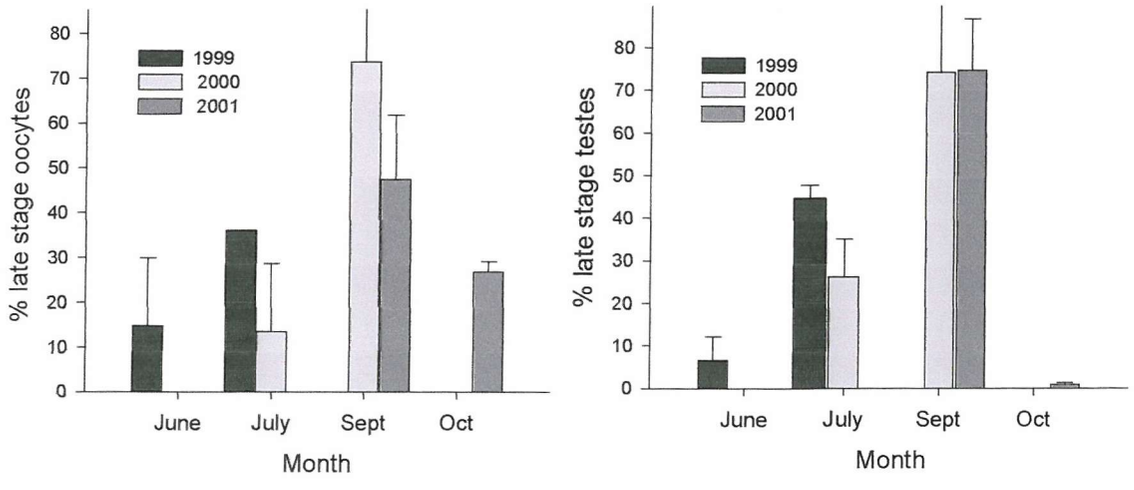
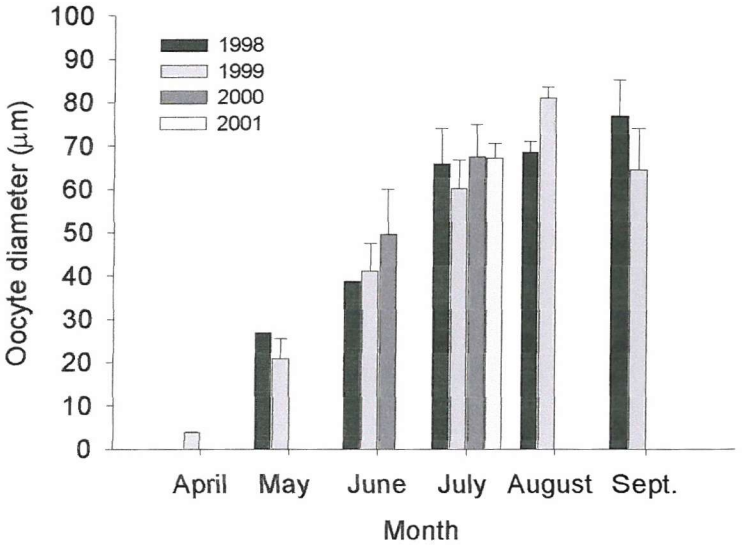


Figure 4.3. A) Oocyte development over time for shallow-water *Oculina* colonies. Error bars represent standard deviation from mean oocyte diameter from 3-5 colonies per month. B) Graph showing the percentage of gametogenic material in the late stages of development: male gonads containing heavily stained testes and females with oocytes >60μm in diameter. Twenty mesenteries were scored for gonad maturity from each of 3 different male and female colonies per month

A



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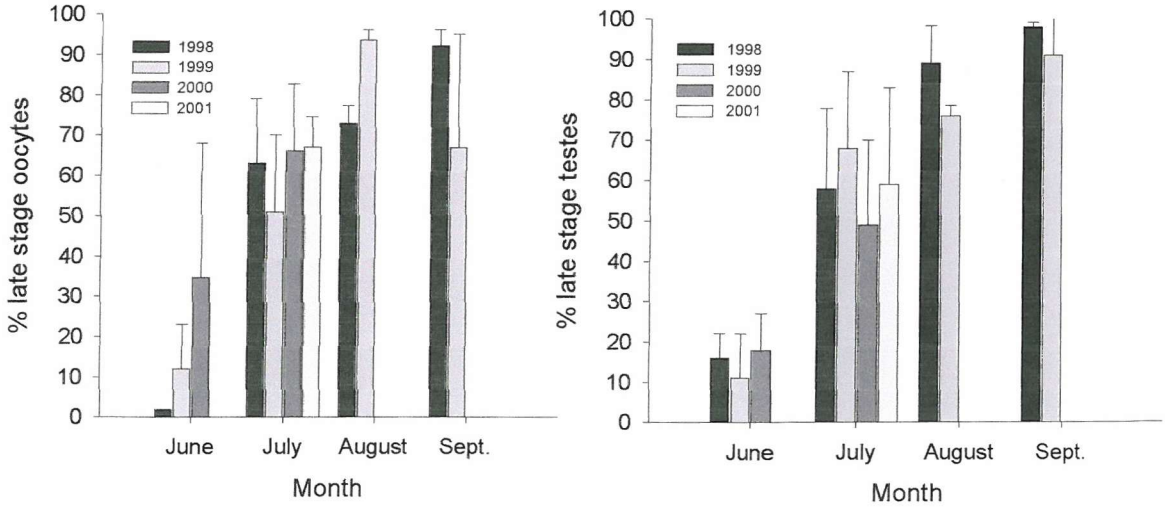


4.4.2 Synchrony of gametogenesis within populations

4.4.2.1 Deep-water populations

Samples were analysed for inter-colony variation in oocyte diameter within sampling date, using one way analysis of variance. When the data failed to meet the conditions of normality and homogeneity of variance, an ANOVA on ranks was performed and the H statistic is presented. All sample months for 1999, 2000 and 2001 showed a significant difference between colonies ($p< 0.001$) (Table 4.1), except for October 2001 ($H=0.81$, $p=0.71$), indicating a lack of synchrony between colonies during the middle of the gametogenic cycle. The colonies remained asynchronous toward the end of the gametogenic cycle, with significant differences between colonies in September 2000 ($F=7.89$, $p=0.001$) and 2001 ($F=13.4$, $p=0.001$).

4.4.2.2 Shallow-water populations

The samples were analysed for inter-colony variation in oocyte diameter within sampling year, using one way analysis of variance. Of the fourteen sample dates, ten showed a significant difference ($p< 0.001$) between colonies (Table 4.2), therefore, as with the deepwater population, there is a high level of asynchrony between colonies for much of the oogenic cycle. The exceptions were April 1999 ($F=3.43$, $p=0.07$), August 1998 ($F=1.57$, $p=0.21$), August 1999 ($F=1.83$, $p=0.14$) and July 2001 ($F=1.75$, $p=0.18$), which again, coincide with the earliest and later stages of development.

4.4.3 Inter-annual gametogenic variation within populations.

4.4.3.1 Deep populations

There was no significant difference in mean oocyte diameter between July 1999 and July 2000 ($F=3.54$, $p=0.20$) or September 2000 and September 2001 ($F=2.21$, $p=0.18$). Changes in oocyte size distribution throughout the reproductive season, and inter-annual variation in oocyte development are shown in Figure 4.4. The limited data indicated no significant inter-annual variation in the timing of oocyte development and development of the male gonads also appeared to be consistent between years.

4.4.3.2 Shallow populations

One-way analyses of variance between sample pairs showed no significant inter-annual differences in oocyte diameter for May, June, July or

Figure 4.4. *Oculina varicosa* oocyte size frequency distribution for deepwater populations, with months from different years. Error bars are the standard deviation from the mean of 3-5 female colonies per month.

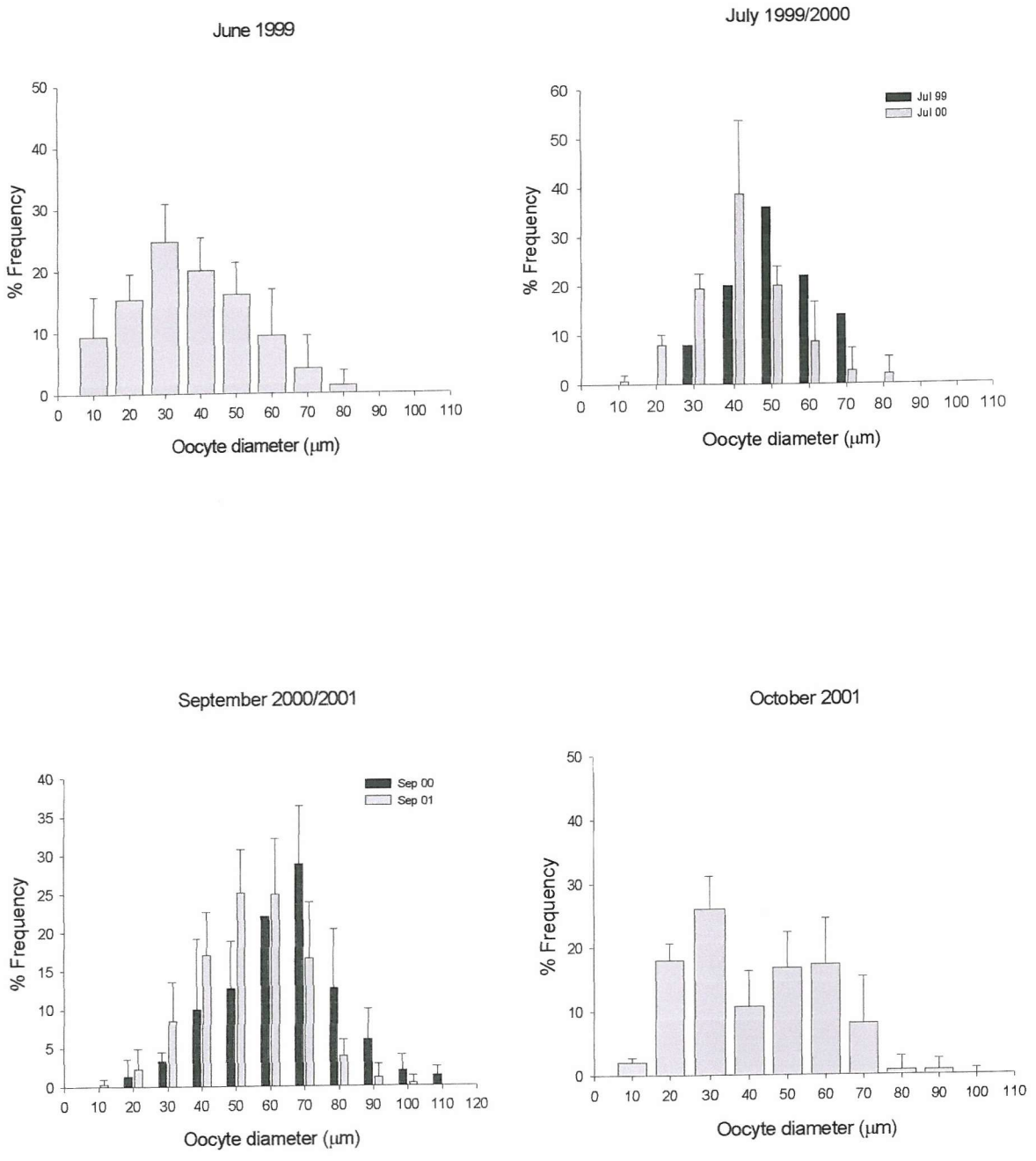


Figure 4.5: *O. varicosa* oocyte size frequency distribution for shallow-water populations, with months from different years. Error bars are the standard deviation from the mean of 3-5 female colonies per month.

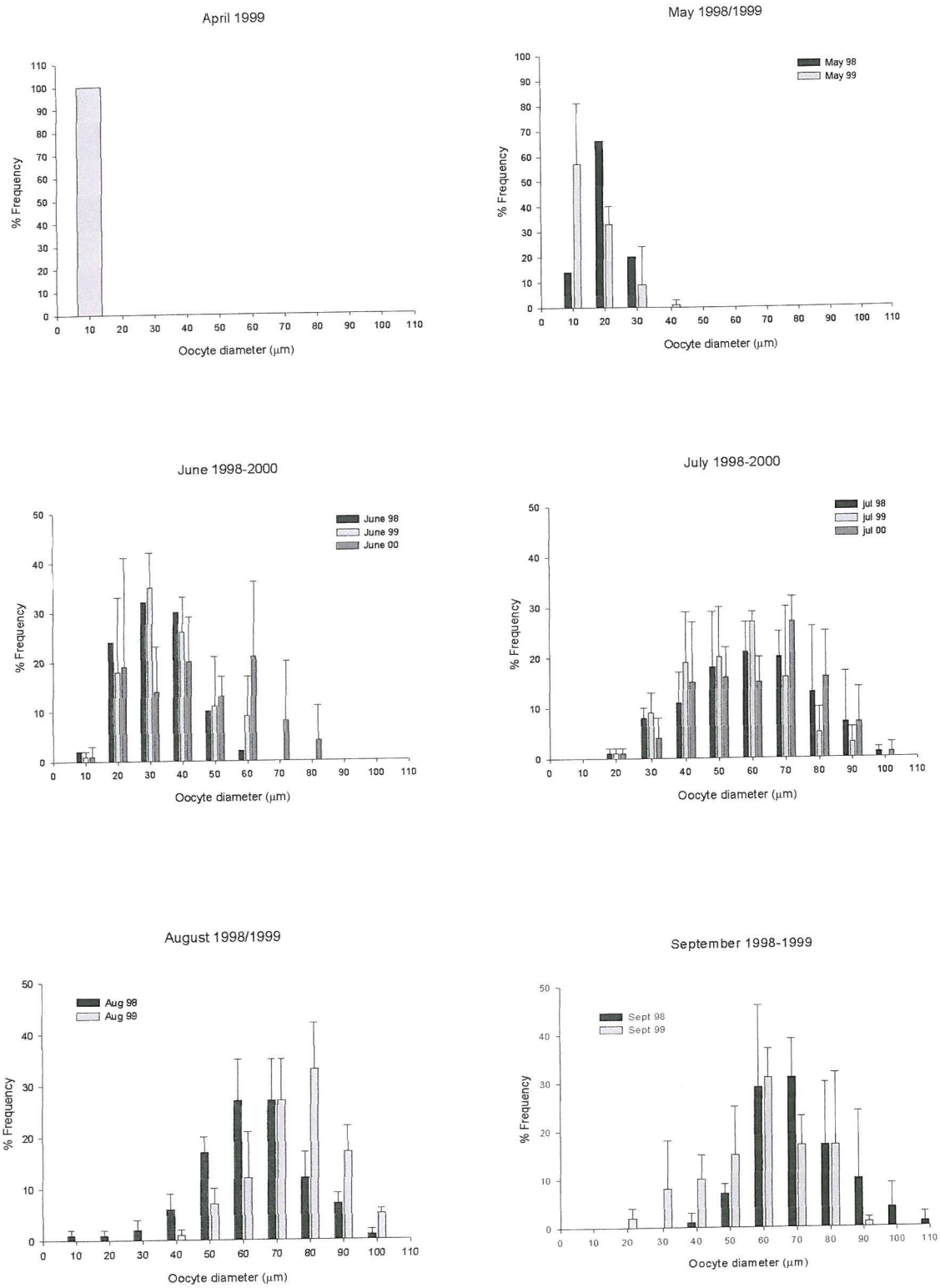


Table 4.1 Results of analysis of variance within deep-water populations using oocyte diameter. Significance level $p<0.05$; an asterisk denotes those months where oocyte size differed significantly between colonies. Except where otherwise labeled, all the test statistics are one way ANOVA (F). Those data sets that failed tests for normality and/or homogeneity of variance were analysed using ANOVA on ranks (H).

Sample month	Test statistic	P
June 1999	F = 11.50	0.00*
July 1999	N/A	0.00*
July 2000	H=10.31	0.01*
Sept 2000	F= 7.89	0.00*
Sept 2001	F=13.42	0.00*
Oct 2001	H=0.81	0.66

Table 4.2 Results of analysis of variance within shallow-water populations using oocyte diameter. Significance level $p<0.05$; an asterisk denotes those months where oocyte diameter differed significantly between samples.

	1998		1999		2000		2001	
Month	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
April			3.44	0.001*				
May			H=34.4	0.001*				
June			H=32.9	0.001*	46.55	0.001*		
July	H=22.0	0.001*	10.49	0.001*	11.62	0.001*	1.75	0.18
Aug.	1.57	0.21	1.83	0.14				
Sept.	H=38.7	0.001*	22.23	0.001*				

Table 4.3 Results of analysis of variance to determine inter-annual differences in oocyte diameter in shallow populations. Significance level $p<0.05$; an asterisk denotes those months where oocyte diameter differed significantly between samples.

Month	Years compared	F	P
May	98, 99	1.27	0.38
June	98, 99, 00	0.66	0.57
July	98, 99, 00, 01	9.47	0.00*
Aug.	98, 99	44.36	0.00*
Sept.	98, 99	2.87	0.17

September (Table 4.3). There was however, a significant difference between samples from August 1998 and 1999 (F=44.36; p=0.017). Figure 4.5 shows changes in oocyte size distribution both within and between years. A two-way analysis of variance was used to compare mean oocyte diameter from all months for 1998, 1999 and 2000. The analysis showed no significant difference between the samples (F=2.86, p=0.12), indicating synchrony of oocyte development across the years under study.

4.4.4 Variation in gametogenesis of deep and shallow populations.

Both deep and shallow data are available for two collection dates, June 1999 and July 2000. One-way analysis of variance was performed on the oocyte diameter data from these samples; no significant difference was found between the populations from June 1999 (F=0.02; p=0.89), but a significant difference was detected in the July 2000 samples (F=18.20; p=0.007). A comparison of reproductive cycles of deep and shallow populations would be very useful if the data sets were more compatible, unfortunately with so little data, inferences into the coherence of cycles across depths would be questionable.

4.4.5 Fecundity variation within populations.

4.4.5.1 Deep-water populations

Regression analysis shows a non significant relationship between fecundity and polyp length for 2000 (r^2: 0.726, F=7.95, p=0.07) and 2001 (r^2: 0.1, F=0.35, p=0.6). Fecundity was standardised to represent eggs mm^{-1} of polyp length, and a one way ANOVA showed a significant difference in fecundity between deep-water colonies collected in 2000 (F=7.028; p=0.006) and 2001 (F=21.80; p=0.001). The results of an unpaired two-tailed t-test show a significant inter-annual difference in fecundity of deep-water colonies from 2000 and 2001 (t=-3.5, p=0.01).

4.4.5.2 Shallow-water population

As with the deep-water populations, Regression analysis shows a non-significant relationship between fecundity and polyp length (r^2: 0.729, F=8.07, p=0.06). Fecundity data were standardised to eggs mm^{-1} of polyp length for data analysis. Results of a one-way ANOVA indicated significant inter-colony fecundity variation (F=8.85; p=0.043).

4.4.6 Comparison of fecundity between populations.

4.4.6.1 Fecundity per polyp.

Shallow water fecundity data are not available for 2000; therefore fecundity comparisons between deep and shallow populations can only be made using the data from 2001. The *a priori* assumption was made that shallow colonies would have a higher fecundity because of the energetic benefit of symbiotic algae. Results of a one-tailed unpaired t-test (assuming unequal variances), show no significant difference ($t=0.579$, $p=0.29$) between the deep and shallow fecundity data for 2001.

4.4.6.2 Fecundity per skeletal surface area.

Measurements of the mean number of polyps per cm^{-2} skeletal surface area (SSA), and standardised (mm polyp $length^{-1}$) polyp fecundity data were combined to produce an estimate of eggs cm^{-2} SSA for the deep (Figure 4.6A), and shallow (Figure 4.6B) 2001 samples. A one-tailed t-test with unequal variances ($t=1.890$; $p=0.048$), indicated that shallow populations have a significantly higher fecundity per unit of skeletal surface area than the deep samples.

