The Reproductive Ecology of Deep-Water Scleractinian Corals

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ABSTRACT
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by Rhian G. Waller

Deep water corals and coral reefs have gained considerable attention recently, both in the public and scientific domain. These corals inhabit depths from relatively shallow at a few tens of metres, to over 6000m, and most have cosmopolitan distributions. These corals can form complex frameworks that attract a variety of invertebrate and vertebrate fauna. Many commercially important species have been observed using these reefs for protection, procreation and feeding, and so have been the target of recent demersal trawling.

This thesis considers the reproduction of eleven species of deep-water scleractinian from the NE Atlantic and Antarctica. Shallow-water reef and solitary coral reproduction has been extensively reported, but basic ecological information on deep-water species is lacking. The gametogenesis and reproductive biology of these eleven species was explored by dissection, histological techniques, and scanning electron microscopy.

Reproductive data obtained indicate that, in common with shallow water scleractinia, there is no strict pattern to their reproductive habit and a variety of modes were observed. The species examined in this study ranged from hermaphroditic species that spawn gametes (*Caryophyllia ambrosia, C. seguenzae, C. cornuformis*); gonochoric species that spawn gametes seasonally (*Lophelia pertusa, Madrepora oculata, Flabellum angulare*); gonochoric species that spawn quasi-continuously (*Fungiacyathus marenzelleri, Flabellum alabastrum*) and brooding species (*Flabellum thouarsii, F. curvatum, F. impensum*). Oocyte size appears to increase as depth increases, and fecundity reduces with depth. The population dynamics of *C. seguenzae* was also examined. There appears to be a large juvenile component to this species population, with stability shown through the three years examined.

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For whatever we lose, like you or me
It's always ourselves we find in the sea

E.E. Cummings (1894-1962)

Maggie and Milly and Molly and May

This thesis is dedicated to my little Squirt, who always loved what I did.
You knew I could do it even when I didn't. I wish you were here to see it.
I miss you so much

Sophie Megan Waller

July 2nd 1986 – August 2nd 2003

Chapter One

General Introduction

1.1 Deep-Sea Biology

The inaccessibility of the deep-sea has long kept it out of reach of scientists, and even now we are still merely scratching the surface of what lies beyond our continental shelves. Both the physical and financial cost of deep-sea exploration left it mainly unexplored until the late 1800s. The apparent stability and predictability of deeper waters, as well as the intense pressure, lack of light and cold temperatures, led scientists 150 years ago to propose that the deep-sea was both a featureless barren and unable to sustain life. The most prominent statement was from Edward Forbes in the 1840's. During dredging operations in the Aegean he observed that life decreased in numbers and size below 130 fathoms (238m) and so proposed the Forbes Azoic theory (Menzies et al., 1973; Mills, 1983). With this theorised lack of life, deeper-waters were largely left untouched by science.

The first major exploration cruises of the deeper areas of the oceans were undertaken by Charles Wyville Thompson on board British naval vessels, the first cruise being the *HMS Lightning* in 1868 and then the *HMS Porcupine* in 1869-1870. Both of these centred on the northeast Atlantic. Their successful dredging brought back clay-mud containing hundreds of invertebrates, as well as many larger skeletal remains, enough to declare the deep-sea was not lifeless, as first thought (Mills, 1983).

The *HMS Challenger* was the first naval ship used to do a worldwide study of the deep ocean. She sailed in late 1872 led by Wyville Thompson and returned to Britain in 1876, with organisms collected from 5700m depth. This proved conclusively there was life in the deep-sea (Mills, 1983). Many research cruises sailed after the *Challenger* expeditions, the French *Talisman*, the Danish *Ingolf*, and the American *Albatross* are a few of the more famous. But the deepest parts of the oceans were only sampled for the first time on the Danish *Galathea* worldwide expedition, 1950-1952. Organisms were obtained from the Philippines Trench at 10,190m showing that the even the deepest parts of the worlds oceans contained some form of life (Mills, 1983; Gage & Tyler, 1991).

Soon after many assumptions were made on the ecology of these deep-sea animals collected. The same assumptions that led to the deep-sea being classed as lifeless, in

turn led to various assumptions on the deep-sea organisms ecology. The apparent uniqueness of environmental variables at these depths (temperature, pressure and lack of light) was thought to cause species specific adaptations not found in their shallow-water equivalents. Their physiology, reproduction and metabolism were all postulated to be significantly different from anything observed before (Somero et al., 1983).

With continued research and a new wave of interest in the deep-sea, many of these assumptions were, and still are, being disproved. Common thoughts on the general nature of the deep-sea have also changed with the discovery of seasonal processes at depth, such as variations in current speeds, benthic storms (Dickson et al., 1986) and the seasonal deposition of phytodetritus (Deuser et al., 1981). The confirmed discovery of hydrothermal vents by the Alvin submersible at the Galápagos Ridge in 1977 made sweeping changes in how scientists regarded energy availability to deep-sea organisms. Here was a completely different environment from the barren sedimented bottom observed before, and these small areas have a high biomass associated with them. Since the discovery of these 'oasis' in deep-waters, more similar fragmented environments have been observed including black and white smokers, cold seeps, and, more recently, cold water coral reefs.

Remotely operated vehicles (ROVs), submersibles, benthic cameras, *in situ* measuring devices and lander technologies have all aided our understanding of the deep environment (Rowe & Sibuet, 1983). The quality and resolution of deep-water data has dramatically increased in the last 30-40 years. Improved equipment for quantitative sampling, such as box cores and multi-corers, have shown that species diversity is not as low as first thought, and in many areas is actually high (Hessler & Sanders, 1967). However, obtaining adequate samples, even with today's technology, is not simple and is often not financially possible. Time-series data on many areas is still unavailable and so a true representation of the ecology of many deep-sea organisms, and even their true diversity, is not yet known. Almost every research cruise today still comes back with new species, whether these be recognised as such at the time. Overall the deep-sea can now be regarded as globally homogeneous, but with local small scale variability, quite different from original hypotheses.

1.2 Reproduction in the Deep-Sea

Early observations of reproduction in most phyla were made as brief references in taxonomic works. The constant environment that was thought to be the deep-sea led many scientists to propose theories on reproduction and development. Yet the widescale exploration that was undertaken in the 19th and early 20th centuries were not suited to studying individual species on a seasonal basis. Even at a time when the reproductive mode and seasonality of many shallow-water invertebrates was well documented, the little data available on deep-sea species led to wide generalisations and predictions. Only recent advancements in technology have made it possible to collect more seasonal data than previously, and to begin hypothesis testing, rather than fortuitous sampling.

Two theories of deep-water reproduction put forward were Orton's (1920) and Thorson's (1950). Orton predicted that, because of the constant environment, there would be no seasonality of reproduction. Without the cues that shallow-water species need to initiate reproduction or spawning, these species would reproduce throughout the year. Thorson predicted that deep dwelling invertebrates would have low fecundities and produce brooded young. This would be because the energy needed to produce many pelagic larvae would not be available in the 'barren' deeper waters, and individuals would put more energy into releasing a fully-formed juvenile, so as to increase infant survivorship.

However, more data are now available that show both these hypotheses to be false. Perhaps the biggest discovery in the ecology of deep-water invertebrates, is the realisation of seasonal processes at depth. Studies in the NE Atlantic have shown that seasonal blooms of surface primary production sink rapidly to the deep-sea floor (Billett et al., 1983; Lampitt, 1985; Thiel, et al. 1989) and can have an effect on the reproductive biology of benthic invertebrates (Tyler et al., 1982a, 1988, 1992; Eckelbarger & Watling, 1995). The seasonal flux of energy-rich phytodetritus to the seafloor can be enough to either initiate gametogenic processes, or even cue spawning of gametes, so settling juveniles have a food source (Eckelbarger & Watling, 1995).

Thorson's rule was widely accepted for many years, though more recent reviews have suggested it be 'laid to rest' (Pearse, 1994). Developments in larval biology and ecology have shown there to be three major forms of larval development in marine invertebrates, pelagic planktotrophic, in which the larvae feed in the plankton; pelagic lecithotrophic, in which food stores sustain the larvae until settlement, and brooding (Thorson, 1946, 1950; Jablonski & Lutz, 1983). Many deep-water invertebrates with large oocyte diameters have been predicted to produce lecithotrophic larvae rather than brood (Tyler et al., 1982b; Tyler & Gage, 1984; Gage & Tyler, 1991). Planktotrophic larvae have also been found to exist at depth (Gage & Tyler, 1981; Tyler et al., 1982b; Tyler & Gage, 1984), something thought impossible by Thorson. The extreme distances involved in traversing to shallow-waters from great depth was thought to be an impossibly wasteful reproductive strategy, though there were planktotrophic theories around in Thorsons time (review in Young, 1994).

The theories of reproduction have changed, from non-seasonal invertebrates with direct development, to seasonality and pelagic development, and more diversification than first thought. Lecithotrophy appears to be predominant (Marshall, 1979; Pearse, 1994), with both seasonality and continuality of reproduction being found (Marshall, 1979). As availability of samples is improving, research directions are changing to explain why these spatial variations might occur, local adaptations and long time series studies.

1.3 The Order Anthozoa

The phylum Cnidaria comprises of hydroids, jellyfish, sea fans, sea pens, anemones and corals, all sharing a common tissue plan, though having variety in body morphology. There are ~9,000 species within this phylum and contain benthic, pelagic, intertidal and deep-sea organisms. This brief review centres on the Scleractinia within the Hexacorallia. There are two major morphologies of Scleractinia, the solitary polyp and the framework creating reef-building.

1.3.1 Classification Table of Hexacorallia

With specific reference to species used within this study (Zibrowius, 1980; Fautin, 2003)

Subclass Hexacorallia
- Order Ceriantharia
- Order Madreporaria
  - Suborder Scleractinia
    - Family Pocilloporidae
      - Genus *Madracis*
    - Family Fungiida
      - Genus *Fungiacyathus*
        - ***Fungiacyathus marenzelleri*** *(NE At)*
      - Genus *Poritidae*
      - Genus *Siderastreidae*
    - Family Faviidae
      - Sub-family Monastrea
      - Genus Trochosmiliidae
      - Genus Mussidae
    - Family Rhizangiidae
      - Genus *Astrangia*
    - Family Oculinidae
      - Genus *Oculina*
      - Genus *Madrepora*
        - ***Madrepora oculata*** *(NE At)*
    - Family Anthemiphylliidae
    - Family Caryophyliidae
      - Sub-family Caryophylliinae
        - Genus *Caryophyllia*
          - ***Caryophyllia ambrosia*** *(NE At)*
          - ***Caryophyllia cornuformis*** *(NE At)*
          - ***Caryophyllia sequenza*** *(NE At)*
        - Genus *Dasmosmilia*
        - Genus *Coenocyathus*
        - Genus *Concentrotheca*
        - Genus *Ceratotrocus*
        - Genus *Trochocyathus*
        - Genus *Tethocyathus*

Genus *Deltocyathus*
Genus *Paracyathus*
Genus *Polycyathus*
Genus *Stephanocyathus*
Genus *Vaughanella*
Genus *Aulocyathus*
Sub-family Turbinoliinae
Genus *Sphenotrocus*
Genus *Peponocyathus*
Sub-family Desmophyllinae
Genus *Desmophyllum*
Genus *Thalamophyllia*
Genus *Hoplangia*
Genus *Lophelia*
Lophelia pertusa *(NE At)*
Sub-family Parasmiliinae
Genus *Coenosmilia*
Genus *Pourtalosmilia*
Genus *Phyllangia)*
Genus *Asterosmilia*
Genus *Solenosmilia*
Family Flabelliidae
Genus *Flabellum*
Flabellum alabastrum *(NE At)*
Flabellum angulare *(NE At)*
Flabellum impensum *(Antarctic)*
Flabellum thouarsaii *(Antarctic)*
Flabellum curvatum *(Antarctic)*
Genus *Monomyces*
Genus *Javania*
Genus *Placotrochides*
Suborder Astrocoeniidae
Family Acroporidae
(Family Agariciidae)
Suborder Meandrinidae
Suborder Thamnasteriidae
Suborder Corallimorpharia

1.3.2. Scleractinian Evolution

Corals have been extant for millions of years and have undergone many changes in morphology. Tabulate and Rugose calcite corals arose in the late Ordovician (490 mya) to form reefs until the end of the Permian (251 mya), when a large extinction event occurred, eliminating most reef-building species (Hallock, 1997) (Fig. 1.1). Modern day, aragonite, scleractinians evolved after this event (~14 million years after - Hallock, 1997; Stanley & Fautin, 2001) from soft bodied anemone-like invertebrates around 230 mya (Oliver, 1980; Veron, 1995). The precise relationships and evolutionary patterns between the Hexacorallians is difficult to determine, as the non-skeletalised forms have left no fossil record.

Older theories of evolution suggested present-day scleractinians evolved from the Rugose corals (review in Veron, 1995; Stanley & Fautin, 2001). However during the 14 million year gap in the Triassic, between the Rugosa and Scleractinia, the global carbonate deposition was suppressed, making it unlikely for any calcite, or aragonite, species to exist (Stanley, 1988). The Rugosa also vary significantly in morphology from Scleractinians, having serial rather than cyclical septa (Oliver, 1980; Veron, 1995), making them unlikely ancestors to the Scleractinia.

Only solitary corals evolved initially (Stanley, 1988; Veron, 1995). Sixty seven genera were widely spread in the late Triassic, this being the highest diversity of scleractinians ever found, though none of these were able to form reefs (Veron, 1995). Rudists (bivalves) were the main reef-builders at this time. These bivalves were probably zooanthellate and formed less cemented reefs with a higher sediment fraction than corals, looking more like present day inshore fringing reefs (Scott, 1988). They contained just 30-60% aragonite in their shells, possibly an advantage over totally aragonite species (Kaufman & Johnson, 1988; Veron, 1995). Corals co-existed throughout this time with Rudists, but were found in different habitats, at greater depths (Veron, 1995).

Another extinction event occurred at the end of the Cretaceous period, an event best known for the extinction of the dinosaurs. Many carbonate-producing animals died out, with only eighteen of the sixty-seven genera of Scleractinia surviving (Veron, 1995). It is thought that a number of species of scleractinians had retreated to deeper

waters and so managed to survive this extinction event (Kaufman & Johnson, 1988; Hallock, 1997). Aragonite-producing corals 230 mya were more predominant than calcite producing animals. After the Cretaceous extinction event, however, the ocean chemistry again changed and so aragonite producing invertebrates did not regain in significant numbers until around 40mya. This time coincided with the recent glaciation (thermohaline circulation, accumulation of seas ice around Antarctica, decreasing abyssal temperatures) and the extinction of Rudists (Veron, 1995).

Molecular evidence suggests that the Scleractinian skeleton may have evolved up to four times (Romano & Cairns, 2000), and so may have originated from several ancestors, rather than a single one. Most genetic data support a single ancestor, but most of these data are also incompatible with the current morphological classification of the Scleractinia (Veron, 1995; Veron et al., 1996). Indeed, Daly et al. (2003) question the taxonomy of the Hexacorallia as a whole. The increasing use of molecular techniques as taxonomic tools is vital to understanding species relationships, but it is also essential to consider morphological taxonomy.

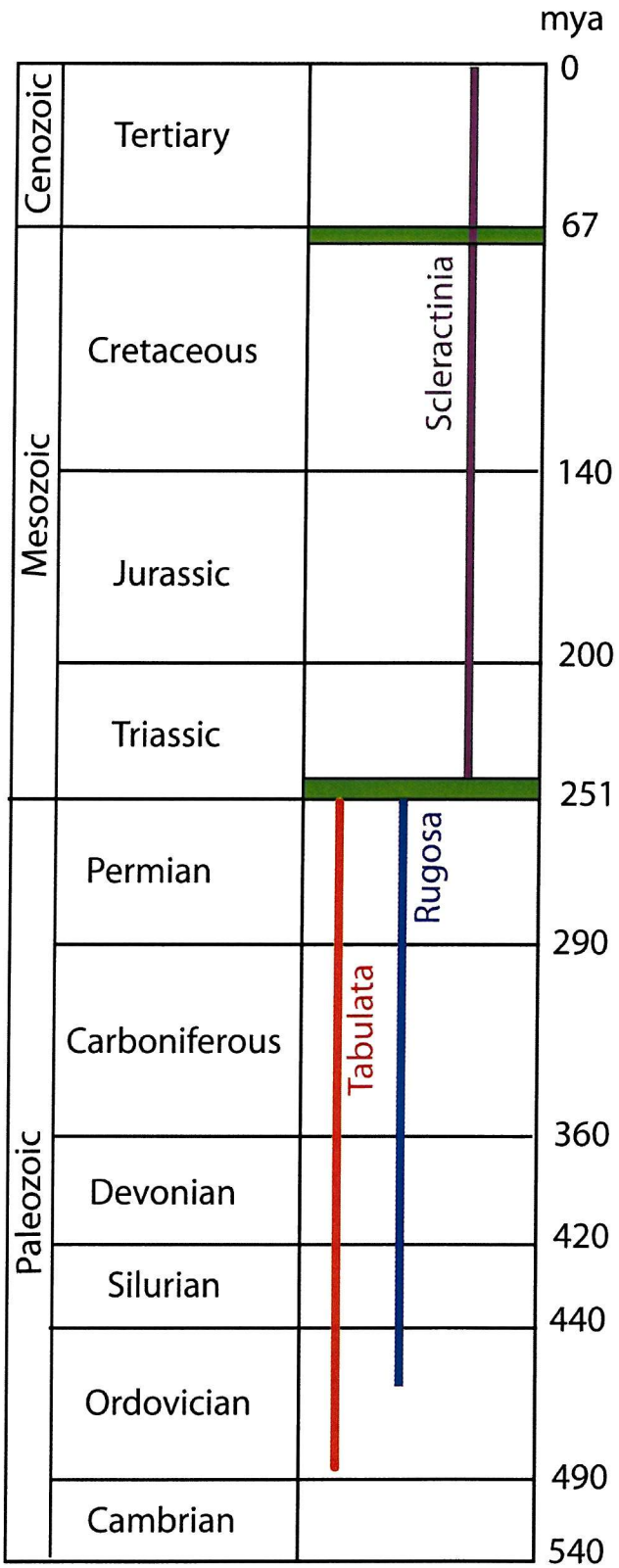


Fig. 1.1 - Geological intervals used in text. Showing the extent of the Tabulate, Rugosa and Scleractinian corals, and major extinction events
Green bar – Extinction events

1.3.3. Scleractinian Reproduction

Brief statements of the reproductive mode of many cnidarians can be found in taxonomic texts (Doumenc, 1975; Fautin, 1983; Tur, 1993). Once thought to be useful characters for taxonomy and evolution, the sheer number of patterns now known, and their environmental plasticity, makes such information of limited use (Fautin, 2002). The biological simplicity of cnidarians is likely to be the reason behind their evolutionary plasticity (Fautin, 1991; Shick, 1991; Veron, 1995). Fadlallah (1983) and Shlesinger et al. (1998) observed that, in corals, there appeared to be little pattern in the relationships between polyp size, oocyte size or mode of reproduction, and to describe a pattern, would have to take into account the many variables found in the Scleractinia.

The importance of asexual versus sexual reproduction is unknown, and a few authors state the case that there is little distinction between the two in the Cnidaria (Budd, 1990; Fautin 2002). Cnidarians appear to be able to adjust their rates of sexual versus asexual and even mode of gametogenesis, dependent on environment (Stimson, 1978; Fadlallah, 1983; Shick, 1991; Fautin, 2002), though how exactly this is done is unknown. I will now discuss the asexual and sexual reproductive biology of the Scleractinia, with reference to information on deep-water species where available.

Asexual Reproduction

Most groups within the Cnidaria are able to undergo some form of asexual reproduction (the notable exception being the Ceriantharia, though this is likely to be due to a lack of studies rather than a true representation – Fautin, 2002). This form of reproduction produces a genetic clone, and so decreases chances of local extinctions in the species by producing large numbers of, locally, well adapted individuals. However it does not increase the gene pool, and so in the long term will not aid evolution or produce clones that are able to withstand changing environmental conditions. Asexual reproduction in colonial scleractinians increases colony size (Szmant-Froelich et al., 1980; Fadlallah, 1983; Hall & Hughes, 1996), whereas in solitary scleractinians asexual reproduction will increase population numbers (Krupp, 1983). Chia (1976) suggested that the Cnidaria can select for asexual reproduction over sexual means in stressful situations, ie. where the energy available is less than that required for sexual reproduction.

Asexual reproduction in scleractinians can occur by polyp division, fragmentation, polyp expulsion and parthenogenesis. Polyp division can either be intratentacular, when one polyp divides into two, or extratentacular, when a new polyp buds from between two polyps. Solitary scleractinians use asexual reproduction to produce clones (Krupp, 1983), once the juvenile reaches a certain size it breaks off from the parent to form a single living unit. The bathyal-abyssal solitary scleractinian *Fungiacyathus marenzelleri* reproduces asexually by intratentacular budding (Waller et al., 2002, this Thesis). Fragmentation in reef-builders can occur when a large piece, containing several polyps, breaks from the parent colony to form a new colony. Many corals may be morphologically adapted to allow this to happen (Bothwell, 1982; Highsmith, 1982), but this has been challenged (Smith & Hughes, 1999). Storms, wave action, fish predation, and even scuba divers can cause pieces of the more fragile plate and branching corals to break. If these fragments land on a solid surface and attach, they can then start a new colony by polyp division. This method is thought to be important for rapid colonisation and reef extension (Bothwell, 1981; Highsmith, 1982; Harrison & Wallace, 1990), but has a high mortality rate, with many fragments not landing on suitable substrata (Knowlton et al., 1981). 'Polyp bailout' has been reported in several species of Pocilloporid in relation to environmental stress (Rinkevich & Loya, 1979; Sammarco, 1982; Richmond, 1997). Polyps may eject from their skeleton and swim, using cillia, until coming into contact with a suitable substrata for settlement. Kramarsky-Winter et al. (1997) also observed polyp expulsion in *Favia favus* and *Oculina patagonica* in response to stress. Similarly, several authors have noted pieces of coral tissue remaining on dead coral skeletons, or oozing out of their calices, and producing new colonies (Rosen & Taylor, 1969; Highsmith, 1982; Krupp, 1983).

Parthenogenesis is the production of asexually derived planulae, and is not common in the Cnidaria (Shick, 1990; Fautin, 2002). Stoddart (1983) found asexually produced planulae in the Scleractinian *Pocillopora damicornis*. The azooxanthellate *Tubastraea diaphne* and *T. coccinea* also produce genetically identical brooded planulae (Ayre & Resing, 1986). There are few studies examining the paternity of Scleractinian planulae, so this pattern may be more widespread than currently thought.

The large number of asexual reproduction strategies in the Scleractinia suggests they are important life history factors. The rates of sexual versus asexual within a single species is unknown.

Sexual Reproduction

Scleractinians do not have 'gonads'. Sperm occurs in cysts held together by a mesogleal envelope, hence spermatocysts. Oocytes develop singularly, but are often found in groups or 'pockets', though there is no direct connection between them. In shallow-water species, several authors have noted that germ cells are most likely to originate from interstitial cells near the septal filaments (lamellae) (Rinkevich & Loya, 1979; Szmant-Froelich et al., 1980; Delvoye, 1982; Fadlallah, 1983).

Hermaphroditism is found more commonly amongst the shallow-water Scleractinia than gonochorism (Fadlallah, 1983; Richmond & Hunter, 1990). Although gonochorism has been suggested as a more primitive strategy than hermaphroditism (Goffredo et al., 2000) it has been shown to be important for genetic diversity of populations (Szmant, 1986). The high numbers of hermaphroditic corals present in shallow waters makes it seem likely that self-fertilisation may be an important factor. Several studies have shown that several Caribbean scleractinian broadcast spawning species are self-incompatible (Knowlton et al., 1997; Szmant et al., 1997; Willis et al., 1997), whereas two species of brooding corals from the same area have been shown to self up to 49% (Brazeau et al., 1998). Stoddart et al. (1988) also observed around 50% of planulae to be self-fertilised in *Goniastrea favulus*. Willis et al. (1997) also observed self fertilisation in three broadcast spawning species from the Great Barrier Reef. Selfing may be an important adaptation to increase fertilisation success in sparsely distributed species (Kojis & Quinn, 1981, 1982; Heyward & Babcock, 1986).

Most shallow-water scleractinians reproduce via a seasonal cue, usually controlled by temperature, tidal or lunar periodicity (Stimpson, 1978; Fadlallah, 1983; Harrison & Wallace, 1990; Richmond & Hunter, 1990). Inter-species synchrony may also occur, forming mass spawning events (Babcock et al., 1986, 1994). A number of species are thought to have chemical attractants that cause gamete release in opposing sexes (Szmant-Froelich et al., 1980; Tranter et al., 1982), but whether this is interspecific,

and therefore the cue to mass spawning, is unknown. Mass spawning has also recently been proposed as a strategy to promote hybridisation of some taxa (Willis et al., 1997; Hatta et al., 1997). Reproductive seasonality has also been observed in deeper waters. Brooke & Young (2003) found *Oculina varicosa* to reproduce seasonally. *Solenosmilia variabilis* and *Goniocorella dumosa* from the Chatham Rise, New Zealand (Burgess & Babcock, in press) and *Lophelia pertusa* and *Madrepora oculata* in the NE Atlantic (Waller & Tyler, in press, this Thesis), also show seasonality.

Brooding was originally thought to be the main mode of reproduction in Scleractinians, but it is now known that fewer species brood planulae than spawn gametes (Fadlallah, 1983; Harrison & Wallace, 1990; Richmond & Hunter, 1990). The ratio of brooding to spawning can, however, change dependent on location, suggesting an environmental adaptation. Richmond and Hunter (1990) found there to be more species that brooded in the Caribbean than elsewhere. Larvae of brooded individuals are thought to be usually sexually produced, even in hermaphroditic species (Ward, 1992; Willis et al., 1997).

Planulae of anthozoans are generally cylindrical in shape and evenly ciliated (Martin & Koss, 2002). In brooding species, planulae are ejected and settle in one or two days (Rinkevich & Loya, 1979; Kojis & Quinn, 1982; Tranter et al., 1982; Shlesinger & Loya, 1985; Szmant-Froelich et al., 1985), a few hours (Goreau et al., 1981) or even immediately (Fadlallah & Pearse, 1982a). Short settlement times may have a selective advantage by retaining propagules and colonising an area adjacent to the parent (Kojis & Quinn, 1982; Szmant, 1986; Harriott, 1992). Some broadcasting species planulae spend around 4 to 6 days in the plankton prior to settlement (Shlesinger & Loya, 1985; Babcock & Heyward, 1986; Sakai, 1997). Planulae produced can either be planktonic, or benthic crawlers. Although there is only a single confirmed case of benthic crawling (Gerodette, 1981), many more species are suspected of using this method (Fadlallah & Pearse, 1982a). The planulae of some species' may remain competent in the plankton for much longer periods of time, eg. *Pocillopora damicornis* planulae can remain competent for 100 days (Richmond, 1988). This may provide an advantage for long distance colonisation of new areas. *Oculina varicosa*, a deep-water species from 500m, under experimental conditions, began probing the

bottom after 10-14 days, suggesting competency (Brooke, 2002). There are no other deep-water larval studies published to date.

There have been reports that depth may affect reproduction in several species of shallow-water anthozoans. Kojis and Quinn (1983) found deeper populations of the Scleractinian *Acropora palifera* to produce half the number of oocytes than shallower populations. A similar situation was also found in *Stylophora pistillata* (Rinkevich & Loya, 1987) and in two *Zoanthus* spp (Karlson, 1981). This trend was also observed in the deep-water coral *Fungiacyathus marenzelleri*. Populations from 2000m (Waller et al., 2002) had a fecundity almost twice as great as those from 4000m (Flint, 2003). These cases are possibly a result of the decreasing food supply found at depth, and so there is less energy available to produce large numbers of gametes (Eckelbarger & Watling, 1995).

This thesis examines the reproductive biology of deep-water scleractinian corals from the NE Atlantic and Antarctica. The only other reproductive studies published to date on deep-sea corals are that on *Oculina varicosa*, off Florida (Brooke & Young, 2003) and that on *Madrepora oculata*, *Enallopsammia rostrata*, *Solenosmilia variabilis* and *Gonicorella dumosa* from the Chatham Rise, New Zealand (Burgess & Babcock, in press).

1.4 Deep-sea Anthozoans

In contrast to shallow-water, there is a considerable gap in our knowledge on all aspects of deep-water Cnidarian ecology. In shallow-water they are near perfect subjects for study, being of simple design, relatively sessile, being present in high numbers and having a cosmopolitan distribution. However the deep-sea poses many problems for the collection of benthic organisms, especially non-colonial species. High spatial variability between individuals can cause sample bias, as can the types of gear used (eg. larger net sizes will only collect the larger individuals of a population, whereas a box core will collect few individuals).

The Anthozoa make up a large proportion of the sessile megafauna (Lampitt et al., 1986) and are found at both bathyal and abyssal depths, yet they are one of the least studied groups to date (Gage & Tyler, 1991). Until recently, taxonomy and distribution were the extent of data available, with some texts commenting on basic ecology (Riemann-Zurneck, 1973, 1978, 1979, 1986, 1987, 1991; Doumenc, 1975).

Ecological studies are still limited. Aldred et al. (1979) observed high densities of the flytrap anemone *Actinoscyphia aurelia* from the highly productive waters off the NW African coast. Van-Praet (1983) undertook one of the first ecology centred studies. He examined feeding in the two NE Atlantic deep-water actiniarians *Paracalliactis stephonsoni* and *Phelliactis robusta*, and observed that phytoplankton food fall may be an important component of their diet. Lampitt & Paterson (1987) observed feeding behaviour in the abyssal anemone *Sicyonis tuberculata* using a time lapse camera. Herring (1991) observed bioluminescence from four species of deep-water anthozoan.

Information on reproduction in deep-sea anthozoans is also limited. For actinarians, Van-Praet et al. (1990) and Van-Praet (1990) have described the gametogenic biology of three species of *Phelliactis*, Bronsdon et al. (1997) described the gametogenic periodicity of the bathyal epizoite *Amphianthus inornata* and the abyssal epizoite hermaphrodite *Kadosactis commensalis*. Muirhead et al. (1986) observed gametogenesis in two zooanthid species, the bathyal *Epizoanthus paguriphilus* and the abyssal *E. abyssorum*. In the Alcyonaria, the gametogenic pattern has been described for a variety of species of pennatulid from the NE Atlantic (Rice et al.,

1992; Tyler et al., 1995) and the Gulf of Maine (Eckelbarger et al., 1998). Cordes et al (2001) described brooding in the deep-sea alcyonacean *Anthomastus ritteri* from the Pacific.

With the recent increase in interest of deep-water corals, there has been a large influx of material on their distribution and the destructive processes occurring in these areas, yet papers on their basic ecology are still lacking.

1.5 Distribution of Deep-Water Scleractinians

There are more species of solitary deep-water Scleractinians than reef-builders (Cairns, 1999), yet knowledge for the distribution of reef-builders far outweighs that of solitary polyps. Solitary species are found on both hard and sedimented substrata, whereas the reef-building species are more limited in their ability to colonise.

Hard substrata are necessary for shallow water reef formation, highly sedimented areas are not favoured because of the risk of polyp suffocation (Dodge & Vaisnys, 1977; Rogers, 1990). Hard substrata also appears necessary in the deep-sea, and may take a variety of forms, from rocky outcrops, worm tubes, mollusc shells (Wilson, 1979, 1997), fossil scleractinian and gorgonian skeletons (Atkins & Schierer, 2003) and even man made structures such as undersea cables (Wilson, 1979) and oil rigs (Bell & Smith, 2000). Deep-water reefs, mounds and banks are commonly found on continental margins and shelf breaks, as well as on seamounts and ridges, where high water flow is present. These flows are thought to be necessary for the delivery of food and larvae to the area, as well as removing waste and excess sediment (Grigg, 1974, 1984).

There are many species of deep-water reef-building scleractinians around the globe. *Goniocorella dumosa* occurs on the Campbell Plateau, New Zealand (Squires, 1965), *Oculina varicosa* on the Florida continental shelf (Reed, 1980; Brooke, 2002), *Solenosmilia variabilis* from the South Pacific, SE Atlantic and Cook Islands (Cairns, 1982; Keller, 1993; Cairns, 1995), *Dendrophyllia cornigera* in the NE Atlantic (Zibrowius, 1980), *Desmophyllum dianthus* off Chile (Cairns, 1982) and *Enallopsammia rostrata* in New Zealand, *E. profunda* from the Western Atlantic and *E. marenzelleri* from the NE Atlantic, New Zealand and Indonesia (Cairns, 1995). The most cosmopolitan deep-water scleractinian species, however, are *Lophelia pertusa* and *Madrepora oculata* (Rogers, 1999).

Many sources of deep-water scleractinian distribution to date may be unreliable. Often taxonomic reports will refer to dead skeletal remains, and so the true distribution may be overestimated (Rogers, 1999). However, the nature of the substrata of choice for scleractinians may also lead to their distribution being highly

underestimated. Rocky outcrops, steep continental shelves and seamounts are all difficult sampling areas for traditional methods, such as trawl nets and sleds. The spatial variability of deep-water corals may also cause sampling bias (Wilson, 1997). Thus, present distributional knowledge is probably a gross underestimation.

In recent years the true significance of deep-water Scleractinians has become more apparent. Deep-water reef-building species, as in shallow-water, form important habitats, creating a framework for species that contribute to the high diversity and biomass associated with these reefs. Koslow & Gowlett-Jones (1998) observed 242 species of invertebrate and 37 species of fish around *Solenosmilia variabilis* reefs on the Tasmanian Seamounts, *Lophelia pertusa* reefs in the NE Atlantic have been shown to support over 1300sp (ACES, 2003), and *Oculina varicosa* bioherms off the Florida continental shelf have been observed with over 350sp of invertebrate (Reed, 1992; Koenig et al., 2000). *Goniocorella dumosa* alao provides habitat for a diverse invertebrate fauna, including solitary scleractinians, sponges, molluscs and alcyonaceans (Cairns, 1995).

With an increased interest in these deep-water scleractinian species, a more accurate picture of global distributions will become apparent. Many areas currently viewed as coral 'hotspots' may in fact only be the precursor to many more in as yet unexplored areas. Many new databases of distributions are being compiled (Fautin, 2003; Morgan & Etnoyer, 2003; Scanlon & Ackermann, 2003) and will become invaluable tools in the future for predicting new coral areas.

1.6 Anthropogenic Impacts in the Deep-Sea

During the past 50 years, man's interest in deep-sea exploitation has increased dramatically (Thiel, 2001, 2003). With oil, mineral and food resources struggling to cope with an exponentially growing human population, there is an increased need to find these resources from another source. The deep-sea has, up until recently, been viewed as an open area, where the dumping of waste and exploitation that is outlawed in shallow waters, occurred on a regular basis.

Concern has been recently raised over anthropogenic impacts occurring in the deep-sea. Activities that may be causing environmental impacts, include mining (Thiel et al., 1992, 2001), waste disposal (Pearse et al., 1979; Kullenberg, 1986), deep-water fisheries (Koslow et al., 2000), hydrocarbon exploration (Creasey & Rogers, 1999) and, in a few minor cases, even science (Tunnicliffe et al., 1990; Herring et al., 1995).

Natural processes within the deep-sea also cause disturbance. Sediment shift is constantly caused by benthic animals burrowing, and feeding, yet cause little overall disturbance (Grassle, 1989). Benthic storms and recurrent strong currents have the ability to cause major impact, yet the adverse affects have been shown to decrease with time and are not very large (Thistle, 1991; Borowski, 2001). A number of large scale sediment re-suspension experiments simulating the effects of benthic disturbance by trawling, on megafauna, have shown the effects to be highly detrimental (Borowski, 2001; Rodrigues et al., 2001).

Scleractinians have recently been at the heart of environmental issues concerning man's use of the deep-sea. In the shallow-water, coral reefs have been destroyed in many areas of the world (Brown, 1997). Pollution, coastal development, detrimental fishing practices and climate change have all had their effect on the shallow reef ecosystem. Only recently have studies targeted the potential impacts of man's various activities on deep-water fauna (Olsgard & Gray, 1995; Probert et al., 1997; Jennings & Kaiser, 1998; Rogers, 1999; Hall-Spencer, 2003; Thiel, 2003). The remainder of this section will be concerned with anthropogenic affects directly of threat to deep-water corals.

1.6.1 Fishing Pressures

Commercial fishing is one of the most important human impacts on the benthic environment. Though bottom fishing was originally sustainable in shallow-water, the reduction in pelagic fisheries around many continents has caused fishermen to move to deeper waters to maintain catches (Koslow, 1997; Creasey & Rogers, 1999). Many life-history characteristics of deep-water fish make them unsuitable for exploitation and particularly vulnerable to over fishing (Koslow, 1997; Creasey & Rogers, 1999; Clark, 2001). Yet today fisheries below 600m form an important component of worldwide commercial fisheries (Clark, 2001).

Numerous studies have shown trawling to negatively affect the benthic environment. The damage of trawling to bottom dwelling invertebrates can attract predators (Kaiser & Spencer, 1994), cause habitat modification (Auster et al., 1996) and polyp suffocation (Dodge & Vaisnys, 1977; Rogers, 1990). Decreases in densities, and increases in damage to anthozoans was observed after a single trawl pass in the Gulf of Alaska (Freese et al., 1999). 15.5% of benthic macrofauna was removed after a single demersal trawl in waters off NW Australia (Moran & Stephenson, 2000). McConnaughey et al. (2000) found there to be a higher diversity in un-fished areas in the Bering Sea, and suggested that recovery of erect fauna, such as sponges and soft corals is slow. Thrush et al. (1998) found distinct, generally negative, relationships between macrobenthic community structure and fishing disturbance.

Deep-water coral reefs appear to play an important role in commercial fisheries. Orange Roughey, Roundnose Grenadier (Rogers, 1999; Mortensen, 2000), Gag and Scamp grouper (Brooke & Young, 2003), Redfish, ling and tusk (Husebø et al., 2002) and many other fisheries species have been observed using deep-water reefs (Koenig et al., 2000; Reed, 2000).

Coral patches were traditionally avoided as fishing areas because of the damage they caused to nets and gear. However, with developments in equipment, such as rockhoppers, 'tangle nets' and stronger nets, more fishermen are realising the potential of these areas as lucrative fisheries (Koslow et al., 2000; Hall-Spencer at al., 2003).

The damage from deep-water trawling can now be seen in several areas around the globe (Probert et al., 1997; Koslow & Gowlett-Jones, 1998; Freese et al., 1999; Bett, 2001; Brooke, 2002; Hall-Spencer, 2003). *Solenosmilia variabilis* reefs on the Southern Tasmanian seamounts have undergone significant trawl destruction (Koslow & Gowlett-Jones, 1998). The target species in this area has been the Orange Roughy (*Hoplostethus atlanticus*) and Oreos (*Pseudocyttus maculatus, Allocyttus niger*), with there being large numbers of coral by-catch (Probert et al., 1997). In the NE Atlantic many *Lophelia pertusa* reefs have been adversely affected by trawling (Kenyon et al., 1999; Rogers, 1999; Fosså et al., 2000; Roberts et al., 2000; Hall-Spencer et al., 2003; Wheeler et al., in press). Aside from the obvious reduction of habitat, the ecological impacts of such damage have yet to be fully realised.

Many shallow-water studies have examined the ecological damage to scleractinian corals exposed to trawling. Dodge & Vaisnys (1977) examined the impacts of dredging in the vicinity of a shallow-water reef in Bermuda. They found that dredging occurring at this site between 1941 and 1943 caused mass mortality of two species of *Diploria.* Ward (1995) found increased mortality and reduced growth in *Pocillopora damicornis* exposed to trawling, off the Western Australian coast. Gametogenesis can also be adversely affected by stress (Brown & Howard, 1985; Rinkevich & Loya, 1989), and so recovery rates can be slow. Sedimentation rates can also have a strong influence on the recruitment rates of Scleractinians, with excess sediment in an area preventing settling (Rogers et al., 1984; Richmond., 1997). Sedimentation is a persistent problem for shallow-water corals, as the energy required to constantly clean the polyp surface takes its toll on the energy available for reproduction and growth (Tomascik & Sander, 1987). Bell & Turner (2000) observed smaller polyps of *Caryophyllia smithii* in habitats with increased sedimentation in comparison to low sedimented habitats. In deep-water corals, laboratory experiments have shown that sedimentation has little effect on growth rates in *Lophelia pertusa* (ACES, 2003).

1.6.2 The Oil Industry

As well as fishing pressures, these deep-water reefs are also potentially under threat from oil and mineral exploration taking place in deeper waters (Rogers, 1999; Bett, 2001). The high carbon content of sediments, and oxygen minimum zones (increasing the burial of organic matter), make the thick, continental substrata often suitable areas for oil formation (Rogers, 1999). However, these areas are also the most common for deep-sea coral formations (Section 1.5). The potential impacts of deep-water exploration and drilling are high. Aside from the extreme locations making them inaccessible should accidents happen (Rogers, 1999), there are also the affects of sedimentation and chemical release. Sedimentation affects on coral polyps have been discussed previously (Section 1.6.1), and so pollution will be targeted here.

Drill cuttings, produced water, drains, seabed engineering and sanitary waste would be the main input of poisonous chemicals into the benthic system close to oil production centres (Rogers, 1999). Drill cuttings are often laden with heavy metals and produced water may contain oil waste (Rogers, 1999). Benthic epifauna and infauna can also be smothered by these cuttings (Messieh et al., 1991). No studies have yet targeted oil pollution on deep-water corals, yet hydrocarbons have been shown to have detrimental affects on the reproduction of shallow-water species (Loya & Rinkevich, 1979; Guzman & Holst, 1993) and even on reproductive synchrony among colonies (Richmond, 1994). Growth may also be depressed (Birkeland et al., 1976) and even mass mortality can occur (Loya & Rinkevich, 1980; Brown, 1996). Oil pollution can remain for many years on reef sites (Loya & Rinkevich, 1979; Brown, 1996) and so the affects can be long term (Loya, 1976). Differing species, however, show different responses to oil pollution (Brown, 1996), so it would be important to assess properly, on a species wide basis, the affects of oil and pollutants on deep-water coral habitats.

The deep-water reef-forming coral *Lophelia pertusa* has been observed colonising several North Sea oil rigs (Bell & Smith, 1999). These authors suggest that the position of these apparently healthy colonies negates against any detrimental affect caused by drilling. However, Roberts (2000) argues, these corals may not, in fact, be subjected to any adverse conditions produced by the rigs, and may only colonise areas

where physical factors remove all pollutants. The colonisation of *L. pertusa* on man made structures has been documented previously (Wilson, 1979) and so these rigs may be forming stepping stones in a larval supply route. However, research is needed into these particular colonies ecology and environmental position, to explore the true effects of pollutants.

1.7 Project Objectives

1.7.1. The ACES Project

The presence of large areas of deep-water corals within the NE Atlantic has been known for many years (Broch, 1922; Joubin, 1922; Dons, 1944; LeDanois, 1948; Zibrowius, 1980), but using new technologies, the true extent of the coral populations within European waters is just being appreciated (Wilson, 1979; Mortensen et al., 1995; Henriet et al., 1998; Mortensen, 2000).

The 5th European Framework Programme project 'Atlantic Coral Ecosystem Survey' (ACES) began in April 2000, running for 3 years, with the main aim of studying the environmental sensitivities of deep-water corals in the European Margin. With most of Europe's coral reefs and mounds lying within Exclusive Economic Zones (EEZs), and the majority of the public unaware that these cold water species exist, the threat from anthropogenic impact is very real. As discussed in Section 1.6, fishing and hydrocarbon production are already happening in these areas, and so the need for a full scientific analysis is paramount. The project had four main objectives, 1) To map the structural and genetic variability, the framework-constructing potential, and the longevity of deep-water coral ecosystems; 2) To assess the hydrographic and other local physical forcing factors affecting the benthic boundary layer (BBL) sediment particle dynamics and particulate organic carbon (POC) supply; 3) To describe the deep-water coral ecosystem, its dynamics and functioning; investigate coral biology and behaviour and assess coral sensitivity to natural and anthropogenic stressors and 4) To assign a sensitivity code, identify major conservation issues (including increasing public awareness) and make recommendations for the rationale use of deep-water resources on the European Margin.

The multinational and multidisciplinary project encompassed five main study areas (Fig. 1.2). Each of these sites lie at differing depths and have a range of morphologies and threats. The Galicia Bank, off NW Spain is at a depth of 700m; the Porcupine Seabight (encompassing the Belgica Mounds and Propellor Mound) has a series of large carbonate mounds between 600-1000m water depth; the Darwin Mounds lie in the NE corner of the Rockall Trough at 900m depth; the Swedish Kosterfjord is the

shallowest site in the study at just 85m and the Sula Reef off the Norwegian continental shelf is the largest coral reef area, at 300m depth.

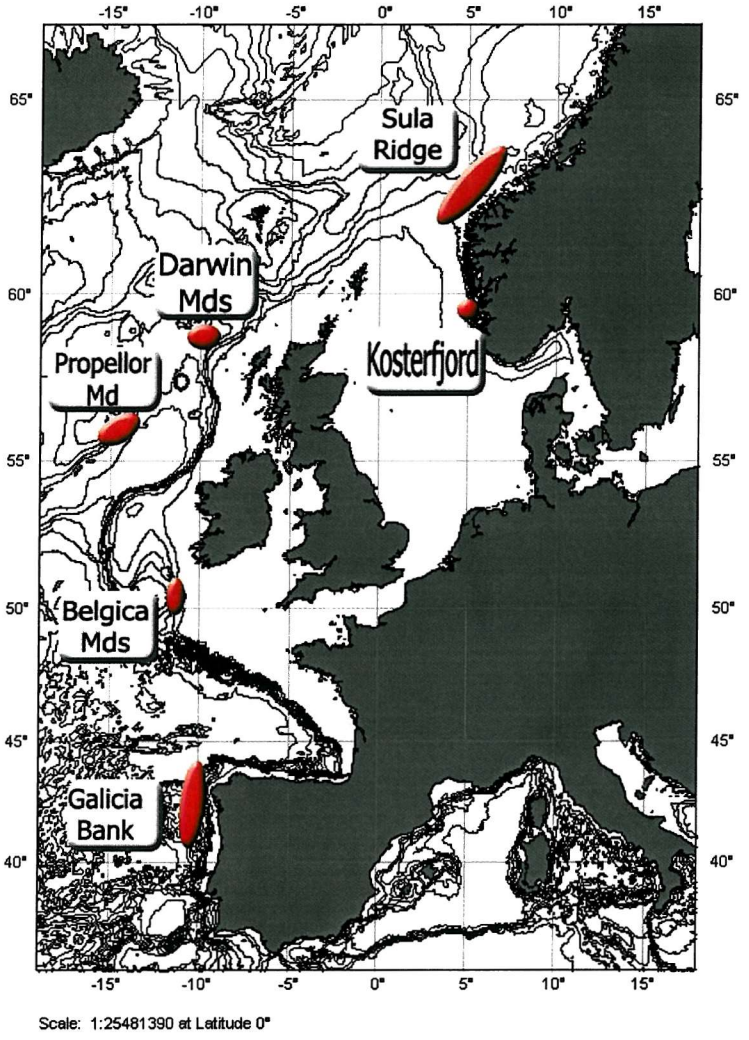


Fig. 1.2 – Map showing the ACES study areas

This reproductive study falls under the third objective, investigating coral biology and was centred on two of the five study areas.

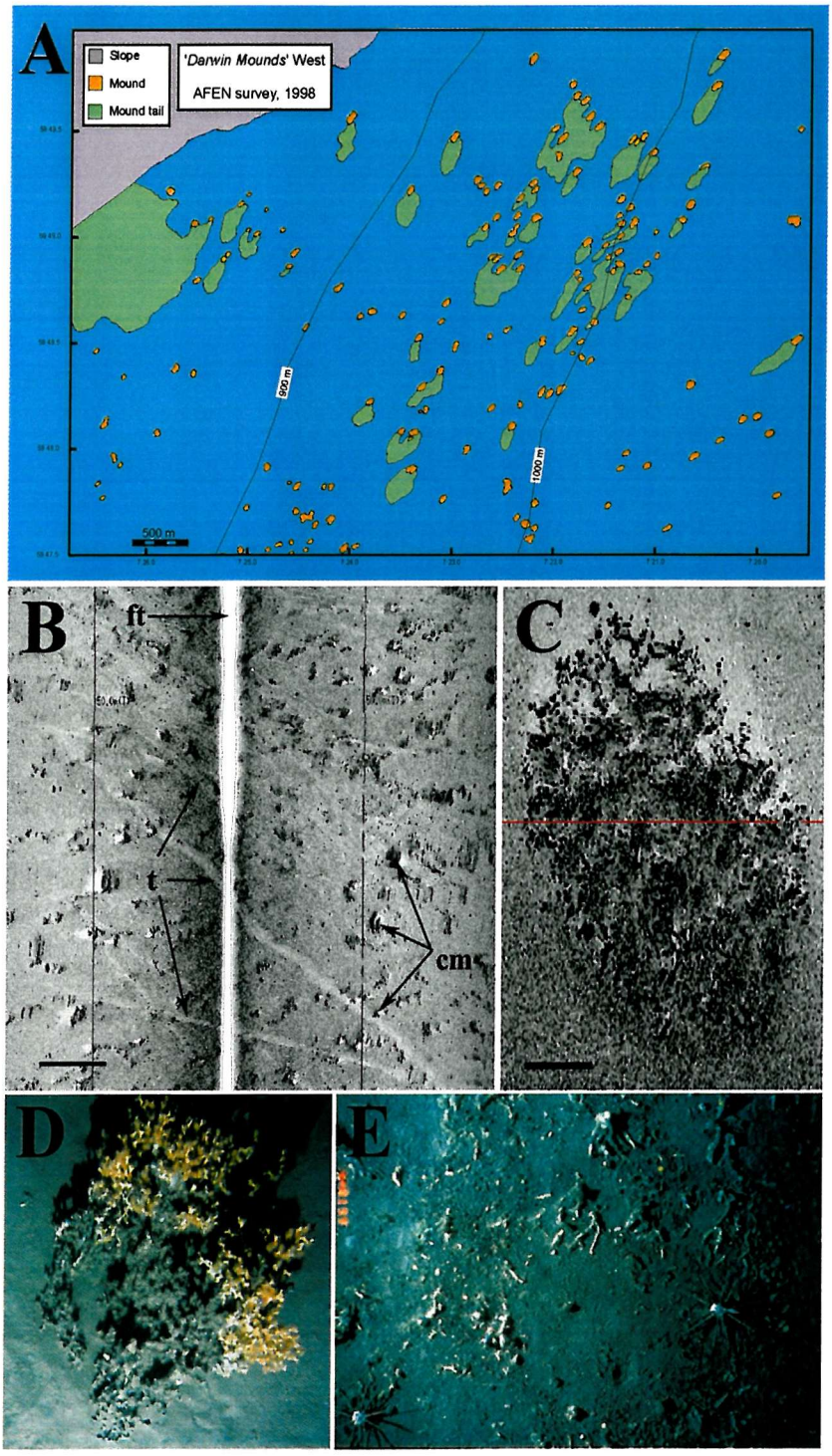


Fig. 1.3 – A, Map of the Darwin Mound area produced from TOBI (35Khz) sidescan data; **B**, Sidescan sonar sonograph showing trawl marks through the Darwin Mound area; **C**, A healthy Darwin Mound; **D**, Seabed photograph of *Lophelia pertusa* from the Darwin Mounds (~6m above seabed); **E**, Seabed photograph showing a mound thought to be damaged by trawling (~6m above seabed)

ft, sidescan fish tow track; **t**, scars from trawl doors; **cm**, coral mounds

scale bars – B, 25m; **C**, 10m

A, D, E courtesy of Brian Bett, SOC; **B, C** courtesy of Andy Wheeler, University College Cork

The Darwin Mounds

The Darwin Mound site is located in the NE corner of the Rockall Trough in an area that spans ~100km^2 and contains over 250 *Lophelia pertusa* and *Madrepora oculata* coral mounds (Fig. 1.3). Each coral mound is between 50 and 100m in diameter and around 5m in relief. The mounds were discovered in 1998 by scientists aboard the RRS *Charles Darwin* during an environmental seabed survey (Bett, 2001). Scientists subsequently found another area in 1999, naming the two areas East and West Darwin Mounds.

The mounds are located on a large sediment drift complex (Stoker et al., 1998) with high current speeds running through the area (35cms^{-1}) and temperatures of ~8°C (Masson et al., 2003; Wheeler et al., in press). The coral mounds have 'tail' features (Fig. 1.3a) that appear on the sidescan sonar but are not distinguishable with seabed photography (Gubbay et al., 2002). These tails occur downstream and are thought to be caused by the high-speed current flowing past the mounds (Gubbay et al., 2002). South of the Darwin Mounds there are large areas of pockmarks that are thought to be caused by fluid escape (Masson et al., 2003). Masson et al. (2003) hypothesised that the Darwin Mounds were originally pockmarks that have been colonised by corals on the rims, eventually covering over the pockmark, and so the area is actually a large pockmark field. Bett (2001) found there to be one coral colony per 4m^2 along the western Darwin Mounds, whilst Masson et al. (2003) observed that 50% of the seafloor was covered by what appears to be coral colonies, making this area an extensive coral field.

The Darwin Mounds have also been subjected, recently, to substantial fishing pressures (Rogers, 1999). The major deep-water fisheries taking place in this area are for blue ling and orange roughy (Gubbay et al., 2002). Wheeler et al. (in press) show there to be significant trawl damage to the Eastern Darwin Mounds, with trawl door scars clearly visible using sidescan sonar, and coral rubble being observed using deep towed cameras (Fig. 1.3 b,e). Box core samples taken in this study contained significant overturned sediments (Bett, 2001). The mounds also fall in an area open for hydrocarbon exploration, though as yet no actual drilling has taken place (Gubbay et al., 2002).

Numerous reports were suggested for the protection of the unique coral formations that make up the Darwin Mounds (Rogers, 2000; WWF-UK, 2001, 2002; Gubbay et al., 2002). In June 2003 the Darwin Mounds were nominated as a special area of conservation (SAC), a process that is still ongoing. However in August 2003 the European Commission Regulation declared the site was immediately to be protected from damaging fisheries activities. This was followed up later in August by announcement by the Department of Trade and Industry (DTI) that any previous and future offshore projects would have to be environmentally reviewed prior to any work taking place. Any activity that is likely to have a significant impact on the ecosystem would not be allowed.

However, as studies have shown, the Darwin Mounds have already undergone significant damage (Wheeler et al., in press). The pressing scientific question is now whether this area has been damaged beyond recovery.

Thérèse Mound – Fig. 1.4

Thérèse Mound is part of the Belgica Mound system, southwest of Ireland, on the northeast edge of the Porcupine Seabight. This area is within the Irish EEZ. The Porcupine Seabight is a N-S orientated embayment on the Irish Atlantic Shelf, with depths ranging from 250m in the north to 4500m at its mouth in the southwest, opening out into the Porcupine Abyssal Plain. Water speed is much less in this area ($\sim$10cms^{-1}) than the Darwin Mounds (Rice et al., 1991). Temperatures fluctuate between 9.5 and 8.5°C (Henriet et al., 1998; DeMol et al., 2002).

The area around the Porcupine slope contains many clusters of hundreds of carbonate mounds, some 2km in diameter and up to 250m in relief (Henriet et al., 1998). Most of these mounds are topped by *Lophelia pertusa* and *Madrepora oculata* corals. The Belgica Mound province was found by scientists aboard the RV *Belgica* in 1997, and is located in the northeast edge of the Porcupine Seabight. The province is made up of 21 outcropping mounds and 14 buried mounds at depths of between 600 to 850m (DeMol et al., 2002). The larger mounds in the area are 50 to 200m high, 1 to 3.5km long and are 260 to 1500m in width. Thérèse Mound has around 100m of relief and

may hold the most spectacular coral colonies of the NE Atlantic (Kenyon et al., 1998).

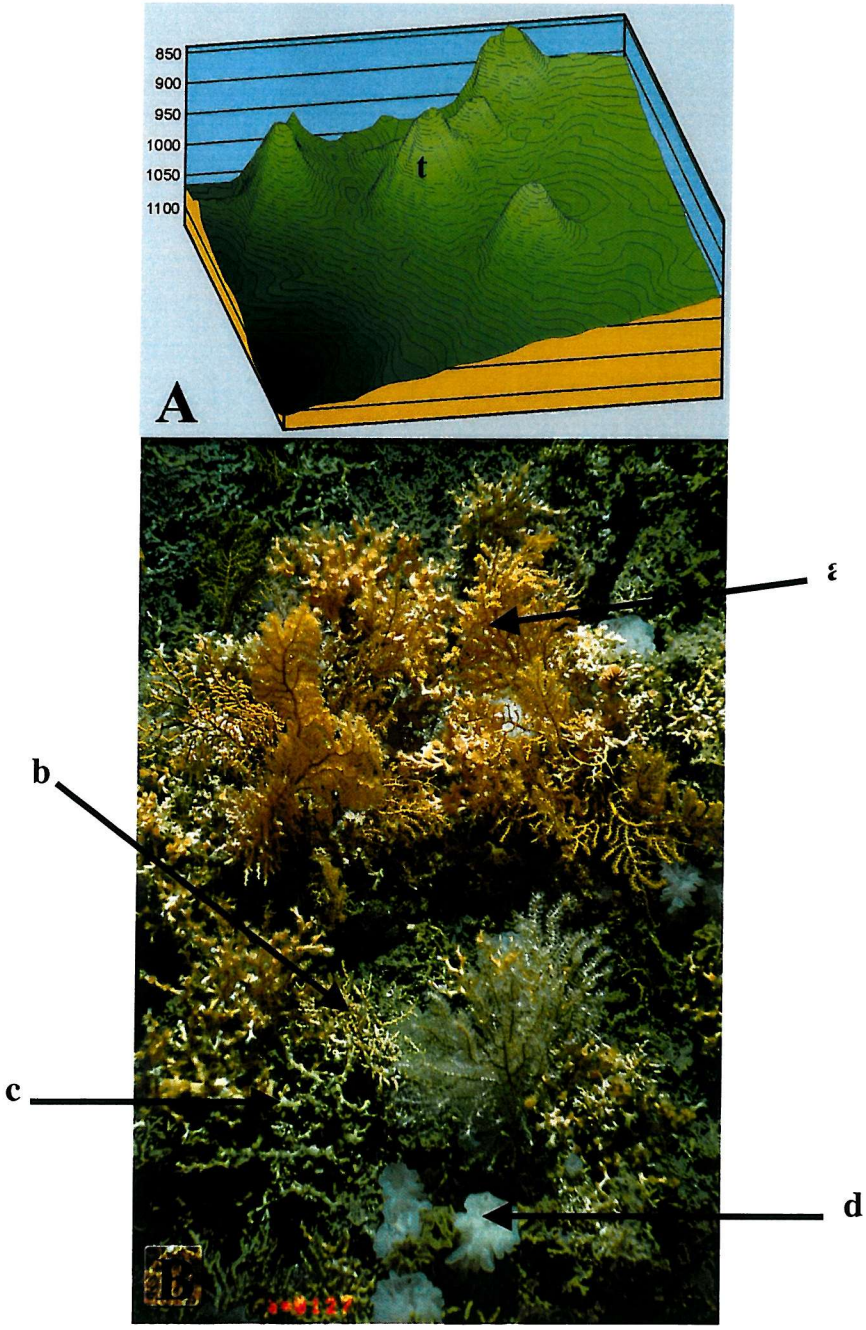


Fig. 1.4 A, Graph of Thérèse Mound in the Belgica Mound Province; **B**, Seabed image of Thérèse Mound (towed ~5m from seabed)
t - Thérèse Mound, Scale in metres; a – Octocoral; b – *Madrepora oculata*; c – *Lophelia pertusa*; d – *Aphrocalistes bocci*
Graph and Image courtesy of Brian Bett

The coral colonies on Thérèse Mound are much larger than those associated with the Darwin Mounds, and this in itself affords some form of protection. Trawl nets that come through these mounds may easily be snagged and broken, and so fishermen tend not to fish these areas, because of the financial cost of losing expensive fishing gear. However, during several cruises a number of fishing gear pieces were found amongst the mounds, suggesting fishermen are still attempting to fish in these areas (Grehan et al., 2003). The coral mound itself however is regarded as being pristine, with little of the widespread destruction seen on the Darwin Mounds being seen in this area.

This area has no protection at present, though key Irish agencies are taking steps to protect these mounds from damage.

1.7.2. The FOODBANCS Project

The Southern Ocean presents a unique environment, where there are constantly low temperatures and seasonal processes, even to depth, which are the most intense in the world. During winter months, when ice cover is extensive, there is very little primary production in surface waters. When the ice melts, extremely large phytoplankton blooms occur, causing a massive influx of phytodetritus to the benthos (Clarke, 1988; Knox, 1994).

The marked seasonality of physical processes can have impacts on the physiology of an organism. Benthic invertebrates in the Antarctic typically show seasonality of growth, reproduction and feeding (Arntz et al., 1994; Brockington & Clarke, 2001) and do not lay down large stores of body lipids in the summer months (Clarke, 1985), though this is the trend and not a strict rule. Metabolism is typically slow. It is thought that the slow rates of metabolism, as a result of the low temperatures, allow invertebrates to survive the long periods without phytodetrital material during the austral winter (Clarke, 1988).

It has been hypothesised that this seasonal input of large amounts of primary production into the Antarctic benthos may persist for longer than that found in the plankton (Knox, 1994; Smith & DeMaster, 1999). This in turn may become a food source that is available for longer periods of time. This may explain why, in a cold environment, organisms would choose not to lay down lipid stores in the food rich summer months (Clarke, 1985).

The FOOD for Benthos on the ANtarctic Continental Shelf (FOODBANCS) project was designed to study a wide variety of chemical and biological aspects of the Antarctic benthos. The programme took place within the Palmer Long Term Ecological Research site (LTER) on the Western Antarctic Peninsula (WAP) shelf. The main objective of this research was to study fully the fate of the large seasonal input of phytodetritus to the seafloor.

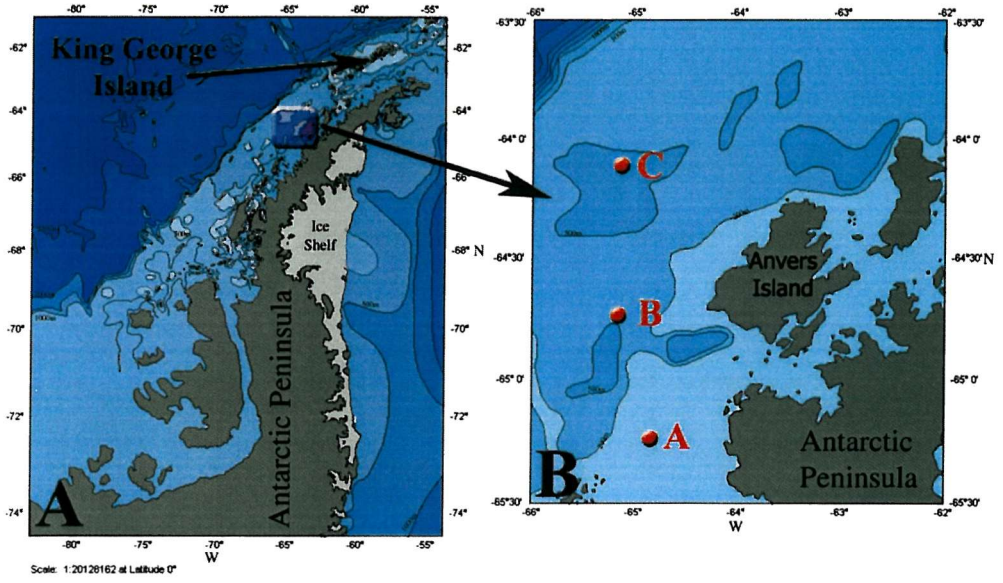


Fig. 1.5 – A, Map showing the WAP shelf area; **B**, Map showing Anvers Island and the three FOODBANCS study sites

Reproduction in shallow-water Antarctic invertebrates has been relatively well studied, especially in the echinodermata (Dell, 1972; Picken, 1980; Hain & Arnaud, 1992; Shilling & Manahan, 1994; Duchene, 1995; Poulin & Feral., 1996; Tyler et al., 2003). There is a reduced occurrence of planktotrophic development, with most organisms producing lecithotrophic or brooded planulae (Clarke, 1986; Pearse et al., 1991; Arntz et al., 1994). Seasonal reproductive processes are thought to be heavily linked to food fall to depth, both in Antarctic waters (Clarke, 1988; Bosch & Pearse, 1990; Arntz et al., 1994; Tyler et al, 2003) and in the deep-sea (Tyler et al., 1982, 1992, 1993).

Five cruises collected samples from three stations at different depths, along the continental shelf (Fig. 1.5). These samples were collected in December 1999, March 2000, June 2000, November 2000 and March 2001, to coincide with the seasonally changing ice cover in this area. One cruise sampled pre-bloom, one post-bloom, one at the end of the summer season, one at the end of the winter period and one at the end of a second bloom. A transect of three sites was located across the continental shelf, close to Palmer station located on Anvers Island.

Thirty seven species of scleractinian are found around the Antarctic and Subantarctic, and most of these are solitary forms rather than reef-builders (Cairns, 1982). The FOODBANCS program collected three species of solitary scleractinians from the WAP shelf and King George Island. These specimens were kindly donated by Prof Craig Smith from the University of Hawaii, and present an interesting comparison to those from the NE Atlantic. Though bentho-pelagic coupling is the main focus of this program, these particular samples are examined as a comparison to those from the NE Atlantic, to examine relationships between the Antarctic deep-water and the NE Atlantic deep-water.

1.8 Study Aims and Objectives

The main aims of this thesis are to –

- To examine the gametogenic biology of deep-water reef-building scleractinians and deep-water solitary scleractinians from the NE Atlantic

- To examine the gametogenic biology of deep-water solitary scleractinians from the Western Antarctic Peninsula

- To investigate differences in reproductive traits between the NE Atlantic and the Western Antarctic Peninsula and to compare results to reproductive patterns of other deep and shallow-water scleractinians and anthozoans

- To make an initial examination of the population structure of deep-water solitary scleractinians

Eleven species were examined during this study, eight from the NE Atlantic and three from the WAP shelf. The comparisons made shall be used to specifically test the following hypotheses.

a) There is no single pattern of reproduction for deep-water scleractinians

b) Solitary scleractinians have not evolved different reproductive habits from reef-building species

c) Skeleton generation is not related to body size (polyp wet weight), and so cannot be used for population dynamics

d) WAP shelf scleractinians are more similar in their reproductive traits to Antarctic fauna than deep-sea fauna

Understanding reproductive processes are essential to aiding the conservation and management efforts centred on deep-water habitats, ongoing in many areas of the

globe. Seasonality, gametogenic quantity and quality, fecundity and larval supply of habitat forming organisms is vital to understanding both how the ecosystem functions and how they would recover after a damaging event. The data presented in this thesis shall be targeted to conservation and management issues.

Chapter Two

Methods

Methods

The methods used throughout this thesis were varied with species. As a consequence, prior to each results section, a brief description of the exact methodology is described for that particular species, as well as full materials listings. This section outlines the basic methods used throughout this thesis, as well as giving a historical overview if their usage.

2.1 Sample Collection

Many samples used for this study were personally collected during three cruises to the Rockall Trough (*RRS Discovery* 248) and the Porcupine Seabight (*RRS Discovery* 248, 260, 266). Samples were also used from the extensive Discovery Collections, held at the Southampton Oceanography Centre, the SAMS Collections, held at the Dunstaffnage Marine laboratory, Oban and from Prof Craig Smith, University of Hawaii. All samples that were used for this study are listed both in Appendix II and prior to the relevant result sections.

All samples were alcohol preserved (formalin-fixed) specimens. In most cases, animals were placed in 30% formalin for a period no longer than a week, then transferred into 70% alcohol. This prevents skeletal calcium carbonate degradation by formalin, but still avoids the over-hardening of soft tissues.

Sampling Methods

Box Core

The box core is an efficient method of collecting a $0.25m^2$ of the seabed, though is heavily affected by the substratum type. A stony bottom will provide a much smaller sample depth than a sedimented substratum. This piece of equipment is mainly used for quantitative sampling of smaller, usually sediment dwelling, species, though during this study proved invaluable for the collection of reef-building scleractinians.

For the two colonial corals in this study, *Madrepora oculata* and *Lophelia pertusa*, once location has been established using single beam sonar, the equipment can bring up a substantial sample, often protruding through the top vents of the box core (Fig.

2.1). The corals did, however, usually wedge open the closing spade, so this technique is entirely non-quantitative for associated faunal studies.

Fig. 2.1 – Box core on the deck of the RRS *Discovery* during cruise 248 to the Porcupine Seabight. The box core is filled with *Lophelia pertusa* and associated fauna, and can be seen 'escaping' from the top vents

For the solitary corals in this study, this method is unsuitable for the collection of large numbers of individuals needed, so a less quantitative method was generally used, such as the Agassiz and Otter Trawl.

Agassiz Trawl

The Agassiz Trawl is a double-sided beam trawl with large twine net and smaller mesh net at cod end to catch smaller fauna. The beam is lowered over the seabed, and so disturbs the sediment and retrieves megabenthos. There are two main problems with this method, exact location of samples and the high occurrence of net breakage from being caught up on rocks and other debris. This method is more suitable for solitary cnidarians, because of the large surface area covered. Larger coral mounds, such as *L. pertusa* / *M. oculata* tend to catch and break the net.

Otter Trawl Semi-Balloon (OTSB)

The Marinovitch semi-balloon otter trawl has been used extensively to collect both benthic invertebrates and fish in the NE Atlantic. Two large v-shaped trawl doors are used to keep a large twine net (with smaller mesh nets at the cod end) open whilst being trawled along the bottom. The OTSB is much larger than the Agassiz trawl, and so can collect species that are wider dispersed. Most of the solitary scleractinians used for this study were collected using this type of net.

2.2 Histological Processing

History

Histology is the process by which tissues are thin sectioned, mounted on a glass slide beneath a thinner glass coverslip, and then stained to show internal structure (Baker et al., 1966). It has been used as an invaluable tool in both hospitals and biological laboratories for looking at the internal structures of whole animals, and for hospital laboratories to look at diseased tissues and organs. There are three main embedding methods used in preparing the tissues prior to thin sectioning - frozen, celloidin and paraffin.

Frozen sections use either fresh tissue or tissue preserved in formalin, quick-frozen and sliced at low temperatures. A cryostat (freeze chamber containing the microtome) is needed to slice the samples after freezing. This method, due to its fast processing, is mainly used in hospitals for rapid diagnosis, though is also useful for identifying lipid areas of tissues (Baker et al., 1966).

Celloidin is a purified form of nitro-cellulose and is used for embedding tissue. This chemical retains a rubbery consistency and so is useful for tissues that are either very hard or made up of several different consistencies (Baker et al., 1966). Although shrinkage is minimised in this technique, it is very slow, the blocks produced must be stored in 70% ethyl alcohol, and sections of less than 10μm cannot be sliced.

The most widely used method, and the method used in this thesis, is the paraffin method. The tissue is made up within blocks of paraffin wax, and then can be thinly sliced, often down ~3μm. Serial sections can also be made easily, unlike the other two methods. Though not the fastest of the three techniques, its main advantage is that the blocks can be stored easily, and so re-examined at a later date.

Tissue Preparation

The following basic steps were used to prepare and embed the tissue in wax ready for processing. Species specific differences in preparation are outlined prior to their results sections in Chapter Three.

Decalcification

Skeletal elements need to be thoroughly removed using a decalcifyer (below), prior to dissection. There were two decalcifiers used for varying skeletal densities.

Bouins Solution - for gentle decalcifying (Fisher Scientific – Picric acid, Formaldehyde)

- Tissue was placed in Bouins until hard parts are dissolved (~12hrs), wash for 12hrs, place in 70% alcohol.

Rapid Decalcifying Solution (RDC) (Cellpath UK – conc Hydrochloric acid, Enzyme stabilisers)

- Tissue was placed in RDC until hard parts removed (>30mins), wash for 12hrs, place in 70% alcohol.

Dissection

Mesenteries of individuals (or whole animals for smaller species) were dissected for both histological analysis and for fecundity estimation. Amount of tissue varied greatly between species and so is detailed prior to their results.

Dehydration

- 95% alcohol - ~4hrs
- 100% alcohol – ~4hrs
- 100% alcohol – ~4hrs
- 100% alcohol - ~overnight

Clearing

- Xylene – between 4-12hrs (depending on tissue size)

Embedding

- Pour off xylene and lightly dry tissue
- Pour molten wax over until just covering
- Place in the wax oven (70°C) for 12-24hrs

Moulding

- Fill mould with clean molten wax
- Place tissue central in mould
- Place cassette on mould
- Top up wax

Staining

All blocks were sliced at 5μm and stained using Masson's Trichrome Stain:-

Histoclear	5mins
100% Alcohol	2mins
70% Alcohol	2mins
Haemotoxylin Z	25mins
Wash – tap water	15mins
Ponceau S	5mins
Wash – tap water	-
Phosphomolybdic Acid	5mins
Light Green	5mins
Wash – tap water	-
100% Alcohol	5mins
100% Alcohol	2mins
Histoclear	5mins

2.3 Image Analysis

Seasonality

For oocyte size-frequency diagrams, between 50 and 100 random oocytes were measured (if present), from either histological slides or dissection, using an Olympus BH2 compound microscope with digital camera attachment. Images were captured using Matrox Rainbow Runner and analysed using SigmaScan Pro version 4 to calculate oocyte diameters ('feret' diameter, the area if the oocyte was a perfect circle, was used). SigmaPlot version 8 was used to construct graphs. Oocytes and spermacysts were also staged to aid determination of seasonality. If possible 10 females and 3-5 males were assessed in this manner (true figures in relevant sections).

Fecundity

For larger animals, 3-5 mesenteries were dissected out and oocytes were hand counted. For smaller individuals, mesenteries were dissected and wax embedded separately. For very small individuals, whole animal was embedded and serially sectioned.

Wax blocks were serially sectioned leaving 10μm below the minimum oocyte diameter between sections (for distance in μm left between slides, see relevant sections) to ensure no oocytes were missed. The total number of mesenteries in each species were determined by dissection. All stages of visible oocyte (previtellogenic, vitellogenic and late vitellogenic) were counted for fecundity estimates.

An average number of oocytes per mesentery was calculated (Realised fecundity) and multiplied by the total number of mesenteries in an individual (Potential fecundity). For the colonial scleractinians, colony fecundity was also estimated using the calculation -

A x B = Colony Fecundity (Oocytes per cm^1)

A = No. polyps per cm^1

B = Potential fecundity

Fecundity data was size corrected using the following equation (L. Peck, pers com).

C12 = R2 x (LNstd – LNobs) + LNfecstd

C12 = Log corrected potential fecundity
R2 = R2 value for total sample
LNstd = Log mass of standard animal
LNobs = Log mass of observed animal
LNfecobs = Log fecundity of observed animal

This size corrected data were then used to construct the average fecundity per month. This allowed monthly samples to be compared without size being a factor. A Mann-Whitney U test was used to compare between mean monthly fecundities. Size corrected data were also used to construct graphs of fecundity verses weight of animal. SigmaPlot version 8 was used for these statistics and graphs.

2.4 Scanning Electron Microscopy (SEM)

History

Light microscopes cannot be used to distinguish objects that are smaller than half the wavelength of light. White light has an average wavelength of 0.55μm, half of which is 0.275μm. Any two lines that are closer together than 0.275μm will be seen as a single line, and any object with a diameter smaller than 0.275μm will be nearly invisible. To see tiny particles under a microscope, a different illumination source must be used, one with a shorter wavelength.

The first electron microscope was invented in Germany in the 1930s by Max Knott and Ernst Ruska. Electrons are speeded up in a vacuum until their wavelength is extremely short (one hundred-thousandth that of white light). Beams of these fast-moving electrons are focused on a cell sample and are absorbed or scattered by the cell's parts so as to form an image on an electron-sensitive photographic plate.

The SEM can be used to look at topography (surface features, texture, hardness, reflectivity), morphology (shape and size of the particles making up the object), composition and also the crystallographic information. For reproductive studies (including this study) the SEM is often used to look at the exact morphological features of different larval stages, features that are largely unseen by light microscopy.

Tissue Preparation

Dissection

The brooded larvae of the Antarctic *Flabellum* spp. were examined using the SEM. During dissection for oocytes, differing stages of larval development were also separated. Two of each stage were dehydrated using one immersion in 95% propan-2-ol, two 4hr submersions in 100% propan-2-ol and then a final overnight submersion in 100% propan-2-ol. These larvae were then critical point dried at the Southampton Hospital Biological Imaging Department.

Critical Point Drying

As the SEM operates under a vacuum, it is impossible to examine wet specimens.

Soft tissue will wrinkle when air dried and so the subsequent SEM picture would not be wholly accurate.

In critically point drying, the alcohol in which the specimens are dehydrated is replaced by liquefied gas, which is then allowed to evaporate, leaving a morphologically intact 'dry' specimen. In this instance liquefied Carbon Dioxide was used in a Polaron CPD 7501 automated critical point drier. The CO_2 was pressurised to 1072 psi and heated to 31°C. Samples were critically point dried for 4hrs and then immediately placed into a dessicator.

Coating

The larvae were then placed on SEM stubs using double sided sticky carbon paper, and coated with gold in a Hummer VI-A Sputter coater. Samples were coated for 10 minutes, leaving a gold coat of around 15nm. This gold coat reflects electrons, allowing features to be visualised.

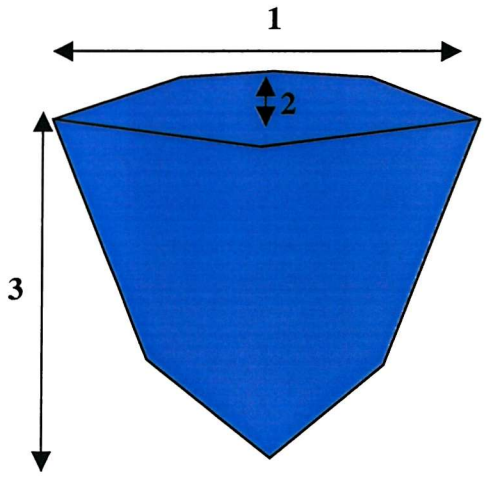
Scanning Electron Microscope

A Leo 1450VP (variable pressure) digital Scanning Electron Microscope was used to take pictures and measure all larval stages of *Flabellum curvatum, F. impensum* and *F. thouarsaii.*

2.5 Population Data

For every solitary species used in this study, skeletal dimensions of at least 20 individuals (depending on sample size) was measured prior to decalcification. Polyp wet weight of these animals was then also taken post-decalcification. The three skeletal dimensions taken are shown in Fig 2.2.

These three dimensions were then plotted against wet weight to examine if there was a significant relationship between skeletal dimensions and the actual polyp. A 'skeletal volume index' estimate was also calculated and plotted against wet weight (Fig. 2.2). In species where there was no relationship, no further data were taken. In species where there was a significant relationship, samples of 100 individuals from as many seasons and years as possible were measured and data plotted as a size-frequency diagram. These seasonal samples were then compared to each other using methods used in Meesters et al., (2001). A Kolmogorov-Smirnov normality test examined normality for all populations. Sample means of different populations were then tested with a Mann-Whitney U-test. Normalality, skewness and kurtosis were described for all graphs constructed. Minitab 14 was used for all statistics.



Skeletal Volume Index = 1 x 2 x 3

Fig 2.2 - Diagram showing the three skeletal dimensions measured on all species of scleractinian used for this study

Chapter Three

Reproduction in Deep-Water Scleractinians

3.1 Reproduction of Deep-water Reef Building Scleractinians

Introduction

Hard substrata are necessary for reef-building corals to attach, though this hard substrate can take a number of forms (Wilson, 1979; Bell & Smith, 1999; Atkins & Scheirer, 2003). Deep-water reefs, mounds and banks are commonly found on continental margins and shelf breaks, as well as on seamounts and ridges. These areas generally have a high water flow associated with them (DeMol, 2002) which are thought to be necessary for the delivery of food and larvae to the area, as well as removing waste and excess sediment (Grigg, 1974, 1984). Highly sedimented areas are rarely colonised by corals, as the risk of polyp suffocation is high (Dodge & Vaisnys, 1977; Rogers, 1990).