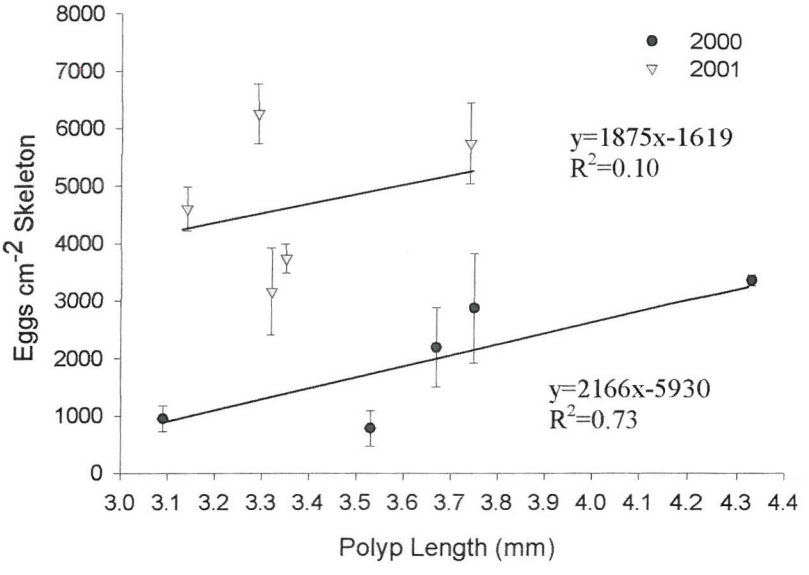
Regression analysis of eggs cm^{-2} SSA against polyp length produced a non-significant relationship for deep-water samples from 2000 ($r^2=0.73$, $F=7.95$, $p=0.07$) and 2001 ($r^2=0.1$, $F=0.35$, $p=0.60$). Regression analysis of shallow water 2000 samples however showed a significant ($r^2=0.44$, $F=10.30$, $p=0.01$) increase in eggs per unit SSA with increasing polyp length. The regression slopes for 2001 were compared for the deep and shallow populations (using a 2-tailed t-test to measure equality of slope coefficient) and no significant difference was found ($t=1.77$, $p>0.05$).

4.4.7 Correlation of spawning events with lunar, tidal and diurnal phase

Spawning events were recorded for both deep and shallow samples from mid-July and early September (Figure 4.7) between 1998 and 2001. A total of 29 events were recorded over the 4-year time period, 26 from shallow samples, and 3 from deep samples. The events were divided into groups according to the moon phase at which they occurred. Four events occurred within 2 days of the Full moon (spring tide), 9 within 2 days of the new moon (spring tide), 4 within 2 days of the first quarter (neap tide) and 8 within 2 days of the 3rd quarter (neap tide). There was no obvious correlation of spawning events with lunar or tidal phase. Twenty-one of these 29

Figure 4.6. The relationship between fecundity (eggs per skeletal surface area) and polyp length for samples taken from A) deep populations of *O. varicosa* in 2000 and 2001 B) shallow populations of *O. varicosa* in 2001. Error bars represent the standard deviation of the mean number of eggs from 5 polyps per colony.

A



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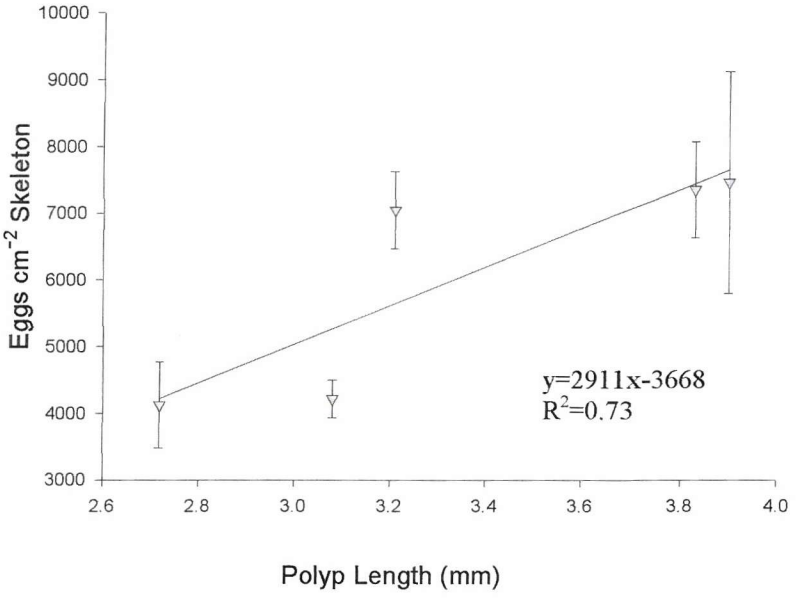


events occurred between sunset and sunrise, and a Chi-squared analysis shows a significant deviation from the expected distribution of 14.5 daytime and 14.5 nighttime events (X^2=9.138; p=0.05). When corals were maintained separately, 13 of 17 spawning events were by males, which differs significantly from the expected ratio of 8.5: 8.5. (X^2=4.77, p=0.05). When corals were held together, a male invariably initiated spawning, with other males and females spawning subsequently.

4.5 Discussion

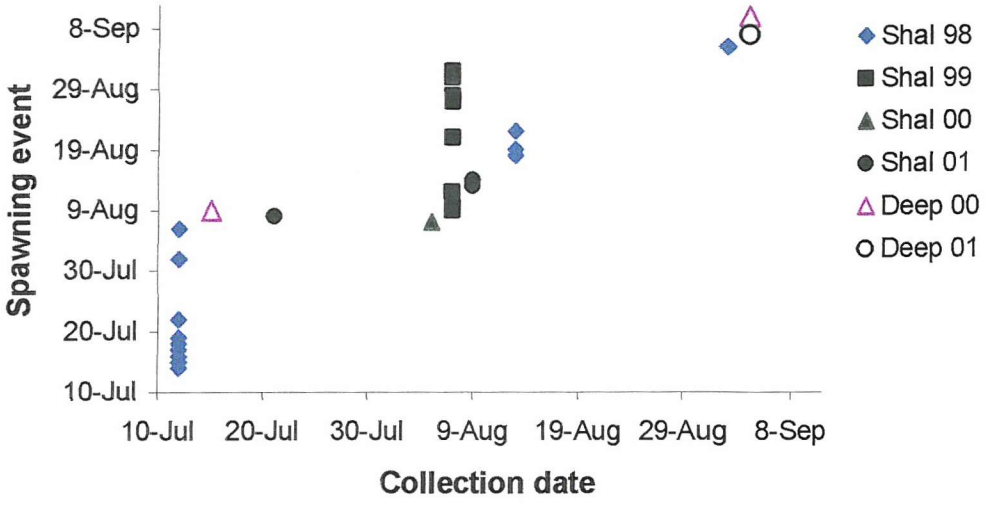
4.5.1 Gametogenesis

Gametogenic cycles in broadcast spawning coral species are all synchronous, to some degree (Fadlallah, 1983a), which is necessary to ensure that the gametes are fertilized. Many tropical hermaphroditic scleractinian species release gametes strictly according to lunar schedules over a short period of time every year (Kojis and Quinn 1981, 1982a, 1982b; Harrison et al. 1984; Simpson 1985; Babcock et al. 1986; Harrison and Wallace 1990). Gonochoristic broadcast spawners tend to have longer reproductive seasons and less tightly synchronized spawning periods than their hermaphroditic counterparts, with gamete release occurring over a number of weeks (Harrison and Wallace, 1990). Evidence from histological preparation of gonads indicates that this is also true for both deep and shallow populations of *O. varicosa*, which show asynchronous inter-colony gametogenic development. Reproductive asynchrony is also apparent in the spawning behaviour, which occurs over several weeks within a single reproductive season at the population level and at least over several days at the level of the colony or individual polyp. Asynchrony of gametogenesis and spawning may provide the population with some security against releasing gametes into unfavorable water conditions or into currents that may carry them away from a suitable settlement habitat.

There was no significant inter-annual variation in gametogenic cycles either within populations or among them. This is surprising considering that the physical environment of both shallow and deep habitats is highly variable and different from each other in unpredictable ways. The available data imply that a constrained environmental or biotic factor is controlling reproductive cycles for both populations.

There is evidence for exogenous control of reproduction in a variety of marine invertebrates, in terms of both proximal (immediate) and ultimate (evolutionary) cues (Giese and Pearse 1974). These include sea temperature, day-length, salinity, food

Figure 4.7. Spawning events from samples from 1998-2001. Each point represents a single spawning event, either by an individual or by a combined group of samples.



supply, lunar phase, tidal cycles and daily light/dark cycles (Giese and Pearse 1974, Himmelman 1980, Babcock et al. 1986, Yankson 1986, Harrison and Wallace 1990, Sasaki and Shepherd 1995, Hardege and Bentley 1997). In corals the evidence is correlative only and specific regulatory mechanisms have yet to be determined. Seasonal change in sea temperature is frequently cited as an important environmental factor that controls gametogenic cycles and planular release in scleractinian corals (Szmant-Froelich et al. 1980, Kojis and Quinn, 1981, Tranter et al. 1982, Fadlallah, 1985; Stoddardt and Black, 1985; Babcock et al. 1986; Harrison and Wallace, 1990). Simpson (1985) however, found that gametogenesis and mass spawning periods differ between populations of the same species on the east and west coasts of Australia, despite similar seasonal temperature regimes. This implies that factors other than temperature might influence reproductive cycles.

Both deep and shallow *Oculina* populations are periodically subjected to influxes of cold upwelled water that is forced up over the shelf edge by meanders of the Florida Current, and which may confound seasonal temperature trends. Other environmental factors such as light levels, sediment load and current regime are also highly unpredictable. Kojis (1986) suggested that changes in day length provide more consistent cues than temperature for corals living in shallow reef flats; for example, initiation of oogenesis in *Acropora palifera* colonies may be initiated by increasing day length, minimum or maximum day-lengths or both. It is possible that gametogenesis of *O. varicosa* is also entrained by changes in day length as this varies predictably from year to year regardless of temperature, lunar/tidal cycles or turbidity. At latitude of 28°N the number of daylight hours undergoes its greatest change increases between April and May, when gametogenesis is initiated, reaching the annual maximum of 14 hours in June, after which the spawning season begins. Differential maturation of males and females has been observed in other gonochoristic broadcast spawning species. In some species (including *O. varicosa*) the male gametogenic cycle begins before the female (Oliver 1985), and in some the reverse occurs (Harriot 1983b; Szmant 1986). The data for *O. varisoca* however, indicate that males and females exhibit concurrent gonad maturation.

4.5.2 Fecundity

Fecundity is a useful index of reproductive effort in marine animals, and in corals is usually expressed as the number of eggs or planulae produced per polyp.

Since only female reproduction is measured however, fecundity underestimates the reproductive effort of the total population. Energy available for reproduction is partitioned amongst the progeny and may be allocated into many small eggs or fewer large eggs and/or planulae. Harrison and Wallace (1990) combined fecundity data from 32 species of scleractinians and they discovered an inverse relationship between fecundity and egg size. Those species with eggs of <250μm in diameter, contained between 100 and 10,000 eggs cm^{-2} of skeletal surface area. *Oculina varicosa* also falls within this fecundity range, with 1,000 to 4,800 eggs cm^{-2} of skeletal surface area. Food supply determines the total energy available to the coral. Energetic demands from growth, calcium deposition, tissue repair and external stresses influence the amount of energy allocated to reproduction and polyp morphology determines how many eggs the mesenteries can physically support. A combination of these factors will therefore produce a fecundity range for each species.

A pre-reproductive juvenile stage is common to all marine invertebrates. In corals the presence of a juvenile stage allows the colonies to reach a size refuge from the high mortality rates associated with small size classes (Kojis and Quinn, 1982a; Szmant, 1986). This pre-reproductive phase commonly lasts between 1 and 6 years, with the ahermatypic or temperate broadcast spawning corals being more precocious than the zooxanthellate and hermatypic broadcasters (Harrison and Wallace 1990). Size at reproductive maturity is quite variable among coral species partly because of differences in the growth rates and morphology (Harrison and Wallace 1990). The size and age at maturity have not been specifically investigated for *O. varicosa*; however all the colonies sampled were at least 10 cm in diameter, and all were fertile. The linear growth rate for deep-water *Oculina* is 16mm per year and 12mm per year for the shallow populations (Reed 1981). This would make a deep-water colony of 10cm diameter approximately 3 years old, and a 10cm diameter shallow-water colony approximately 4 years old, which fits within the age parameters proposed by Harrison and Wallace (1990). Interactions between colony age and size influenced maturity and fecundity for *Goniastrea favulus* and *Montastrea annularis* (Kojis and Quinn 1985).

Once reproductive status is attained, the colony fecundity usually increases both as a function of increasing number of polyps and increased fecundity per polyp with age. As polyps increase in age, their fecundity can increase either by adding mesenteries or by increasing the number of eggs per mesentery. In three faviid species,

Goniastrea aspera, *G. favulus*, and *Platygyra sinensis*, the number of eggs per mesentery increased sigmoidally during adolescence but stabilized after 10-15 years (Harrison and Wallace, 1990). A similar phenomenon in *O. varicosa*, could account for some of the variability observed in inter-colony fecundity, as the colonies sampled were not of uniform age and size. Since the spawning season of *O. varicosa* continues over several weeks, the variation in fecundity may also be explained by release of oocytes by some colonies prior to collection. It must also be noted that the data sets are small and the high degree of variability between colonies may be masking any differences between the populations.

Fecundity varied inter-annually between the deep populations, which implies that some environmental or biotic factor is influencing either inter-annual energy allocation or energy supply at the colony and population level. There was no significant difference between mean fecundity per polyp for the deep and shallow populations, which is surprising since the shallow populations contain algal symbionts in the tissues, giving them an energetic advantage over the deep populations. Possible explanations are that fecundity is genetically constrained, so the same processes are controlling fecundity in both populations, or the shallow populations are using photosynthetic energy for other purposes. In order to withstand the high levels of wave energy in the shallow habitats, the *O. varicosa* colonies need to be more robust than deep colonies, which increases the cost of calcification. The average annual temperature at the shallow water site is also higher than at the deep reefs thereby increasing respiratory demand over the deep-water colonies. A variety of natural and anthropogenic sub-lethal stresses can affect fecundity. These include high sediment loads (Kojis and Quinn 1984), thermal stress (Kojis and Quinn 1984; Jokiel 1985), pollution (Rinkevich and Loya 1977), low salinity and irradience (Jokiel 1985), mechanical damage and fragmentation (Kojis and Quinn 1981, 1985). Fragmentation is a common mechanism of asexual reproduction in corals, especially in the deep *Oculina* populations where the growth form causes the colonies to fall apart. In some species of coral, the polyps at the growing tips of the colony or at sites of injury are non-reproductive (Rinkevich and Loya 1979b,c; Kojis and Quinn 1981). In *O. varicosa* colonies however, gametes were present even in the smallest polyps (<2mm diameter) at the branch tips. It is outside the realm of this project to investigate the processes that drive fecundity in this species, but they pose some interesting ecological questions

4.5.3 Spawning

The timing of gametogenesis did not differ significantly between the two populations, however the deep-water samples generally spawned later than their shallow-water counterparts. It is possible that the females may retain mature oocytes until they detect an appropriate spawning cue, therefore gamete development may be uncoupled from the occurrence of spawning cues. Alternatively, since female gametogenesis was determined by oocyte size, developmental differences would not have been detected if final oocyte maturation did not involve a size increase.

Temperature has been suggested as an important factor in the timing of gamete release, with different species spawning in response to a specific temperature range. For example, spawning in *Astrangia danae* (Szmant-Froelich et al. 1980) and planular release in *Rhizopsammia minuta* (Fadlallah 1983b) are correlated with maximum seawater temperature. Gametogenesis and gamete spawning in *Caryophyllia smithii*, are possibly controlled in the field by a gradual drop followed by a rise in seawater temperature, respectively (Tranter et al. 1982). In *Agaricia agaricites*, rising seawater temperature initiates planular release and leads to increase in planular production (Fadlallah 1983b). For *O. varicosa*, at least in a laboratory situation, this does not seem to be the case. Samples were maintained at ambient laboratory temperature, and spawning occurred, often several days after collection, without changing water temperature.

Lunar schedules of spawning and planular release are well documented (Fadlallah 1983b, Harriot 1983a, Babcock et al. 1994). This may also be true for *O varicosa* in the field; however the only available spawning records are from samples held under laboratory conditions, which show no correlation to lunar or tidal phase.

There is evidence that spawning in corals can be stimulated by gonadal material of the opposite sex; placing small pieces of male gonadal tissue in female polyps, and *vice versa*, induced spawning in *Astrangia danae* (Szmant-Froelich et al. 1980). Sperm released by the males of the broadcasting coral *C. smithii*, may stimulate the release of ripe eggs by the female, although the initial cue for sperm release was not defined (Tranter et al 1982). As mentioned previously, female samples of *O. varicosa* were very rarely observed to spawn before a male had released sperm. In the absence of predictable environmental cues, male-stimulated spawning of females is a possible mechanism for ensuring simultaneous gamete release. Chemical receptor cells in the oral epidermis of females are probably responsible for detecting sperm,

which causes contractions of the oral disc and consequent expulsion of ova. Spawning coordination by males is probably not crucial for brooding species, but may be so for species of gonochoristic broadcasting spawners that must spawn simultaneously to achieve egg fertilization. However, the initial cue for sperm release in *O. varicosa* is not known.

4.6 Conclusions

Oculina varicosa is a gonochoristic broadcast-spawning species with an annual reproductive cycle. Gametogenesis begins in early spring and terminates in a protracted spawning season that lasts from late July until early September. The patterns and timing of reproduction are very similar for both deep and shallow populations. Fecundity levels for both populations are similar and are comparable to shallow-water tropical species, which is surprising since the deep-water populations are without the energetic benefit of zooxanthellae. There is evidence for inter-annual fecundity variation within the deep-water population, but more data are required before conclusions can be drawn regarding fecundity patterns and comparisions between populations. There is evidence for male-induced spawning in females, but again, information regarding spawning patterns and cues is limited as spawning was only observed in the laboratory. This research provides basic information regarding reproduction in both deep and shallow populations of *O. varicosa*; however more field observations and experimental manipulations are required to determine how reproductive cycles, fecundity and spawning are controlled in this species.

Chapter 5: Embryogenesis and larval biology of *Oculina varicosa*

5.1 Introduction

5.1.1 Temperature and larval development

The optimum temperature for growth and survival of most adult scleractinians is 25°C to 29°C, although a number of corals in non-tropical habitats have adapted to lower temperatures (Crossland 1981). There is very little information available however, on environmental tolerances of coral embryos and planulae. The response of coral larvae to temperature perturbations has potentially important consequences for understanding effects of global warming and El Nino-Southern Oscillation (ENSO) events on coral distribution. Abnormal embryos of the tropical scleractinian *Diploria strigosa* were observed at temperatures above 30°C (Bassim et al. 2002). Elevated (33°C) temperature significantly increased the mortality and metamorphosis of *Porites astreoides* larvae (Edmunds et al. 2001). Gross photosynthesis was significantly reduced by both elevated and depressed (26°C) temperatures, and respiration varied proportionately with temperature in that species (Q_{10} of approximately 2). The authors concluded that elevated temperatures could increase larval mortality, shorten larval longevity and cause premature metamorphosis. Alternatively larval life (therefore dispersal potential) may be extended at sub-lethal low temperatures, which has implications for distribution and genetic homogeneity of cold-water and deep-water species.

5.1.2 Oculina varicosa *habitat*

The deepwater *Oculina* reefs grow on the edge of the Florida shelf (Smith 1981, 1987), where periodic upwelling events force cold oceanic water up over the shelf edge. During the summer months, the Florida Current is closer to the coastline (occasionally passing directly over the deep reefs) than during the winter, and the northerly volume transport is at its annual maximum (Smith 1981). This combination of features causes the upwelling events to be more pronounced during the summer months than during the winter. On the nearshore reefs, strong upwelling events during July and August can cause the water temperature to drop to those more closely representing winter temperatures. During upwelling, increased current speed causes re-suspension of fine particles, resulting in increased turbidity and considerably reduced light penetration over the deep reef habitat. The near-shore environments are also variable and unpredictable; as well as impacts from upwelling events, they are

also subject to turbulence from storms, freshwater influx from the Indian River, and seasonal temperature variations. Consequently, an *O. varicosa* larva could hatch for example, in stationary water at 29°C and 5 m depth, or in a $15ms^{-1}$ current in water of 11°C, and 80m from the surface. Environmental extremes may have a deleterious effect on embryogenesis or development of *O. varicosa* larvae; alternatively, this sub-tropical species may be fully adapted to be able to cope with the habitats that they exploit. It is important to understand how sexual offspring of this species behave under variable conditions in order to assess the potential importance of sexual recruits to re-colonisation success.

5.1.3 Larval behaviour

Invertebrate larvae are usually so small that the effects of viscosity overwhelm inertial forces (low Reynolds number) and, when swimming stops, forward motion ceases and the larva will either stay neutral, sink, or float depending on its bouyancy. Invertebrate larvae cannot swim against ocean currents, and so they use behavioural responses to physical stimuli to control their vertical position in the water column. This behaviour allows larvae to avoid predation, optimise feeding, control dispersal distance and regulate spread with respect to siblings and adults (Young 1995). Orientation behaviour of planktonic organisms is classified as either a tactic or a kinetic response, depending on the nature of the cue. Kinesis or a kinetic response is not directional and may be stimulated by cues such as temperature or salinity, which are scalar in nature. With a taxis or tactic response the organism responds to a cue that contains directional or vector information, for example light (phototaxis) or gravity (geotaxis). Light and gravity are the two major vectors to which larvae orient in the marine environment (Young 1995). Thorson (1964) observed that early stage larvae of many phyla are attracted to light and postulated that such behaviour would facilitate movement away from benthic predators and into faster moving surface currents where phytoplankton concentrations are higher and dispersal is more effective. Forward (1988) noted that most of the studies of phototaxis have been done using light regimes that bear little resemblance to those found in the field. He tentatively suggested that most positive light responses reported in the literature are laboratory artifacts and should not be used to infer behaviour in the field. Conroversy also exists over the view that long distance dispersal is advantageous and some research suggests that retention within natal habitats is a preferred strategy (Done 1982, Sammarco and Andrews 1988).

5.1.4 Larval nutrition

For free spawning invertebrates, egg size is a good indicator of developmental mode: large eggs develop into lecithotrophic larvae and small eggs typically develop into planktotrophic larvae (Jaeckle 1995). There is a great deal of variation in the sizes of eggs and planulae of scleractinian corals, but some systematic trends are apparent; for example species in the families of Acroporidae and Mussidae typically have large eggs (400-800μm diameter), while those of Agariciidae, Pocilloporidae and Fungiidae are generally small (100-250μm). The eggs of *O. varicosa* are also small (~100μm diameter) and probably do not have large lipid reserves since they are negatively buoyant, as are the embryos and planulae. Planulae of some species contain zooxanthellae, which are known to translocate metabolites into larval tissues to supplement maternal reserves (Richmond 1987), but despite the small size of some scleractinian eggs, the possibility that the larvae require external nutrition has rarely been addressed. In one documented example of planktotrophy, planulae of the cold water scleractinian, *Caryophyllia smithii*, supplemented maternal egg reserves by extruding mucous strands to trap small food particles, which were subsequently ingested. These larvae increased in size from 100μm on hatching, to 1mm at metamorphosis. Feeding behaviour was stimulated in *Fungia scutaria* by the presence of homigenised *Artemia*, which were subsequently ingested into the mouth (Schwartz et al. 1999). Particulate matter has also been observed moving into the oral pore of *Cyphastrea ocellina* planulae (Wright 1986), and coral larvae may also be able to use dissolved organic material as a source of energy (Fadlallah 1983). *Oculina varicosa* larvae are similar to *C. smithii* larvae (small, azooxanthellate and long-lived), and may also require ingestion of exogenous food to complete their development.

5.2 Objectives

1) The first objective was to obtain viable gametes from samples of deep-water and shallow-water *Oculina varicosa* adults, in order to describe embryogenesis and larval development.
2) This objective investigates the effect of temperature on duration of embryogenesis, and determines lethal temperatures for embryonic development.
3) This is a continuation of the previous objective, and investigates the effect of temperature on larval survival and duration of larval phase.

4) This objective investigates the influence of temperature on swimming speed, as temperature directly influences metabolism, ciliary activity and hence larval motility.
5) This objective investigates ontogenetic changes in larval geotactic and phototactic behaviour.
6) The final objective investigates whether food availability affects larval survival and duration.

5.3 Materials and Methods:

5.3.1 Embryogenesis and larval development

Small pieces from several (6-12) adult colonies were collected from the shallow nearshore ledges (27°32.54'N, 79°58.73'W) and shelf-edge reefs (27°32.8'N, 79°58.8'W) during periods of potential spawning activity, as determined from histological information. The samples were maintained in large (1000ml) glass bowls of 20µm-filtered seawater under ambient light and temperature conditions. When a spawning event occurred, gametes were collected immediately using glass pipettes and transferred to small (200ml) glass bowls of 0.45µm-filtered seawater for fertilisation. The fertilised embryos were observed throughout embryogenesis and photographs of each stage were taken using an Optronics digital camera attached to an Olympus light microscope. The majority of the embryos were maintained at ambient laboratory temperature (23°C), but some were also used to examine the effects of temperature on embryonic development. Studies of gametes and embryos from deep-water samples were not possible as the few spawning events that occurred did so late at night, and were not observed.

Larval cultures were maintained in 2-litre glass jars of 0.45µm-filtered seawater, at a stocking density of 5 larvae per ml. To prevent fouling, 75% of the culture-water was replaced every second day. Stock cultures were incubated at 20°C, and provided larvae for the physiological and behavioural experiments described in this chapter. Samples of larvae were preserved on hatching and at weekly intervals thereafter. These samples were observed under light microscope and scanning electron microscope in order to document the development of ciliary bands and other structures. Larval swimming behaviour was described from observations under an Olympus SZH10 dissecting microscope at x20 magnification.

5.3.2 Effect of temperature on embryogenesis

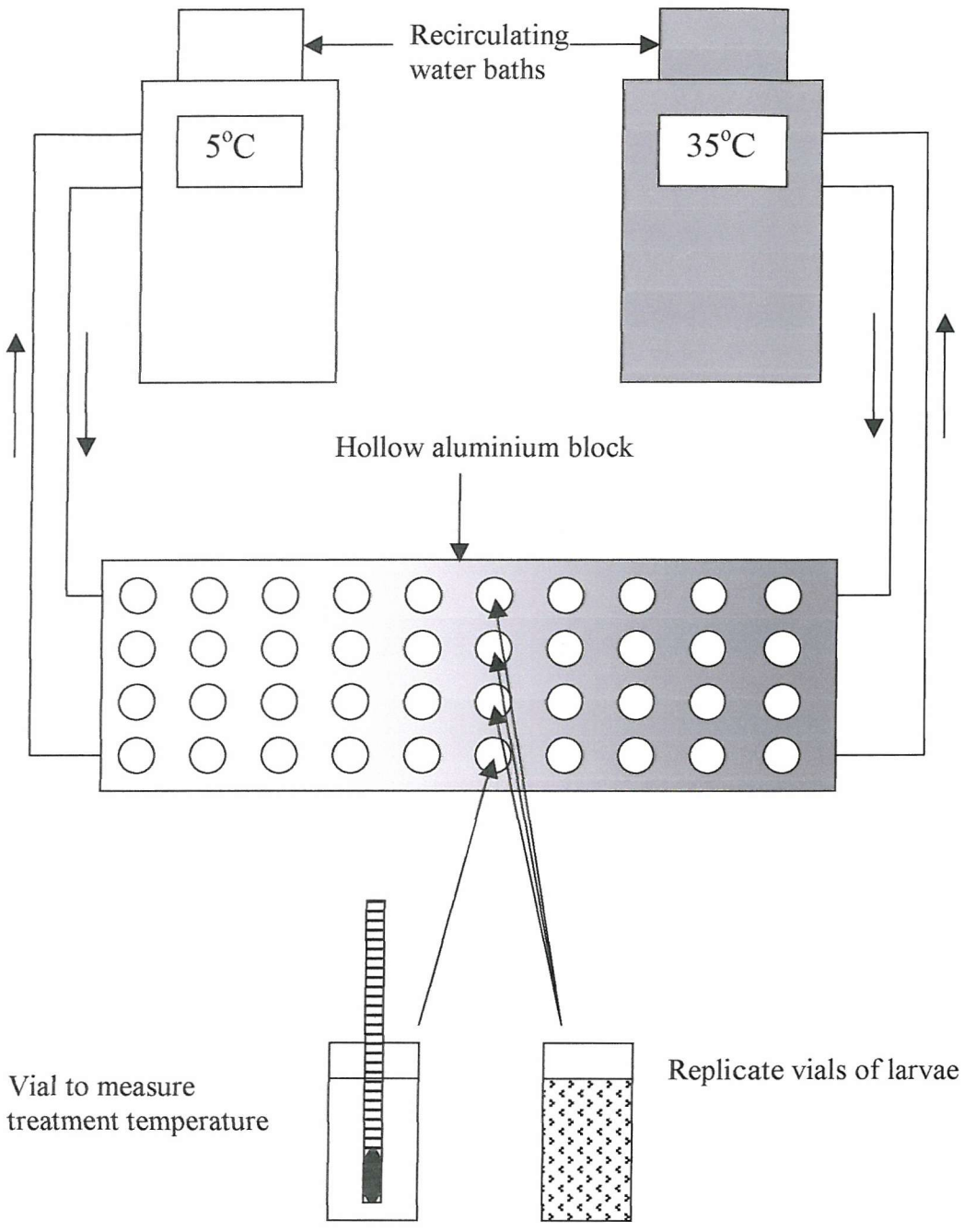
Samples of 100 newly fertilised eggs from shallow water adults were placed in 16 scintillation vials, each containing 40 ml of 45μm-filtered seawater. Replicate (4) vials were incubated at 11°C, 17°C, 25°C, and 30°C in an aluminium temperature gradient block (Figure 5.1). Temperatures were maintained using two water baths at opposite ends of the block; one set at 5°C and the other at 35°C. Treatment temperatures were monitored with thermometers set in vials of fluid, similar in volume to the treatment vials containing larvae. Embryonic developmental stage was scored after 3 hours, to document the effect of temperature on early embryos, and after 18 hours to determine longer-term effects on development. The data were translated into mean number of cell divisions per replicate of each treatment, and analysed using a one way ANOVA, followed by a Student-Newman-Keuls test for pairwise comparisons.

5.3.3 Effect of temperature on larval survival

Samples of 100 deep-water larvae (age 3 days) were placed in each of 20 scintillation vials containing 40ml of filtered seawater. Four replicate vials per treatment were incubated in an aluminium temperature gradient block (as described in the previous experiment) at 10°C, 15°C, 20°C, 27°C and 35°C. The culture water was filtered using a 0.22μm membrane filter to remove bacteria, and was replaced every 2 days to avoid fouling of the treatments. Larvae were counted every 3-4 days for 18 days, after which time, the experiment was terminated. The experiment was also run using shallow water larvae (age 4 days), and treatment temperatures of 11°C, 17°C, 22°C, 26°C and 31°C. These were slightly different from the deep-water larval experiment because temperatures were difficult to pre-set accurately using the temperature gradient block.

Regression analysis was used to produce an equation for age-dependent mortality for each temperature. The regression coefficients of different treatments were then compared using a modified students t-test (Zar 1984), to analyse the effect of temperature on larval mortality. The regression coefficients from the different experiments were analysed separately, and then compared to test for effect of larval source (deep or shallow) on temperature response.

Figure 5.1: Schematic of temperature gradient block used in experiments on temperature effects of embryonic and larval development.



5.3.4 Effect of temperature on larval swimming speed

Larvae from active, healthy cultures were used for these experiments; the deep-water larvae were 9 days old and the shallow water larvae were age 12 days, however their ages were considered sufficiently close that developmental artifacts could be ignored. Larvae were counted into 40ml scintillation vials of 0.45µm-filtered seawater at a stocking density of 5 larvae ml^{-1}. The larval vials (3 replicates per treatment) were placed in incubators at the experimental temperatures: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C to acclimate for six hours prior to data collection. The larvae were then removed from their vial and placed in a small (5cm diameter) petri-dish under an Olympus SZH10 dissecting microscope at x50 magnification. Larval swimming was recorded for 2 minutes onto Hi-8 videotape using a hand-held video camera connected to the microscope. After a larva had crossed the field of view, it was removed from the dish with a glass pipette to avoid re-sampling. A scale was included with each recording for assessment of distance. After 2 minutes, the larvae were removed to avoid artifacts of temperature change. To measure swimming speed, a sheet of acetate was taped to the screen of a Panasonic video monitor (29cm screen), and for each sample the distance travelled by ~20 individual larvae was recorded at 2-second intervals until they disappeared from view. The average swimming speed of each larva was then calculated from these measurements. The swimming speeds of deep and shallow larvae were compared using a paired t-test.

5.3.5 Ontogenetic changes in phototactic and Geotactic responses

This series of experiments was conducted to investigate ontogenetic changes in geotactic and photoactic responses in larvae from shallow-water *O. varicosa* populations. Deep-water larvae were not available in sufficient numbers for a comparative study, but personal observations indicate that they exhibit similar behavioural patterns to their shallow water counterparts.

5.3.6 Geotaxis experiment

Newly-hatched larvae from a single culture were divided into groups of 100 and placed in 50 ml acrylic tissue-culture vials (10 cm x 5cm x 2cm), containing 45µm-filtered seawater. The vials were flat on all sides and divided into 5 equal horizontal sections using a fine permanent marker. The location of larvae in the vial could be observed quite easily with red illumination without affecting behaviour patterns. Four

replicate vials were placed in an incubator at 25°C on a diurnal (12h light: 12h dark) cycle. Another 4 vials were kept at the same temperature in the dark. Larvae were examined at noon and midnight for the first 48 hours, and thereafter at 5-6 day intervals, for a period of 21 days. The number of larvae in each of the 5 sections of the vial was counted; those in the top section were scored as negatively geotactic and larave in the bottom were scored as positively geotactic. The data were analysed using a two way ANOVA with age and location in the vial as the factors. The data were analysed using a Pearson product moment correlation test (p=0.05) to determine whether a significant (p=0.05) correlation exists between age and treatment, and between treatments.

5.3.7 Phototaxis experiment

These experiments were conducted using larvae of 12 hours, 24 hours, 2 days, 8 days, 14 days and 23 days of age. Replicate groups of 25 larvae were taken from a single culture and dark-adpated for 3 hours, and all experiments were done between noon and 4pm to reduce behavioural variation caused by diurnal rhythms. A Kodak slide projector with a 300-watt lamp provided light stimulus for this experiment, which was maintained in a darkened room under dim red light until the start of each trial. The light was filtered with a hot mirror (Baird Atomic) and an IR absorbing filter to remove heat. Light intensity was controlled using neutral density filters and measured with a radiometer (EG&G model 500). A small lucite trough (15cm x 3cm x 3cm) containing 45µm-filtered seawater was placed inside a water bath (to reduce light reflection off the back wall of the trough), and a removable partition was inserted to divide the trough into 5 equal sections.

The larvae were gently placed in the center section with a glass pipette, and upon light stimulation the partitions were removed allowing the larvae to swim freely in the trough. After a 5-minute exposure to the light, the separator grid was inserted into the trough, and the number of larvae in each section was scored. A long exposure time was used because although the larvae swim rapidly, the younger ones swim to the surface initially, and disperse horizontally thereafter. At each light intensity, three replicate trials were run using a different batch of larvae per trial. A larva was scored as exhibiting positive photo-taxis if it was found in the section closest to the light source and negative phototaxis if it was in the farthest section from the light. Five different light intensities were used together with a dark control in which the experimental protocol was followed without light stimulation. Results were analysed using a series of paired t-tests to

compare the responses of larvae in the dark trials with those at different light intensities, for all the age categories tested.

5.3.8 Effect of food and temperature on larval survival and settlement.

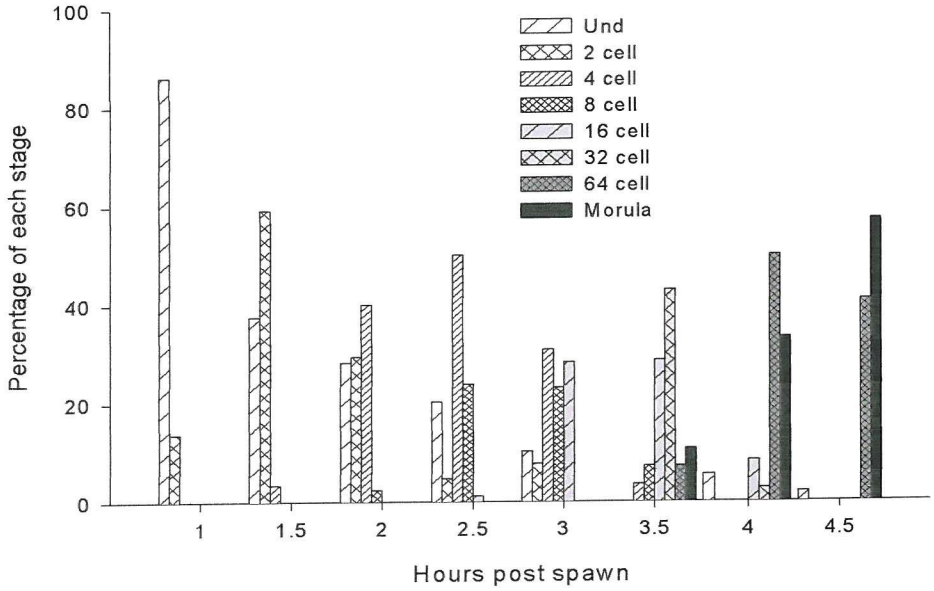
This experiment was designed to test the effect of food and temperature on larval survival and time to settlement. Larvae from shallow water adults, at two days of age were divided into 16 groups of 200 larvae, and each group was cultured in small (250ml) glass bowls. The larval cultures were maintained in seawater, which had been filtered using a 0.22μm millipore-filter to remove all but the smallest bacteria. Half of these cultures were provided with a small (7μm) green flagellate, *Isochrysis galbana* (Tahitian strain) and a larger (10μm) brown diatom, *Chaetoceros gracilis* to supplement their diet. The cultures were innoculated at a rate of 4.5 x 10^3cells^{-1}larva^{-1}day for each algal species. Each food treatment was then divided between two incubators, one at 25°C and the other at 16°C with four replicate cultures for each treatment. Every 4 days the larvae were counted, the culture water was changed, and fresh algae were added. The experiment was maintained under a diurnal regime (12h dark: 12h light), and a two way ANOVA was used to determine the effects of algal food and temperature on larval survival.

5.4 Results:

5.4.1 Embryogenesis and larval development

The eggs were small (~100μm) spherical and mature; germinal vesicle breakdown had occurred either prior to release or shortly thereafter. The ultrastructure of the sperm was not determined, but observation with a light microscope (x40) revealed a simple conical shaped head, which is characteristic of gonochoristic scleractinian sperm (Harrison and Wallace 1990). Embryonic development was documented using fertilized eggs from shallow water samples at 23°C (Figure 5.2). Light micrographs were taken at each successive embryonic stage (Figure 5.3a-f), until the larvae hatched (Figure 5.3g). A single polar body was extruded within 30 minutes to one hour of spawning, but since the male and female gametes were not collected separately, in was unclear whether polar body extrusion occurred prior to or after fertilization. Cell cleavage occurred at intervals of approximately 45 minutes until the embryos reached the 32-cell stage, after which time the cleavage pattern becomes complex and difficult to track. Cell division was complete and equal in both

Figure 5.2: Early development of shallow-water *O. varicosa* embryos at 22°C. Cleavage occured approximately every 45 minutes.



animal and vegetal poles (holoblastic), and embryos developed in a semi-spiral cleavage pattern, which became irregular until the hollow blastula formed 4-5 hours later. Gastrulation of the majority of anothzoans occurs by invagination (Mergner 1971), where the endodermal cells fold inwards from an essentially spherical blastula. During gastrulation in this species the blastulae became flattened and concave, and were initially mistaken for abnomal embryos however, they re-formed into a spheroid shape and produced normal larvae. This pattern of gastrulation has also been observed in embryos of the tropical zonathid *Protopalythoa* sp. (Babcock and Ryland 1990).

On hatching, *Oculina varicosa* planulae were completely ciliated and slightly elongated anterior-posteriorly (Figures 5.3h and 5.4a). The oral pole is wider than the aboral pole, which is directed anteriorly whilst swimming. Planulae were composed of an ectoderm and endoderm separated by a mesoglea. *Oculina varicosa* planulae were azooxanthellate, but contained many nematocycts cells, which fired in response to physical stimulus (Figure 5.4f). Initially the larvae were rounded in shape and moved towards the water surface where they swam rapidly in a spiral fashion by rotating around their longditudinal axis.