Though fewer than solitary species, there are many deep-water reef-building scleractinians around the globe (Cairns, 1999). For example, *Enallopsammia rostrata* can be found in multiple locations in New Zealand (Cairns, 1995) and the NW Atlantic (Atkins & Schierer, 2003), *E. profunda* from the Western Atlantic and *E. marenzelleri* from the NE Atlantic, New Zealand and Indonesia (Cairns, 1995). *Goniocorella dumosa* occurs on the Campbell Plateau, New Zealand (Squires, 1965), *Oculina varicosa* on the Florida continental shelf (Reed, 1980; Brooke, 2002), *Solenosmilia variabilis* from the South Pacific, SE Atlantic and Cook Islands (Cairns, 1982, 1995; Keller, 1993), *Dendrophyllia cornigera* in the NE Atlantic (Zibrowius, 1980), *Desmophyllum dianthus* off Chile (Cairns, 1982). *Lophelia pertusa* and *Madrepora oculata* are found throughout the Atlantic, Pacific, Indian and even Antarctic Oceans (Zibrowius, 1980; Cairns, 1995; Rogers, 1999). These last two species are thought to be the most widely distributed species of reef-building scleractinian (Rogers, 1999), though have, however, recieved considerable attention and so distribution estimates are likely to be highly skewed.

Deep-water reefs are recognised as important biomes for many commercial and non-commercial species (Rogers, 1999). Nearly 1300 species of invertebrate and fish have been described from the branches of *L. pertusa* in the NE Atlantic ocean, and this figure will increase as more are identified (ACES, 2003). This is a comparable

biodiversity to shallow water reef systems, and has proved in recent years to sustain many commercial important species (see Chapter One).

This section examines the reproductive biology of two species of cosmopolitan deep-water reef-building scleractinians, *Lophelia pertusa* and *Madrepora oculata* from the NE Atlantic Ocean. Colour morphs of *Lophelia pertusa* from the North Sea area also briefly examined.

3.1.1 The Reproduction of Two Deep-water Reef Building Scleractinians from the NE Atlantic

Introduction

Lophelia pertusa (Linné, 1758) is a cosmopolitan scleractinian. It can be found from depths of 50m in the Norwegian fjords (Rogers, 1999), to 3600m on the Mid-Atlantic Ridge (Bett et al., 1997) and in most of the worlds oceans. Though many previous taxonomic references refer to the dead remains of *L. pertusa,* new reefs are constantly being discovered. *Madrepora oculata* Linné 1758, is less cosmopolitan than *L. pertusa* and has only been found as a co-species with other reef-forming scleractinians.

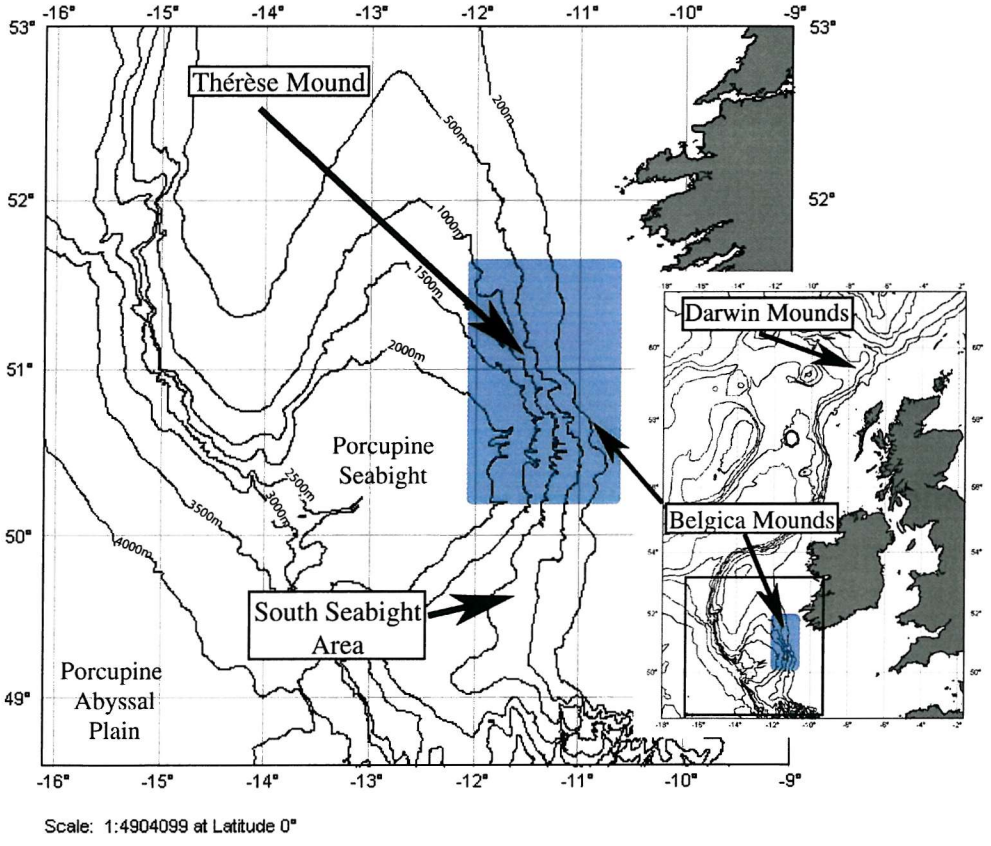


Fig. 3.1 – NE Atlantic Ocean. Showing bathymetry and location of Darwin Mounds, Thérèse Mound (within the Belgica Mounds) and the Southern Seabight Area

M. oculata and *L. pertusa* are frequently found together off New Zealand (Cairns, 1995), the Aegean Sea (Vafidis et al., 1997), SE Atlantic (Keller, 1993) and the NE Atlantic (Keller, 1993; Bett et al., 1997; Wilson, 1997; Rogers, 1999). The present study looks into three sites at bathyal depths in the NE Atlantic Ocean (Fig. 3.1). The Darwin Mounds are series of several hundred mounds, around 100m diameter and 5m height, found within the NE segment of the Rockall Trough (Bett, 2001). Thérèse Mound is a large coral mound found within the Belgica Mound system in the Eastern Porcupine Seabight. The third site was an opportunistic sample on the south side of the Porcupine Seabight (SPS). All the sites are between 800m and1000m depth.

Materials and Methods

All specimens were obtained using a 0.25m^2 box core, 3m Agassiz Trawl, or an Otter Trawl Semi-Balloon, between 2000 and 2002 from the RRS *Discovery* in the NE Atlantic (Table 3.1, Fig. 3.1). Selected specimens of each species were greater than 30cm colony width, and are referred to as putative colonies.

Species	Area	Depth	Cruise	Latitute / Longitute	Date
L. pertusa	Darwin Mds	980m	D248	59° 48.88N 07° 17.99W	16/07/00
	Thérèse Md	870m	D248	51° 25.67N 11° 46.41W	06/08/00
		870m	D266	51° 25.67N 11° 46.41W	30/9/02
		870m	D260	51° 25.67N 11° 46.41W	08/03/02
	South Porcupine Seabight	785-925m	D260	44° 40.00N 11° 30.70W	18/10/02
M. oculata	Thérèse Md	870m	D248	51° 25.67N 11° 46.41W	06/08/00
		870m	D260	51° 25.67N 11° 46.41W	08/03/02
	South Porcupine Seabight	785-925m	D266	44° 40.00N 11° 30.70W	18/10/02

Table 3.1 - Samples used for this study

For histological processing, whole colonies of *Lophelia pertusa* or *Madrepora oculata* were decalcified. *L. pertusa* polyp tissue was weighed post-decalcification. 20 individual polyps from each colony, of each species, were separated and histologically processed. *M. oculata* polyp diameter was measured post-histological

processing, using SigmaScan Pro V. 4. For *M. oculata* few polyps produced more than 50 oocytes and so data were averaged between individuals within a colony.

For fecundity estimation of *L. pertusa*, 5 polyps from a single colony were dissected from colonies collected in August, and in October. The wet weight of each polyp was measured and the total number of mesenteries recorded. 5 mesenteries were dissected and embedded using the procedure above. Blocks were serially sectioned and all previtellogenic, vitellogenic and late vitellogenic oocytes were counted in each mesentery. For fecundity estimation of *M. oculata*, serially-sectioned slides from 5 to 10 female polyps were examined and all oocytes counted. All polyp measurements were size-corrected to the average animal size for that species (Chapter 3). The tin foil method was used for calculating polyps per cm^2 skeletal area (Marsh 1970). This method uses a piece of tin foil of known size that is wrapped around a coral skeleton without overlapping. The number of polyps that lie within the tin foil are then counted and divided by the size of the foil to give a measurement per cm^2. $10cm^2$ foil was used for *L. pertusa* and $5cm^2$ for *M. oculata*.

3.1.2 Colour Dimorphism in *Lophelia pertusa*

Introduction

Within the NE Atlantic it has been noted that *L. pertusa* can be found in several differing morphologies (Freiwald, 1998; ACES, 2003). These include polyp colour, skeletal and skeletal colour morphologies. It is unknown what exactly causes these morphological differences, but is thought to be related to environmental conditions (Wilson, 1979; Veron, 1995; Freiwald, 1998).

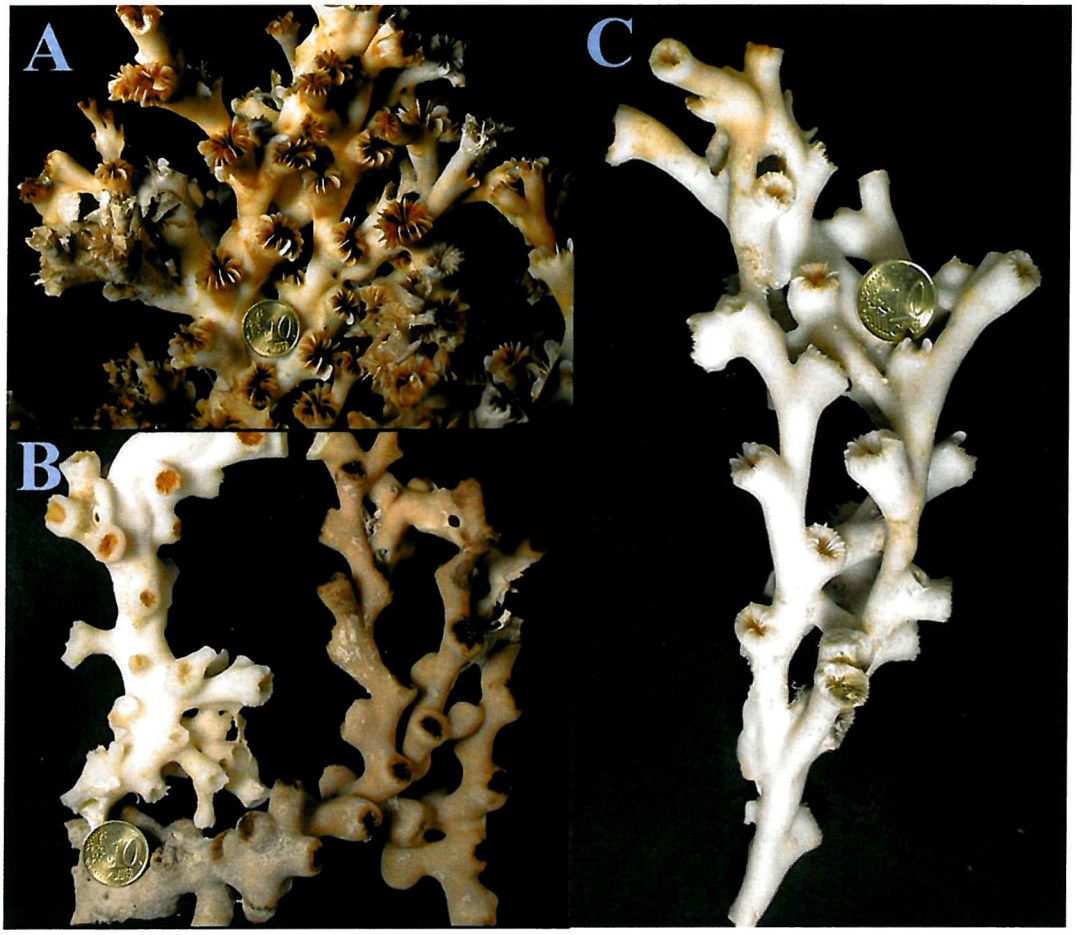


Fig 3.2 *Lophelia pertusa* morphologies from the NE Atlantic; **A**, Extrovert; **B**, Introvert; **C**, Elongate
Pictures courtesy of Andre Friewald, Erlangen University
Coins – 2cm diameter

Polyps of differing colour can be observed side by side on a single reef site, however a colony can only be one colour (Andre Freiwald, pers. com.). Many Cnidaria have differing tissue colours within the same species, *Actinia equina*, the common Beadlet

anemone, is an example where there appears to be no physiological or environmental reason behind the three varying colours found side by side on the seashore (Shick, 1991). Three polyp colours of *L. pertusa* have been noted thus far, orange, white and a newly discovered yellow in the Swedish Fjords (T. Lundalv, pers. com).

Skeletal differences appear to have a more obvious ecological answer. Each skeletal morph (though not skeletal colour) is found in differing areas, with different physical factors. Three major morphologies have been observed in the NE Atlantic (ACES, 2003) (Fig. 3.2). These three morphologies have been named as major morphotypes – extrovert, introvert and elongate. These three morphotypes have been found in differing areas – extrovert is common at shallower depths (<300m), the introvert type is common in the Porcupine Seabight (Propeller Mound) and the Mediterranean, and the elongate type appears to be characteristic of the deep Rockall Bank, Belgica Mounds, Stjersund Reef and the North Sea oil platforms (ACES, 2003). Each colony of *L.pertusa* is a single shape, and so does not change form through life.

However another morphological variation is observed in *L. pertusa*. There are two skeletal colour differences found, white and orange/brown (ACES, 2003; pers obs). Both of these colour forms can be found side by side within the NE Atlantic, but a single colony is only ever one colour (Fig. 3.3). Each of the three major polyp morphotypes have been observed in both white and orange skeletal morphologies (pers. obs.), and can generally be found within a single area (ACES, 2003). Morphologically there are differences between these two colours, the white morph is made up of much smaller aragonite fibres than the orange morph (Andre Freiwald, pers com), though as these colours appear in the same areas there appears to be no obvious environmental reason for the difference.

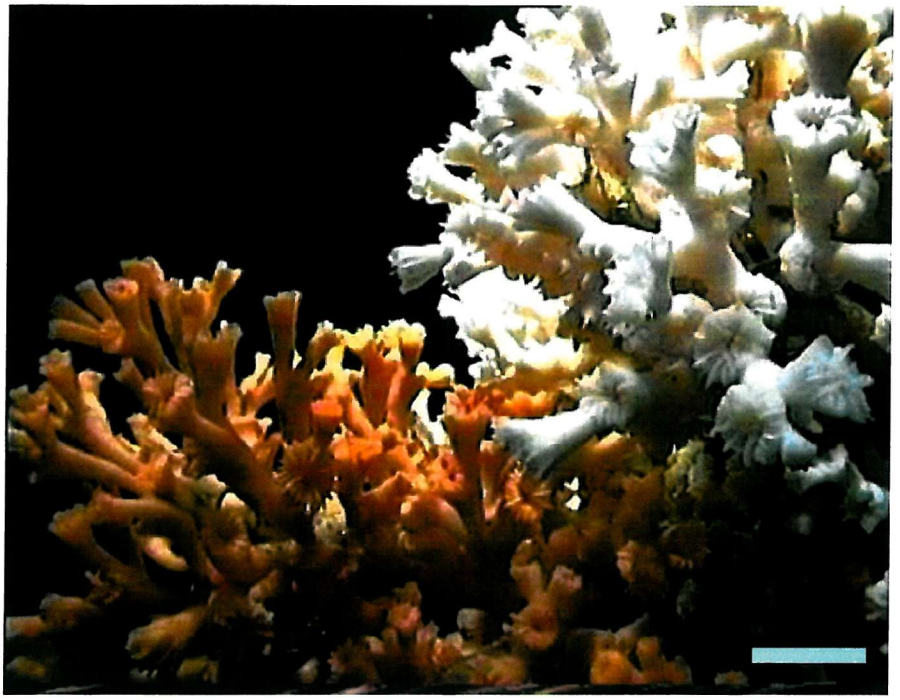


Fig. 3.3 - White and orange skeletal colour morphs of *Lophelia pertusa* from the Sula Ridge
Picture courtesy of Andre Friewald, Erlangen University
scale bar – 5cm

Waller and Tyler (in press, this Thesis) commented on the predominance of the orange skeletal morph in the Porcupine Seabight. They also reported a lack of males found within the study area, only a single male colony was recovered. This male colony was also the only white skeletal morph to be recovered during this study, with all orange morphs being either female or non-reproductive.

There were insufficient samples available from the Porcupine Seabight to test sufficiently the hypothesis that the colour morphologies are due to sexual dimorphism. However samples were kindly donated by Murray Roberts and Susan Gass (Scottish Association of Marine Biology, Oban) from two North Sea oil platforms (Fig. 3.4). These samples consisted of just one collection season, but sufficient numbers of the two colour morphs were provided for a preliminary study. This study tests the hypothesis that the colour variation seen in *Lophelia pertusa* skeletons is a result of sexual dimorphism.

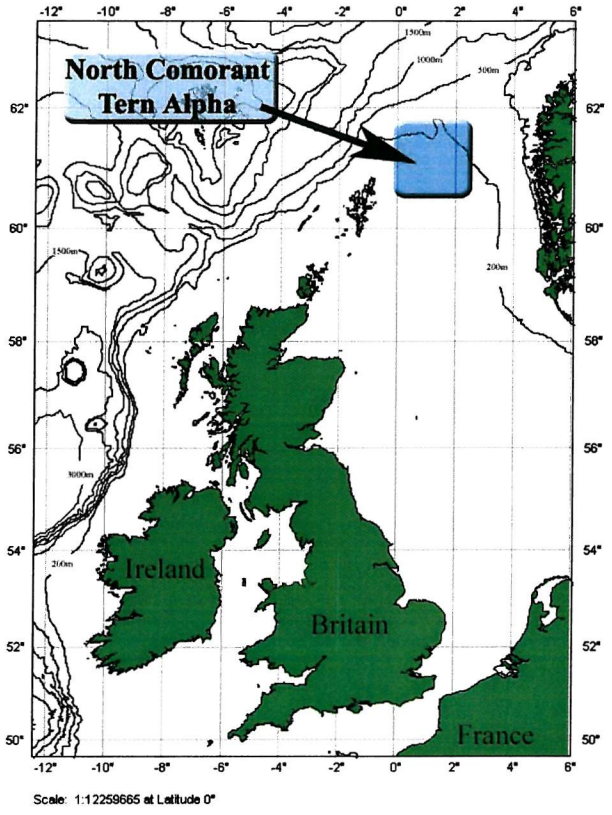


Fig. 3.4 - Map showing area of North Comorant and Tern Alpha oil platforms

Materials and Methods

Lophelia pertusa colonies were collected by commercial ROV from the North Cormorant and Tern Alpha Shell oil platforms in the North Sea. Six colonies were subsampled (Table 3.2), four of white skeletal morphology and three of orange skeletal morphology (all were orange polyp colour). Three polyps from each colony were histologically processed, and three slides were made up from each sample to observe sex.

Platform	Latitude / Longitude	Date	Depth	Morph
Comorant	1° 15N 61° 24E	15/6/03	102m	White
Comorant	1° 15N 61° 24E	18/6/03	102m	White
Comorant	1° 15N 61° 24E	16/6/03	109m	White
Comorant	1° 15N 61° 24E	16/6/03	109m	Orange
Tern	0° 92N 61° 28E	23/6/03	153m	White
Tern	0° 92N 61° 28E	23/6/03	107m	Orange

Table 3.2 - Samples of *Lophelia pertusa* used in this study

3.1.3 Reproduction Results

Lophelia pertusa

Reproductive Morphology

L. pertusa has two major skeletal colour forms, white and orange (see Section 3.1.2). The white form appears less prolific in the areas sampled, and was only obtained from the South Seabight during the October 2002 cruise.

L. pertusa is gonochoristic with all mesenteries fertile. Gametes are in two to three pockets throughout the mesentery and 2 to 3 oocytes or spermatocysts wide (Fig. 3.5b to e). These pockets contain gametes at the same stage of development. The number of mesenteries per polyp varied from between 13 and 24.

Gametogenesis

Gametes were observed in colonies sampled during August, September and October from the Porcupine Seabight only. No gametes were found in colonies from the Darwin Mound site or from March in the Porcupine Seabight. Males were only found in the October sample, and only in the white skeletal form.

Oogenesis

All female colonies had orange skeletons. Only three stages of oogenesis were observed, but a fourth is inferred from previous azooxanthellate coral studies (Fig. 3.5b to e).

Stage I - Oogonia, – <5μm diameter; bud from the mesenterial lamellae

Stage II - Previtellogenic Oocytes – 5μm to 30μm diameter; small oocytes with thin wall and an basophilic cytoplasm (Fig. 3.5c)

Stage III - Vitellogenic Oocytes – >30μm diameter (Fig. 3.5c, d)

(Inferred) Stage IV - Late Vitellogenic Oocytes – >140μm diameter; oocytes become heavily granulated and have a thick cortical granular layer around the periphery of the ooplasm.

The March sample revealed 2 small oocytes (~50μm feret diameter) that appeared to be reabsorbing. These were not measured or counted for fecundity estimations. Previtellogenic oocytes were observed in August, but not September or October.

Spermatogenesis

Only one sample containing males was found, during October 2002. All spermacysts were at the same stage of development (Fig 3.5e). Using the same staging scale as Waller et al. (2002) (See Section 3.2.2), all spermacysts appear to be at stage 2. Numerous spermatocytes were present, with a few spermatozoa beginning to congregate around the lumen (Fig. 3.5e).

Fecundity

Fecundity estimates were size corrected to 0.1775g polyp wet weight. There was no significant difference between fecundity estimations for August and October (U=8, P=<0.05). Fecundity for the March sample is taken as 0, as no developing oocytes or oogonia were observed, and the only oocytes present were few and being reabsorbed. Individual polyps in August had an average fecundity of 3146 oocytes per polyp (±1688), and in October had an average fecundity of 2308 oocytes (±818).

No evidence of gametogenesis was found in polyps below 0.08g, suggesting this is the size of first reproduction. However there was also no significant relationship between weight of polyp and fecundity (R^2=0.4, P=<0.05). Colony fecundity was recorded at 3327 oocytes per cm^2. This would give a colony of 30cm^2 (the approximate average size in this study) a total fecundity of 99,831 oocytes.

Reproductive Periodicity

All individuals within a month sample were synchronously developing (Appendix III), and so oocyte size-frequency graphs were collated. Oocyte size-frequency analysis shows there was a single cohort of developing oocytes in the population (Fig. 3.6). There appears to be rapid growth of oocytes, feret diameter doubles between August and October (41.3μm for Aug, 64.3μm for Sept and 88.7μm for Oct). Maximum oocyte size observed 139μm.

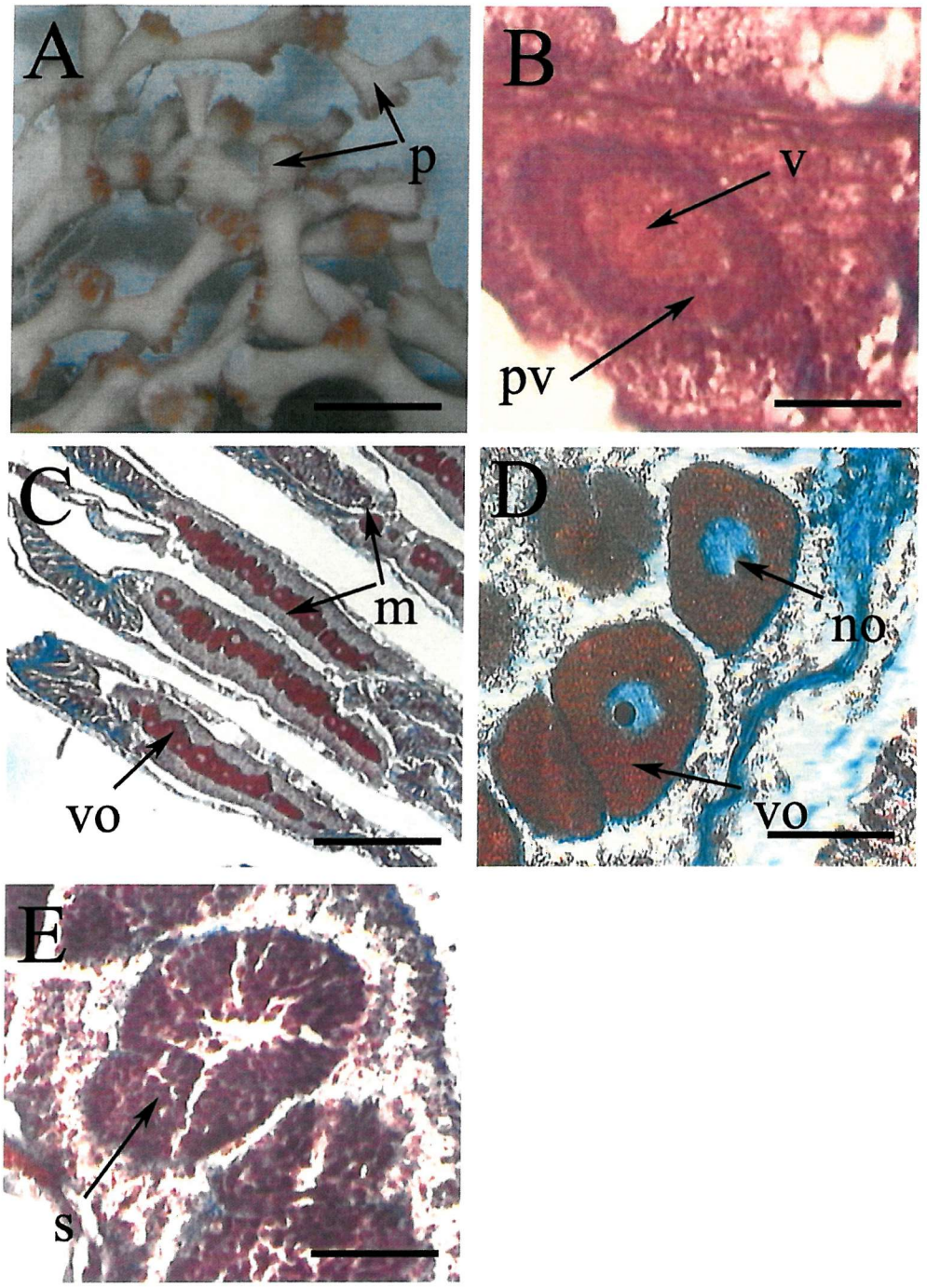


Fig. 3.5 - **a.** *L. pertusa* colony ; **b.** Previtellogenic oocyte undergoing vitellogenesis; **c.** Female mesenteries containing vitellogenic oocytes; **d.** Vitellogenic oocytes; **e.** Stage 2 spermacyst

p-polyp; **v**-vitellogenesis; **pv**-previtellogenic; **vo**-vitellogenic oocyte; **m**-mesentery; **no**-nucleolus; **s**-spermatocytes

Scale bars **a** 2cm; **b** 25μm; **c** 125μm; **d** 50μm; **e** 50μm; Stained with Masson's Trichrome

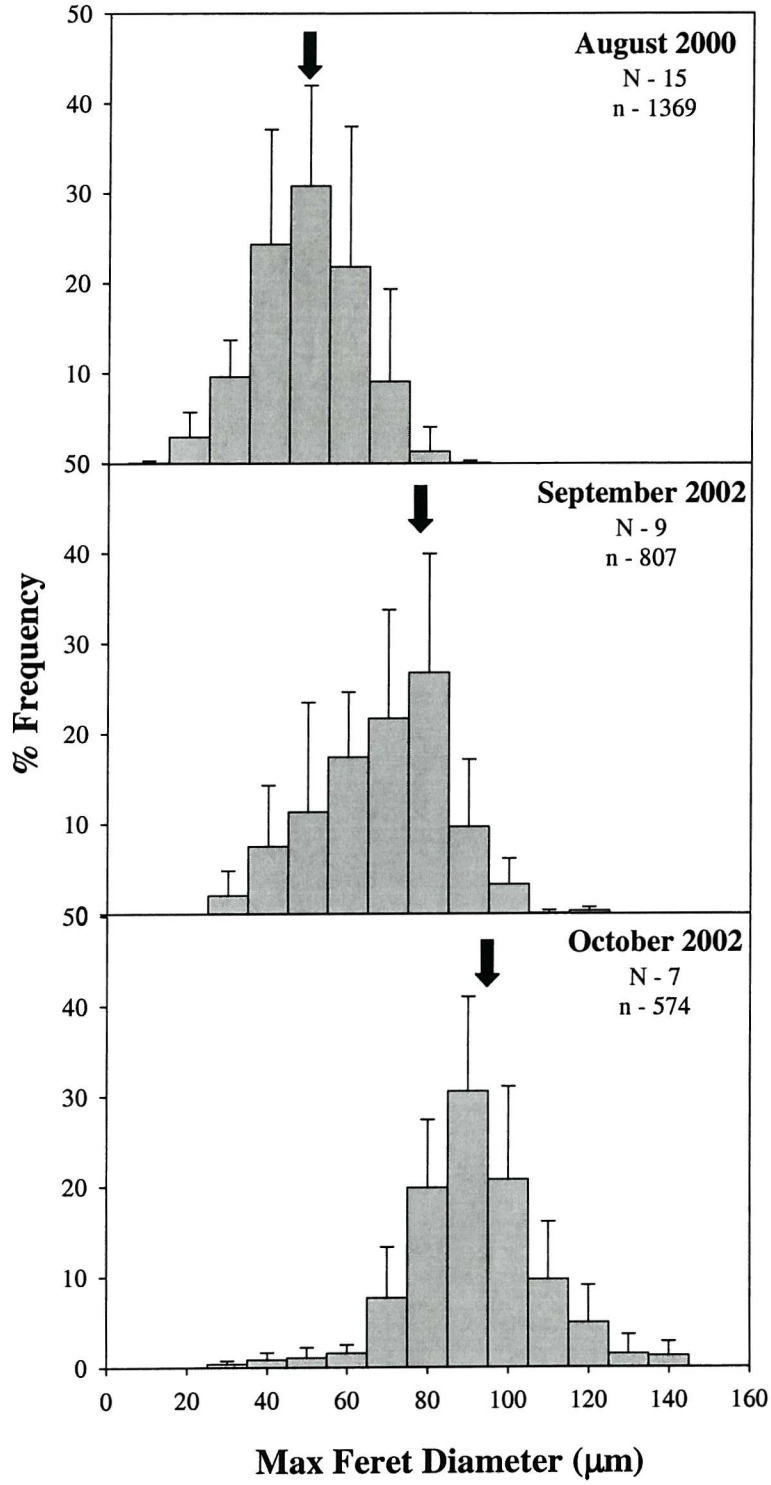


Fig. 3.6 – *Lophelia pertusa* sample mean oocyte size-frequency diagrams for August 2000, September 2002 and October 2002

N – Total number of polyps; **n** – Total number of oocytes measured; **Arrow** – mean oocyte diameter; error bars - ±SD

Madrepora oculata

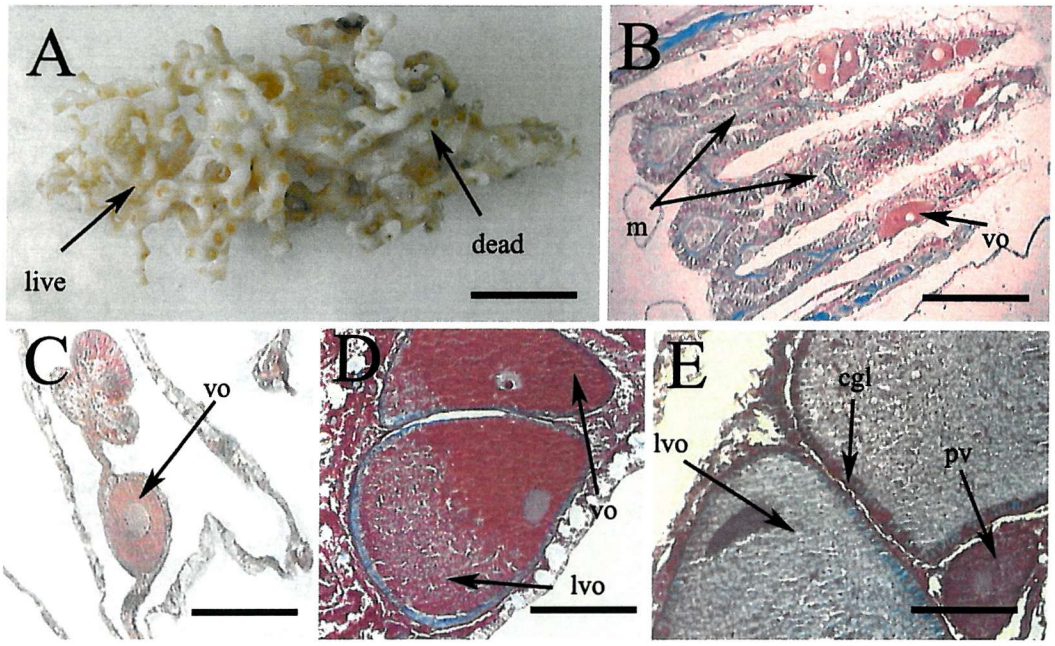


Fig. 3.7 – a. *Madrepora oculata* colony showing dead and live polyps; **b.** Female polyp showing position of vitellogenic oocytes; **c.** Vitellogenic oocyte within mesentery; **d.** Vitellogenic oocyte undergoing change to late vitellogenic oocyte; **e.** Late and previtellogenic oocytes
m-mesentery; **vo**-vitellogenic oocyte; **lvo**-late vitellogenic oocyte; **cgl**-cortical ganular layer; **pv**-previtellogenic oocyte
Scale bars **a** 2cm; **b** 400μm; **c** 300μm; **d** 150μm; **e** 250μm; Stained with Masson's Trichrome

Madrepora oculata

Reproductive Morphology

There are also white and orange skeletal forms of *M. oculata*, but, as with *L. pertusa*, the white morph appears less prolific. No white forms were obtained in our sampling programme. *M. oculata* is gonochoristic, with all mesenteries fertile (Fig. 3.7).

Gametogenesis

No male *M. oculata* were found during this study. In the female previtellogenic, vitellogenic and late vitellogenic oocytes were present within a single mesentery in the October sample

Stage I – Oogonia – >37μm diameter; small female gametes budding off the mesenterial lamellae

Stage II – Previtellogenic - 37μm to 200μm diameter; small oocytes with a thin wall and a basophilic cytoplasm (Fig 3.7e)

Stage III – Vitellogenic - 200μm to 350μm diameter; larger granulated oocytes with a thin wall (Fig 3.7b-d).

Stage IV – Late Vitellogenic - >350μm +. Large, highly granulated, with a thick cortical granule layer, presumably ready for spawning (Fig 3.7d-e).

Fecundity

The average fecundity varied over the months studied (Fig 3.8). The lowest fecundity recorded was during March, with an average of 10 oocytes per polyp (±8.26). In August 28 oocytes per polyp (±8.26) were recorded and October 68 oocytes per polyp (±24.15).

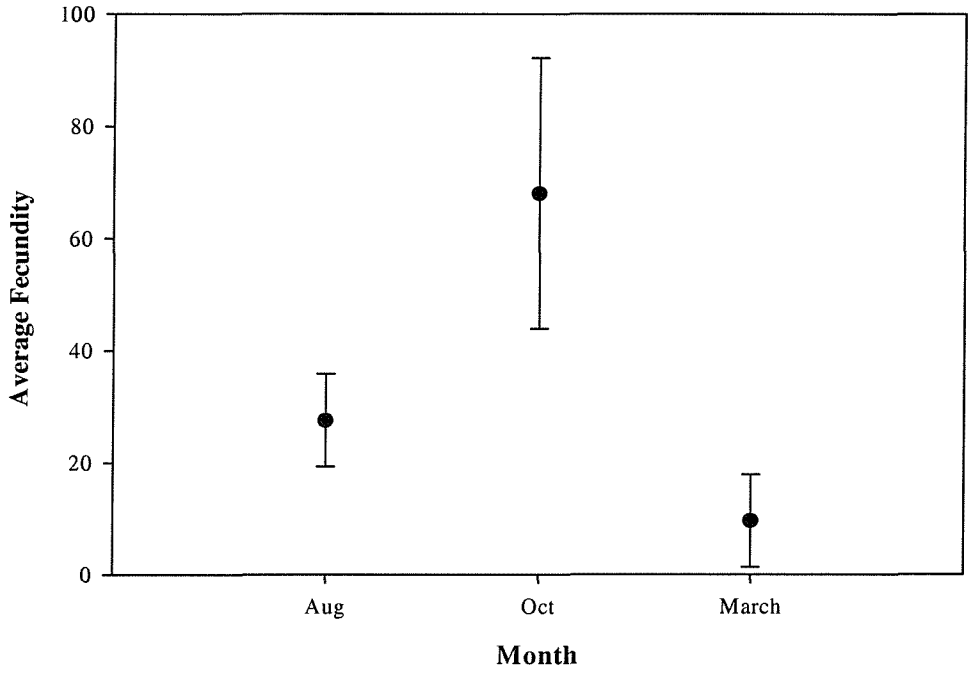


Fig. 3.8 – *Madrepora oculata* mean fecundity for August 2000, October 2002 and March 2002
All animals size corrected to 1661μm polyp diameter; error bars - ±SD

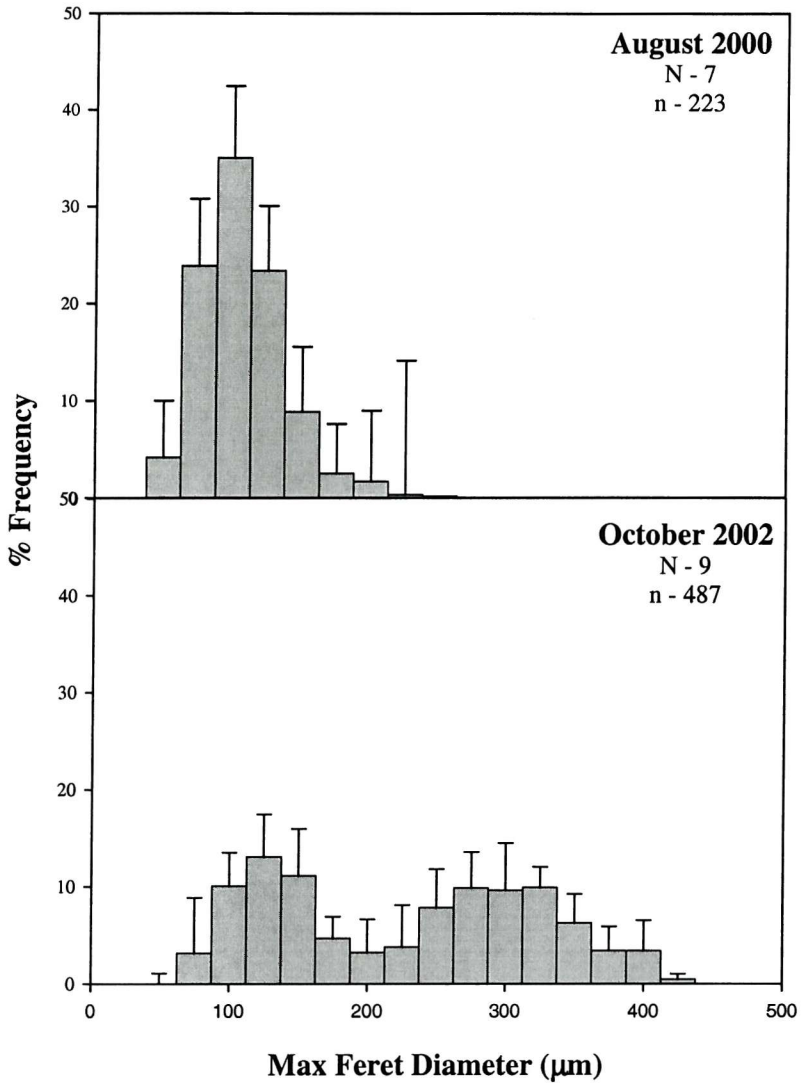


Fig. 3.9 – *Madrepora oculata* sample mean oocyte size-frequency diagrams for August 2000 and October 2002
N – Total number of polyps; **n** – Total number of oocytes measured; error bars - ±SD

The smallest reproductive individual had a polyp diameter of 1.24mm. However non-reproductive individuals were found at a polyp diameter of up to 1.7mm. This suggests that, in these cases, a factor other than polyp size is controlling reproduction. There was no significant relationship between polyp diameter and fecundity (R^2=0.07, P=<0.05).

Colony fecundity was recorded at 36 oocytes cm^2 for March, 104 oocytes cm^2 for August and 256 oocytes cm^2 for October. This gives a colony of around $30cm^2$ a fecundity of 7684 oocytes in October.

Reproductive Periodicity

Females oocyte size-frequency plots were collated within a month, as individuals appeared synchronous (see Appendix III for individual plots). One cohort could be seen in the August sample (Fig. 3.9), whereas in the October sample two distinct cohorts can be seen. Oocyte growth appears rapid, more than doubling in size between August and October. Maximum oocyte size is 405μm. The March sample did not produce enough oocytes to construct oocyte size-frequency diagrams (i.e. >50).

3.1.4 Colour Morphology Results and Discussion

None of the *Lophelia pertusa* colonies analysed were reproductive. This could be because of the time of year they were sampled, July. Waller and Tyler (in press, this Thesis) showed *L.pertusa* in the Porcupine Seabight to be beginning reproduction in the late summer, and spawning over the winter. These samples were at much shallower depth than those from the Porcupine Seabight, and so it would be expected that some degree of difference in reproductive habit would occur, especially when considering that scleractinians are renowned for their reproductive plasticity (Fadlallah, 1983; Richmond & Hunter, 1990; Richmond, 1997). Another factor that may be affecting reproduction is colony size. In many species of shallow water reef-building scleractinians, colonies will not begin to reproduce until after a size-dependent juvenile phase is completed (Kojis & Quinn, 1982; Szmant, 1986; Hall & Hughes, 1986). Polyp size has also been attributed to reproduction (Harriot, 1983; Van Veghel & Kahmann, 1994; Schlesinger et al., 1998). However, polyp wet weight was not significantly different from the samples used for the previous study (U=23000.5, P=0.8693), and so this is unlikely to be a factor. It is likely colony size may govern reproduction in this species (also see section 3.1.5).

There are also further factors that must be considered in discussing these particular corals reproductive habit. These samples of *L. pertusa* were collected from an oil platform in the North Sea, whereas those in the study by Waller and Tyler (in press, this Thesis) were in the Porcupine Seabight, a relatively 'clean' habitat compared to the North Sea. Numerous shallow water studies have looked at the effects of chemicals and oils on the reproduction of corals (Loya & Rinkevich, 1979; Loya & Rinkevich, 1980; Harrison & Ward, 2001) as well as other factors (Patrick & Duke, 1990; Guzman & Jimenez, 1992; Koop et al., 2001). There is the possibility that these corals reproduction may be being affected by oils and chemicals released by these platforms.

However, should these colonies be on the same schedule as those from the Porcupine Seabight, it is likely that reproduction does not begin for this seasonal spawner until August. The study undergone by Waller and Tyler (in press, this Thesis) was not a full monthly study, and so the exact date reproduction begins is still unknown.

3.1.5 Discussion

There are two main reproductive patterns in marine invertebrates, the production of small numbers of large oocytes, and the production of large numbers of small oocytes (Gage & Tyler, 1991). In shallow reef building scleractinians, Harrison and Wallace (1990) showed there was an inverse relationship between oocyte size and fecundity. In deeper water scleractinians, Brooke (2002) observed *O. varicosa* producing large numbers (1,000 to 4,800 oocytes cm^2 skeletal area) of small oocytes (<100μm diameter) at 500m in Florida. In the bathyal scleractinians from the NE Atlantic, *Lophelia pertusa* produces relatively large numbers of small oocytes (3327 oocytes cm^2 140μm maximum diameter) whereas *M. oculata* produces small numbers (256 oocytes cm^2 skeletal area) of large oocytes (405μm max).

The presence of previtellogenic oocytes in the August sample *L. pertusa*, followed by rapid oocyte development, suggests that oogenesis is likely to be initiated in late summer. However, without samples obtained during the summer, this can only be speculated. The oocyte size-frequency samples for *L. pertusa* for August, September and October show an increasing mean oocyte size. This, together with the absence of developing gametes, and the observation of two reabsorbing oocytes in the March sample of *L. pertusa* suggests that this species has an annual gametogenic cycle with spawning around January/February. Such a gametogenic pattern in a bathyal species would be coincident with other deep-sea seasonally-spawning species in the NE Atlantic (Tyler and Young, 1992). Conversely the data for oocyte size-frequency, and the large oocyte size, in *M. oculata* may support a seasonal pattern of gametogenesis. There is, however, evidence of multiple cohorts of developing oocytes (Fig. 3.9). This would in turn suggest a episodicity of reproduction, rather than true seasonality.

Most shallow-water scleractinians have a cue for spawning (Fadlallah, 1983). In shallow water species this can be temperature, lunar phases, tidal cycles or, in synchronous spawners, the presence of other gametes in the water. For deep-water species the cues are less obvious. There is little temperature change at depths below 800m, and there is no solar radiation. Studies in the NE Atlantic have shown that seasonal blooms of surface primary production sink rapidly to the deep-sea floor (Billett et al., 1983; Lampitt, 1985; Thiel et al., 1989) and can have an effect on the

reproductive biology of benthic invertebrates (Billett et al., 1983; Tyler et al., 1992; Tyler et al., 1993; Eckelbarger & Watling, 1995). Seasonal phytoplankton blooms in the Porcupine area reach the deep benthos in July (Lampitt et al., 2001), and this could be the cue for *L. pertusa* and *M. oculata* to initiate (or complete) gametogenesis. At this time there would be the extra food available to begin the, energetically expensive, production of gametes. It is unkown what cnidarians feed on but they are unable to scavenge actively for food, and so it is likely they rely heavily on food fall to these bathyal depths.

A lecithotrophic larvae would appear the most suitable for such conditions. The extra energy put into producing larger planulae serves as a food store until settlement takes place. In the deepwater coral *Oculina varicosa*, settlement has been observed to occur between 20 and 35 days in the laboratory (Brooke, 2002). Lecithotrophic larvae of echinoderms have also long been shown to be suitable for long distance dispersal (Shilling & Manahan, 1994).

In *M. oculata*, polyp size overlapped for non-reproductive and reproductive polyps, suggesting that a factor other than size controls gametogenesis. It has been reported in shallow water corals that total colony size can control reproduction (Kojis & Quinn, 1982; Szmant, 1986; Hall & Hughes, 1996). A colony would asexually reproduce, extending total size, until large enough to overcome juvenile size-related mortality, and then proceed to sexual reproduction. Due to collection methods in the present study this was not observed, though is likely to be an important aspect of the ecology of these two species, as both species do not appear to show polyp size related fecundity.

No reproductively-active polyps were observed in *L. pertusa* taken from the Darwin Mounds. This area has been observed to be extensively damaged by trawling operations (See Chapter 1). Although colonies appeared comparable in size to those taken from the Porcupine Seabight, around 49% of the colonies from here were composed of dead polyps. This is compared to an average of 8.3% dead polyps for the Thérèse Mound and 2% for the Southern Porcupine Seabight site (pers. obs.). Scleractinian colonies have to reach a certain size before reaching reproductive maturity (Kojis & Quinn, 1982; Szmant, 1986; Hall & Hughes, 1996). It is

hypothesised that the constant trawling in this area may keep the *L. pertusa* colonies at a size below that necessary for gametogenesis to occur. Rinkevich and Loya (1989) observed that removal of even 23% of a colony of *Stylophora pistillata* causes sterility for up to a year. There is also the possibility of polyp suffocation from increased sediment suspension. Dodge and Vaisnys (1977) have shown that extended dredging operations have had a destructive effect on shallow-water coral communities. Genetic analysis of populations in the Darwin Mounds area also support these findings, and show that there is little sexual reproduction occurring (LeGoff – Vitry & Rogers, in press). Microsatellite and ITS1 and 2 sequences were used to examine ten sample sites around the European Margin. These authors found that these populations were not one panmictic population, but actually small sub-populations. Of these ten sites examined, the Darwin Mounds had the least genetic diversity, and asexual was the predominant mode of reproduction occurring.

Shallow reefs have long been under threat from man's misuse (Brown, 1987). Pollution, coastal development, detrimental fishing practices and climate change have all had their effect on the reef ecosystem. Reduction of colony size through damage has been shown to raise mortality rates (Loya & Rinkevich, 1979; Rinkevich & Loya, 1989; Ward, 1995), increase disease (Rogers et al., 1988) and reduce reproductive output (Loya & Rinkevich, 1980; Szmant-Froelich, 1985; Zakai et al., 2000). *Seriatopora hystrix* initiates a 'polyp bail-out' response to environmental stress, leaving large areas of empty skeleton (Sammarco, 1982). Pollutants can also have detrimental effects; heavy metals have been found within coral skeletons (Guzman & Jimenez, 1992). Harrison and Ward (2001) found elevated levels of ammonia and phosphates reduced fertilisation, and areas that have been oil-contaminated can stop colonisation, cause reduced reproductive output, lower planulae life expectancy, cause abnormal behaviour (Loya & Rinkevich, 1980), and abortion of planulae (Loya & Rinkevich, 1979).

The Great Barrier Reef was declared a World Heritage Site in 1981, to protect and preserve the area for future generations. There have been suggestions for the Darwin Mounds to become a Marine Protected Area, thus preventing any unauthorised use (Gubbay et al., 2002). The suspension of fishing operations in the Darwin Mound area has recently occurred (see Chapter One), and is necessary to allow *L. pertusa* and *M.*

oculata colonies to recover to a sexually viable size. The Porcupine Seabight area, although a healthier community, is also under pressure from fishing and exploration operations. Numerous fishing gears have been observed over Thérèse Mound (Grehan et al., 2003). Lessons that have been learned from shallow water reefs must be implemented in deep-water situations, to save these important habitats.

Should this species be sexually dimorphic, it would be vitally important to design management strategies around this factor. By conserving a single colour in one area would be highly detrimental to both the whole local population, and to populations further afield that may be seeded by these colonies. This part of the study urgently needs repeating, as knowledge of the precise reproductive ecology of deep-water reef building corals is essential to producing any management strategy.

3.2 Reproduction of Deep-Water Solitary Scleractinians

Introduction

Azooxanthellate scleractinian corals are both diverse in morphology and cosmopolitan, being found in all the worlds oceans (Zibrowius, 1980). Species are either reef-builders, such as *Lophelia pertusa*, or occur as solitary polyps. Deep-water solitary species are more diverse in morphology, can inhabit sedimented and rocky substrata, and have been found at greater depths than reef-builders (Cairns, 1979; Zibrowius, 1980). The numbers of azooxanthellate solitary corals outnumbers reef builders (Zibrowius, 1980; Cairns, 1982), and yet knowledge of their ecology or spatial distribution is limited.

The reproductive questions posed for solitary corals compared to colonial scleractinians differ. As discussed in the previous section, large reef building scleractinians both form and function as one structure, even though each polyp is a separate entity. Sexual maturity may not be reached until the whole unit reaches a determined size, damage to the unit can have colony-wide reproductive consequences, and even cause mass colony mortality. Whereas each polyp of a colonial species is a genetic clone, solitary corals form just a single unit and so, maybe, much more genetically diverse. Their local distribution is largely unknown, yet it is this factor that could prove the most important in their reproductive biology. What reproductive patterns have evolved in these corals to overcome their lack of mobility and the great vastness that is the deep-sea environment?

The major taxa of deep-water solitary corals includes the genus *Fungiacyathus* (Family Fungiacyathidae), *Caryophyllia* (Family Caryophylliidae) and *Flabellum* (Family Flabellidae). This section describes the reproductive biology of nine species of deep-water solitary scleractinian. Six of these species were collected from the NE Atlantic at differing depths, three species were collected from waters to the west of the Antarctic Peninsula.

3.2.2 Gametogenesis in *Fungiacyathus marenzelleri*

Introduction

Fungiacyathus marenzelleri (Vaughan, 1906) is a solitary, deep-sea scleractinian, which is known to occur from 730m to 5870m depth (Zibrowius, 1980) and has a wide distribution, occurring in the Atlantic (Zibrowius, 1980) and the Pacific (Lauerman et al., 1996). Although the morphology of this species has been well-described (Cairns, 1979; Zibrowius, 1980), little is known of its ecology or life history biology. Three species of *Fungiacyathus* inhabit the NE Atlantic, *F. crispus*, *F. fragilis* and *F. marenzelleri*. *F. crispus* is the smallest, reaching a maximum of 9mm diameter and is found at the shallowest depths (<1500m) (Zibrowius, 1980). *Fungiacyathus fragilis* inhabits depths from 366m to 2200m, and coexists with *F. marenzelleri* at ~2000m (Zibrowius, 1980). *Fungiacyathus fragilis* and *F. marenzelleri* have similar morphologies and so from depths around 2000m must be carefully distinguished by septal differences outlined in Zibrowius (1980).

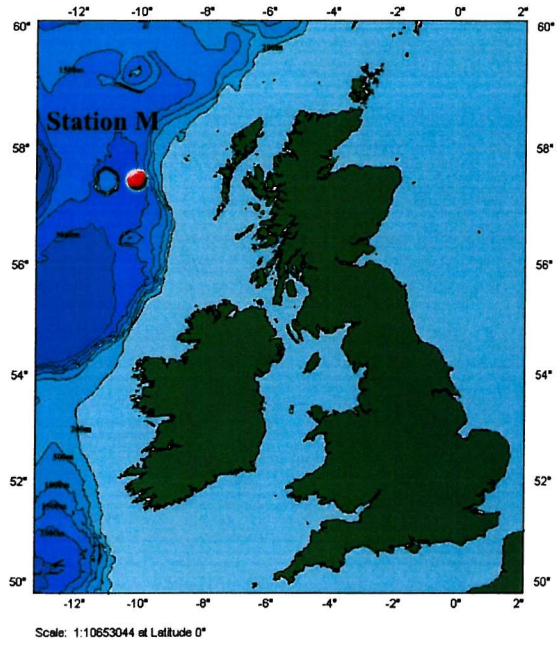


Fig. 3.10 - Map of Station M in the NE Atlantic

Materials and Methods

All specimens were obtained using either a 3m Agassiz Trawl, or an Otter Trawl Semi-Balloon (14m headrope), from RRS *Challenger* in the area around Station 'M' (57° 18'N, 10°11'W)(Gage and Tyler 1982) (Fig. 3.10; Table 3.4). This station is at a depth of 2200m.

After decalcification, mesenteries from 20 whole individuals total, from varying months, were counted and had their structure noted. Roughly a quarter of the polyp

tissue was histologically processed. All wax blocks were serially sectioned at 5µm, leaving 75µm in between slides.

The number of oocytes in the two mesenteries were averaged and that number is hereby referred to as the 'realised fecundity'. 'Potential fecundity' is the realised fecundity multiplied by the number of mesenteries. Mean realised fecundity was calculated for each sample.

		Area		
Cruise No.	**Date**	**Longitude**	**Latitude**	**No. analysed**
Challenger 1A/79	15 Jan 79	57° `20N	10° `27W	20
Challenger 75	17 Feb 91	57° `19N	10° `23W	20
Challenger 4/80	03 Mar 80	57° `16N	10° `17W	30
Challenger 6A/81	12 April 81	57° `22N	10° `19W	20
Challenger 9/80	29 May 80	57° `18N	10° `16W	20
Challenger 10/83	24 July 83	57° `07N	09° `23W	0 - Poor condition
Challenger 12B/81	18 Aug 81	57° `21N	12° `02W	20
Challenger 15A/81	19 Oct 81	57° `27N	11° `10W	16
Challenger 86	19 Nov 91	57° `18N	10° `24W	25
Challenger 74	14 Dec 90	57° `15N	10° `21W	15

Table 3.4 – Table of colonies analysed

Results

Mesenterial Structure

48 mesenteries are present in the mesentery, arranged in cycles of four reducing in number towards the anterior. The mesenterial mesogloea is thick at the periphery of the polyp, and reduces in width towards the small central oral cavity.

All individuals of *Fungiacyathus marenzelleri* examined were gonochoristic. Sex ratios averaged over the nine months analysed show a 1:1 ratio, varying only slightly

among months ($\chi 2$ =0.882, P=0.01). Reproductive tissue was found in high densities at the base of the individual, reducing in occurrence towards the anterior. Only five individuals did not have either oocytes or spermacysts present on all mesenteries. Each mesentery produces embedded reproductive structures, though there is no obvious external morphological difference between males and females.

Asexual Reproduction

Fungiacyathus marenzelleri undergoes asexual reproduction by a form of fission (Fig. 3.11), followed by complete detachment of the new polyp when it reaches a certain size (>6mm diameter, as this was the smallest found).

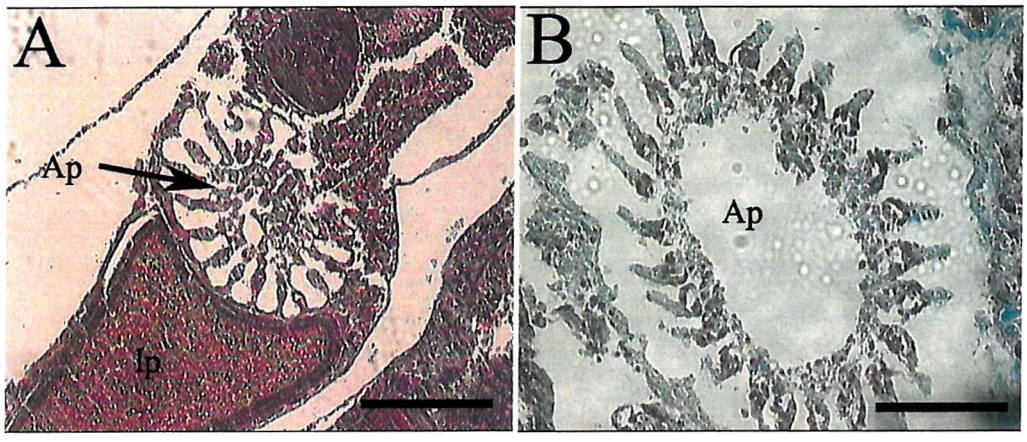


Fig. 3.11 - **A**. Early asexually produced bud in female *F. marenzelleri* polyp; **B**. Early asexually produced bud in *F. marenzelleri* polyp

Ap, Asexual polyp; **Lv,** Late vitellogenic oocyte; **Scale bars** - 40µm; Stained with Masson's Trichrome

The new polyp grows on the anterior surface of the coral. Both sexes were observed to undergo fission. The asexually produced polyp buds from a single mesentery (Fig. 3.11) and is composed of mesenteries with a central oral area. No more than one bud was found on any individual. There was a low incidence of asexual reproduction in the samples studied. The maximum number of individuals undergoing asexual fission in any month sample was two, with four months having no individuals budding. There was no temporal pattern in the monthly incidence of asexual reproduction in polyps.

Sexual Reproduction

Spermatogenesis

Ovoid spermacysts occurred throughout, and in all, the mesenteries. Each mesentery contained many spermacysts at varying stages of differentiation. There appeared to be no specific arrangement of spermacysts within the mesenteries. Spermatogenesis can be divided into four stages :-

Stage One (Early, Fig. 3.12a) - Loosely packed aggregations of spermatocytes contained within a cell membrane. Empty lumen can be seen.

Stage Two (Maturing, Fig. 3.12a, b) - Some spermatozoa present, still loosely packed. Lumen less distinct.

Stage Three (Mature, Fig. 3.12c) - Densely packed with spermatocytes and lumen packed with spermatozoa.

Stage Four (Spent, Fig. 3.12d) - Relict spermatozoa can be seen.

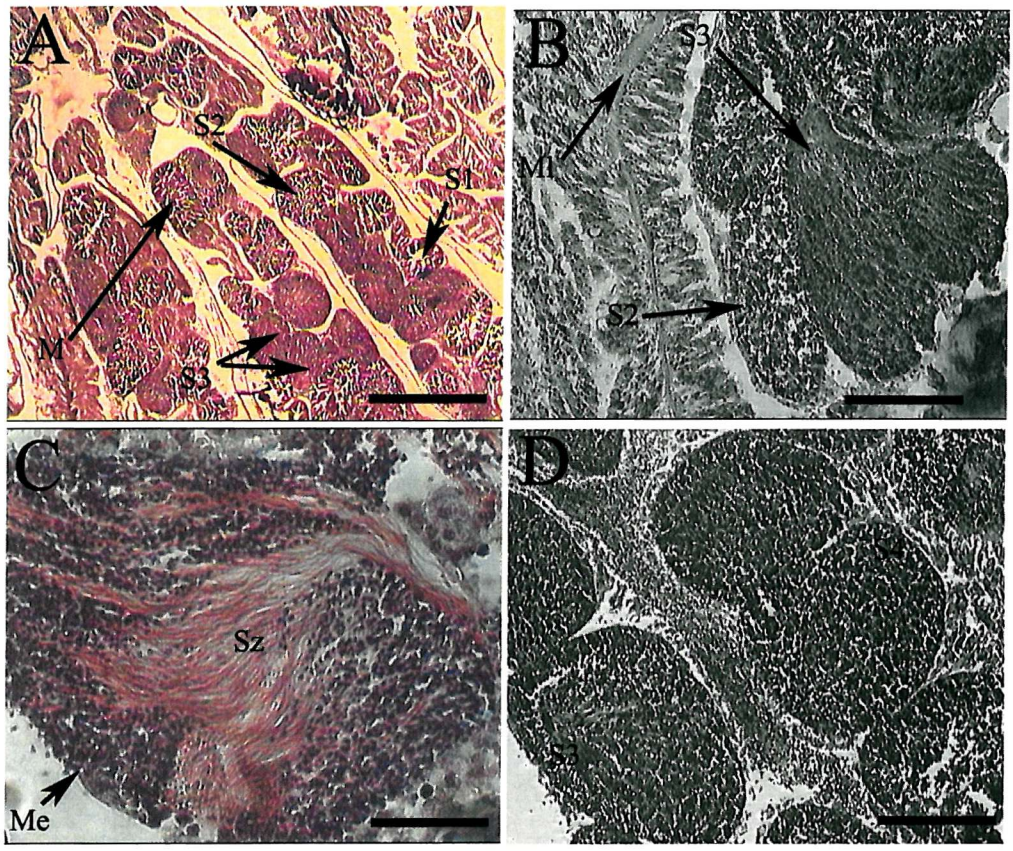


Fig. 3.12 - **A**. Arrangement of spermacysts within *F. marenzelleri* mesenteries; **B**. Stage II and III spermacysts; **C**. Stage III spermacysts; **D**. Stage III and IV spermacysts

M, Mesentery; **SI**, Stage I spermacyst; **S2**, Stage II spermacyst; **S3**, Stage III spermacyst; **Me**, Mesogloear envelope; **Sz**, Spermatozoa; **Sc**, Spermacyst; **S4**, Stage 4 spermacyst; **Scale bars** - **A**, 200μm; **B**, 40μm; **C**, 20μm; **D**, 40μm

Stained with Masson's Trichrome

Oogenesis

Oogonia arise from the mesogloea of the mesentery and develop into 'previtellogenic' oocytes at <28µm diameter (Fig. 3.13a). These early oocytes, which have a central nucleus, are attached to the lamellae of the mesentery and are surrounded by follicle cells. Previtellogenic oocytes then undergo vitellogenesis at ~150µm diameter, and continue to accumulate yolk up to the maximum of 750µm diameter (Fig. 3.13a to d), and are classed as 'vitellogenic'. At >300µm a cortical granular layer develops (Fig. 3.13c). These oocytes were classed as 'late vitellogenic'. Large yolk vacuoles and a prominent nucleolus in the central nucleus can now also be observed.

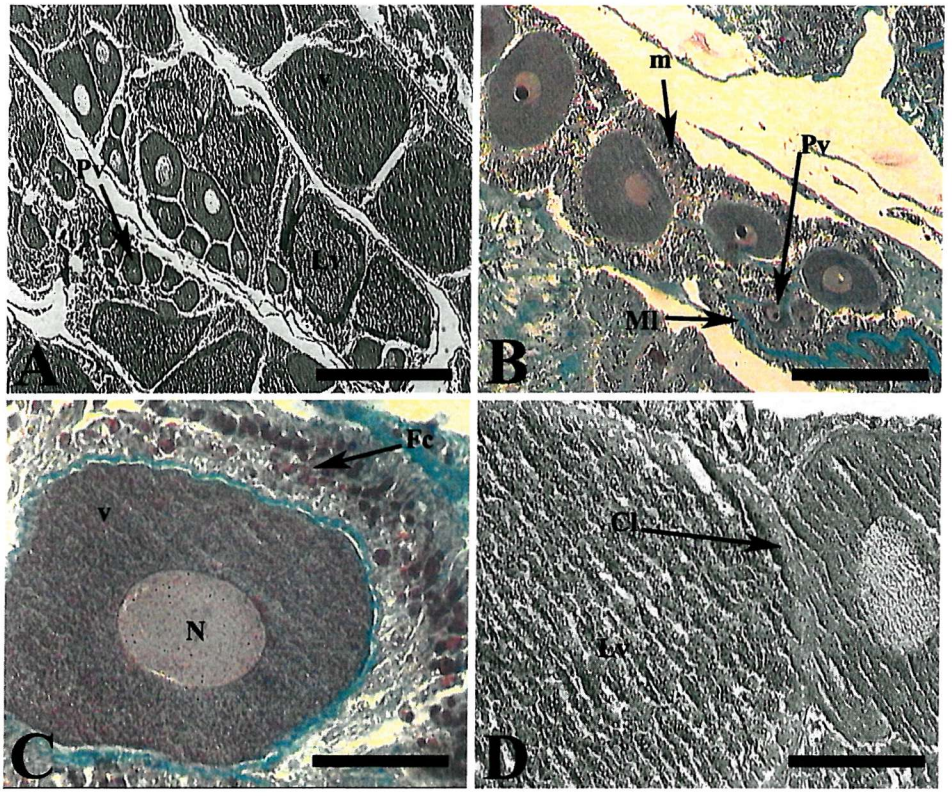


Fig. 3.13 - **A.** *F. marenzelleri* mesentery with previtellogenic, vitellogenic and late vitellogenic oocytes**; B.** Section showing young oocyte attachment to *F. marenzelleri* mesentery; **C.** Vitellogenic oocyte; **D.** Thick cortical layer

Fc, Follicle cells; **N**, Nucleus; **V**, vitellogenic oocyte; **M**, Mesentery; **Ml**, Mesentery Lamellae; **Pv**, Previtellogenic oocyte; **Lv**, Late vitellogenic; **Cl**, Cortical layer; **Scale bars - A**, 200µm, **B**, 200µm, **C**, 20µm, **D**, 20µm
Stained with Masson's Trichrome

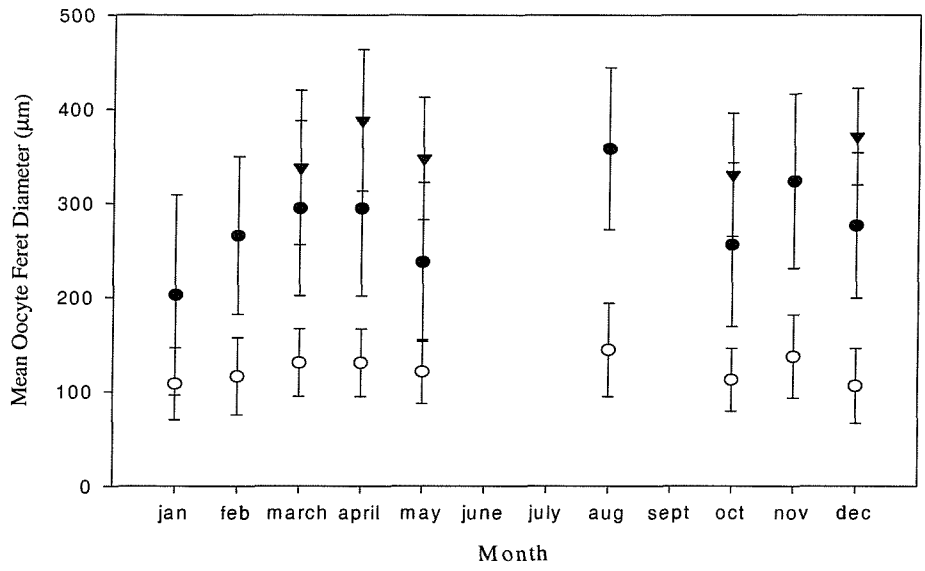


Fig. 3.14 – *F. marenzelleri* Monthly mean previtellogenic, vitellogenic and late vitellogenic oocyte diameters

(▼ - Late Vitellogenic; ● - Vitellogenic; ○ - Previtellogenic; Error bars = ±1sd)

Oocytes of varying stages occurred throughout and in all the mesenteries (Fig. 3.13a), though ten polyps (from all examined) in total contained only previtellogenic or vitellogenic oocytes. No planulae or brooded individuals were found during histological examination.

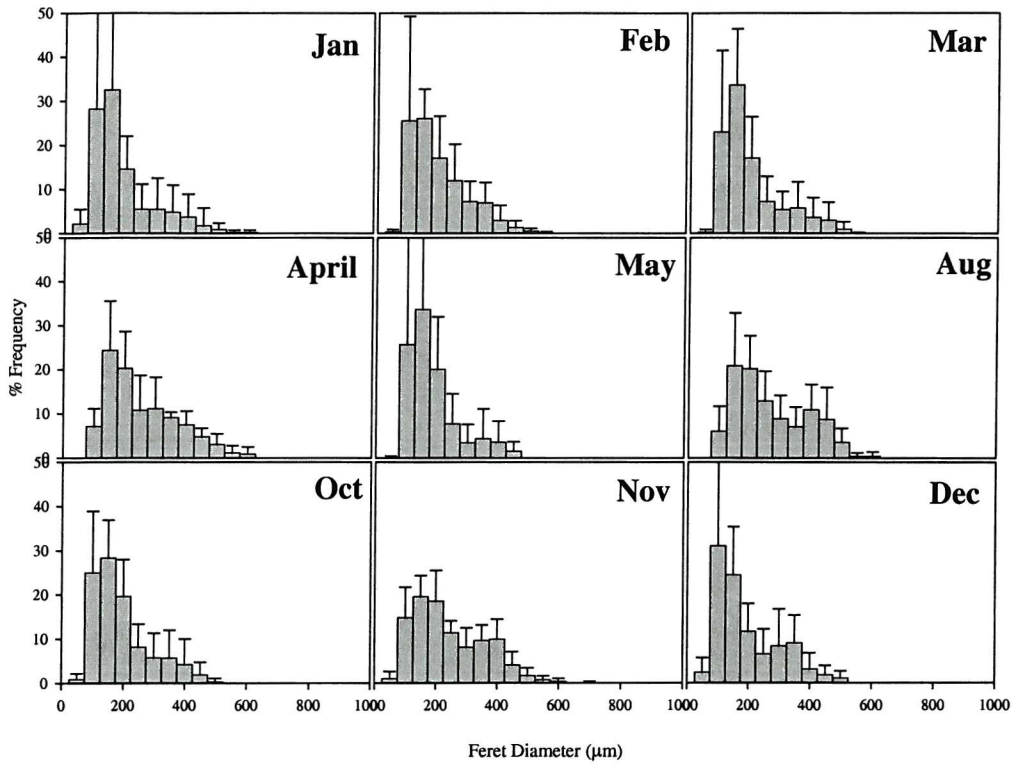


Fig. 3.15 – *F. marenzelleri* oocyte size-frequency distribution for mean monthly samples (Error bars = ±1 sd)

Analysis of the distribution of the three stages of oocyte development (Fig.4) or the oocyte size-frequency (Fig.3.15) gave no evidence of a marked annual reproductive periodicity. Individuals within a month were synchronous and so individual frequencies were collated (see Appendix III for individual plots). This suggests that *Fungiacyathus marenzelleri* is a quasi-continuous reproducer. All three classes of oocytes appear to develop simultaneously, though during February, August and November no late vitellogenic oocytes were found, suggesting they had spawned prior to, or during, collection. The presence of all levels of sperm development in spermacysts within the same mesentery (overlapping gametogenesis) suggests a quasi-continuous release of gametes. The lack of late vitellogenic oocytes in the summer months suggest there may be a variation in the intensity of gamete production rather than a true seasonality (*sensu* Harrison, 1988).

Fecundity

The realised fecundity of each individual within a month's sample was averaged, producing colony fecundity. This number varied significantly between months (Fig.3.16). This suggested an annual periodicity in fecundity with maximum fecundity being reached during April, and spawning during May/June, supporting the suggestion of a variation in intensity of reproduction within a year. The average potential fecundity (realised fecundity multiplied by the number of mesenteries [48]), for all ten months, was 2892 (±44.435).

The size of first reproduction was around 10mm polyp diameter, as both non-reproductive and reproductive individuals were found at this size. No reproduction was observed below this size. Fecundity was size-dependent (Fig 3.17) and conformed to a second order polynomial curve ($R^2 = 0.7132$).

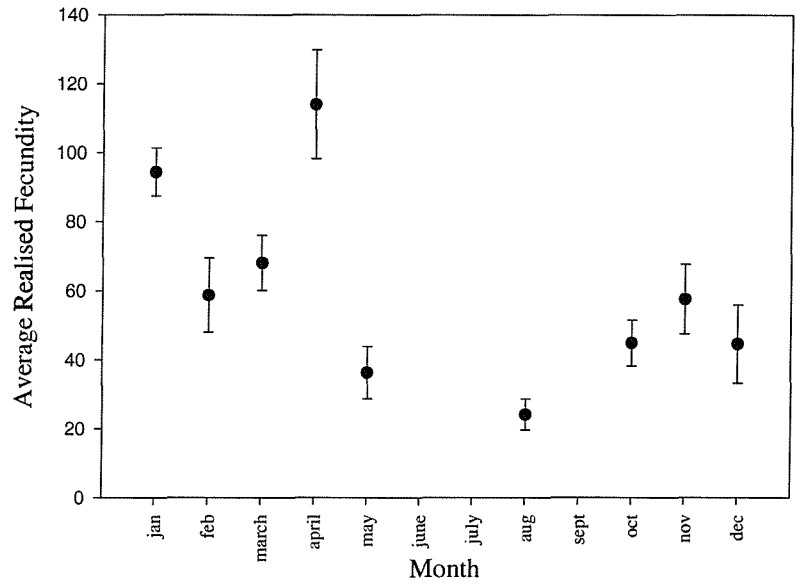


Fig. 3.16 – *F. marenzelleri* mean realised fecundity for monthly samples
(Error bars = ±1 sd) Size corrected to 1.834cm skeletal polyp diameter

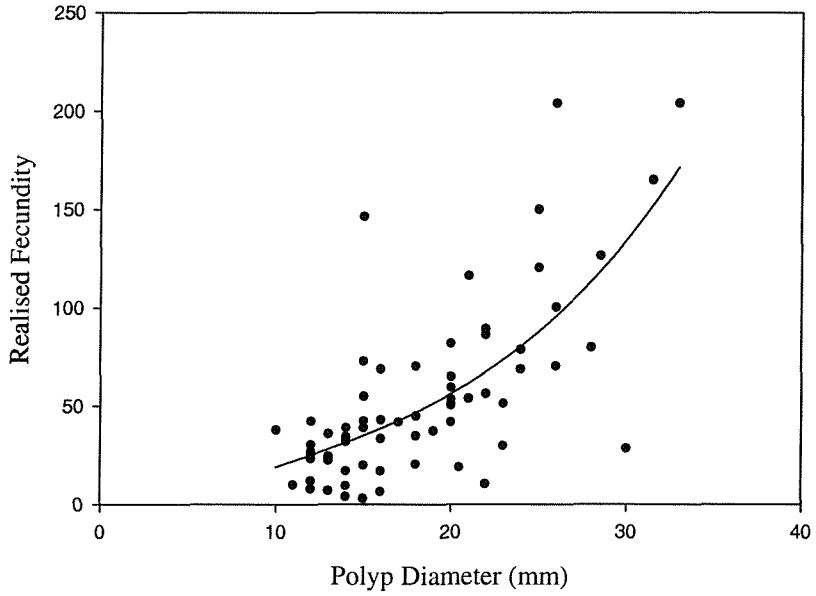


Fig. 3.17 - *F. marenzelleri* skeletal polyp diameter plotted against average realised fecundity with a fitted second order polynomial curve
(N-83, R^2=0.7132 [$y=32.071x^2 - 63.089x + 53.235$]) Size corrected to 1.834cm skeletal polyp diameter

Discussion

All samples were obtained from Station 'M' in the Rockall Trough (Gage & Tyler, 1982). This station lies between the Anton Dohrn Seamount and the Hebrides Shelf in the North East Atlantic, an area known for its high species diversity (Gage, 1979). Examination of the reproductive biology of a wide variety of marine invertebrates has shown that different species show quasi-continuous, seasonal or opportunistic reproductive patterns (Tyler & Young, 1992). In addition, there are good environmental data from this site (Dickson et al., 1986; Ellett et al., 1986; Holliday et al., 2000).

In shallow water scleractinians, gametes develop within the lamellae of the mesenteries and subsequently migrate into the mesogloea, to develop as oogonia (Szmant-Froelich et al., 1980; Fadlallah, 1983). Oogonia of *Fungiacyathus marenzelleri* were first observed attached to the lamella. We believe that the gametes of *F. marenzelleri* also originate in this area and then migrate into the mesogloea, in common with other corals and anthozoans (Fadlallah & Pearse, 1982; Fadlallah, 1983).

All the developing individuals examined were found to be gonochoristic, whereas the majority of scleractinians are hermaphrodites (Fadlallah, 1983; Harrison et al., 1984; Szmant, 1986). The random mixture of males and females amongst size classes mitigates against sequential hermaphroditism. Although gonochorism has been suggested as a more primitive strategy than hermaphroditism (Goffredo et al., 2000) it has been shown to be important for genetic diversity of populations (Szmant, 1986). There are certain restrictions, however, in being a gonochoristic individual; spawning must occur synchronously and densities of both individuals and gametes must be sufficiently high to allow successful fertilization. Because of their lack of mobility as an adult, an individual that settles and grows at very low density is less likely to pass its genes on to the next generation, than an individual in a high-density population. The spatial relationships between individuals of *Fungiacyathus marenzelleri* are critical in understanding this species' reproductive success. Lauerman et al. (1996) report densities of 0.0181 to 0.293m^2at 4100m depth off California.

Asexual reproduction in reef-building corals is an important adaptation for rapid colonisation of available areas, as competition for space is high (Szmant-Froelich et al., 1980; Fadlallah, 1983; Hall & Hughes, 1996). In solitary species this need would appear to be limited, especially within the deep-sea sedimentary environment where space is not limited. The solitary coral *Fungia scutaria*, which lives in shallow reef areas, has been found to asexually reproduce by budding and possibly from tissue fragments (Krupp, 1983). Population genetic studies have also shown that most of a local population is of the same genotype (Krupp, 1983). In *Fungiacyathus marenzelleri* asexual proliferation appears in such low densities to suggest that this would be the second mode of reproduction, with sexually produced polyps comprising the majority of the population.