Measurements of planulae at 24 hours post hatch showed an average length of 161.5μm (sd=15.8) for larvae from deep-water adults, and 141.64μm (sd=16.54) for larvae from shallow-water adults. After 3-4 days (Figure 5.4b), the larvae were more elongated and began swimming throughout the water column using various different swimming patterns such as straight directional, large circular, and sigmoidal movements. After 7-10 days (Figure 5.4c) an apical tuft of longer cilia formed on the posterior pole and the larvae displayed an apparent benthic probing or creeping behavior. The planulae are very flexible and often change shape during this phase. Late stage anthozoan larvae (Figure 5.4d) transform into two different types, the edwardsia and the halcampoides, which are classified by the number of divisions that partition the gastrovascular cavity; the edwardsia larva has eight septa while the halcampoides has twelve (Martin and Koss 2002). More than eight faint septal divisions were observed in the late stage planulae indicating that the larvae belong to the halcampoides class (Figure 5.4e).

Some of the larvae attached temporarily to the substratum by the aboral pole, but began to swim freely again a short time later. The few planulae that underwent metamorphosis did so on the sides of glass culture vessels after approximately 21 days.

Figure 5.3. Light micrographs of *Oculina varicosa* embryos and larvae: A) 2-cell stage, B) 4-cell stage, C) 8-cell stage, D) 16-cell stage, E) morula stage, F) blastula stage, G) newly hatched ciliated larva, H) larva at age 1 week.

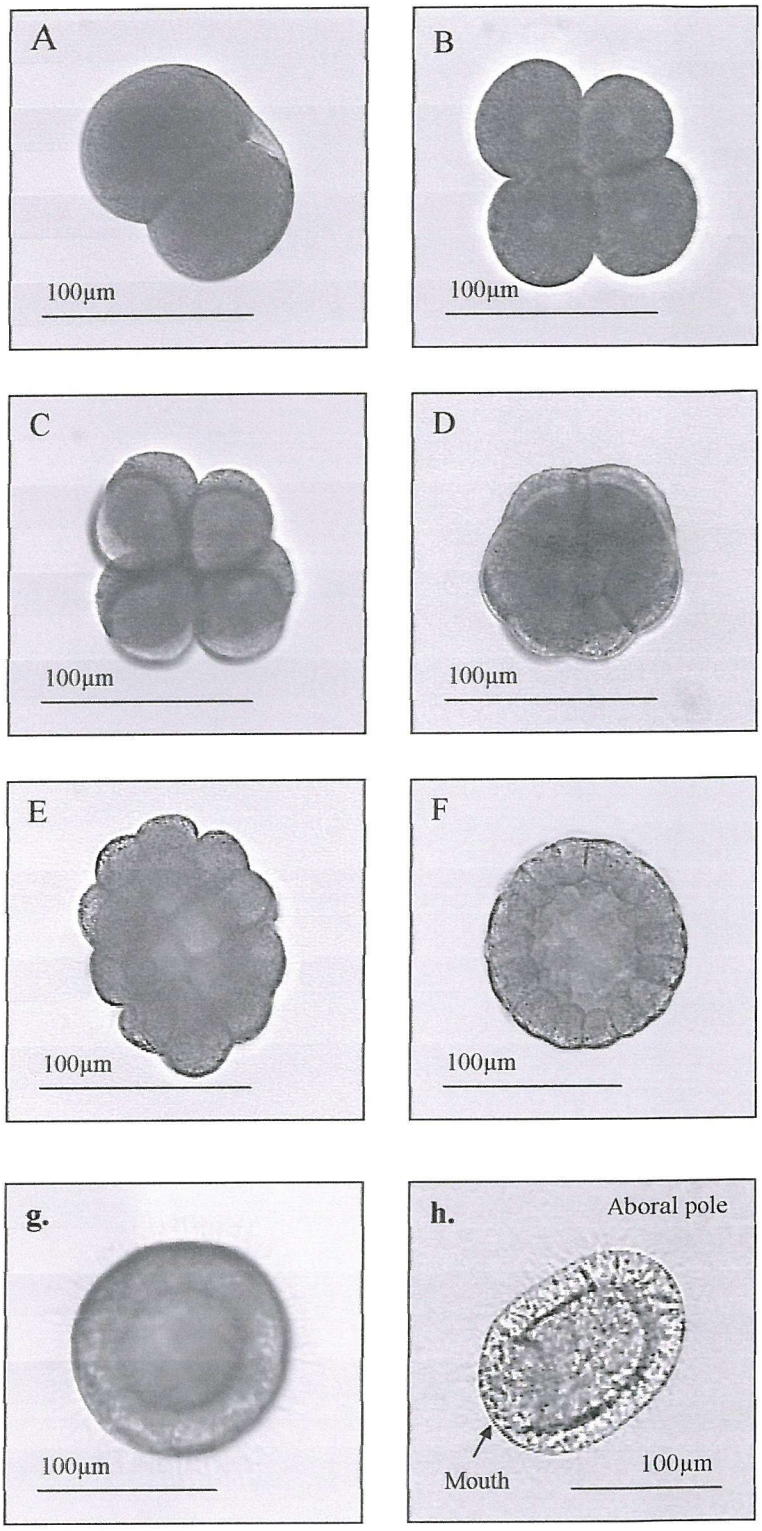
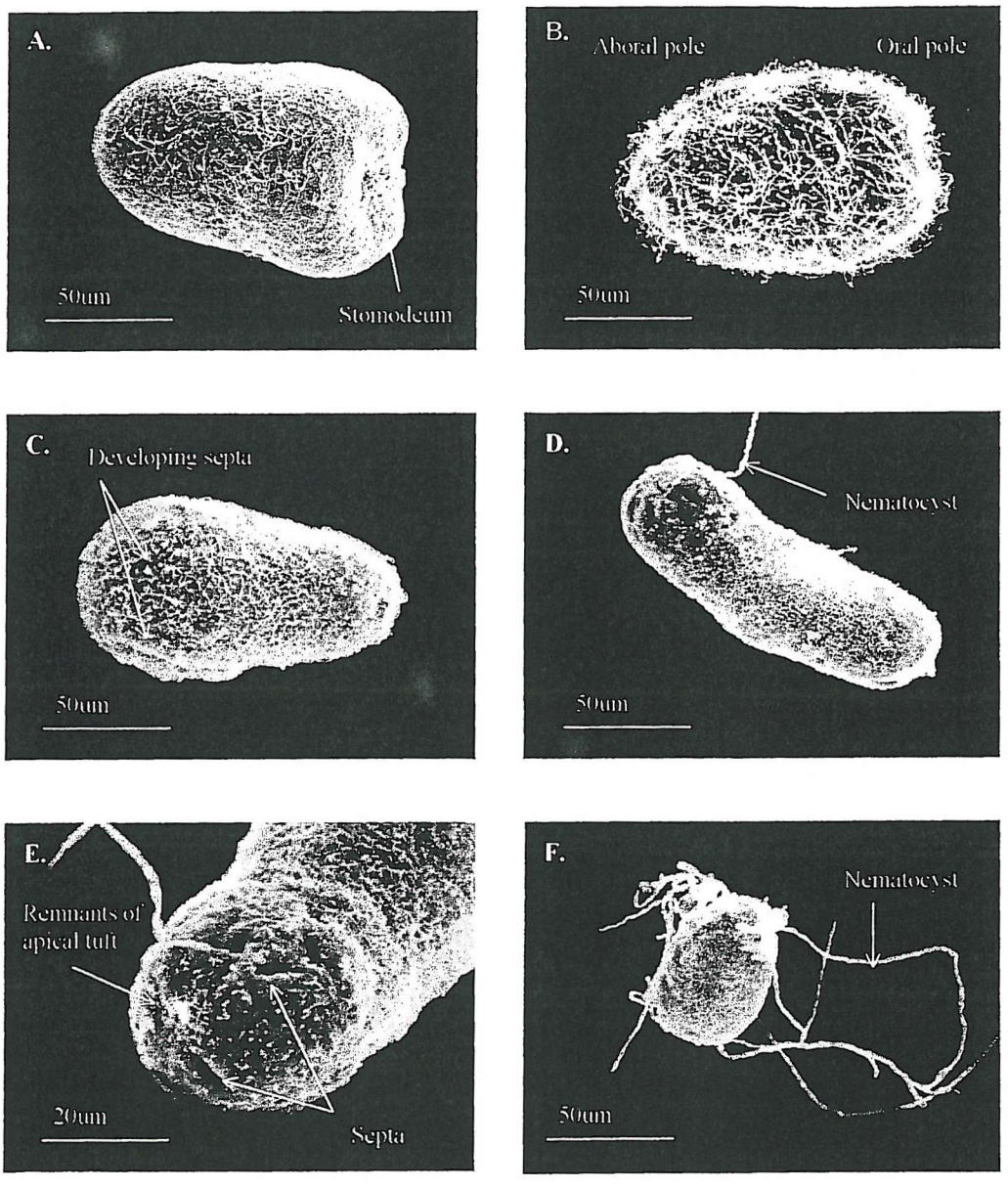


Figure 5.4. Scanning electron micrographs of *Oculina varicosa* larvae at various developmental stages. A) early planula; B) age 4 days with fully developed cilia; C) age 9 days with septa beginning to develop; D) age 16 days, larvae are elongated; E) aboral end of a 16-day planula; F) planula showing fired nematocysts.



The polyps were small in diameter (mean=85.71; sd=13.99) and between 2 and 6 extended tentacles were observed on several occasions. The newly-settled polyps were extremely fragile and difficult to clean, and all died after a few days because of bacterial fouling.

5.4.2 Effect of temperature on embryogenesis.

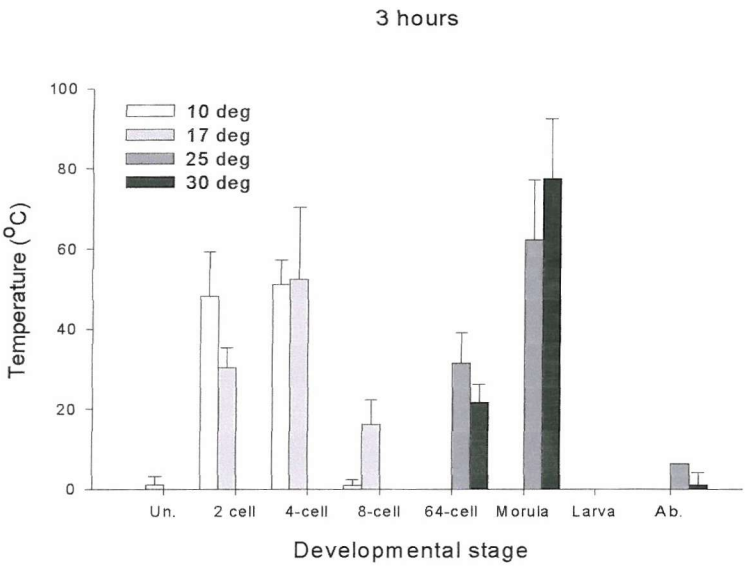
This experiment shows a positive relationship between temperature and embryonic development. When maintained at 25°C and 30°C, embryos developed rapidly reaching the morula stage after 3 hours of incubation. Cultures incubated at 10°C were composed primarily of embryos with 2 and 4 cells, with less than 2% at the 8-cell stage. In the 17°C culture, 16% of the embryos had progressed through another cell division to the 8-cell stage (figure 5.5a).

After 18 hours, all of the cultures at the 25°C and 30°C temperatures contained 100% actively swimming larvae. Those maintained at 17°C had arrested at the morula stage, and those at the lowest temperature (10°C) had still not progressed beyond 8-cells (Figure 5.5b). Those embryos with arrested development were incubated at 25°C to see whether they could be induced to develop further. The 17°C culture proceeded with development and after 18 hours contained 39% apparently normal but very slow-swimming planulae, and 61% abnormal embryos. Embryos from the 10°C culture did not develop, and all were either abnormal or dead after 36 hours.

After 3 hours of development, the mean number of cell divisions was calculated for each treatment. These were compared using a one way ANOVA of the data, which showed a significant difference between treatments ($F=109.2$, $p<0.001$). Pair-wise comparisons of the treatments were performed using a Student-Newman-Keuls test ($p=0.05$). There was no significant difference in development rate between the two coolest treatments (10°C and 17°C) and the two warmest treatments (25°C and 30°C). Comparisons of 10°C and 17°C with 25°C and 30°C however, showed significant differences from each other (Table 5.1a). After 18 hours, the ANOVA showed a significant difference overall ($F=581.8$, $p<0.001$) and, except for the two warmest treatments, all pairwise comparisons were significantly different (Table 5.1b).

Figure 5.5 Effect of temperature on embryonic development using shallow water *O. varicosa* embryos. A) after 3 hours; B) after 18 hours. The stages are: undeveloped (Un.), 2-cell, 4-cell, 8-cell, 64-cell morula, bastula, larva and abnormal (ab.). The error bars represent positive standard deviations from the mean of four replicate vials.

A)



B)

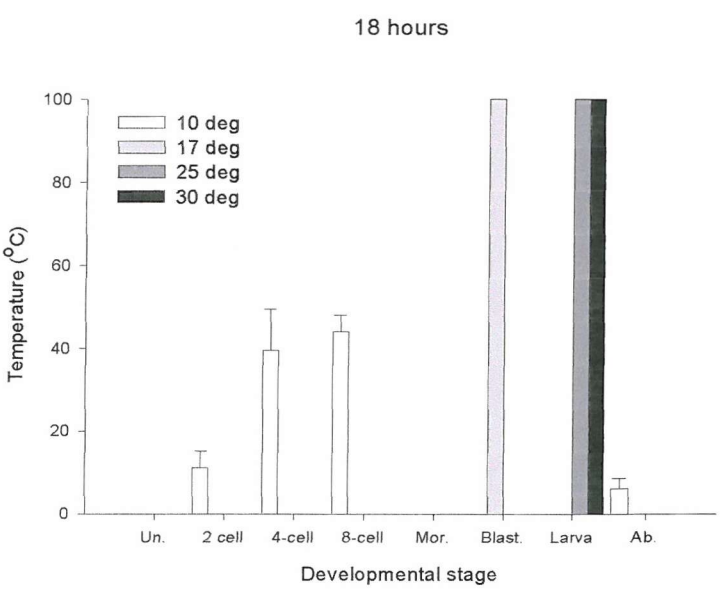


Table 5.1 Results of a One Way ANOVA and Student-Newman-Keuls test to determine whether temperature has a significant effect on embryonic development after A) 3 hours, and B) 18 hours

A)

Variance	DF	Sum Sq.	Mean Sq.	F	P
Between Treatments	3	8280301.6	2760100.5	109.2	<0.0001
Residual	8	202290.7	25286.3		
Total	11	8482592.2			

Pairwise comparisons: $p<0.05$

	17°C	25°C	30°C
10°C	No	Yes	Yes
17°C		Yes	Yes
25°C			No

B)

Variance	DF	Sum Sq.	Mean Sq.	F	P
Between Treatments	3	1266681.2	422227.1	581.1	<0.0001
Residual	8	5805.3	725.7		
Total	11	1272486.5			

Pairwise comparisons: $p<0.05$

	17°C	25°C	30°C
10°C	Yes	Yes	Yes
17°C		Yes	Yes
25°C			No

5.4.3 Effect of temperature on larval survival and duration of the larval phase

5.4.3.1 Deep larvae

Survival levels were high for the first 12 days of the experiment (>78%), in all except the warmest treatment. After 8 days, larvae in the 35°C treatment experienced rapid mortality, and by the end of the experiment most of the larvae from this treatment had died. The coldest treatment showed highest survival, with an average of 89% (sd=4.24) when the experiment was terminated. Graphical representation of the results (Figure 5.6a) shows a decrease in survival with increasing temperature; however, when the regression slopes of each temperature (Table 5.2a) were compared (using a t-test to measure equality of slope coefficients), none of the treatment pairs were significantly different (Table 5.2b). The experiment was terminated at 18 days and although lethal low temperatures were not determined, the mortality at 35 °C was almost 100% by the end of the experiment.

5.4.3.1 Shallow larvae

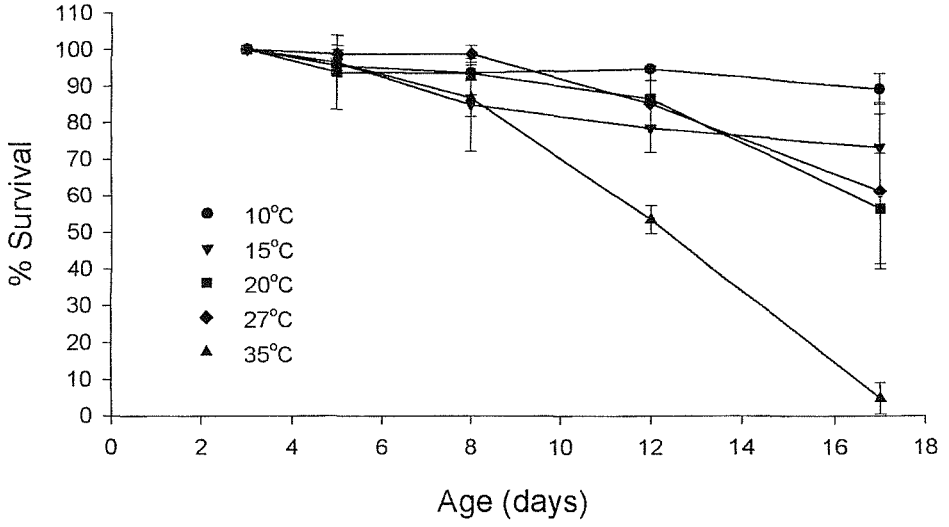
As with the deep-water cultures, survival was high (>80%) for the first 15 days of the experiment. After 18 days, larvae in the 31°C treatment began to experience some mortality, and by the end of the experiment all the larvae from this treatment had died. The coldest treatment showed highest survival, with an average of 60% (sd=3.53) when the experiment was terminated. Graphical representation of the results (5.6b) and shows a decrease in duration of larval life with increasing temperature. When the regression slopes of each temperature (Table 5.3a) were compared (using a t-test to measure equality of slope coefficients), none of the treatment pairs were significantly different (Table 5.3b). After 42 days none of the treatments showed signs of larval settlement, and the experiment was terminated. Lethal high and low temperatures were not determined, but a detrimental effect was seen in the highest temperature, as with the deepwater experiment. Regression lines were compared between the closest matching treatments from the deep and shallow larval experiments. There was no significant difference between the survival of deep and shallow larvae over the range of temperatures tested.

5.4.4 Influence of temperature on larval swimming speed

The results of this experiment show that larval swimming speed is temperature dependent in larvae from both populations, reaching a maximum at 25°C for both deep

Figure 5.6 Effect of temperature on survival of *O. varicosa* larvae; A) from deep populations; B) from shallow populations. The error bars represent standard deviations from the mean of four replicate vials

A)



B)

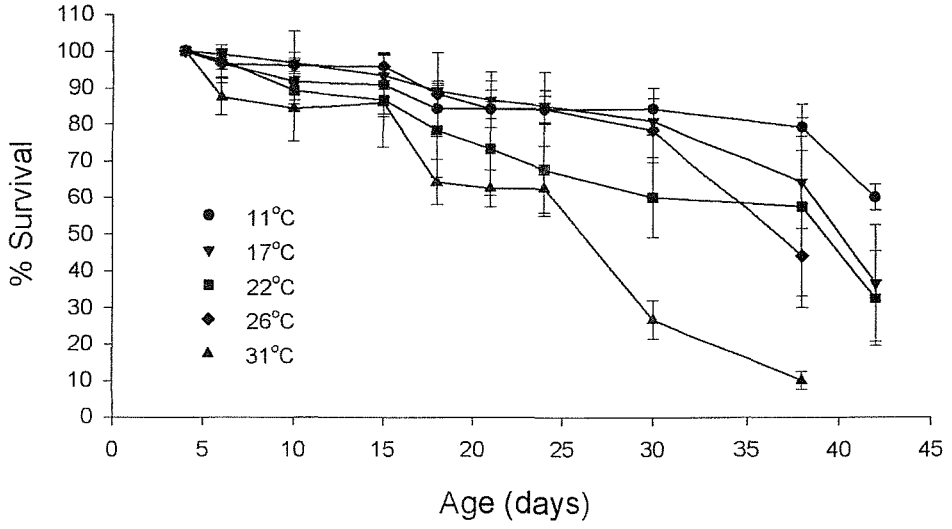


Table 5.2 Statistical analysis of the effect of temperature on deep-water larval survival A) results of linear regression analysis B) comparison of regression slopes of the different treatments using students t-test to test equality of regression coefficients. For $\nu=6$, critical t-value for a two tailed test ($p=0.05$) is 2.447. NS: not significant

A)

Treatment	Regression equation	R^2	p value
10°C	100.21-0.64x	0.812	<0.001
15°C	104.39-1.99x	0.941	<0.001
20°C	111.53-2.84x	0.867	<0.001
27°C	113.69-2.78x	0.880	<0.001
35°C	130.67-6.94	0.949	<0.001

B)

Treatment	15°C	20°C	27°C	35°C
10°C	0.254 NS	0.305 NS	0.242 NS	0.825 NS
15°C		0.059 NS	0.000 NS	0.593 NS
20°C			0.057 NS	0.524 NS
27°C				0.572 NS

Table 5.3 Statistical analysis of the effect of temperature on shallow-water larval survival A) results of linear regression analysis B) comparison of regression slopes of the different treatments using students t-test to test equality of regression coefficients. For $\nu=14$, critical t-value for a two tailed test ($p=0.05$) is 2.145. NS: not significant

A)

Treatment	Regression equation	R^2	p value
11°C	101.70-0.78x	0.830	<0.001
17°C	111.86-1.38x	0.838	<0.001
22°C	106.81-1.57x	0.949	<0.001
26°C	110.62-1.37x	0.814	<0.001
31°C	111.98-2.56x	0.929	<0.001

B)

Treatment	17°C	22°C	26°C	31°C
11°C	0.077 NS	0.458 NS	0.245 NS	1.094 NS
17°C		0.367 NS	1.361 NS	0.986 NS
22°C			0.192 NS	0.641 NS
26°C				0.805 NS

(1.9 mms^{-1}) and shallow (1.6 mms^{-1}) larvae. Both larval types also displayed lower motility at the experimental extremes of 5°C and 35°C (Figure 5.7a). Swimming speed approximately doubles with 10°C temperature increase between 5°C and 25 °C for both deep (Q_{10} ≈1.89) and shallow (Q_{10} ≈1.98) larvae. The reduction in swimming speeds observed at the higher experimental temperatures, were reflected in the Q_{10} values for deep (-2.87) and shallow (-3.48) larvae. A paired t-test showed no significant difference between the response curves for larvae from the different populations (t=2.45; p=0.054).

The estimated position of the larvae in the water column after 6, 12 and 24 hours of vertical migration was calculated using the average swimming speed at each temperature for both deep and shallow larvae. These calculations demonstrate that even the optimal swimming speed is not sufficient for the larvae from deep reef habitats to undergo a diurnal vertical migration. Larvae spawned into shallow water habitats however could easily undergo daily migrations to the surface, even at the most extreme temperatures (Figure 5.7b).

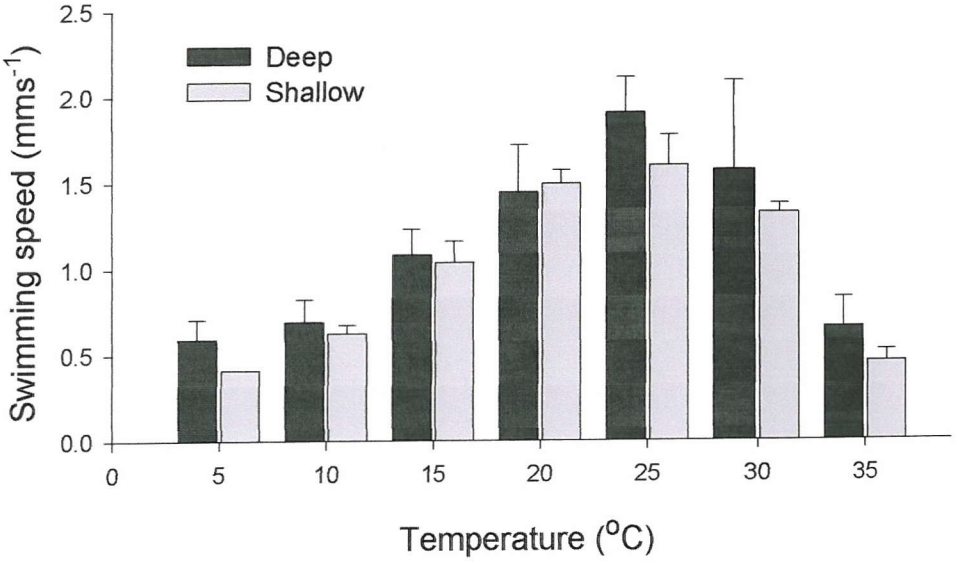
5.4.5 Larval geotactic and phototactic responses

5.4.5.1 Geotaxis

Larvae observed at the top of the experimental vials are displaying a negative geotactic response, and those at the bottom are positively geotactic. Graphical presentation of the data indicates a definite ontogenetic shift in geotactic response after about 2 days post hatch, regardless of whether larvae were maintained in a diurnal cycle (Figure 5.8a), or in the dark (Figure 5.8b). Under both culture conditions, the larvae exhibited strong negatively geotactic behaviour for the first 24 hours after hatching. At approximately 48 hours, their behaviour pattern changed, and larvae began to show positive geotaxis, which continued until the experiments were terminated. A paired t-test showed no significant difference in larval response between the diurnal and dark treatments, and these data were combined to increase the power of subsequent analysis. A two-way ANOVA shows that there is no significant effect of age alone (F=0.00, p=1.0); however location in the culture vial has a significant effect on larval response (F=38.50, p<0.001). There is also a significant interaction between age and location (F=6.40, p<0.001) therefore geotactic response is age-dependent.

Figure 5.7. Effect of temperature on: A) swimming speed, and B) potential vertical migration of both deep and shallow *Oculina varicosa* larvae. For both experiments the data represent the average and standard deviation of 3 replicate trials with 20 larvae/trial. The dashed line in figure 5.6B represents a hypothetical sea surface.

A)



B)

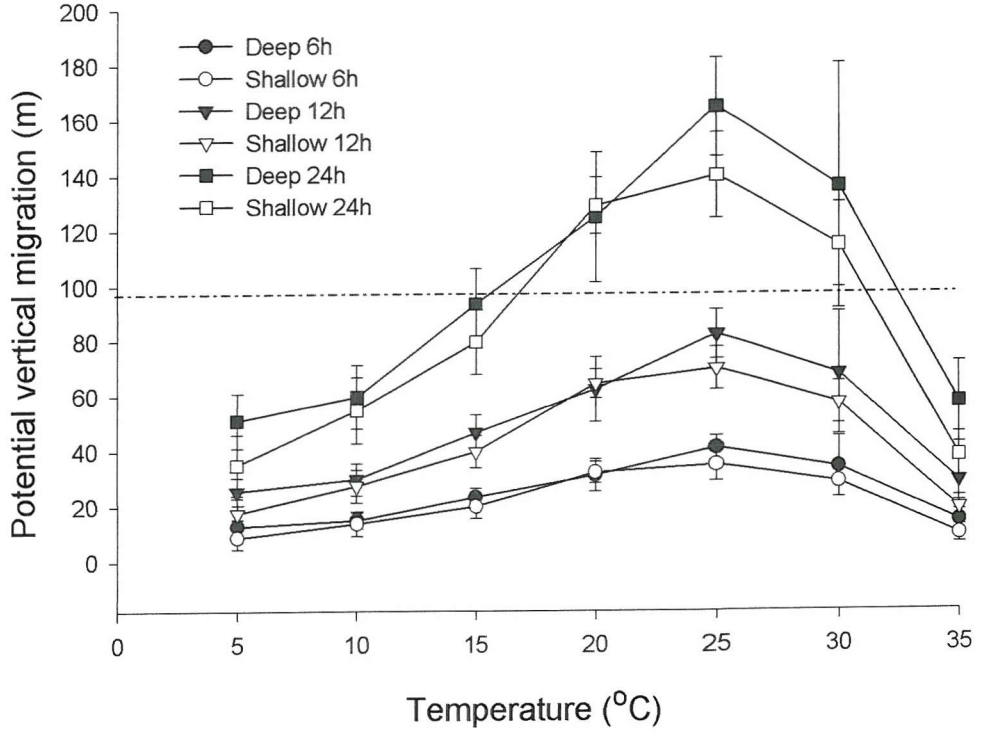
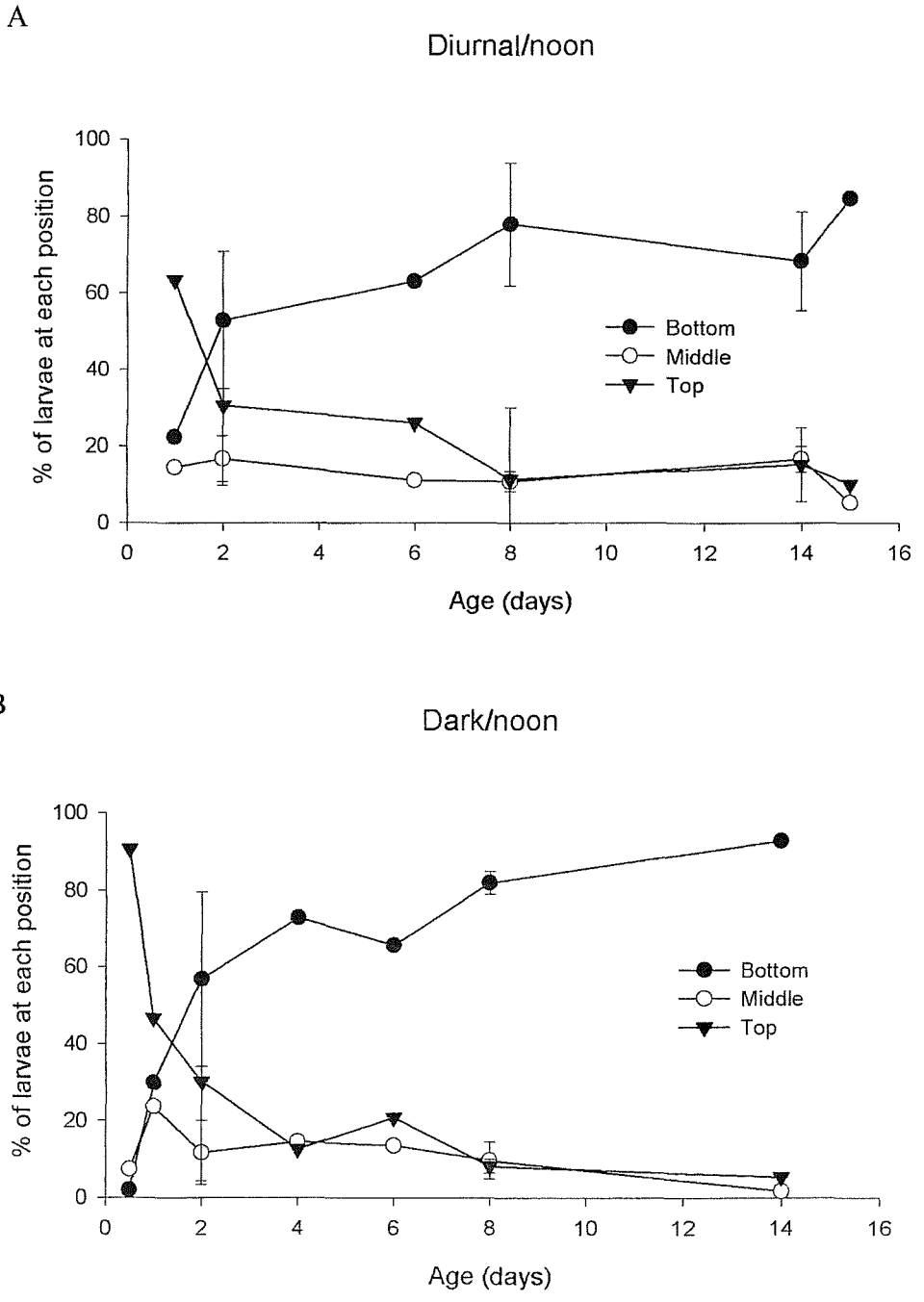


Figure 5.8. Ontogenetic change in geotactic response of *Oculina* larvae from shallow water populations A) experiment maintained under a diurnal (12h light:12h dark) regime B) experiment maintained in the dark. Top, middle and bottom denote location in experimental chamber. Numbers are mean values of multiple (3) spawns, with associated standard deviations.



5.4.5.1 Phototaxis

The larvae did not appear to show any kind of consistent photo-response at any age during the experiment (Figure 5.9a-f). A two way ANOVA showed no significant difference between the percentage of photo-positive responses at different light intensities within age groups, ($F=1.68$, $p=0.18$) or between age groups ($F=2.43$, $p=0.063$). Neither age nor light intensity has a significant effect on the number of larvae exhibiting a positively phototactic response. Photonegative responses also showed no significant difference between light intensities within age groups ($F=1.14$, $p=0.37$), but significant differences between age groups (2.82, $p=0.04$). Larval age therefore had a significant effect on photonegative response.

5.4.6 Effect of food availability on larval survival and lifespan.

The results of a two-way analysis of variance showed a significant effect of temperature and food on larval survival ($F=12.8$, $p<0.001$). A Student-Newman-Keuls test of pair-wise comparisons show that both food availability and temperature can have a significant effect on larval survival (Table 5.4). The cultures maintained at 16°C with no food had the best survival rate, and the addition of food seemed to have a generally detrimental effect on survival especially in the warm treatment where cultures became fouled and contaminated with protozoa (Figure 5.10).

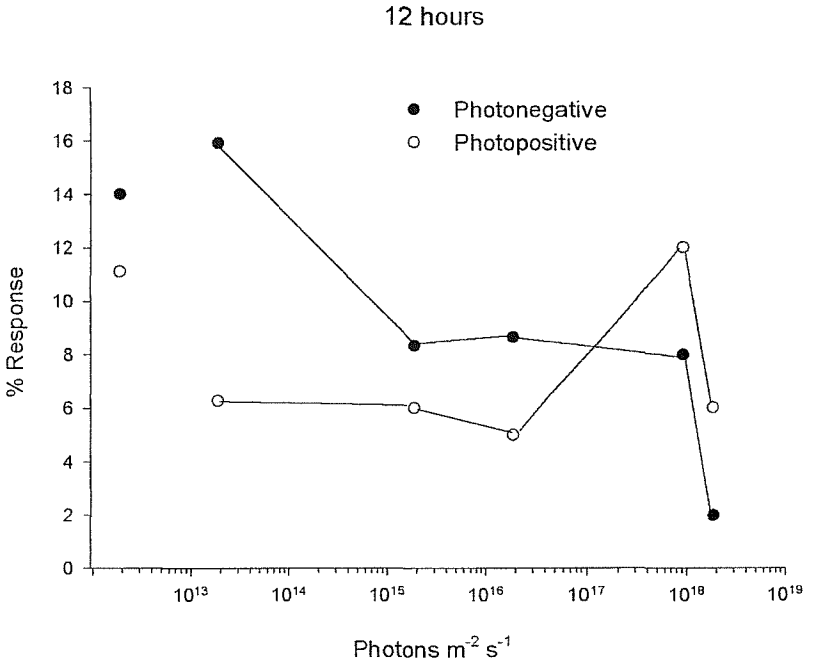
5.5 Discussion

5.5.1 Embryogenesis and larval development

Studies of the brooding scleractinians *Favia fragrum* (Szmant-Froelich 1985) and *Monomyces rubrum* (Heltzel and Babcock 2002) indicate that embryonic development proceeds via solid stereoblastulae, with gastrulation occurring through delamination rather than invagination of the hollow bastula. This trend is also seen in the brooding alcyonarians *Parerythropodoim fulvum fulvum* (Benayahu and Loya 1983) and *Xenia macrospiculata* (Benayahu and Loya 1988). Internal fertilization may constrain larvae to develop this way because of space restrictions (Babcock and Ryland 1990). Externally fertilized species such as *Astrangia danae* (Szmant-Froelich et al. 1980), *Favia pallida Goniastrea favulus* and *Montipora digitata* (Babcock and Heyward 1986), which have no space restrictions, support this premise and develop via hollow blastulae.

Figure 5.9A-F. % response of dark-adapted *O. varicosa* larvae to a range of light intensities. Each graph represents a specific larval age: A) 12 hours, B) 24 hours, C) 2 days, D) 8 days, E) 14 days, F) 23 days. Unconnected data points represent dark controls. Error bars represent standard deviation from the mean of 3 spawns.

A)



B)

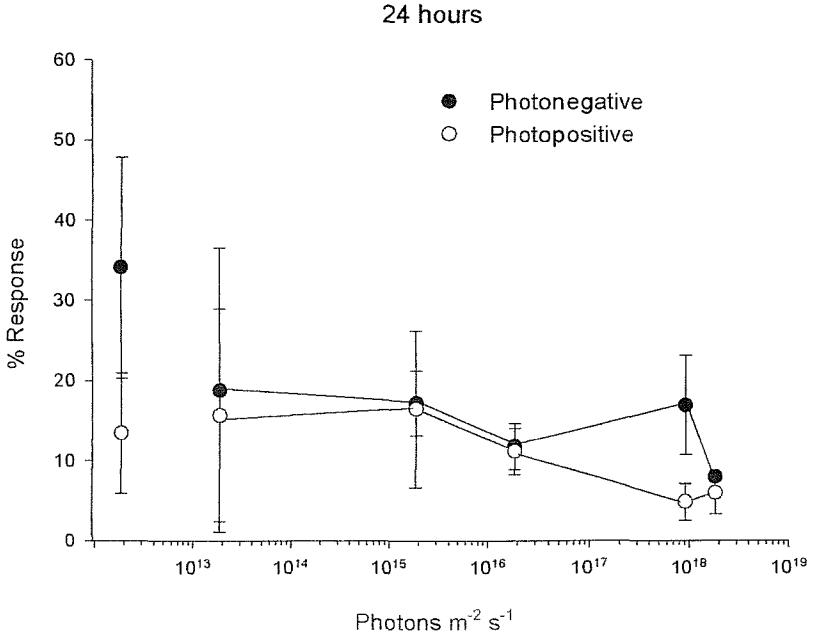
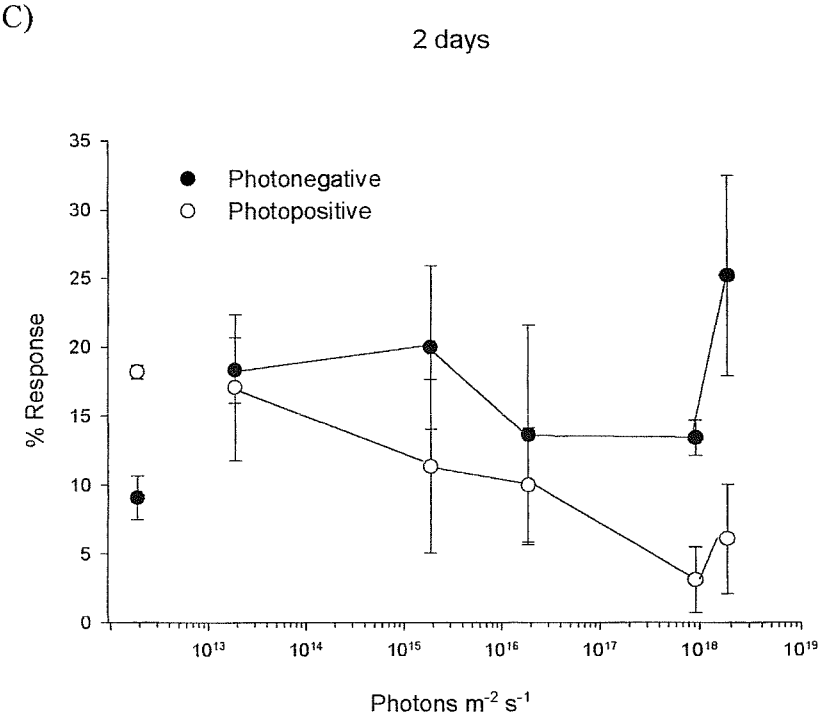


Figure 5.9 continued:

C)



D)