Planulation in deep-sea species is difficult to observe without *in vitro* cultures. However, histological evidence suggests that *Fungiacyathus marenzelleri* spawns gametes rather than broods. This is inferred by both the lack of planulae, and the varying stages of gamete development within a single polyp. The release of eggs and sperm as the normal mode of reproduction in the Cnidaria has been well documented for a number of species (Kojis & Quinn, 1981; Bothwell, 1982; Fadlallah & Pearse, 1982; Fadlallah, 1983; Harrison et al., 1984; Szmant, 1986; Harrison, 1990; Richmond & Hunter, 1990; Richmond, 1997). Stimson (1978) proposed that deeper-living (deep fore-reef) corals should broadcast gametes to facilitate a wide dispersal range required at depths. Rinkevich and Loya (1979) proposed that large-polyped species would spawn large numbers of large eggs. Szmant (1986) also noted that the larger-polyp species of Caribbean corals were gonochoristic broadcasters. The extra energy required for growth, defence and maintenance of the large polyp would mean the energy required to produce a brooded planulae would be unavailable. *Fungiacyathus marenzelleri* appears to fit both of these hypotheses. The large egg size suggests a lecithotrophic development rather than planktotrophic early development (Fadlallah, 1983; Gage & Tyler, 1991). Lecithotrophic development, contrary of earlier suggestions, is now recognised as an adaptation for wide dispersal in oligotrophic environments (Shilling & Manahan, 1994).

Body size, in most invertebrates and some vertebrates, usually has a major effect on the reproductive output (Gage & Tyler, 1991; Hall & Hughes, 1996). *Fungiacyathus*

marenzelleri has a strong size-dependent reproduction with a size of 10mm diameter for first reproductive output (Fig. 3.17). The fecundity for a single polyp is high when compared to both the polyp and colony fecundities of other scleractinian species. The Great Barrier Reef coral, *Goniastrea retiformis* has a polyp fecundity of 46 oocytes and a colony fecundity of around 360 oocytes (Hall & Hughes, 1996).

The majority of scleractinian corals have some form of reproductive periodicity, usually lunar or temperature controlled (Fadlallah, 1983). The individuals of *Fungiacyathus marenzelleri* sampled for this study came from ~2200m depth, an area below the permanent thermocline, and so there is little seasonal fluctuation in either temperature or salinity (Holliday et al., 2000). There are, however, data that suggest a marked seasonal flux of surface primary production to the deep-sea bed at this station, and elsewhere in the NE Atlantic, that has an influence on the reproductive biology of both infaunal and epibenthic invertebrates (Billett et al., 1983; Tyler et al., 1992, 1993). Although there is some evidence for a seasonal variation in intensity of reproduction in *F. marenzelleri* most of the evidence such as large egg size and lack of significant oocyte variation between samples suggest quasi-continuous reproduction. This reproductive strategy may benefit a deep-sea solitary coral, as an increased number of eggs broadens the chances of fertilisation and aids wide dispersal (Szmant & Froelich, 1980).

Flint (2003) examined *F. marenzelleri* from the NE Pacific, at a site ~2000m deeper than in this section. She observed the reproductive habit was unchanged, with gonochorism, spawning of gametes and oocyte sizes being the same. However the fecundity calculated for the Pacific site was around half the fecundity of this study from the NE Atlantic. The decreased food fall to this greater depth is likely to affect the energy available for reproduction, and so fecundity will decrease (Eckelbarger & Watling, 1995).

3.2.3 Reproduction in three NE Atlantic *Caryophyllia* spp.

Introduction

The reproductive biology is known for two shallow water species of *Caryophyllia*. The Devonshire Cup Coral, *Caryophyllia smithii*, can be found around the British coast (as well as throughout the Atlantic) from just a few metres depth, to over 1200m (Zibrowius, 1980). A gonochoristic seasonal reproducer, shallow *C. smithii* spawns gametes during March, and produces a planktotrophic larvae (Tranter et al., 1982), although there are reports of brooding in this species (Hiscock and Howlett, 1977). *Caryophyllia clavus* also broods its young, though little else is known (Fadlallah, 1983).

In this section we report on the gametogenic development and fecundity of three deep-water species of *Caryophyllia* inhabiting different, but overlapping depths in the NE Atlantic Ocean. *C. ambrosia* Alcock 1898 (Fig. 3.18a) lives between 1100 and 3000m depth, *C. sequenzae* Duncan 1873 (Fig. 3.18b) lives between 960 and 1900m depth whilst the most shallow species is *C. cornuformis* Pourtales 1868 (Fig. 3.18c) living between 435 and 2000m (Zibrowius, 1980). *C. ambrosia* is the most cosmopolitan of these three species, occurring in the Atlantic, Pacific and Indian Oceans. *C. cornuformis* has been found at numerous locations around the Atlantic, and *C. sequenzae* has only been found in the Eastern Atlantic.

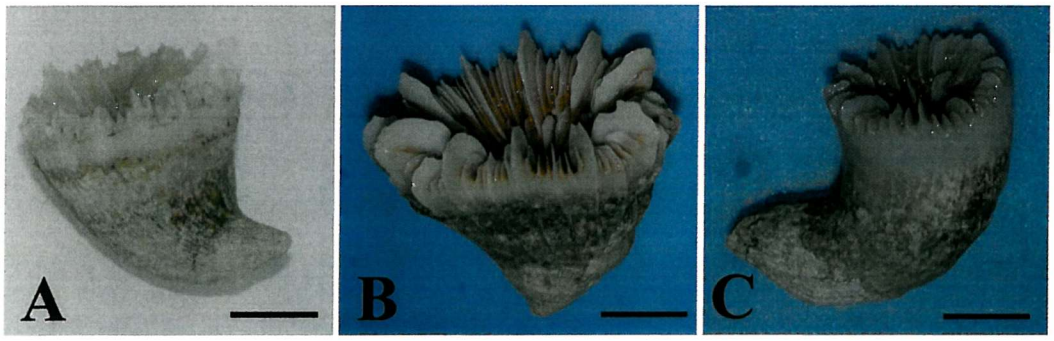


Fig. 3.18 – A. *Caryophyllia cornuformis*; **B.** *Caryophyllia seguenzae*; **C.** *Caryophyllia ambrosia*
Scale bars - A, 1cm; **B**, 2cm; **C**, 2cm

Materials and Methods

Samples were collected by either 3m Agassiz beam trawl or Otter Trawl Semi-Balloon (14m leadrope) from the research ships RRS *Challenger*, RRS *Charles Darwin* and RRS *Discovery* between 1978 and 2002 (Table 3.5) from the Porcupine Seabight and, for *C. cornuformis*, the Rockall Trough. After decalcification, all polyps were wet-weighed and mesenteries from 20 whole individuals, from each species (differing seasons), were counted and had their structure noted.

For histological preparation the whole polyps of *C. cornuformis* and 3 mesenteries of each of 15 individuals of *C. seguenzae* and *C. ambrosia* were processed. All wax blocks were serially sectioned at 5μm, leaving 50μm in between slides.

PRIMER 5 was used to statistically analyse, and allow grouping, of oocyte size-frequency plots.

Species	Date	Cruise	Depth	Latitude/Longitude
C. seguenzae	12.2.98	RRS *Discovery*	1278m	58° '58N 07° '57W
	24.4.78	RRS *Discovery*	1404m	50° '30N 12° '00W
	7.11.80	RRS *Darwin*	1250m	51° '04N 11° '50W
	19.10.02	RRS *Discovery*	1240m	49° '50N 12° '05W
C. ambrosia	20.6.85	RRS *Darwin*	2440m	51° '00N 12° '59W
	4.8.81	RRS *Challenger*	2713m	51° '05N 11° '48W
	21.3.02	RRS *Discovery*	2500m	49° '36N 12° '11W
	4.9.79	RRS *Discovery*	2315m	51° '00N 12° '03W
	1.10.02	RRS *Discovery*	2452m	50° '04N 12° '45W
C. cornuformis	10.3.93	RRS *Challenger*	1650m	57° '07N 09° '30W
	11.8.92	RRS *Darwin*	2017m	57° '00N 09° '58W

Table 3.5 – Samples used for this study

Results

Gametogenesis in the genus *Caryophyllia*

Caryophyllia ambrosia (Fig. 3.19a-e)

Five monthly samples were available for analysis. *C. ambrosia* is a cyclical hermaphroditic, where one sex appears to be dominant at any one time. Individuals are termed 'female' and 'male' hermaphrodites pertaining to the dominant sex (with 'Intermediate' in those that appear similar). PRIMER cluster analysis of the size frequency data (Fig. 3.20) show the three groupings to be significantly different from each other (R^2=>0.496; P=>0.001). Size-frequency diagrams were therefore collated (for full diagrams see Appendix III). Gametes of both sexes develop from the mesenterial lamellae (Fig. 3.19a). Both male and female gametes were found in several pockets throughout the same mesentery, and increase in density towards the actinopharynx.

Oogenesis

Oogenesis can be divided into four stages:-

Stage I – Oogonia (<50μm) are observed budding from the mesenterial lamellae

Stage II – Previtellogenic oocytes (<250μm) containing a large nucleus (Fig. 3.19a)

Stage III – Vitellogenic oocytes (<500μm) showing evidence of yolk accumulation (Fig. 3.19b, c)

Stage IV – Late Vitellogenic oocytes (>500μm) that have a thick cortical granular layer just inside the oolemma, large yolk granules and prominent nucleolus in the central nucleus (Fig. 3.19d)

Oocytes within a single individual were at the same stage of development whereas individuals from the same sample showed asynchrony in oocyte development. Oocyte size-frequency diagrams show this asynchrony and the unimodal development of oocytes (Fig. 3.21). The maximum oocyte diameter observed was 700μm suggesting lecithotrophic development. Hermaphroditic individuals (Fig. 3.19e), containing developing oocytes, were found in all sizes of polyp.

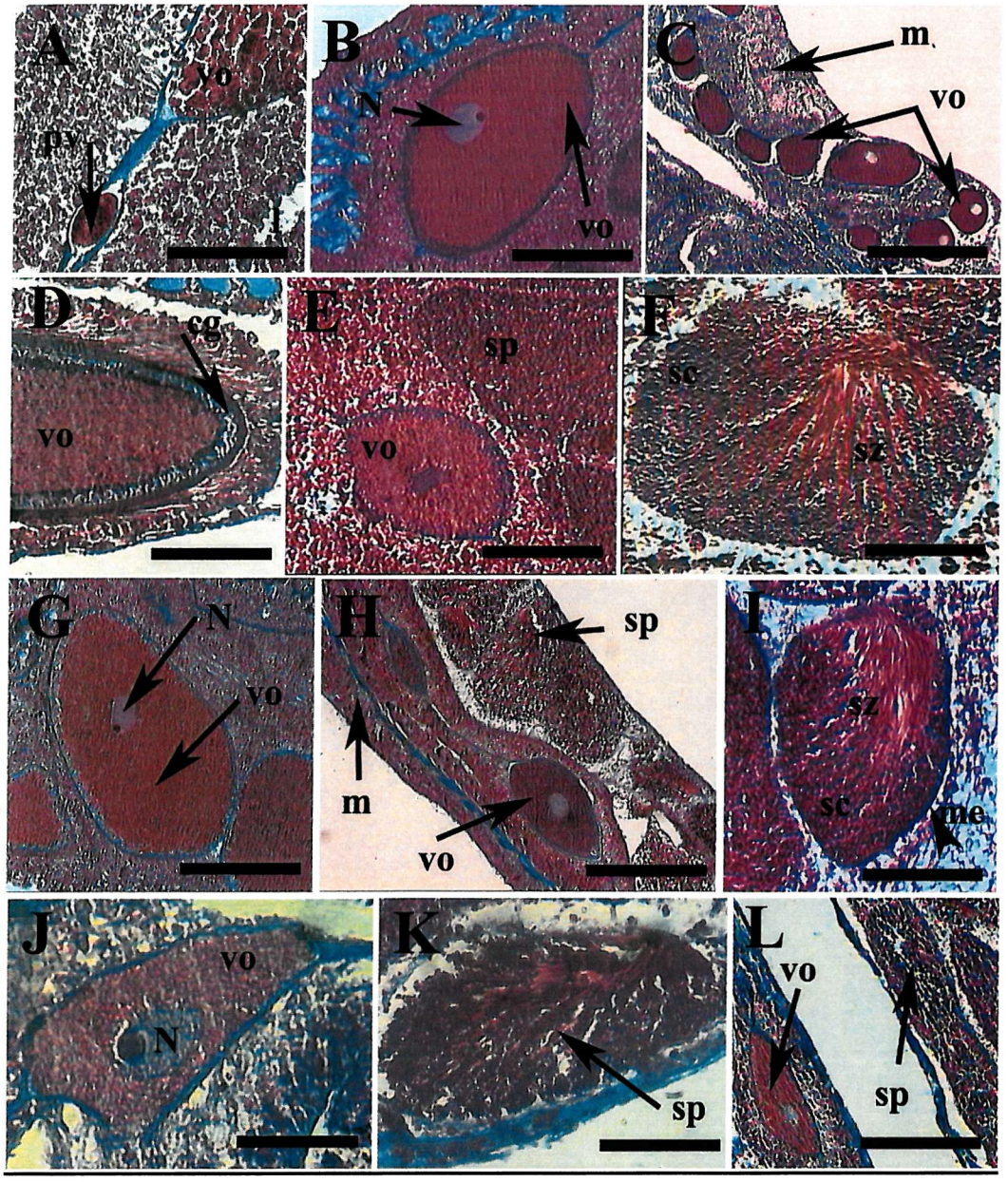


Fig. 3.19 – A, *C. ambrosia* previtellogenic and vitellogenic oocytes connected by the mesenterial lamellae; **B**, *C. ambrosia* vitellogenic oocyte; **C**, *C. ambrosia* female mesentery; **D**, *C. ambrosia* late vitellogenic oocyte showing cortical granular layer; **E**, *C. ambrosia* hermaphroditic mesentery; **F**, *C. ambrosia* stage III spermacyst; **G**. *C. seguenzae* vitellogenic oocytes; **H**, *C. seguenzae* hermaphroditic mesentery; **I**. *C. seguenzae* stage III spermacyst; **J**. *C. cornuformis* vitellogenic oocyte; **K**. *C. cornuformis* stage III spermacyst ; **L**. *C. cornuformis* hermaphroditic mesenteries

Pv, Previtellogenic oocyte; **Vo**, Vitellogenic oocyte; **L**, mesenterial lamellae; **N**, Nucleus; **M**, Mesentery; **Cg**, Cortical granular layer; **Sp**, Spermacyst; **Sc**, Spermatocytes**; Sz,** Spermatozoa; **Me**, Mesogloeal envelope; **O**, Oocyte;
Scale bars - A, 200µm; **B,** 200µm; **C**, 400µm; **D**, 100µm; **E,** 200µm; **F,** 100µm, **G**, 100µm; **H**, 200µm; **I**, 100µm, **J,** 50µm; **K,** 50µm; **L,** 100µm
Stained with Masson's Trichrome

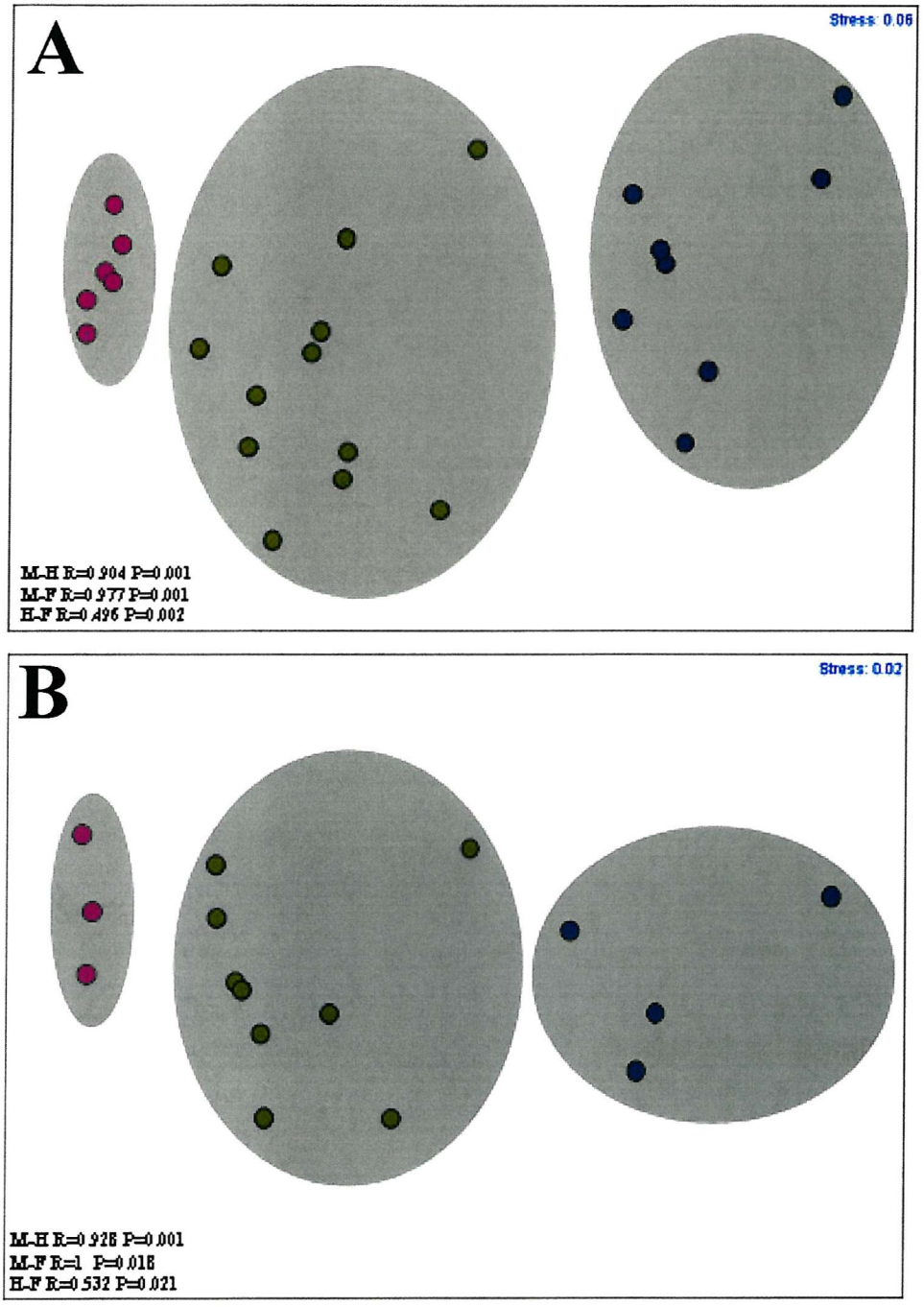


Fig. 3.20 – **A**, PRIMER cluster analysis and ANOSIM statistic for *C. ambrosia*; **B**, PRIMER cluster analysis and ANOSIM statistic for *C. seguenzae*

Pink circles, Female hermaphrodites; **Green circles**, Intermediate hermaphrodites; **Blue circles**, Male hermaphrodites

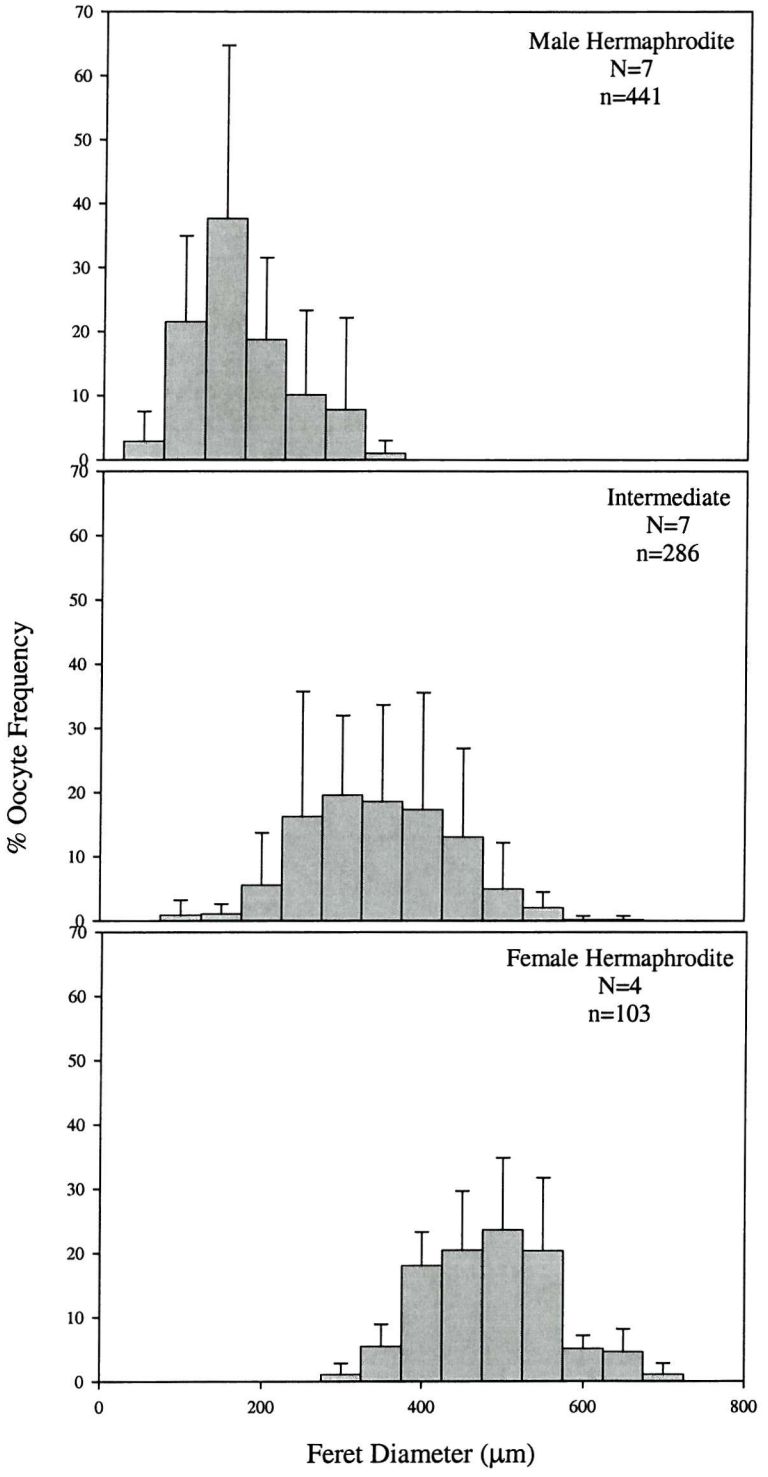


Fig. 3.21 – C. ambrosia sample mean oocyte size-frequency distribution . Plots split into 'male hermaphrodites', 'intermediate' and 'female hermaphrodites' based on PRIMER 5 analysis

Error bars - ±1sd

Spermatogenesis

Four stages of spermatogenesis were identified

Stage I, Early - Loosely packed aggregations of spermatocytes contained within a spermacyst. Empty lumen can be observed

Stage II, Maturing - Some spermatozoa are present, starting to fill the lumen but loosely packed

Stage III, Mature - Well developed spermatocyte layer and lumen packed with spermatozoa (Fig. 3.19f)

Stage IV, Spent - Relict spermatozoa can be seen

Spermacysts were observed to be in a similar stage of development within a single individual. Hermaphrodites that were predominantly female had fewer, smaller spermacysts, at earlier stages, than male hermaphrodites. The presence of mainly late-stage spermacysts in most individuals suggests rapid development or long-term storage of sperm.

***Caryophyllia sequenza* (Fig. 3.19g,h,i)**

Four monthly samples were analysed during this study. *C. seguenzae* is also a cyclical hermaphrodite. PRIMER 5 cluster analysis was used to separate 'male', 'female' and 'intermediate' individuals (R2=>0.532; P=>0.001). Gametes of both sexes develop from the mesenterial lamellae. Both male and female gametes were found in several pockets throughout the same mesentery, and increase in density towards the actinopharynx.

Oogenesis

Oogenesis is very similar to that of *C. ambrosia*, with differing oocyte sizes:-

Stage I – Ovoid oogonia are <60μm

Stage II – Previtellogenic oocytes are < 125μm

Stage III – Vitellogenic oocytes are <350μm (Fig. 3.19g)

Stage IV - Late Vitellogenic oocytes are >350μm reaching a maximum size of 450μm

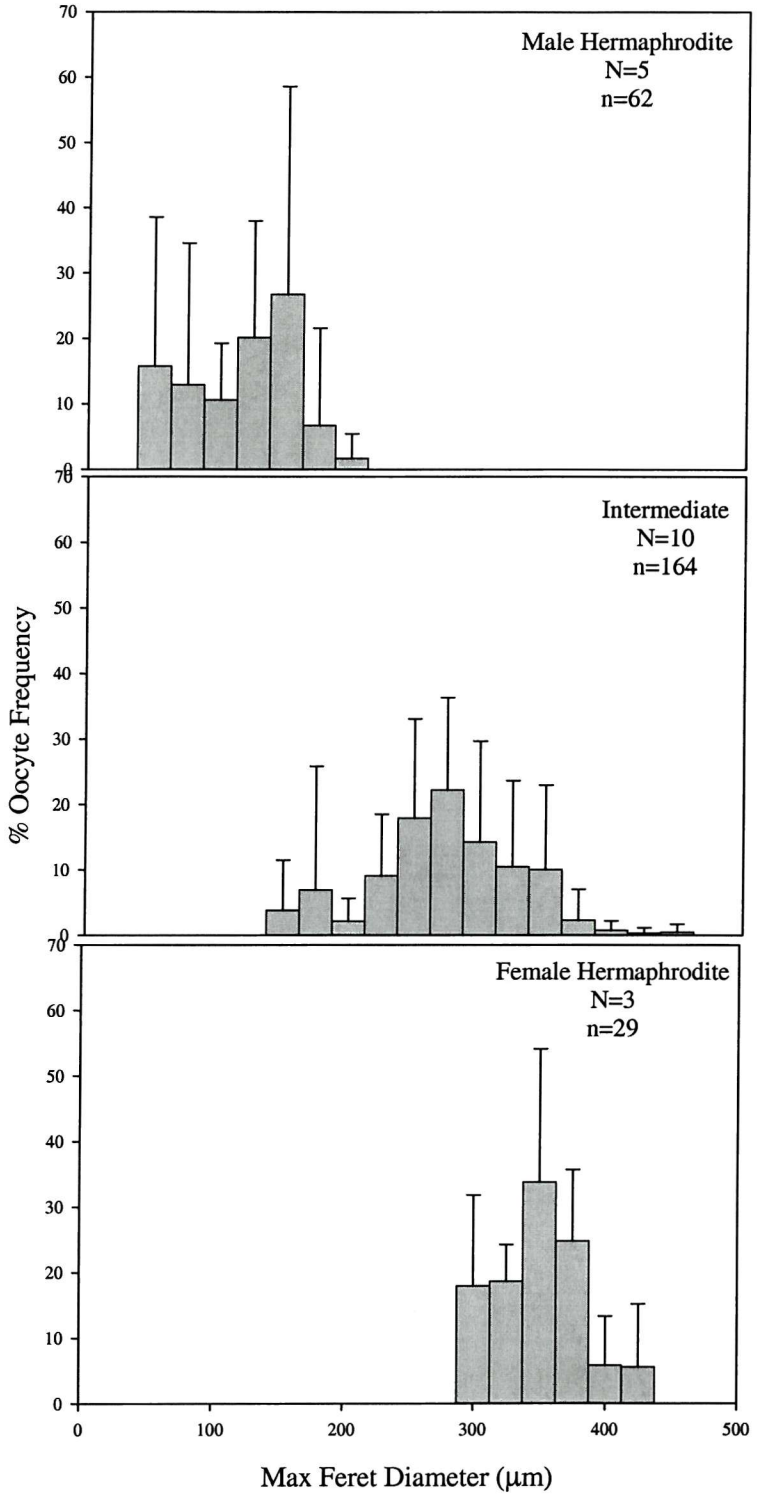


Fig. 3.22– C. seguenzae sample mean oocyte size-frequency distribution plots for *C. seguenzae*. Plots split into 'male hermaphrodites', 'intermediate' and 'female hermaphrodites' based on PRIMER 5 analysis
Error bars = ±1sd

Individuals within a month sample were asynchronous (see Appendix III for individual plots). Oocytes within a single individual were at the same stage of development and hermaphroditic individuals (Fig. 3.19h) were found in all size classes. Oocyte size-frequency diagrams (Fig. 3.22) show the unimodal development, and the asynchrony of these data. The maximum oocyte diameter observed was 450μm suggesting lecithotrophic development.

Spermatogenesis

Spermatogenesis in *C. seguenzae* follows the same stages as *C. ambrosia.* Spermacysts were observed to be in a similar stage of development within a single individual and, as in *C. ambrosia*, these were mainly late stages (Fig. 3.19i). Hermaphrodites that were predominantly female had fewer, smaller spermacysts, at earlier stages, than male hermaphrodites.

***Caryophyllia cornuformis* (Fig. 3.19j,k,l)**

Only two months of samples were obtained for this species and these were not in good condition. It is thought that formalin penetration into the lower extremities was poor and so tissue was not preserved adequately to gain reliable fecundity results or numerous oocyte diameters.

This species is also thought to be a cyclical hermaphrodite, with gametes of both sexes developing from the mesenterial lamellae. There were insufficient oocytes in measurable condition to develop oocyte size-frequency histograms for this species, and so sizes were averaged for every individual and then for the two samples (Fig. 3.23). Maximum oocyte, measurable, size is 350 μm, and so a lecithotrophic larvae is inferred. Individuals from the same sample had varying average oocyte diameters, this suggests asynchronous gamete development within the population.

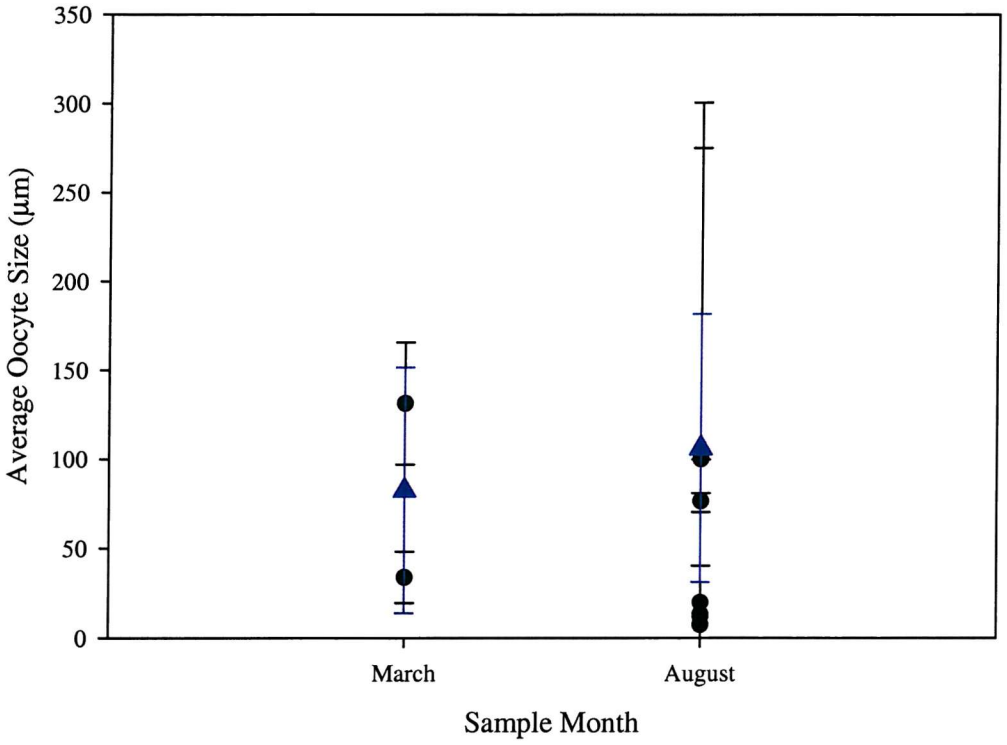


Fig. 3.23 – *C. cornuformis* average oocyte diameters for months analysed

•, Individual average oocyte diameters; •, Average oocyte diameter for month; Error bars - ±1sd

Fecundity

Fecundity was calculated from both 'female' and 'male' hermaphrodites in both *C. ambrosia* and *C. seguenzae*. There were insufficient samples to obtain a fecundity estimate for *C. cornuformis*. There was no significant difference in fecundity between these two phases in either species (*C. ambrosia* – U=43.5, P=0.078; *C. seguenzae* – U=36.0, P=0.2986). This suggests that production is rapid, with a full fecundity of oocytes even in male hermaphrodites.

C. ambrosia

Potential fecundity was calculated as a minimum of 200 oocytes per polyp and a maximum of 2750 oocytes per polyp. After size correction there was no significant difference between average fecundity between the four months analysed (U=>8.5, P=>0.155), suggesting a quasi-continuous production of gametes. Fecundity did not

increase with wet weight of polyp (R^2 = 0.005, P=0.05), and the size of first reproduction could not be discerned.

C. seguenzae

Potential fecundity was calculated at a minimum of 52 oocytes per polyp and a maximum of 940 oocytes per polyp. After size correction there was no significant difference between average fecundity between the four months analysed (U=54, P=0.006), suggesting a quasi-continuous production of gametes.

Fecundity is size-dependent (Fig. 3.24; R^2=0.728, P=<0.05). Non-reproducing individuals were not included in the regression analysis, as they were observed in many size classes.

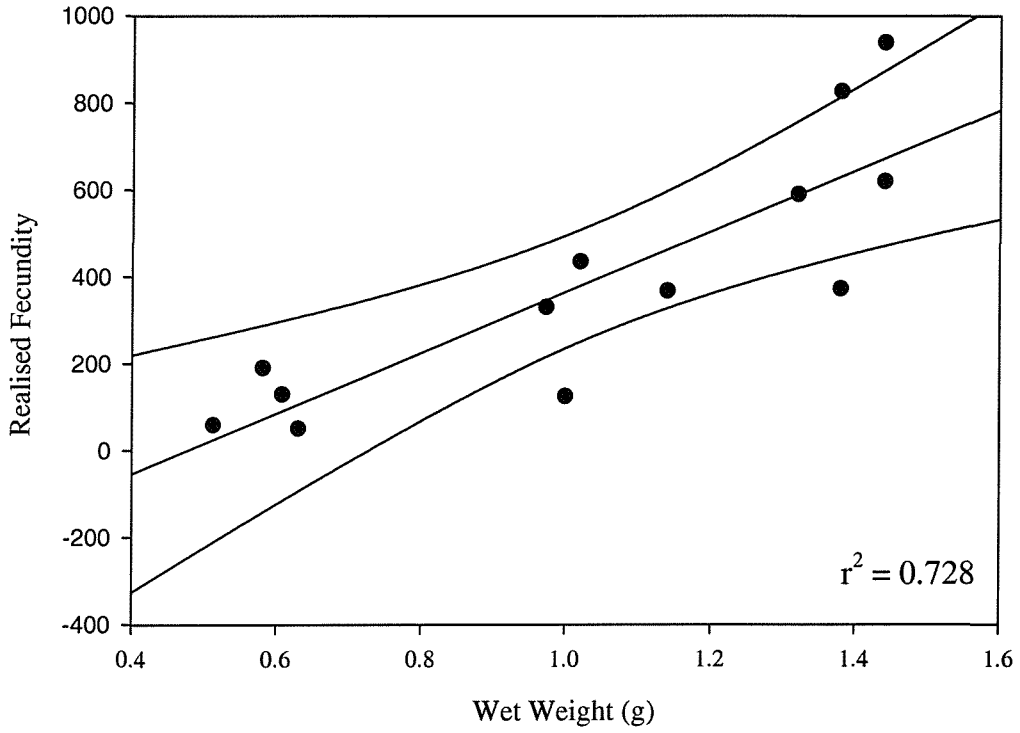


Fig. 3.24 – *C. seguenzae* polyp wet weight plotted against realised fecundity, fitted with regression line

N=13; R^2 = 0.728; P=<0.05; 99% confidence intervals; f=y0+a*x ; Size corrected to 1.398g polyp wet weight

Discussion

All three deep-water scleractinian species of *Caryophyllia* show asynchronous, cyclical hermaphroditism with evidence of seasonality. The majority of scleractinians for which the reproduction is known are hermaphroditic (Fadlallah, 1983; Harrison et al., 1984; Szmant, 1986; Goffredo et al., 2000). However in cyclical hermaphroditism, a single individual is unable to produce viable gametes of both sex at the same point in time, so self fertilisation cannot occur. Selfing is thought to be an important mode of fertilisation in hermaphroditic corals, and can ensure fertilisation success (Brazeau et al., 1998). However this mode does not allow for the genetic diversity and evolution of a species (Veron, 1995).

The large egg size of these three species suggest lecithotrophic rather than planktotrophic development (Marshall, 1979; Fadlallah, 1983; Gage & Tyler, 1991). Lecithotrophic development, contrary to earlier suggestions, is now recognised as a beneficial adaptation for wide dispersal in oligotrophic environments (Shilling and Manahan, 1994). Most deep-sea scleractinians studied to date appear to have lecithotrophic larvae (Waller et al., 2002, in press; Waller & Tyler, in press), with just one species thought to produce a planktotrophic larvae (Brooke & Young, 2003). Though planulation in deep-sea species is difficult to observe without *in vitro* cultures, histological evidence suggests that all three species of *Caryophyllia* in this study spawn gametes rather than brood. This is inferred by both the lack of planulae, and the varying stages of gamete development within the population (which would mean the likelihood of observing planulae within the polyp would be high). The spawning of gametes as the normal mode of reproduction in the Cnidaria has been well documented for a number of shallow species (Kojis & Quinn, 1981; Bothwell, 1982; Fadlallah & Pearse, 1982; Fadlallah, 1983; Harrison et al., 1984; Szmant, 1986; Harrison, 1990; Richmond & Hunter, 1990; Richmond, 1997). *C. smithii*, a shallow water congener, also broadcast spawns and is externally fertilised (Tranter et al., 1982). Rinkevich and Loya (1979) proposed that large-polyp species would be unlikely to brood, as the extra energy required for growth, defence and maintenance of the large polyp would mean the energy required to produce a brooded planulae would be unavailable. Stimson (1978) also suggested that deep fore-reef scleractinians may broadcast gametes in order to aid the wide dispersal distance

required at depths. These two theories fit with the data acquired during this study. All four species of *Caryophyllia* broadcast gametes (except for a report of possible brooding in *C. smithii* (Hiscock & Howlett, 1977)), and the oocyte size increases with polyp size.

Species	Depth	Max Oocyte Diameter	Max Fecundity (per polyp)	Sex	Gamete Release	Larvae Type
C. smithii	2-1200m	150μm	'several thousand'	Gonochoristic	Seasonal	Planktotrophic
C. cornuformis	435-2000m	350μm	ND	Cyclical Herm	Quasi-cont	Lecithotrophic
C. seguenzae	960-1900m	430μm	940	Cyclical Herm	Quasi-cont	Lecithotrophic
C. ambrosia	1100-3000m	700μm	2750	Cyclical Herm	Quasi-cont	Lecithotrophic

Table 3.6 – Showing comparisons of depth range and reproductive ecology of four species of *Caryophyllia*. (*C. smithii* data from Tranter et al., 1982)
ND – No Data; Herm – Hermaphrodite; Quasi-cont - Quasi Continuous;

Gametes of shallow water scleractinians are thought to develop within the lamellae of the mesenteries and subsequently migrate into the mesogloea, to develop as oogonia (Szmant-Froelich et al., 1980; Fadlallah, 1983). Oogonia and spermacysts of all three species of *Caryophyllia* studied were first observed attached to the mesenterial lamella, and so this is also believed to be the case with these deep-water species, and others such as *Fungiacyathus marenzelleri* (Waller et al., 2002), *Lophelia pertusa* and *Madrepora oculata* (Waller & Tyler, In press, this Thesis).

Caryophyllia smithii is gonochoristic and a seasonal reproducer, producing gametes between January and March (Tranter et al., 1982). This is a very different strategy than the three deep-water species in this study, even though they are congeners. It is not unusual for scleractinian species within the same genus to have differing reproductive patterns (Fadlallah, 1983; Harrison & Hunter, 1990). The environment these three deep water species inhabit is very different from the shallow water where *C. smithii* resides. Seasonal processes, such as changing temperatures and lunar cycles may have an affect on this species, as in most shallow water scleractinians (Fadlallah,

1983). However, below the thermocline, these processes do not have any effect, perhaps contributing to their quasi-continuous nature.

Fecundity and oocyte size both increase with depth in three species of deep-water *Caryophyllia* (Table 3.6). Spawning and larval type also appears to shift between seasonal planktotrophic to quasi-continuous lecithotrophic development as the depth increases. Environmental conditions have often been shown to influence reproductive patterns. Many species of shallow scleractinians have been shown to use lunar periodicity and seasonal temperature differences to time their reproduction (Fadlallah, 1983), whereas reproduction in several deep-water invertebrate species may be cued in the NE Atlantic by a seasonal phytodetrital pulse (Tyler et al., 1982; Billett et al., 1983; Tyler et al., 1992, 1993). Brooding was also originally thought to be an ideal strategy for the deep-sea environment as it takes a developing planulae to late stages before releasing it to the 'harsh' environment (reviewed in Gage & Tyler, 1991), though this is yet to be found in the deep-water Anthozoa.

The asynchronous nature between individuals of these three species suggests that either there is a near constant presence of spawned gametes in the water, or that individuals spatially close to one another are synchronous while the population as a whole is asynchronous, or both. Because individuals were trawled, their small scale spatial dispersal is not known. There are also no data available on the density of any of the deep-water *Caryophyllia* corals. Population densities of deep-water solitary corals are likely to play an important role in their reproductive patterns and ecology. Densities of both individuals and gametes in spawning species must be sufficiently high to allow successful fertilization yet densities of fauna are generally low in the deep-sea (Gage and Tyler, 1991).

These three species are all non-seasonal. Cnidarians are generally regarded as opportunistic omnivores, and so this feeding method perhaps gives these large-polyp species (in comparison to reef builders such as *L .pertusa* or *M. oculata*, Waller & Tyler, in press, this Thesis) enough available energy to produce gametes year round. Releasing gametes into the water column year-round will provide a constant supply of larvae and so increase the chances of species survival.

3.2.4 Reproduction in two NE Atlantic *Flabellum* spp. - *F. alabastrum, F. angulare*

Introduction

Flabellum alabastrum, Moseley, 1873 and *Flabellum angulare*, Moseley, 1876 are solitary deep-water scleractinians found only within the Atlantic (Cairns, 1999). *F. alabastrum* inhabits a wide depth range, being found from 401 to 2250m in the NE Atlantic (Zibrowius, 1980). By comparison, *F. angulare* has a much narrower distribution, 1647 to 2857m (Zibrowius, 1980).

Five monthly samples for *F. alabastrum* were collected from Station 'M' in the Rockall Trough (see section 3.2.2) and three month samples for *F. angulare* were collected from the Porcupine Seabight in the NE Atlantic (Table 3.7). This section examines the gametogenesis and reproductive periodicity of these two deep-water solitary scleractinians.

Materials and Methods

Fifteen individuals from each species from each season were decalcified. All individuals were then wet weighed. Individuals of both species were then examined to determine sex, as gametes were large enough to be visible. Females had three mesenteries dissected and histologically processed. All three mesenteries of each individual were serially sectioned to give fecundity estimates, leaving 100μm between slides

Species	Date	Cruise	Depth	Latitude/Longitude
F. alabastrum	21.2.1991	Challenger 75/91	1908m	56°'56N 09°'50W
	10.3.1993	Challenger 101	1650m	57°'07N 09°'30W
	31.7.1983	Challenger 10/83	1705m	57°'56N 12°'21W
	17.8.1981	Challenger 12B/81	2190m	56°'00N 13°'58W
	21.11.1991	Challenger 86/91	1370m	56°'34N 09°'31W
F. angulare	1.10.2002	Discovery 266	2443-2452m	50°'04N 12°'45W
	11.3. 2002	Discovery 260	2412m	49°'57N 12°'42W
	21.9.2000	Discovery 249	2454-2467m	50°'02N 12°'42W

Table 3.7 - Samples used for this study

Results

Flabellum alabastrum

F. alabastrum is a gonochoristic species, with gametes visable when the tissue is decalcified. The total sex ratio is 1:1 with little deviation within months. Putative size of first reproduction was 0.247g polyp wet weight.

Oogenesis

Oogenesis can be divided into four stages:-

Stage I – Oogonia (unobserved, but <100μm), expected to bud from the mesenterial lamellae

Stage II – Previtellogenic oocytes (<300μm), containing a large nucleus

Stage III – Vitellogenic oocytes (<800μm), larger yolk granules can be observed (Fig. 3.25a)

Stage IV – Late Vitellogenic oocytes (>800μm), rarely observed, thick cortical granular layer present and a prominent nucleolus in the central nucleus observed.

Previtellogenic and vitellogenic oocytes were found within most females observed, late vitellogenic and oogonia were only observed rarely. Females weighing less than 0.5g polyp wet weight, contained only previtellogenic oocytes and so were not included in percentage size-frequency plots, as these are considered juveniles. The maximum oocyte size observed was 925μm diameter, suggesting lecithotrophic larval development.

Spermatogenesis

In all months examined spermacysts were at a late stage of development, with spermatozoa tails clearly visible. Spermacysts appeared similar in morphology to that described for other deep-water scleractinians (Waller et al.., 2003, in press; Waller & Tyler, in press).

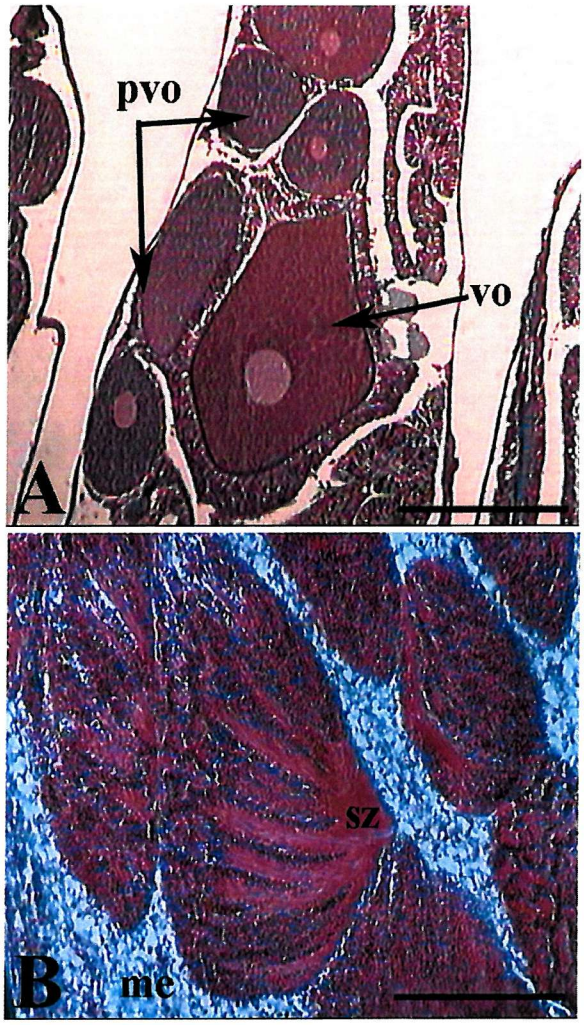


Fig. 3.25 – *F. alabastrum*, **A**, Female mesentery showing previtellogenic and vitellogenic oocytes; **B**, Male mesentery
pvo, previtellogenic oocyte; **vo**, vitellogenic oocyte; **sz**, spermatozoa; **me**, mesogloeal envelope
Scale bars - A, 500μm; **B**, 250μm; Stained with Masson's Trichrome

Fecundity

Fecundity is size-dependent and rises with polyp wet weight ((Fig. 3.26) $R^2 = 0.54$; P=0.01). There was no significant difference between the average fecundity for the months analysed (Fig. 3.27).

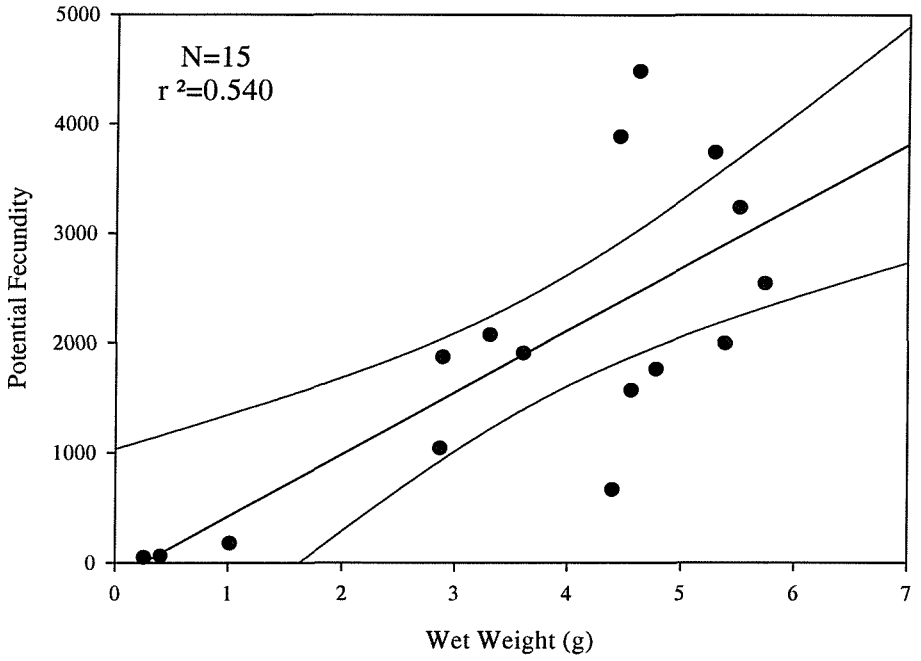


Fig. 3.26 - *F. alabastrum* potential fecundity plotted against decalcified polyp wet weight with a fitted regression line
95% confidence limits, f=y0+a*x, Size corrected to 3.690g polyp wet weight

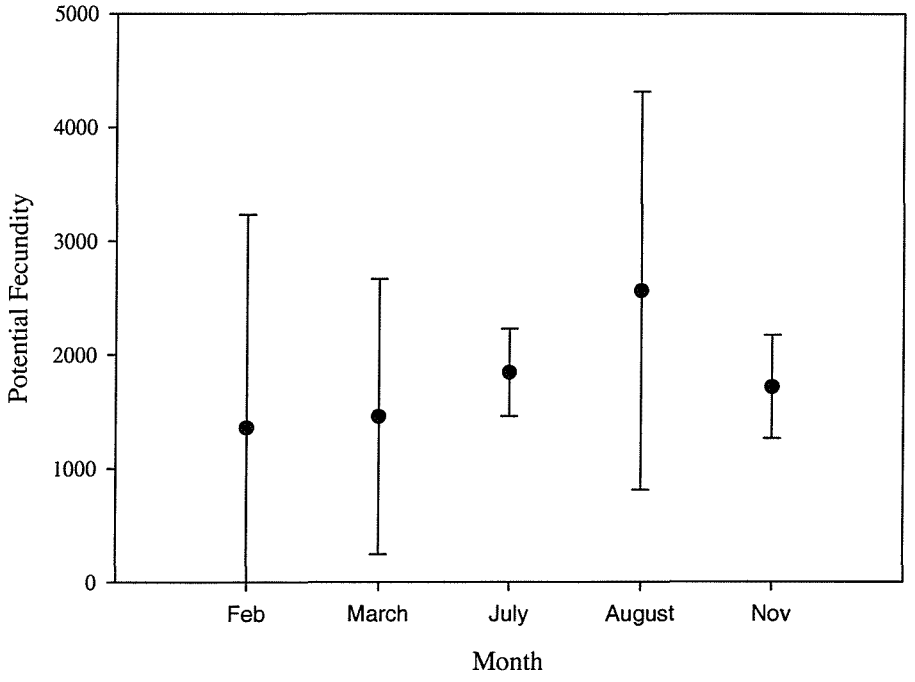


Fig. 3.27 - *F. alabastrum* average potential fecundity per month analysed
error bars - ±SD, Size corrected to 3.690g polyp wet weight

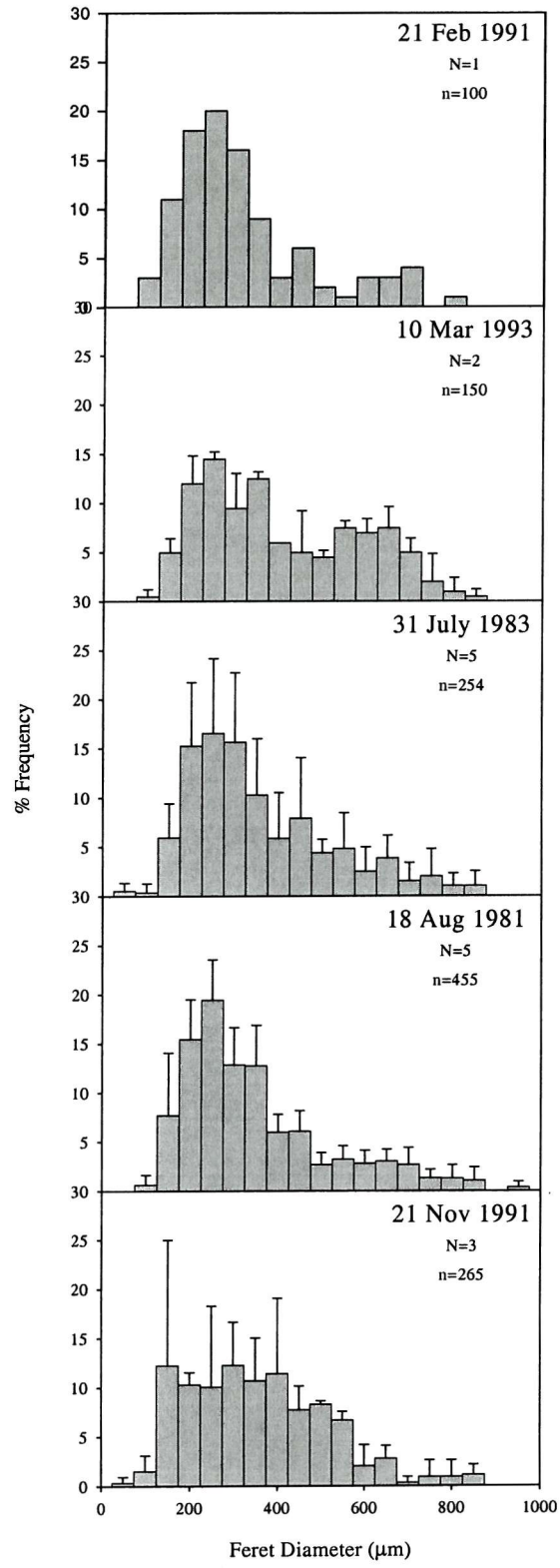


Fig. 3.28 – *F. alabastrum* sample mean oocyte size-frequency diagrams
error bars - ±SD

Periodicity

Samples within a month sample were synchronous and so individual plots within a month sample were collated (for individual plots see Appendix III). Oocyte size-frequency diagrams (Fig. 3.28), show little difference between monthly samples. Two cohorts are developing and individuals are synchronous within populations. This suggests a quasi continuous life history, with gametes being produced regularly. Male individuals were found only in the late stages of sperm development, this would also suggests a quasi-continuous strategy.

Flabellum angulare

F. angulare is also a gonochoristic species with a 1:1 sex ratio (X^2=0.719, P=0.01), deviating little within monthly samples. Gametes were also clearly visible post decalcification within and throughout mesenteries. Size of first reproduction is putatively 1.379g polyp wet weight.

Oogenesis

Oogenesis can be divided into four stages, similar to *F. alabastrum*. Oogonia (<100μm), as in *F. alabastrum*, were also rarely observed. Previtellogenic (<350μm) and vitellogenic (<900μm) were present in all female individuals examined. Late vitellogenic oocytes were also rarely observed (>900μm). The maximum oocyte diameter observed was 1015μm, strongly suggesting a lecithotrophic larval development in this species.

Spermatogenesis

There were four stages of spermatogenesis observed in *F. angulare*.

Stage I, Early – Loosly packed aggregations of spermatocytes within spermacyst. Empty lumen.

Stage II, Maturing – Some spermatozoa present, still largely empty lumen.

Stage III, Late – Lumen packed with spermatozoa

Stage IV, Spent – relict spermatozoa can be seen

Mainly maturing and late stages of spermatogenesis could be observed in single individuals, though all males in September were at stages one and two.

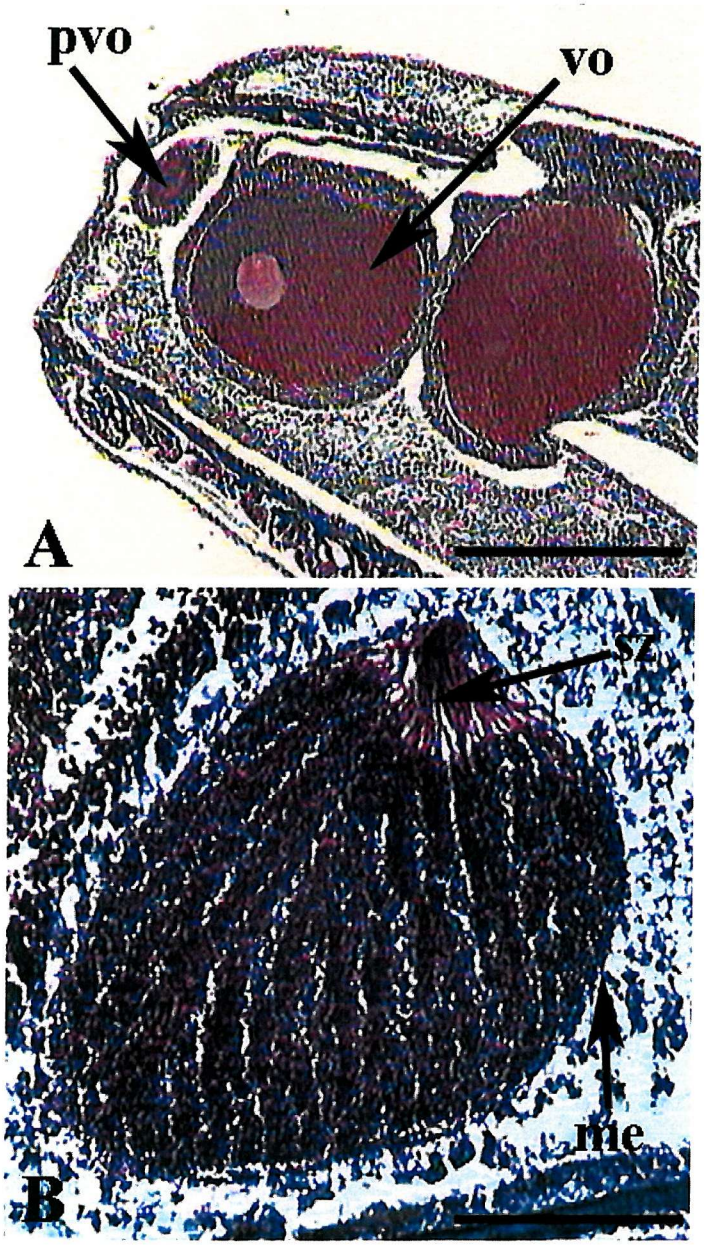


Fig. 3.29 – *F. angulare*, **A**, female mesentery; **B**, male spermatocyst
pvo, previtellogenic oocytes; **vo**, vitellogenic oocytes; **sz**, spermatozoa tails; **me**, mesogloeal envelope
scale bars, **A**, 600μm; **B**, 350μm; Stained with Masson's Trichrome

Fecundity

Fecundity is size dependent in *F. angulare*, increasing numbers of oocytes are produced as wet weight increases ((Fig 3.30) R^2=0.528, P=0.01). Figure 3.31 shows a marked periodicity in fecundity, with a significant increase in oocytes produced

between October and March (U=39.0, P=0.329) and a significant decrease in September (U=30, P=0.036).

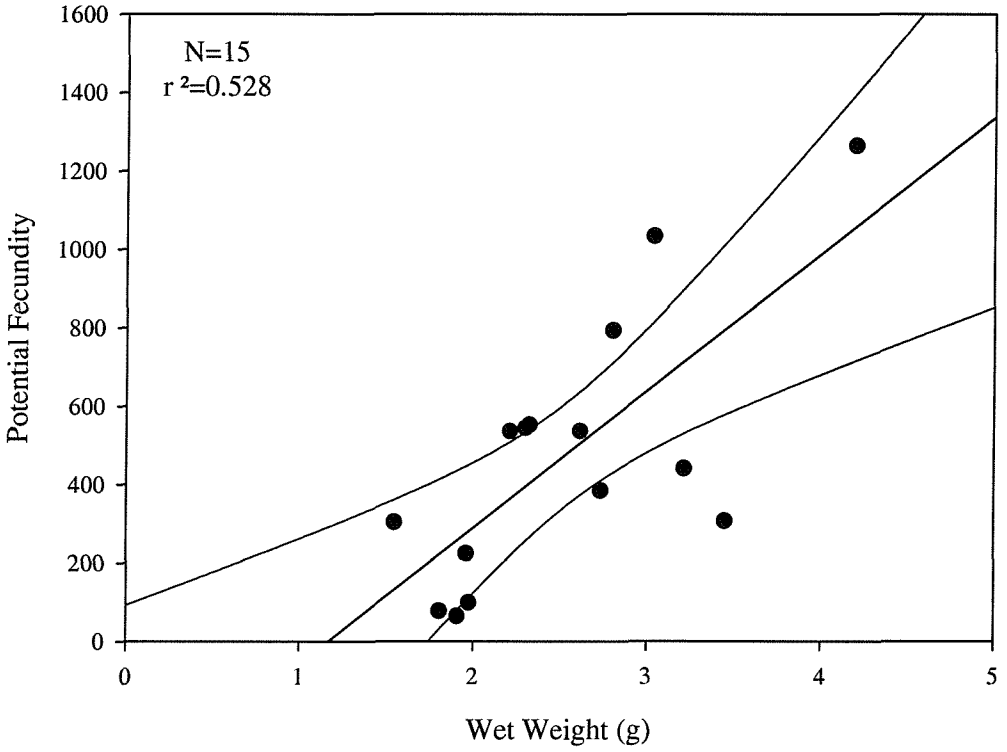


Fig. 3.30 - *F. angulare* potential fecundity plotted against polyp wet weight, with a fitted regression line and 95% confidence limits. f=y0+a*x, Size corrected to 2.538g polyp wet weight

Periodicity

Individual oocyte-size-frequency diagrams were collated monthly, as individuals were synchronous (for individual plots see Appendix III). Oocyte size-frequency diagrams show a similar bimodal peak in March and September, but less of a second peak in October (Fig. 3.32). These data coincide with the fecundity data, showing a release of oocytes around September, and therefore the lack of late vitellogenic oocytes in October. This suggests either seasonality or periodic production of gametes.

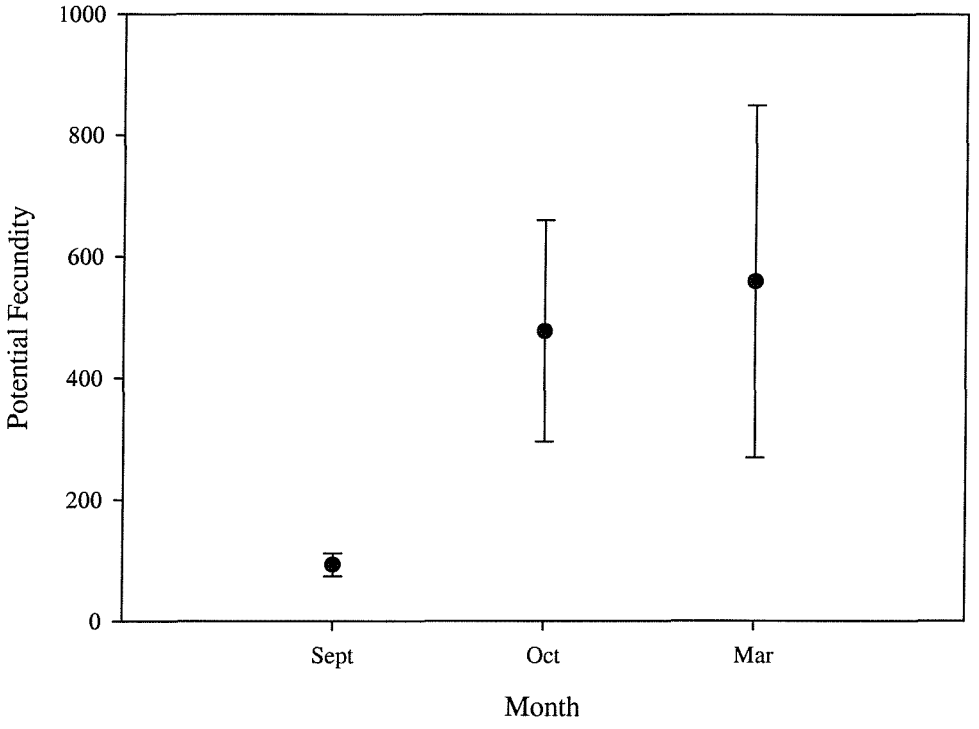


Fig. 3.31 – *F. angulare* mean potential fecundity for each month analysed
error bars - ±1sd, Size corrected to 2.538g polyp wet weight

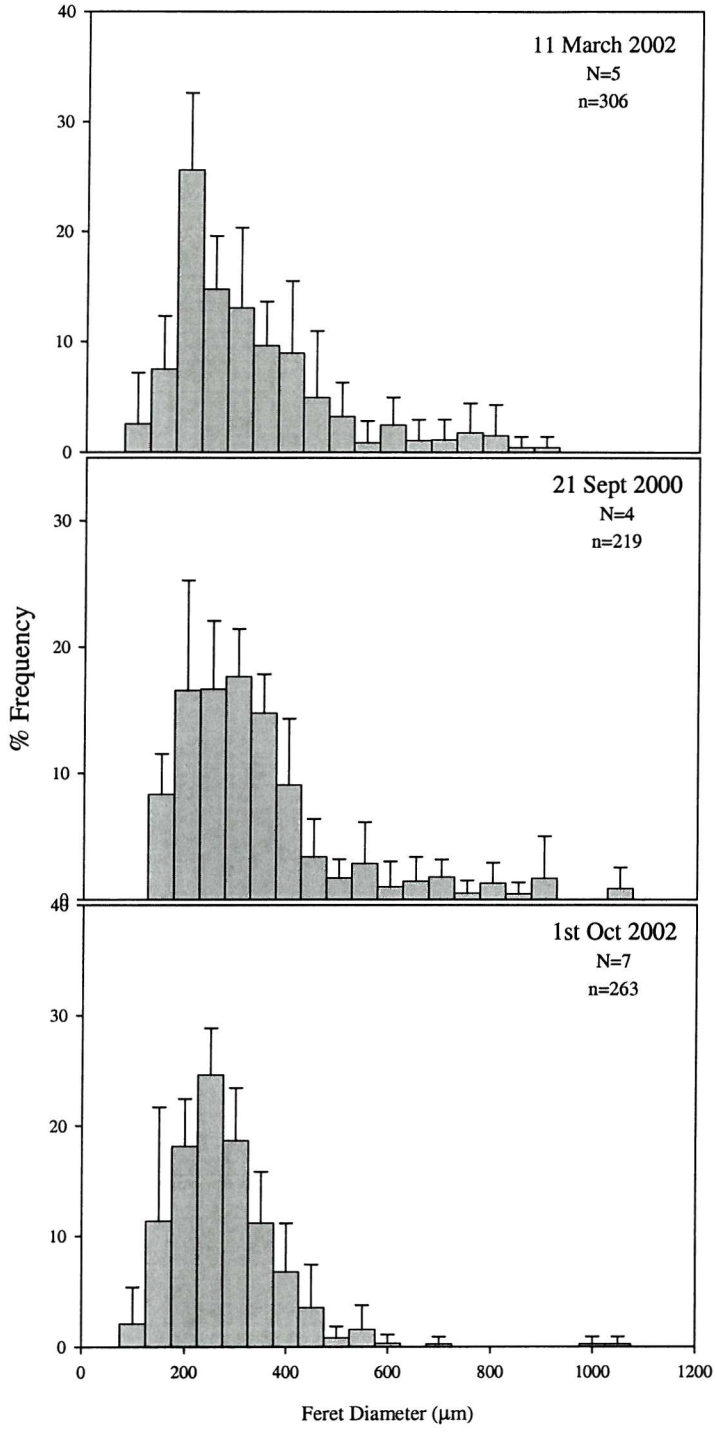


Fig. 3.32 – *F. angulare* sample mean oocyte size-frequency diagrams
error bars - ±SD

Discussion

Both species in this study were gonochoristic, with no hermaphroditic individuals being found. The random selection of males and females within size classes mitigates against sequential hermaphroditism. No brooded planulae were observed, and so it is hypothesised both species spawn gametes. Gonochorism appears to be more common than hermaphroditism in deep-water scleractinian species studied to date, *Fungiacyathus marenzelleri* (Waller et al., 2002), *Oculina varicosa* (Brooke & Young, 2003), *Lophelia pertusa* and *Madrepora oculata* (Waller & Tyler, in press, this Thesis). Richmond and Hunter (1990) found the majority of shallow water scleractinian species to be hermaphroditic, with over three times as many species being hermaphrodites than gonochorists.

Most scleractinians have some form of reproductive periodicity, usually either lunar or temperature dependent (Fadlallah, 1983; Richmond & Hunter, 1990). Within this study there appears to be a split within this genus. *F. alabastrum*, the shallower species, was quasi-continuous, and *F. angulare* was seasonal. There were two cohorts present throughout all samples of *F. alabastrum*, and no marked difference in fecundity. Spermatogenesis also appeared to be constantly in the late stages of development, these factors all contribute to the conclusion of quasi-continous production of gametes into the water column. Vitellogenic oocytes in *F. angulare,* however, were present in larger numbers in March and September, than in October, and fecundity was significantly lower in September than in March or October. This suggests vitellogenic oocytes were spawned throughout late September. Previtellogenic oocytes are then produced to develop the fecundity found in October. Though these species are found below the permanent thermocline, there have been many instances of reproductive cues in the deep-sea (Billet et al., 1983; Tyler et al., 1992, 1993) and have even been related to deep-water scleractinians (Waller & Tyler, in press, this Thesis). Seasonal phytoplankton blooms reach the benthos in the Porcupine Seabight occur around July (Lampitt et al., 2001), and so in *F. angulare*, which is hypothesised to spawn in late August/September, this food fall could be the seasonal cue often found in scleractinians (Fadlallah, 1983; Richmond & Hunter, 1990). However, *F. angulare* is hypothesised to produce lecithotrophic larvae owing to the large oocytes observed. These larvae are more energetically expensive to

produce because they contain their own food source and so do not feed while in the plankton. Eckelbarger and Watling (1995) proposed that increased food in the environment could affect reproduction in three ways; 1, it can initiate gametogenesis followed soon after by spawning; 2, it can initiate spawning for planktotrophic larvae; or 3, it can initiate and synchronise gametogenesis, with a spawning event occur after a time period. Using this model it is likely that this increase in food availability initiates vitellogensis (previtellogenic oocytes appear to be present throughout the year) and is soon after followed by a spawning event.

F. alabastrum produces relatively small numbers (550 oocytes per polyp (oop)) of large oocytes (1015μm diameter) and *F. angulare* produces large numbers (2800oop) of smaller oocytes (925μm diameter). This is a trait seen regularly in both scleractinians (Harrison & Wallace, 1990; Brooke, 2002; Waller & Tyler, in press, this Thesis) and other deep-water invertebrates (Gage & Tyler, 1991). For the deep-sea scleractinians known to date, both of these oocyte sizes are relatively large, and fecundity in *F. angulare* is high (Waller, in press, this Thesis).

All samples of *F. angulare* were obtained from the Porcupine Seabight, though *F. alabastrum* is also collected in this area, there were insufficient numbers for an adequate study. *F. angulare* is generally collected deeper than *F. alabastrum* (Zibrowius, 1980). During trawls from *RRS Discovery* between 2000 and 2002 (pers .obs.) it was interesting to note the lack of crossover in these two species, where a trawl was full of one species there was generally a lack of the other, and vice versa, even though Zibrowius (1980) has shown their depth ranges to be very similar. This suggests some dominance of *F. alabstrum* to shallower depths than *F. angulare.* A more detailed visual survey in this area would provide interesting insights into the population ecology of these species.

Scleractinians are renowned for their reproductive plasticity. Within a genus there are often differing reproductive patterns (Fadlallah, 1983; Szmant-Froelich, 1984; Szmant, 1986; Richmond & Hunter, 1990), and even the same species having differing patterns in different locations (Richmond & Jokiel, 1984). So it is not unusual within the Cnidaria for *F. alabastrum* to be quasi continuous and *F. angulare*

to be seasonal, and is likely to be a consequence of environmental conditions being different at these different depths (Eckelbarger & Watling, 1995).

3.2.5 Reproduction of *Flabellum* spp from the Western Antarctic Shelf – *Flabellum curvatum, F. impensum, F. thouarsaii*

Introduction

Flabellum thouarsii, Milne, Edwards and Haime 1848, *F. curvatum*, Moseley 1881, and *Flabellum impensum*, Squires 1962, are three of the nine species of the genus *Flabellum* to be found in Antarctic waters. *F. thouarsii* can be found between 71 and 600m in depth, around the southeast of South America, from Rio de la Plata, Uruguay, down to Cape Horn, the Falkland Islands and the Scotia Ridge (Cairns, 1982). *F. curvatum* has a similar distribution (being also found off the Burdwood Bank and South Georgia) and can be found between 115 and 1137m depth (though more commonly around 400-800m)(Cairns, 1982). *F. impensum* has the largest depth range and the greatest distribution of the three species examined here. This species is circumpolar, including the Shetland Islands, South Orkney Islands and the South Sandwich Islands (Cairns, 1982). *F. impensum* is found between 46 and 2260m depth, though more commonly between 100 and 1000m (Cairns, 1982).

These scleractinian samples provided a unique opportunity for comparison with the scleractinians discussed previously, including those from the genus *Flabellum* from the NE Atlantic (Section 3.2.4). Many invertebrates from Antarctica have had their reproduction reported and distinct trends have been observed that differ from the general deep-sea (Thorson, 1950; Picken, 1980; Arntz et al., 1994). Brooding and lecithotrophy have been found in many Antarctic invertebrates and though spawning and planktotrophic development have been observed, the general trend is towards the former. Reproduction in a number of Antarctic anthozoans has been reported. Gonochorism and brooding has been found in a number of octocorals (Orejas et al., 2001, 2002; Orejas, 2001), as well as lecithotrophy (Stanwell-Smith et al., 1999; Johnson, 2003).

The FOODBANKS program (as discussed in Chapter one) took place on the Western Antarctic Peninsula between 1999 and 2001 (Fig. 3.31). Five cruises collected samples from three stations at different depths, along the continental shelf, as well as one sample from King George Island. *F. thouarsii* and *F. curvatum* were collected

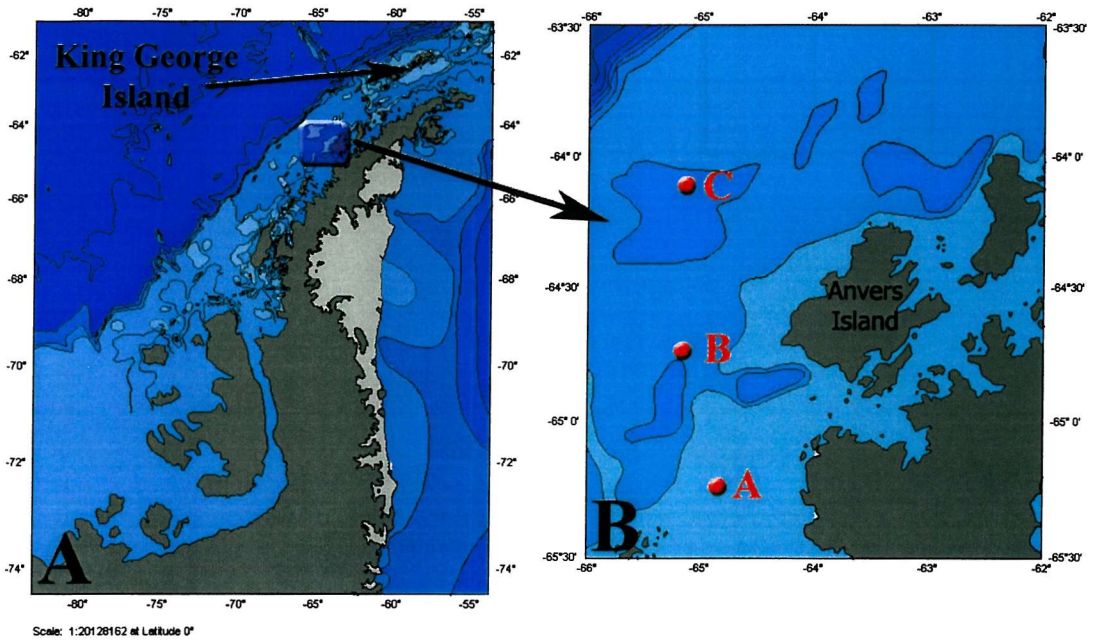


Fig. 3.31 - A, Western Antarctic Peninsula, showing King George Island and the FOODBANKS study area; **B**, Sites A,B and C of the FOODBANKS study area

from sites A, B and C, whereas samples of *F. impensum* (and a single sample of *F. thouarsii*) were collected from King George Island.

Materials and Methods

All samples were collected by Otter Trawl from the research vessels *RV Palmer* and *RV Laurence M Gould* (Table 3.8). Numbers of animals available to this study was limited. Samples collected from three different stations, on single cruises, were collated, as there were insufficient numbers to examine comparisons between sites. Differing seasons were however, kept separated. For Latitude and Longitude of individual samples, see Appendix II.

Samples were decalcified for ~24hrs for the largest individuals. Once decalcified, sex could easily be determined (and even prior to decalcification if tentacles were fully extended allowing vision into the enteron). One section of mesentery from all male individuals was histologically processed. Females were dissected, with three mesenteries being used for fecundity estimates. Small pieces of mesentery were histologically processed initially. However, oocytes contained very large lipid droplets, and so results could not be used for either image analysis or fecundity. Three

mesenteries had the oocytes completely dissected out for fecundity estimates. These oocytes were used for image analysis (dissecting further mesenteries if 100 oocytes were not present), using a video camera attached to an Olympus dissecting microscope. Fecundity estimations were not size-corrected as there were insufficient replicates.

Planulae were dissected out of mesenteries, and a representative of each larval stage for each species was processed for the scanning electron microscope (see Chapter 2).

Species	No.	Date	Area	Depth
F. thouarsii	5	March 2000	A,C	500-650m
	3	July 2000	C	550-580m
	7	November 2000	KGI	270-300m
F. curvatum	1	November 1999	A	500-650m
	3	March 2000	B	550-700m
	2	July 2000	C	550-580m
	6	March 2001	A,B	500-700m
F. impensum	19	November 2000	KGI	270-300m

Table 3.8 – Samples used for this study

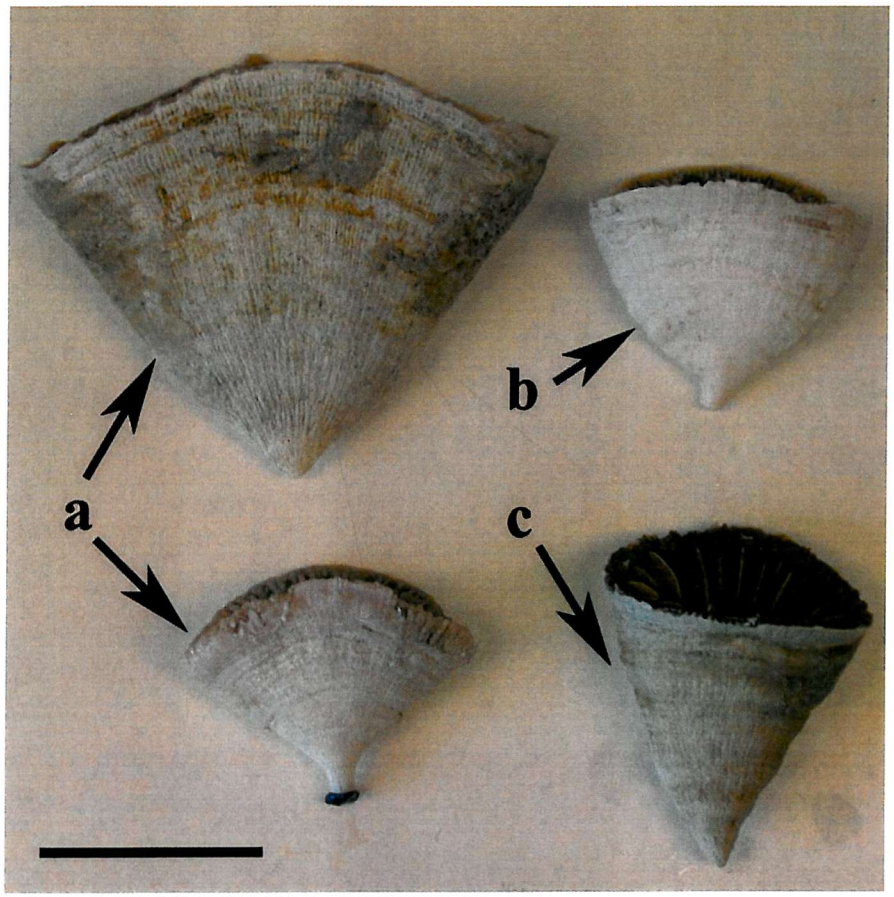


Fig. 3.34 - Antarctic *Flabellum* spp. used in this study, **A**, *F. impensum, juvenile below*; **B**, *F. thouarsii;* **C**, *F. curvatum*
Scale bar – 5cm

Results

All three species are gonochoristic, brooding species. Male and female individuals were found in all species examined, and they were found in a variety of size classes.