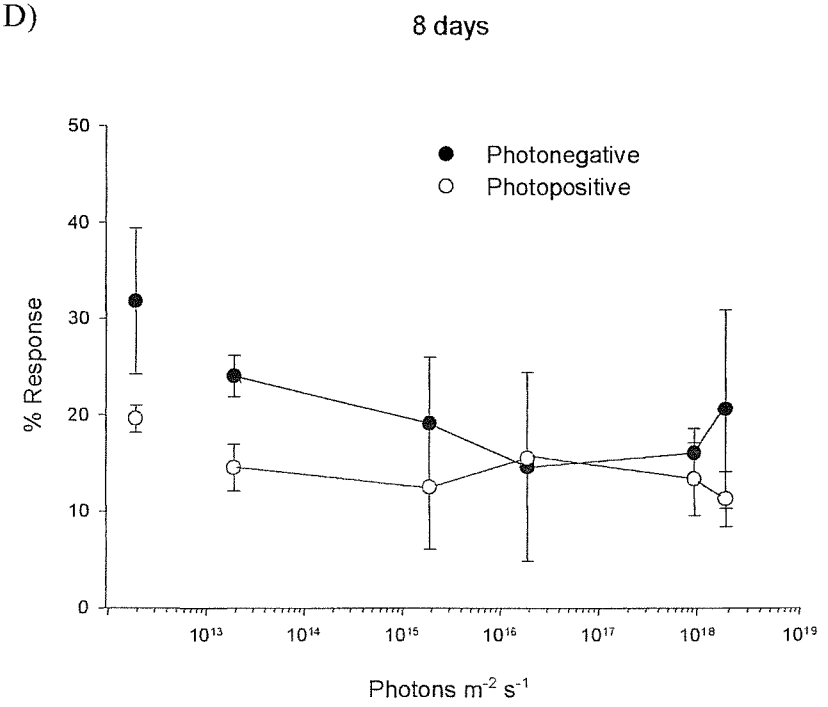
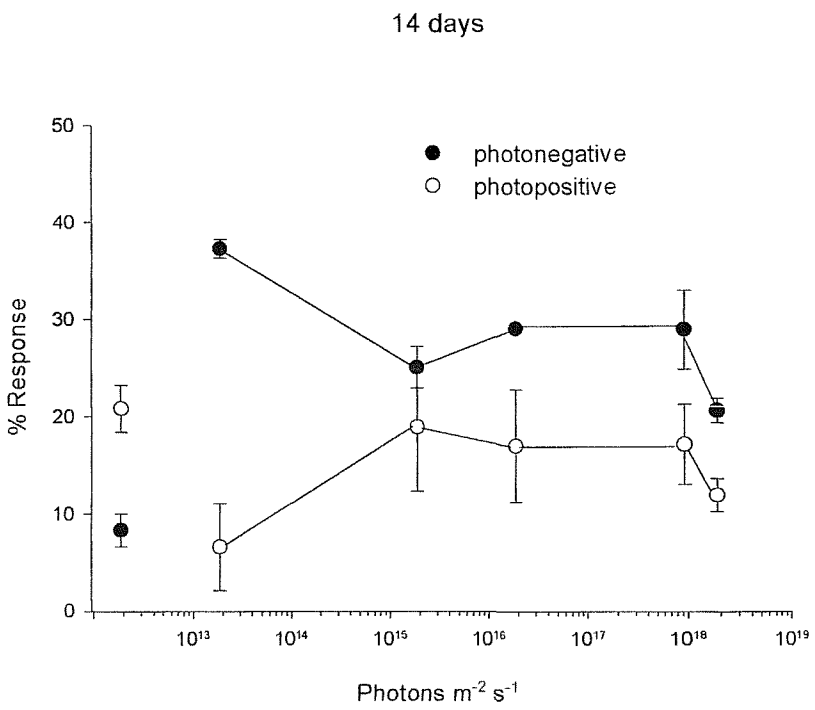


Figure 5.9 continued:

E)



F)

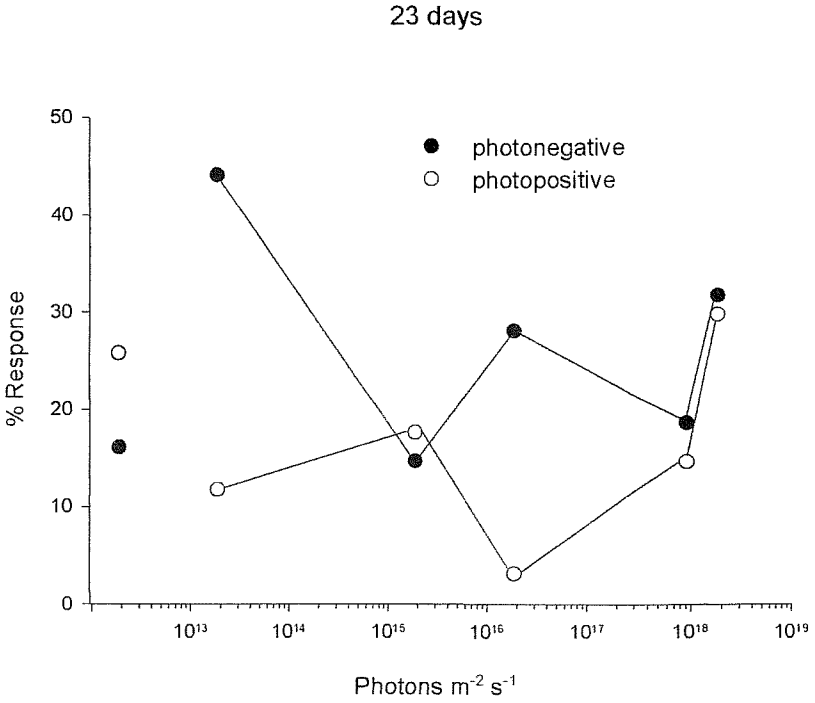


Figure 5.10: Effect of food and temperature on survival of *O. varicosa* larvae over time. The figure legends represent the treatment temperatures (16 °C and 25°C), and food treatment (F: food provided and NF: no food provided). Error bars represent standard deviations from the means of 4 replicates.

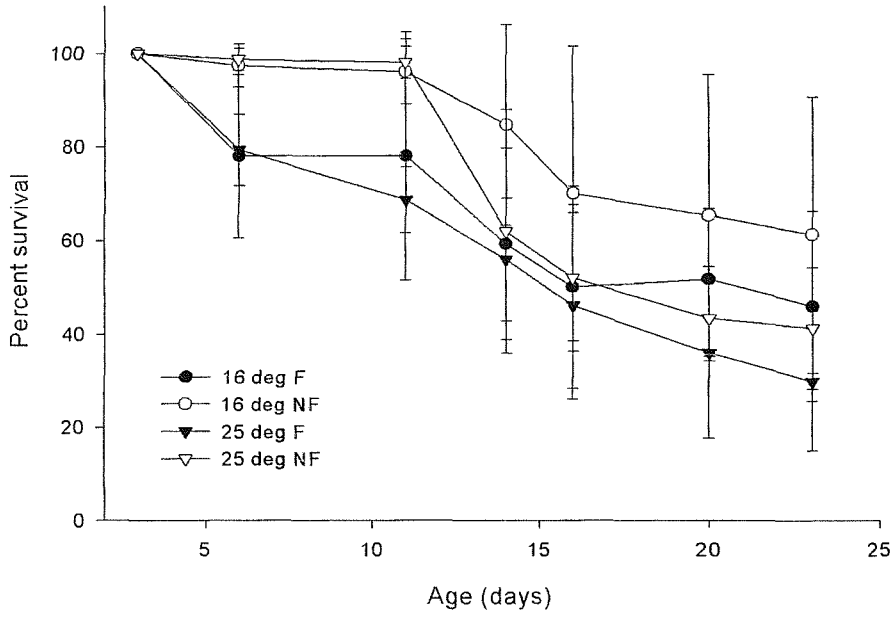


Table 5.4: Results of A) repeated measures one way ANOVA, and B) Student-Newman-Keuls test to determine whether temperature and additional algal food has a significant effect on larval survival. The treatments are 16 °C and 25 °C with algal food (F) and without food (NF).

A)

Source of Variance	DF	SS	MS	F	P
Between Subjects	6	11219.4	1869.9		
Between Treatments	3	1910.2	636.7	12.8	0.0001
Residual	18	898.6	49.9		
Total	27	14028.2			

B)

Comparison	P<0.05
16°C-NF vs 25°C-F	Yes
16°C-NF vs 16°C-F	Yes
16°C-NF vs 25°C-NF	Yes
25°C-NF vs 25°C-F	Yes
25°C-NF vs 16°C-F	No
16°C-F vs 25°C-F	No

Embryogenesis and larval development appear to proceed more slowly in brooding corals for example, *Balanophyllia elegans* has a brooding cycle that lasts 14-15 months (Fadlallah and Pearse 1982). Brooded embryos of *Favia fragrum* take about 4 days to develop into planulae and stay within the polyp for approximately 3 weeks before being released (Szmant-Froelich et al. 1985). In contrast, embryogenesis can be quite rapid in externally fertilized embryos, with swimming larvae developing within 6-8 hours in *Astrangia danae* (Szmant-Froelich et al. 1980) and 48 hours in *Platygyra sinensis* (Babcock and Heyward 1986).

A detailed ultra-structural study of embryogenesis was not conducted for *O. varicosa,* but the embryos conformed to both the type and speed of development described above for other broadcast spawning species. Heltzel and Babcock (2002) postulated that externally fertilized embryos rapidly progressed to the swimming larval stage in order to minimise time spent in the plankton and facilitate faster settlement, thereby reducing mortality. This advantage is somewhat negated in species such as *Oculina varicosa* that produce long-lived larvae. These swimming planulae, however, have an advantage over the passive embryos in that they can control (to a limited extent) their position in the water column via behavioural and sensory mechanisms, and thus may avoid some of the mortality associated with a planktonic existence.

5.5.2 Temperature effects on embryogenesis

Episodic upwelling over the Florida shelf may cause the water temperature to drop below 15°C periodically during the *Oculina* spawning season. Evidence from the temperature block experiment indicates that an upwelling event during embryogenesis may cause aberrance or death to the developing embryos. During the spawning months (July-September), the *average* temperatures on both the deep and shallow reefs were higher than the lower limit for normal embryonic development. There were several occasions, however, when the temperature at the deep reef dropped below 20°C, which could have had a detrimental effect on any *O. varicosa* embryos in the plankton.

5.5.3 Temperature effects on larval survival

The larval temperature block experiment demonstrated quite clearly that the larvae of *O. varicosa* have a higher tolerance of extreme temperatures than the embryos over an extended period of time. The effect of temperature on recruitment was not determined however, since despite the duration of the experiment, none of the

larvae in any of the treatments displayed settlement behaviour. In non-experimental cultures, *O. varicosa* larvae have settled and metamorphosed after approximately 20 days (personal observation). Decreasing temperature lowers metabolic rate and could account for a delay in settlement in the cold treatments; however, factors other than temperature are clearly responsible for inhibiting settlement in the warm treatments.

Many marine invertebrate larvae are capable of extending their competency period and delaying settlement until suitable conditions are available, and since the experimental conditions were so unlike those the larva would encounter in the field, delayed metamorphosis may explain the extended life-span of the experimental larvae. Alternatively, the small size of these larvae may mean that they require an external food source and that larval energy levels were insufficient to complete development.

5.5.4 Temperature effects on larval swimming

Larval swimming speed decreases as temperature is reduced, a phenomenon which can be explained by the combined effects of higher water viscosity and slower metabolic rate with lower water temperatures. As water temperatures increase above 25°C, motility decreases as the larvae may experience respiratory distress and temperature inhibition. Impaired swimming speed may reduce the ability of the larvae to control their vertical position in the water column, which in turn can increase their risk of mortality and loss owing to unfavourable currents. Larvae spawned in the shallow reef areas would be potentially quite capable of daily migrations; however, those spawned by the deep populations, even at the optimum temperature (25°C), would not reach the surface within 12 hours. These calculations ignore the effects of advective currents, but show that with the same physiological responses, larval diurnal patterns may differ as a function of habitat. Differences in *O. varicosa* larval diurnal patterns undoubtedly have complex ecological consequences, but would require a full study to determine how larval dispersal and survival is affected.

5.5.5 Geotactic and photoactic responses of O. varicosa *larvae*

The geotaxis experiments showed no diurnal pattern of larval migration either when maintained in the dark or on a diurnal cycle. An age-related behaviour pattern, however, was consistently observed in laboratory cultures, where *O. varicosa* larvae invariably swam in a spinning motion towards the surface upon hatching, and remained there for 12-18 hours. After this time, they began to swim throughout the water column

and subsequently became demersal. This behaviour has been observed in other coral planulae (Atoda 1953, Harrigan 1972, Lewis 1974, Szmant-Froelich et al. 1985) and is usually attributed to changing phototactic and geotactic responses although in most cases the tactic responses had not been tested experimentally (Harrison and Wallace 1990). Kawaguti (1941) studied tactic responses in planulae of *Achrelia horrescens, Euphyllia glabrescens, Pocillopora damicornis and Seriatopora hystrix*. All exhibited negative geotaxis and positive phototaxis, but became negatively phototactic in bright light. Phototaxis varied between species and correlated well with zooxanthellae density in the planulae, as well as observed distribution of adults. In temperate coastal environments the light level can change unpredictably and a particular light level could occur over a range of locations in the water column, depending on the conditions. Light therefore would not be a reliable way of controlling vertical position in variable habitats. Larvae of *O. varicosa* are azooxanthellate, as are most other species with externally developed planulae (Kojis and Quinn 1981); however, tactic responses probably still play an important role in settlement and distribution of this species.

5.5.6 Feeding in O. varicosa *larvae*

Most coral larvae that spend short periods in the plankton are lecithotrophic, using maternal lipid stores from the egg as an energy source. Larvae, which do not have large energy stores, probably need an exogenous food supply to survive for extended periods in the plankton. The results of the larval feeding experiment were inconclusive; two types of algae were provided as an external food source for the planulae, but algal cells appeared to bounce off the oral cilia rather than being ingested. The planulae of *Caryophyllia smithii* feed by trailing mucous strings from the oral pole, which entangle food particles and ingest them into the larval gastric cavity. Excess mucus and undigested particles are egested back via the mouth. Although *O. varicosa* has never been observed to feed, larvae have been seen trailing mucous and expelling material thorough the stomodeum (pers. observation) so it appears that ingestion is physically possible. They also have some of the same characteristics as *C. smithii* larvae, which are the product of external fertilization, are azooxanthellate and are similar in size (130μm) to *O. varicosa* larvae.

5.6 Conclusions

Decreased water temperature does not cause larval mortality, but may damage embryos or delay the onset of metamorphosis. Larvae have survived under experimental conditions for more than one month, but under certain conditions have metamorphosed after 20 days. The planulae of *O. varicosa* began probing the bottom of culture vessels after 10-14 days, which may mean that they are competent to metamorphose; however until larvae are observed under field conditions the natural duration of larval life cannot be known. Colder water temperatures also reduce larval motility and may prevent larvae from optimising migration cycles or predator avoidance behaviour.

For oceanic plankton, there is a risk of predation and unfavorable transport associated with the upper part of the water column. Conversely, staying near the reef also presents the risk of being consumed by adult corals or other benthic predators. The behaviour that was observed in *O. varicosa* larvae could facilitate an initial escape from predation and a means of dispersal followed by a benthic migration in search of appropriate settlement substrata. Although controlled laboratory experiments can provide insight into response to stimuli, behaviour patterns in the field are probably the result of complex interactions of several cues, and extrapolations of this data to field situations must be made with reservation.

Successful settlement and metamorphosis occurred in all of the feeding treatments despite high larval mortality from fouling. Other cultures that were filtered to remove all bacteria had no fouling and very little mortality; however, not a single larva metamorphosed. These observations, although inconclusive, imply that fine filtration removed something that the larvae needed for completion of development. These larvae may be capable of consuming bacteria or taking up DOM as external food source, as well as (or instead of) particulate food. Whether *O. varicosa* larvae require an exogenous food source to complete larval development and metamorphosis, is still undetermined and warrants further research.

Chapter 6: Larval dispersal and recruitment

6.1 Introduction

6.1.1 Larval competence and metamorphosis

Apart from their role as a food supply for others higher up the food chain, the ultimate purpose of larval existence is to settle, metamorphose and become a reproductive member of the adult community. The transition from larva to viable adult is completed by a very few of the original propagules. There is a great deal of loss between spawning and settlement, and the challenges of metamorphosis and post larval survival contribute to this attrition.

Towards the end of the planktonic period, the larvae become 'competent' to settle. The onset of competency may involve behavioural changes in response to environmental cues, and/or physiological changes that allow the larva to choose a settlement site. The relative importance of each behavioural and physiological mechanism is a species-specific trait (Heyward and Negri 1999, Raimondi and Morse 2000). Like other larvae, scleractinian planulae can presumably discriminate between different substrata. However, there are conflicting reports regarding the specificity and conditions required for settlement. Bacterial and algal films (Harrigan 1972, Loya 1976 a, b), extracts of crustose or coralline algae (Heyward and Negri 1999, Raimondi and Morse 2000) and phorbol ester TPA (Henning et al. 1996) have all been used to induce metamorphosis in scleractinians. Settlement also occurred when larvae of *Acropora millepora* were exposed to calcified reef rubble, demonstrating that metamorphosis was possible in the absence of encrusting algae, and other studies indicate that larvae will settle quite readily on unconditioned substrata (Lewis, 1974). Goreau (1981) demonstrated spatial aggregation and preference for horizontal substrata by larvae of *Porites porites*. Gregarious settlement may also occur in response to stimuli of adults and juveniles of conspecifics (Doyle, 1975). The common denominator in substratum selection by scleractinian larvae is the requirement for hard rocky substrata, which are necessary for adhesion of skeletal material.

Duration of planular life is species-specific and can vary from days to weeks (Szmant-Froelich et al. 1980; Babcock, 1984, Harrison et al. 1984, Schlesinger and Loya 1985, Stoddart and Black 1985, Szmant-Froelich et al. 1985). Coral planulae, like many other invertebrates, have demonstrated delayed metamorphosis in the laboratory (Szmant-Froelich et al. 1980, Kojis and Quinn 1981, Fadlallah and Pearse

1982b). There are risks associated with planktonic life; however, delaying metamorphosis until a suitable substratum is available potentially increases recruitment success of the larva.

6.1.2 Hydrodynamics of the Florida Shelf

Although larvae have some influence over their position in the water column, they cannot swim against oceanic flow and are subject to the hydrodynamics of the system in which they were released. Dispersal of *Oculina varicosa* larvae will therefore be strongly influenced by the currents and tides of the Florida shelf system. The proximity of the Florida Current creates a prevailing northerly flow in the surface waters of Florida shelf. The strongest flows are closest to the center of the Florida Current, which follows the edge of the continental shelf along the East Coast of North America, as it turns into the Gulf Stream. Periodically, the Florida Current meanders shoreward of the shelf edge causing an upwelling of cold, nutrient-rich water over the edge of the shelf at 100m depth (Smith 1981, 1983). Upwelling takes place throughout the year, creating an east-west component to the current, but during the summer months the upwelling occasionally intrudes into the near-shore ledges, inundating the shallow colonies with cold nutrient rich water (Smith 1983). Occasionally the flow of the Florida Current becomes unstable and warm-core eddies spiral off into the shallow reefs. The upwelling events and eddies created by the Florida current, although somewhat unpredictable, are a potential route for larval transport between deep and shallow water populations of *Oculina*.

6.1.3 Status of the Oculina *reefs*

The *Oculina* banks have been damaged to a considerable extent; in most places, the previously thick bushes of coral have been reduced to rubble. The only intact *Oculina* thickets were found in a small (4 ha) area known locally as Jeff's Reef, and a larger area just to the north called Chapman's Reef (Brooke pers. observation, September 2001). The causes of the damage are open to speculation, but include natural episodic die-off, freshwater seepage, disease, and destruction from illegal trawling activities. Some of these damaged areas were observed in the early 1980s and have not recovered. The fecundity of *O. varicosa* is quite high, and the larvae spend 2-3 weeks in the plankton, so the elements of re-colonisation potentially exist within the reproductive biology of the species. The dead coral rubble may not be suitable for *O. varicosa* settlement since it is a low relief substrate and easily covered with sediment. Small pieces of rubble may also

tumble in high current, sloughing off any new recruits. It is also possible that shelf currents infrequently transport larvae from the intact reefs in the south to the denuded areas of the banks, and that many larvae are lost in the complex hydrodynamics. A complex three-dimensional model of shelf hydrodynamics incorporating larval behaviour is needed to predict scenarios of larval transport, but is unfortunately outside of the scope of this project. Deployment of recruitment substrata and mapping of simple current flows may provide insight into potential larval transport.

6.2 Objectives

1) Measure near-bottom current speed and direction at deep reefs using an acoustic doppler current meter. Use these measurements together with duration of larval life to predict patterns of potential larval dispersal and recruitment.
2) Deploy settlement substrate to evaluate recruitment of coral colonies in deep and shallow-water populations.

6.3 Materials and Methods

6.3.1 Measurement of current speed and direction

An acoustic doppler current meter (Falmouth Scientific model 2D-ACM) was deployed on the northwest quadrant of Jeff's Reef (27°32.54 N; 79°58.73 W), between April 2000 and March 2001. The meter recorded temperature, current speed and current direction. The current speed and direction during coral spawning season, together with larval duration (from laboratory cultures) were used to estimate the track of larvae released during each spawning event observed in 2000.

6.3.2 Deployment of larval recruitment substratum

6.3.2.1 Shallow reef recruitment project

The recruitment substrata used in this experiment were small (20cm wide x 15cm high) hemisperical structures called 'reefballs', produced by the Reefball Foundation (www.reefball.org). These structures are used to create artificial reefs, and have been used worldwide in various commercial, educational and research projects. The reefballs are made from a low pH (~8.5) concrete, in contrast to regular concrete, which is alkaline, and the surface of the structure is very rugose. Eighteen reefballs were deployed using scuba on a shallow ledge (5-7m depth) at Pepper Park (27°29.924'N, 80°17.429'W). A map of the site showing reefball locations is shown in Figure 6.1.

The reefballs were attached to the substratum using quick setting cement. The substratum was pre-cleaned with a wire brush and a 'fist sized' ball of wet cement was placed in the center of the patch. The reefball was immediately placed on top of the cement and pushed hard in to place. Excess cement was smoothed over the edge of the reefball. The cement begins to dry in approximately 6 minutes from mixing so the deployment was very-time sensitive. The experiment was deployed on a ledge with abundant coral colonies during the third week of August. This is just after the peak of spawning season for the shallow populations and just before the peak for the deep-water populations. Larvae should have been present in the water column when the experiment was deployed. The reefballs were labelled with set number (1-6) and replicate number (1-3), and closely examined every month, (or as frequently as weather permitted) for evidence of *Oculina* settlement.

6.3.2.2 Jeff's reef recruitment project

The recruitment substrata deployed at Jeff's reef (Figure 6.2) were part of a larger reef restoration project, funded by the National Marine Fisheries Service. The project has been running since 1996 and the objective is to provide elevated stable substrata for coral larval settlement and promote reef regeneration. In 1996-1999, large cubic structures constructed from concrete building blocks (Figure 6.3A) were deployed at various sites on the reef tract (Figure 6.3C). The blocks at Jeff's Reef were observed for evidence of coral recruitment in June 1999 (using the Johnson Sea Link research submersible), and March 2001 and September 2001(using the Clelia research submersible). In 2000 and 2001, a total of 240 Bay Balls (0.9m wide x0.6m high, 250kg) (Figure 6.3B) were deployed in clusters of 25 at a damaged area of the reef tract called Sebastian Pinnacles (27°50.974′ N, 79°57.698′ W)(Figure 6.3D). The reefballs were also observed for signs of recruitment in September 2001.

6.3 Results

6.3.1 Current speed and direction at Jeff's Reef

During the months of August and September, when spawning events occur, the speed and direction of the bottom currents at Jeff's Reef indicate the initial dispersal direction for the coral larvae. The north-south and east-west components of the bottom currents at the reef are highly variable during the *Oculina* spawning season. Figure 6.4

Figure 6.1. Site-map of shallow Pepper Park site showing location of reefballs, deployed in August 2000.

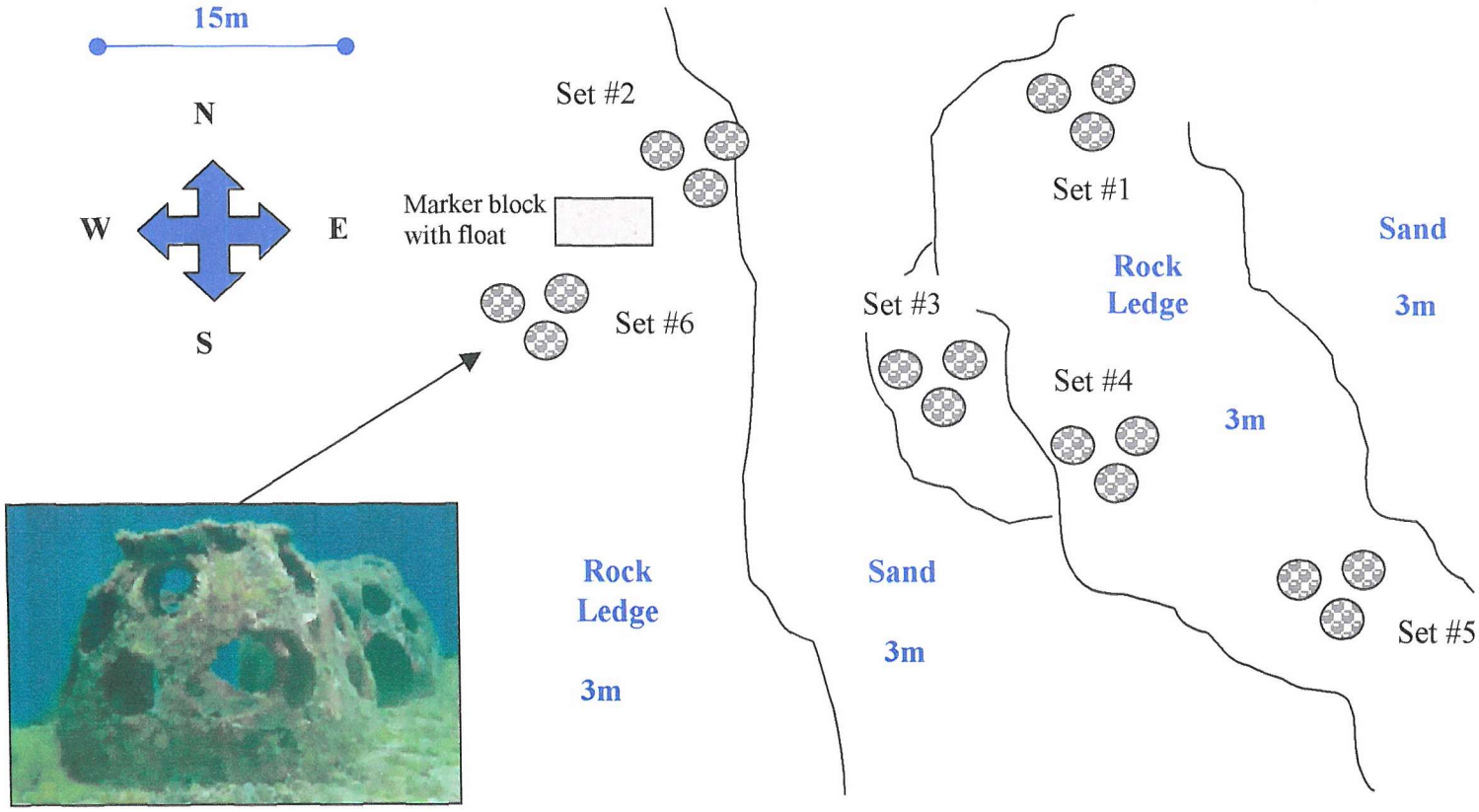


Figure 6.2. Site map of Jeff's Reef showing locations of blocks deployed between 1996-1998 and current meter deployed from April 2000-April 2001. Depths are also marked (m) at various points on the reef, and a scale bar (25m) for reference.

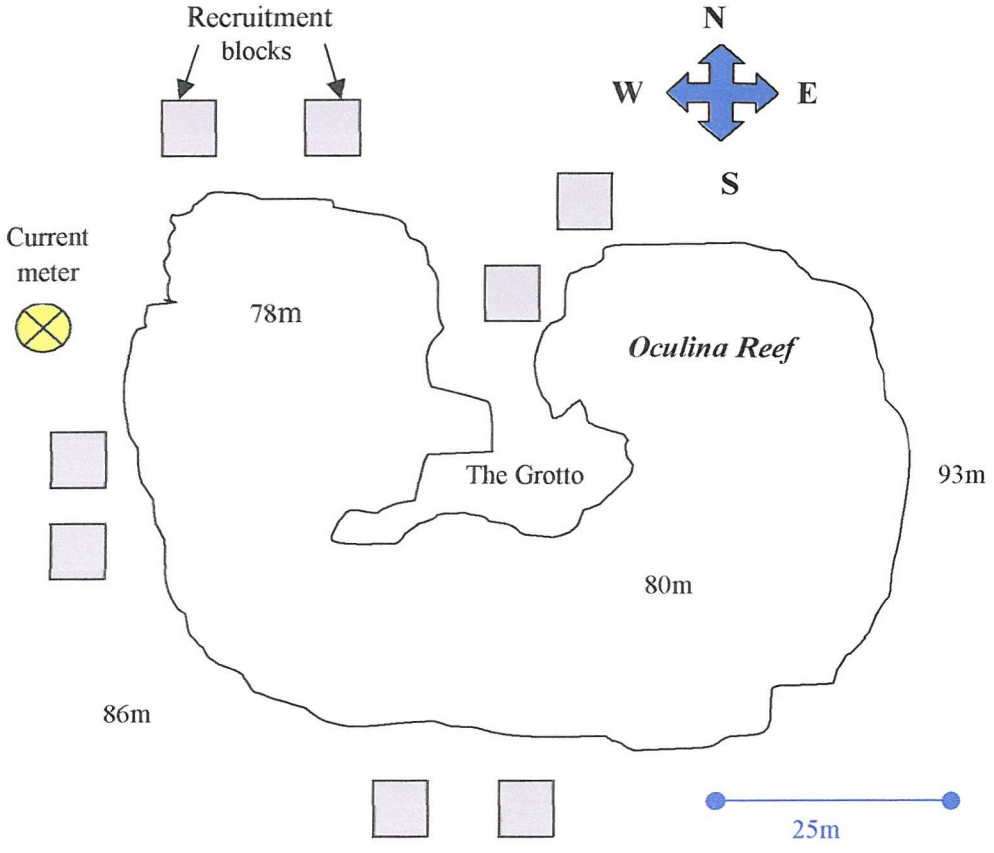
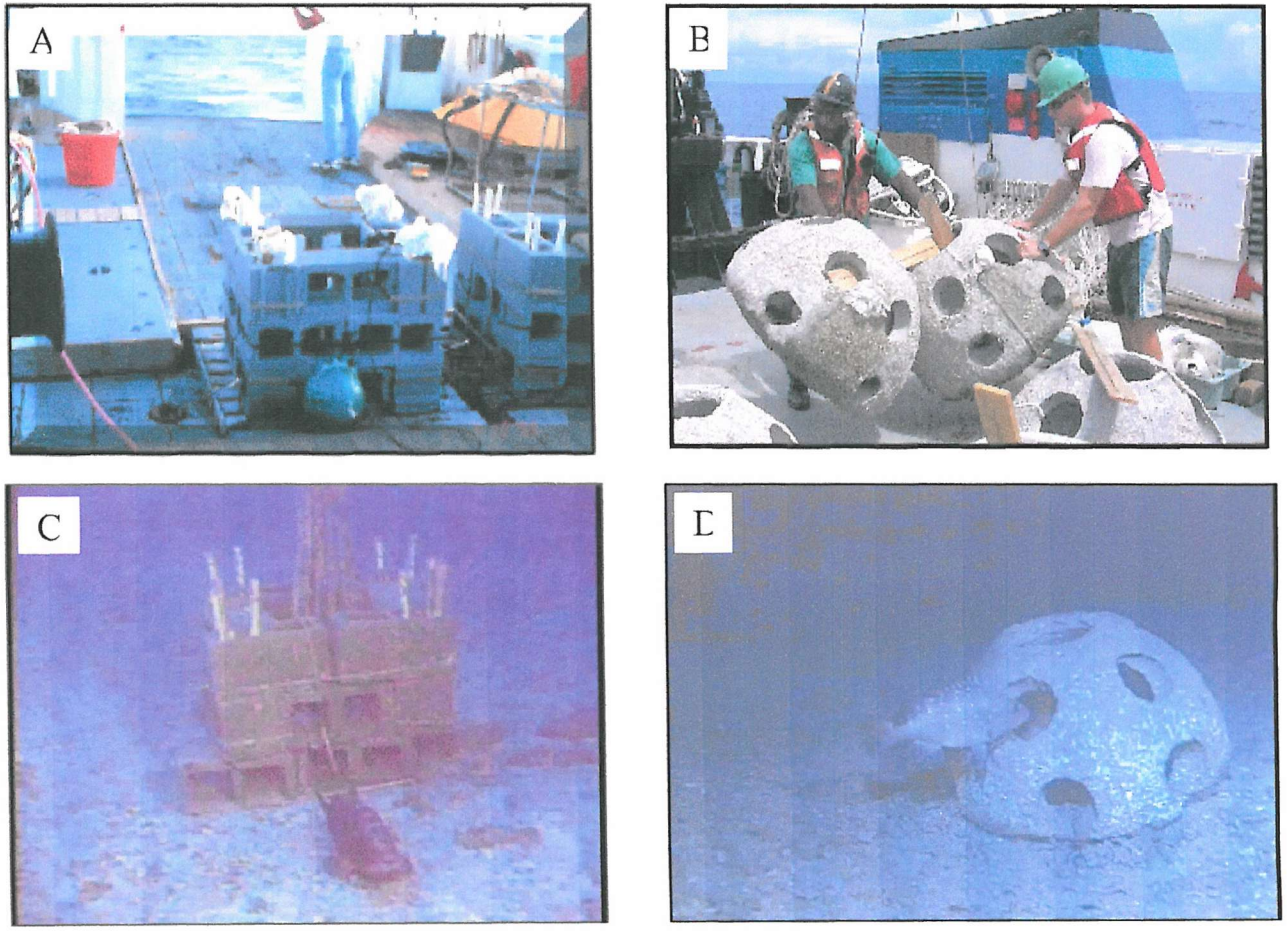


Figure 6.3. Images of recruitment modules deployed on the deep reef tract A) Concrete blocks used from 1996-1998 (total 56 blocks) on the deck of the ship. B) In the water after 2 years with attendant grouper. C) Reefballs about to be deployed (total 240). D) Reefball at Sebastian pinnacles, after 1 year. Note the depauperate substrate.



shows that the prevailing directions of the currents are north and east, with occasional south and west components. These westerly currents coincide with a decrease in temperature, which is indicative of an upwelling event (Figure 6.5). Historic data (Reed 1980, Smith 1981, 1983, 1987) also show a high degree of variability in current speed and direction, with a prevailing Northerly flow. At an average near-bottom current speed of 4.2 cm s^{-1} (during August and September), the larvae could be carried a total of 76.1km (45.7 miles) during their 3 weeks of larval planktonic existence. The estimated trajectories of larvae released from two spawning events (August 7 and September 9, 2000) are shown as progressive vector diagrams (Figure 6.6). If the larvae reach the upper water column, as they may, their transport would be influenced by the Florida Current, which also has a strong prevailing northerly flow with occasional localized reversal from gyres and eddies. These estimates indicate that *O. varicosa* larvae can potentially reach almost any location within the range of this species on the Florida Continental Shelf, but that the potential for them to be lost to open ocean is also high.

6.3.2 Recruitment onto shallow substrate

Observation of the shallow substrata showed no sign of recruitment by any scleractinian on any of the reefballs. Settlement by other organisms was abundant; for example sponges, hydroids, oysters, barnacles, polychates worms, bryozoans, gorgonians, serpulid worms, tunicates and other encrusting taxa. The recruits were not documented rigorously or identified to species name since this would have been an extremely time consuming task and although interesting, peripheral to the study objectives.

6.3.3 Recruitment onto deep substrate

The opportunity to examine the deep recruitment blocks and reefballs occurred infrequently and during submersible operations close observation of recruitment was limited by either current speed or low visibility. In 1999, a small colony (approximately 5cm in diameter) was observed on one of the older blocks. The colony was a maximum of 3 years of age (since the block was deployed in 1996) but looked much younger. The other blocks were surveyed briefly before the dive ended owing to deteriorating conditions, and no further signs of recruitment were found. None of the reefballs showed

Figure 6.4. Filtered trajectory (2 weeks moving average) of current speed and direction between April 2000 and March 2001 at 80m depth Jeffs Reef.

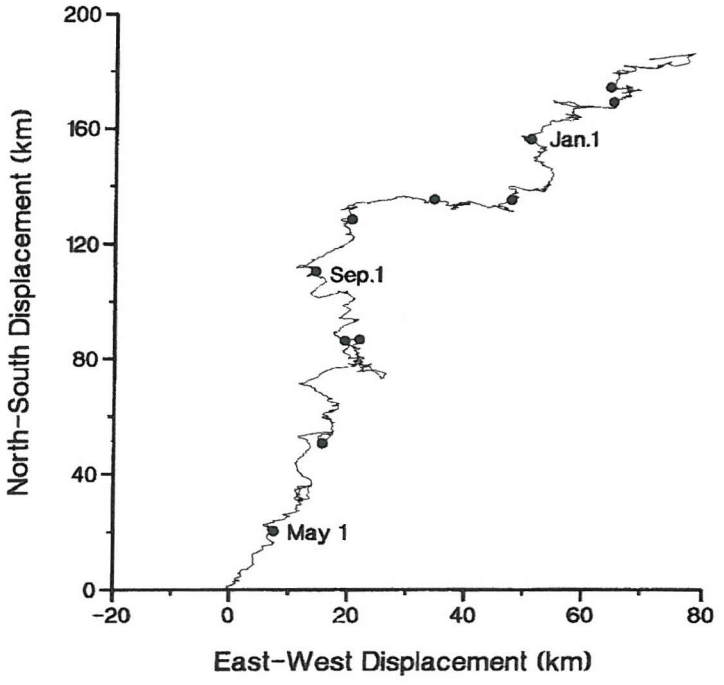


Figure 6.5. Temperature at 80m on Jeff's Reef between April 2000 and March 2001

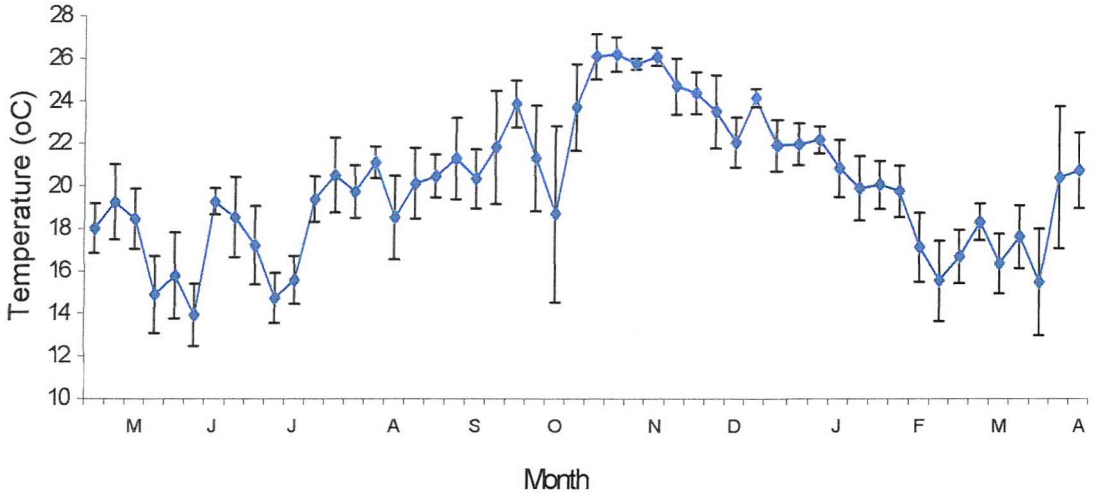
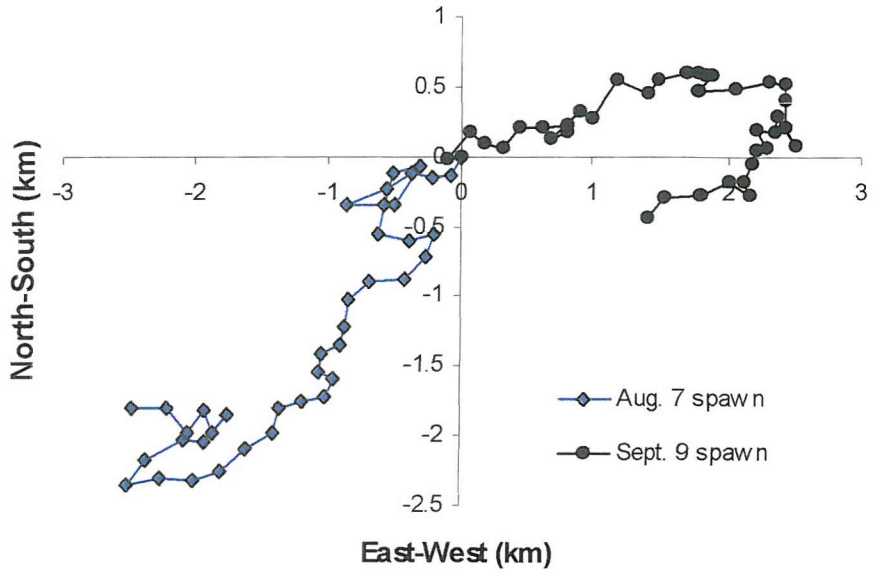


Figure 6.6. Estimated trajectory of *O. varicosa* larvae released from Jeff's Reef on two separate spawning events, August 8 and September 9, 2000. Track was estimated using current speed and distance values from the current meter data. This track assumed that the larvae did not migrate into the upper water column, but remained under the influence of the bottom currents.



any signs of recruitment, but new colonies would have been just one year old and difficult to identify unless very close. In summary, the level of recruitment onto the deep-water modules is still unknown and will probably remain so until the colonies are sufficiently large to be seen easily from an ROV or submersible.