Spermatogenesis

Spermatogenesis in all three species appeared to follow the same pattern, with all four stages being present in most individuals (Fig. 3.35). Spermacysts were present throughout and on all mesenteries. Stages were as follows –

Stage I – Early – empty lumen clearly visible, loosely packed aggregations of spermatocytes contained within a cell membrane

Stage II – Maturing – lumen still mainly empty, but some spermatozoa tails visible

Stage III – Late – lumen filled with spermatozoa, densely packed

Stage IV – Spent – relict spermatozoa can be seen

Oogenesis and Brooding

Oogenesis occurs in three stages in all three species for this study:-

Oogonia – Not visible during dissection

Previtellogenic oocytes – whiter in colour, small, generally ovoid

Vitellogenic – pink/orange in colour, large lipid droplets can be seen, ovoid to elongate

No late vitellogenic oocytes were found in any females examined.

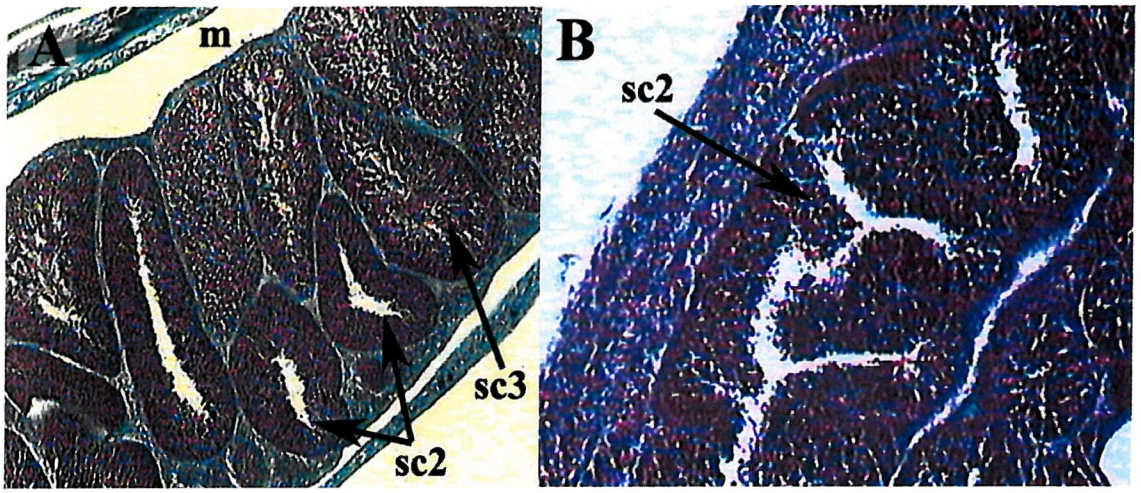


Fig. 3.35 - A, *F. thouarsii* male mesentery; **B**, *F. impensum* male mesentery
m, mesentery; **sc3**, stage 3 spermatocyst; **sc2**, stage 2 spermatocyst; Stained with Masson's Trichrome

Brooded planulae were found in all species, within the gastrodermis, as well as loose within the gastrovascular cavity.

Flabellum thouarsii

Round previtellogenic oocytes were found up to 500μm diameter. Maximum size for vitellogenic oocytes was 4807μm diameter, these oocytes being more elongate and orange than the previtellogenic. Large lipid deposits could be seen easily with a light microscope, and if an oocyte was split, an oily layer would form on the water surface.

Oocyte size-frequency diagrams (Fig. 3.36) show there to be two cohorts in March and June, but only a single cohort in November. There were low numbers of replicates for this study, but these data suggest a seasonal spawning period between June and November, and prolonged or periodic gameteogenesis.

Three stages of brooded planulae were found within the tissue of *F. thouarsii* (Fig. 3.38). On dissection there appeared to be developing embryos or planulae in most mesenteries. The maximum number of planulae found within a single mesentery was two. Planulae were ciliated, though not heavily.

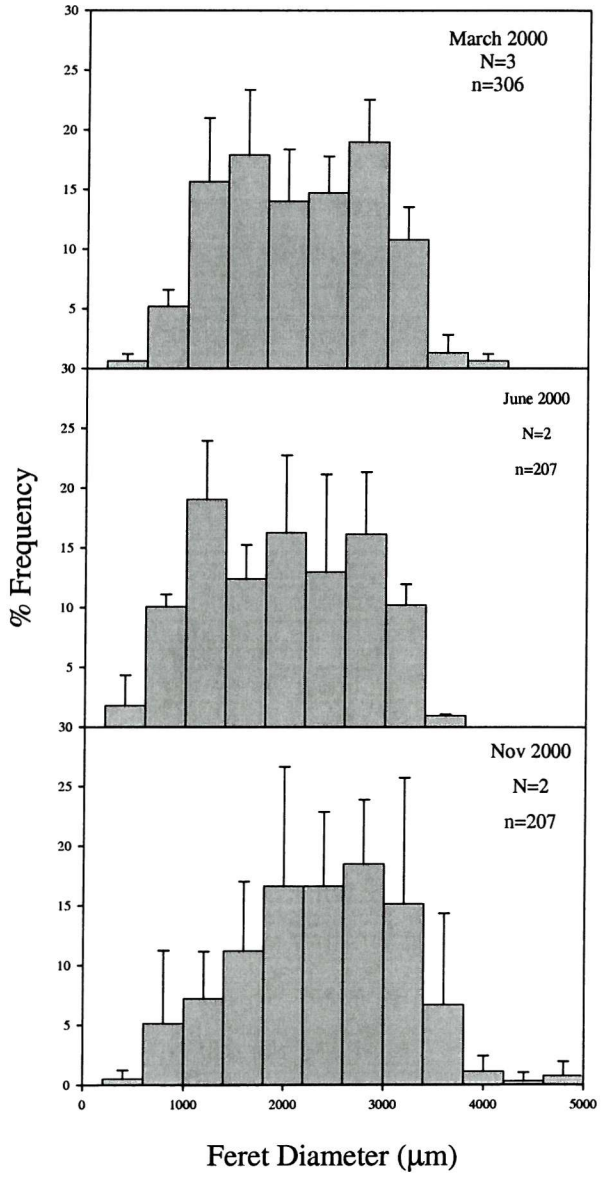


Fig. 3.36 – *F. thouarsii* sample mean oocyte size-frequency diagrams for three months
error bars - ±1SD

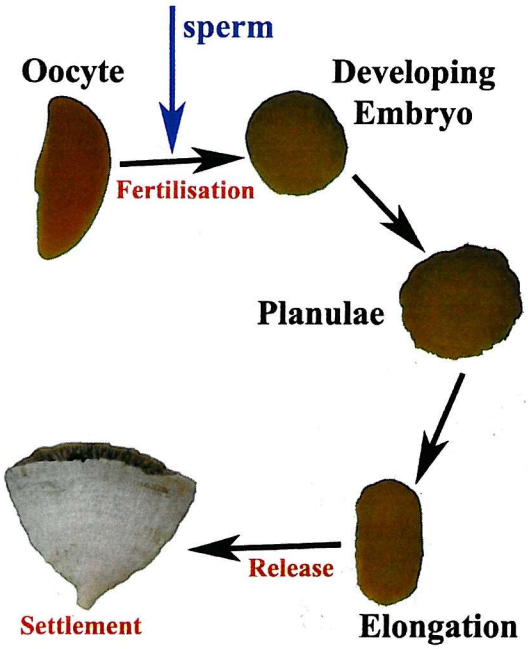


Fig. 3.37 - Life cycle of *F. thouarsii*

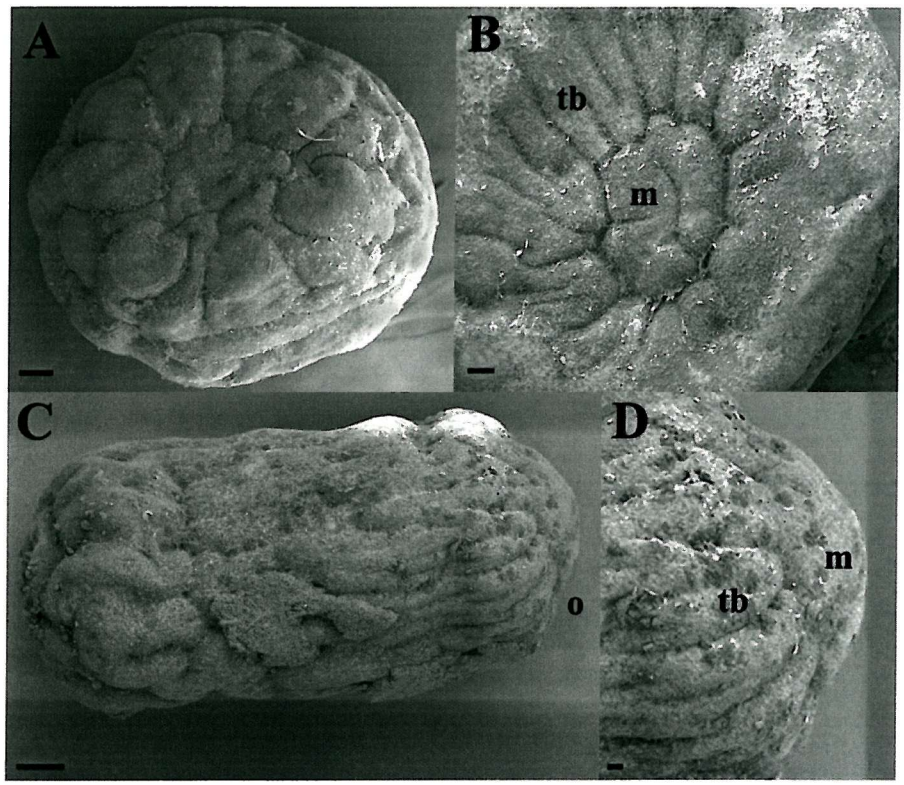


Fig. 3.38 - SEM pictures of *F. thouarsii* larvae, **A**, early flattened planulae; **B**, oral area of early larvae; **C**, Late elongated larvae; **D**, oral area of elongated larvae
tb, tentacle bud; **m**, mouth; **o**, oral end
scale bars - **A**, 200µm; **B**, 100µm; **C**, 200µm; **D**, 30µm

Flabellum curvatum

Previtellogenic oocytes were found up to around 800μm, and were circular and light orange in colour. Vitellogenic oocytes were also circular, and reached a maximum feret diameter of 5124μm. Oocytes were a darker ruby colour than the other two species examined.

Oocyte size-frequency diagrams (Fig. 3.39) show a wide distribution of sizes, with little difference between the months sampled. This suggests a quasi continuous life history. Three stages of planulae development were found (Fig. 3.40) within the mesenteries of females (Fig. 3.41), with up to four planulae being found per mesentery. Planulae were ciliated, though not heavily, and cilia could only be observed in high magnification on the SEM (Fig. 3.42).

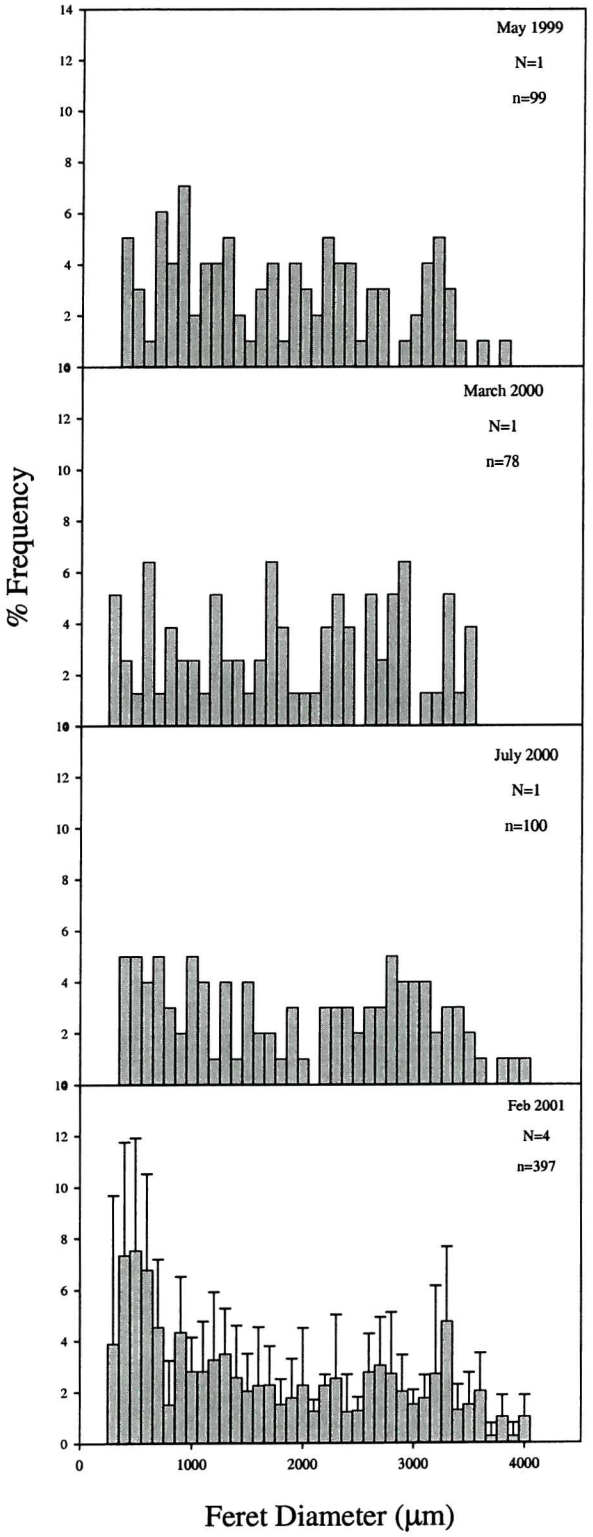


Fig. 3.39 – *F. curvatum* oocyte size-frequency diagrams
error bars - ±1SD for Feb 2001

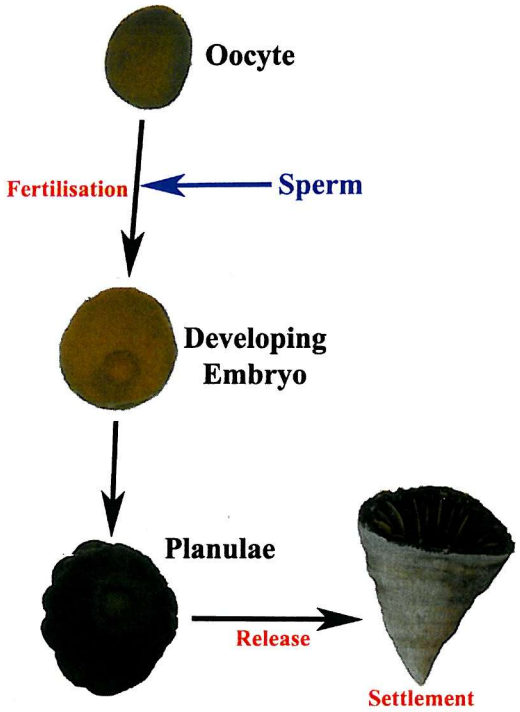


Fig. 3.40 - Life cycle of *F. curvatum*

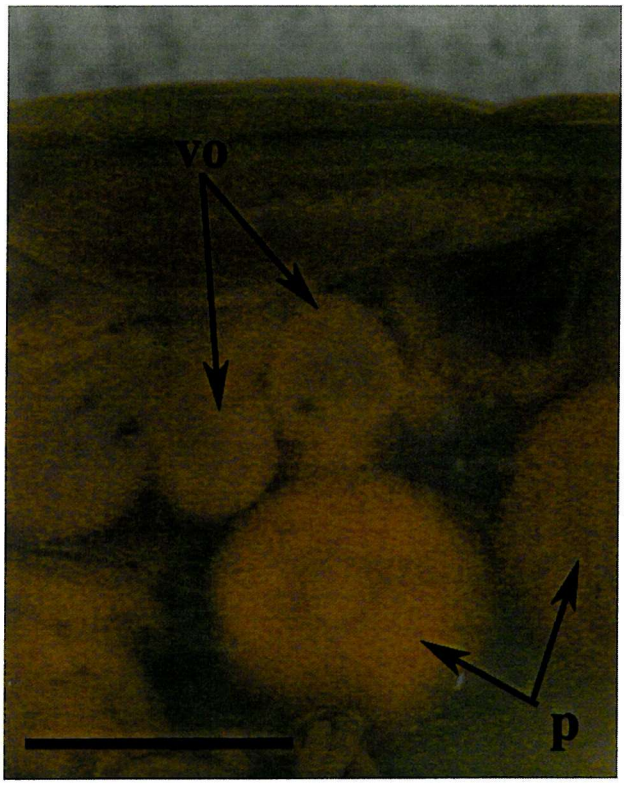


Fig. 3.41 Mesentery of *F. curvatum*, showing planulae embedded into the gastroderm
scale bar - 5000µm

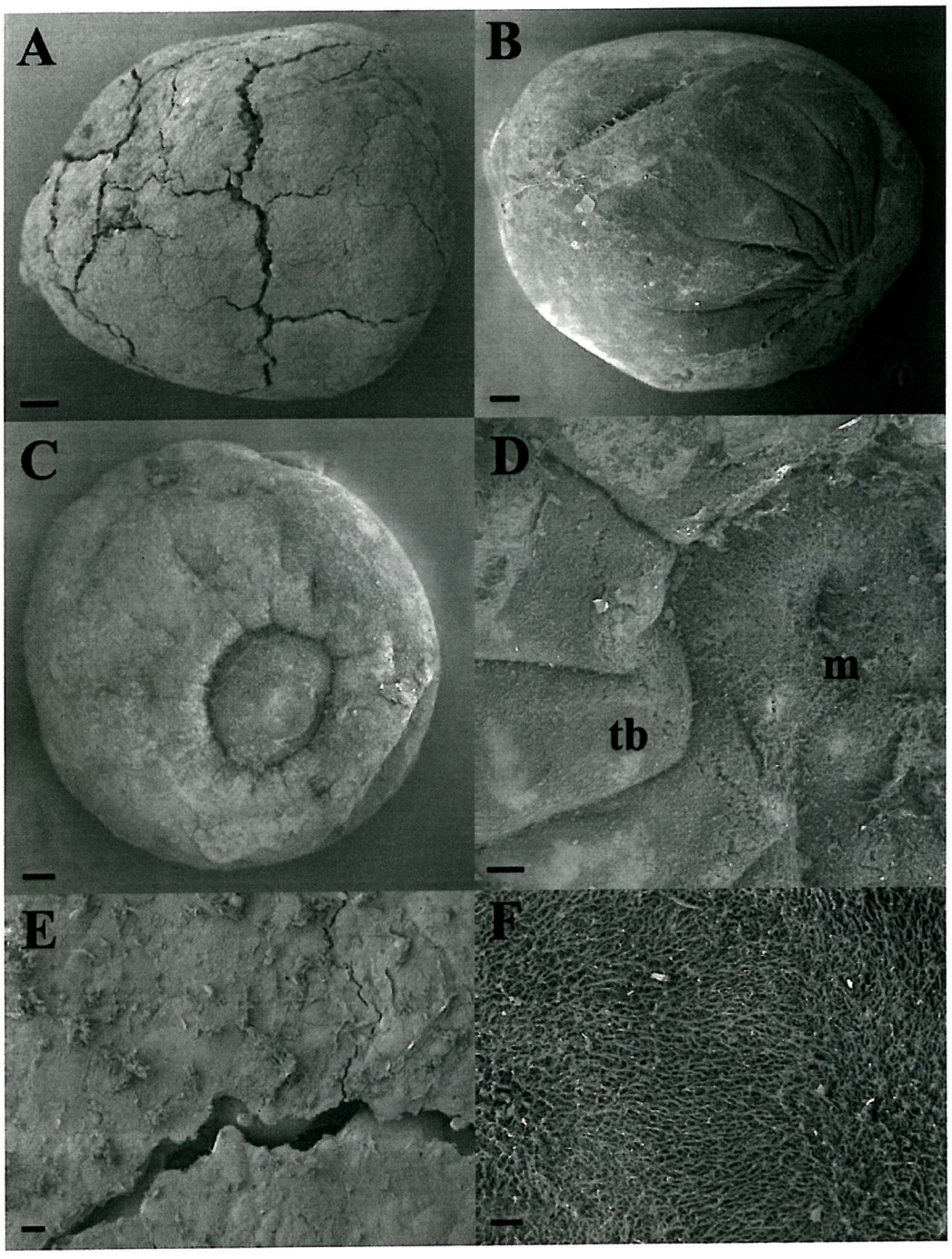


Fig. 3.42 - SEM photographs of *F. curvatum*, **A**, Vitellogenic oocyte; **B**, Early developing embryo; **C**, Planulae larvae; **D**, Oral area of planulae; **E**, Oocyte surface; **E**, Planulae surface
o, oral axis; **tb**, tentacle bud; **m**, mouth
Scale bars, A, 200μm; **B,** 200μm; **C,** 200μm; **D,** 100μm; **E,** 10μm; **F,** 20μm

Flabellum impensum

Round, white, previtellogenic oocytes were found up to around 1000μm. Vitellogenic oocytes were also spherical and deep orange in colour. The maximum feret diameter was 5167μm. Large lipid droplets could also be easily seen through a light microscope, and an 'oil slick' would also appear if oocytes were damaged.

Only one month of samples were analysed for *F. impensum*, November 2000, as they were not collected in large numbers on any previous, or later, cruise. The oocyte size frequencies for this month (Fig. 3.43) show there to be a large number of late stage oocytes, and a smaller second peak in the early stage oocytes.

With only a single month sample it is difficult to suggest any reproductive periodicity in this species.

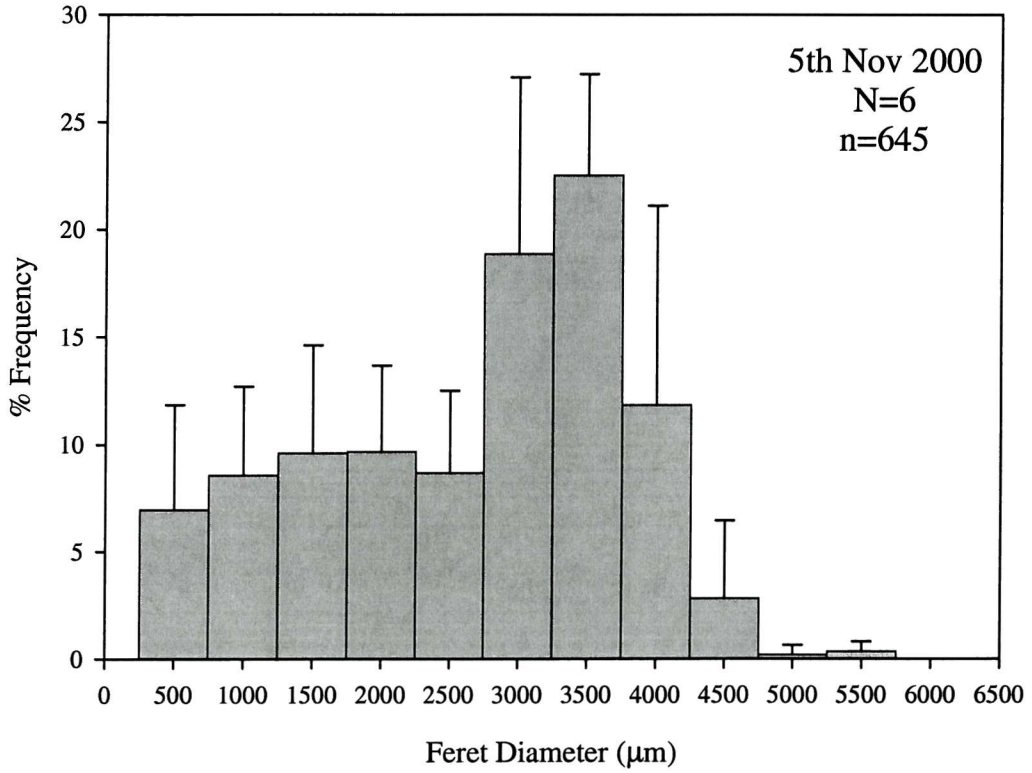


Fig. 3.43 - Oocyte size-frequency diagram for *F. impensum*
error bars - SD

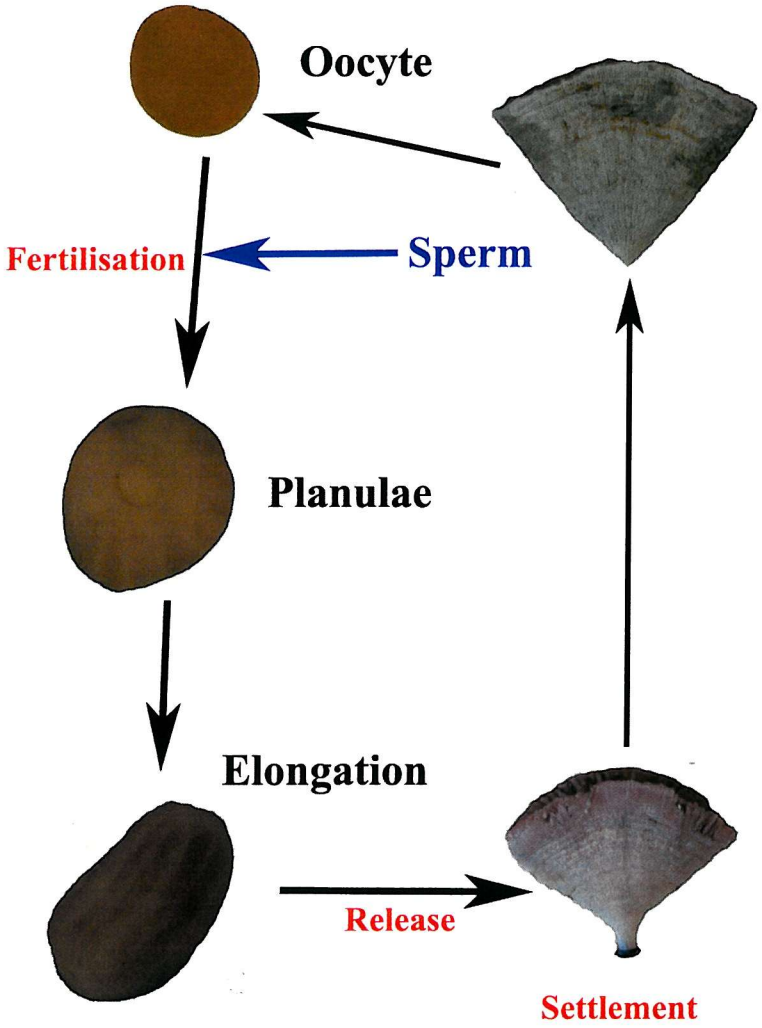


Fig. 3.44 Life cycle of *F. impensum*

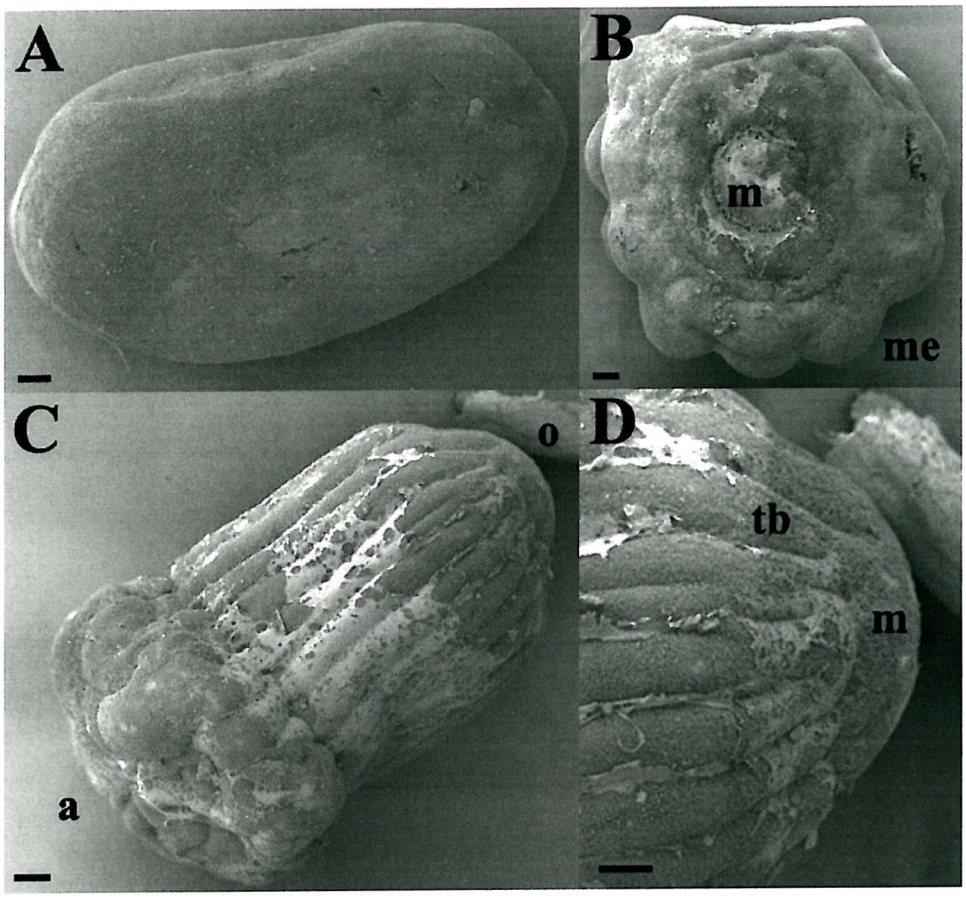


Fig. 3.45 – A, F. impensum oocyte, **B**, Developing embryo; **C**, Elongated planulae; **D**, Planulae oral area
m, mouth; **me,** mesenteries; **a**, aboral axis; **o,** oral axis; **tb**, tentacle bud

Fecundity

As there were few individuals of each species to calculate fecundity, no intra-monthly analysis has been performed.

In all three species, mean fecundity was high, *F. thouarsii*, 2412 oocytes per polyp (oop) (±1554), *F. curvatum*, 1618oop (±1071) and *F. impensum*, 1270oop (±884) potential fecundity. *F. thouarsii* and *F. impensum* both showed size-dependent fecundity (Fig. 3.46, 3.47).

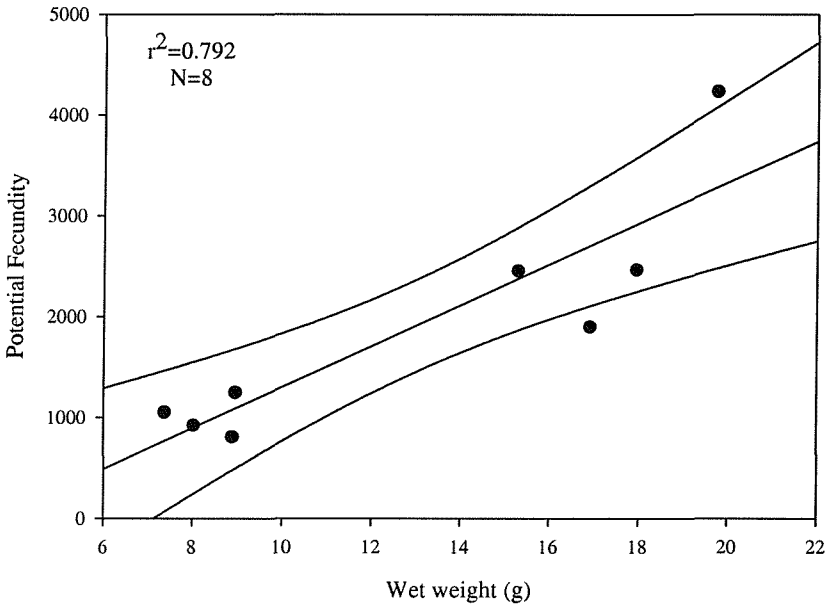


Fig. 3.46 - *F. thouarsii* potential fecundity plotted against wet weight, with a fitted regression line and 95% confidence limits
Regression - f=y0+a*x; P=0.05

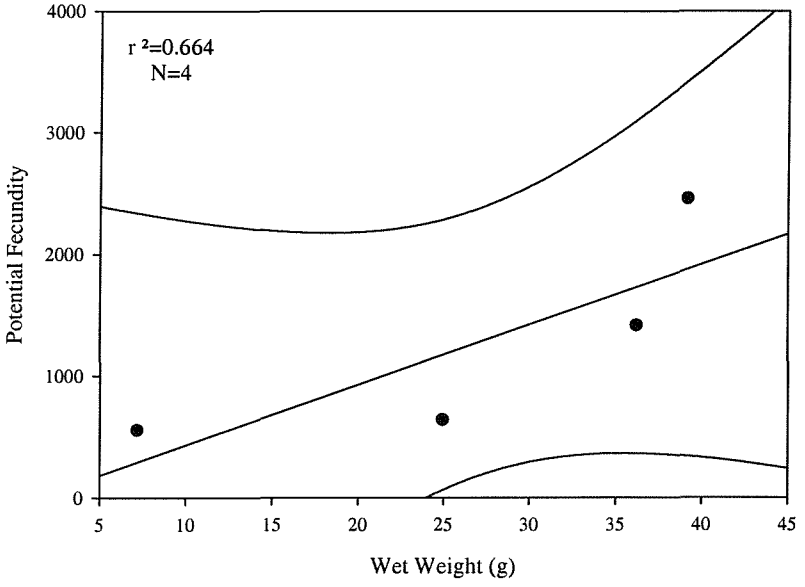


Fig. 3.47 - *F. impensum* potential fecundity plotted against wet weight with a fitted regression line and 95% confidence limits
regression -, f=y0+a*x, P=0.05

Discussion

Flabellum thouarsii, *F. curvatum* and *F. impensum* are all gonochoristic and brood their young. Males and females were found of all three species in a variety of size classes and so sequential hermaphroditism is ruled out.

These are the first deep-water scleractinians to be found to brood, though this has been reported in a small number of shallow water species (Fadlallah, 1983; Richmond & Hunter, 1990). Brooding has also been noted previously in *F. curvatum* from shallow depths (80 to 160m) off Argentina (Squires, 1962). These larvae appeared similar to those from this study, with measurements of ~4mm length and a flattened tympanoid form, with numerous tentacle buds. This study also had few samples, and , unsuccessfully, attempted to observe attachment of the planulae to the substrata.

Brooded planulae were found, in all three species, inside the gastrodermis and what appeared to be free inside the gastrovascular cavity. However it must be noted that, as these species are large, many were heavily trawl damaged, and though whole specimens were specifically targeted, it still cannot be ruled out that planulae were lost during collection. Decalcification can also damage fine tissues, especially with long submersion times, as was needed in these cases. The gastrodermis could have been damaged during decalicification, releasing the planulae into the enteron.

This study has shown a first insight into the reproductive period of these three species. *F. thouarsii* appears to be periodic or prolonged, and *F. curvatum* appears to be quasi-continuous. However there were such few numbers of seasons and replicates that this is really a preliminary estimate. A more detailed study is needed into the seasonality of reproduction in these three species to more fully determine their life history.

There are advantages to brooding in the deep sea, the main one being the production of a more viable planulae to increase infant survivorship. The fecundity of these three species appears high, and with brooding being so energetically expensive compared to spawning gametes, this would seem unusual. However, the body weight of *F. thouarsii* compared to the NE Atlantic species *F. angulare* (which has a similar fecundity, but spawns gametes) is over 5 times as heavy (average size *F. thouarsii* –

14.23g; average size *F. angulare* – 2.53g). This would appear to make the fecundity of *F. thouarsii* (and indeed *F. curvatum* and *F. impensum*) to be low compared to other species. Large polyp size can also be regarded as a sink for energy. Rinkevich and Loya (1979) and Szmant (1986) suggested that the energy required for maintence of a large polyp would mean energy being unavailable to brood planulae, and so may explain why in shallower water, only species with small polyps brood. A factor also is the highly seasonal supply of phytodetritus in Antarctica, providing large amounts of food over a short time period.

Shallow-water Antarctica and the general deep-sea appear to have many similarities, including similarity of temperatures and a seasonal input of phytodetritus. As a consequence of these physical and biotic similarities, many, often conflicting, evolutionary theories have been posed regarding the origins of both deep-water and Antarctic fauna (reviewed in Lipps & Hickman, 1982).

The reproductive biology of many shallow water Antarctic invertebrates has been described in great detail (review in Galley, 2003). Polar invertebrates are generally regarded as having both a slow metabolism and reproductive rates (Clarke & Johnston, 1999). Distinct trends have been observed, including a high proportion of brooding and lecithotrophy (Thorson, 1950; Picken, 1980; Arntz et al., 1994).

Traditional, the deep-sea is regarded as beginning at the end of the continental shelf, at depths below 200m (Gage & Tyler, 1991). However, in Antarctic waters, because continental dispersion and ice scouring, the continental shelf extends to depths of around 800m (Clarke, 1996, 2003). Clarke (1996) suggested that the Antarctic deep-sea should not begin until around 2000m depth, as it is not until this point that physical factors are distinctly different from shallower depths in this area.

The samples for this study were collected at around 500m on the continental shelf off of the Western Antarctic Peninsula. Should the more traditional view of deep-sea be regarded, these organisms should follow a reproductive pattern of other deep-water scleractinians. However all three of these species were found to have high fecundity, very large egg sizes and brood their young. *F. thouarsii* appears to be seasonal and may release planulae just prior to a phytoplankton bloom event (Smith et al., 1995)

that may provide food for developing larvae (Eckelbarger & Watling, 1995). These are traits that more closely follow the Antarctic benthos. When also comparing them to two species of the same genus that live in the deep northeast Atlantic (Section 3.2.4), *F. alabastrum* and *F. angulare*, their reproductive patterns are significantly different. The two species from the Atlantic spawn (one seasonally and one quasi-continuously) gametes, whereas the three Antarctic species brood. The high variability of reproductive traits between scleractinia genera is well documented (Fadlallah, 1983; Richmond & Hunter, 1990; Richmond, 1997).

There are similarities between these five species, all have high fecundity and large oocyte sizes when compared to other deep water (and shallow water) scleractinians, and this maybe a trait of the genus *Flabellum*.

3.3 General Discussion and Conclusions

Eleven species of deep water scleractinian have been examined for this study, within which almost the whole breadth of reproductive modes found in the Cnidaria have been observed. Two species of gonochoristic seasonal spawners; two gonochoristic quasi-continuous spawners, one gonochoristic periodic spawner; three hermaphroditic quasi continuous spawners; one gonochoristic seasonal brooder, one gonochoristic quasi continuous brooder. In addition, asexual reproduction was observed in *Fungiacyathus marenzelleri*. Table 3.8 shows a full summary of the findings of this study, and of other published studies to date.

In order to compare all scleractinian samples, a fecundity ratio was calculated. This is the average fecundity divided by the average decalcified wet weight (for fecund animals) in each species. This produces a ratio of the average number of visible oocytes per average body weight. Figure 3.48 shows the fecundity ratio data for each species (except *Madrepora oculata*, as this is a species with a small polyp that was unable to be weighed) plotted against their depth distribution. This graph shows there to be a trend towards an increasing fecundity with increasing depth. More interestingly, nearly all of the genus *Flabellum* group together, having a larger number of oocytes per volume than the other species in this study. *F. marenzelleri* has the lowest ratio, perhaps a consequence of its physiological ability to colonise the greatest depths in this study.

The general rule for the deep sea is continuous reproduction with lecithotrophic larvae, though many exceptions do occur (Marshall, 1979; Gage & Tyler, 1991; Scheltema, 1994). The species in this study show a mixture of both seasonal and quasi-continuous reproduction, yet many were collected from the same areas and depths, where the environmental conditions would be presumed to be similar. Tyler and Young (1992) discuss varying reproductive patterns in invertebrates collected from the same area and depth in the NE Atlantic. They propose a hypothesis suggesting seasonal reproducers in the NE Atlantic invaded during the last glaciation (>20,000 years ago) to the deep sea, an area previously colonised by non-seasonal reproducers (that possibly invaded during previous glaciations and have evolved for

continuous lecithotrophic development). Three species of the nine from the NE Atlantic in this study were seasonal reproducers (seasonally periodic in the case of *M. oculata*), and these three cover a depth range con-current with the other five species.

Tyler and Young's (1992) hypothesis could be supported by the data presented here. Two species of *Flabellum* from the NE Atlantic were examined in this study, and each had a differing periodicity, *F. alabstrum* is quasi-continuous and *F. angulare* is seasonal, yet both also inhabit similar depth distributions. It is possible that these two species invaded at differing times. However it is also well documented, and has been discussed previously, that scleractinians have extremely plastic reproductive traits (Fadlallah, 1983; Harrison & Hunter, 1990; Richmond, 1997), and so it is possible that environmental conditions are responsible for such differences. Molecular evidence may find the answers to questions of evolution.

In those seasonally-reproducing species from the NE Atlantic in this study, *L. pertusa* and *M. oculata* appear to spawn early in the year, whereas *F. angulare* appears to spawn around September. With the seasonal deposition of phytodetritus in this area being around late July, it appears that each of these species uses this pulse for differing reproductive purposes. Eckelbarger and Watling (1995) suggested several patterns of reproduction related to the seasonal pulse in the NE Atlantic. In fast oocyte producing species (autosynthetic), the pulse may initiate gametogenesis with rapid oogenesis, closely followed by a spawning event. Whereas, with a slow oocyte producing species (heterosynthetic) the pulse may initiate a spawning event when there is sufficient food for developing larvae. *L. pertusa* and *M. oculata* appear to fall into the autosynthetic category, whereas *F.angulare*, which appears to spawn soon after the pulse, may be heterosynthetic. In the Antarctic samples, though preliminary, *F. thouarsii* appeared to be a seasonal reproducer, with double cohorts being observed in March and June samples, but a single cohort being present in November. This may suggest hetrosynthetic oocyte production, releasing brooded larvae just prior to the pulse. With slow metabolism and reproduction being a feature of life in Antarctica (Thorson, 1950; Picken, 1980; Arntz et al., 1994), it would not be unexpected for all three *Flabellum* spp. observed to be heterosynthetically derived.

Species	Area	Depth	Sex	Reproduction						Notes	Ref
				Max Oocyte	Fecundity	Method	Production	Release	Larvae		
Fungiacyathus marenzelleri (Vaughan, 1906)	Rockall Trough	2000m	Gonochoristic	750µm	2900 oop	Spawner	Continuous		(I) Lecithotrophic		1
F. marenzelleri	California	4100m	Gonochoristic	750µm	1290 oop	Spawner	Continuous		(I) Lecithotrophic		2
Lophelia pertusa (Linnaeus, 1758)	Porcupine Seabight	900m	Gonochoristic	140 µm	3146 oop / 3327 ocm2	(I) Spawner	June/Aug	Jan/Feb	(I) Lecithotrophic		3
L.pertusa	Trondheim Fjord	147m	Gonochoristic	60 µm (mean)						Nov. only	4
Madrepora oculata Linnaeus 1758	Porcupine Seabight	900m	Gonochoristic	350 µm	10 opp / 256 ocm2	Spawner	Periodic		(I) Lecithotrophic	Female Only	3
M. oculata	Chatham Rise	800-1000m	Gonochoristic							Male Only	7
Caryophyllia cornuformis Pourtales 1868	Porcupine Seabight	435-2000m	Hermaphrodite (cyclical)	340 µm	-	Spawner	Continuous		(I) Lecithotrophic		5
Caryophyllia ambrosia Alcock 1898	Porcupine Seabight	1100-3000m	Hermaphrodite (cyclical)	630 µm	2900 oop	Spawner	Continuous (sequential)		(I) Lecithotrophic		5
Caryophyllia seguenzae Duncan 1873	Porcupine Seabight	960-1900m	Hermaphrodite (cyclical)	450 µm	940 oop	Spawner	Continuous (sequential)		Planktotrophic		5
Oculina varicosa Lesueur 1821	Florida	3-100m	Gonochoristic	100 µm	2115-4693 ocm2	Spawner	early summer	late summer/fall	Lecithotrophic?		6
Flabellum alabastrum Moseley, 1876	Porcupine Seabight	1800-2250m	Gonochoristic	1010 µm	~550 oop	Spawner	Continuous		(I) Lecithotrophic		
Flabellum angulare Moseley, 1876	Porcupine Seabight	1647-2875m	Gonochoristic	814 µm	~2800 oop	Spawner	Continuous	September	(I) Lecithotrophic		
Enallopsammia rostrata (De Pourtales, 1878)	Chatham Rise	800-1000m	Gonochoristic	400 µm	144 oop	Spawner	Continuous		(I) Lecithotrophic	One sample	7
Solenosmilia variabilis Duncan, 1873	Chatham Rise	800-1000m	Gonochoristic	165 µm	290 oop	Spawner	Seasonal			One sample	7
Goniocorella dumosa (Alcock, 1902)	Chatham Rise	800-1000m	Gonochoristic	135 µm	480 oop	Spawner	Seasonal			One sample	7
Flabellum thouarsii	W Antarctic Peninsula	500m	Gonochoristic	4800 µm	2412 oop	Broods	Seasonal?		Brooded		

Species	Area	Depth	Sex	Reproduction					Notes	Ref
				Max Oocyte	Fecundity	Method	Production	Release / Larvae		
Flabellum curvatum	W Antarctic Peninsula	500m	Gonochoristic	5120 µm	1618 oop	Broods	Continuous?	Brooded		
Flabellum impensum	W Antarctic Peninsula	500m	Gonochoristic	5167µm	1270 oop	Broods	?	Brooded		

Table 3.9 - Reproductive data known for deep water scleractinian species.

Key – (I), Inferred

References – **1**, Waller et al.., 2002; **2**, Flint (2003); **3**, Waller & Tyler, in press; **4**, Brooke, pers com; **5,** Waller et al.., submitted; **6**, Brooke, 2003; **7**, Burgess & Babcock, in press

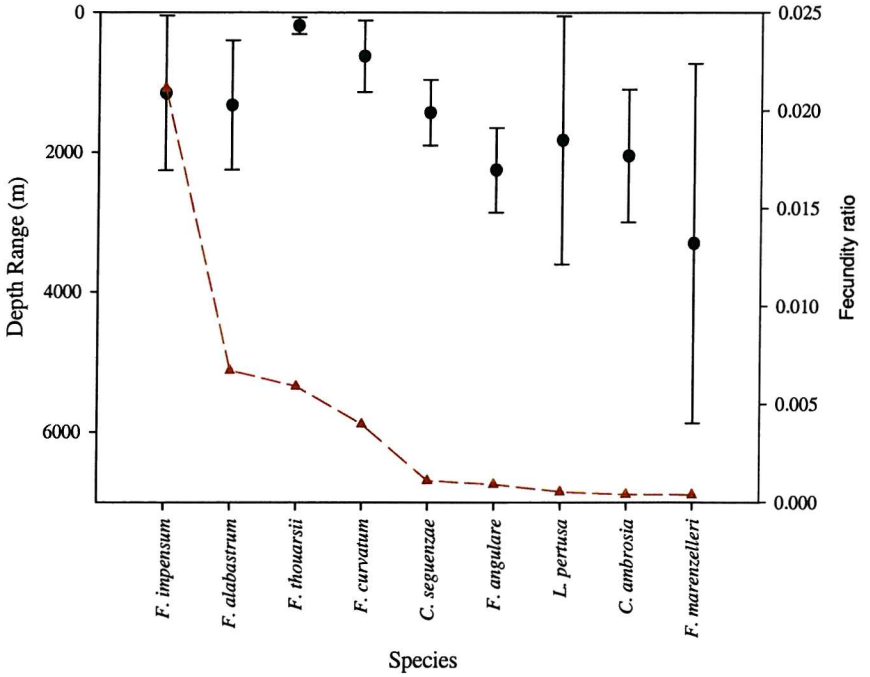


Fig. 3.48 – Graph showing the fecundity ratio and the depth range for each species analysed in this study
Red triangles show fecundity ratio; Black circles show depth distribution
Depth data compiled from Zibrowius (1980) and Cairns (1982)

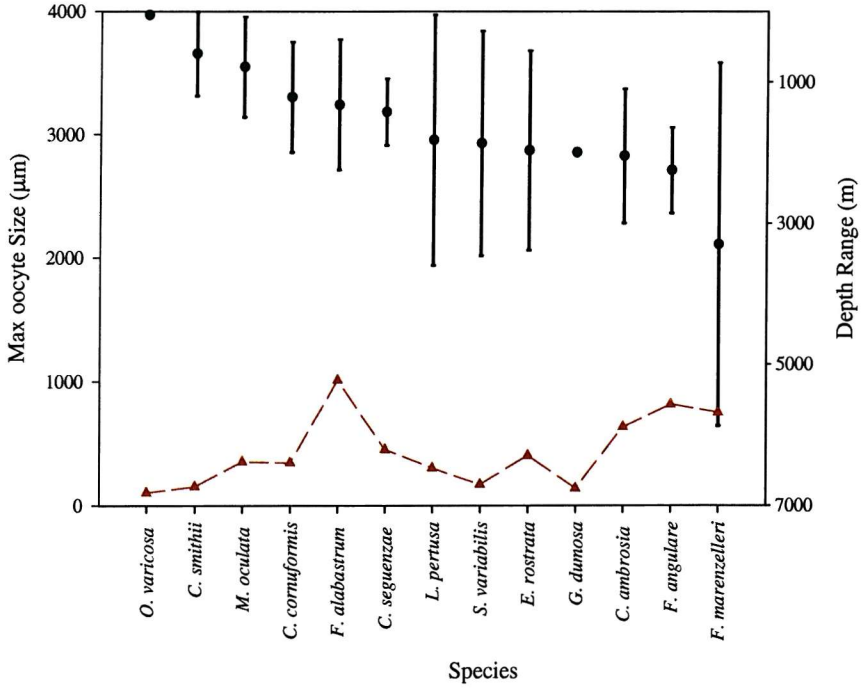


Fig. 3.49 – Graph showing the maximum oocyte size and the depth range species in this study. Antarctic species omitted as they were off scale
Red triangles show fecundity ratio; Black circles show depth distribution, bars indicate depth ranges
Depth data compiled from Zibrowius (1980) and Cairns (1982); *O. varicosa* data from Brooke & Young, (2003); *S. variabilis*, *E. rostrata* and *G. dumosa* from Burgess & Babcock, in Press

There is also a trend in the deep sea for oocyte size to increase as depth increase, thereby producing a lecithotrophic larvae (Gage & Tyler, 1991; Young, 1994). Lecithotrophic larvae are also common in many polar invertebrate species (Clarke, 1979; Picken, 1980; Arntz et al., 1994). All species in this study appear to be over the threashold for a lecithotrophic larvae, 200μm (Gage & Tyler, 1991). *L. pertusa* is the closest to this, but due to the lack of collection at vital spawning periods and its rapid development, it is hypothesised that the maximum oocyte diameter is actually much higher than the 140μm found in this study. Fig. 3.49 plots the maximum oocyte size for deep water scleractinian species, against their depth distribution. Although not a perfect correlation, there is a distinct trend for larger oocyte diameters as the depth increases. Antarctic species were not included in this diagram, as their oocyte diameters were significantly higher than any of those found in other species and resolution of the graph would be lost. However the trend is the same between these three species.

A number of deep-water anthozoans have had their reproduction reported. *Actinoscyphia aurelia* has an oocyte diameter of ~150μm and is thought to have a high fecundity (Aldred et al., 1979). The epizoite zooanthids *Epizoanthus paguriphilus* and *Epizoanthus abyssorum* have oocyte diameters of 180μm and 280μm respectively (Muirhead et al., 1986). Van-Praet et al. (1990) examined reproduction in *Phelliactis robusta* and *P. hertwigi* and observed maximum oocyte diameters of 210μm and 180μm respectively. Van-Praet (1990) also examined the deep water anemone *Paracalliactis stephsonsi* and found oocyte diameters of ~180μm. *Hormathia digitata* and *Allanatis parasitica* from the Arctic have oocyte diameters between 300μm and 400μm, with the former possibly being a brooder (Riemann-Zurneck, 1994). *Hormathia nodosa* also produces large oocytes, ~800μm diameter (Riemann-Zurneck, 1994). Bronsdon (1997) observed the two epizoic actinarians *Amphianthis inornata* and *Kadosactis commensalis* to have oocyte diameters of ~205μm and ~150μm respectively. Bronsdon (1997) also examined the pennatulid *Umbellula lindahli* and found a maximum oocyte diameter of

~800μm and fecundity of ~2000 mature oocytes per individual, and so a potential fecundity of ~30,000 oocytes. *Anthipathes fiordensis* from New Zealand was observed to have a colony fecundity of ~16.9 million oocytes, and a maximum oocyte diameter of 140μm (Parker et al., 1997). The brooding alcyonacean *Anthomastus ritteri* contains over 4000 oocytes and larvae, and mature oocyte diameters of 376μm (Cordes et al., 2001).

These other deep-water anthozoans, when combined with data from this study, show a wide range of both oocyte sizes and fecundities (though the majority of the studies discussed above lack fecundity data). The lack of a distinct 'pattern' for the Cnidaria is one echoed in the shallow-water environment.

In comparison to shallow water species, deep water scleractinians appear to form different trends, though it must be stated that while reproductive information on deep-sea species is in its infancy, information on their shallow water counterparts is expansive (Fadlallah, 1983; Harrison & Hunter, 1990; Richmond, 1997). 75% of the shallow species known to date are hermaphroditic, yet gonochorism appears to be more dominant in deeper waters. Gonochorism has been suggested as a more primitive strategy than hermaphroditism (Goffredo et al., 2000), though is more important for maintaining genetic diversity (Szmant, 1986) perhaps essential to survival in the deep-sea. In sedentary species (such as the Scleractinia), and the wide expanse that is the deep-sea, it might be assumed that hermaphroditism be more common. Broadcasting gametes on a seasonal basis, mainly cued by tidal, lunar or temperature, is the most common strategy in shallow water scleractinians. Quasi-continuous production of gametes was observed more frequently in this study, and indeed in other studies of deep water scleractinians (Burgess & Babcock, in press). As discussed previously, this fits with other patterns observed in deep water. Brooding is uncommon in shallow-water species, and was only observed in Antarctic deep water species from this study, which, as discussed earlier, appear to fit patterns more attributed to Antarctic shallow water than the general deep sea.

Chapter Four

Deep-Water Solitary Scleractinians:

The Polyp and its Calice

4.1 The Polyp and its Calice

Introduction

In the previous chapter I have addressed the issues of sexual reproduction, and so have observed the process by which new individuals may be brought into a population. This chapter is an initial investigation into the population structure of solitary deep-water corals. The turnover of populations is an important process, as it aids us in understanding how quickly they might recover from disturbance (Chadwick-Furman et al., 2000).

There is little information on the population structure of scleractinians, probably owing to their complex life histories (Hughes & Jackson, 1985; Babcock, 1991; Chadwick-Furman et al., 2000). Both sexual and asexual processes can lead to new individuals within cnidarian populations. In colonial scleractinians, asexual proliferation leads to the increase in colony size, and therefore represents growth. However there is a second process that is attributed to growth, the formation of a new aragonite skeleton, calcification. It is possible that in colonial corals this process is less important to growth than asexual processes (which may also include calcification).

However, in solitary corals it is solely by calcification that a polyp can increase dimensions. In asexual reproduction, a separate (but genetically identical) entity is created and therefore does not contribute to growth as in colonial forms. Calcification is controlled by many environmental variables, the main one is thought to be the calcium carbonate saturation of seawater (Gattuso et al., 1998). It is a biologically-driven, physical chemical process that has proved difficult to quantify (Tambutté et al., 1996; Gattuso et al., 1998). There are many ongoing arguments over the state of the carbonate saturation in seawater, and the effect of global warming upon it (Buddemeier, 1994, 2001; Kleypas et al., 1999; Idso et al., 2000). Ultimately it will have an effect on coral reefs around the globe, shallow and deep.

In shallow-water, zooxanthellae are thought to provide the chief source of energy for the process of calcification (Idso et al., 2000). However, deep-water scleractinians do not contain zooxanthellae, and so calcification and growth is thought to be much

slower at depth (Goreau, 1961; LeTissier, 1990). In zooxanthellate corals, linear extension rates have been observed to slow with depth, probably a function of lowered light levels (Villiniski, 2003). The calcium carbonate saturation also decreases with decreasing temperature (Idso et al., 2000, Howe & Marshall, 2002), this is likely to also affect growth rates, as well as possibly being a contributing factor to the lack of large reefs below 2000m.

Scleractinians also exhibit a wide range of interspecific morphological differences, making population information difficult to obtain (Chadwick-Furman et al., 2000). As discussed in Chapter 3 Section 1.2, *Lophelia pertusa* can be found in various morphologies, as can a number of shallow-water reef building species (Veron, 1995). These morphologies tend to occur in different areas, and consist of forms that are more suitable for the differing environmental conditions. Veron (1995) comments on the appearance of large colonies of several shallow-water species in some areas, whereas in others only smaller colonies are ever found. Potts (1984) transplanted pieces of *Acropora palifera* and *A. cuneata* onto differing sites along Heron Island, GBR, each with a different physical environment. He observed rates of growth to vary greatly between areas. Todd et al. (2001) also found differences in polyp size in *Favia speciosa* dependent on location. Even in single colonies there can be morphological differences from the base of a branch to its tip (Veron & Wallace, 1984). In the solitary azooxanthellate coral *Caryophyllia smithii*, skeletal density was observed to increase with increasing depth (Bell & Turner, 2000). These cases all suggest strong environmental controls on growth and morphology.

Growth appears not be related to age in some species of scleractinian. Babcock (1991) demonstrated that the sizes of three species of shallow-water reef building corals do not relate to age as partial mortality is not taken into account. He observed that shrinkage of colonies (by partial mortality) can drastically affect the size structure of the population, but, did not affect the age structure. He also observed that these species had both age and size-specific fecundity (with a reduction in fecundity in older corals because of size reduction). Some species, however are more constrained in their morphology. For example, some species rarely undergo partial mortality, fission or fusion that may distort age-fecundity relationships (Loya, 1976; Grigg, 1984; Chadwick & Loya, 1990; Babcock, 1991; Johnson, 1992; Chadwick-Furman et

al., 2000). Chadwick-Furman et al. (2000) found age-related growth in *Fungia granulosa*, and was able to apply the Beverton and Holt population dynamic model to populations of these species in the northern Red Sea. The Beverton and Holt population model has proved an invaluable tool for managing exploited populations of scleractinians (Grigg, 1984; Ross, 1984). This method, however, relies on growth rates, unobtainable during the present study.

Examining the population structure of any organism is related to the determination of their sexual output. By assessing the numbers of reproducing individuals in a population, management strategies can be implemented to protect populations. As discussed above, there are many factors that contribute to the growth and morphology in scleractinians. By understanding the relationships between sexual output and size in corals, it would be possible to predict the reproductive output of a population as a whole.

The physical variables of an organism on reproduction has to be assessed prior to population dynamics. Heyward and Collins (1985) examined the growth and reproduction of *Montipora digitata* and found that the number of oocytes per cm^2 was high. Yet they failed to examine if fecundity was related to the size of the colony, the size of the polyp or age of colony. Chapter Three examined the fecundity of several species of solitary and reef building scleractinian. Neither *Lophelia pertusa* nor *Madrepora oculata* had size-specific fecundities, suggesting something other than polyp size was mainly governing fecundity in these cases. Several authors have noted that colony size in shallow-water scleractinians has limitations on fecundity (Kojis & Quinn, 1985; Szmant-Froelich, 1985; Hall & Hughes, 1996) and the initiation of reproduction (Fan & Dai, 1995; Hall & Hughes, 1996). This factor was discussed in Chapter Three, and was unable to be established for the two reef-forming species in this thesis because of collection methods.

However, most solitary species in this study did show size-specific fecundity. Therefore it would be possible to measure the individual polyp sizes and gain an insight into the reproductive output of the population as a whole. There are, however, several measurements that can be taken and related to fecundity: per polyp (Kruger & Schleyer, 1998), wet weight (this study), polyp diameter (Van Veghal & Kahmann,

1994; Shlesinger et al., 1998) and skeletal dimensions (Glynn et al., 1994). However, the relationship between all these features is virtually unknown. Shlesinger et al (1998) observed that there appeared to be no universal relationship between coral size, polyp size, oocyte size and mode of reproduction. Wet weight of polyp is ultimately directly related to fecundity, as the larger an individual, the larger the oocytes, or the more oocytes, that may be produced. However, whether this is directly related to skeletal size (calice) is unknown. Though it is obvious a polyp of 10cm could not reside in a 5cm calice, this, in theory, could occur vice-versa, should the environmental conditions dictate for a heavily calcified skeleton for protection. Bell and Turner (2000) found heavier calcified polyps of *C. smithii* in deeper locations around Ireland. Calcification rates of deep-water scleractinians are unknown, and even in shallow-water are poorly reported (Howe & Marshall, 2002). By examining the relationships between a polyp and its skeleton, and an individual and its fecundity, it would be possible to assess the best method to examine population dynamics on a species by species basis.

This study examines the relationships of polyps to the skeleton (calice) in five species examined previously in Chapter Three.

4.2 Materials and Methods

Species	No. Individuals	Collected	Details
C. ambrosia	58	Reproductive study	
C. cornuformis	31	Reproductive study	
C. seguenzae	45	Reproductive study	
C. seguenzae	52	26th March 1982,	1343-1370m, OTSB, *RRS Darwin*
C. seguenzae	100	8th July 1979	1365-1415m, OTSB, *RRS Challenger*
C. seguenzae	100	October 2002	1360-1240m, OTSB, *RRS Discovery*
F. angulare	30	Reproductive study	
F. alabastrum	30	Reproductive study	

Table 4.1 – Numbers of species used in the population analysis
Reproductive study – contains a mix of individuals from the reproductive study, Chapter Three

Five species of solitary scleractinian were used for this study. For the initial analysis, individuals that were used for the reproductive study (Chapter Three), had their skeletal dimensions measured (see Chapter Two) prior to decalcification, and were wet weighed after. *C. seguenzae* was the only species present in sufficient numbers in the Discovery Stores (SOC) to do further size frequency analysis, following the statistical method in Meesters et al. (2001), detailed in Chapter Two.

4.3 Results

For the solitary species *Caryophyllia ambrosia, C. cornuformis, C. sequenzae, Flabellum angulare* and *F. alabastrum*, three skeletal dimensions (see Chapter 2, Section 5) and wet weight were measured. Each of these measurements, and a volume index estimate (see Chapter Two) were correlated against wet weight (see Appendix IV). Skeletal volume in all cases provided the highest R^2 values, and so is presented here.

Caryophyllia spp

C. ambrosia

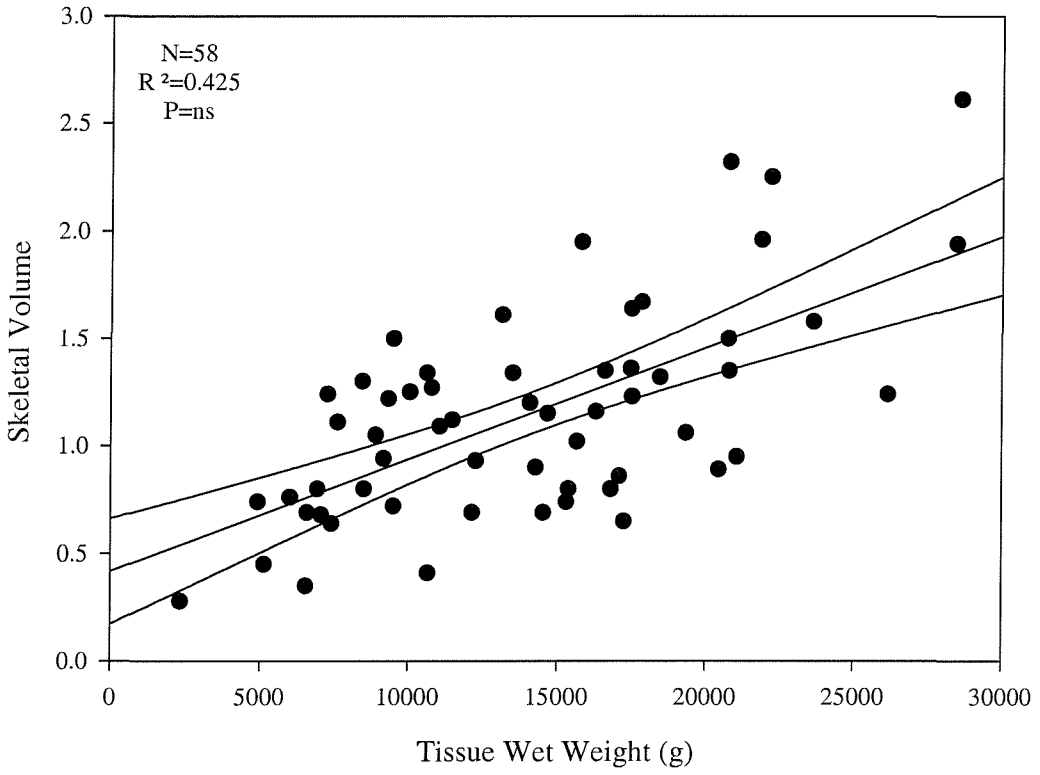


Fig. 4.1 – Wet weight against skeletal volume index in *C. ambrosia* with regression analysis showing 95% confidence intervals
N – Number of individuals measured
f=y0+a*x

There was no significant correlation between wet weight and skeletal volume index in *C. ambrosia* ($R^2 = 0.421$, P=ns).

C. cornuformis

There was a significant correlation between polyp wet weight and skeletal volume index in *C. cornuformis* (R^2=0.7498, P=>0.01). This species is rare in collections, and so there were limited numbers of individuals within a months sample for the analysis. As a consequence no further statistical analysis could be done on this species.

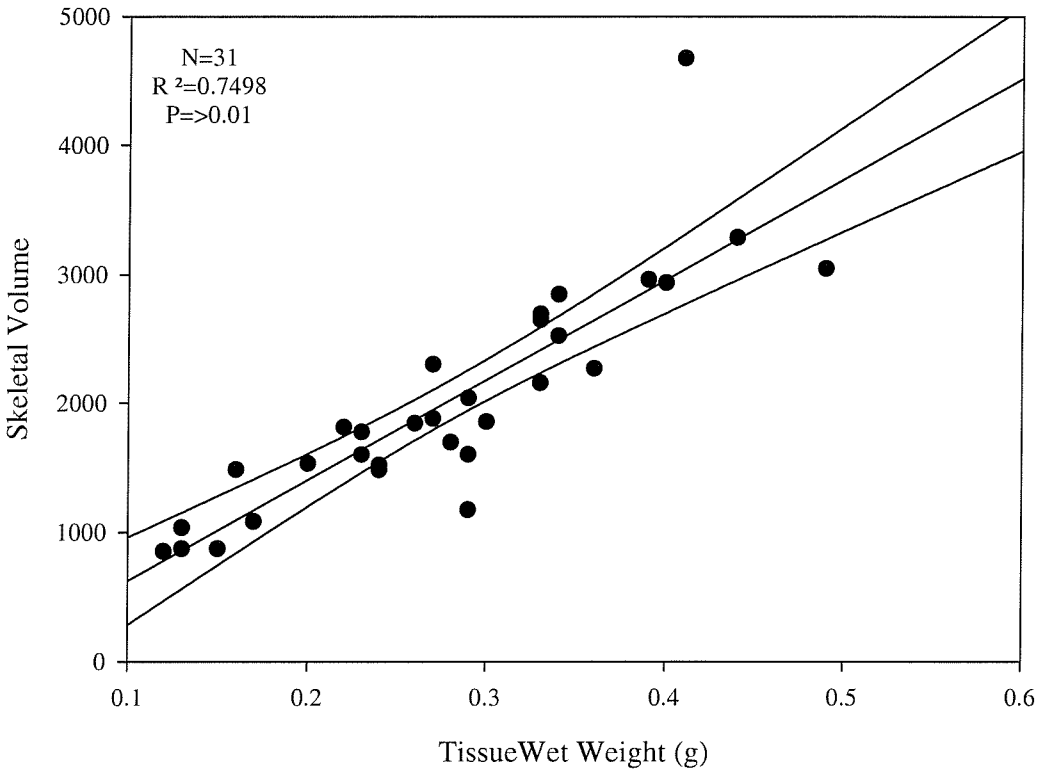


Fig. 4.2 – Wet weight against skeletal volume index in *C. cornuformis* with regression analysis showing 95% confidence intervals
N- number of individuals measured
f=y0+a*x

C. seguenzae

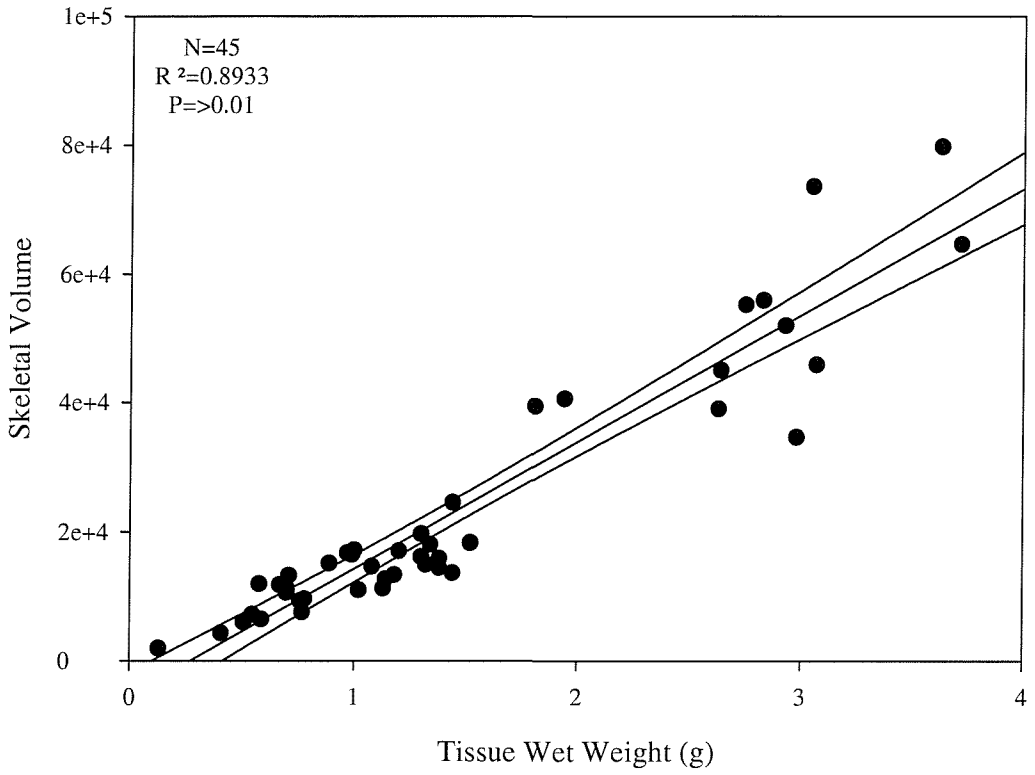


Fig. 4.3 – Weight wet against skeletal volume index in *C. seguenzae* with regression analysis showing 95% confidence intervals
N – Number of individuals measured
f=y0+a*x

There is a significant correlation between polyp wet weight and skeletal dimensions in *C. seguenzae* (R^2=0.8933, P=>0.01). Three population samples from the Porcupine Seabight (1979, 1982 and 2002) were measured and binned into size-frequency classes (Fig. 4.4). Gaussian 3 parameter peak curves were fitted using SigmaPlot 8.0 and were significant in all three plots (1979 – R^2=0.932; 1982 – R^2=0.555; 2002 – R^2=0.894, P=>0.01). Kolmogorov-Smirnov normality tests were performed on all three data sets, none were normally distributed (1982 – K-S=0.182, P=0.111; 1979 – K-S=0.299, P=<0.010; 2002 - K-S=0.338, P=<0.010). Skewness and kurtosis of each colony are represented in Table 4.1. All colonies were positively skewed, and kurtosis showed a sharper than normal distribution in all three. Mann-Whitney U-Tests showed there to be no significant difference between the populations (P=0.6925, P=0.5064, P=7397).

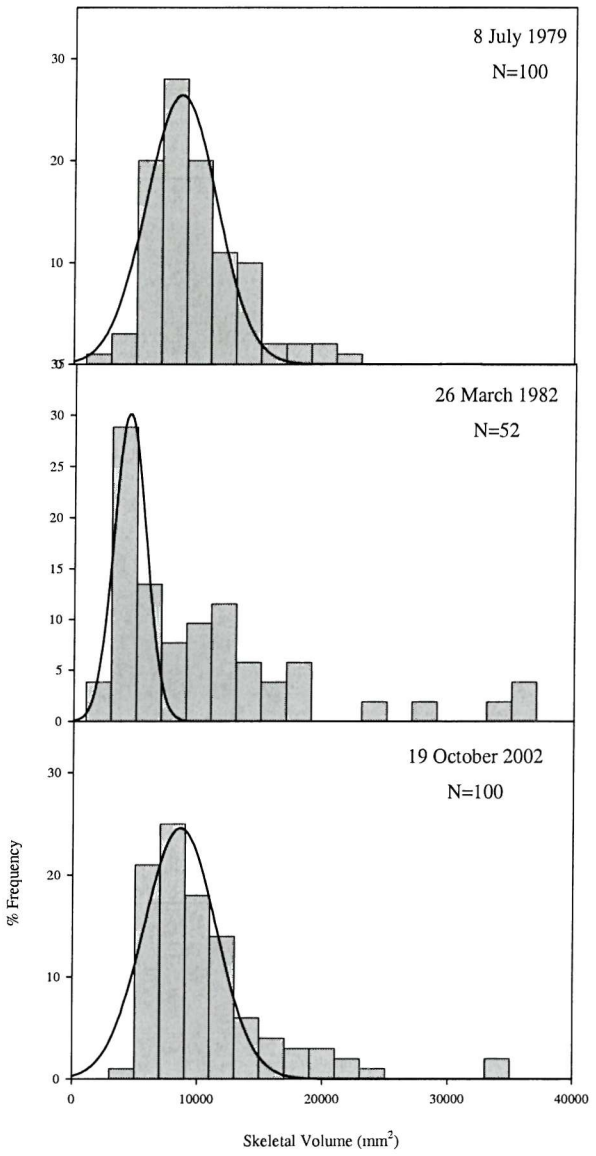


Fig. 4.4 – Size frequency diagrams for *C. seguenzae*, with Gaussian 3-Parameter Peak curves.
N – number of individuals measured
1979, R^2=0.932, P=0.102; 1982, R^2=0.555, P=0.005; 2002, R^2=0.894, P=0.02

Variable	Maximum %	Skewness	Kurtosis
1979	25.00	1.53	1.02
1982	28.85	2.26	6.27
2002	28.00	1.64	1.63

Table 4.2 – Skewness and kurtosis values for *C. seguenzae* populations

Flabellum spp.

F. angulare

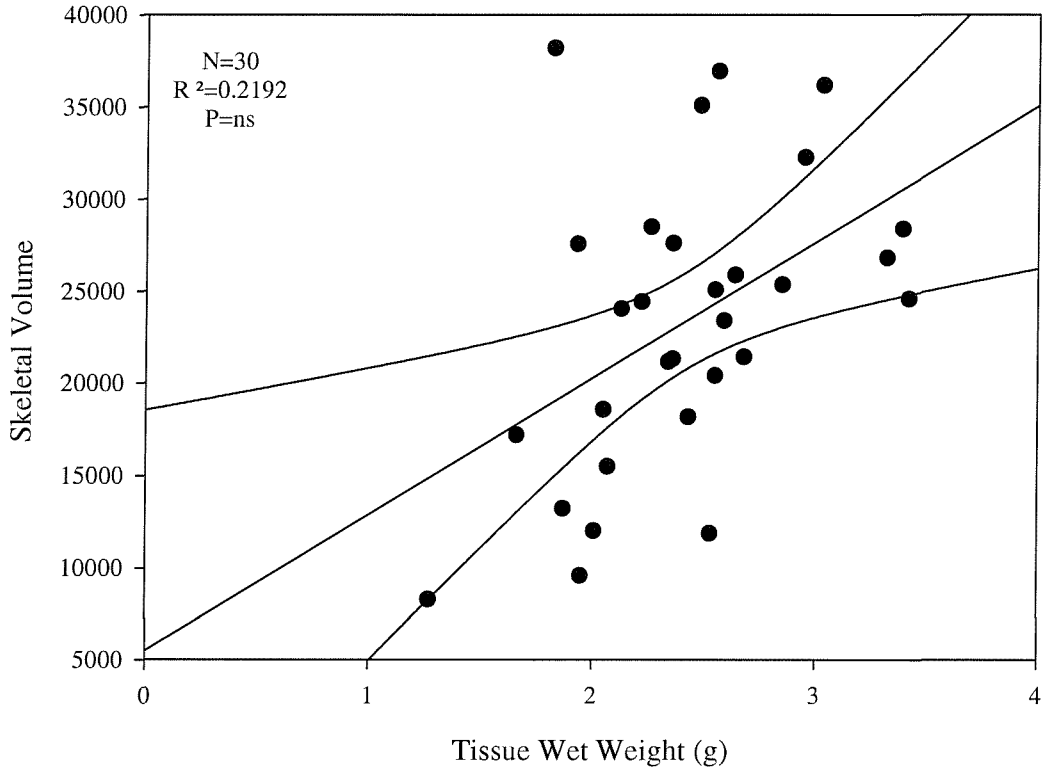


Fig. 4.5 – Wet weight against skeletal volume index in *F. angulare* with regression analysis showing 95% confidence intervals
N - number of individuals measured
f=y0+a*x

There was no correlation between wet weight and skeletal volume index in *F. angulare* ($R^2 = 0.2192$).

F. alabastrum

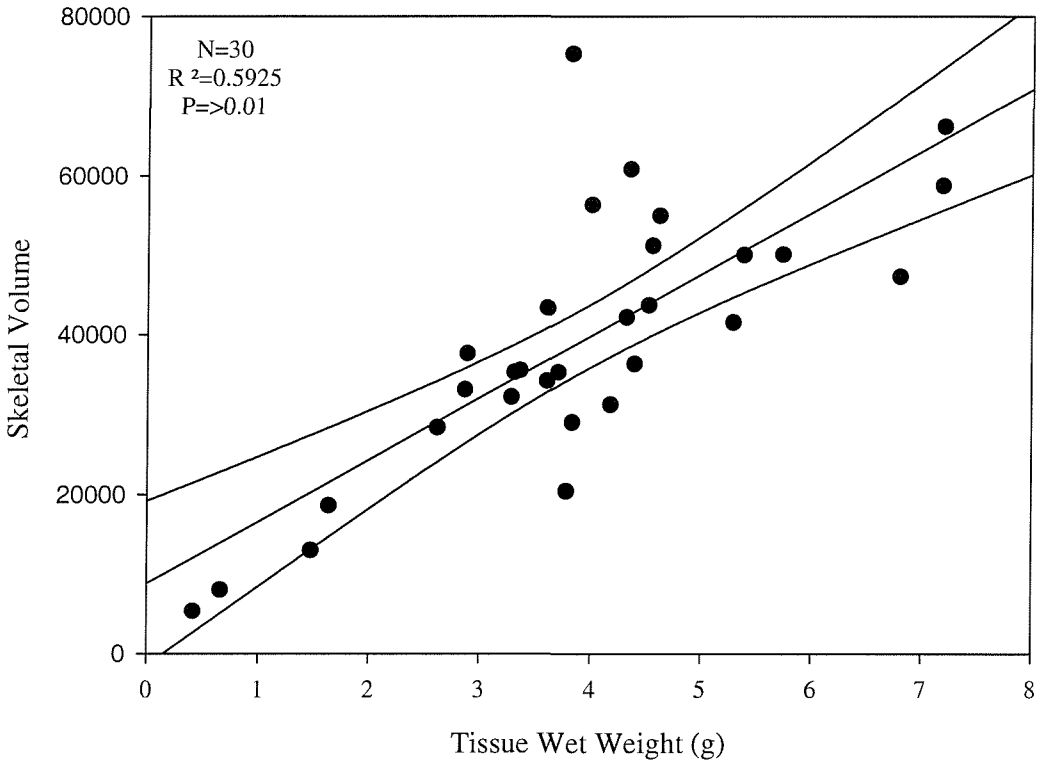


Fig. 4.5 – Wet weight against skeletal volume index in *F. alabastrum* with regression analysis showing 95% confidence intervals
N - number of individuals measured
f=y0+a*x

There is a significant relationship between wet weight and skeletal volume index (R^2=0.5925, P=>0.01). Unfortunately there were insufficient individual numbers in single populations to measure size-frequencies in this species.

4.4 Discussion

Three of the five species used in this population analysis, *C. cornuformis*, *C. seguenzae* and *F. alabstrum*, showed significant relationships between their actual polyp size and skeletal sizes. In addition, all three species also showed size-specific fecundity (Chapter Three).

There is little data available on size structures of populations of deep-water invertebrates, and none on deep-water scleractinians. Gage & Tyler (1991) found the population dynamics of three species of deep-water mollusc from the Rockall Trough to be similar to shallow-water counterparts. Ingram & Hessler (1987) analysed size-frequency data from the deep-sea amphipod *Eurythenes gryllus* and formulated a growth rate from these data. Ragonese & Bianchini (1996) studied size-frequency plots of the deep-sea shrimp *Aristeus antennatus*, estimating growth, recruitment and mortality. Sarda & Cartes (1993) found depth related differences in the sizes for four species of decapod from the Meditteranean. A number of deep-water echinoderms have also been analysed (see Gage & Tyler, 1991).

The increased interest, and extensive collections made, around chemosynthetic environments have led to several population studies in these areas. Thiebaut et al. (2002) examined the tube worm *Riftia pachyliptia* from differing sites in the East Pacific Rise. These populations appeared not to differ from one another and displayed a high population turnover. (Semi) continuous juvenile recruitment was found in two species of alvinellid polychaete from the Juan De Fuca and Explorer Ridge vent sites (McHugh, 1989). Large numbers of small juveniles have been recorded at chemosynthetic sites (Van Dover et al., 2001) and Smith et al. (2000) studied the inner areas of brine seeps in the Gulf of Mexico and found these to more suited to high recruitment in the mussel *Bathymodiolus childressi* compared to the outer rims. These limited studies have shown the usefulness of population studies in deep-water sites, yet the number of individuals needed on a regular basis are, almost, impossible to obtain from these areas because of the limited, and expensive, availability of equipment.

Growth in scleractinians can be controlled by many environmental factors (Potts, 1984; Veron, 1995; Todd et al., 2001), and so has proved difficult to quantify. It is therefore unsurprising that some species have proven unrelated to their skeletal dimensions. An individual living in an area of high flow would be expected to have a more heavily calcified skeleton for survival purposes than one that is in a low flow area (Veron, 1995; Bell & Turner, 2000). The expensive energy cost of calcification would be minimised in the low flow areas. It is then possible that this extra energy is then used for growth or reproduction. Fan and Dai (1995) found different non-reproductive 'juvenile' sizes for scleractinians in differing areas, suggesting they were related to the unfavourable location of one site. Shlesinger et al. (1998) suggested that massive colonies may survive physical disturbances by increasing their energy for growth and subsequently reducing the energy available for reproduction. A more in depth study is required into solitary and reef-building scleractinians, to fully assess the morphological differences in skeletal elements, and relate them to reproductive output.

With the exception *C. seguenzae,* insufficient individuals were collected to complete a full population analysis. *C. seguenzae* showed a non-normal distribution that was more peaked and skewed to the right. This shows a large percentage of the population to be smaller individuals, and so juvenile recruitment is probably very important in this population, with relatively high adult mortality probably also occurring. Chadwick-Furman et al. (2000) observed a similar population structure in the solitary mushroom coral *Fungia granulosa.* Meesters et al. (2001), studying colonial corals, found that in degraded areas, populations tended to have lower recruitment rates and therefore a higher number of larger individuals. *C. seguenzae* also had a significant relationship between wet weight and fecundity (Chapter Three, R^2=0.728, P=0.05), with a maximum fecundity of 940 oocytes per polyp (oop). At an average polyp size of around 0.75g (correlated using fecundity/wet weight scatter plot (Chapter Three) and wet weight/skeletal volume scatter plot(This Chapter)), this would give and average individual in these populations studied to have a fecundity of approximately 200 oop. At a maximum fecundity of 940 oop, this gives this population a very low fecundity compared to that possible. More populations from more areas need to be studied to observe if this is the trend in all situations. This trend of large numbers of

smaller individuals does not appear to have changed with time in the Porcupine Seabight, and may relate to a large juvenile recruitment rate.

Veron (1995) shows the morphological variations of the reef-building *Pocillopora damicornis* on the Great Barrier Reef when subjected to different environmental regimes. Bell and Turner (2000) have also observed morphological variations in a solitary scleractinian subjected to different environmental conditions. By observing deep-water populations in different areas it would be possible to observe if the environmental conditions dictate how large an individual can grow, and what morphological features it may have. A further extension to this analysis would be to also date the individuals within the population. It would be interesting to observe if the dates of individuals is related to skeletal body size, as has been found in several shallow-water studies (Loya, 1976; Grigg, 1984; Chadwick & Loya, 1990; Johnson, 1992; Chadwick-Furman et al., 2000).

Fungiacyathus marenzelleri also did not undergo this type of population analysis. This was in error, as initially in the reproductive study, polyp diameter was found to have a better correlation with fecundity, and so was used instead of wet weight. However, Flint (2003) did measure wet weight and skeletal diameter in this species (volume would be difficult to calculate as it has a flattened morphology) and found there to be a significant relationship (R^2=0.5719, P=<0.0001).

Ultimately the importance of the population structure of corals (and other organisms) is related to reproduction. Knowing the diversification of size classes within a population allows us to estimate the reproductive output for that population and therefore develop a management strategy for individual species needs. This study has been designed to study specifically solitary scleractinians. Reef-building forms are more difficult to study population structure, as it has long been argued whether colonial species are reproductively integrated or isolated (Harrison & Wallace, 1990; Soong & Lang, 1992). Many colonial invertebrates are polymorphic (differing areas having differing roles) and show integration between individuals within the colony (Soong & Lang, 1992). Coral colony size has also been observed to be related to fecundity rather than polyp size in some shallow species (Kojis & Quinn, 1985; Szmant-Froelich, 1985; Soong & Lang, 1992; Hall & Hughes, 1996; Sakai, 1998).

This would suggest some form of integration within the colony. However, Harriot (1983), Van Veghel and Kahmann (1994) and Schlesinger et al. (1998) suggested that polyp size rather than colony size constrained reproduction. Hall and Hughes (1996) summated the situation by stating that in any reproductive study, both colony and polyp fecundities should be taken into account, as colony fecundity is not a simple linear function of the number of modules.

For species in which colony size did reflect fecundity, it would be possible to study populations by measuring colony size. Buoyant weight has been used successfully to measure growth of reef-building in response to stress (Davies, 1990, 1995; Koop et al., 2001) and is perhaps a more suitable strategy than dimensions owing to the complex structures colonial corals form. However, removing a large colony from the substrata for weighing may not be easy, especially with present deep-water technology. Both *L. pertusa* and *M. oculata* did not show polyp size-specific fecundity, and collection methods did not allow for the sizes of colonies to be observed. For future reproductive and population studies it would be important to take this factor into account, as from many shallow-water studies, it is likely to be a vital factor.

Chapter Five

Overview and Conclusions

5.1 Overview

Eleven deep-water scleractinian species have been analysed for this reproductive study, covering a range of morphological features (Fig. 5.1) and habitat. Eight of these species have been examined under the auspices of the EU project Atlantic Coral Ecosystem Survey (ACES). The other three species have been studied under the NSF funded FOOD for the Benthos on the ANtarctic Continental Shelf (FOODBANCS) project.

The key findings of this study are:-

5.1.1 *Lophelia pertusa – Fig. 5.1a*

L. pertusa is a cosmopolitan reef-building scleractinian that was examined from 800 to 1000m in the NE Atlantic Ocean. This species was found to be gonochoristic and have seasonal gametogenesis with spawning occurring early in the year. *L. pertusa* has a high fecundity and small oocyte size (3146 oocyte per polyp (oop), maximum feret diameter 140μm). Spermatogenesis also appears to be seasonal, with all spermacysts at the same stage within an individual, though only one sample was obtained. No planulae were observed, suggesting spawning rather than brooding. Fecundity does not relate to body weight. Samples taken from the Darwin Mounds in the NE corner of the Rockall Trough were found to be non-reproducing. This is believed to be a result of the extensive trawl damage occurring in this area, keeping colony sizes below that required for reproduction.

5.1.2 *Madrepora oculata – Fig. 5.1b*

M. oculata is also a cosmopolitan reef-building scleractinian that was examined from 900m depth in the Porcupine Seabight, NE Atlantic. This species was found to be a periodic reproducer, which in this instance, appeared to be spawning in the early part of the year. Spawning of gametes is inferred by the lack of brooded planulae, and small numbers of large oocytes are produced (10oop, max feret diameter 350μm). There was no relationship between fecundity and polyp diameter. No males were found in this study.

5.1.3 *Fungiacyathus marenzelleri – Fig. 5.1c*

F. marenzelleri has a wide depth distribution as well as being a cosmopolitan solitary azooxanthellate species. This species was examined from 2500m in the Rockall Trough, and was found to be gonochoristic and to spawn gametes on a quasi-continuous basis. Large numbers of large oocytes are produced by each polyp (2900 oop, 750μm max feret diameter). Asexual reproduction was also observed, occurring by intratentacular budding of the juvenile from the anterior of the polyp. There was a significant relationship between fecundity and polyp diameter.

5.1.4 *Caryophyllia ambrosia – Fig. 5.1d*

C. ambrosia is a bathyal cosmopolitan species, found throughout the Atlantic, Pacific and Indian Oceans. This species was examined from ~2400m in the Porcupine Seabight, west of Ireland. This species was found to be a cyclical hermaphrodite, with asynchronous individuals, spawning gametes quasi-continuously. Oocytes were large (630μm max feret diameter), as was fecundity (2900oop). This species did not show size-dependent fecundity, and also showed no relationship between wet weight of polyp and skeletal dimensions. This suggests calcification may be more related to environmental conditions rather than as a function of polyp size.

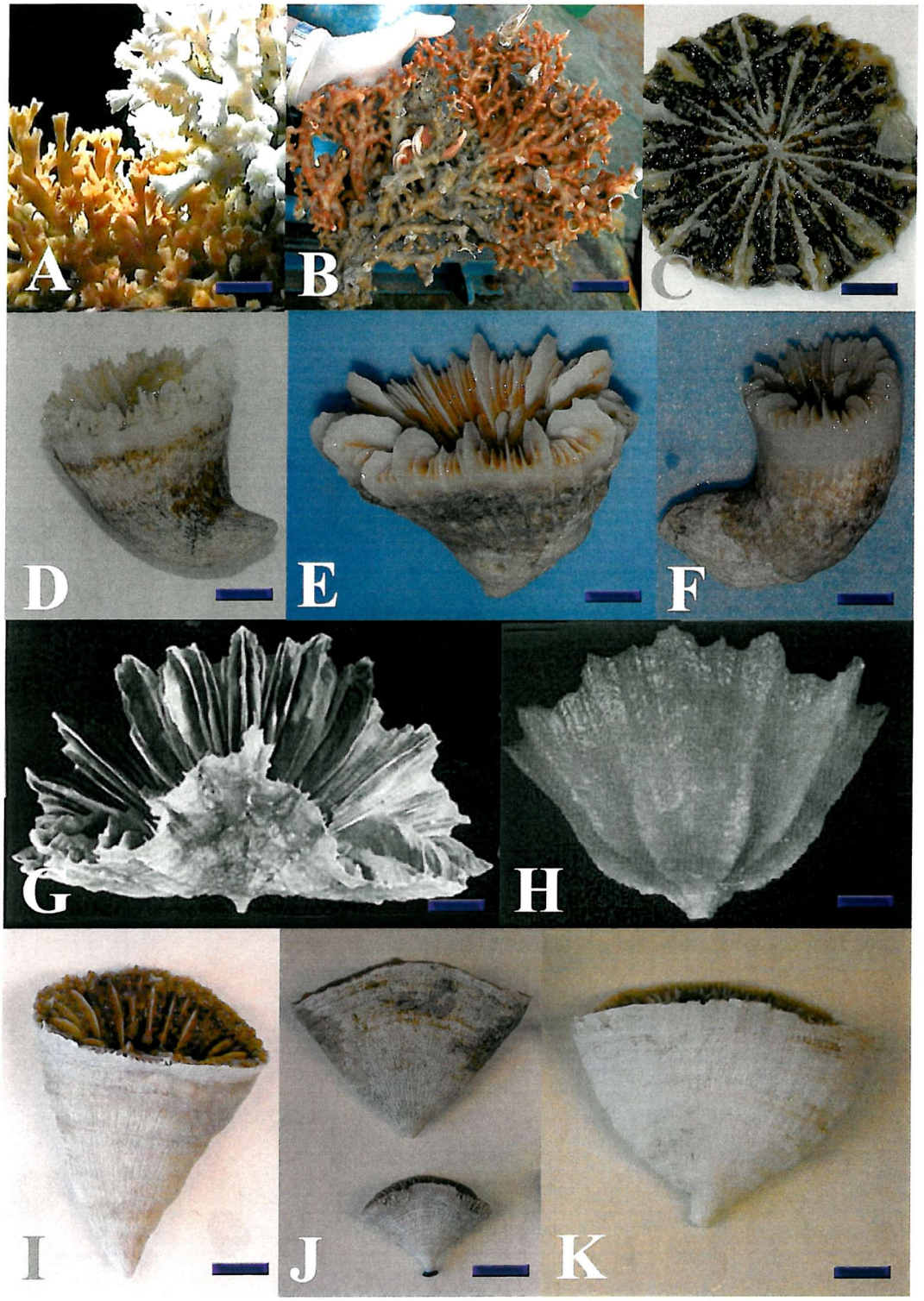


Fig. 5.1 – Species examined in this study, **A**, *Lophelia pertusa* (white and orange morph); **B**, *Madrepora oculata*; **C**, *Fungiacyathus marenzelleri*; **D**, *Caryophyllia ambrosia*; **E**, *C. seguenzae*; **F**, *C. cornuformis*; **G**, *Flabellum angulare*; **H**, *F. alabastrum*; **I**, *F. curvatum*; **J**, *F. impensum*; **K**, *F. thouarsii*
Scale bars – A, 2cm; **B**, 5cm; **C**, 1cm; **D**, 3cm; **E**, 3cm; **F**, 2cm; **G**, 1cm; **H**, 1cm; **I**, 2cm; **J**, 1cm; **K**, 2cm
A, courtesy of Andre Freiwald; **G,H** from Zibrowius, 1980

5.1.5 *Caryophyllia seguenzae – Fig. 5.1e*

C. seguenzae is only found within the East Atlantic at bathyal depths. This species was examined from ~1300m in the Porcupine Seabight, west of Ireland. *C. seguenzae* was also found to be a cyclical hermaphrodite, with asynchronous individuals within the population. Spawning of gametes appeared to be quasi-continuous. Oocytes were large (450μm max feret diameter) though fecundity (940oop) was less than *C. ambrosia.* Fecundity was size-dependent, and polyp wet weight also correlated with skeletal dimensions. Measurements taken of three populations from three different years (1979, 1982, 2002) from the same area showed the population to be dominated by smaller individuals, and did not change between among the three populations. This suggests a high level of recruitment and adult mortality in the population, not changing significantly between these three years.

5.1.6 *Caryophyllia cornuformis – Fig. 5.1f*

There were few individuals from this study, so only a basic reproductive overview could be obtained. This is another cosmopolitan bathyal species, collected from the Rockall Trough at a depth of ~1800m. This species is thought to also be a cyclical hermaphrodite, with a maximum oocyte size of 450μm.

5.1.7 *Flabellum alabastrum – Fig. 5.1g*

F. alabstrum is found only within the Atlantic at bathyal depths. This species was collected from ~1800m depth in the Rockall Trough, west of Britain. This species is gonochoristic and spawns gametes quasi-continuously. Small numbers of large oocytes were produced (550oop, 1010μm max feret diameter) and male spermatocysts always appeared to be in the late stages of development. There was size-dependent fecundity in *F. alabstrum*, and a significant relationship between polyp wet weight and skeletal dimensions. However there were insufficient samples to measure for population analysis.

5.1.8 *Flabellum angulare – Fig. 5.1h*

F. angulare is also only found within the Atlantic at bathyal depths, and was collected for this study from the Porcupine Seabight, west of Ireland at depths of ~2400m. This species is also gonochoristic, and appears to spawn seasonally. Smaller oocytes were present at larger numbers than *F. alabastrum* (814μm, 2800oop), and this species also shows size-dependent fecundity. There was no relationship between polyp wet weight and skeletal dimensions.

5.1.9 *Flabellum curvatum – Fig. 5.1i*

F. curvatum was collected from the continental shelf off the Western Antarctic Peninsula at a depth of ~500m. This species is gonochoristic and broods planulae within the mesenteries. Oocyte size was very large (5120μm max feret diameter) and fecundity was, relatively, low (1618oop). This species appears to be quasi-continuous, though more samples are needed to assess periodicity. Males were found at all four stages within a single mesentery, also suggesting a quasi-continuous periodicity.

5.1.10 *Flabellum impensum – Fig. 5.1j*

F. impensum was also collected from ~260m depth on the continental shelf off the Western Antarctic Peninsula. This species is gonochoristic and similarly broods its planulae. There were insufficient samples to observe periodicity, but fecundity was also relatively low (1270oop) with a very large oocyte size (5167μm max feret diameter). Males also contained several stages of spermatocysts within a single mesentery.

5.1.11 *Flabellum thouarsii – Fig. 5.1k*

F. thouarsii was also collected from ~500m depth on the continental shelf off the Western Antarctic Peninsula. This is a gonochoristic species, that also exhibits brooding. *F. thouarsii* appears to have periodic or prolonged reproduction, and produces the most oocytes (2412oop) and also the smallest (4800μm). Male corals had several stages of speramtocysts present within a single mesentery.

5.2 Conclusions

5.2.1 The ACES Project

The ACES project concluded in April 2003, and proved a highly successful study. Before the start of ACES in 2000, the knowledge of deep water corals was still in infancy. Individual and national projects had undergone a handful of camera and submersible surveys (Wilson, 1979; Messing et al., 1990; Mortensen et al., 1995; Freiwald et al., 1997). The majority of our knowledge of these cold water corals came from dredge hauls and fishermen, and so provided an incomplete picture of distributions, ecology and the state of the reefs and mounds. The fate of cold water corals was brought into the public arena by Greenpeace in 1997, when it challenged the UK government over the exploration licensing (predominately the oil industry licensing) in the Atlantic. Greenpeace claimed the licensing was unlawful because of the lack of protection for cold water corals (mainly *Lophelia pertusa*) in the area, and also the lack of protection for the Hebridean Islands from the potential impacts of the oil industry (Greenpeace, 1997). There was little scientific knowledge available on the status or dynamics of these ecosystems, and so the UK government lost.

The ACES project concluded with 18 offshore and 73 inshore cruises to numerous locations in the NE Atlantic. During the lifetime of ACES, the number of confirmed cold water coral sites within European waters rose from 416 to 816, and the number of associated faunal species rose from 800 to 1300 (Rogers, 1999; ACES, 2003). Microsatellite markers were used to examine the gene flow of *Lophelia pertusa* and *Madrepora oculata* (LeGoff & Rogers, in Press). Extensive mapping of deep-water coral sites took place from the Galicia Bank to the Norwegian Sula Reef, and a strong tidal current regime was discovered and is thought to be the prime nourishment source for coral banks and mounds (ACES, 2003). The reproductive biology of *L. pertusa* and *M. oculata*, as well as other deep water corals within Europe was investigated and found to be, potentially, sensitive to anthropogenic impacts (Waller & Tyler, in Press).

Moreover, the ACES results have been widely distributed to the public audience, and have been used to allow the designation of the Darwin Mounds as the first Marine Protected Area (MPA) in the UK. The deep-sea ecosystem seabed maps have been

distributed to stakeholders and decision makers in the hope of stimulating political implementation of sustainable use of deep-water habitats in the future (ACES, 2003). The multidisciplinary nature of the ACES project has made this study and invaluable tool for understanding the complete habitat and history of deep-water corals, and resulted in a new understanding of these unique ecosystems.

5.2.2 The FOODBANCS Project

Although the samples used in this study were not directly related to the FOODBANCS study, it is pertinent to also mention the success of this program. The FOODBANCS finished in 2001, with the final of five cruises to the Western Antarctic Peninsula. This study did show there to be strong evidence of uniform banks of organic carbon within the Antarctic sediment (DeMaster et al., 2003). They found microbial degradation rates and biological abundances of both meiofauna and macrofauna to be stable throughout, despite the high variability of particulate organic carbon in the water column. Galley (2003) observed that the 'Foodbank' provided sufficient nutrients for the physiological and metabolic needs of four out of six species studied through the Antarctic Program.

There were insufficient samples of scleractinians taken for a full analysis of the FOODBANCS hypothesis. They did, however, provide unique comparisons to the NE Atlantic species of *Flabellum* studied, as well as providing an opportunity to examine whether these deep-water Antarctic species followed patterns of reproduction for deep water organisms, or Antarctic organisms.

5.2.3 Hypotheses

The hypotheses posed in Chapter One can now be answered:-

A) There is no pattern of reproduction in deep-water scleractinians

The data presented in this thesis show each species to have its own distinctive pattern, however there are some 'general' trends that can be proposed. Fecundity appears to decrease with depth, and oocyte diameter appears to increase with depth. Neither of these are 'perfect' correlations. Reproductive mode also shows a pattern not consistent with shallow water counterparts. Gonochorism appears the trend in these deep-water species, whereas in shallow-water species, hermaphroditism is more proliferent. So this null hypothesis can be accepted, as there is no strict pattern.

B) Solitary scleractinians do not employ different reproductive habits than reef-building species

There appeared to be similarities in reproductive patterns in reef-building and solitary corals. Both reef building species were gonochoristic, several solitary species were also gonochoristic, both showed seasonality or periodicity. This null hypothesis can be accepted in that both reef-building and solitary forms appear to have no patterns unique to their form.

C) Skeleton generation is not related to body size, and so cannot be used for population dynamics

This null hypothesis can be rejected based on the data presented here. A method for further studying populations of deep-water scleractinians has been tested and most solitary scleractinians used in this part of the study did show a relationship between body size and skeletal dimensions. Data were presented for *C. seguenzae* that demonstrated the ability for skeletal dimensions to be used in population analysis of deep water scleractinians, and was related to reproductive output. These data can prove important for further scientific work on the conservation of deep-water corals. However not all species showed this relationship, highlighting the importance of investigating skeletal size, polyp size and reproductive output for differing species before a population model can be used.

D) The Western Antarctic Peninsula shelf scleractinians are more similar in their reproductive traits to Antarctic fauna, than deep-sea fauna

The three species of deep water Antarctic *Flabellum* were all observed to brood planulae and have large oocyte sizes. The other deep-water scleractinians in this study showed neither brooding, nor such extreme oocyte sizes. The trends in deep water fauna were discussed as being broadcasting, and the trends for Antarctic fauna were discussed as being brooding. The trends amongst shallow water scleractinians were also discussed as being broadcasting rather than brooding. On this basis the null hypothesis is accepted, as these three solitary species are more similar to Antarctic fauna than deep-water fauna.

5.2.2 Final Conclusion

This study has shown the benefits of individual species' study, rather than wide generalisations. Each species has a life history trait unique to itself, with no two having similar fecundities or oocyte size. Within the genus *Flabellum*, the only apparent similarity is that gonochorism prevails. This trait is not unusual within the Scleractinia however. Scleractinians are plastic in most elements of their biology, their simple body plan, yet complex structure leads to many problems with studying life history and population dynamics. This study has provided a baseline survey of eleven species, which, with more samples, can be built upon to provide a more complete life history of these species.

This study has also provided vital information for the conservation efforts occurring worldwide at present. Only by understanding many aspects of the life history of deep water scleractinians can we hope to protect them adequately from damage. Though this study has examined these numerous species extensively, there are still many more questions to be answered about these species' life histories. The extensive damage seen on shallow water reefs, that is still occurring in many places, might not be comparable to the damage already sustained by some reefs in deeper waters. 'Out of sight, out of mind' but hopefully not for much longer. Many new deep-water coral programmes are being proposed across the globe. More no-fish zones are being implemented in deeper waters and increased technology is also making these habitats more visible to both scientists and the general public.

5.3 Study Limitations and Further Research

The larval biology of many deep water scleractinians is as yet unknown, yet is vital to conservation efforts. This study has been able to assess the quantity and infer the type of larvae for the species studied. However, understanding fertilisation success, juvenile mortality, competence and settling ecology of larvae are important aspects of understanding a species. Only a combination of gametogenesis, larval ecology and population structure will provide a true representation of the importance of reproduction to a population.

During this programme three attempts were made to induce spawning in *Lophelia pertusa* and *Madrepora oculata* on the *RRS Discovery* cruises 248, 260 and 266. All of these were unsuccessful, and as histological evidence later showed, these three cruises were all at times when gametogenesis was not sufficiently advanced for spawning to occur. To be able to induce spawning in these species, in these locations, would mean collection times of late January/February. As anyone who has braved the Porcupine Seabight even in October or March well knows, the thought of travelling out there in January is not a pleasant one, not to mention the financial cost of many lost days. Should we wish to examine the larval ecology of these species, there are two options, the first is to brave the weather, and the second is to take this study to a more enclosed site. However, it is important to stress that, as discussed in length in this thesis, scleractinians can have variable reproductive characters dependent on environmental conditions. So, by taking this study to another area would require another full seasonal histological examination to determine spawning behaviour before studying the larvae.

This study has merely scratched the surface of the true dynamics of populations of deep water scleractinians. Only by knowing the importance of reproduction compared to sizes of species, be that skeletal size of actual polyp size, can we start to understand how populations function. In the solitary species studied it has been found that some species have relationships and some do not. As submersible and ROV technology becomes more refined, in species where a correlation exists, it would become possible, with an extensive initial survey, to use this method to observe population

changes non-destructively. To be able to extend the project to population dynamics of colonial corals would also be of benefit. As reviewed, many shallow-water colonial corals rely on size of the total colony to increase their fecundity, and not individual polyp weight. It would be interesting to take this further, taking measurements of the total colony and polyp weight, and examining which plays the most important role in constraining reproduction in individual species. The vast number of reproductive patterns in deep-water scleractinians, suggests that not all species will follow colony size-dependent reproduction, but will probably yield their own methods. Individual species comparisons are all important.

The rates of sexual versus asexual reproduction is virtually unknown in single species of scleractinians, and yet, as discussed previously, is an essential part of Cnidarian life-history. Newly developed genetic techniques, such as microsatellites and AFLP's may now be able to provide answers to these questions. At present the differences between these two modes of propogation, and even growth in colonial corals, are difficult to determine. Examining populations and individuals, studying further rates of sexual and asexual reproduction would allow us to delve further into the strategies these corals employ to survive the deep-sea, as well as being directly relevant to shallow water situations.

Most of this further work would require submersible or ROV technology. To be able to place individual colonies in context was something unachievable during this study. The destructive nature of trawling meant all corals collected could neither be placed into individual sub-populations, or, in the case of reef-building species, be even isolated into individual colonies. The inability to place individuals severely limits any reproductive study, especially one on a family that can be as reproductively plastic as the Scleractinia. In the shallow water, even a few meters down slope can cause morphological variations in skeletal structure in the same species (Veron, 1995), yet trawls can typically cover over 500m of relief, sometimes much larger. The nature of trawling also did not allow equality of numbers to be collected in deeper waters (which is by no means ensured by submersible/ROV technology, but chances are increased) that would allow a fuller, statistically sound, reproductive study to be completed.

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Appendices

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Appendix I

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Ib - **Waller, RG and Tyler PA (in Press)** – The reproductive biology of two deep-sea reef building scleractinians from the NE Atlantic Ocean. ***Coral Reefs***

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REPORT

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Reproductive ecology of the deep-sea scleractinian coral *Fungiacyathus marenzelleri* (Vaughan, 1906) in the northeast Atlantic Ocean

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Abstract The reproductive biology and its seasonality were examined in the deep-water, solitary coral *Fungiacyathus marenzelleri* from 2,200 m depth in the NE Atlantic, using histological techniques. A total of 186 corals were collected using either an Otter trawl (semi-balloon) or Agassiz trawl from the research vessel RRS *Challenger* between 1979 and 1991. All individuals were gonochoric, with all 48 mesenteries being fertile. A high fecundity was recorded (2,892 ±44.4 oocytes per polyp) with strong size-dependency. This species is a quasi-continuous reproducer with overlapping gametogenesis for both spermacysts and oocytes. No planulae were observed and broadcasting of gametes is inferred, with the large egg size (max. 750-μm diameter) suggesting lecithotrophic development. There is a non-significant trend towards a synchronous spawning event during June/July. Asexual fission was observed, though in low numbers.

Keywords Ahermatypic · Azooxanthellate · Solitary coral · Gametogenesis

Introduction

Zooxanthellate corals form one of the best-recognized marine ecosystems on earth. These corals, however, are limited to the warm, sunlit waters of the tropics and subtropics, and by depth. Below the permanent thermocline in many parts of the world's oceans, particularly at bathyal depths, large accumulations and small patches of colonial, azooxanthellate corals, such as *Lophelia pertusa*, occur (see Rogers 2000). Solitary ahermatypic corals can also be found on soft sediments at both bathyal and abyssal depths.

Information on reproduction in deep-sea anthozoans is limited. For actinarians, Van Praet et al. (1990) have described the gametogenic biology of two species of *Phelliactis*; Bronsdon et al. (1993) described the gametogenic periodicity of the bathyal epizoite *Amphianthus inornatus* and the abyssal epizoite hermaphrodite *Kadosactis commensalis*. Muirhead et al. (1986) observed gametogenesis in two zooanthid species, the bathyal *Epizoanthus paguriphilus* and the abyssal *E. abyssorum*. In the Alcyonaria, the gametogenic pattern has been described for a variety of species of pennatulid (Rice et al. 1992; Tyler et al. 1995). From these limited studies, it appears that there is no gametogenic pattern 'typical' of deep-sea anthozoans.

Information on reproduction in shallow-water scleractinians is much more extensive. The majority of species are hermaphroditic, with just 25% known to be gonochoric (Fadlallah 1983; Harrison and Wallace 1990; Richmond and Hunter 1990; Hall and Hughes 1996; Richmond 1997). The pattern of reproduction is also known for several solitary corals from shallow depths, of which the majority are gonochoristic, brooding species (Fadlallah 1983). Goffredo et al. (2000, 2002) reported on the hermaphroditic brooder *Balanophyllia europaea* and Krupp (1983) reported that *Fungia scutaria* is a gonochoristic solitary coral that spawns gametes during the late summer. More recently, there has been interest in the reproduction of corals from high latitudes (Harii et al. 2001). However, nothing is known at present about the gametogenic biology of deep-sea scleractinians.

In this paper we report on the gametogenic development of the deep-water coral *Fungiacyathus marenzelleri* in the NE Atlantic Ocean. *F. marenzelleri* (Vaughan, 1906) is a solitary, deep-sea scleractinian, which is known to occur from 730 to 5,870 m depth

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(Zibrowius 1980) and has a wide distribution, occurring in the Atlantic (personal observations) and the Pacific (Lauerman et al. 1996). Although the morphology of this species has been well described (Cairns 1979; Zibrowius 1980), little is known of its biology, ecology, or life history. Three species of *Fungiacyathus* inhabit the NE Atlantic, *F. crispus*, *F. fragilis*, and *F. marenzelleri*. *F. crispus* is the smallest, reaching a maximum of 9 mm in diameter and is found at the shallowest depths (< 1,500 m) (Zibrowius 1980). *F. fragilis* inhabits depths from 366 to 2,200 m, and coexists with *F. marenzelleri* at ~2,000 m (Zibrowius 1980). *F. fragilis* and *F. marenzelleri* have similar morphologies and so from depths around 2,000 m must be carefully distinguished by septal differences described by Zibrowius (1980).

Methods

All specimens were obtained using either a 3-m Agassiz trawl or a semi-balloon Otter trawl with 14-m aperture, from RRS *Challenger* in the area around station 'M' (57°18′N, 10°11′W; Gage and Tyler 1982; Table 1). This station is at a depth of 2,200 m. The material was preserved in 4% formalin and later transferred to 70% alcohol. The July 1983 sample was fully processed, but the data were not used due to the poor condition of the preserved tissue of the specimens. For histological processing, all individuals were submerged for approximately 4 h in rapid decalcifying solution (conc. HCl) until no carbonate skeleton remained. They were then rinsed in running tap water for 24 h to remove acid traces.

Individuals were dehydrated by three, 4-h submersions in 100% propanol, followed by clearing with Histoclear for approximately 12 h. The diameter of each individual was then measured and recorded. Around a quarter of the polyp tissue was embedded by being left for 6–12 h in molten histology wax at 70 °C, which was then poured into standard molds. All wax blocks were serially sectioned at 5 μm, leaving 75 μm in between slices, which were mounted on slides and stained with Masson's Trichrome stain.

For fecundity estimation, all slides of female tissue were examined. After decalcification, mesenteries from 20 whole individuals, from varying months, were counted and had their structure noted. Sections of each individual were then examined using an Olympus BH2 compound microscope with video camera attachment. Images were captured using Matrox Rainbow Runner and analyzed using SigmaScan Pro version 4 to calculate oocyte diameters ('feret' diameter, the area if the oocyte was a perfect circle, was used). Spermatogenesis and oogenesis were staged. All previtellogenic, vitellogenic, and late vitellogenic oocytes were counted in two mesenteries from each female. The number of oocytes in the two mesenteries were averaged and that number is hereby referred to as the 'realized fecundity.' 'Potential fecundity' is the realized fecundity multiplied by the number of mesenteries. Mean realized fecundity was calculated for each sample.

Results

Mesenterial structure

Forty-eight mesenteries are present, arranged in cycles of four, reducing in number towards the anterior. The mesenterial mesoglea is thick at the periphery of the polyp, and reduces in width towards the small central oral cavity. All individuals of *F. marenzelleri* examined were gonochoric. Sex ratios averaged over the 9 months analyzed show a 1:1 ratio, varying only slightly among months ($\chi^2 = 0.882$; $P = 0.01$). Reproductive tissue was found in high densities at the base of the individual, reducing in occurrence towards the anterior. Only five individuals did not have either oocytes or spermacysts present on all mesenteries. Each mesentery produces embedded reproductive structures, though there is no obvious external morphological difference between males and females.

Asexual reproduction

F. marenzelleri undergoes asexual reproduction by a form of fission (Fig. 1), followed by complete detachment of the new polyp when it reaches a certain size (> 6 mm diameter, as this was the smallest found). The new polyp grows on the anterior surface of the coral. Both sexes were observed to undergo fission. The asexually produced polyp buds from a single mesentery (Fig. 1) and is composed of mesenteries with a central oral area. No more than one bud was found on any individual. There was a low incidence of asexual reproduction in the samples studied. The maximum number of individuals undergoing asexual fission in any month's sample was two, with 4 months' samples having no budding individuals. There was no temporal pattern in the monthly incidence of asexual reproduction in polyps.

Sexual reproduction

Spermatogenesis

Ovoid spermacysts occurred throughout, and in all the mesenteries. Each mesentery contained many spermacysts at varying stages of differentiation. There appeared

Table 1 Colonies analyzed

Cruise no.	Date	Longitude	Latitude	No. analyzed
Challenger 1A/79	15 Jan 79	57°20′N	10°27′W	20
Challenger 75	17 Feb 91	57°19′N	10°23′W	20
Challenger 4/80	03 Mar 80	57°16′N	10°17′W	30
Challenger 6A/81	12 April 81	57°22′N	10°19′W	20
Challenger 9/80	29 May 80	57°18′N	10°16′W	20
Challenger 10/83	24 July 83	57°07′N	09°23′W	0 – poor condition
Challenger 12B/81	18 Aug 81	57°21′N	12°02′W	20
Challenger 15A/81	19 Oct 81	57°27′N	11°10′W	16
Challenger 86	19 Nov 91	57°18′N	10°24′W	25
Challenger 74	14 Dec 90	57°15′N	10°21′W	15

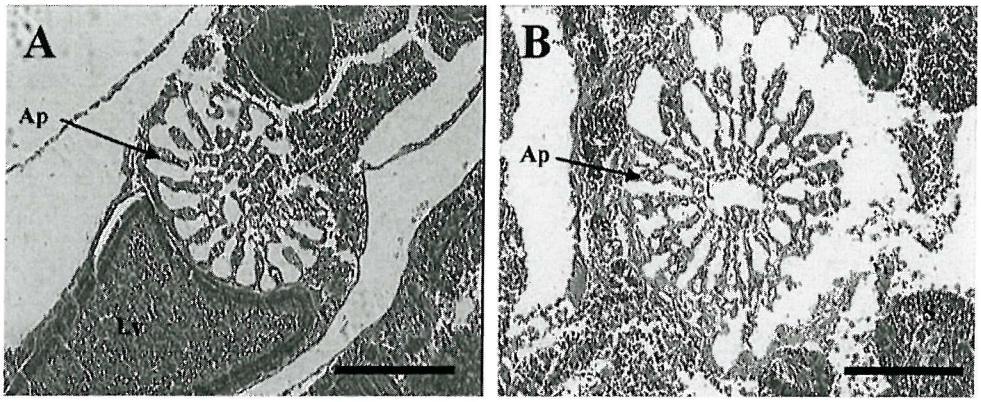

Fig. 1 **a** Early asexually produced bud in female *F. marenzelleri* polyp; **b** early asexually produced bud in male *F. marenzelleri* polyp. *Ap* Asexual polyp; *Lv* late vitellogenic oocyte; *S* spermacyst; *scale bars* 40 μm

to be no specific arrangement of spermacysts within the mesenteries. Spermatogenesis can be divided into four stages:

Stage 1

Loosely packed aggregations of spermatocytes contained within a cell membrane. Empty lumen can be seen (Early, Fig. 2a).

Stage 2

Some spermatozoa present, still loosely packed. Lumen less distinct (Maturing, Fig. 2a,b).

Stage 3

Densely packed with spermatocytes and lumen packed with spermatozoa (Mature, Fig. 2c).

Stage 4

Relict spermatozoa can be seen (Spent, Fig. 2d).

Oogenesis

Oogonia arise from the mesoglea of the mesentery and develop into 'previtellogenic' oocytes at <28 μm (Fig. 3a). These early oocytes, which have a central nucleus, are attached to the lamellae of the mesentery and are surrounded by follicle cells. Previtellogenic oocytes then undergo vitellogenesis at ~150 μm diameter, and continue to accumulate yolk up to the maximum of 750 μm diameter (Fig. 3a–d), and are classed as 'vitellogenic.' At <300 μm a cortical granular layer is devel-

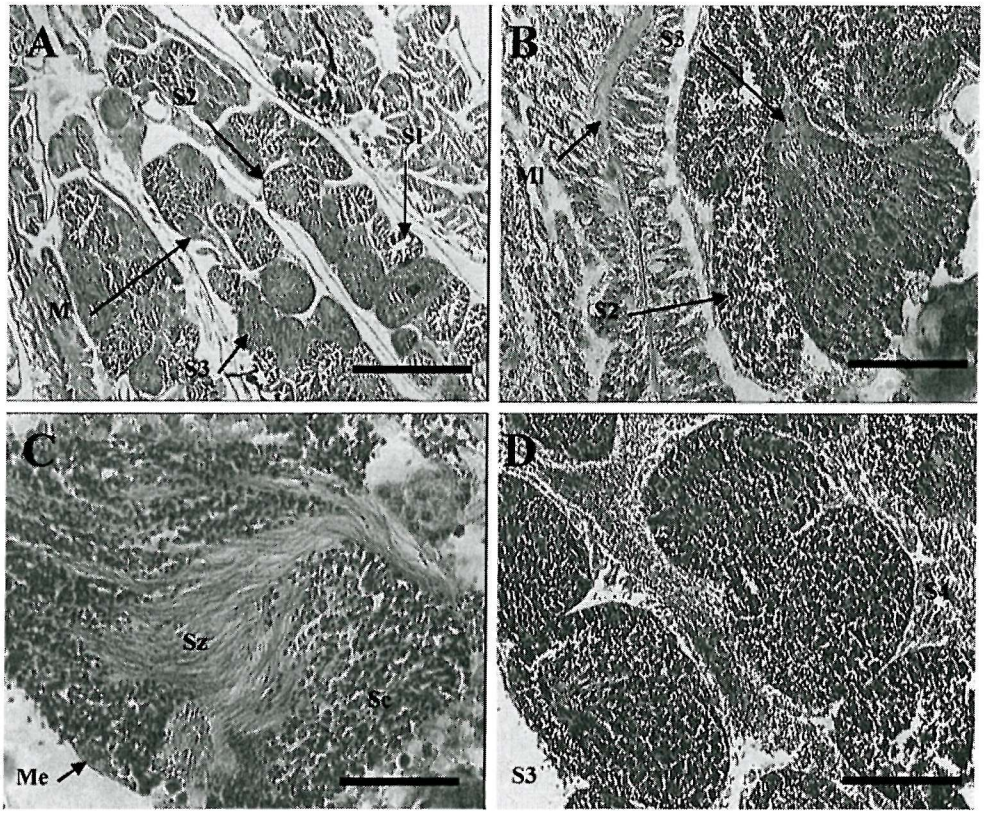

Fig. 2 **a** Arrangement of spermacysts within *F. marenzelleri* mesenteries; **b** stage II and III spermacysts; **c** stage III spermacysts; **d** stage III and IV spermacysts. *M* Mesentery; *S1* stage I spermacyst; *S2* stage II spermacyst; *S3* stage III spermacyst; *Me* mesoglear envelope; *Sz* spermatozoa; *Sc* spermacyst; *S4* stage 4 spermacyst; *scale bars* **a** 200 μm; **b** 40 μm; **c** 20 μm; **d** 40 μm

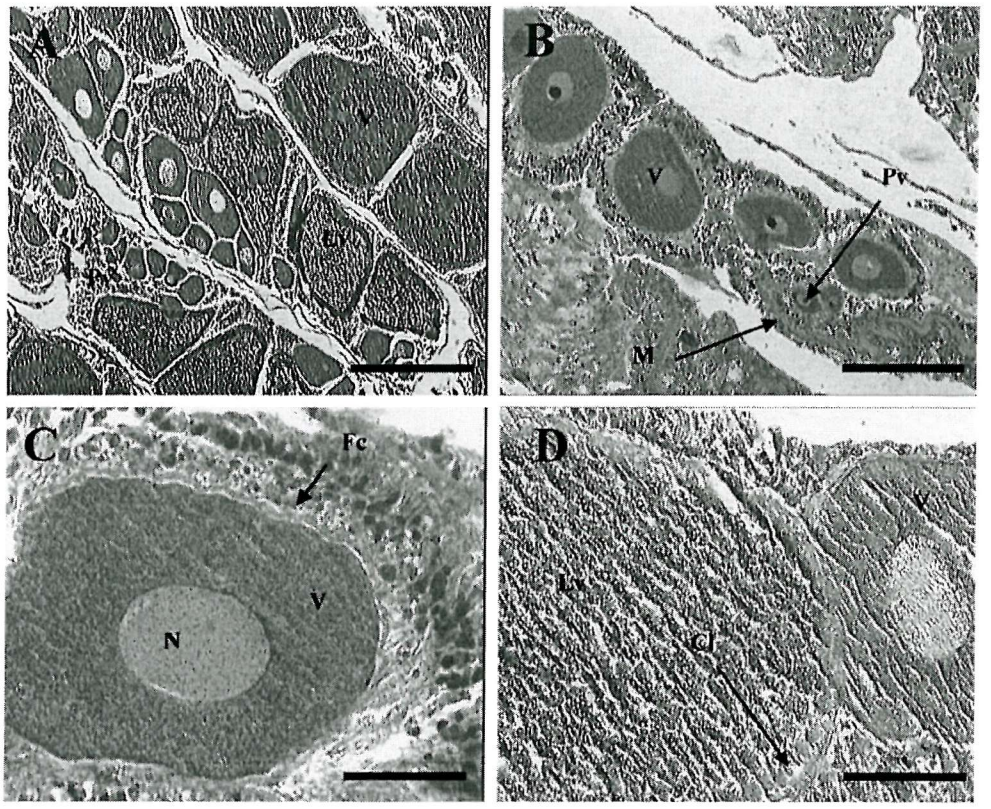

Fig. 3 **a** *F. marenzelleri* mesentery with previtellogenic, vitellogenic, and late vitellogenic oocytes; **b** section showing young oocyte attachment to *F. marenzelleri* mesentery; **c** vitellogenic oocyte; **d** thick cortical layer. *Fc* Follicle cells; *N* nucleus; *V* vitellogenic oocyte; *M* mesentery; *Ml* mesentery lamellae; *Pv* previtellogenic oocyte; *Lv* late vitellogenic; *Cl* cortical layer; *scale bars* **a** 200 μm; **b** 200 μm; **c** 20 μm; **d** 20 μm

oped (Fig. 3c). These oocytes were classed as 'late vitellogenic.' Large yolk vacuoles and a prominent nucleolus in the central nucleus can now also be observed.

Oocytes of varying stages occurred throughout and in all the mesenteries (Fig. 3a), though ten polyps (of all those examined) contained only previtellogenic or vitellogenic oocytes. No planulae or brooded individuals were found during histological examination.

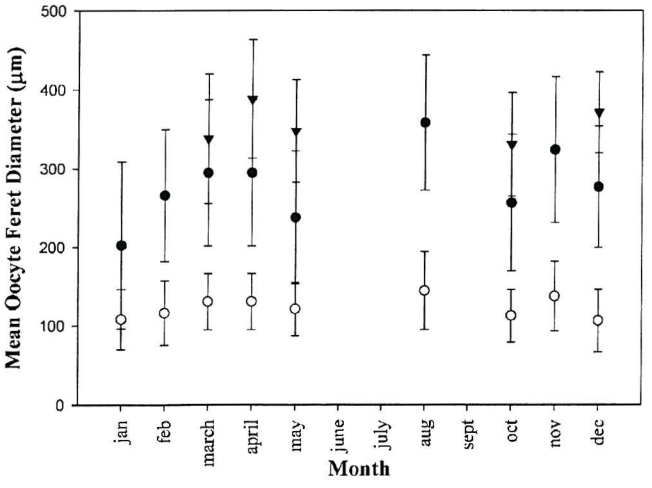

Fig. 4 Monthly mean previtellogenic, vitellogenic, and late vitellogenic oocyte diameters for *F. marenzelleri* sampled between 1979 and 1991. *Triangles* Late vitellogenic; *solid circles* vitellogenic; *open circles* previtellogenic; *error bars* ±1 SD

Analysis of the distribution of the three stages of oocyte development (Fig. 4) or the oocyte size/frequency (Fig. 5) gave no evidence of a marked annual reproductive periodicity. There was no significant difference between any of the mean oocyte diameters for the different months. This suggests that *F. marenzelleri* is a quasi-continuous reproducer. All three cohorts appear to develop simultaneously, though during February, August, and November no late vitellogenic oocytes were found, suggesting they had spawned prior to, or during, collection. The presence of all levels of sperm development in spermacysts within the same mesentery (overlapping gametogenesis) suggests a quasi-continuous release of gametes. The lack of late vitellogenic oocytes in the summer months suggests there may be a variation in the intensity of gamete production rather than a true seasonality (sensu Harrison 1988).

Fecundity

The realized fecundity of each individual within a month's sample was averaged, producing an estimate of mean, per colony fecundity. This number varied significantly among months (Fig. 6). The data suggest an annual periodicity in fecundity, with a maximum being reached during April, and spawning during May/June, suggesting a variation in intensity of reproduction within a year. The average potential fecundity [realized fecundity per mesentery multiplied by the

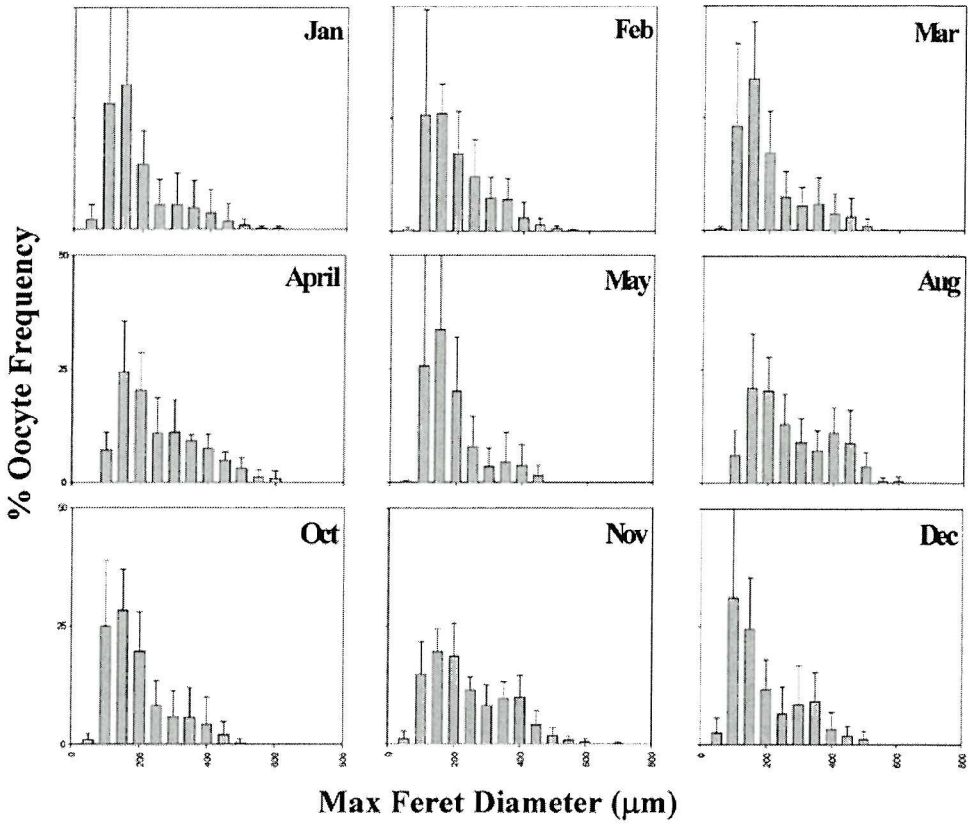

Fig. 5 Oocyte size/frequency distribution for *F. marenzelleri* monthly samples from the Rockall Trough, NE Atlantic Ocean. *Error bars* ±1 SD

number of mesenteries (48)] for all 10 months was 2,892 (±44).

The size at first reproduction was approximately 10 mm polyp diameter, because both non-reproductive and reproductive individuals were found at this size. No reproduction was observed below this size. Fecundity is size dependent (Fig. 7) and rises as a second-order polynomial ($r^2 = 0.713$).

Discussion

This is the first reproductive investigation of the solitary coral *F. marenzelleri*, and of any deep-water scleractinian. All samples were obtained from station 'M' in the Rockall Trough (Gage and Tyler 1982) between the Anton Dohrn Seamount and the Hebrides Shelf in the northeast Atlantic, an area known for its high species diversity (Gage 1979). Examination of the reproductive biology of a wide variety of marine invertebrates has shown that different species show quasi-continuous, seasonal, or opportunistic reproductive patterns (Tyler and Young 1992). In addition, there are good environmental data from this site (Dickson et al. 1986; Ellett et al. 1986; Holliday et al. 2000).

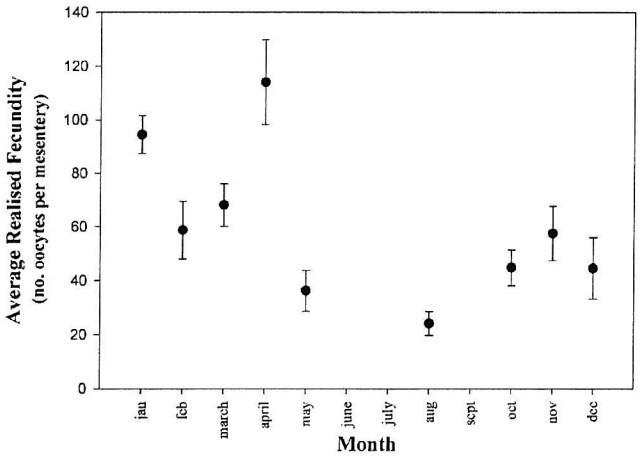

Fig. 6 Average realized fecundity of *F. marenzelleri* monthly samples collected between 1979 and 1991. *Error bars* ±1 SD

In shallow-water scleractinians, gametes develop within the lamellae of the mesenteries and subsequently migrate into the mesoglea, to develop as oogonia (Szmant-Froelich et al. 1980; Fadlallah 1983). Oogonia of *F. marenzelleri* were first observed attached to the lamella. We believe that the gametes of *F. marenzelleri*

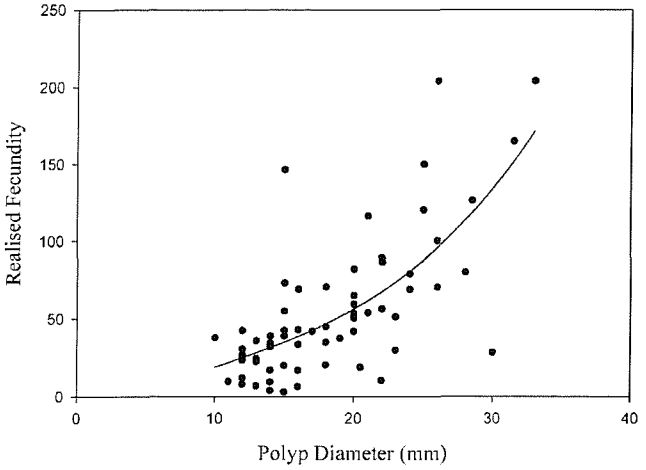

Fig. 7 Polyp diameter of *F. marenzelleri* plotted against average realized fecundity with a fitted second-order polynomial curve. [$n=83$, $r^2=0.7132$ ($y=32.071x^2-63.089x+53.235$)]

also originate in this area and then migrate into the mesoglea, in common with other corals and anthozoans (Fadlallah and Pearse 1982; Fadlallah 1983).

All the developing individuals examined were found to be gonochoric, whereas the majority of scleractinians are hermaphrodites (Fadlallah 1983; Harrison et al. 1984; Szmant 1986). The random mixture of males and females among size classes mitigates against sequential hermaphroditism. Although gonochorism has been suggested as a more primitive strategy than hermaphrodism (Goffredo et al. 2000), it has been shown to be important for genetic diversity of populations (Szmant 1986). There are certain restrictions, however, in being a gonochoristic individual: spawning must occur synchronously and densities of both individuals and gametes must be sufficiently high to allow successful fertilization. Because of its lack of mobility as an adult, an individual that settles and grows at very low density is less likely to pass its genes on to the next generation than an individual in a high-density population. The spatial relationships between individuals of *F. marenzelleri* are critical in understanding this species' reproductive success. Lauerman et al. (1996) report densities of 0.0181 to 0.293 m at 4,100 m depth off California.

Asexual reproduction in reef-building corals is an important adaptation for rapid colonization of locally available areas, since competition for space is high (Szmant-Froelich et al. 1980; Fadlallah 1983; Hall and Hughes 1996). In solitary species this need would appear to be limited, especially within the deep-sea sedimentary environment where space is not limited. The solitary coral *Fungia scutaria*, which lives in shallow reef areas, has been found to asexually reproduce by budding, and possibly from tissue fragments (Krupp 1983). Population genetic studies have also shown that most of a local population is of the same genotype (Krupp 1983). In *F. marenzelleri* asexual proliferation appears in such low densities to suggest that this would be the secondary mode of reproduction, with sexually produced polyps comprising the majority of the population.

Planulation in deep-sea species is difficult to observe without in vitro cultures. However, histological evidence suggests that *F. marenzelleri* spawns gametes rather than broods. This is inferred by both the lack of planulae and the varying stages of gamete development within a single polyp. The release of eggs and sperm as the normal mode of reproduction in the Cnidaria has been well documented for a number of species (Kojis and Quinn 1981; Bothwell 1982; Fadlallah and Pearse 1982; Fadlallah 1983; Harrison et al. 1984; Szmant 1986; Harrison and Wallace 1990; Richmond and Hunter 1990; Richmond 1997). Stimson (1978) proposed that deeper-living (deep fore-reef) corals should broadcast gametes to facilitate a wide dispersal range required at depths. Rinkevich and Loya (1979) proposed that large polyped species would spawn large numbers of large eggs. Szmant (1986) also noted that the larger-polyp species of Caribbean corals were gonochoristic broadcasters. The extra energy required for growth, defense, and maintenance of the large polyp suggests that the energy required to produce a brooded planula would not be available. *F. marenzelleri* appears to fit both of these hypotheses. The large egg size suggests a lecithotrophic development rather than planktotrophic early development (Fadlallah 1983; Gage and Tyler 1991). Lecithotrophic development, contrary to earlier suggestions, is now recognized as an adaptation for wide dispersal in oligotrophic environments such as the deep sea (Shilling and Manahan 1994).

Body size, in most invertebrates and some vertebrates, usually has a major effect on reproductive output (Gage and Tyler 1991; Hall and Hughes 1996). *F. marenzelleri* has a strong size-dependent reproduction with a size of 10 mm diameter for first reproductive output. The fecundity of a single polyp is high when compared to both the polyp and colony fecundities of other scleractinian species. The Great Barrier Reef coral *Goniastrea retiformis* has a polyp fecundity of 46 oocytes and a colony fecundity of around 360 oocytes (Hall and Hughes 1996).

The majority of scleractinian corals have some form of reproductive periodicity, usually lunar or temperature controlled (Fadlallah 1983). The individuals of *F. marenzelleri* sampled for this study came from ~2,200 m depth, an area below the permanent thermocline, and so there is little seasonal fluctuation in either temperature or salinity (Holliday et al. 2000). There are, however, data that suggest a marked seasonal flux of surface primary production to the deep sea bed at this station, and elsewhere in the NE Atlantic, which has an influence on the reproductive biology of both infaunal and epibenthic invertebrates (Billett et al. 1983; Tyler et al. 1992, 1993). Although there is some evidence for a seasonal variation in intensity of reproduction in *F. marenzelleri*, most of the evidence, such as large egg size and lack of significant oocyte variation between

samples, suggests quasi-continuous reproduction. This reproductive strategy may benefit a deep-sea solitary coral, as an increased number of eggs broadens the chances of fertilization and aids wide dispersal (Szmant-Froelich et al. 1980).

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Ib

IN PRESS – CORAL REEFS

The reproductive biology of two deep-water, reef-building scleractinians from the NE Atlantic Ocean

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Abstract

The reproductive ecology of colonies of *L. pertusa* (Linné, 1758) and *M. oculata* Linné, 1758 from the Porcupine Seabight (Thérèsa Mound and South Porcupine Seabight site) and the Darwin Mounds (NE Rockall Trough – *L. pertusa* only) was investigated using histological techniques. Samples of *L. pertusa* exhibited seasonal reproduction, whereas the evidence for *M. oculata* is equivocal but suggests multiple cohorts of gamete production. *L. pertusa* produces a single cohort of around 3000 oocytes, whereas *M. oculata* produces two cohorts, with a total fecundity of around 60 oocytes. Maximum observed oocyte size in *L. pertusa* was 140μm and in *M. oculata* was 405μm. From these oocyte sizes and the timing of reproduction, a lecithotrophic larva is expected, though not observed. This seasonality of reproduction fits with the phytodetrital food fall occurring around July in the Seabight area.

L. pertusa was found to be non-reproductive at the Darwin Mound site. Though unable to be specifically tested, this suggests that the increased trawling activity in this area might be keeping colonies below sexually viable sizes, as seen in numerous shallow water situations. All areas in the NE Atlantic are coming under threat from increased fishing and commercial exploration practices. This study shows that these highly seasonal reproducers could be sensitive to these fishing operations and care must be taken so as not to repeat the destruction that has occurred on shallower reefs.

Introduction

Fig 1

Recently there has been an increase in recognition of the importance of deep-water reefs as fragmented, biodiverse communities at bathyal depth around the globe (Rogers, 1999). Though deep-sea scleractinians have been known for many decades (Cairns, 1979; Zibrowius, 1980), there has been little observation of their ecology owing to the difficulty and expense of deep-water collections.

Deep water reefs, mounds and banks are commonly found on continental margins and shelf breaks, as well as on seamounts and ridges, where high water flow is present. As in shallow-water, these flows may be necessary for the delivery of food and larvae to the area, as well as removing waste and excess sediment (Grigg, 1974; Grigg, 1984). Hard substrata are also necessary for reef formation in shallow-water, as highly sedimented areas are not favoured because of the risk of polyp suffocation (Dodge & Vaisnys, 1977; Rogers, 1990). Many deep-water reefs have been observed to begin from settlement on worm tubes, coral rubble or mollusc shells, and so this need for hard substrate may also be necessary for these deeper living scleractinians (Wilson, 1979).

There are many species of deep-water, reef-building scleractinians (Zibrowius, 1980). *Goniocorella dumosa* occurs on the Campbell Plateau, New Zealand (Squires, 1965), *Oculina varicosa* on the Florida continental shelf (Reed, 1980; Brooke, 2002), *Solenosmilia variabilis* from the South Pacific, SE Atlantic and Cook Islands (Cairns, 1982; Keller, 1993; Cairns, 1995), *Dendrophyllia cornigera* in the NE Atlantic (Zibrowius, 1980), *Desmophyllum dianthus* off Chile (Cairns, 1982) and *Enallopsammia rostrata* in New Zealand, *E. profunda* from the Western Atlantic and *E. marenzelleri* from the NE Atlantic, New Zealand and Indonesia (Cairns, 1995) are

just a few. The most cosmopolitan deep-water scleractinian species are *Lophelia pertusa* and *Madrepora oculata* (Rogers, 1999). *L. pertusa* is found in the Pacific, Atlantic, Indian and Antarctic oceans, *M. oculata* in the Pacific, Atlantic and Indian oceans.

Deep-water reefs are recognised as important biomes for many commercial and non-commercial species (Rogers, 1999). Nearly 1300 species of invertebrates and fishes have been found inhabiting the branches of *L. pertusa* in the NE Atlantic ocean, and it is thought this figure will increase (M. Roberts, pers com). *L. pertusa* is a cosmopolitan scleractinian. It can be found from depths of 50m in the Norwegian fjords (Rogers, 1999), to 3600m on the Mid-Atlantic Ridge (Bett & et al., 1997) and in most of the worlds oceans. Though many of these taxonomic references refer to the dead remains of *L. pertusa,* many new reefs are being discovered around the globe.

M. oculata is less cosmopolitan than *L. pertusa* and has only been found as a co-species with other reef forming scleractinians. *M. oculata* and *L. pertusa* are frequently found together off New Zealand (Cairns, 1995), the Aegean Sea (Vafidis et al., 1997), SE Atlantic (Keller, 1993) and the NE Atlantic (Keller, 1993; Bett et al., 1997; Wilson, 1997; Rogers, 1999).

There are few data on reproduction of deep-water scleractinians. The reef building *Occulina varicosa* is a seasonal gonochoristic broadcast spawner, with small oocytes (<100μm) and a high fecundity (1,000 to 4,800 oocytes per cm^{-2} skeletal area) (Brooke 2002). *Fungiacyathus marenzelleri* is a solitary deep-water scleractinian found in sedimentary environments in the NE Atlantic. This species is a non-seasonal gonochoristic broadcast spawner, with high fecundity (2900 oocytes per polyp) and large oocytes (750μm) (Waller et al., 2002). Three deep-water *Caryophyllia* species from the NE Atlantic Ocean have also been examined (Waller et al., in press).

Caryophyllia ambrosia, *C. seguenzae* and *C. cornuformis* all exhibited cyclical hermaphroditism.

The present study focuses on these *L. pertusa* and *M. oculata* from three sites at bathyal depths in the NE Atlantic Ocean (Fig. 1). The Darwin Mounds are series of several hundred mounds, each approximately 100m diameter and 5m high, found within the NE segment of the Rockall Trough (Bett, 2001). Thérèse Mound is a large coral mound found within the Belgica Mound system in the Eastern Porcupine Seabight. The third site is an area on the south side of the Porcupine Seabight (SPS). All the sites are between 800m-1000m depth. This study is part of the interdisciplinary European project 'Atlantic Coral Ecosystem Survey' (ACES). This project began in April 2000, to examine the biology, oceanography and geology of the reefs and mounds at bathyal depths round the European margin.

Methods

Field sampling

Table 1

All specimens were obtained using a $0.5m^2$ box core, 3m Agassiz Trawl, or an Otter Trawl Semi-Balloon (OTSB), between 2000 and 2002 from the RRS *Discovery* in the NE Atlantic (Table 1, Fig. 1). Corals from Thérèse Mound were solely collected by box core, corals from the Darwin Mounds were collected by Agassiz Trawl and the South Seabight area was a sample collected by OTSB. Differing methods were used for the different environmental conditions found at the sites. For Thérèse mound the corals are too large to trawl, as the net is easily broken. The Darwin mounds were unable to be box cored, though this was repeatedly attempted, because of their small size. The South Seabight area was a fortuitous coral sample from an OTSB trawling for benthic invertebrates. As this sample was also collected within the Porcupine

Seabight, and at similar depths to Thérèse mound, these two samples are pooled herein so as to give a better overall picture of reproduction in the Seabight. No sample site was samples more than twice in a season, limiting the availability of material.

The corals were preserved in 4% formalin and later transferred to 70% alcohol. Specimens of each species were selected at greater than 30cm colony width, and are referred to herin as putative colonies. Large specimens were selected to try to ensure the collection of sexually viable material. In trawled corals, colonies were selected that had dissimilar appearance (i.e. skeletal shading, orientation of polyps, thickness of skeleton). This was to try and minimise the sampling of the same colonies within the trawl haul, though this cannot be ruled out entirely. Any major differences in appearance (such as colouration) was noted.

Histology

For histological processing, large pieces of the putative colonies of *Lophelia pertusa* (Fig. 2a) and *Madrepora oculata* (Fig. 4a) were submerged for approximately 4 hours in rapid decalcifying solution (conc. HCL) until no carbonate skeleton remained. They were then rinsed in running tap water for 24hrs to remove acid traces. *L. pertusa* polyp tissue were weighed at this stage.

Twenty individual polyps of varying sizes from each colony were separated and dehydrated by three, four-hour submersions in 100% propan-2-ol, followed by clearing with xylene for a maximum of 12hrs. Whole polyp tissue was embedded in molten histology wax at 70°C for 6 to 12h, then poured into standard moulds. All wax blocks were serially sectioned at 5μm, leaving 50μm in between slides, and then stained with Masson's Trichrome stain. Remaining polyps were stored in 70% propan-2-ol. Sections of each individual were examined using an Olympus BH2

compound microscope with video camera attachment. Images were captured using Matrox Rainbow Runner and analysed using SigmaScan Pro version 4 to calculate oocyte feret diameter ('feret' diameter is the diameter of an oocyte if it was perfectly round). Between 50 and100 random oocytes were measured from each individual polyp. Percentage size frequency charts were constructed for each individual polyp and subsequently polyps were combined within and between colonies to give a percentage oocyte size-frequency and variance for each month. For *M. oculata,* few polyps produced more than 50 oocytes and so data was pooled between polyps. Spermatogenesis and oogenesis were staged to determine their sexual state.

Fecundity

For fecundity estimation of *L. pertusa*, five polyps were dissected from August, and October. The wet weight of each polyp was measured and the total number of mesenteries recorded. Five mesenteries were dissected and embedded using the procedure above. Blocks were serially sectioned and all visible previtellogenic, vitellogenic and late vitellogenic oocytes were counted in each mesentery. For fecundity estimation of *M. oculata*, serially-sectioned slides from 5 to 10 female polyps were examined and all oocytes counted. All polyp measurements were size-corrected to the average polyp size for that species. The tin foil method was used to calculate the number of polyps per cm^{-2} skeletal area (Marsh 1970). An area of $10cm^2$ foil was used for *L. pertusa,* and $5cm^2$ for *M. oculata.* This number of polyps per cm^{-2} was then used to calculate number of oocytes per unit area of skeletal surface.

Results

Lophelia pertusa

Morphology

L. pertusa has two major skeletal colour morphologies; white and orange. The white morph appears less prolific in the areas sampled, as it was only obtained from the South Seabight during the October 2002 cruise.

This species is gonochoristic with all mesenteries fertile. Gametes are in two to three pockets throughout the mesentery and 2-3 oocytes/spermacysts wide (Fig. 2b to e). These pockets contain gametes at the same stage of development. The number of mesenteries per polyp varied from between 13 and 24.

Gametogenesis

Gametes were observed in colonies sampled during August, September and October from the Porcupine Seabight only. No gametes were found in colonies sampled in August from the Darwin Mound site, or in March from the Porcupine Seabight. Males were only found in the October sample at this site, and only in the white morph. All results, therefore, are only from the Porcupine Seabight.

Fig 2

Female

All female colonies were of the orange morph. Only three stages of oogenesis were observed, but a fourth is inferred from previous azooxanthellate coral studies (Fig. 2b to e). Oocytes appear to develop from the lamellae of the mesentery.

Stage I - Oogonia, – <5μm. diameter bud from the mesenterial lamellae

Stage II - Previtellogenic Oocytes – 5μm to 30μm diameter Small oocytes with thin wall and an basophilic cytoplasm (Fig 4c)

Stage III - Vitellogenic Oocytes – >30μm diameter (Fig. 2c, d)

(Inferred) Stage IV - Late Vitellogenic Oocytes – >140μm. Oocytes become heavily granulated and have a thick cortical layer around the periphery of the ooplasm.

The March sample revealed two small oocytes (~50μm) that appeared to be reabsorbing. These were not measured or counted for fecundity estimations. Previtellogenic oocytes were observed in August, but not September or October.

Male

Only one sample of a male colony was found, in the October 2002 sample. All spermacysts were at the same stage of development (Fig 2e). Using the same staging scale as Waller et al. (2002), all spermacysts appear to be at stage 2. Numerous spermatocytes were present, with a few spermatozoa beginning to congregate around the lumen (Fig. 2e).

Fecundity

There was no significant difference between fecundity estimations for August and October ($U=8$; $P<0.05$). It is expected that further oogonia have developed into oocytes between August and October. Fecundity for the March sample is taken as 0, as no developing oocytes or oogonia were observed and the only oocytes present were few and being reabsorbed. Individual polyps in August had an average fecundity of 3146 oocytes per polyp (±1688), and in October had an average fecundity of 2308 oocytes (±818).

No evidence of gametogenesis was found in polyps below 0.08g, suggesting this is the size of first reproduction. There was no significant relationship between weight of polyp and fecundity ($R^2=0.4$ $P=<0.05$). Colony fecundity was recorded at

approximately 3300 oocytes per cm^{-2} (±1103). This would give a colony of around $30cm^2$ an approximate total fecundity of 99,800 oocytes.

Reproductive Periodicity

Fig 3

Oocyte size-frequency analysis shows there was a single cohort of developing oocytes in the population (Fig. 3). There appears to be rapid growth of oocytes, feret diameter doubles between August and October (41.3μm for Aug, 64.3μm for Sept and 88.7μm for Oct). Maximum oocyte size is 139μm.

Madrepora oculata

Morphology

There are also white and orange skeletal morphs of *M. oculata* (ACES, 2003), none were obtained in our sampling programme. *M. oculata* is gonochoristic, with all mesenteries fertile (Fig. 5). Oocytes develop from the lamellae of the mesentery.

Gametogenesis

Fig 4

No male *M. oculata* were found during this study. In the female previtellogenic, vitellogenic and late vitellogenic oocytes were present within a single mesentery in the October sample

Stage I – Oogonia – >37μm. Small female gametes budding off the mesenterial lamellae

Stage II – Previtellogenic - 37μm- 200μm. Small oocytes with thin wall and an basophilic cytoplasm (Fig 4e)

Stage III – Vitellogenic - 200μm - 350μm. Larger granulated oocytes with thin wall (Fig 4b-d).

Stage IV – Late Vitellogenic - >350μm +. Large, highly granulated, with thick cortical granule layer, presumably ready for spawning (Fig 4d-e).

Fecundity

Fig 5

The average fecundity varied over the months studied (Fig 5). The lowest fecundity recorded was during March, with an average of 10 oocytes per polyp (±8.26). In August, 28 oocytes per polyp (±8.26) were recorded and in October 68 oocytes per polyp (±24.15).

The smallest reproductive individual had a polyp diameter of 1.24.mm. However non-reproductive individuals were found up to a polyp width of 1.7mm. There was no significant relationship between polyp diameter and fecundity (R^2=0.07 P=<0.05).

Colony fecundity was recorded at 36 oocytes cm^{-2} (±2) for March, 104 oocytes cm^{-2} (±6.2), for August and 256 oocytes cm^{-2} (±12) for October. This gives a colony of around 30cm^{-2} surface area, a fecundity of 7680 oocytes in October.

Reproductive Periodicity

Fig 6

One cohort could be seen in the August sample (Fig. 6), whereas in the October sample two distinct cohorts could be seen. Oocyte growth appears rapid, more than doubling in size between August and October. Maximum oocytes size is 405μm. The March sample did not produce enough oocytes for size-frequency analysis.

Discussion

There are two main reproductive patterns in marine invertebrates, the production of small numbers of large oocytes, and the production of large numbers of small oocytes (Gage & Tyler, 1991). In shallow reef building scleractinians, Harrison

and Wallace (1990) demonstrated an inverse relationship between oocyte size and fecundity. In deeper water scleractinians Brooke (2002) observed *O. varicosa* producing large numbers (1,000 to 4,800 oocytes cm^{-2} skeletal area) of small oocytes (<100μm diameter). In the bathyal scleractinians from the NE Atlantic sampled here, *Lophelia pertusa* produces relatively large numbers of small oocytes (an average of 3300 oocytes cm^{-2}, 140μm maximum diameter) whereas *M. oculata* produces small numbers (an average of 256 oocytes cm^{-2} skeletal area) of large oocytes (405μm max).

The presence of previtellogenic oocytes in the August sample of *L. pertusa*, followed by rapid oocyte development (with only mature vitellogenic oocytes being observed in September and October), suggests that oogenesis is likely to be initiated in late summer. The oocyte size-frequency samples for *L. pertusa* for August, September and October show an increasing mean size of oocyte. This, together with the absence of developing gametes, and the observation of two reabsorbing oocytes in the March sample of *L. pertusa* suggests that this species has an annual gametogenic cycle with spawning around January/February. Such a gametogenic pattern in a bathyal species would be coincident with other deep-sea seasonally-spawning species in the NE Atlantic (Tyler and Young 1993). Oocyte size-frequency diagrams, and the large oocyte size in *M. oculata,* also suggests a seasonal pattern of gametogenesis. There is, however, evidence of multiple cohorts of developing oocytes not usually found within seasonal reproducers. This would in turn suggest a periodicity of reproduction, rather than true seasonality. *M. oculata* may be environmentally cued into producing and spawning gametes, when conditions are suitable for reproduction.

Most shallow-water scleractinians spawn in response to environmental cues (Fadlallah, 1983, Richmond, 1997). In shallow water species, this can be temperature, lunar phases, tidal cycles or, in synchronous spawners, the presence of other gametes

in the water. For deep water species the cues are less obvious. There is little temperature variation at depths below 800m, and there is no solar radiation. Studies in the NE Atlantic have shown that seasonal blooms of surface primary production sink rapidly to the deep-sea floor (Billett et al., 1983; Lampitt 1985; Thiel et al. 1989) and can have an effect on the reproductive biology of benthic invertebrates (Billett et al., 1983; Tyler et al., 1992; Tyler et al., 1993). Seasonal phytoplankton blooms in the Porcupine area occur in July (Lampitt et al., 2001), and this could be the cue for *L. pertusa* and *M. oculata* to initiate gametogenesis. At this time there would be a substantial increase in the availability of particulate organic matter, and so may coincide with the, energetically expensive, production of gametes in both these species. No samples were obtained during this time, and so the precise date of the beginning of reproduction in both these species from this area is unknown.

It is also unknown precisely what these species feed on, though as they are sessile and unable to scavenge actively for food, it is likely that they may rely heavily on food fall to these bathyal depths. A lecithotrophic larvae would appear the most suitable for such conditions. The extra energy and nutrients put into producing larger planulae serves as a food store until settlement takes place. In the deepwater coral *Occulina varicosa*, settlement has been observed to occur between 20 and 35 days in the laboratory (Brooke, 2002). Lecithotrophic larvae have also long been shown to be suitable for long distance dispersal (Shilling & Manahan, 1994). Brooding would also appear to be a suitable life strategy for both *L. pertusa* and *M. oculata*, providing extra protection from the deep-sea environment until planulae are more mature, though no planulae were observed in this study.

In *M. oculata*, polyp size overlapped for non-reproductive and reproductive polyps, suggesting that a factor other than size controls gametogenesis. It has been

reported in shallow water corals that total colony size can determine mode of reproduction (Kojis & Quinn, 1982; Szmant, 1986; Hall & Huges, 1996). A small colony would reproduce asexually, extending total size until large enough to overcome size-related mortality, and then proceed to sexual reproduction. Due to collection methods in this study this aspect was not observed, though is likely to be an important aspect of these two species' ecologies, as both do not show polyp size related fecundity. Using box cores and trawls, total colony size is unable to be established as the sample is broken, sometimes into many pieces, before retrieval. In-situ methods, such as submersibles and remotely operated vehicles, are required to observe this factor in deep-water scleractinian ecology.

No reproductively-active polyps were observed in *L. pertusa* taken from the Darwin Mounds. This area has been observed to be extensively damaged by trawling operations (Bett, 2001, Hall-Spencer et al, 2003; Wheeler et al., in press). Wheeler et al (in press) observed multidirectional trawl door scars using a high resolution sidescan sonar, and 28 trawl scars were observed within a 5km towed video camera track. Many areas within the Darwin Mounds were also assessed to be 100% trawled between trawl door scars (Wheeler et al., in press). Many scleractinian colonies have to reach a certain size before reaching reproductive maturity (Szmant-Froelich, 1985; Szmant, 1986; Richmond, 1997). Brown and Howard (1985) also observed that a stress can also reduce reproductive output, and even cause death in some cases (Ward, 1995; Rinkevich & Loya, 1989). It is hypothesised that the intense trawling in this area may keep the *L. pertusa* colonies at a size that is below that necessary for gametogenesis to occur. Rinkevich and Loya (1989) observed that removal of even 23% of a colony of *Stylophora pistillata* causes sterility for up to a year. Trawling has been shown to both remove corals, and disturb colonies from these mounds (Wheeler

et al., in press). There is also the possibility of polyp suffocation, from increased sediment suspension. In shallow water, Dodge and Vaisnys (1977) have shown that extended dredging operations have had a destructive effect on coral communities. Genetic analysis of populations in the Darwin mound area also support these findings, showing that there is little sexual reproduction occurring (LeGoff-Vitry, et al., 2004). In this study using microsattelite markers and the ITS1 and ITS2 regions, the Darwin Mounds had the least genetic diversity of any fo the sites in the NE Atlantic.