6.4 Discussion

The strongest cross-shelf transport events occur during the late summer when the *Oculina* are spawning. It is unclear how much of the recruitment onto the near-shore reefs comes from deep-water larvae, but the mechanism for transport exists in these upwelling events. The upwelled water is less likely to be forced close to shore at Cape Canaveral where the shelf is wide, than further South in Fort Pierce where the shelf is quite narrow. Since the along-shelf water movement is dominated by the Florida Current, the prevailing flow is in a northerly direction; however there are occasional direction reversals that can potentially transport larvae north or south between both deep and shallow reef tracts.

In a large scale recruitment experiment on Helix Reef in Australia, Sammarco and Andrews (1988, 1989), discovered that most of the recruitment of scleractinian coral larvae occurred within 300m of the natal reef. Settlement was generally lower in pocilloporids than acroporids. Most of the juveniles that settled further away from the reef (acroporids) were derived from corals with externally fertilized eggs and a planktonic larval development period of 24-72 h, implying higher effective dispersal capabilities in these species. Highly localized circulation patterns appeared to retain most of the larvae near the reef and larval dispersal of corals in this experiment was a small-scale phenomenon.

There are few field studies on coral larval settlement, substrate selection and survival of very young corals. Evidence from field studies suggest that before corals are able to settle on new surfaces, they need to be conditioned for at least a year (Grigg and Maragos 1974, Loya 1976, Schuhmacher 1977), although the substrates used in these studies were not natural reef substrates so conditioning periods may not be representative of natural reefs. Pearson (1981) studied the long-term recovery of reef areas on the GBR damaged by *Acanthaster plancii* and concluded that that several dacades are required for recovery of most coral communities affected by major natural disasters. There is less evidence available on manmade disturbances but indications are that full recovery may be prolonged or prevented altogether because of permanent

change in the environment and or a continuation of chronic low level disturbances. On reef surfaces with structural damage, recovery is likely to be retarded if less surface area is available for settlement ie stuctural complexity is reduced.

During the spawning season of *O. varicosa* in 2001, current meter data show highly variable current speed and direction. Some gametes may be spawned into currents that transport the larvae to suitable settlement sites, whereas others may be carried to unsuitable habitat or out to sea and lost. It is probable that larvae settling outside their depth of origin are able to survive, grow and reproduce, in which case, the potential source of recruits into damaged reef areas, may come from the near-shore ledges outside the *Oculina* protected area. The observed level of recruitment over the study period was extremely low at the deep sites, and non-existent in the shallows. Explanations for this include: 1) the recruitment modules were not suitable for coral larval settlement, or were not sufficiently conditioned for planuae to settle. 2) Larval recruitment is naturally very low in this species 3) Recruitment is episodic and dependent on favourable currents, which may not have occurred during the study period. 4) New colonies have recruited but are not sufficiently large to see. 5) The artificial substrate does not have a sufficient level of structural complexity at the microscale level.

Reefballs have proven to be suitable substratum for coral settlement in other locations and although the deep modules were not examined closely, the shallow reefballs were, on several occasions. Low or episodic recruitment rates for this species seems the most likely explanation for the lack of new colonies, especially in the context of the highly variable and unpredictable flow over the *Oculina* habitat.

6.5 Conclusions

Oculina varicosa is a broadcast spawning species with high colony fecundity and a long-lived planktonic larval stage. The larvae are active swimmers, and can adjust their position in the water column; they are also able to tolerate the wide range of temperatures that may occur in the deep reef habitat. These characteristics are conducive to re-colonization of the deep *Oculina* habitat, but there is little information available on other important processes such as larval supply, recruitment rates and post recruitment survival. Unfavorable larval transport, insufficient suitable substrata, low natural recruitment rates, and continued disturbance from illegal fishing activity may all be prohibitive to reef regeneration.

An estimate of larval trajectories from two spawning events in 2000, indicated that larvae could be transported to widely separated, and not necessarily favorable locations. A three-dimensional model of the complex hydrodynamic regime of the Florida shelf would enable prediction of larval transport within and between *Oculina* populations. An effort to restore parts of the *Oculina* banks is currently in progress, and large concrete recruitment modules have been deployed in damaged areas, but to date very little recruitment has been observed.

There are several Second World War wrecks at 40-80m to the northwest of Jeff's Reef, which are covered with abundant *Oculina* colonies. Even with sporadic recruitment and slow growth rates, the *Oculina* habitat could potentially recover, given sufficient time; however, stringent enforcement of trawling regulations is also required to prevent continued damage.

Chapter 7: Summary, Synthesis, Limitations and Further Research

7.1 Summary of findings

7.1.1 Genetic analysis

The objective of this research was to determine whether the deep and shallow populations of *Oculina varicosa* are truly conspecific, using variation of the internal transcribed spacer (ITS) genes of nuclear ribosomal DNA (rDNA).

The internal transcribed spacers of various Oculinidae were amplified using primers 1S and A4 (ITS-1) and FA and RA (ITS-2). The amplification of ITS-1 was more successful than ITS-2, which showed a high level of polymorphism. The *Oculina varicosa* deep and shallow populations were compared with samples of congeneric *O. diffusa* and *O. patagonica*. The deepwater corals, *Madrepora oculata* and *Lophelia pertusa* were included as outliers. The consensus tree from the manipulated data separated outgroups *Lophelia pertusa* and *Madrepora oculata*, from the other sequences. The remaining samples, including the congeneric *Oculina diffusa* and *O. patagonica*, show no species grouping. The results from ITS-2 were less robust but produced a similar result.

In summary, the *O. varicosa* populations were conspecific, but the other two *Oculina* species also fell into the same groups, which opens up many more questions regarding the species classification of the Western Atlantic Oculinidae.

7.1.2 Energetic considerations

The objective of this chapter was to gain insight into differences in growth and energy allocation between deep and shallow water populations. Calcification rates were measured under controlled conditions, and transplants were deployed to determine *in situ* growth rate. Seasonal changes in lipid content were recorded and primary productivity was measured for the zooxanthellate shallow colonies.

The laboratory growth rate studies showed that light had a significant effect on calcification rate in zooxanthellate samples, but that temperature did not. Calcification rate was also dependent on sample source, therefore the presence of symbionts appeared to confer an advantage on the coral. The lipid content increased throughout the summer months and peaked just prior to the spawning season for both populations. Primary productivity and respiration rates were comparable to shade-adapted warm water corals.

7.1.3 Reproductive ecology

The overall objective of this set of experiments was to describe ecologically relevant aspects of *Oculina* reproductive ecology, including reproductive strategy, gametogenic cycles, fecundity, embryology, larval biology and settlement rates.

Oculina varicosa is a gonochoristic, broadcast spawning species with a gametogenic cycle that begins in the spring and ends in the late summer with a protracted spawning period from July to September. The timing of gametogenesis did not differ significantly between the two populations, although the deep-water samples generally spawned later than their shallow-water counterparts. Spawning did not correlate with lunar phase or tidal cycle, but occurred most frequently between sunset and sunrise. Spawning cues were not investigated extensively, but there is observational evidence for male-induced epidemic spawning.

Fecundity was high for both populations and falls within the range recorded for warm water species with comparable egg sizes. The fecundity of the shallow water population was significantly higher than the deepwater samples for the single year of data available, and there was also a significant difference between years for the deep-water samples. The eggs are small and give rise to rapidly developing embryos, and ultimately small, planktonic larvae. Laboratory experiments showed that larvae are active swimmers and stay in the plankton for up to three weeks. Their behaviour shows ontogenetic changes in geotactic responses, and they do not appear to respond to light. The larvae showed no evidence of feeding behaviour although the small egg size and long planktonic existence suggest that these may be feeding larvae.

Embryogenesis, larval development and larval swimming speed are all temperature sensitive. The lower lethal temperature was ~17°C for embryogenesis, but larval survival was high at temperatures ranging from 10°C to 30°C. Larval swimming speed increased predictably with temperature (Q_{10}~2) in larvae from both populations, until respiratory inhibition occurred above 30°C. Settlement rates were low in the laboratory and no research was possible regarding juvenile biology. From the very limited information available, natural recruitment rates appear to be very low, in both deep and shallow populations. Not all of the individual objectives were achieved to the fullest extent possible, but I believe these results create a strong foundation of knowledge for further research.

7.2 Synthesis of results

The habitats exploited by *O. varicosa* are very dynamic, and have extremely variable environmental conditions such as temperature, light, sediment load and current speed and direction. The hydrodynamic regime not only drives the physical characteristics of the habitat, but may also represent a mechanism for genetic exchange between the deep and shallow populations. The results of the genetic analysis support the existence of this mechanism since the two *O. varicosa* populations were genetically homogenous. The genetic analysis also provides evidence that the other *Oculina* species are more closely related than currently defined morphologically. There is no reproductive information available on *O. diffusa*, but Fine et al (2001) showed that *O. patagonica* has a very similar reproductive biology to *O. varicosa*, with great deal of potential for large-scale exchange of genetic material. The coral appears to have adapted well to the range of shelf edge conditions. Adults from both populations of *O. varicosa* can withstand the low temperatures and high sedimentation rates that occur periodically throughout the habitat, and even without symbionts produce a comparable number of oocytes to tropical species with a similar egg size. The annual seasonal gametogenic cycles cause the larvae to be released during periods of the potential high flow of summer upwelling events. After the initial swim towards the surface, the behavioural characteristics of the larvae however may serve to keep them close to the benthos and retained within the immediate area. Alternatively, the strong currents often associated with the shelf edge may cause many larvae to be lost from the reef system. There is insufficient information available at present to model larval dispersal patterns within and between the populations, but this is an avenue that needs further exploration. The ecology of the coral and the genetic evidence indicate that unassisted recovery of the reef is possible. Recruitment of new colonies, either on artificial substrate or into denuded areas has been minimal, and it is possible that the reef structure has been damaged so severely that the small-scale flow regimes are no longer conducive to larval settlement. Although the reefs are protected, there is evidence of human activity in the reserve. This creates confusion when trying to evaluate the success of the protected status of the *Oculina* habitat. There is an urgent need for research of deepwater resources as their exploitation currently far exceeds our ability to conserve them.

7.3 Limitations to research success

7.3.1 Sample acquisition

Most of the limitations that affected the research in this thesis were simply a result of the nature of the *Oculina* habitat. The deep-water banks, although only 100m in depth were nonetheless extremely challenging to work in. The high velocity Florida current combined with an unpredictable shelf-edge water mass precluded technical diving on the *Oculina* Banks as it was considered too dangerous an operation. This would have been the preferred method for making small collections, deploying experiments and examining recruitment substrata.

The alternatives to scuba diving; submersibles and remotely operated vehicles (ROV's), were expensive, temperamental and very sensitive to ocean conditions. Deep-water sample collections were often opportunistic, therefore spatial and temporal resolution of samples was not ideal. Conditions at the shallow-water habitat, although only 6-8m deep also precluded sample collection on many occasions, especially in the winter and early spring months. The seas can be very rough on the exposed Atlantic shore, and even in calm seas the visibility is frequently less than 1m. The numerous gaps in the shallow water sample collections are almost always attributable to the weather.

7.3.2 Genetic analysis

The High Salt DNA Extraction technique was used to avoid potential problems caused by mucous in cnidarian tissues. The salt however, can interfere with the later stages of the extraction if it is not completely removed. Approximately half of the original samples provided usable results, usually because the DNA quality was poor. *Oculina* does not produce a lot of mucous unless it is under stress. In future the samples could be left to acclimate (and cease mucous production) in tanks before preservation, and alternative DNA extraction techniques could be used. The primers used for ITS-1 worked quite well, but those for ITS-2 need to be re-designed. The shortage of data for ITS-2 considerably weakens the conclusions from the analysis. The ITS-1 data places all the species of *Oculina* studied in the same group, which casts doubt on the species definitions for the entire *Oculina* genus. Without another sequence for confirmation, however the results must be considered with caution.

7.3.3 Oculina *growth and respiration*

The growth and respiration experiments were an incomplete look at energy budgets for *O. varicosa*. The study was limited by the availability of the equipment used to measure respiration and primary productivity, and several more trials would have strengthened the analysis. The original objective was to assess the contribution of zooxanthellae to the coral's energy budget (CZAR). However the storage freezer for the zooxanthellae and coral tissue extracts expired one weekend, and the samples were ruined. The same freezer also contained some of the tissue samples that were destined for lipid quantification; these would have considerably strengthened the lipid content study.

7.3.4 In situ *growth rates.*

The transplant pieces were too small and exposed to survive the winter storms in the shallows, and too close to the ground to resist the heavy sedimentation at the deep reef. The experimental methodology needs to be reconsidered in the light of severe environmental conditions.

7.3.5 *Gamete supply*

Oculina varicosa spawns quite readily in the laboratory when the gametes are ripe, but spawning is unpredictable and can occur several days after collection from the field. It was not feasible therefore to observe them through the night for extended periods of time. The deepwater samples spawned only 3 times and once appeared to be premature since the numbers were low and the larvae not healthy. Deep-water embryology was not observed and there were few comparative larval studies between populations. This is not considered a serious limitation because there were no apparent differences between the deep and shallow planulae. The original list of objectives included studies of fertilisation kinetics. Although the males would spawn separately the females did not spawn without a male present, and the eggs were too contaminated with sperm to run the experiments.

7.3.6 *Larval settlement*

Larvae were offered a wide array of substrata: pre-conditioned glass slides, various types of tile, old adult skeleton, pieces of rubble from the reef and clamshells. These substrata were combined with extracts of various local coralline and crustose

algae, with filtered water where adults were cultured and with filtered water directly from the reef. The experiments either fouled immediately or the larvae continued their demersal existence. The few larvae that did settle and metamorphose, decided to do so on the sides of the glass culture vessels. Relocation attempts resulted in instant decimation and efforts to remove bacteria and debris from around the polyps had the same effect. Either the larvae were not competent to settle during the experiments, or the conditions were not acceptable. The use of artificial settlement-induces may be the next avenue of investigation.

7.3.7 Hydrodynamic data

Estimates of larval trajectory were severely limited by the paucity of hydrodynamic information. Ideally there would have been an array of current meters along the deep and shallow habitats, and suspended at intervals in the water column over the deep reefs. Current meters however are very expensive and even if they were available, the risk of loss from environmental and human interference in these habitats was too high to justify the cost.

7.4 Avenues for further research

7.4.1 Oculina *taxonomy and clonal variability of the deep reefs*

It is apparent from the results of the ITS analysis that the classification of the genus *Oculina* should be revisited using molecular techniques. The ITS-2 sequences need to be analysed to corroborate the ITS-1 results for *O. diffusa* and *O. patagonica*, and the ITS regions of other *Oculina* species should also be analysed.

Information on natural recruitment rates is needed to assess recovery potential of the *Oculina* banks, however conventional ecological techniques are not easily applicable to the deep shelf habitat. Molecular techniques can provide insight into ecological processes. For example, high clonal variability within a reef area would imply influx of genetic material from outside the system, whereas a low variability indicates that the system was created primarily from asexual growth and colony fragmentation.

Microsatellite analysis has been used to determine clonal variability of *Lophelia pertusa* reefs (Rogers. In prep). Microsatellites are small tandem repeat sequences of a few base pairs in length. They are non-coding sequences and are selectively neutral. They also vary at the level of the individual and therefore have a high resolution in

determining clonal variation. Microsatellite analysis could be applied equally well to determine clonal variation of the *Oculina* reef system.

7.4.2 Energy budgets for cold water corals

Energy budgets in cold-water corals have rarely been studied, in fact most aspects of cold-water coral biology has been somewhat neglected in favour of the more dramatic and visible tropical coral reefs. Deep-water corals pose more of a challenge than shallow cold-water species for obvious reasons, but since deepwater coral biology is in its infancy, there are lots of interesting questions that should be addressed. Most of the deepwater corals studied so far have quite a high fecundity, so reproductive output is not being compromised by their environmental conditions. These corals rely on planktonic food alone for their nutrition, and skeletal deposition occurs without the benefit of light enhanced calcification. Energy resources and allocation into growth and reproduction are important aspects of the ecology of the coral and warrant further investigation.

7.4.3 Reproduction in deep-water corals

Most of the very basic research on reproductive ecology has not been done on most temperate or deepwater species. Deepwater corals invariably live in high-flow low-temperature environments, which suggests that most deepwater corals are probably slow growing with low recruitment rates, and equally slow recovery potential. The *Oculina* habitat is a model for the slow to non-existent recovery of an impacted reef. Given that most deepwater framework building corals are under direct threat from destructive fishing practices, this is an area that needs to be addressed urgently. Unfortunately research on most deepwater bioherms is still at the stage of mapping and species description.

7.5 Closing thoughts

Morphological and genetic evidence has confirmed, within reasonable doubt, that both the deep and shallow *Oculina* populations are *Oculina varicosa*. There are striking differences between the morphologies of individual colonies and of their role in the structure of their respective habitats. There are also distinct biological and ecological similarities between the populations. Assuming that one of the populations arose from the other, do the deep reefs represent the true species niche, with the

shallow colonies merely products of vagrant larvae from the deep populations? Alternatively, have shallow water populations migrated seaward to avoid competition with photosynthetic phyla on the shallow ledges? The adults and larvae can both tolerate a range of environmental conditions, so opportunistic exploitation of different habitats is quite possible.

One hypotheses is that the deep reefs were originally shallow nearshore habitat which was "drowned' with rising sea level, and adapted to the changing environmental conditions. During the numerous glacial advances and retreats of the Pleistocene, the climatic changes had a great impact on the coastal and marine species of the Southeast. During glacial episodes, sea levels were much lower and the shelf edge of modern day was the ancient Florida coastline. The shelf edge *Oculina* reefs were apparently derived from ancient pleistocene shoreline (Koenig et al. 2000). The shallow reefs could have been established from larvae carried north from the Caribbean and as the sea level rose during the glacial retreat, the corals adapted to deeper and deeper waters. Meanwhile, the ledges of the shelf became colonised by vicariant larvae as the shoreline moved progressively west with rising sea level.

In this scenario, the deep reefs were originally formed under much more shallow conditions, with a completely different hydrodynamic regime. The physiology and behaviour of the deep and shallow larvae are very similar, but the hydrodynamic regimes that they enter are very different. There may be undiscovered larval behaviours or patterns of shelf-edge flow that retain the deep-water larvae within the *Oculina* Banks. Alternatively, if larvae swim upwards (as they do in the laboratory) and enter the Florida Current, they will probably be lost from the habitat. Large *Oculina* colonies have been observed on relatively recent shipwrecks in deep water, which lends some hope for the re-colonization of the damaged habitats. However observations of artificial substrata show almost no settlement and given the unpredictable nature of the shelf hydrodynamics, re-colonisation rates may be extremely slow.

If left alone for a few thousand years the banks will probably recover, such is the resilience of nature. Unfortunately illegal trawling continues in the reserve to satisfy human taste for delicacies such as rock shrimp and scallops. The only intact reef tracts are at the southern end of the reserve and it they would be destroyed rapidly if trawlers ventured that far. Despite their protected status, the outlook is not good for the fragile *Oculina* Banks.

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Appendix I Details of solutions used for high salt DNA extraction.

1m Tris HCl
30.27 g Tris base
200 ml distilled water
11.5 ml conc HCl

0.5M EDTA
46.43 g EDTA
200 ml distilled water

20% SDS
50 g lauryl sulphate
200 ml distilled water
Conc HCl to adjust to Ph 7.2

TNE buffer
2.5 ml 1M Tris HCl (pH 8)
5 ml 0.5M EDTA (pH 8)
50 ml 0.5M NaCl
192.5 ml distilled water

6M NaCl
87.5g NaCl
250 ml distilled water

0.5M NaCl
7.29 g NaCl
250 ml distilled water

Proteinase K
200 mg proteinase K
10 ml PCR water

Extraction buffer
600 µl TNE
24 µl 20% SDS
5 µl proteinase K