Acknowledgments

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Figure captions

Fig. 1 – NE Atlantic Ocean. Showing bathymetry and location of Darwin Mounds in the Rockall Trough, Thérèse Mound within the Belgica Mounds, and the Southern Seabight Area

Fig. 2 - **a.** *L. pertusa* colony ; **b.** Previtellogenic oocyte undergoing vitellogenesis; **c.** Female mesenteries containing vitellogenic oocytes; **d.** Vitellogenic oocytes; **e.** Stage 2 spermacyst

p-polyp; v-vitellogenesis; pv-previtellogenic; vo-vitellogenic oocyte; m-mesentery; no-nucleolus; s-spermatocytes

Scale bars **a** 2cm; **b** 25μm; **c** 125μm; **d** 50μm; **e** 50μm

Fig. 3 – *Lophelia pertusa* oocyte-size frequency diagrams for samples from August 2000, September 2002 and October 2002

N – Total number of polyps; **n** – Total number of oocytes measured; **Arrow** – mean oocyte diameter

Fig. 4 – **a.** *Madrepora oculata* colony showing dead and live polyps; **b.** Female polyp showing position of vitellogenic oocytes; **c.** Vitellogenic oocyte within mesentery; **d.** Vitellogenic oocyte undergoing change to late vitellogenic oocyte; **e.** Late and previtellogenic oocytes

m-mesentery; vo-vitellogenic oocyte; lvo-late vitellogenic oocyte; cgl-cortical ganular layer; pv-previtellogenic oocyte

Scale bars **a** 2cm; **b** 400μm; **c** 300μm; **d** 150μm; **e** 250μm

Fig. 5 – *Madrepora oculata* average fecundities for August 2000, October 2002 and March 2002

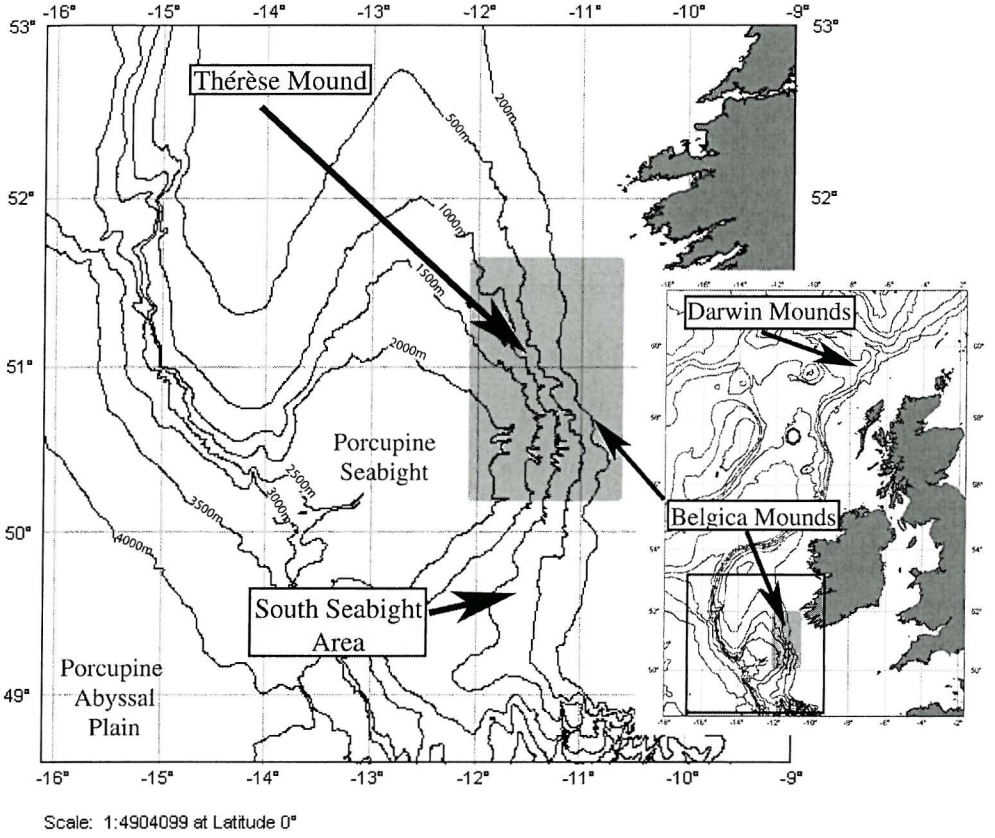
All animals size corrected to 1661μm polyp diameter

Fig. 6 – *Madrepora oculata* oocyte size-frequency diagrams for August 2000 and October 2002

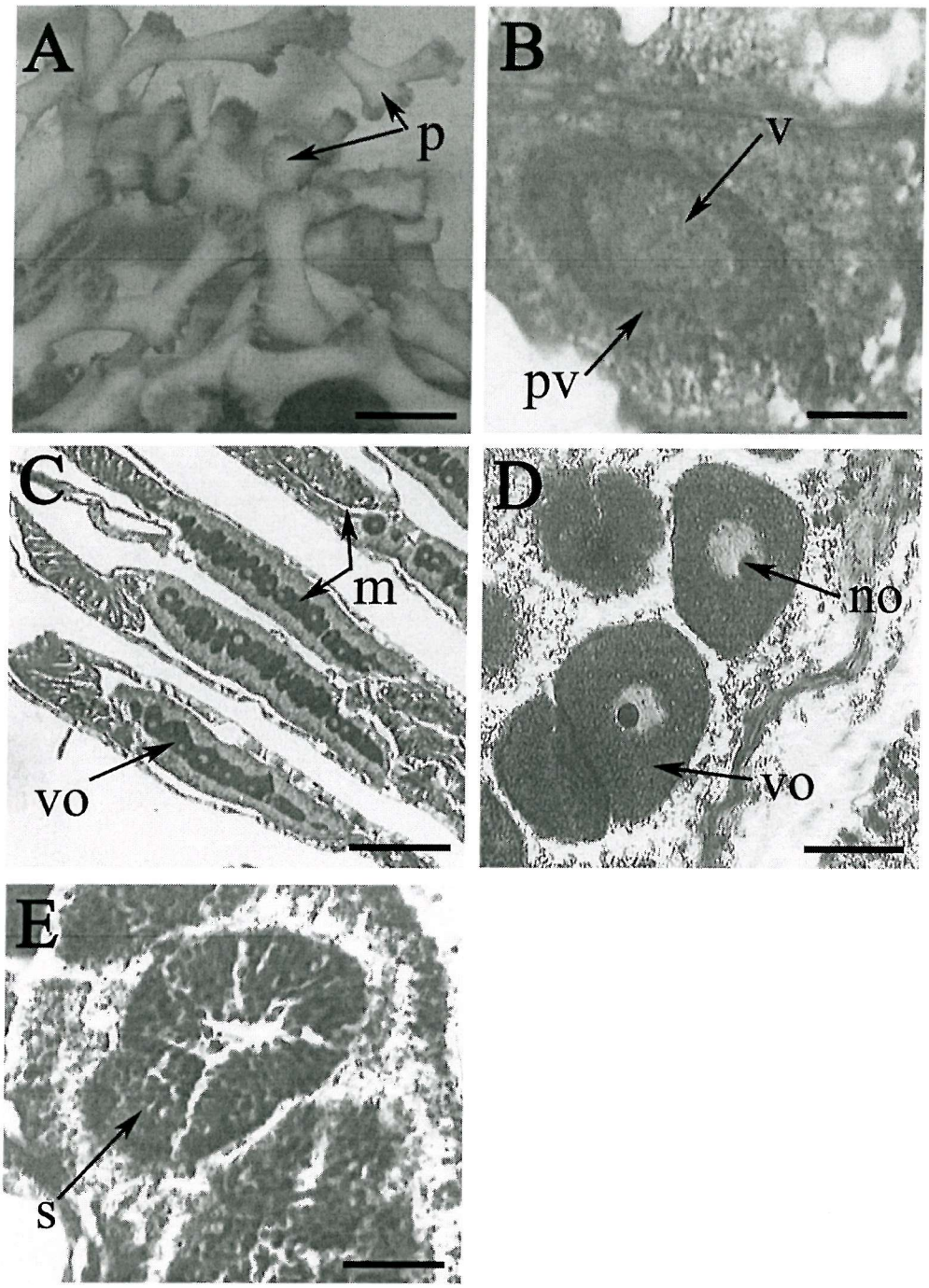
N – Total number of polyps; **n** – Total number of oocytes measured; **Arrow** – mean oocyte diameter

Species	Area	Depth	Cruise	Co-ordinates	Date
L. pertusa	Darwin Mounds	980m	248	59'N 48.88 07'W 17.99	16/07/00
	Thérèse Mound	870m	248	51'N 25.67 11'N 46.41	06/08/00
		870m	266	51'N 25.67 11'N 46.41	30/9/02
		870m	260	51'N 25.67 11'N 46.41	08/03/02
	South Porcupine Seabight	785-925m	260	44'N 40.00 11'W 30.70	18/10/02
M. oculata	Thérèse Mound	870m	248	51'N 25.67 11'N 46.41	06/08/00
		870m	260	51'N 25.67 11'N 46.41	08/03/02
	South Porcupine Seabight	785-925m	266	44'N 40.00 11'W 30.70	18/10/02

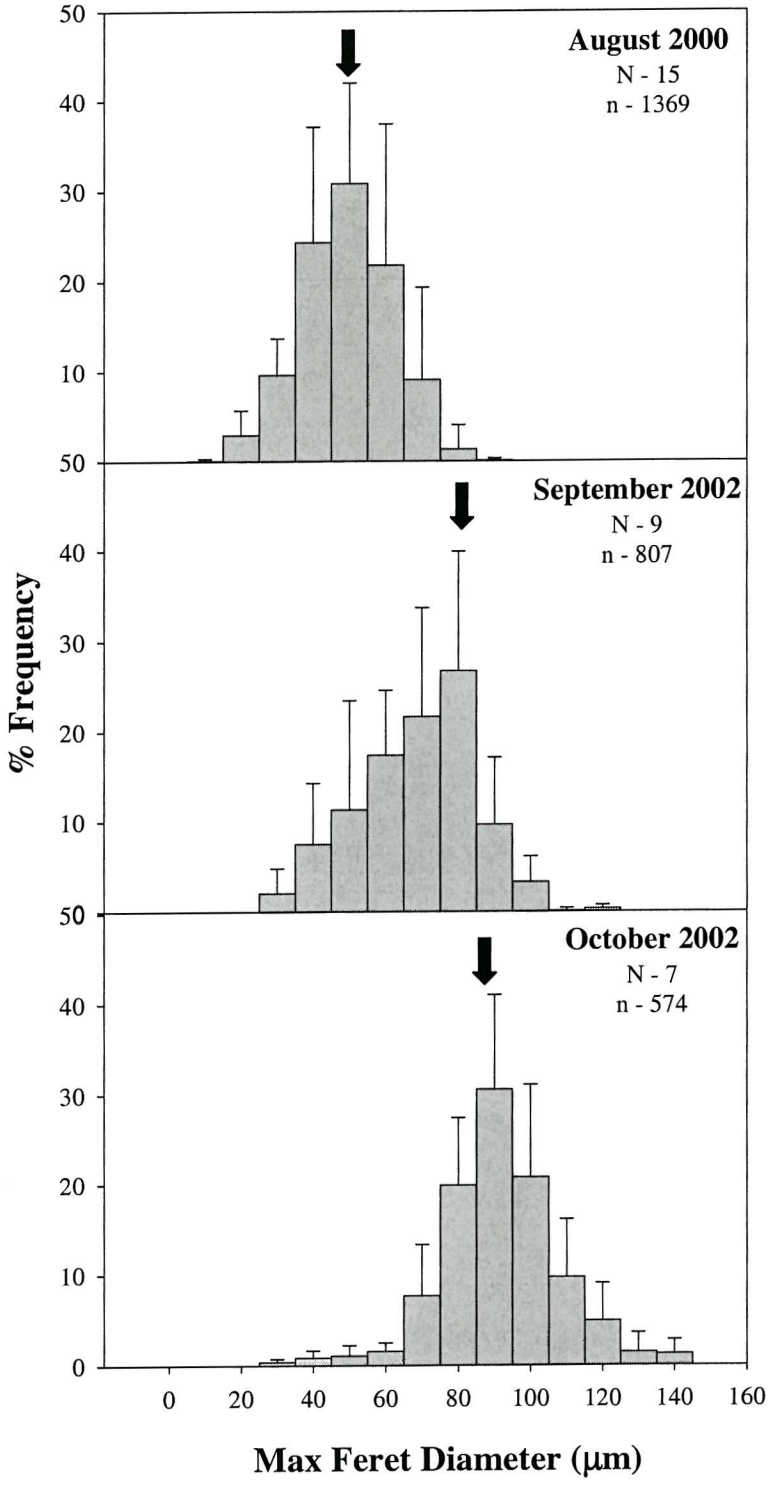
Waller & Tyler, Table 1. Collections made of *L. pertusa* and *M. oculata*



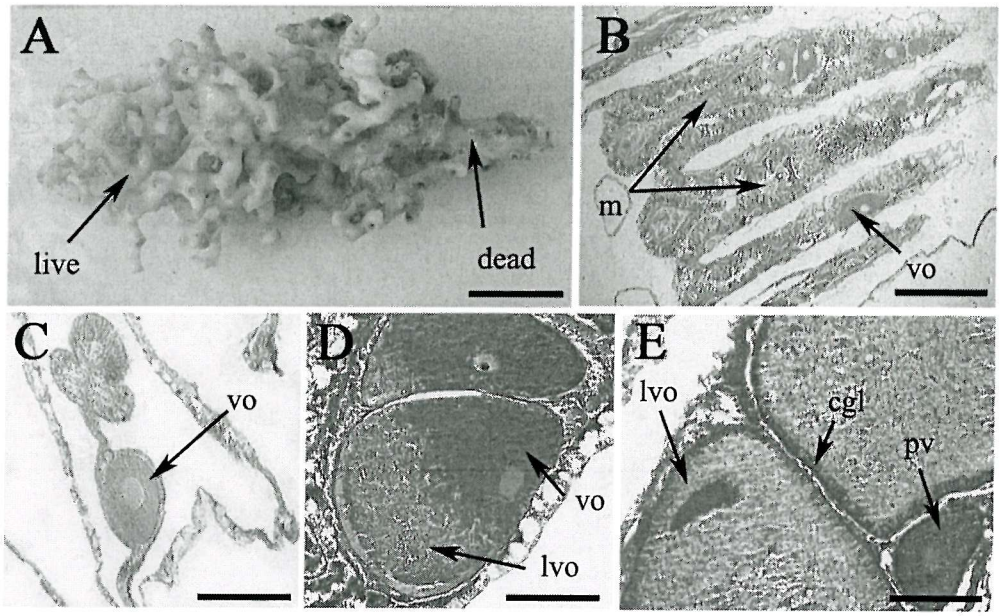
Waller & Tyler Fig 1



Waller & Tyler Fig 2



Waller & Tyler Fig 3



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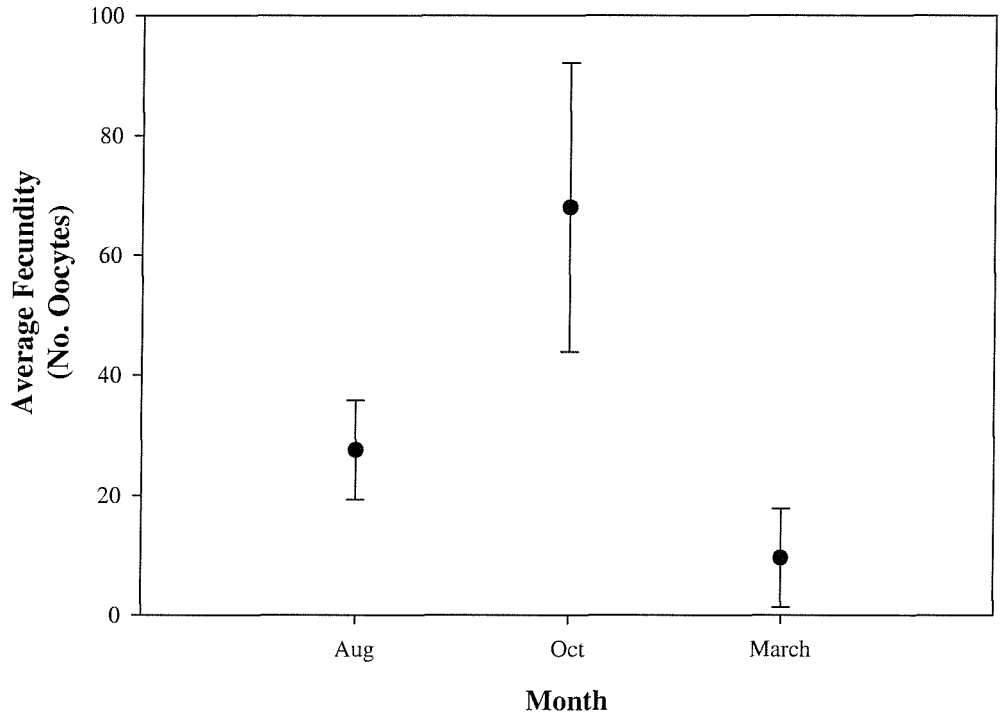
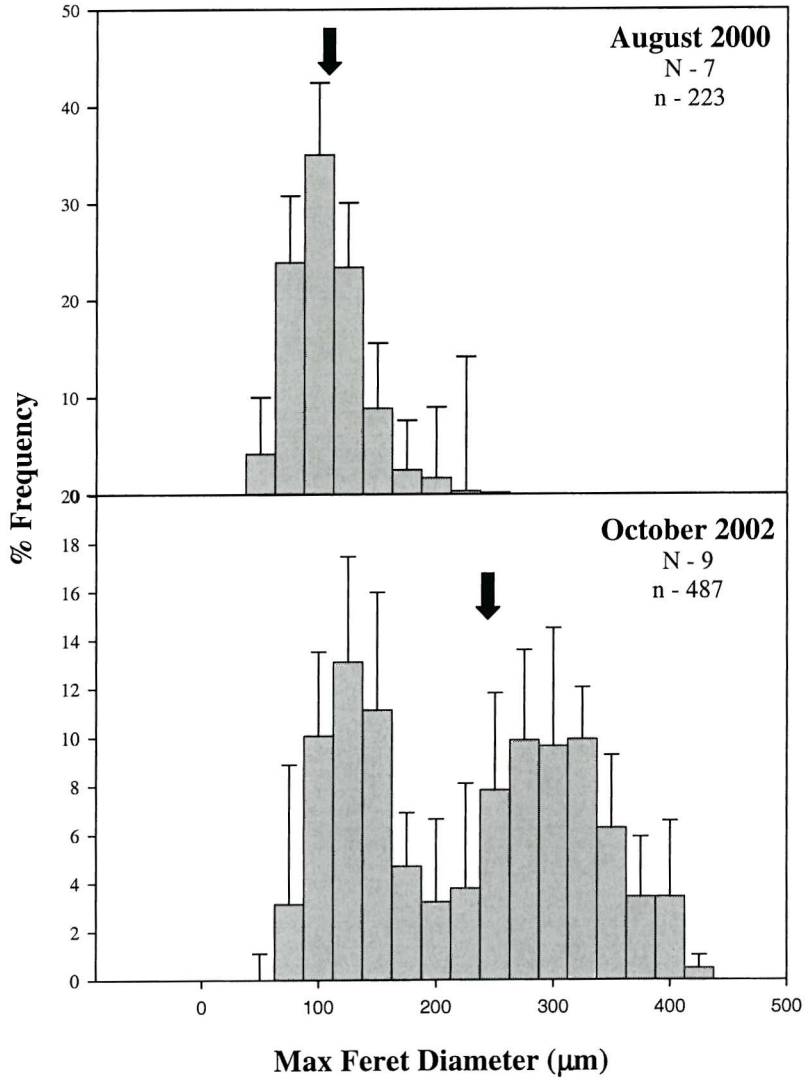


Waller & Tyler Fig 5



Waller & Tyler Fig 6

Ic

IN PRESS – CORAL REEFS

Sexual reproduction in three hermaphroditic deep-sea *Caryophyllia* species (Anthozoa: Scleractinia) from the NE Atlantic Ocean.

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Keywords:- ahermatypic, azooxanthellate, solitary coral, gametogenesis, cyclical hermaphrodite

Abstract

The reproductive biology and gametogenesis of three species of *Caryophyllia* were examined using histological techniques. *Caryophyllia ambrosia*, Alcock 1898, *C. cornuformis*, Pourtales 1868 and *C. seguenzae*, Duncan 1873, were collected from the Porcupine Seabight and Rockall Trough in the NE Atlantic Ocean. These three ahermatypic solitary corals inhabit different depth ranges: *C. ambrosia* - 1100-3000m, *C. sequenzae* - 960-1900m, and *C. cornuformis* - 435-2000m. All three species are hermaphroditic. Hermaphroditism is cyclical, with only one sex of gametes viable in any individual at any point in time, although gametes of both sexes are found together within a single mesentery. Once the viable gametes are spawned the next sex of gametes continues to grow until mature, and so gametogenesis is a continuous cycle. Oocytes and spermacysts, in all species, increased in density towards the actinopharynx. Maximum fecundity for *C. seguenzae* was 940 oocytes per polyp, and for *C. ambrosia* 2900 oocytes per polyp. Fecundity could not be established for *C. cornuformis*. In all three species individuals were asynchronous within populations, and production of gametes is quasi-continuous throughout the year. All species are hypothesised to have lecithotrophic larvae owing to their large oocyte sizes (*C. cornuformis* max - 350μm; *C. sequenzae* max – 430 μm; *C .ambrosia* max – 700 μm) Both the average oocyte size and fecundity increased in species going down the depth gradient of the NE Atlantic.

Introduction

Deep-water scleractinian corals are both diverse in morphology and cosmopolitan, being found in all the worlds oceans (Zibrowius 1980). Species are either reef-builders, such as *Lophelia pertusa*, or occur as solitary polyps. Reef-building species form an important habitat for numerous vertebrate and invertebrate species, many of increasing commercial value (Rogers, 1999; Mortensen, 2000). Deep water solitary species are more diverse in morphology, can inhabit sedimented and rocky substrata, and have been found at greater depths than reef-builders (Cairns, 1979; Zibrowius, 1980).

Species in the phylum Cnidaria show varying combinations of reproductive patterns - sexual, asexual, free-spawning, brooding, external fertilisation, internal fertilisation, gonochorism, hermaphroditism, seasonal, continuous and periodic life histories have all been found in this diverse group (Fadlallah, 1983; Harrison & Wallace, 1990). The Scleractinia are typical of this variety. Most information on coral reproduction is derived from studies of shallow-water species, whilst only four studies thus far have described reproduction in deep-water scleractinians. Waller et al. (2002) examined the gametogenic biology of *Fungiacyathus marenzelleri* from the Rockall Trough (NE Atlantic), Brooke (2002) reported on the reef building *Oculina varicosa* from the east coast of Florida, Waller and Tyler (accepted) studied the reef builders *Lophelia pertusa* and *Madrepora oculata* from a variety of sites in the NE Atlantic ocean and Burgess and Babcock (in press) examined *Enallopsammia rostrata*, *Solenosmillia variabilis*, *Goniocorella dumosa* and *Madrepora oculata* from the Chatham Rise. *F. marenzelleri* and *E. rostrata* are non-seasonally breeding gonochoric species, *O. varicosa*, *L. pertusa*, *S. variabilis* and *G. dumosa* are seasonal gonochoric species and *M. oculata* is a periodic gonochoric species. Six of these

species appear to have oocyte sizes that indicate lecithotrophic larval development, with *O. varicosa* being the exception.

Additional information is available on the reproductive ecology of other deep-water anthozoans. Van Praet et al. (1990) described the gametogenic biology of two actinarian species of the genus *Phelliactis*, Bronsdon et al. (1993) described reproductive processes in the actinarian epizoites *Amphianthus inornatus,* and *Kadosactis commensalis*. Muirhead et al. (1986) observed gametogenesis in two Epizooanthus species, the bathyal *E. paguriphilus* and the abyssal *E. abyssorum*. In the Alcyonaria, Rice et al. (1992) and Tyler et al. (1995) described the gametogenic patterns for a variety of deep-water pennatulids.

The pattern of reproduction has also been described for several shallow water solitary corals, of which the majority are gonochoric, brooding species (Fadlallah 1983). *Balanophyllia elegans* is a non-seasonal brooder (Fadlallah & Pearse, 1982a) and *Paracyathus stearnsii* is a seasonal spawner (Fadlallah & Pearse, 1982b). *Fungia scutaria* is gonochoric and spawns gametes during the late summer (Krupp, 1983), whilst Goffredo et al. (2000; 2002) observed *Balanophyllia europaea* to be a hermaphroditic brooder. The reproductive biology is also known for two shallow water species of *Caryophyllia*. The Devonshire Cup Coral, *Caryophyllia smithii*, can be found around the British coast (as well throughout the Atlantic) from just a few metres depth, to over 1200m (Zibrowius, 1980). A gonochoric seasonal reproducer, shallow *C. smithii* spawns gametes during March, and produces a planktotrophic larvae (Tranter et al., 1982), though there are reports of brooding in this species (Hiscock and Howlett, 1977). *Caryophyllia clavus* also broods its young, though little else is known (Fadlallah, 1983).

In this paper we report on the gametogenic development and fecundity of three deep-water *Caryophyllia* inhabiting different, but overlapping depths in the NE Atlantic Ocean. *C. ambrosia* (Fig.1a) lives between 1100 and 3000m depth, *C. sequenzae* (Fig. 1b) lives between 960 and 1900m depth whilst the most shallow species, *C. cornuformis* (Fig. 1c), lives between 435 and 2000m (Zibrowius, 1980). *C. ambrosia* is the most cosmopolitan of these three species, occurring in the Atlantic, Pacific and Indian Oceans. *C. cornuformis* has been found at numerous locations around the Atlantic, and *C. sequenzae* has only been found in the Eastern Atlantic.

Fig .1

This study is part of the interdisciplinary European project 'Atlantic Coral Ecosystem Survey' (ACES). This project began in April 2000, to examine the biology, oceanography and geology of scleractinians at bathyal depths round the European margin.

Methods

Samples were collected by either 5m Agassiz beam trawl or Otter Trawl Semi-Balloon (14m) from the research vessels RRS *Challenger*, RRS *Charles Darwin* and RRS *Discovery* between 1978 and 2002 (Table 1). Samples were collected from the Porcupine Seabight and the Rockall Trough for

Table

C. cornuformis. The material was preserved in 4% formalin and later transferred to 70% alcohol. Before histological preparation, all individuals were submerged for approximately 4 hours in rapid decalcifying solution (conc. HCL) until no carbonate skeleton remained. They were then rinsed in running tap water for 24hrs to remove acid traces. Polyps were then wet-weighed prior to dissection. After decalcification, mesenteries from 20 whole individuals from each species (collected in different seasons), were counted and their structure noted.

For histological preparation the whole polyps of *C. cornuformis* and 3 mesenteries of each of 15 individuals of *C. seguenzae* and *C. ambrosi*a were dehydrated by three, four-hour, submersions in 100% propan-2-ol, followed by clearing with xylene for approximately 12hrs. These were then embedded by being left for 6-12 hours in molten histology wax at 70°C, and poured into standard moulds. All wax blocks were serially sectioned at 5μm, leaving 50μm in between slides, and stained with Masson's Trichrome stain.

Sections of each individual were then examined using an Olympus BH2 compound microscope with video camera attachment. Images were captured using Matrox Rainbow Runner and analysed using SigmaScan Pro version 4 to calculate oocyte diameters. 'Feret' diameter (the area if the oocyte was a perfect circle) was used as this normalises the often irregular outline of the oocytes that results from close packing in the mesentery. 'Plymouth Routines in Multivariate Ecological Research'(PRIMER) 5 was used to statistically analyse, and allow grouping, of oocyte size-frequency data. Multidimensional Scaling (MDS) and Analysis of Similarity (ANOSIM) was also used. Spermatogenesis and oogenesis were staged.

To estimate fecundity, all female and hermaphroditic slides were examined. All previtellogenic, vitellogenic and late vitellogenic oocytes were counted in three mesenteries from each female. The number of oocytes in the three mesenteries were averaged (realised fecundity) then multiplied by the total number of mesenteries within the individual (potential fecundity).

Results

Gametogenesis in the genus *Caryophyllia*

Caryophyllia ambrosia

Five monthly samples were available for analysis. *C. ambrosia* is a cyclical hermaphroditic, where one sex appears to be dominant at any one time. Individuals are termed 'female' and 'male' hermaphrodites pertaining to the dominant sex (with 'Intermediate' used to describe those at similar stages). PRIMER MDS and ANOSIM analysis (Fig 2) show the three groupings to be significantly different from each other (R^2=>0.496; P=>0.001). Gametes of both sexes develop from the mesenterial lamellae (Fig. 3a). Both male and female gametes were found in several clusters throughout the same mesentery, and increase in density towards the actinopharynx.

Fig. 2

Oogenesis

Oogenesis can be divided into four stages:-

Stage one – Oogonia (<50μm) are observed budding from the mesenterial lamellae; Stage two – Previtellogenic oocytes (<250μm) containing a large nucleus (Fig. 3a); Stage three – Vitellogenic oocytes (<500μm) showing evidence of rapid yolk accumulation (Fig. 3b, c); and Stage four – Late Vitellogenic oocytes (>500μm) that have a thick cortical granular layer just inside the oolemma, large yolk granules and prominent nucleolus in the central nucleus (Fig. 3d)

Fig. 3

Oocytes within a single individual were at the same stage of development whereas individuals from the same sample showed asynchrony in oocyte development. Oocyte size frequency diagrams show this asynchrony and the unimodal development of oocytes (Fig. 4). The maximum oocyte diameter observed was

Fig. 4

700μm. Hermaphroditic individuals (Fig. 3e), containing developing oocytes, were found in all sizes of polyp.

Spermatogenesis

Four stages of spermatogenesis were identified:-

Stage one (early) - Loosely packed aggregations of spermatocytes contained within a spermacyst. Empty lumen can be observed; Stage two, (maturing) - Some spermatozoa are present, starting to fill the lumen but loosely packed; Stage three, (mature) - well developed spermatocyte layer and lumen packed with spermatozoa (Fig. 3f); and Stage four, (spent) - relict spermatozoa can be seen

Spermacysts were observed to be in a similar stage of development within a single individual. Hermaphrodites that were predominantly female had fewer, smaller spermacysts, at earlier stages, than male hermaphrodites.

Caryophyllia sequenza

Four monthly samples were analysed during this study. *C. seguenzae* is also a cyclical hermaphrodite, as in *C. ambrosia*. A PRIMER MDS and ANOSIM plot (Fig. 2) was used to separate male, female and intermediate hermaphroditic individuals (R^2=>0.532; P=>0.001). Gametes of both sexes develop from the mesenterial lamellae. Both male and female gametes were found in several pockets throughout the same mesentery, and increase in density towards the actinopharynx.

Oogenesis

Oogenesis is very similar to that of *C. ambrosia*, with differing oocyte sizes:-

Stage One – Ovoid oogonia are <60μm; Stage two – Previtellogenic oocytes are < 125μm; Stage three – Vitellogenic oocytes are <350μm (Fig. 3g); Stage four - Late Vitellogenic oocytes are >350μm reaching a maximum size of 450μm

Oocytes within a single individual were at a similar stage of development whereas individuals from the same sample showed asynchrony in oocyte development. Oocyte size frequency diagrams show this asynchrony and the unimodal development of oocytes (Fig. 5). The maximum oocyte diameter observed was 450μm. Hermaphroditic individuals, containing developing oocytes, were found in all sizes of individual (Fig. 3h).

Fig. 5

Spermatogenesis

Spermatogenesis in *C. seguenzae* follows the same stages as *C. ambrosia.* Spermacysts were observed to be in a similar stage of development within a single individual and, as in *C. ambrosia*, these were mainly late stages (Fig. 3i). Hermaphrodites that were predominantly female had fewer, smaller spermacysts, at earlier stages, than male hermaphrodites.

***Caryophyllia cornuformis* (Fig. 3j,3k,3l)**

Only two monthly samples were obtained for this species, and these were not in good condition. It is thought that formalin penetration into the lower extremities

was poor, and so tissue was not preserved adequately to gain reliable fecundity estimates or numerous measurements of oocyte diameter.

Fig. 6

This species is also a hermaphrodite, which may also be cyclical ,though insufficient samples were available for a conclusive assessment. There were insufficient oocytes in measurable condition to develop oocyte size frequency histograms for this species, and so sizes were averaged for every individual and then for the two samples (Fig. 6). Maximum oocyte size is 350 μm. Individuals from the same sample had varying average oocyte diameters.

Fecundity

Fecundity was calculated from both 'female' and 'male' hermaphrodites in both *C. ambrosia* and *C. seguenzae*. There was no significant difference in realised fecundity between these two phases in either species (*C. ambrosia* – U=43.5, P=0.078; *C.seguenzae* – U=36.0, P=0.2986).

C. ambrosia

Potential fecundity was calculated as a minimum of 200 oocytes per polyp and a maximum of 2750 oocytes per polyp. After size correction there was no significant difference in average fecundity among the four months analysed (U=>8.5, P=>0.155), indicating nearly continuous production of gametes over this period. Fecundity did not increase with wet weight of polyp (R^2 = 0.227; P=0.05), and there was no distinct size of first reproduction within the size range of individuals observed.

C. seguenzae

Potential fecundity was calculated at a minimum of 52 oocytes per polyp and a maximum of 940 oocytes per polyp. After size correction there was no significant difference between average fecundity between the four months analysed (U=54, P=0.006), suggesting a quasi-continuous production of gametes.

Fig. 7

Fecundity is size-dependent (Fig. 7; R^2=0.728; P=<0.05). Non-reproducing individuals were not included in the regression, as there was no distinct size of first reproduction.

Discussion

All three of these deep water scleractinian species show asynchronous, cyclical hermaphroditism with no evidence of seasonality. The majority of scleractinians for which the patterns of reproduction is known, are hermaphroditic (Fadlallah 1983; Harrison et al. 1984; Szmant 1986), with this being a more dominant strategy than gonochorism (Goffredo, 2000). In cyclical hermaphroditism, a single individual is unable to produce viable gametes of both sexes at the same point in time, so self fertilisation cannot occur. Selfing is thought to be an important mode of reproduction in hermaphroditic corals that can ensure fertilisation success (Brazeau et al., 1998), however it does not allow for genetic diversity and evolution of a species (Veron, 1995).

The large egg size of these three species suggest lecithotrophic rather than planktotrophic early development (Fadlallah 1983; Gage and Tyler 1991). Lecithotrophic development, contrary to earlier suggestions, is now recognised as a beneficial adaptation for wide dispersal in oligotrophic environments, such as the deep sea (Shilling and Manahan, 1994). Most deep-sea scleractinians studied to date (seven out of eight species) appear to have lecithotrophic larvae (Waller et al., 2002; Waller and Tyler, accepted; Burgess & Babcock, in press). Though planulation in deep-sea species is difficult to observe without *in vitro* cultures, histological evidence suggests that all three species of *Caryophyllia* in this study spawn gametes rather than brood. This is inferred by both the lack of planulae, and the varying stages of gamete development within the population (which would mean the likelihood of observing planulae within the polyp would be high). The spawning of gametes as the normal mode of reproduction in the Cnidaria has been well documented for a number of shallow species of coral (Kojis and Quinn 1981; Bothwell 1982; Fadlallah and Pearse

1982; Fadlallah 1983; Harrison et al. 1984; Szmant 1986; Harrison 1990; Richmond and Hunter 1990; Richmond 1997). *C. smithii*, a shallow water congeneric, also broadcast spawns and is externally fertilised (Tranter et al., 1982) although there is a report of possible brooding (Hiscock & Howlett, 1977),. Rinkevich and Loya (1979) proposed that large-polyp species would be unlikely to brood, as the extra energy required for growth, defence and maintenance of the large polyp would mean the energy required to produce a brooded planulae would be unavailable. Stimson (1978) also suggested that deep fore-reef scleractinians may broadcast gametes in order to aid the wide dispersal distance required at depths. These two theories fit with the data acquired during this study. All of these *Caryophyllia* species broadcast gametes, and the average oocyte size increases with polyp size.

Gametes of shallow water scleractinians are thought to develop within the lamellae of the mesenteries and subsequently migrate into the mesoglea, to develop as oogonia (Szmant-Froelich et al. 1980; Fadlallah 1983). Oogonia and spermatocysts of all three species of *Caryophyllia* studied here were first observed attached to the mesenterial lamella, and so this mode of development is also believed to be the case with these deep water corals. This has also been observed in other species of deep-water scleractinian, i.e. *Fungiacyathus marenzelleri* (Waller et al., 2002), *Lophelia pertusa* and *Madrepora oculata* (Waller and Tyler, accepted).

Caryophyllia smithii is gonochoric and a seasonal reproducer, producing gametes between January and March (Tranter et al., 1982). This is a very different strategy from the three deep water species examined in this study even though they are all congeners. It is not unusual for scleractinian species within the same genus to have differing reproductive patterns (Fadlallah, 1983; Harrison & Hunter, 1990) and

Table

so it is possible that this change in reproductive strategy is environmentally rather than phylogenetically constrained.

Average fecundity and average oocyte size (although this pattern is not apparent in *C. smithii*) both increase with depth in these three species of deep-water *Caryophyllia* (Table 2). Spawning and larval type also appears to shift between seasonal planktotrophic to quasi-continuous lecithotrophic development as the depth increases. Environmental conditions have often been shown to influence reproductive strategies. Many species of shallow scleractinians have been shown to use lunar periodicity and seasonal temperature differences to time their reproduction (Fadlallah, 1983, Richmond, 1997), whereas reproduction in several deep water invertebrate species may be cued in the NE Atlantic by a seasonal phytodetrital pulse (Billett et al., 1983; Tyler et al., 1992; Tyler et al., 1993). Brooding was also originally thought to be an ideal strategy for the deep-sea environment as it takes a developing planulae to late stages before releasing it to the 'harsh' environment (Gage & Tyler, 1991), though this is yet to be found in the deep water Anthozoa.

The asynchronous pattern of reproduction observed in these three species suggests that there is a near constant presence of spawned gametes in the water, while it is possible that individuals in close proximity to one another are synchronously spawning. Because individuals were trawled, their small scale spatial distribution is not known. There are also no data available on the density of any of the deep water *Caryophyllia spp.* corals. Densities of both individuals and gametes in broadcast spawning species must be sufficiently high to allow successful fertilization, yet because of low food availability, densities of fauna are generally regarded as low in the deep sea (Gage and Tyler, 1991). Population densities of deep water solitary

corals are likely to play an extremely important role in their reproductive strategies and ecology.

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Species	Date	Vessel	Depth	Latitude/Longitude
C. seguenzae	12.2.98	RRS *Discovery*	1278m	58° '58N 07° '57W
	24.4.78	RRS *Discovery*	1404m	50° '30N 12° '00W
	7.11.80	RRS *Darwin*	1250m	51° '04N 11° '50W
	19.10.02	RRS *Discovery*	1240m	49° '50N 12° '05W
C. ambrosia	20.6.85	RRS *Darwin*	2440m	51° '00N 12° '59W
	4.8.81	RRS *Challenger*	2713m	51° '05N 11° '48W
	21.3.02	RRS *Discovery*	2500m	49° '36N 12° '11W
	4.9.79	RRS *Discovery*	2315m	51° '00N 12° '03W
	1.10.02	RRS *Discovery*	2452m	50° '04N 12° '45W
C. cornuformis	10.3.93	RRS *Challenger*	1650m	57° '07N 09° '30W
	11.8.92	RRS *Darwin*	2017m	57° '00N 09° '58W

Table 1 – Details of deep-water *Caryophyllia* species sampled for this study

Species	Depth	Max Oocyte Diameter	Max Fecundity (per polyp)	Sex	Gamete Release	Larvae Type
**C. smithii*	2-1200m	150μm	'several thousand'	Gonochoric	Seasonal	Planktotrophic
C. cornuformis	435-2000m	350μm	N/D	(Cyclical) Hermaphrodite	(Quasi continuous)	(Lecithotrophic)
C. seguenzae	960-1900m	430μm	940	Cyclical Hermaphrodite	Quasi continuous	(Lecithotrophic)
C. ambrosia	1100-3000m	700μm	2750	Cyclical Hermaphrodite	Quasi continuous	(Lecithotrophic)

Table 2 – Comparisons of depth range and reproductive ecology of four species of Caryophyllia. (**C. smithii* data from Tranter et al., 1982)

N/D = No data; Data in parenthesis hypothesised

Figure Captions for Waller et al.

Fig. 1 – Specimens of three deep-sea solitary scleractinian corals from the NE Atlantic, **A**. *Caryophyllia cornuformis*; **B**. *C. seguenzae*; **C**. *C. ambrosia*
Scale bars = **A**, 1cm; **B**, 2cm; **C**, 2cm

Fig. 2 – A, PRIMER MDS plot and ANOSIM statistic for *C. ambrosia*; **B,** PRIMER MDS plot and ANOSIM statistic for *C. seguenzae*.
circles, female hermaphrodites; **triangles**, intermediate; **diamonds**, male hermaphrodites

Fig. 3 – A, *C. ambrosia* previtellogenic and vitellogenic oocytes connected by the mesenterial lamellae; **B**, *C. ambrosia* vitellogenic oocyte; **C**, *C. ambrosia* female mesentery; **D**, *C. ambrosia* late vitellogenic oocyte showing cortical granular layer; **E**, *C. ambrosia* hermaphroditic mesentery; **F**, *C. ambrosia* stage III spermacyst; **G**. *C. seguenzae* vitellogenic oocytes; **H**, *C. seguenzae* hermaphroditic mesentery; **I**. *C. seguenzae* stage III spermacyst; **J**. *C. cornuformis* vitellogenic oocyte; **K**. *C. cornuformis* stage III spermacyst ; **L**. *C. cornuformis* hermaphroditic mesenteries
Pv, Previtellogenic oocyte; **Vo**, Vitellogenic oocyte; **L**, mesenterial lamellae; **N**, Nucleus; **M**, Mesentery; **Cg**, Cortical granular layer; **Sp**, Spermacyst; **Sc**, Spermatocytes; **Sz**, Spermatozoa; **Me**, Mesogleal envelope; **O**, Oocyte;
Scale bars = **A**, 200μm; **B**, 200μm; **C**, 400μm; **D**, 100μm; **E,** 200μm; **F**, 100μm, **G,** 100μm; **H,** 200μm; **I**, 100μm, **J**, 50μm; **K**, 50μm**; L**, 100μm

Fig. 4 – Oocyte size-frequency distribution plots for *C. ambrosia*. Plots split into 'male hermaphrodites', 'intermediate' and 'female hermaphrodites' using PRIMER
Error bars = ±1sd; **N**= number of individuals; **n**= number of oocytes measured

Fig. 5 – Oocyte size-frequency distribution plots for *C. seguenzae*. Plots split into 'male hermaphrodites', 'intermediate' and 'female hermaphrodites' using PRIMER
Error bars = ±1sd; **N**= number of individuals; **n**= number of oocytes measured

Fig. 6 – Average oocyte diameters in *C. cornuformis* for two months analysed
circles, Individual average oocyte diameters; **triangles**, Average oocyte diameter for month; Error bars = ±1sd

Fig. 7 – *C. seguenzae* polyp wet weight plotted against realised fecundity, fitted with regression line

N= number of individuals; **N**=13; 99% confidence intervals; f=y0+a*x; Size corrected to 1.398g polyp wet weight

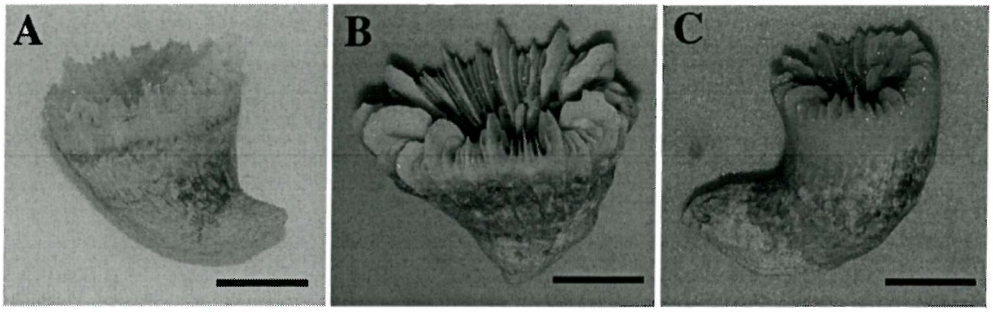

Fig. 1 - Waller et al.

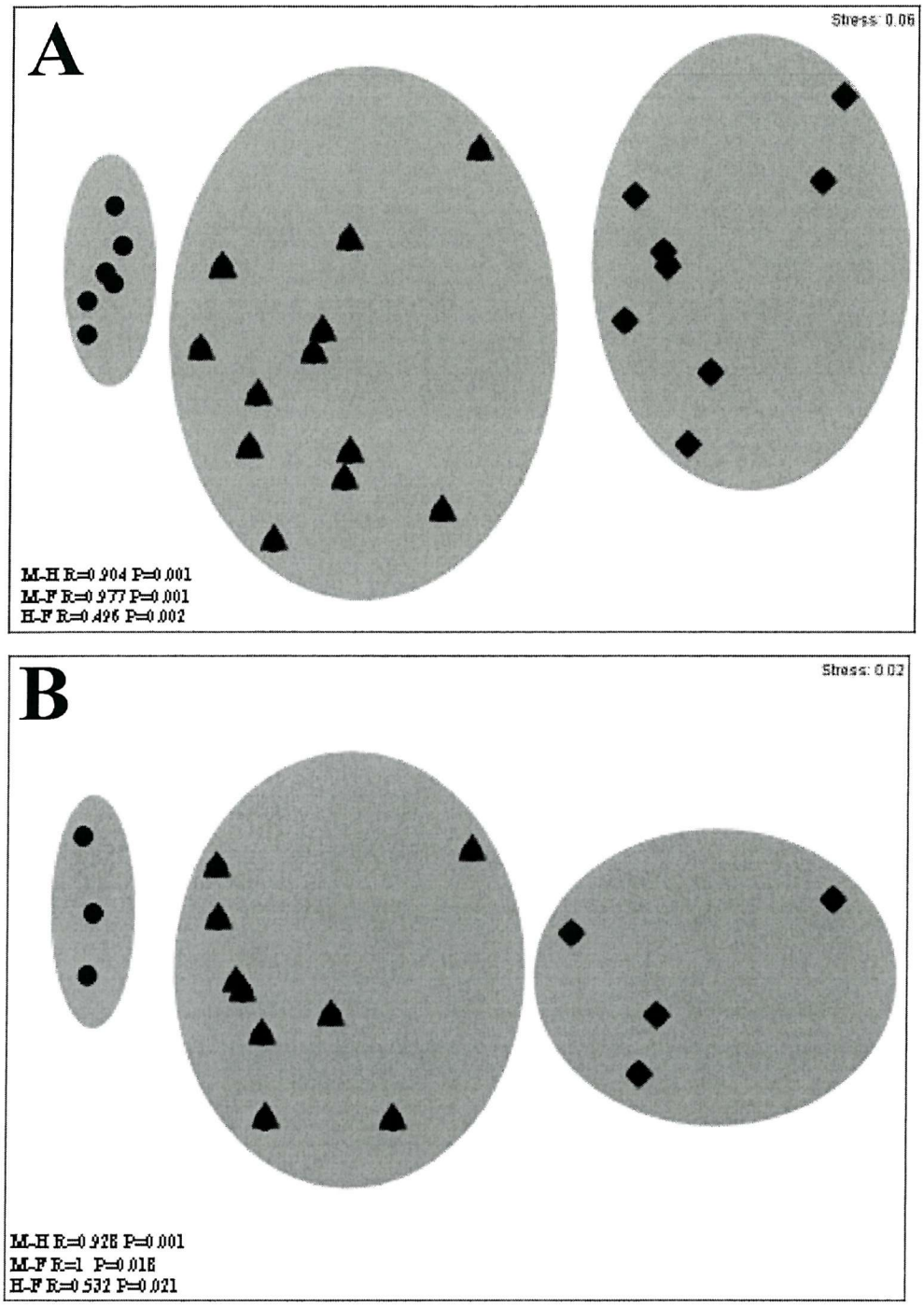

Fig. 2 – Waller et al.

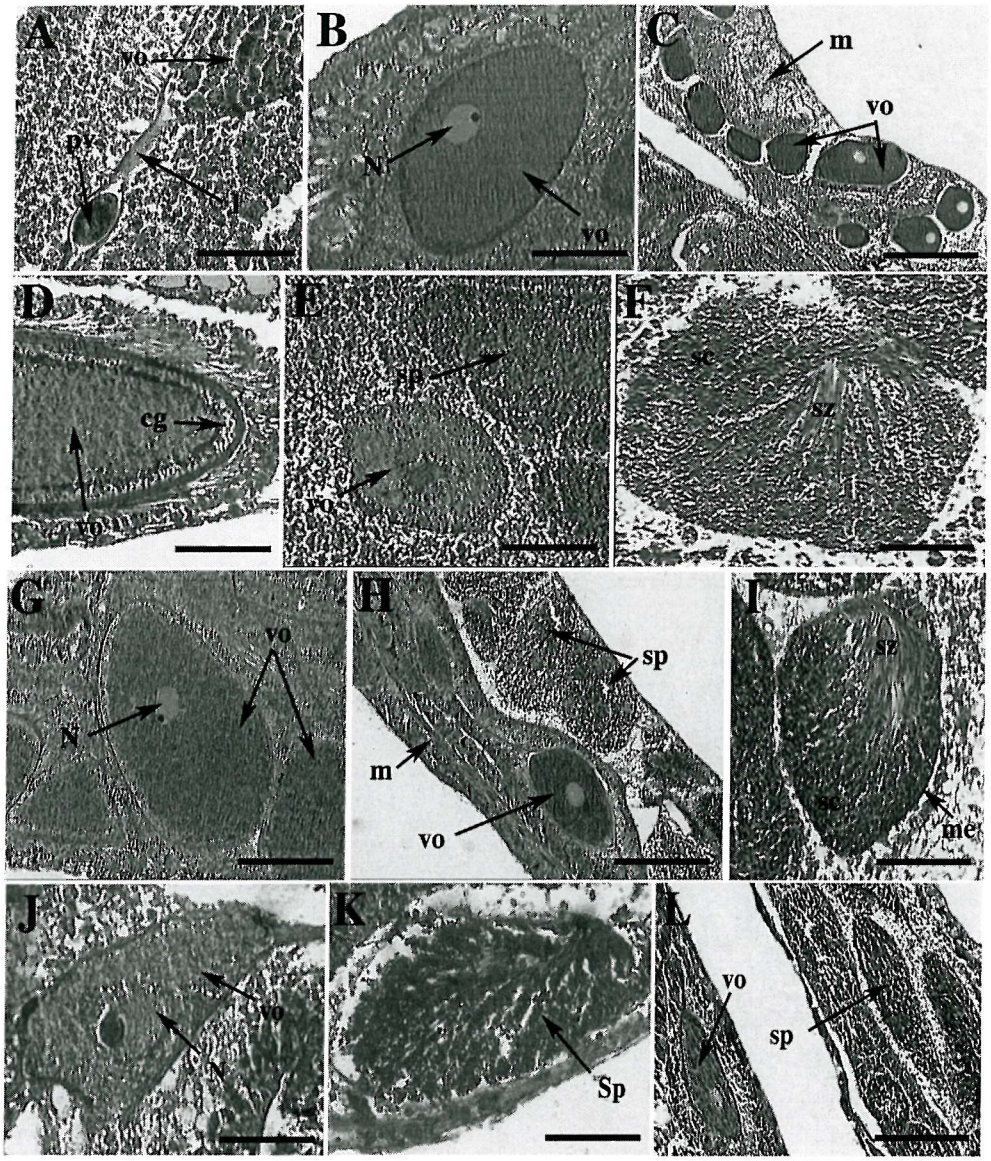

Fig. 3 – Waller et al.

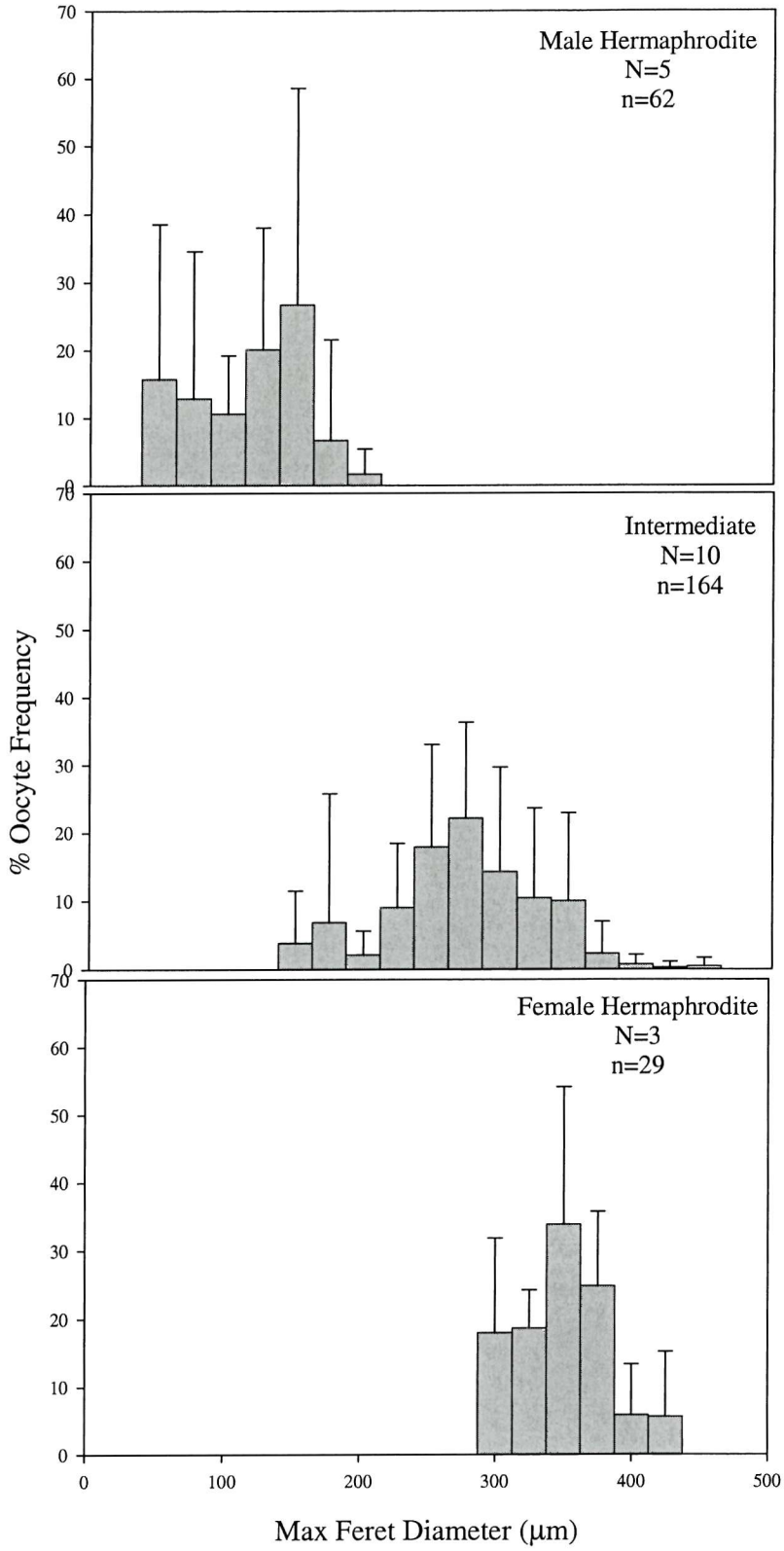

Fig. 4 – Waller et al.

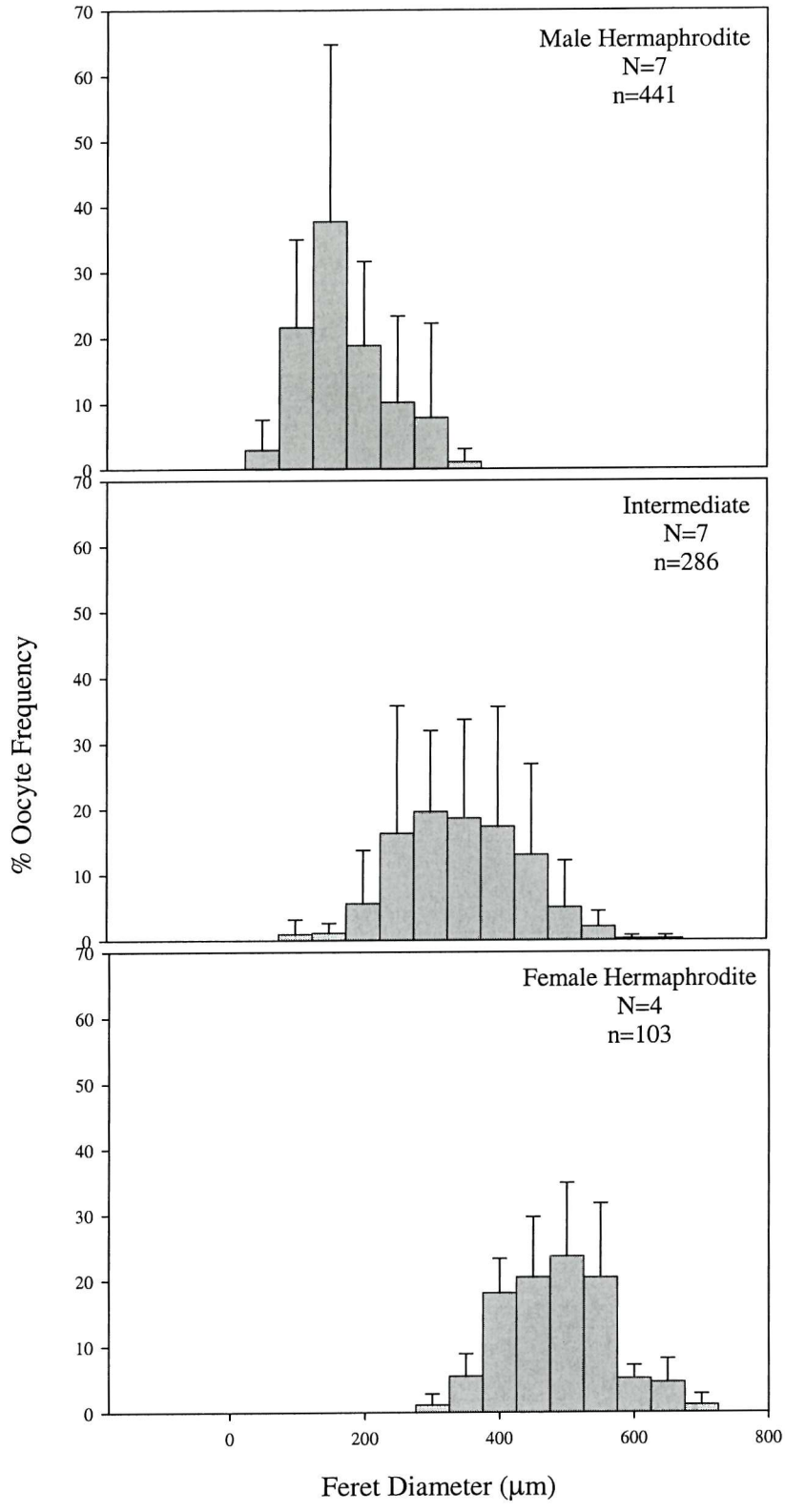

Fig. 5 – Waller et al.

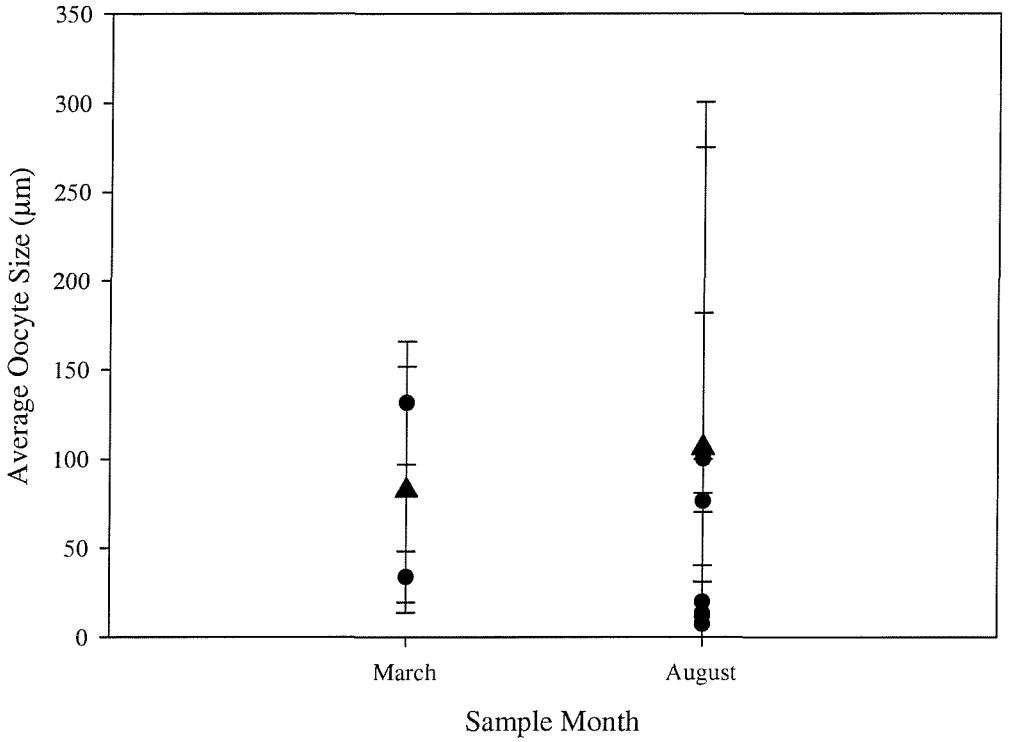

Fig. 6 – Waller et al.

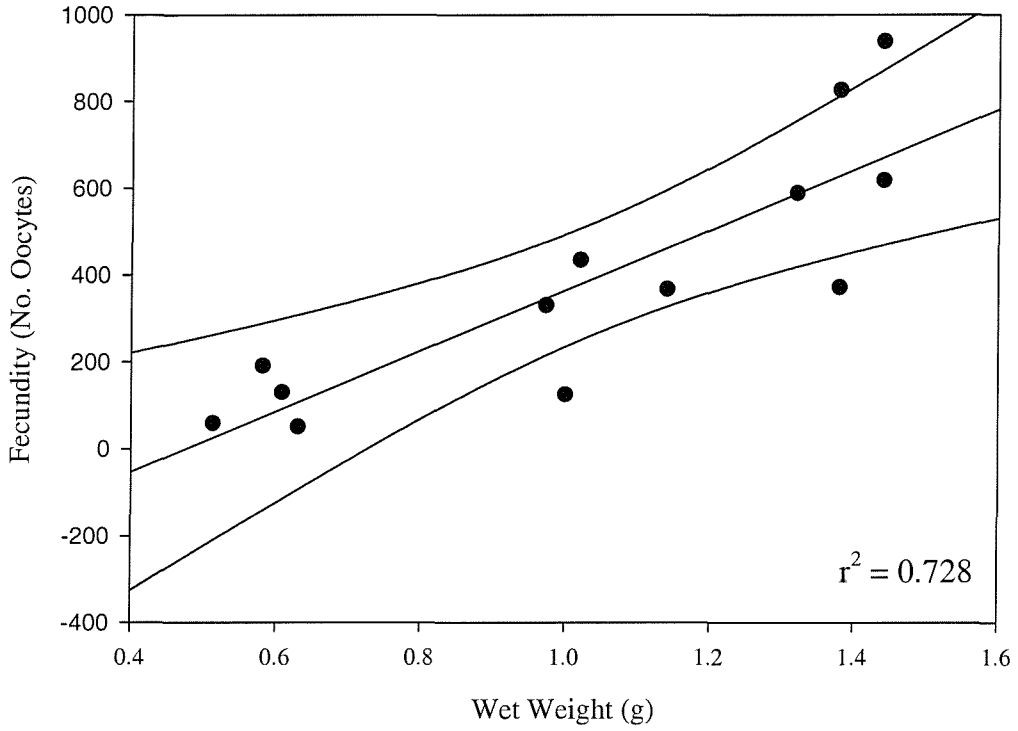

Fig. 7 – Waller et al.

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Deep-Water Scleractinia (Cnidaria: Anthozoa): Current knowledge of reproductive processes

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Abstract

Little is known of the basic biology and ecology of the numerous species of deep-water scleractinians found in all the world's oceans. Of all the biological processes, reproduction is the most fundamental. Without knowledge of a species' reproduction, we know little about how they survive both the environment that is the deep-sea, and the increasing anthropogenic effects of man's exploration for new fisheries and energy reserves.

This review collates current knowledge of the reproductive processes of deep-water scleractinians. Only fifteen deep-water species as yet have had their reproduction described in the literature. Gametogenesis, reproductive seasonality and larval ecology are summarised and compared briefly to shallow-water counterparts.

A summary table of all deep-water scleractinian species, for which the reproductive strategy is known, and their sample locations, is presented here. It is hoped this knowledge will be used as a basis for further understanding of how the deep-sea species of this order survive and disperse. These data are also directly applicable to the conservation and management of these deep-sea ecosystems.

Introduction

The presence of scleractinian corals living at depths beyond 100m has been known for many decades, and yet there is still a paucity of information on their basic biology and ecology. In recent years the ecological value of deep water scleractinians has become more apparent. Reef building species, similar to those found in shallow-water systems, form important habitats, creating oases on continental shelves and slopes, seamounts and ridges. These habitats support a wide diversity of life. Koslow and Gowlett-Jones (1998) observed 242 species of invertebrates and 37 species of fish associated with *Solenosmilia variabilis* reefs on the Tasmanian Seamounts, whilst *Lophelia pertusa* reefs in the NE Atlantic have been shown to support over 1300 species of associated fauna (ACES, 2003). In addition, over 350sp of invertebrates were collected from *Oculina varicosa* bioherms off the Florida continental shelf (Reed, 1992; Koenig et al., 2000).

Of more economic importance to man, many commercial species of fish have been found using these reefs for feeding, protection and reproduction (Koslow & Gowlett-Jones, 1998; Rogers, 1999; Mortensen, 2000). Of particular importance are Orange Roughy, Roundnose Grenadier (Rogers, 1999; Mortensen, 2000), Gag and Scamp grouper (Brooke & Young, 2003) whist many other fisheries species have also been observed using these reefs (Koenig et al., 2000; Reed, 2000).

As traditional pelagic and shallow-water fisheries decline, more fishermen are using deep-water benthic trawls to maintain catches. Large deep-water demersal trawlers now operate in areas of high fish densities, which are often related to coral structures (Wheeler et al., in Press). Deep-water reefs were avoided in the past by fishermen because of the damage they caused to expensive nets. However, with developments in equipment, such as rockhoppers and stronger nets, more fishermen

are realising the potential of these areas as lucrative fisheries (Koslow et al., 2000; Hall-Spencer at al., 2003). The damage to deep-water coral ecosystems from trawls has been recently observed in many areas around the globe (Probert et al., 1997; Koslow & Gowlett-Jones, 1998; Bett, 2001; Brooke, 2002; Hall-Spencer et al., 2003; Wheeler et al., in Press). The Orange Roughey fishery is a classic example. This fish species is found in close association with Tasmanian seamount coral communities, and exploitation has already caused the mass destruction of large reef areas (Koslow & Gowlett-Jones, 1998). Within the European margin there has also been active destruction by trawlers occurring within the Darwin Mounds (Wheeler et al., in Press). These authors report 28 trawl scars observed in a $5km^2$ are of seabed, and many damaged coral mounds.

As well as fishing pressures, deep-water reefs are also potentially under threat from oil and mineral exploration (Rogers, 1999; Bett, 2001). Such activities have the potential to cause excessive sedimentation from drilling mud, as well as the production of poisonous chemicals, although no active extraction is occurring at present (Rogers, 1999). Only recently have studies targeted the potential impacts of man's various activities on deep-water fauna (Olsgard & Gray, 1995; Probert et al., 1997; Jennings & Kaiser, 1998; Rogers, 1999; Hall-Spencer et al., 2003).

Understanding reproductive processes is essential to aiding conservation and management practices. Reproductive seasonality, gamete quality, fecundity and larval supply of habitat-forming organisms are vital to understanding both ecosystem function and recovery potential following a damaging event. This review presents a summary of the limited information on these basic biological processes in both reef-building and solitary deep-water scleractinians.

Methods

This review is a synthesis of known research on the reproductive processes of deep-water scleractinians. Information was gathered from both published literature and personal communication from colleagues, and is summarised in Table 1.

Table 1.

Sexuality

It is generally not possible to distinguish the sex of individual scleractinian polyps or colonies from external morphology. In a few shallow-water hermatypic species, eggs and sperm are different colours, and so may be distinguished in the polyps just prior to spawning (Fadlallah, 1983). After fixation and decalcification, ripe oocytes may be observed within the mesenteries if they are large in size (Brooke, 2002; Waller, 2003).

The majority of shallow-water scleractinians are hermaphroditic (Fadlallah, 1983; Richmond & Hunter, 1990). Of the deep-water scleractinians studied to date, only three species were observed to be hermaphroditic. These are solitary corals, all species of the genus *Caryophyllia* (Waller et al., in Press). All three species show a novel form of hermaphroditism, cyclical sexuality. Here the spermacysts and oocytes develop within the same mesentery, in a continuous cycle, but not synchronously between individuals. Only sperm or eggs are released at any one time, thus preventing self-fertilisation. Both sperm and eggs would therefore be present near-continually in the water column, whereas gonochorism would require ripe males and females to spawn synchronously to ensure fertilisation.

Twelve of the fifteen species of deep-water scleractinian examined to date are gonochoric (Table 1) compared to 75% of the shallow species known being

hermaphroditic (Fadallah, 1983). Gonochorism has been proposed as a more primitive adaptation than hermaphroditism (Goffredo et al., 2000), and may be more important for maintaining genetic diversity (Szmant, 1986).

Gametogenesis

Scleractinians do not have 'gonads' in the traditional metazoan sense of the term. Sperm develop in cysts held together by a mesogleal envelope, hence the term spermacysts. Oocytes develop singularly, but are often found in groups or 'pockets', though there is no direct connection between them.

In shallow water species, several authors have noted that germ cells are most likely to originate from interstitial cells near the septal filaments (lamellae) (Rinkevich & Loya, 1979; Szmant-Froelich, 1980; Delvoye, 1982; Fadlallah, 1983), but there are no data as yet for deep water species. Gametes of many deep-water species reported appear to develop in the lamellae of the mesentery, eventually migrating into the mesoglea (Waller et al., 2002,in press; Brooke, 2002; Waller 2004). Oogonia in *Lophelia pertusa* and *Madrepora oculata* have been observed still attached to the lamellae (Waller & Tyler, in Press), and even some larger vitellogenic oocytes appear to have a form of attachment (Waller, 2004).

Stages of oogenesis in deep-water species studied appear to follow those of shallow-water species (*sensu* Fadlallah, 1983). The first stage consists of oogonia attached to the lamellae. These grow into previtellogenic oocytes and migrate into the mesoglea of the mesentery. Previtellogenic oocytes then accumulate large quantities of lipid-rich yolk and form vitellogenic oocytes. The final stage is late vitellogenesis, where a thick cortical granular layer is formed on the outside of the oocyte, prior to release.

Gamete Release

There are just three records of brooding in deep-water scleractinians from the Antarctic deep continental shelf. Waller (2004) observed brooded planulae in the mesenteries of *Flabellum impensum*, *F. curvatum* and *F. thouarsii*. All other species reported, both reef building and solitary, appear to spawn their gametes into the water column for external fertilisation (Table 1). Waller (2004) however suggests that these three Antarctic species, though found at 500m, follow reproductive patterns more common amongst Antarctic fauna than deep-water. Broadcast spawning is also more prevalent than brooding in shallow-water species of scleractinian (Richmond & Hunter, 1990). Indeed, Stimpson (1978) suggested deeper-living corals should spawn gametes rather than brood, to aid wide dispersal needed to find suitable substratum.

Gametogenesis can either be quasi-continuous (Waller et al., 2002; Flint, 2003; Waller et al, in Press; Burgess & Babcock, this issue;), periodic (Waller & Tyler, in Press) or seasonally-controlled (Brooke, 2003; Waller & Tyler, in Press). The majority of shallow-water scleractinians have some form of seasonality, usually controlled by temperature or lunar periodicity (Fadlallah, 1983; Harrison & Wallace, 1990; Richmond & Hunter, 1990). Seasonality in the deep-sea environment has been debated for a number of years (see Gage & Tyler, 1990). In most areas, these deep-sea species live below the permanent thermocline, so there is little seasonal fluctuation in temperature. However, there is a seasonal fluctuation in phytodetritus to depth that has been associated with seasonal reproduction in several species of echinoderms and bivalves (Billet et al., 1983; Tyler et al., 1992, 1993; Rice & Lambshead, 1994). Waller & Tyler (in Press) also suggest the seasonal reproduction found in *Lophelia*

pertusa, and the periodic reproduction found in *Madrepora oculata* from the NE Atlantic may also be related to the phytodetrital fall.

Larval Development

There have been numerous studies concerning the larval biology and development of shallow-water scleractinians (Rinkevich & Loya, 1979; Szmant-Froelich et al., 1980; Fadlallah & Pearse, 1982a; Tranter et al., 1982). These corals have proved to be ideal candidates for larval ecology, spawning freely when cued by either heat shock (Szmant-Froelich, 1980; Fadlallah, 1983) or the addition of gametes from the opposing sex (Szmant-Froelich, 1980; Tranter et al., 1982).

Deep water scleractinians, as yet, have proved more difficult to spawn. Trials of heat and cold shock were applied to numerous colonies of *Lophelia pertusa* and *Madrepora oculata* during three cruises in the NE Atlantic (RRS *Discovery* cruise reports 248, 260 & 266) with no success.

Brooke (2002) had more success with *Oculina varicosa*, with deep-water colonies observed broadcast spawning after collection. Brooke & Young (2003) is the only detailed study to date on the larval ecology of a deep water scleractinian. *O. varicosa* produced ciliated planula larvae and patterns of embryogenesis were observed to be similar to those of shallow water species (Brooke & Young, 2003). These planulae were small (~100μm) when compared to the maximum oocyte sizes of other species studied (Table 1), and thus likely to be planktotrophic (Brooke & Young, 2003).

Settlement

Brooke (2002) found deep-water *O. varicosa* larvae to remain active for over a month, but the larvae also probed the bottom after 10-14 days, suggesting they are ready to metamorphose. The wide dispersal of many species of deep-water scleractinian would suggest the competency periods of larvae to be long (Rogers, 1999), but there are as yet no data to support this.

Brooded planulae tend to have shorter competency times, settling immediately after one or two days (Fadlallah & Pearse, 1982a; Szmant-Froelich et al., 1985). Broadcasting species tend to settle after 4-6 days (Shlesinger & Loya, 1985; Babcock & Heyward, 1986, Sakai, 1997). Some brooded planula have a longer larval life. Richmond (1997) estimated larval competency to be over 100 days in *Pocillopora damicornis*. It is possible that this extended period is likely to be due to the zoozanthellae found in this brooded planulae providing extra energy reserves (Richmond, 1997). Deep-water species are, however without zooxanthellae, therefore the larval planktonic phase may be shorter, there is however, as yet no data.

It is thought that the larvae of deep-water scleractinians require a hard substratum, such as a rock outcrop, or shell or worm tube in sandy areas, for settlement to occur (Wilson, 1979a; Rogers, 1999). Many colonies of *Lophelia pertusa, Desmophyllum cristagalli, Enallopsammia rostrata* have been observed attached to rocky outcrops or dead gorgonian and scleractinian skeletons in the Northern Atlantic (Adkins & Scheirer, 2003).

Discussion

In shallow waters the pattern of reproduction is highly variable both between and within species (Fadlallah, 1983; Richmond & Hunter, 1990).These interspecific differences are thought to be due to environmental adaptations, and so are also likely

to occur within deep-water species. Within the genus *Caryophyllia*, *C. clavus* and *C. smithii* are gonochoric species (Tranter et al, 1982; Fadlallah, 1983), however three species of deep water *Caryophyllia* have all been shown to be hermaphroditic (Waller et al., in Press). Of the five species of *Flabellum* studied, three brood their young, and two broadcast (Waller, 2004).

In the western Atlantic, Brooke (2002) found shallow-water colonies of *O. varicosa* to have the same seasonal pattern of reproduction as the deeper-living colonies suggesting little environmental control in this case. Flint (2003) observed *Fungiacyathus marenzelleri* from 4000m in the NE Pacific to have the same seasonal cycle as that. described by Waller et al. (2002) at 2000m in the NE Atlantic. However, fecundity was significantly different, with the deeper individuals producing fewer oocytes, possibly a result of reduced food availability at these greater depths.

With there is evidence for both phylogenetically and environmentally constrained reproduction, reproductive studies in definitive areas are required to allow an adequate assessment of conservation potential. Understanding reproductive processes of a species in a single area, though providing a baseline, cannot be guaranteed to be the pattern observed in other areas.

The main aim of this review is to demonstrate the paucity of biological information that is available on deep-sea corals. Reproduction is one of the most fundamental biological processes and an understanding of it must be attained to complete any conservation or management effort. The intense trawling damage now observed around globally (Probert et al., 1997; Koslow & Gowlett-Jones, 1998; Bett, 2001; Brooke, 2002; Hall-Spencer et al., 2003; Wheeler et al., in Press) has highlighted the need to attain ecological and biological data quickly, before these

areas are damaged beyond repair. Without this information we would have no insight as to this habitats ability, and speed, of re-colonisation after a damaging event.

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Species	Area	Depth	Sex	Reproduction						Notes	Ref
				Max Oocyte	Fecundity	Method	Production	Release	Larvae		
Fungiacyathus marenzelleri (Vaughan, 1906)	Rockall Trough	2000m	Gonochoric	750µm	2900 opp	Spawner	Continuous		(I) Lecithotrophic		1
F. marenzelleri	California	4100m	Gonochoric	750µm	1290 oop	Spawner	Continuous		(I) Lecithotrophic		2
Lophelia pertusa (Linnaeus, 1758)	Porcupine Seabight	900m	Gonochoric	140 µm	3146 opp / 3327 ocm2	(I) Spawner	June/Aug	Jan/Feb	(I) Lecithotrophic		3
L.pertusa	Trondheim Fjord	147m	Gonochoric	60 µm (mean)						Nov. only	4
Madrepora oculata Linnaeus 1758	Porcupine Seabight	900m	Gonochoric	350 µm	10 opp / 256 ocm2	Spawner	Periodic		(I) Lecithotrophic	Female Only	3
M. oculata	Chatham Rise	800-1000m	Gonochoric							Male Only	8
Caryophyllia cornuformis Pourtales 1868	Porcupine Seabight	435-2000m	Hermaphrodite (cyclical)	340 µm	-	Spawner	Continuous		(I) Lecithotrophic		5
Caryophyllia ambrosia Alcock 1898	Porcupine Seabight	1100-3000m	Hermaphrodite (cyclical)	630 µm	2900 oop	Spawner	Continuous (sequential)		(I) Lecithotrophic		5
Caryophyllia seguenzae Duncan 1873	Porcupine Seabight	960-1900m	Hermaphrodite (cyclical)	450 µm	940 oop	Spawner	Continuous (sequential)		Planktotrophic		5
Oculina varicosa Lesueur 1821	Florida	3-100m	Gonochoric	100 µm	2115-4693 ocm2	Spawner	early summer	late summer/fall	Lecithotrophic?		6
Flabellum alabastrum Moseley, 1876	Porcupine Seabight	1800-2250m	Gonochoric	1010 µm	~550 oop	Spawner	Continuous		(I) Lecithotrophic		7
Flabellum angulare Moseley, 1876	Porcupine Seabight	1647-2875m	Gonochoric	814 µm	~2800 oop	Spawner	Continuous		(I) Lecithotrophic		7
Enallopsammia rostrata (De Pourtales, 1878)	Chatham Rise	800-1000m	Gonochoric	400 µm	144 oop	Spawner	Continuous		(I) Lecithotrophic	One sample	8
Solenosmilia variabilis Duncan, 1873	Chatham Rise	800-1000m	Gonochoric	165 µm	290 oop	Spawner	Seasonal			One sample	8
Goniocorella dumosa (Alcock, 1902)	Chatham Rise	800-1000m	Gonochoric	135 µm	480 oop	Spawner	Seasonal			One sample	8
Flabellum thouarsii	W Antarctic Peninsula	500m	Gonochoric	4800 µm	2412 oop	Broods	Seasonal?		Brooded		
Flabellum curvatum	W Antarctic Peninsula	500m	Gonochoric	5120 µm	1618 oop	Broods	Continuous?		Brooded		
Flabellum impensum	W Antarctic Peninsula	500m	Gonochoric	5167	1270 oop	Broods	?		Brooded		

Table 1. Reproductive data known for deep water scleractinian species.

Key – (I), Inferred

References – 1, Waller et al., 2002; 2, Flint, 2003; 3, Waller & Tyler, In Press; 4, Brooke, pers com; 5, Waller et al., In Press; 6, Brooke & Young, 2003; 7, Waller, 2004; 8, Burgess & Babcock, this issue

Appendix II

Samples Used in Thesis

PSB – Porcupine Seabight

Station M – Rockall Trough

A,B,C – Refer to FOODBANCS sites, see Chapter Three

BC – Box Core

AT – Agssiz Trawl

OTSB – Otter Trawl Semi-Balloon

Species	Call	No. Individuals	Date	Cruise	Area	Sample No.	Lat	Long	Depth	Equip	Notes
L. pertusa	LpA	50	22nd July 2000	D248	Darwin Mounds	13831#1	59 48.88N	07 17.99W	900m	BC	colony
	LpB	40	8th Aug 2000	D248	Therese Mound	13881#3	51 25.66N	11 46.33W	863m	BC	colony
	LpE	10	29th Sept 2002	D266	Therese Mound	15044#1	51 25.8N	11 46.4W	865m	BC	colony
	LpF	60	8th Aug 2000	D248	Therese Mound	13874#2	51 25.67N	11 46.41W	865m	BC	colony
	LpG	17	18th Oct 2002	D266	South Seabight	15063#1	49 40N	11 30W	746-921m	OTSB	colony
	LpH	60	18th Oct 2002	D266	South Seabight	15063#1	49 40N	11 30W	746-921m	OTSB	odd pieces
	LpI	15	8th March 2002	D260	Therese Mound	14299#1	51 25N	11 46W	870m	BC	colony
	LpJ	15	18th Oct 2002	D266	South Seabight	15063#1	49 40N	11 30W	746-921m	OTSB	White colony
	LpK	5	8th Aug 2000	D248	Therese Mound	13874#2	51 25.67N	11 46.41W	865m	BC	colony
	Lmorph1	3	15/6/03	/	Comorant	/	1.15N	61.24E	102m	ROV	White
	Lmorph2	3	18/6/03	/	Comorant	/	1.15N	61.24E	102m	ROV	White
	Lmorph3	3	16/6/03	/	Comorant	/	1.15N	61.24E	109m	ROV	White
	Lmorph4	3	16/6/03	/	Comorant	/	1.15N	61.24E	109m	ROV	Orange
	Lmorph5	3	23/6/03	/	Tern	/	0.92N	61.28E	153m	ROV	White
	Lmorph6	3	23/6/03	/	Tern	/	0.92N	61.28E	107m	ROV	Orange
M. oculata	MoA	152	8th Aug 2000	D248	Therese Mound	13881#2	51 25.66N	11 46.33W	863m	BC	colony
	MoB	20	18th Oct 2002	D266	Therese Mound	15063	49 40N	11 30W	746-921m	BC	colony
	MoC	20	8th Aug 2000	D248	Therese Mound	13874#2	51 25.67N	11 46.41W	865m	BC	colony
	MoD	20	8th March 2002	D260	Therese Mound	14299#2	51 25N	11 46W	870m	BC	colony

Species	Call	No. Individuals	Date	Cruise	Area	Sample No.	Lat	Long	Depth	Equip	Notes
	MoE	20	18th Oct 2002	D266	South Seabight	15063#1	49 40N	11 30W	746-921m	OTSB	colony
F. marenzelleri	FungA	20	15th Jan 1979	Chall 1A/79	Station M		57 20N	10 27W	2200m	AT	
	FungB	20	17th Feb 1991	Chall 75	Station M		57 19N	10 23W	2195m	AT	
	FungC	20	3rd March 1980	Chall 4/80	Station M		57 16N	10 17W	2225m	AT	
	FungD	20	12th April 1981	Chall 6A/81	Station M		57 22N	10 19W	2170m	AT	
	FungE	20	29th May 1980	Chall 9/80	Station M		57 18N	10 16W	2200m	AT	
	FungF	17	24th July 1983	Chall 10/83	Station M		57 07N	09 23W	1047m	AT	
	FungG	20	18th Aug 1981	Chall 12B/81	Station M		57 01N	12 02W	1862m	AT	
	FungH	18	18th Aug 1981	Chall 15A/81	Station M		57 27N	11 10W	1800m	AT	
	FungI	20	19th Nov 1981	Chall 86	Station M		57 18N	10 24W	2220m	AT	
	FungJ	16	14th Dec 1990	Chall 74	Station M		57 15N	10 21W	2240m	AT	
C. cornuformis	CcornA	9	10th March 1993	Chall 101	PSB		57 07 N	09 30 W	1650m	AT	
	CcornB	22	11th Aug 1992	Darwin 71/92	PSB		57 07 N	09 58 W	2017m	AT	
C. ambrosia	CambA	17	20th June 1985	Darwin	PSB	52213#1	51 00N	12 59W	2440m	BN1.5/3	
	CambB	12	4th Aug 1983	Chall 10/83	PSB		51 05N	11 48W	2713m		
	CambC	15	21st March 2002	D260	PSB	14322#1	49 36N	12 11W	2550m	OTSB	
	CambD	15	4th Sept 1979	Discovery	PSB	10106#1	51 00N	12 03W	2315m		

Species	Call	No. Individuals	Date	Cruise	Area	Sample No.	Lat	Long	Depth	Equip	Notes
C. seguenzae	CsegA	10	12th Feb 1998	D230	PSB		58 58N	07 57W	1278m	OTSB	
	CsegB	15	24th May 1978	Discovery	PSB	9779#1	50 30N	12 00W	1404m	BN1.3/3	
	CsegC	20	7th Nov 1980	Darwin	PSB	50903#1	51 04N	11 50W	1250m	OTSB	
	Cseg Pop1	52	26th March 1982	Darwin	PSB	51403#1	50 14N	09 50W	1370m	OTSB	
	Cseg Pop2	100	8th July 1979	Challenger	PSB	50611#1	51 20N	11 00W	1415m	OTSB	
	Cseg Pop3	100	19th October 2002	D266	PSB	13066#1	56 02N	09 05W	1240m	BN1.3/3	
F. angulare	FangA	15	1st Oct 2002	D266	PSB	15052#1	50 04N	12 45W	2443-2452m	OTSB	
	FangB	10	11th March 2002	D260	PSB	14032#1	49 57N	12 42W	2412m	OTSB	
	FangC	10	21st Sept 1999	D249	PSB	13910#1	50 05N	12 42W	2454-2467m	OTSB	
F. alabastrum	FlabA	15	21st Feb 1991	Chall 75/91	Station M		56 56 N	09 50 W	1908m	AT	
	FlabB	9	10th March 1993	Chall 101	Station M	450	57 07 N	09 30 W	1650m	AT	
	FlabC	10	31st July 1983	Chall 10/83	Station M		57 56N	12 21W	1705m	AT	
	FlabD	10	17th August 1981	Chall 12B/81	Station M		56 00N	13 58W	2190m	AT	
	FlabE	10	21st Nov 1991	Chall 86/91	Station M		56 34 N	09 31 W	1370m	AT	
F. thouarsii	FlatA	10	5th Nov 2000	FBIV	KGI	CRS 717	61 49 S	58 48 W	260m	OTSB	
	FlatB	3	16th March 2000	FBII	A	CRS551	65 10S	64 47W	550m	OTSB	

Species	Call	No. Individuals	Date	Cruise	Area	Sample No.	Lat	Long	Depth	Equip	Notes
	FlatC	2	10th March 2000	FBII	C	CRS 490	64 11S	65 24W	566m	OTSB	
	FlatD	2	13th June 2000	FBIII	A	CRS 598	65 10S	64 47W	550m	OTSB	
	FlatE	1	11th June 2000	FBIII	C	CRS 582	64 11S	65 29W	550m	OTSB	
F. curvatum	FlacA	1	5th Dec 1999	FBI	A	CRS 466	65 08.52S	64 44.03W	410-600m	OTSB	
	FlacB	3	16th March 2000	FBII	A	CRS551	65 10S	64 47W	550m	OTSB	
	FlacC	2	11th June 2000	FBIII	C	CRS 582	64 11S	65 29W	550m	OTSB	
	FlacD	4	27th Feb 2001	FBV	A	CRS 734	65 10S	64 47W	550m	OTSB	
	FlacE	2	2nd March 2000	FBV	B	CRS 754	64 50.64S	65 21.98W	602-700m	OTSB	
F. impensum	FlipA	16	5th Nov 2000	FBIV	KGI	CRS 717	61 49 S	58 48 W	260m	OTSB	

Appendix III

IIIa NE Atlantic Reef Building

Lophelia pertusa

Madrepora oculata

IIIb NE Atlantic Solitary

Fungiacyathus marenzelleri

Caryophyllia ambrosia

Caryophyllia seguenzae

Caryophyllia cornuformis

Flabellum alabastrum

Flabellum angulare

IIIc Antarctic Scleractinians

Flabellum curvatum

Flabellum thouarsii

Flabellum impensum

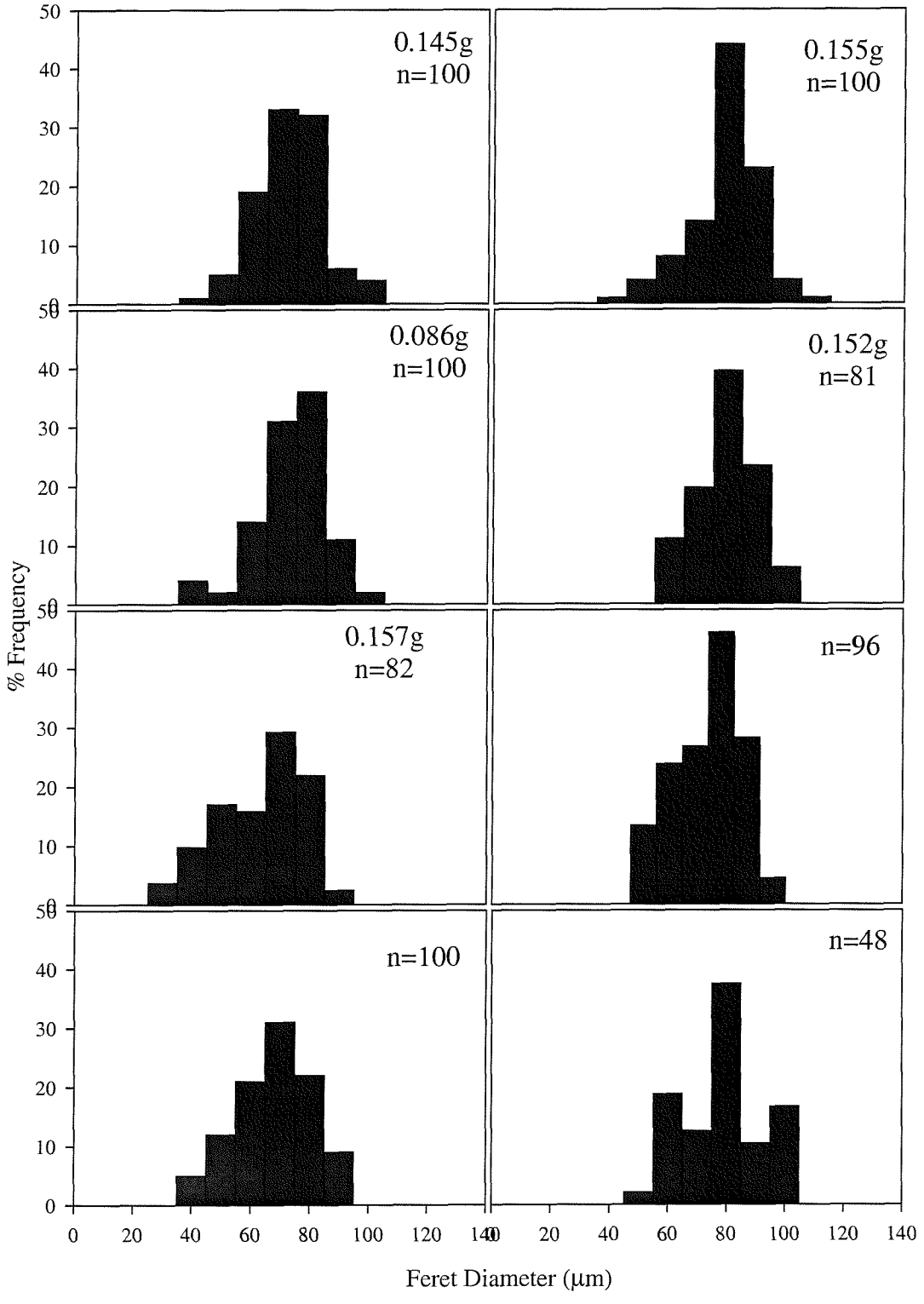
g = Polyp decalcified wet weight

mm / cm = polyp decalcified diameter

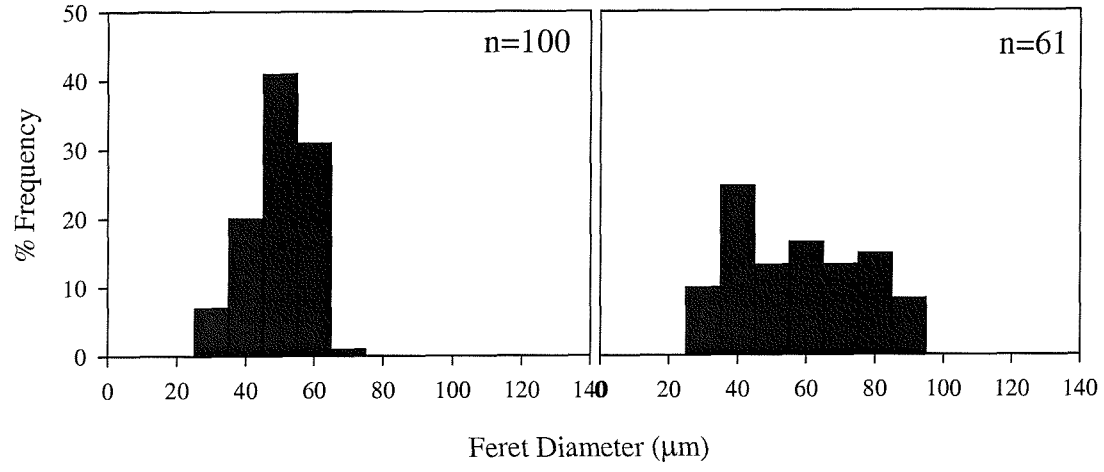
n= Number of oocytes measured

IIIa

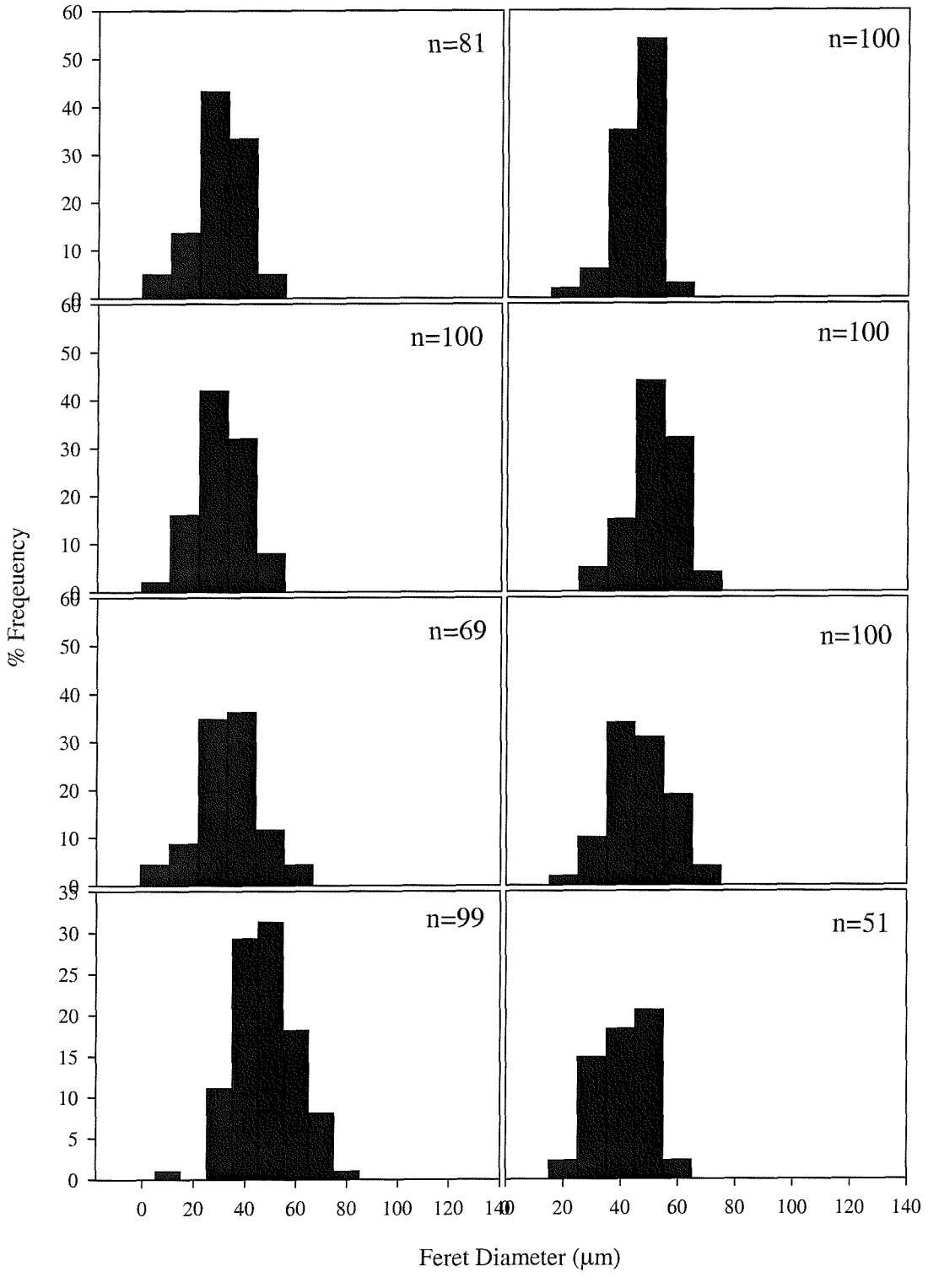
NE Atlantic Reef Building



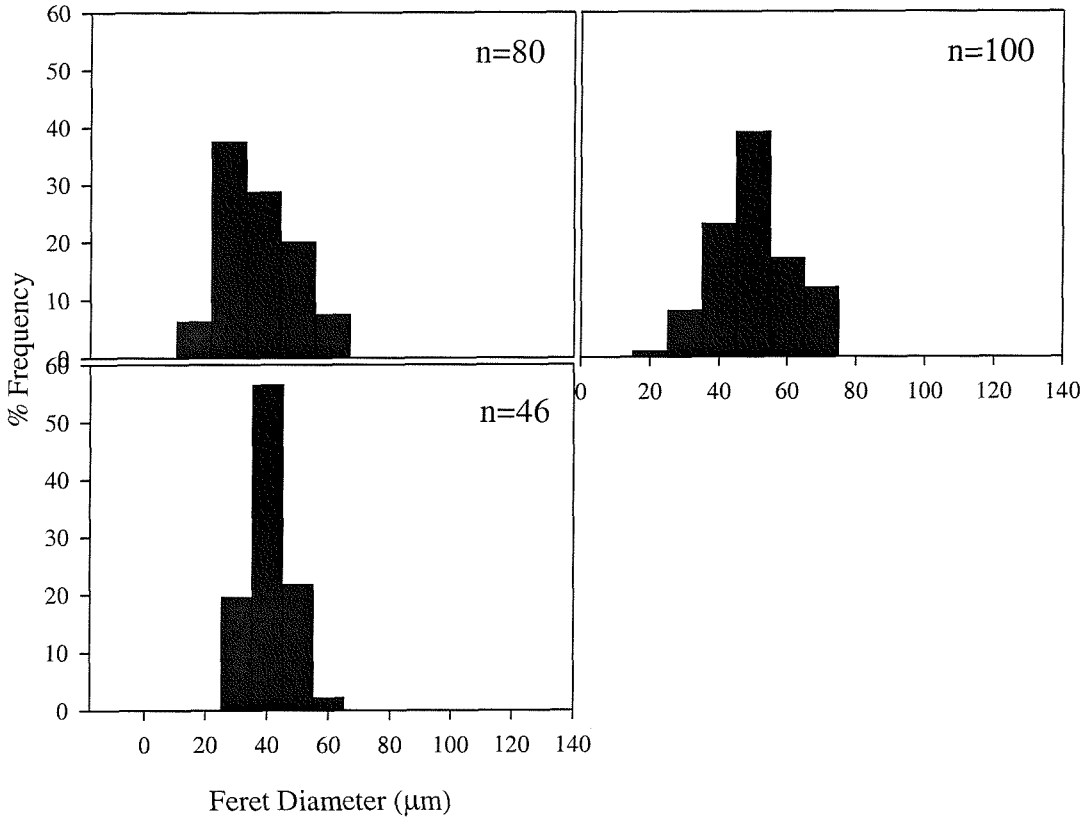
NE Atlantic – *L. pertusa* – E (1 of 2) (29th September 2002)



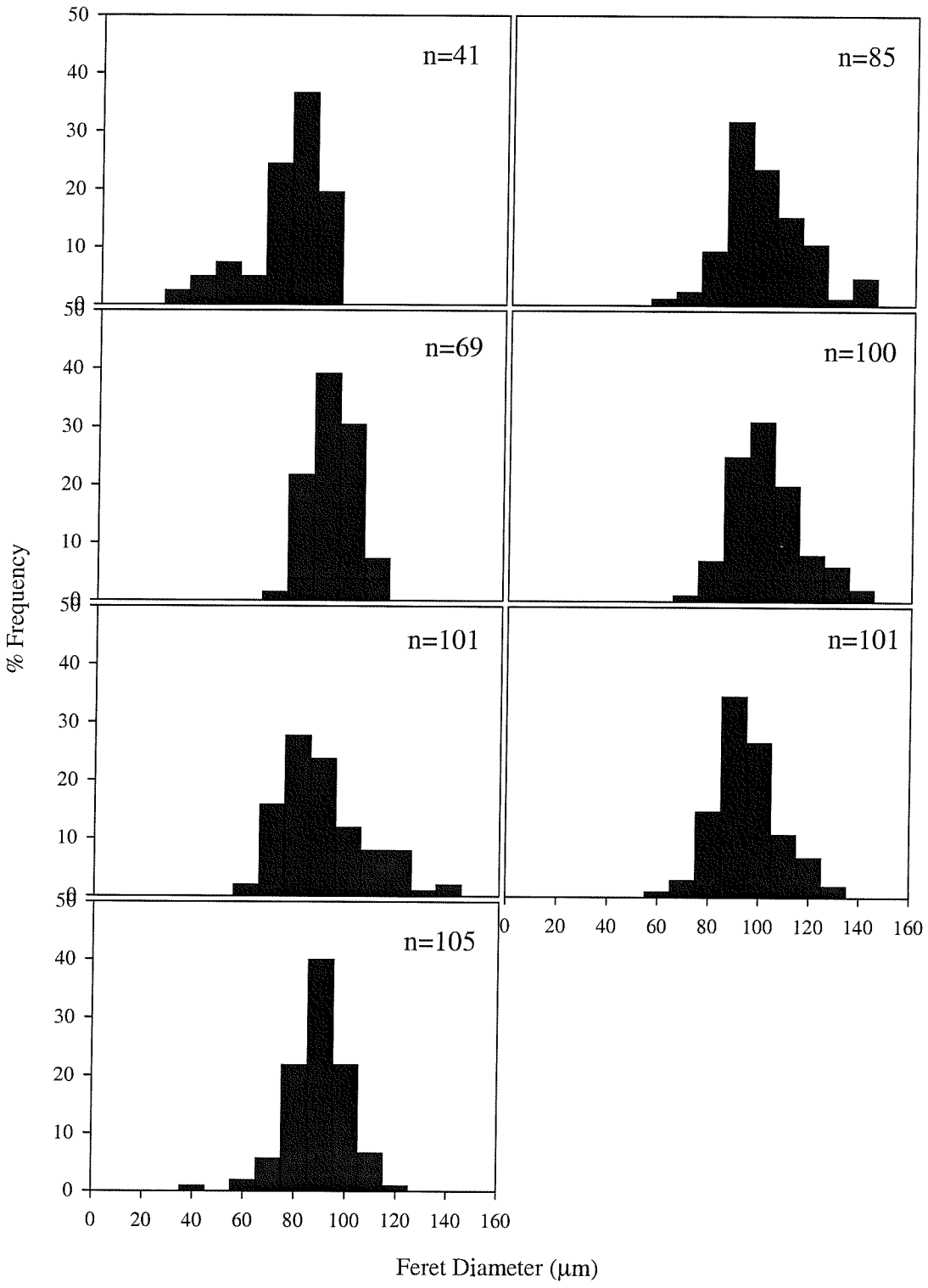
NE Atlantic – *L. pertusa* – E (2 of 2) (29th September 2002)



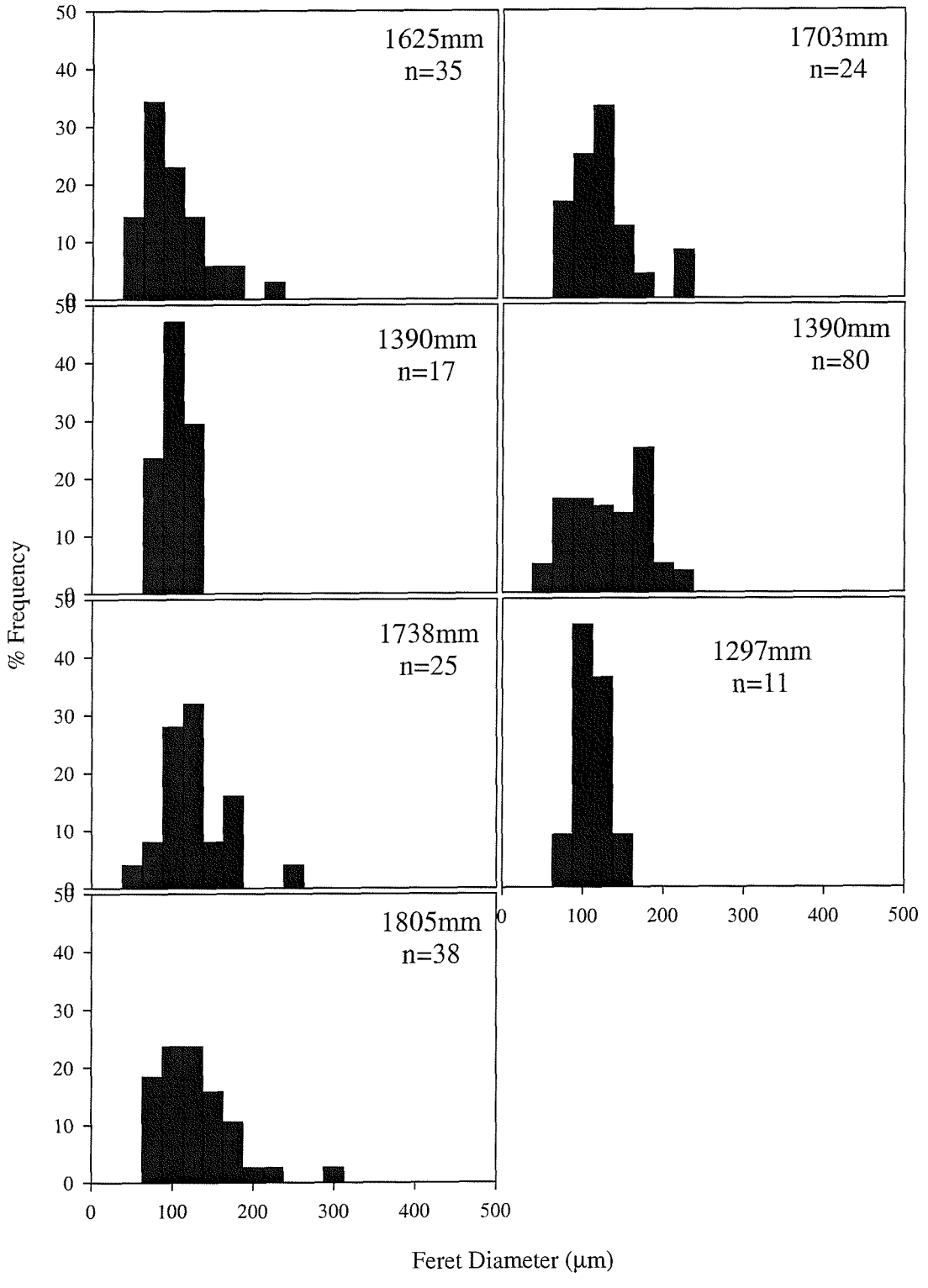
NE Atlantic – *L. pertusa* –F(1 of 2) (8th August 2000)



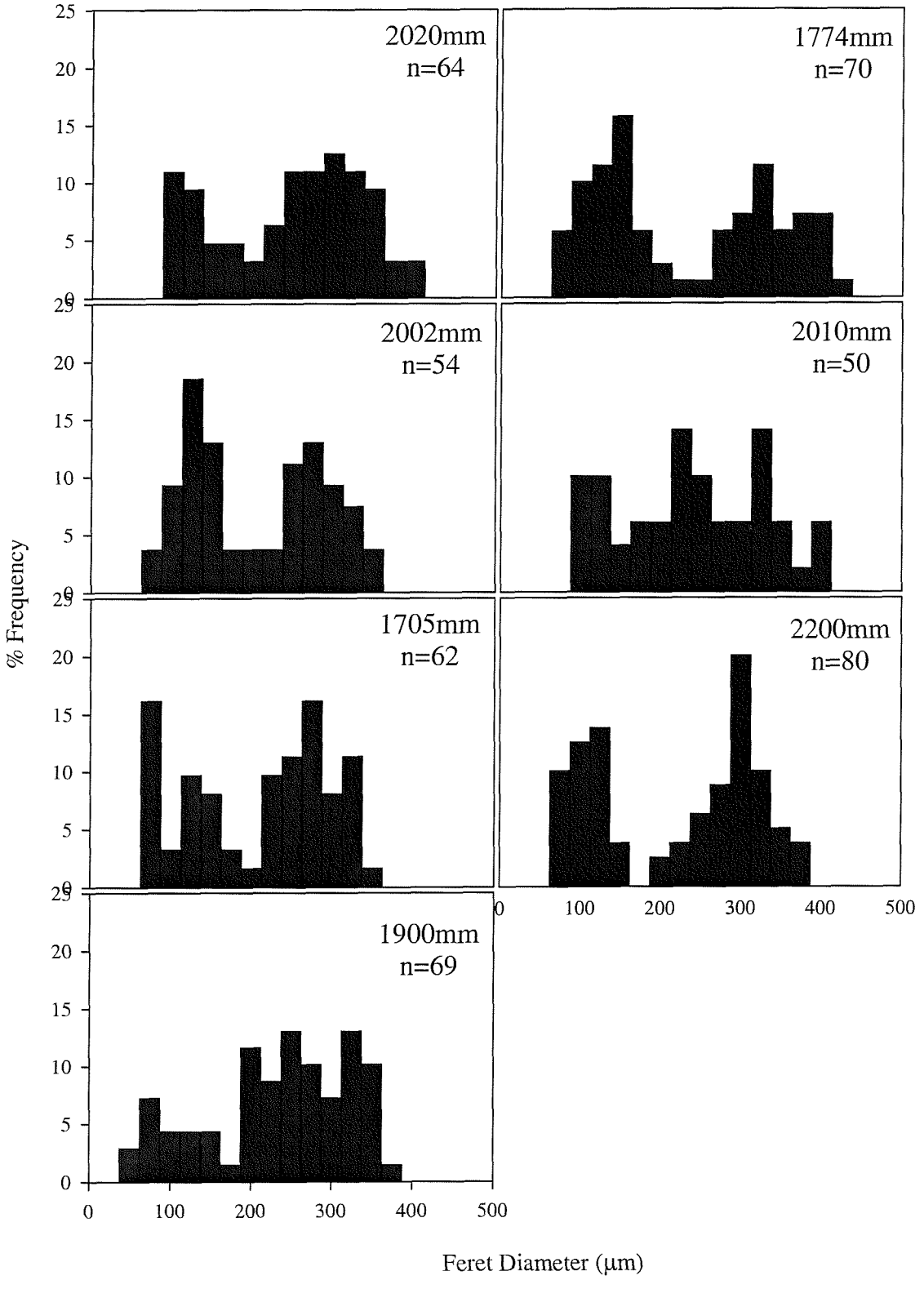
NE Atlantic – *L. pertusa* –F (2 of 2) (8th August 2000)



NE Atlantic – *L. pertusa* –G (12th October 2002)



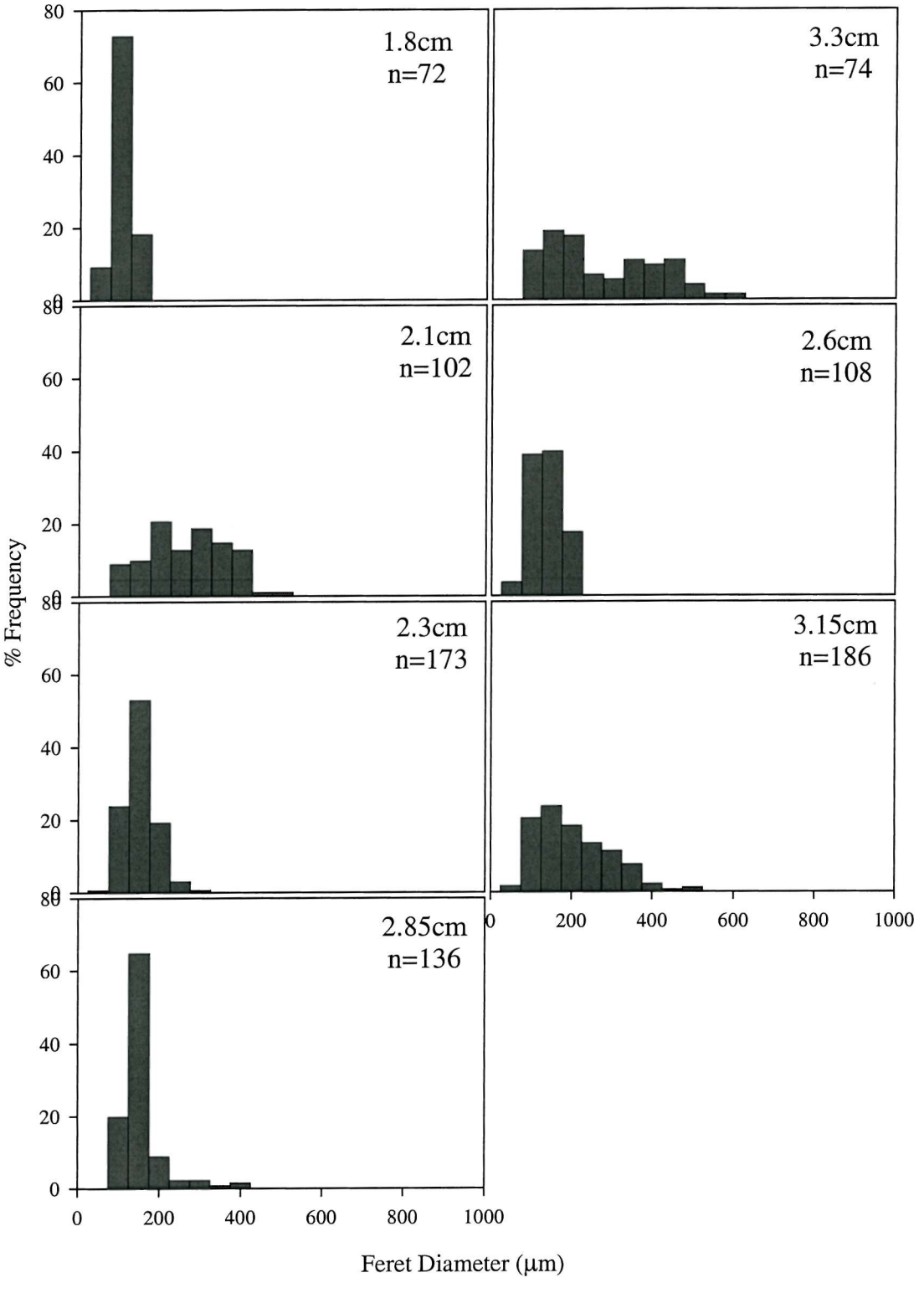
NE Atlantic – *M. oculata* – A (8th August 2000)



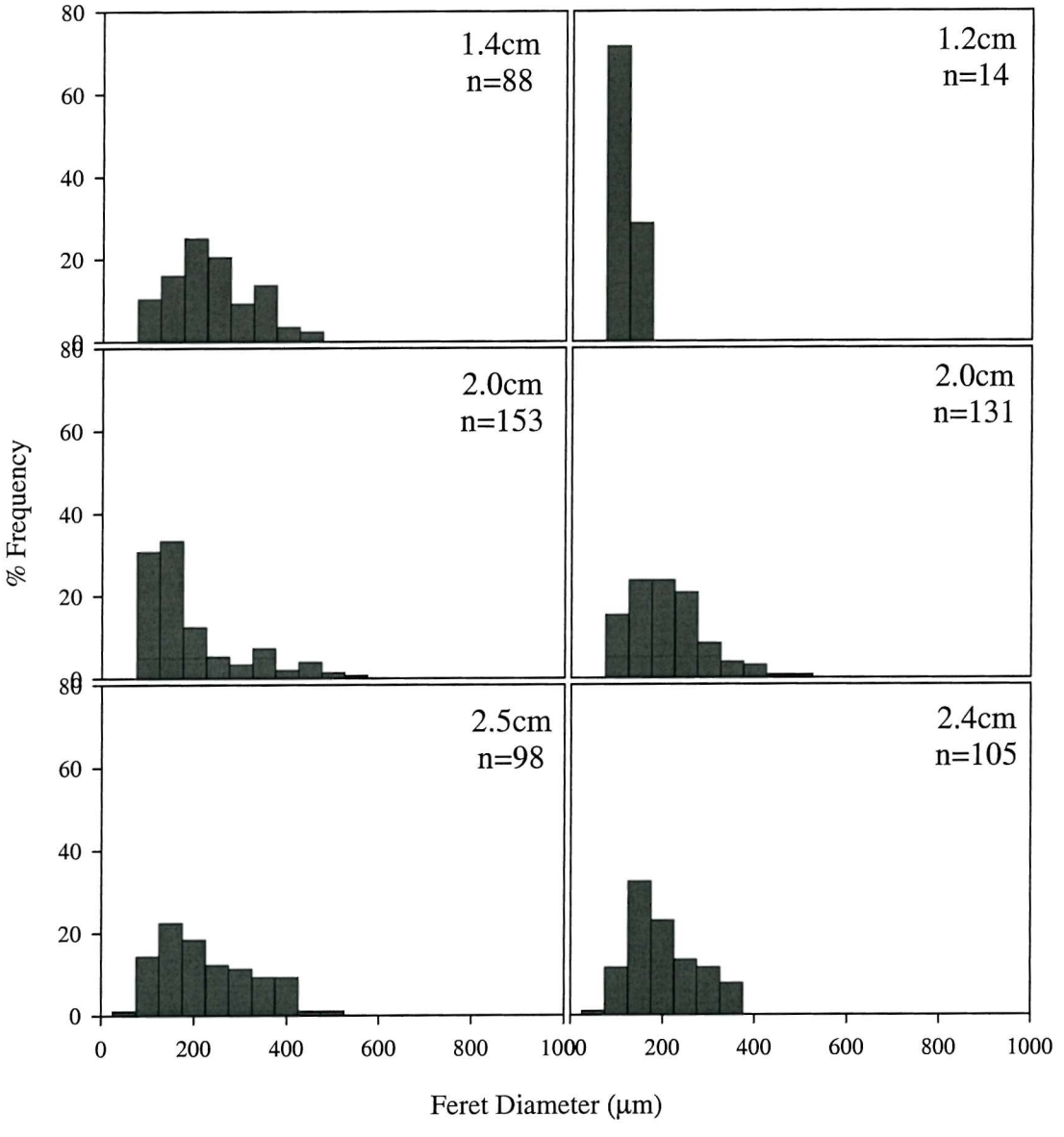
NE Atlantic – *M. oculata* – E (18th October 2002)

IIIb

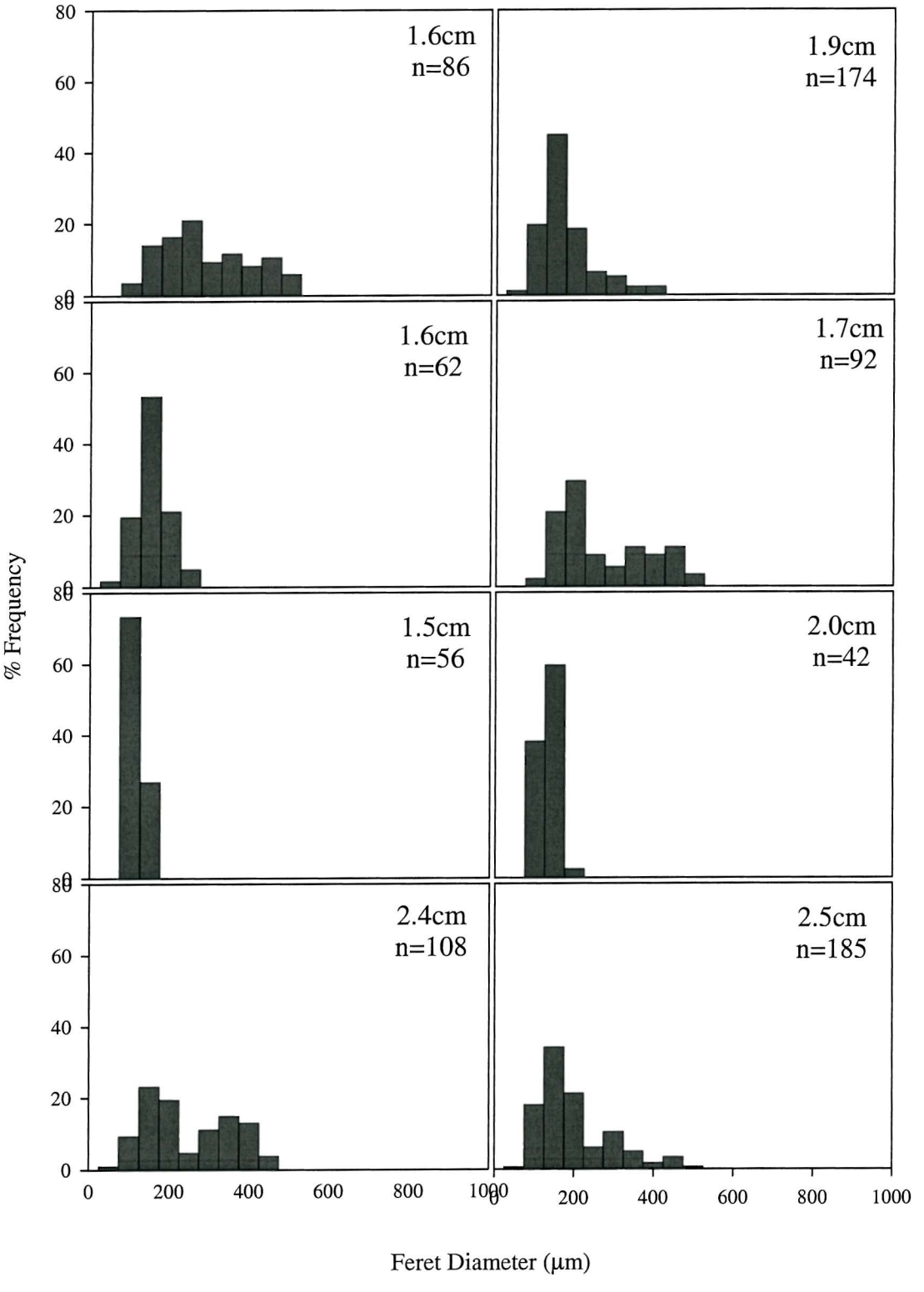
NE Atlantic Solitary



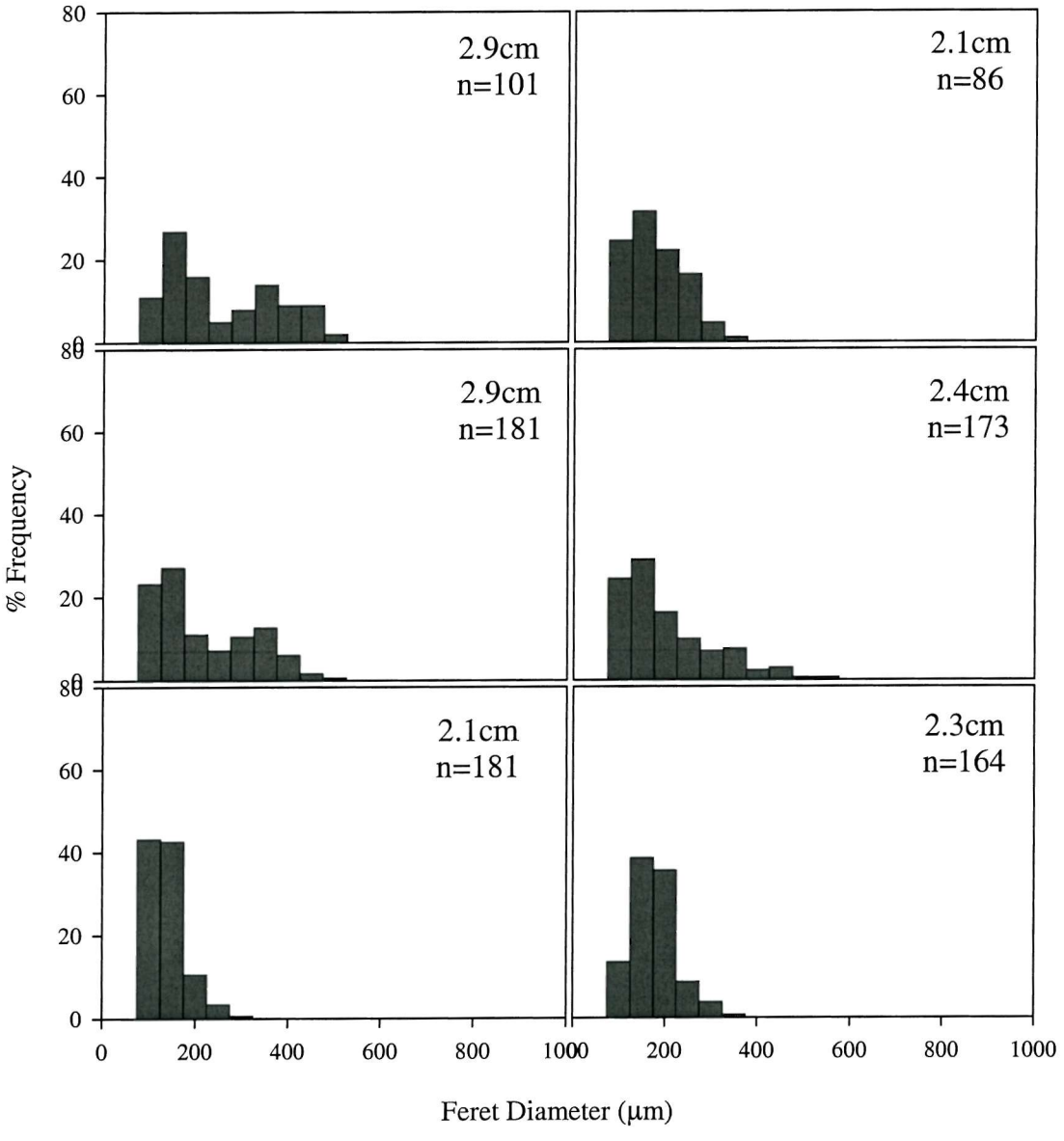
NE Atlantic – *F. marenzelleri* – A (15th January 1979)



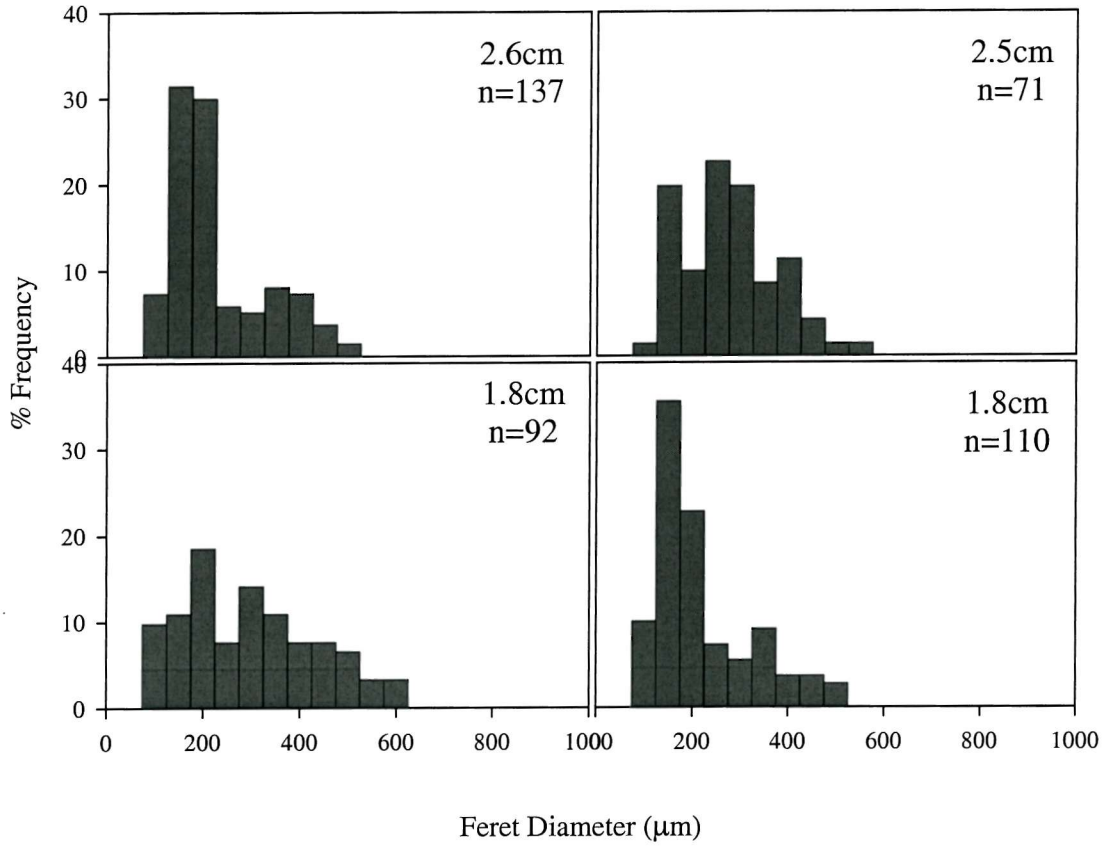
NE Atlantic – *F. marenzelleri* – B (17th February 1991)



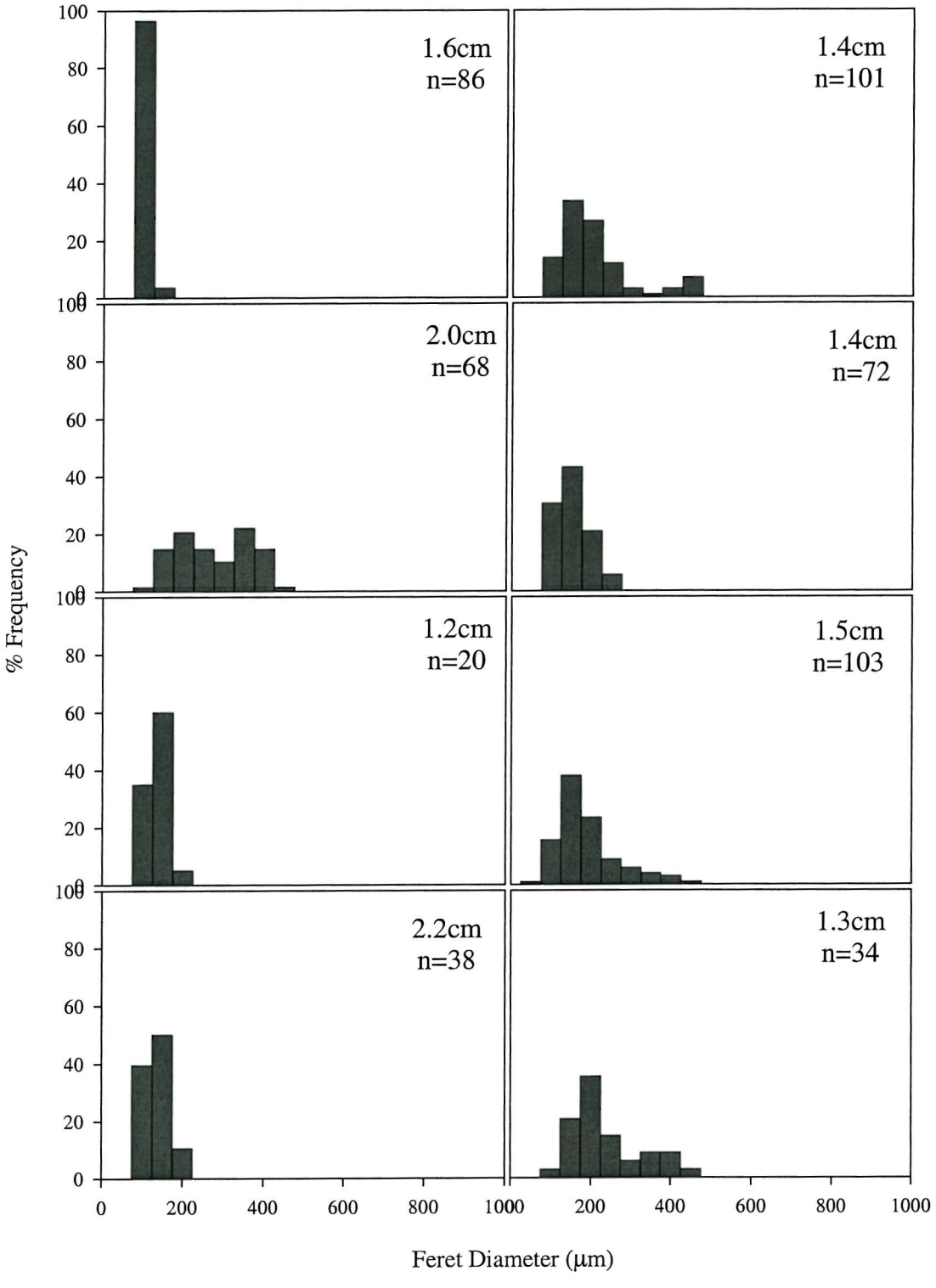
NE Atlantic – *F. marenzelleri* – C (1 of 2) (3rd March 1980)



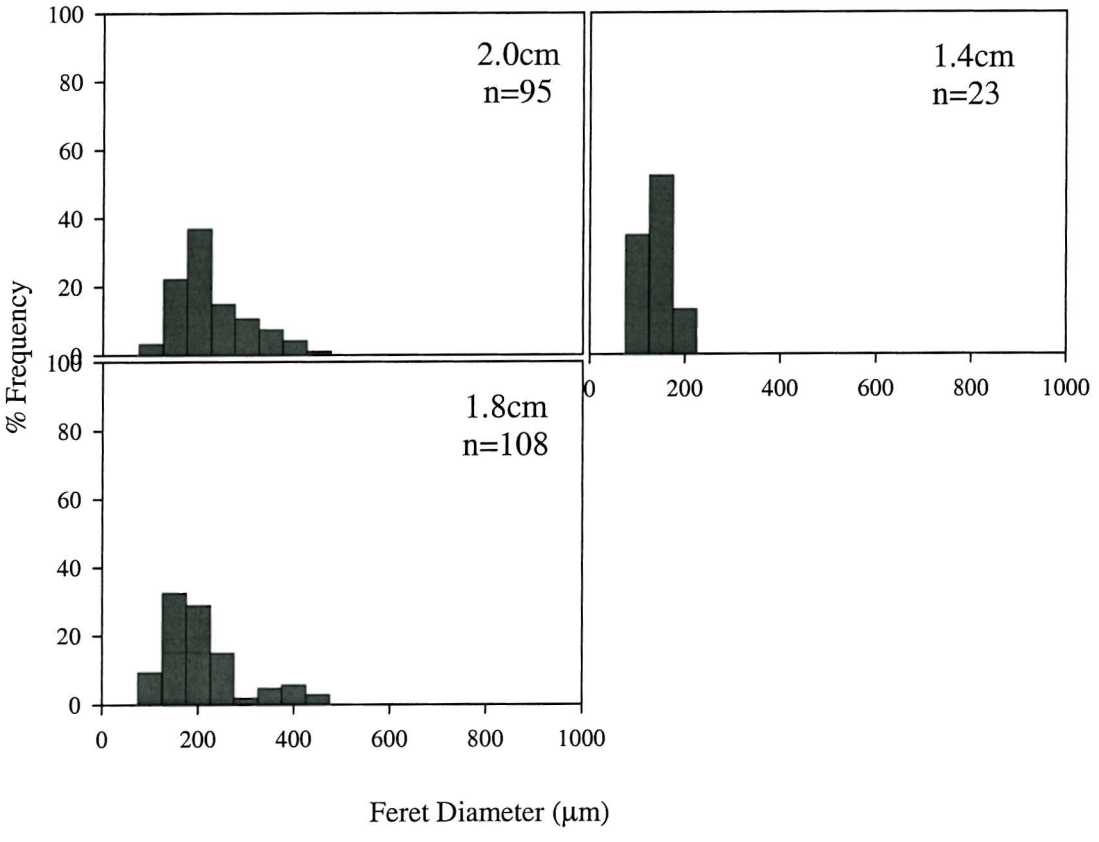
NE Atlantic – *F. marenzelleri* – C (2 of 2) (3rd March 1980)



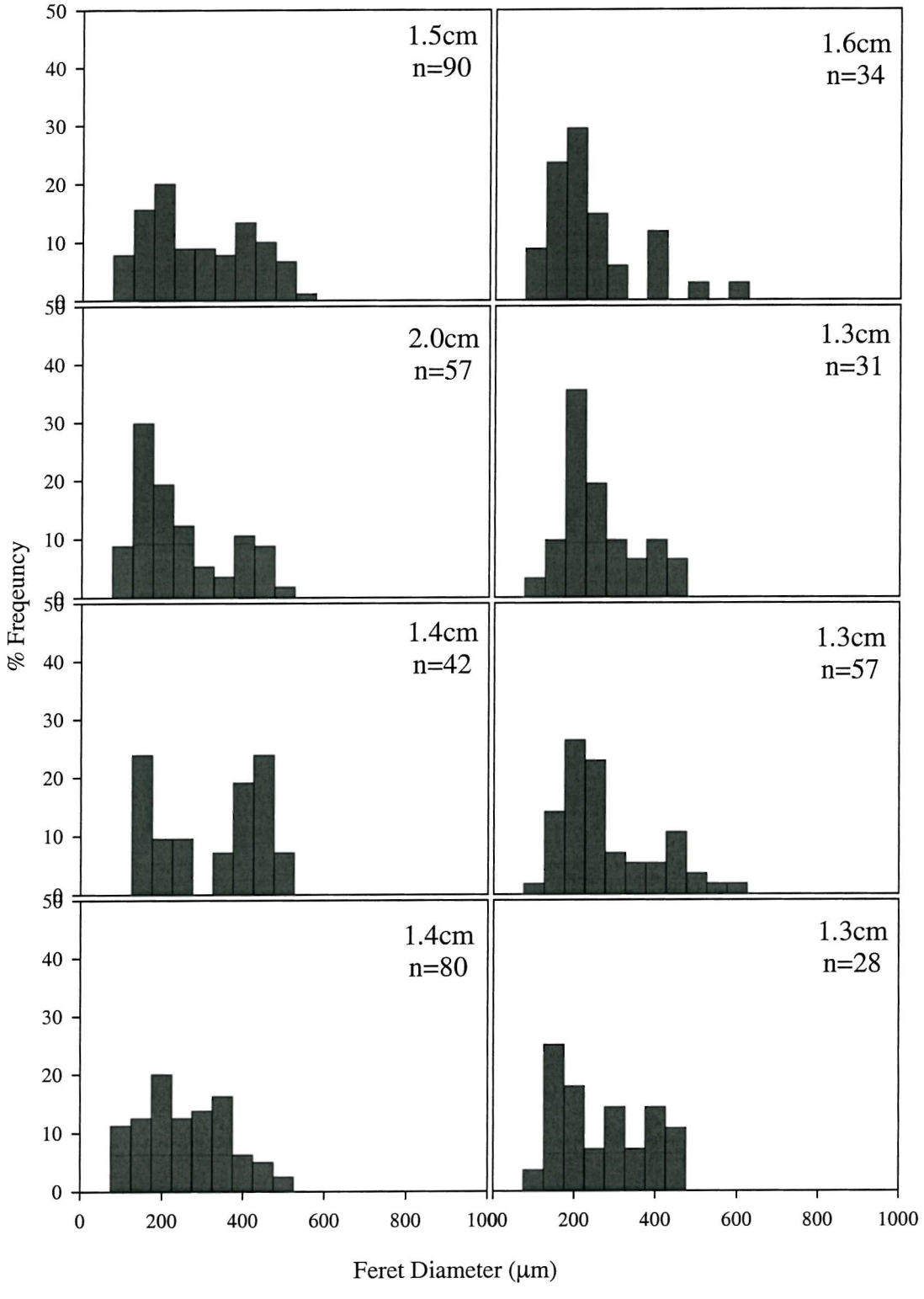
NE Atlantic – *F. marenzelleri* – D (12th April 1980)



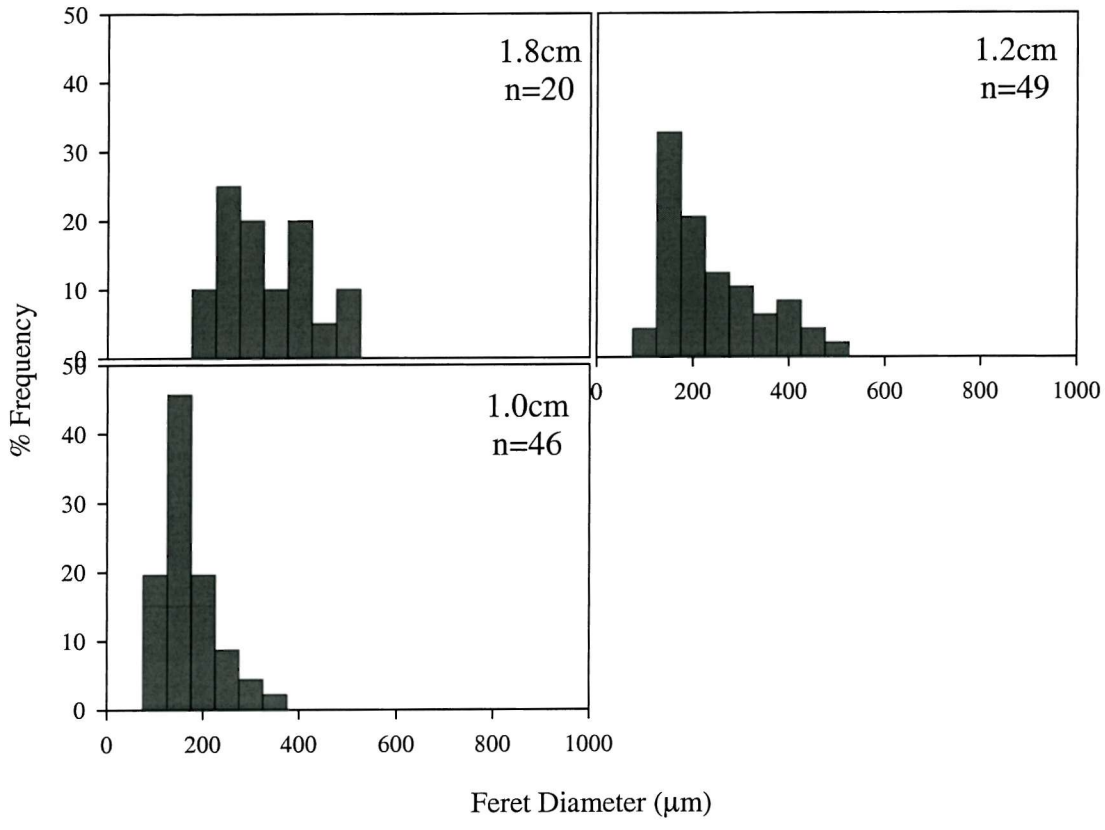
NE Atlantic – *F. marenzelleri* – E (1 of 2) (29th May 1980)



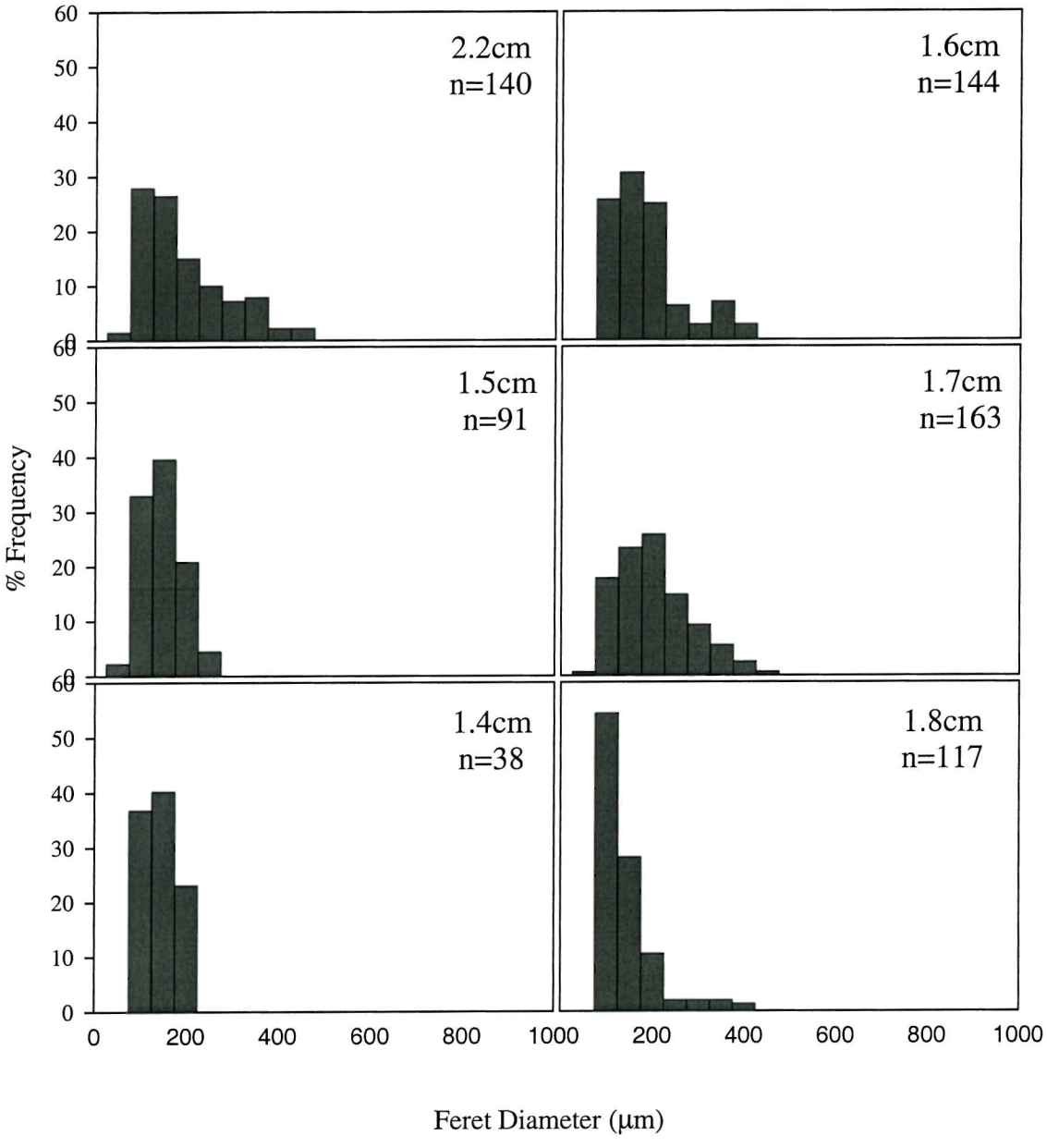
NE Atlantic – *F. marenzelleri* – E (2 of 2) (29th May 1980)



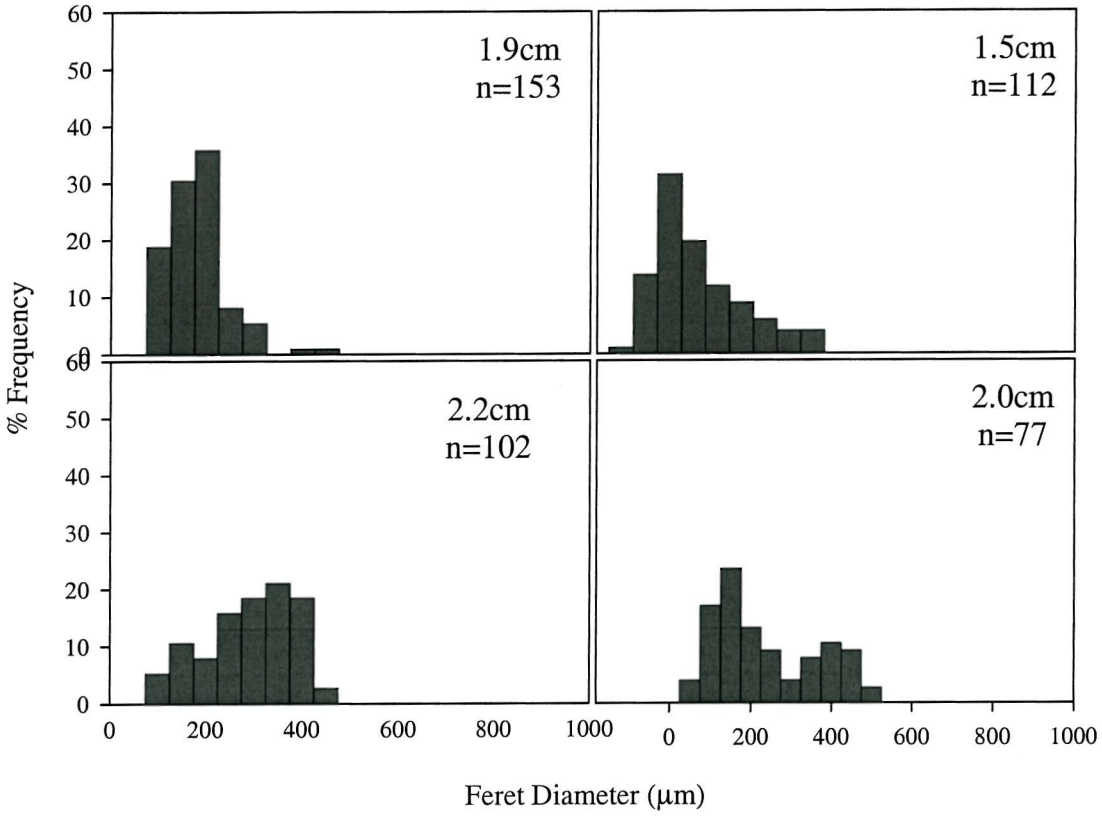
NE Atlantic – *F. marenzelleri* – G (1 of 2) (18th August 1981)



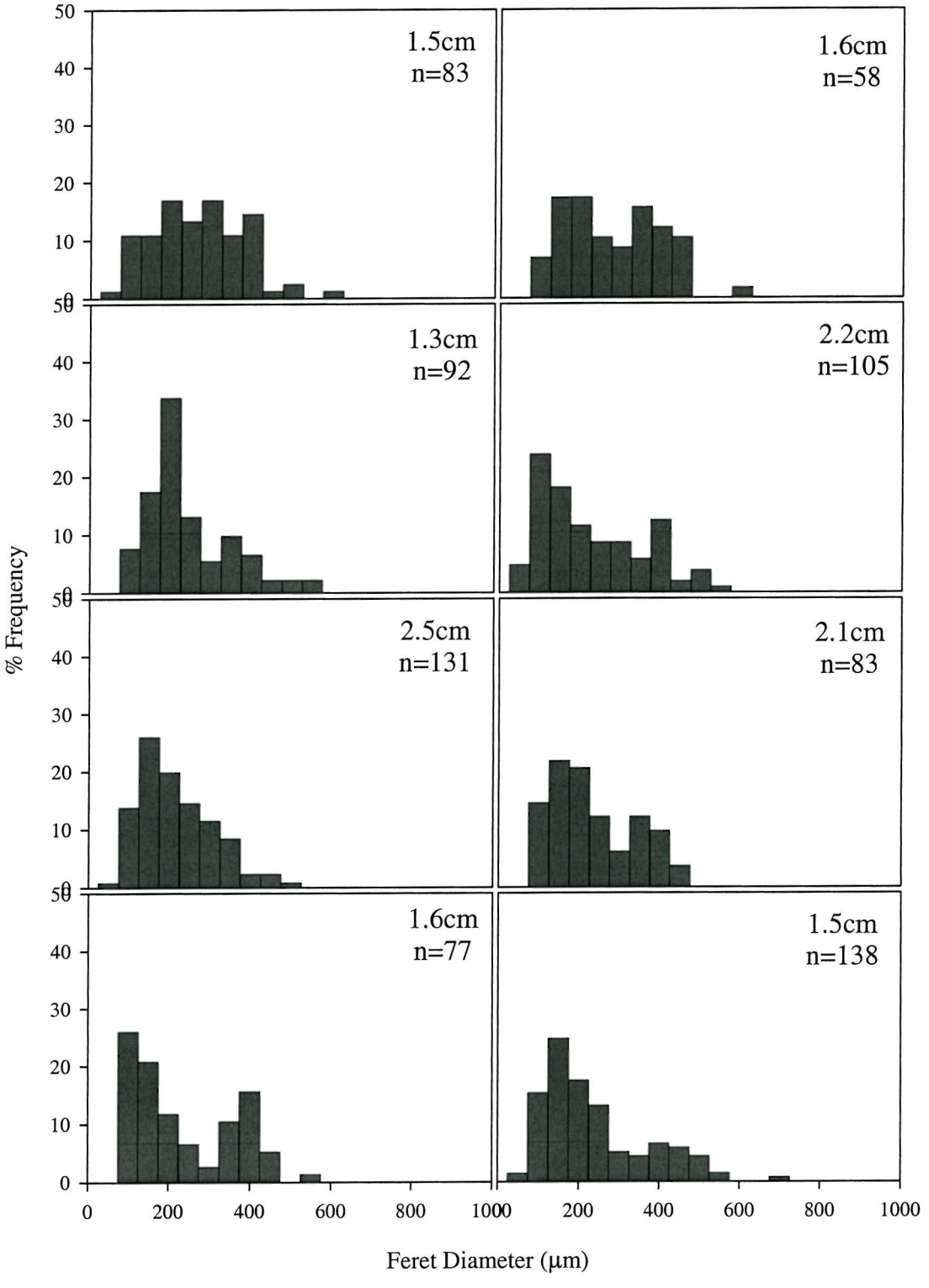
NE Atlantic – *F. marenzelleri* – G (2 of 2) (18th August 1981)



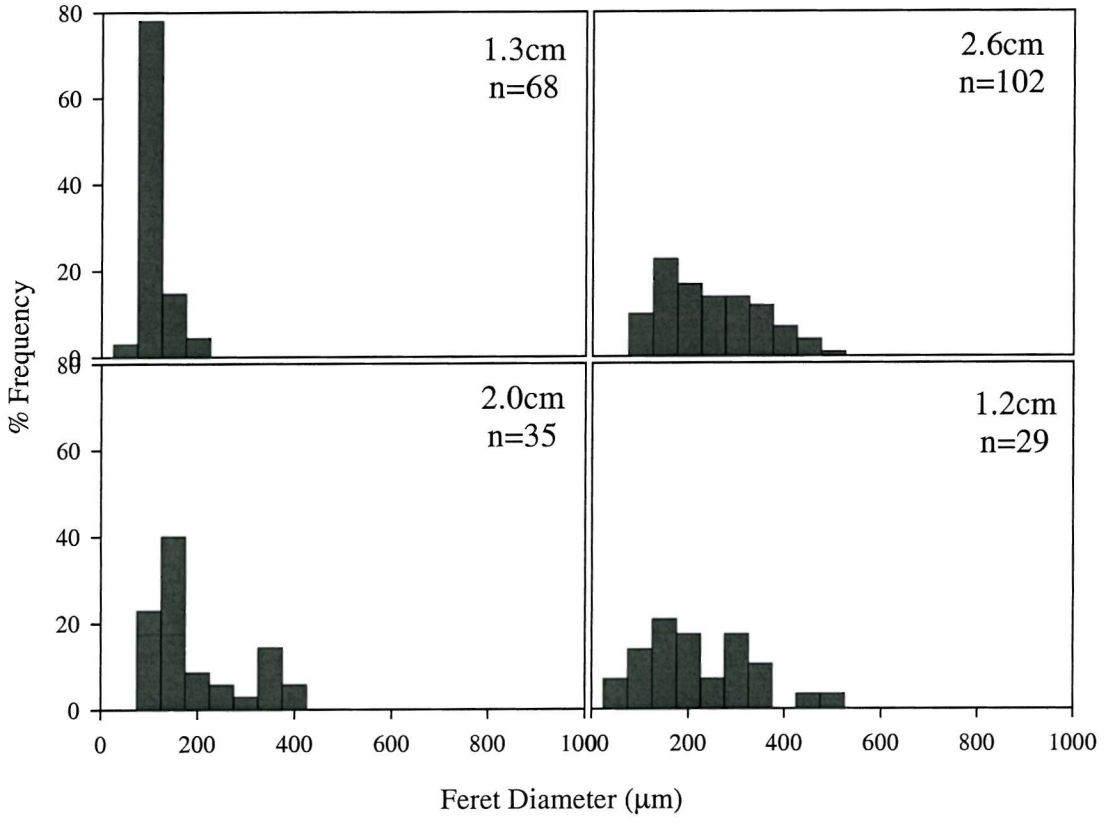
NE Atlantic – *F. marenzelleri* – H (1 of 2) (19th October 1981)



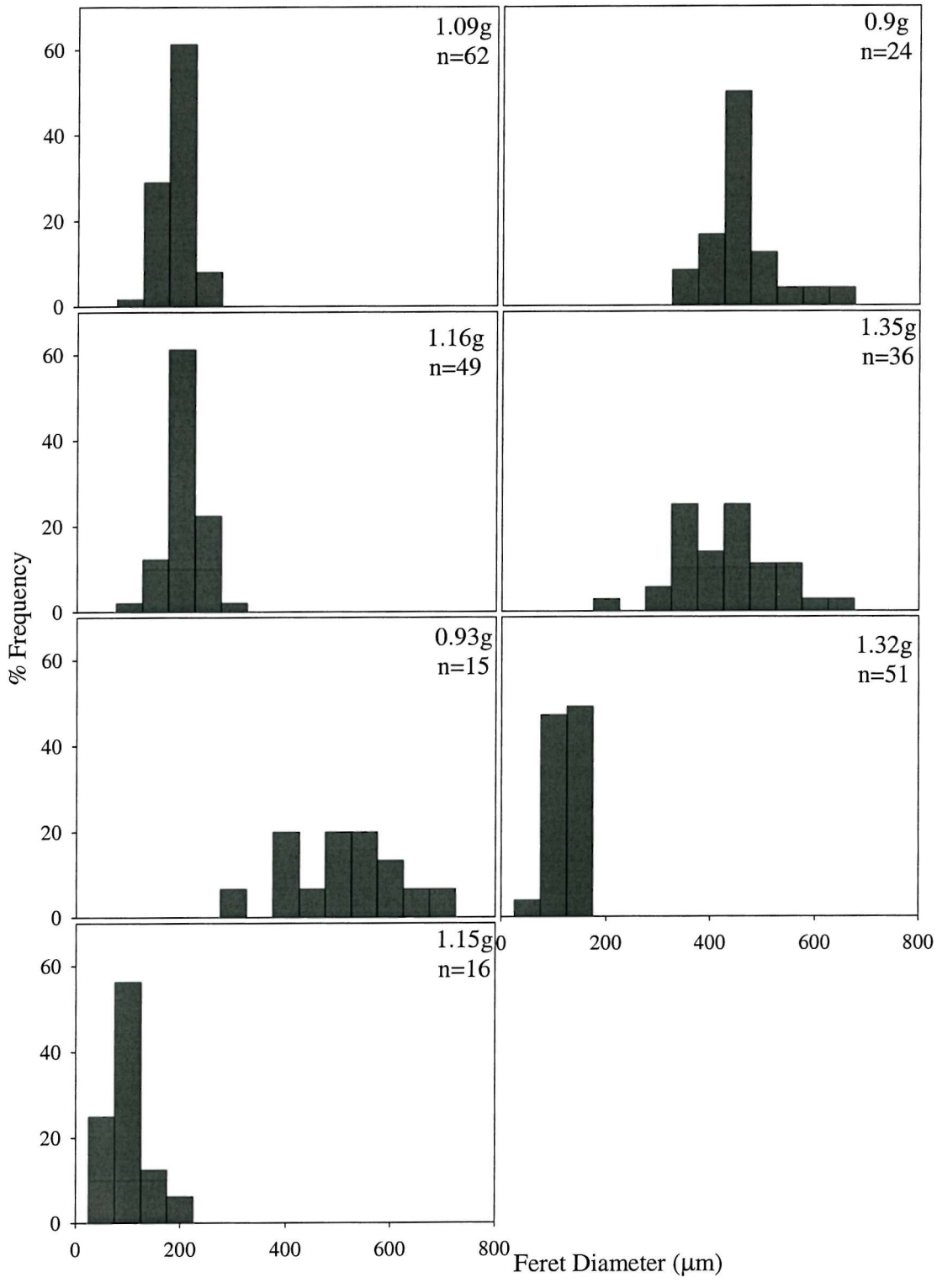
NE Atlantic – *F. marenzelleri* – H (2 of 2) (19th October 1981)



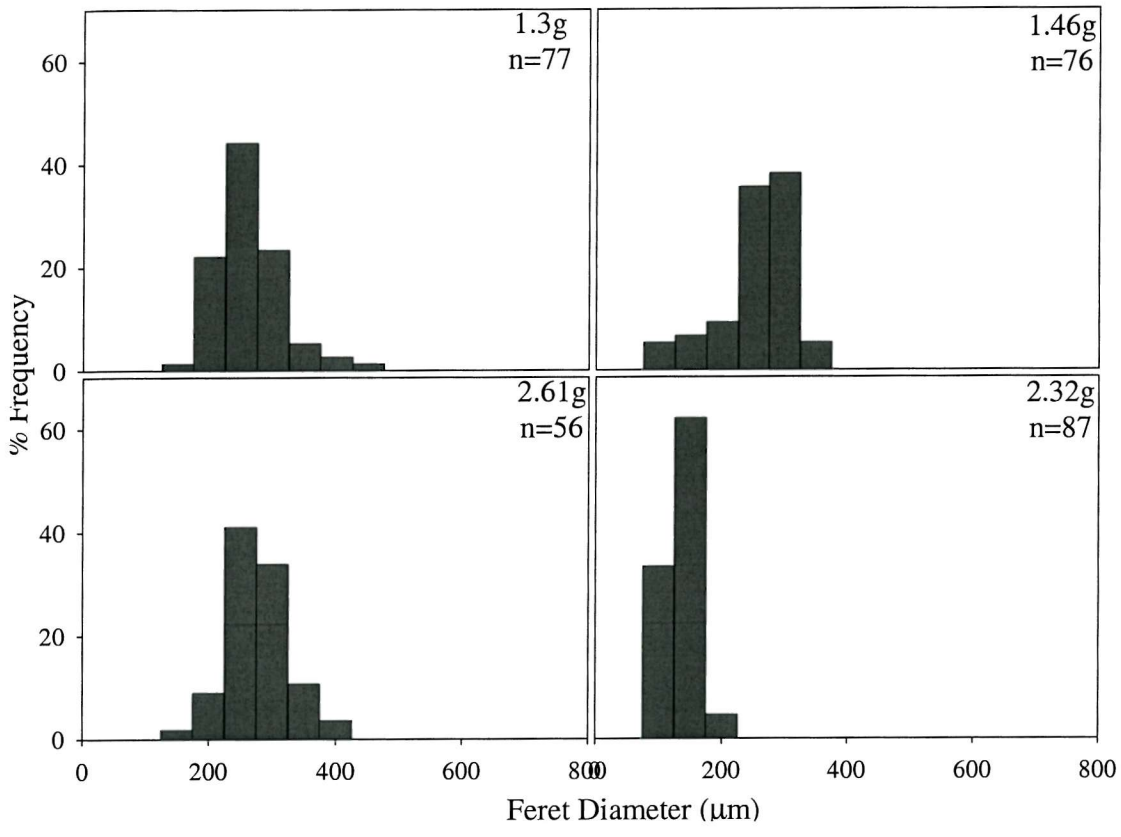
NE Atlantic – *F. marenzelleri* – I (19th November 1991)



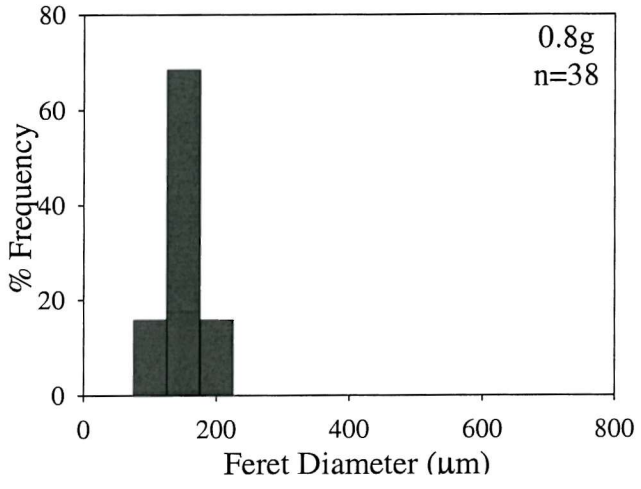
NE Atlantic – *F. marenzelleri* – J (14th December 1990)



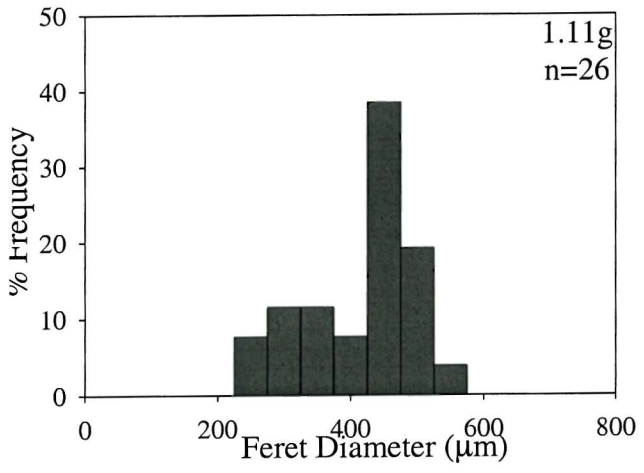
NE Atlantic – *C. ambrosia* – A (20th June 1985)



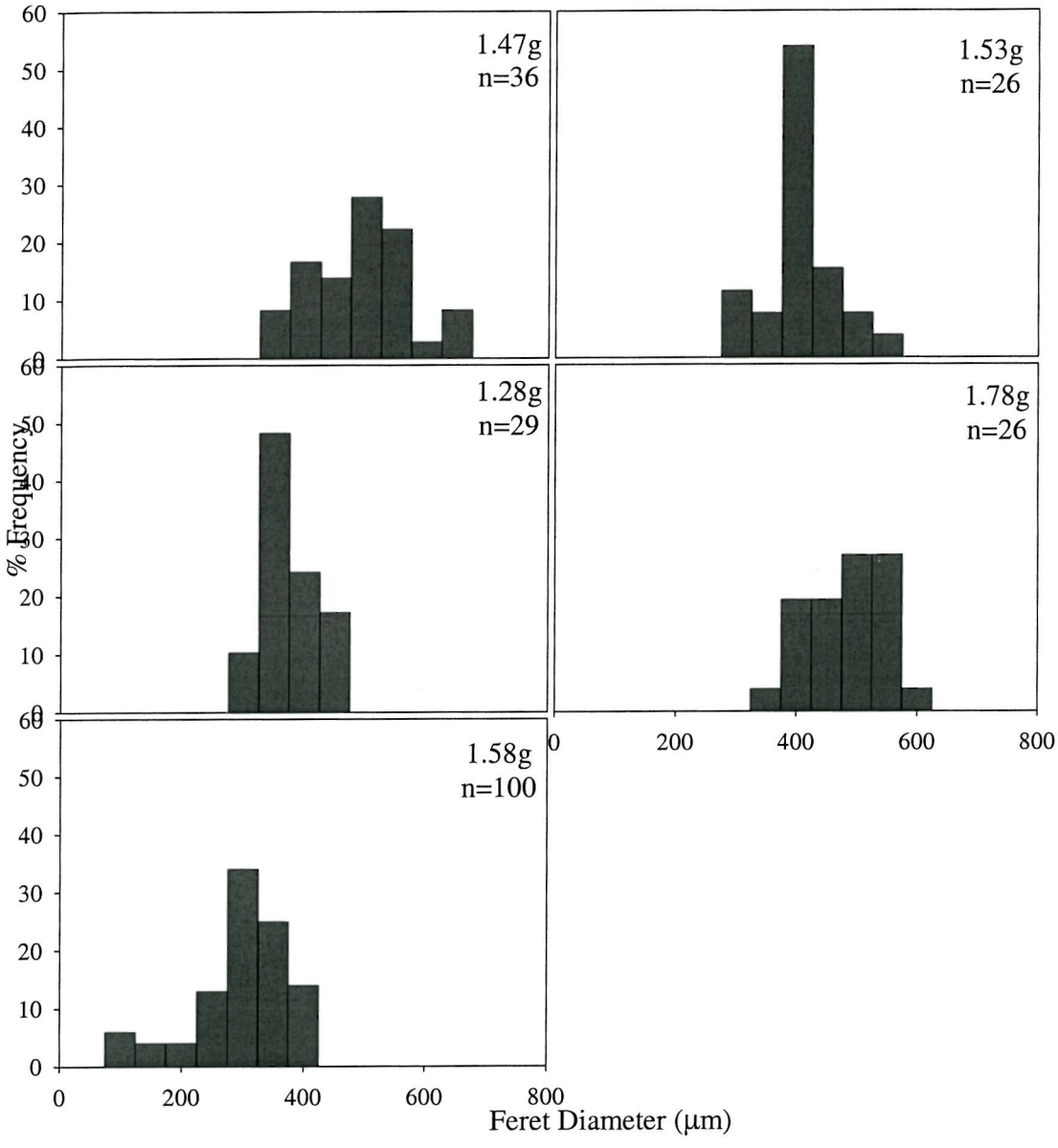
NE Atlantic - *C. ambrosia* – B (4th August 1983)



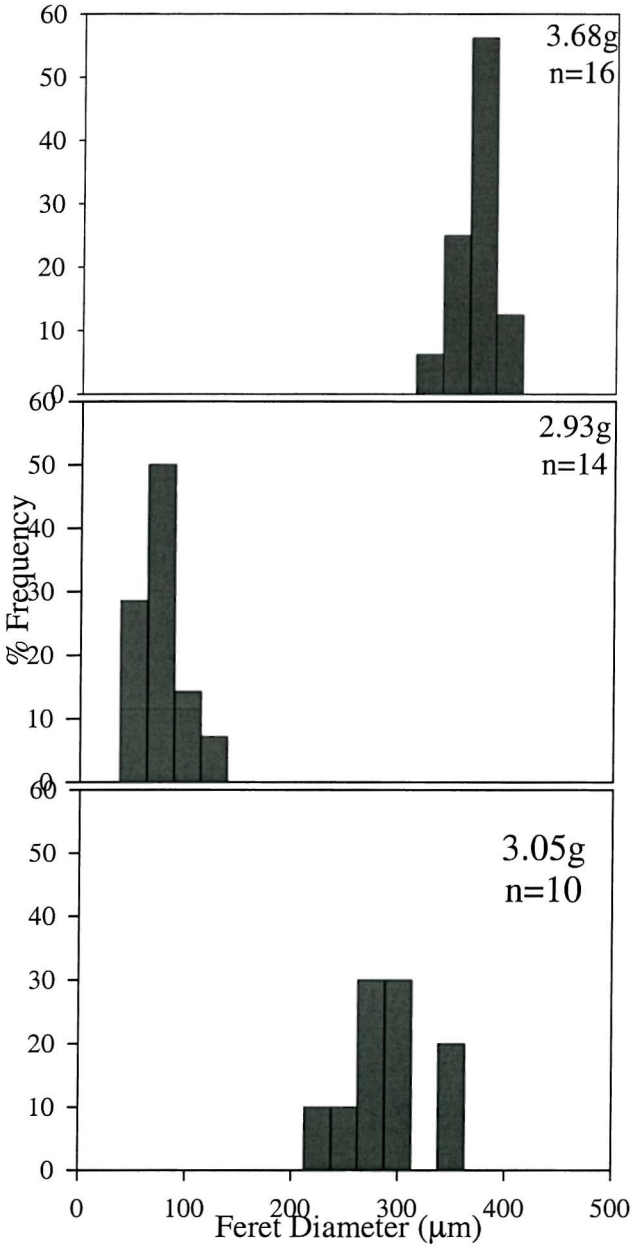
NE Atlantic - *C. ambrosia* – C (21st March 2002)



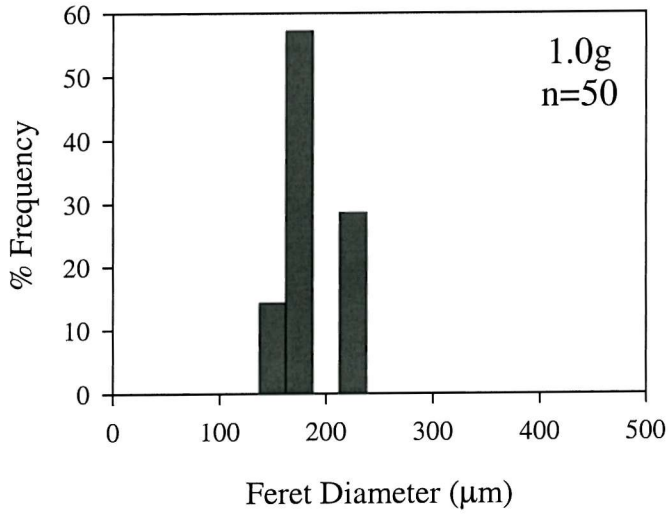
NE Atlantic - *C. ambrosia* – D (4th September 1979)



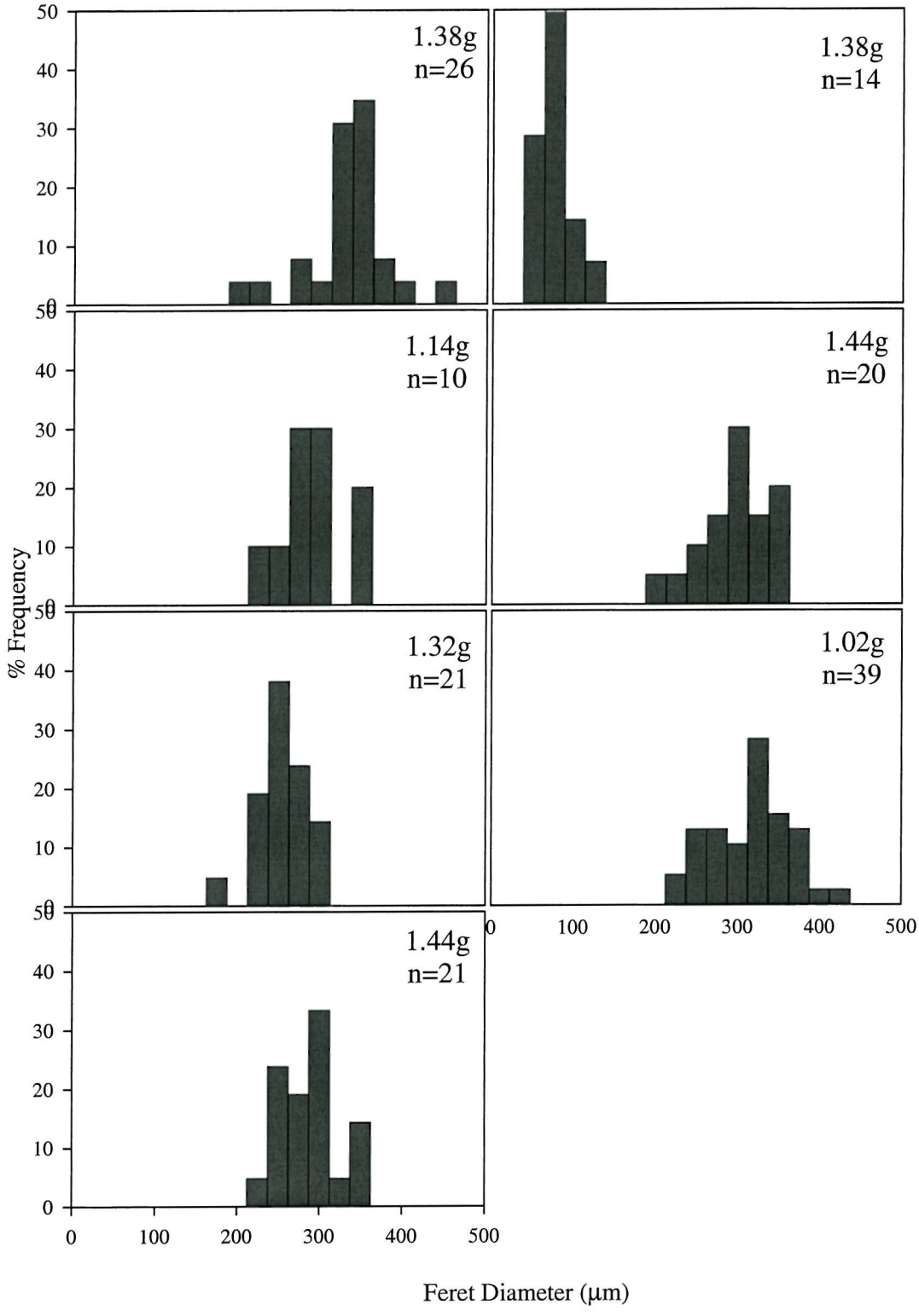
NE Atlantic - *C. ambrosia* – E (1st October 2002)



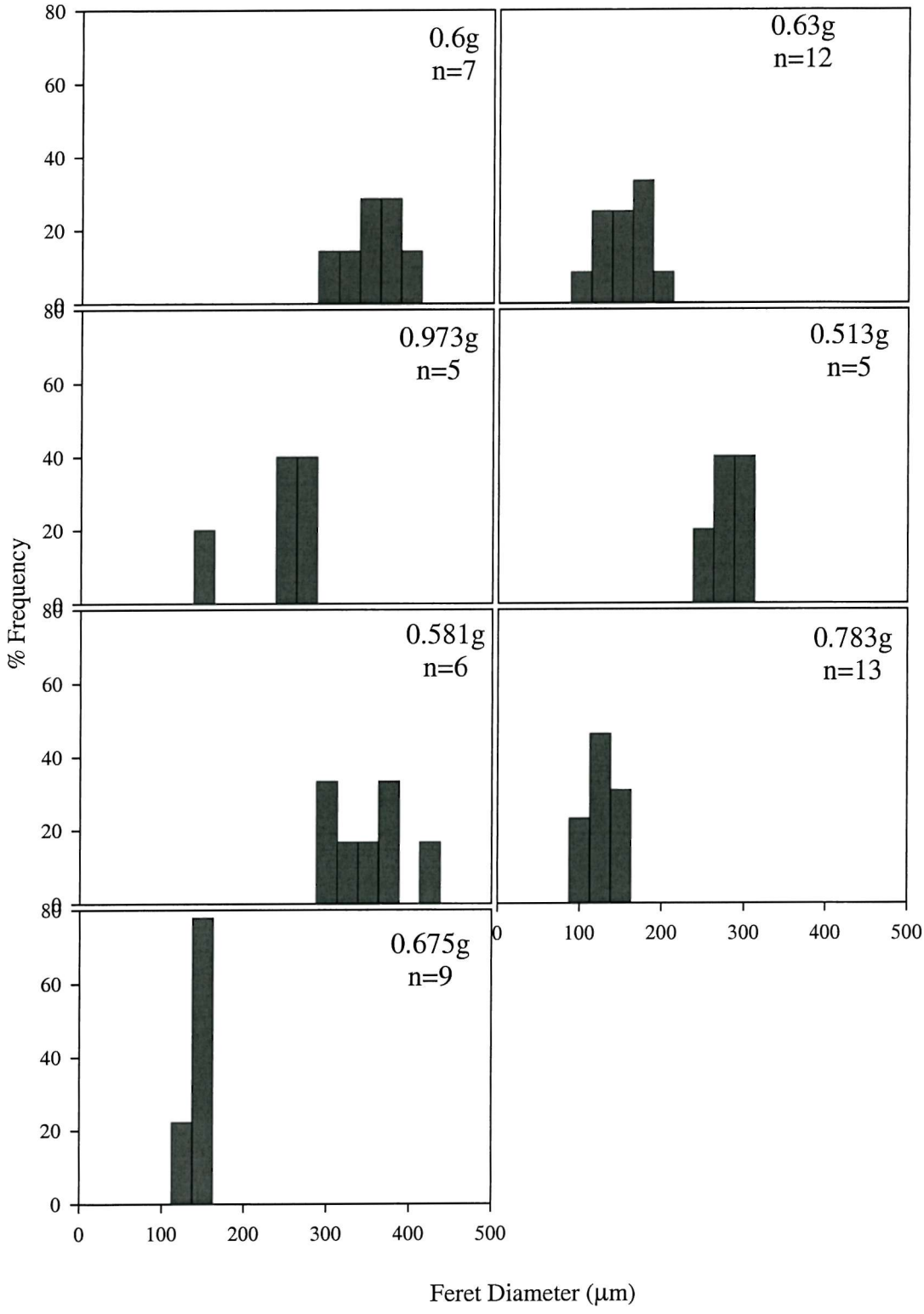
NE Atlantic - *C. seguenzae* – A (12th February 1998)



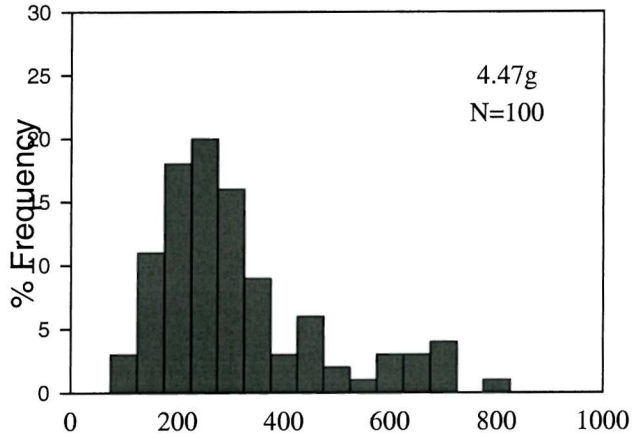
NE Atlantic - *C. seguenzae* – B (24th April 1978)



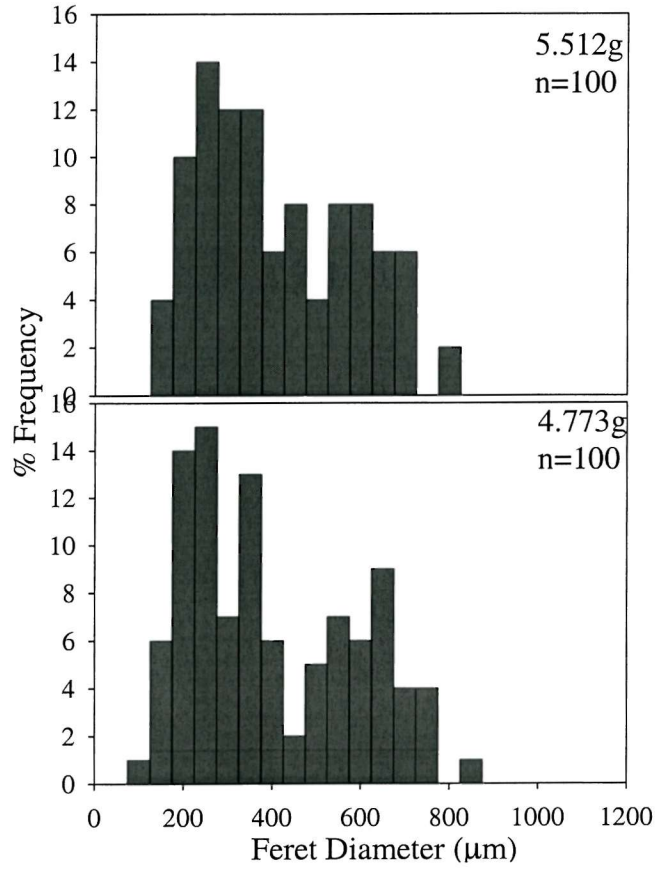
NE Atlantic - *C. seguenzae* – C (7th November 1980)



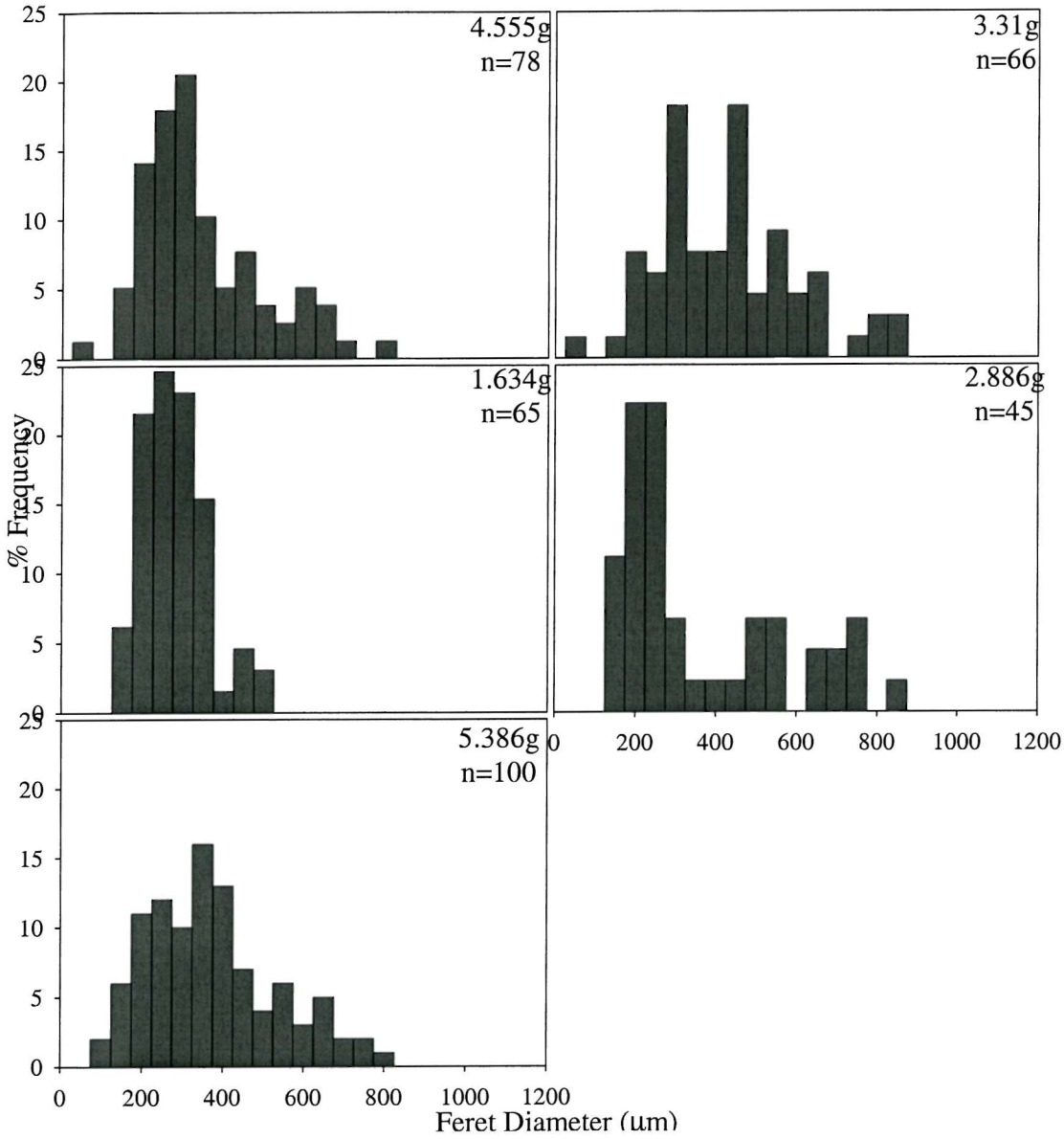
NE Atlantic - *C. seguenzae* – D (9th October 2002)



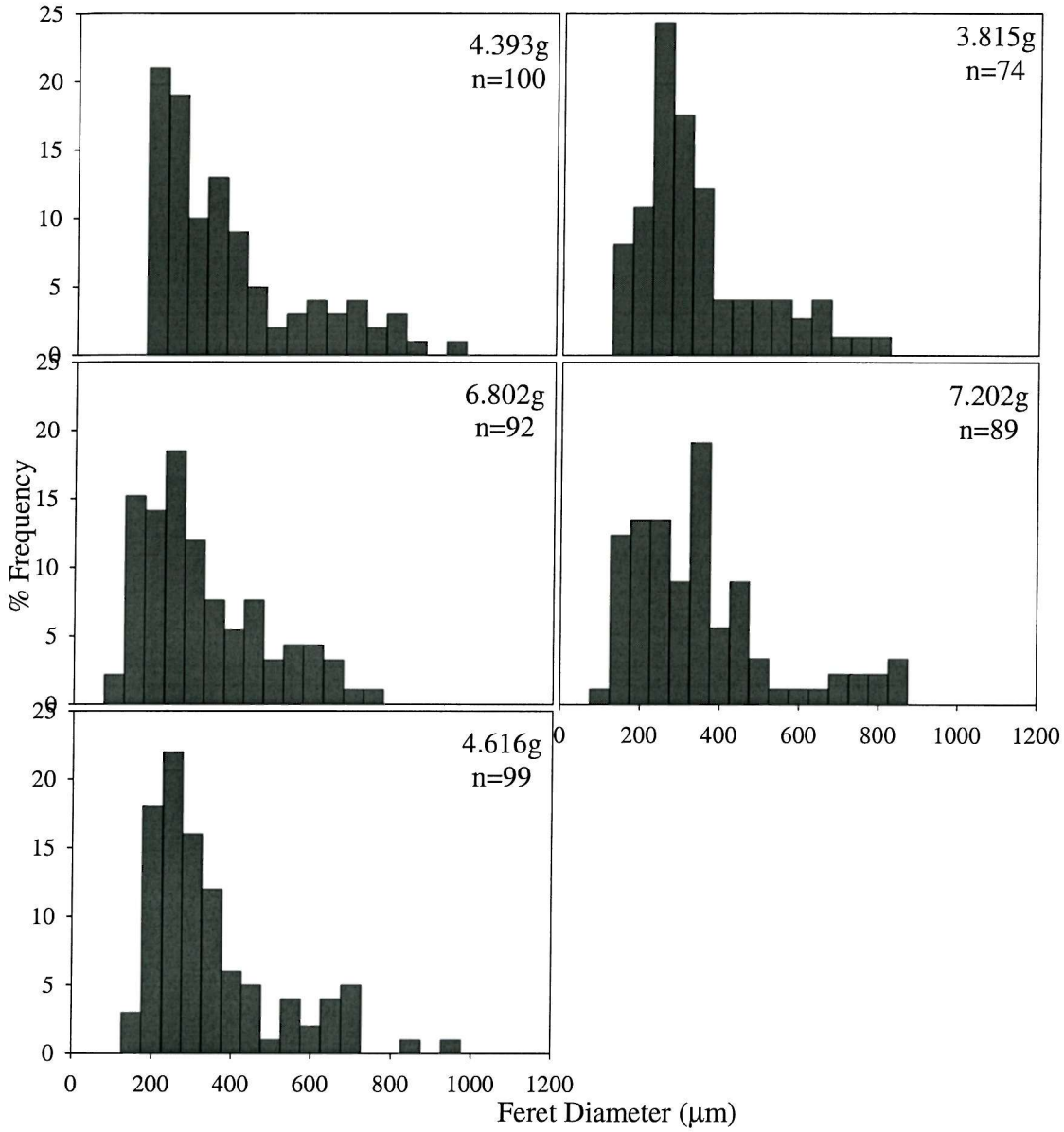
NE Atlantic – *F. alabastrum* – A (21st February 1991)



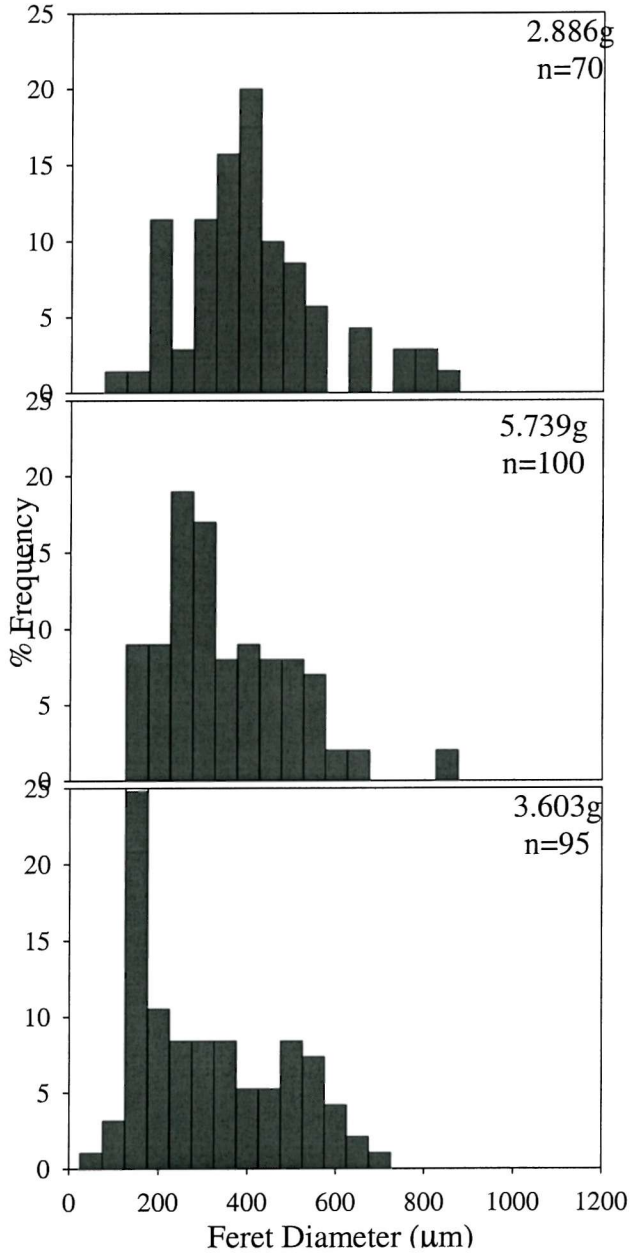
NE Atlantic – *F. alabastrum* – B (10th March 1983)



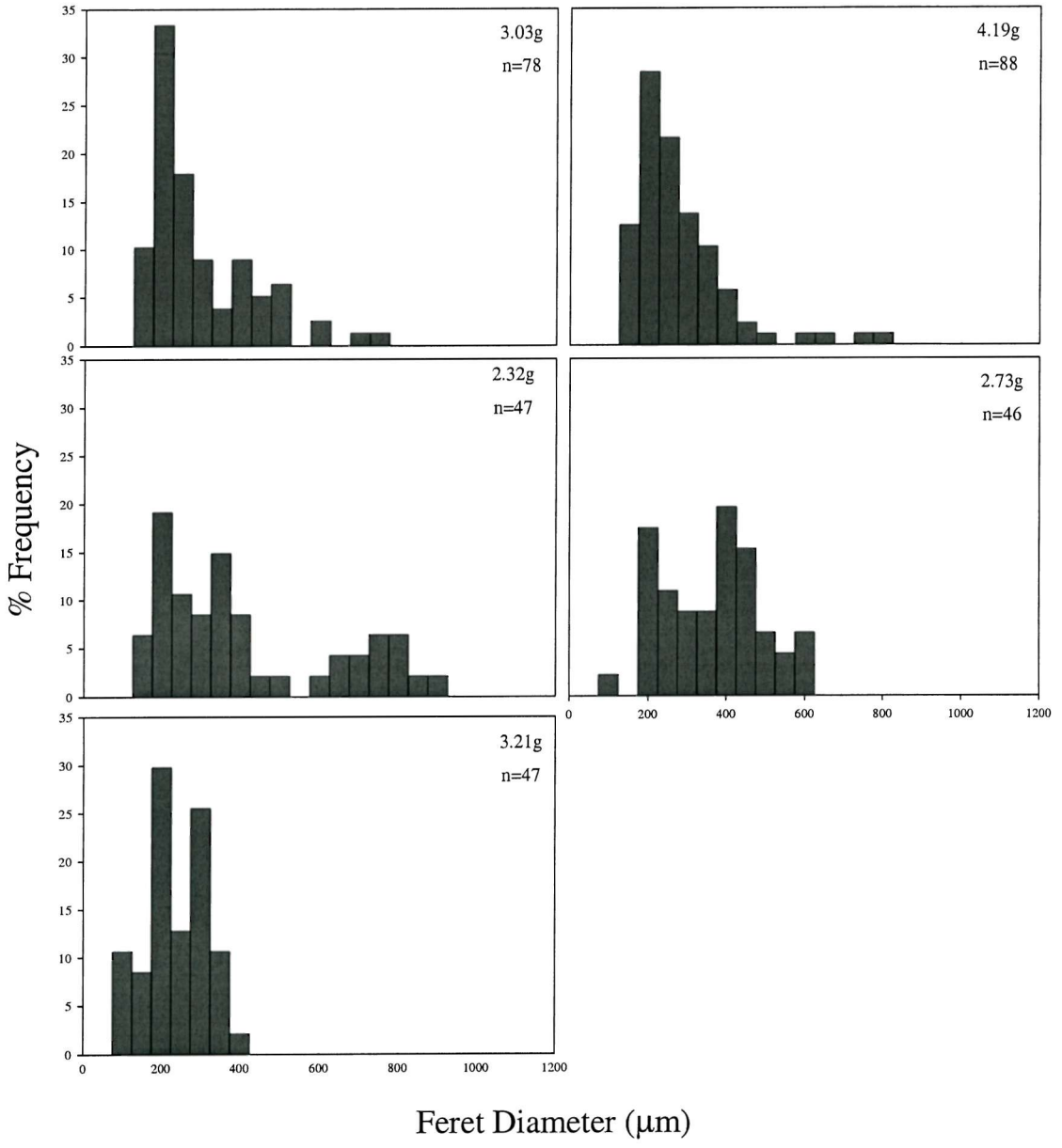
NE Atlantic – *F. alabastrum* – C (31st July 1983)



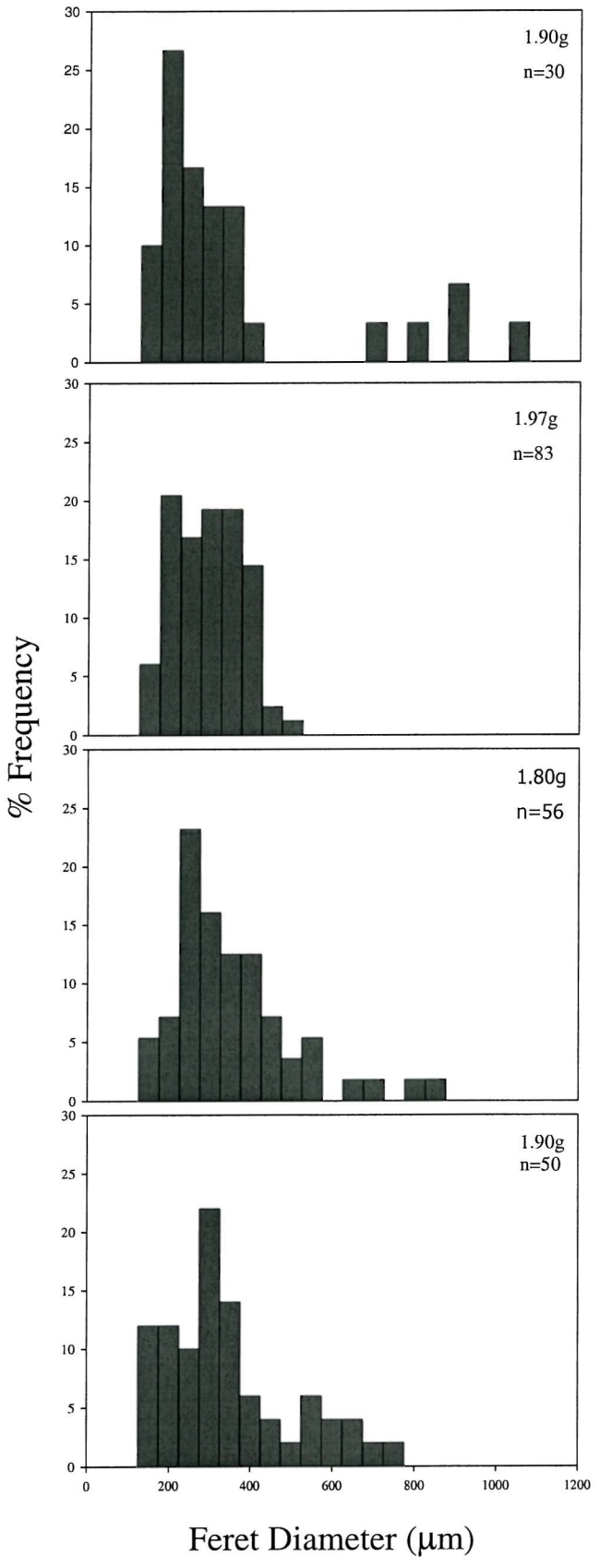
NE Atlantic – *F. alabastrum* – D (18th August 1982)



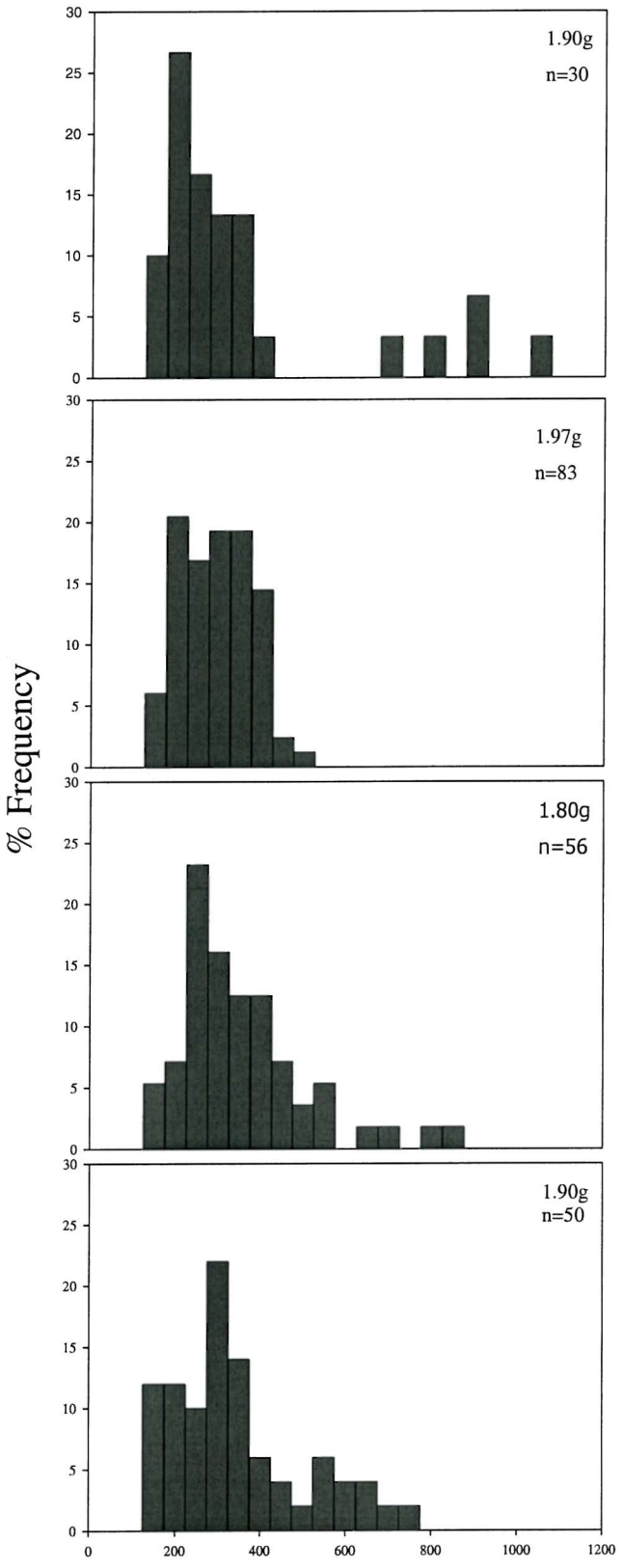
NE Atlantic – *F. alabastrum* – E (21st November 1991)



NE Atlantic – *Flabellum angulare* – A (1st October 2002)



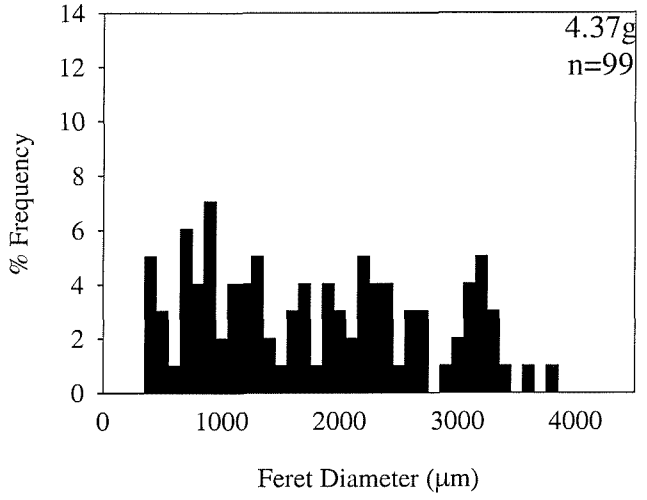
NE Atlantic – *Flabellum angulare* – B (11th March 2002)



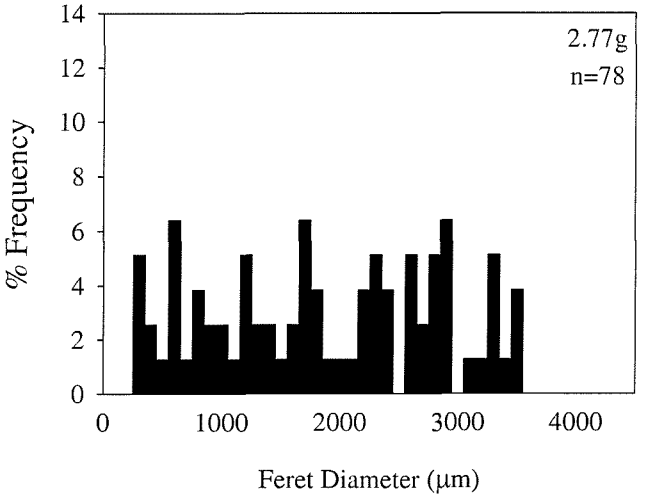
NE Atlantic – *Flabellum angulare* – C (21st September 2000)

IIIc

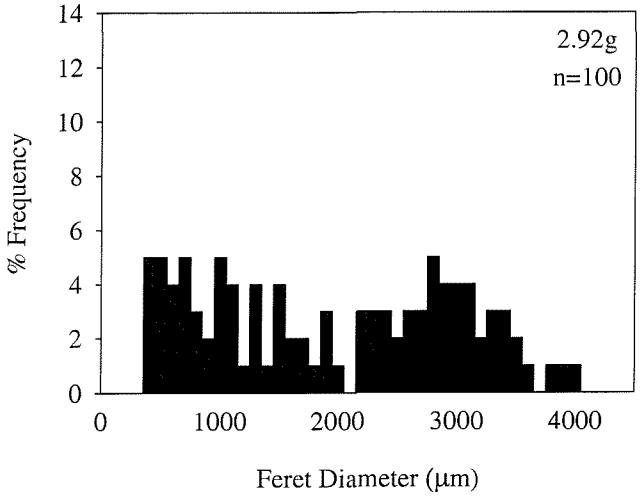
Antarctic Scleractinia



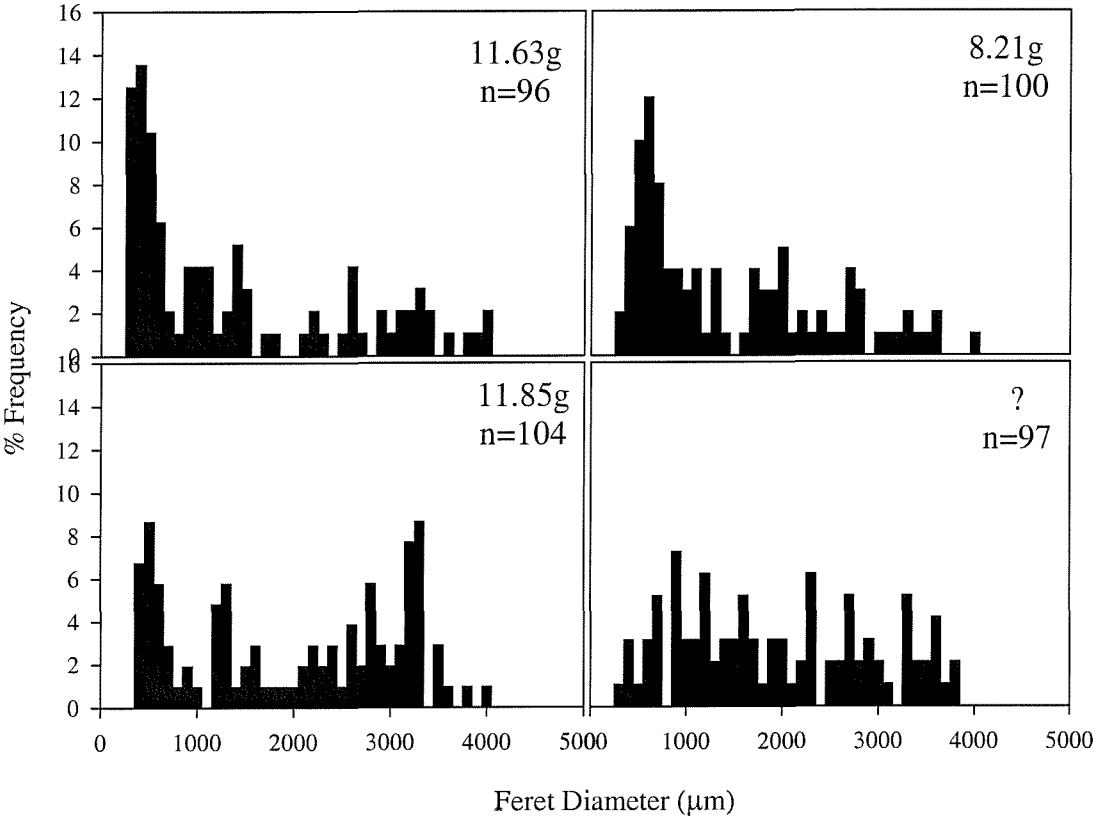
Antarctica – *Flabellum curvatum* A (5th November 1999)



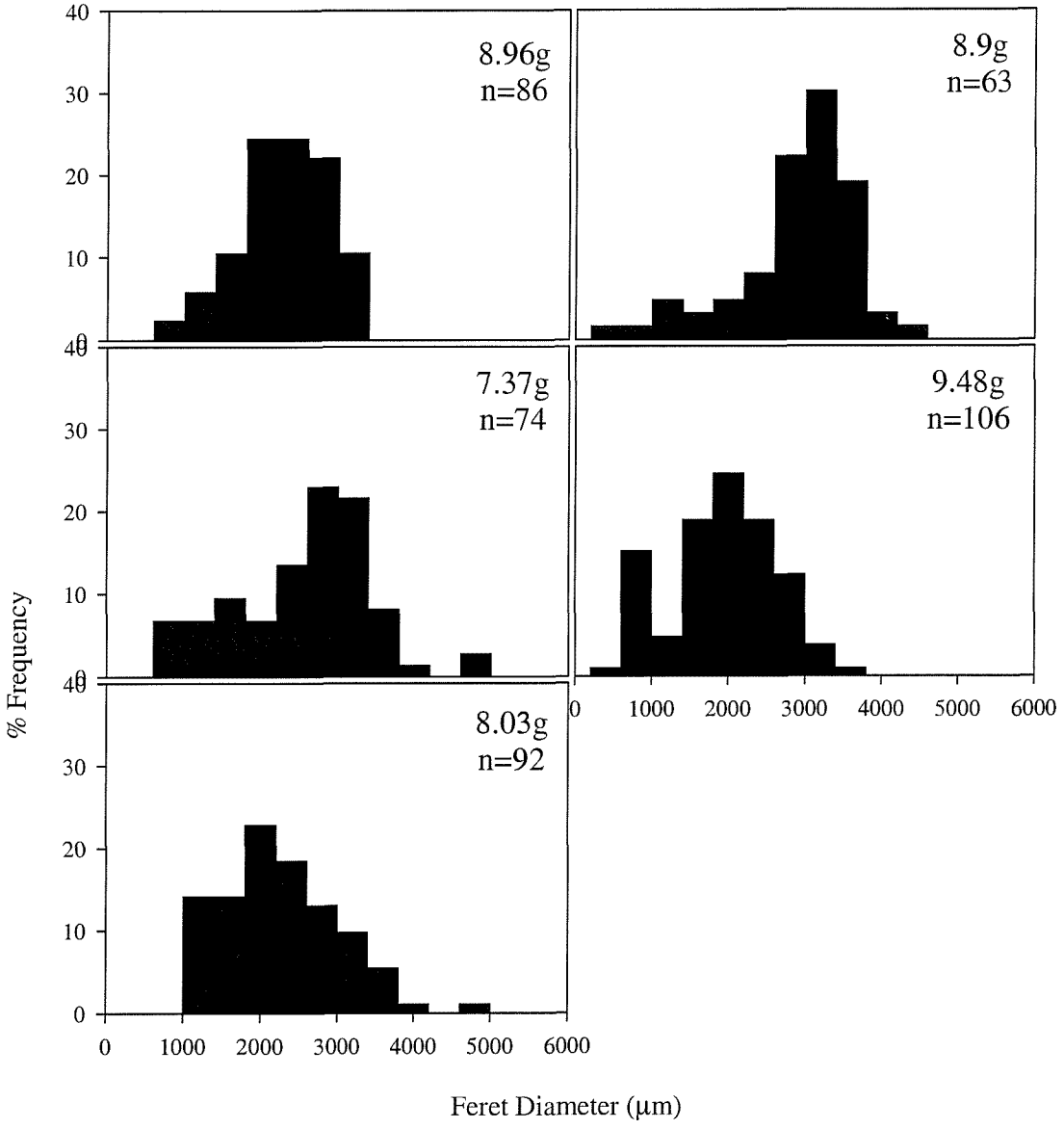
Antarctica – *Flabellum curvatum* B (16th March 2000)



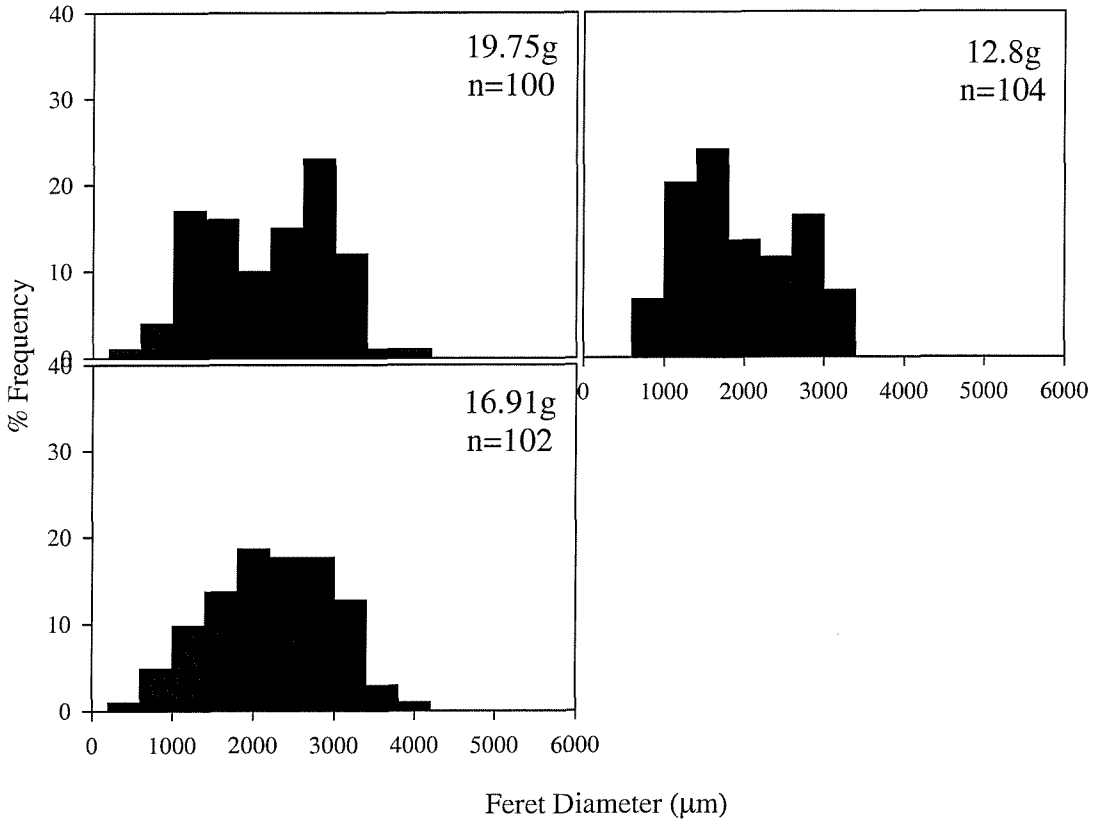
Antarctica – *Flabellum curvatum* C (11th July 2000)



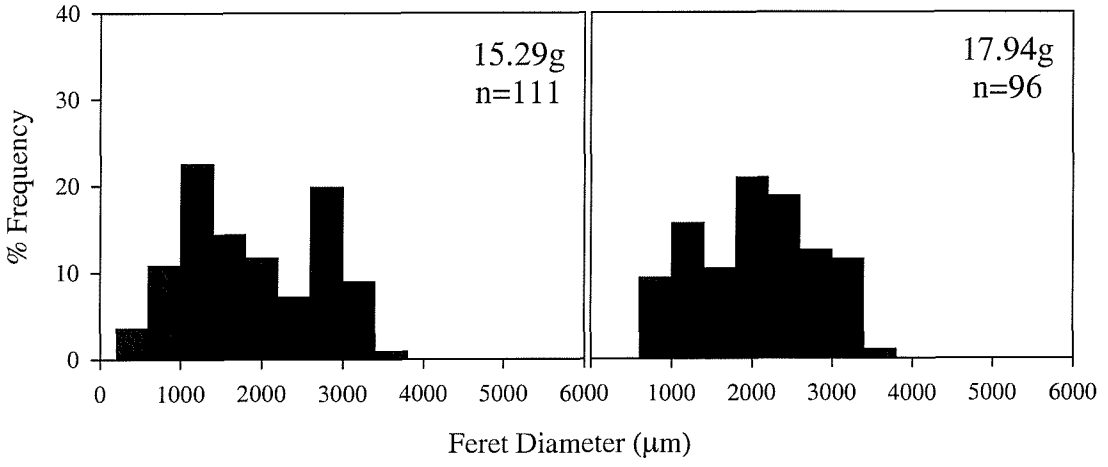
Antarctica – *Flabellum curvatum* E (2nd March 2001)



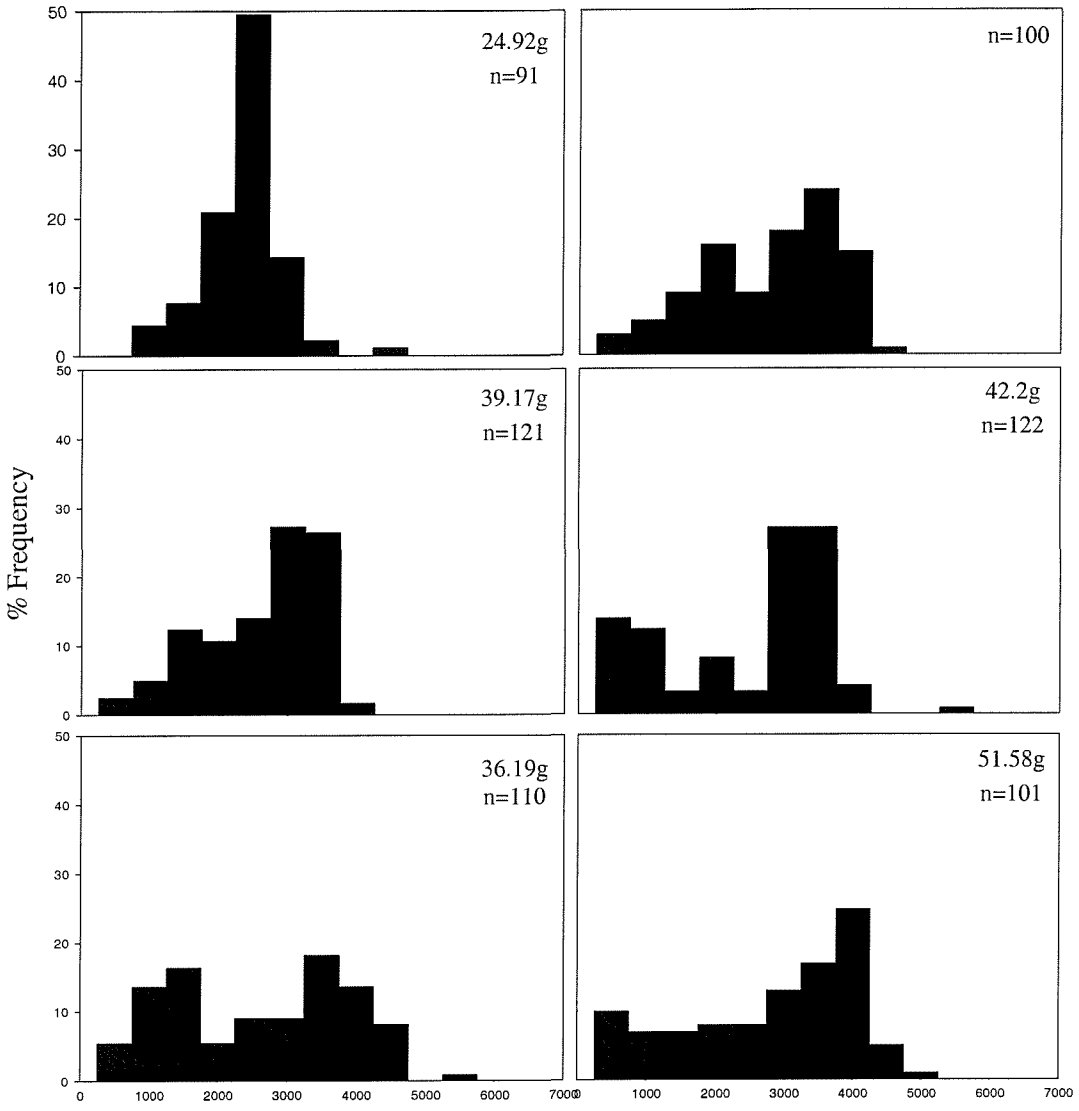
Antarctica – *Flabellum thouarsii* A (5th November 2000)



Antarctica – *Flabellum thouarsii* B/C (16th/10th March 2000)



Antarctica – *Flabellum thouarsii* D/E (13th/11th June 2000)



Antarctica - *Flabellum impensum* (5th November 2000)

Appendix IV

C. ambrosia

C. cornuformis

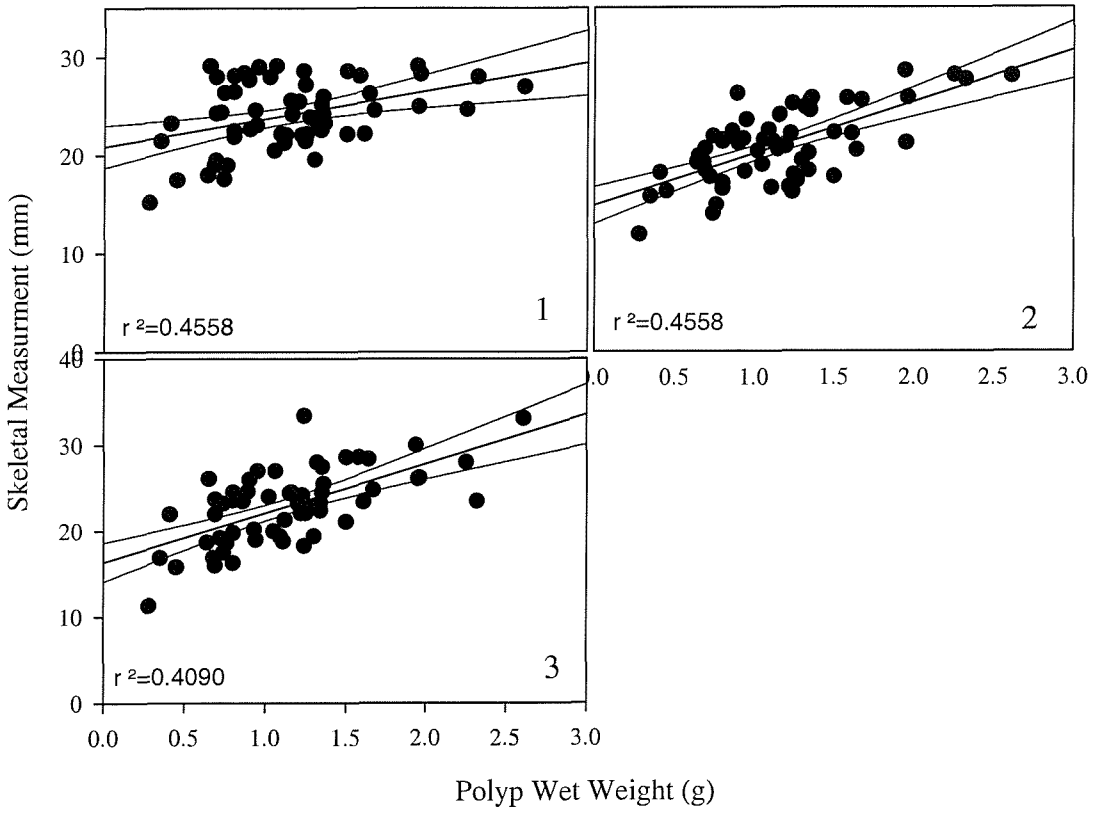
C. seguenzae

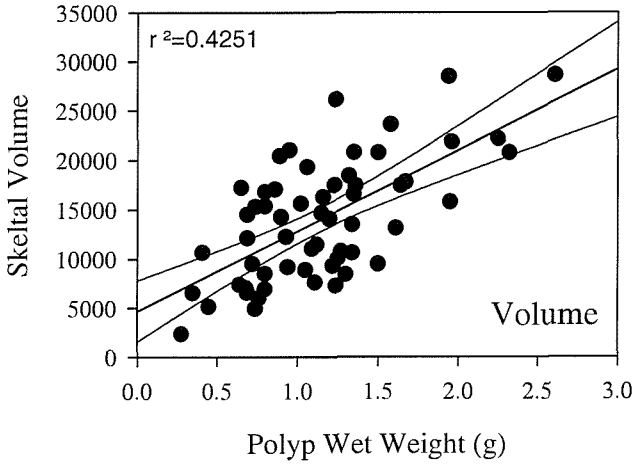
F. angulare

F. alabastrum

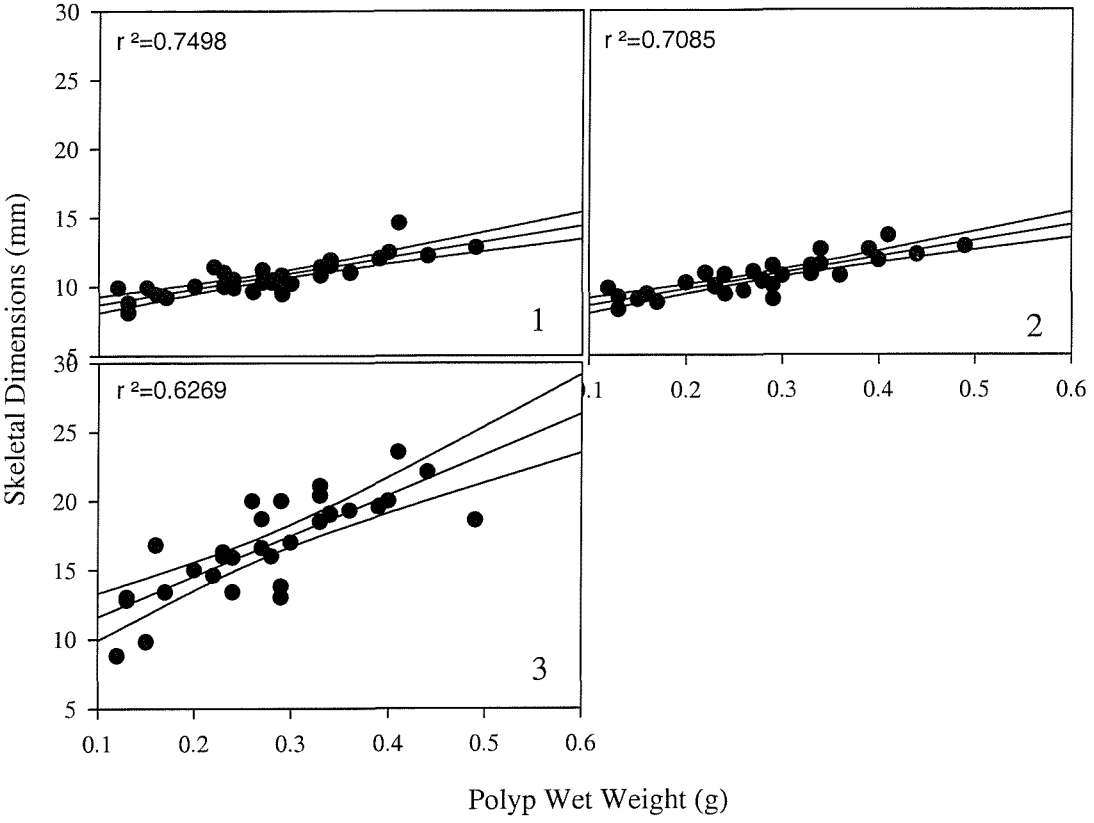
n=Number of individuals measured

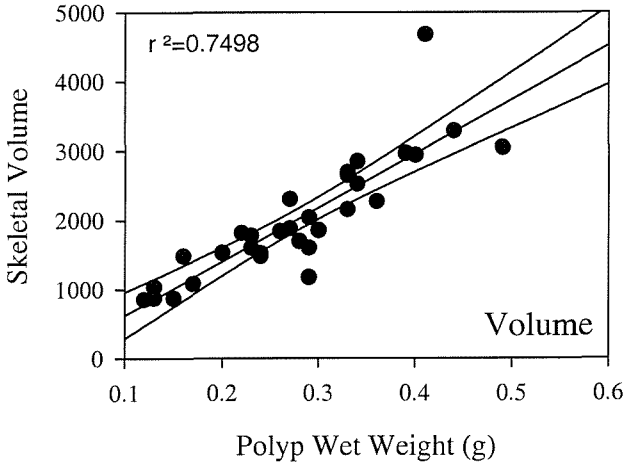
1,2,3 = Skeletal Dimensions – see Chapter 2



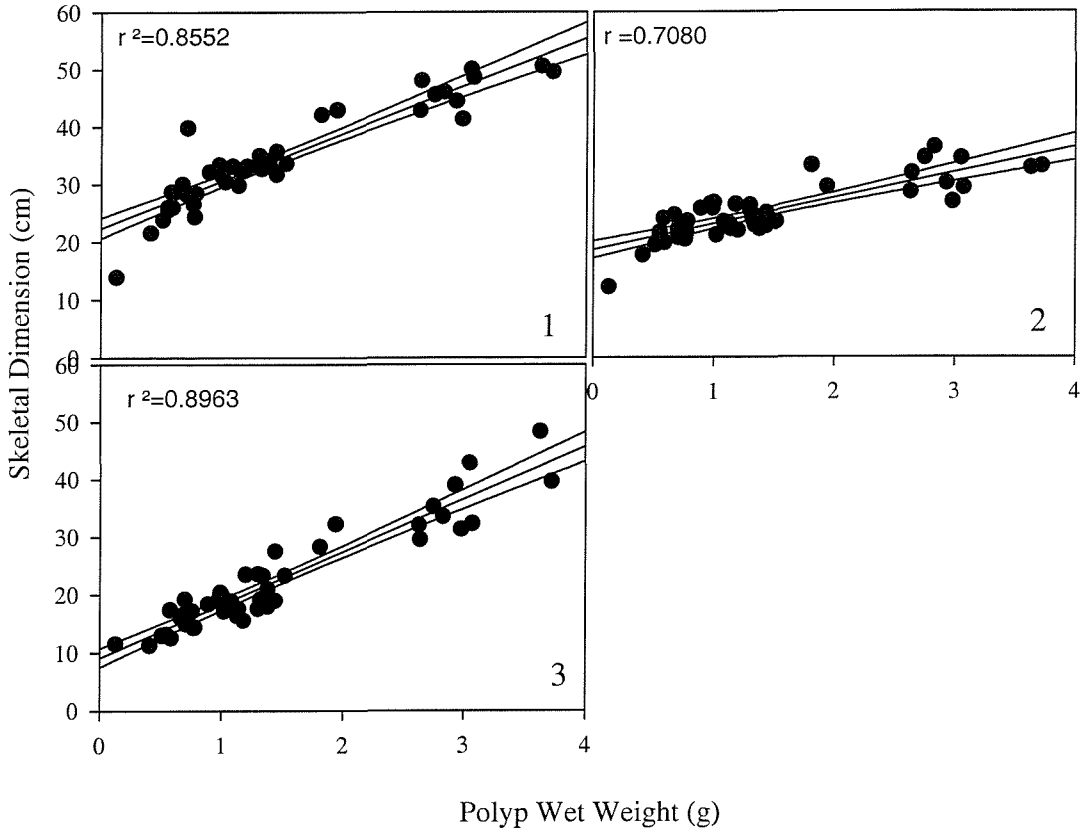


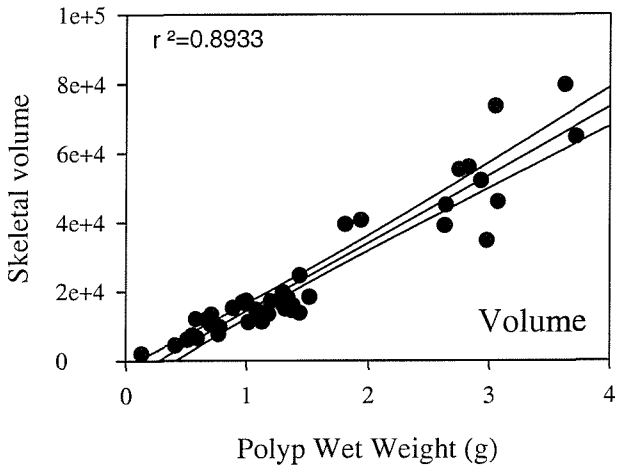
C. ambrosia – Polyp Wet Weight vs Skeletal Dimensions
95 % Confidence Intervals; n=58; p=ns; f=y0+a*x



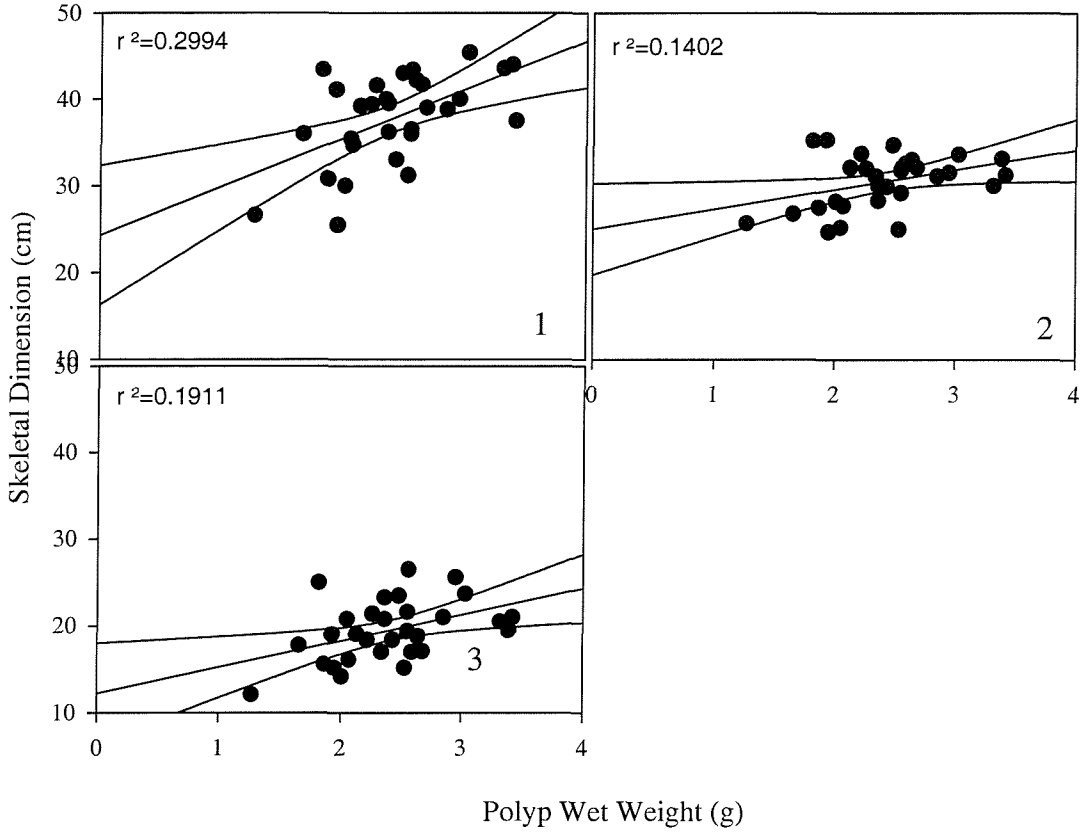


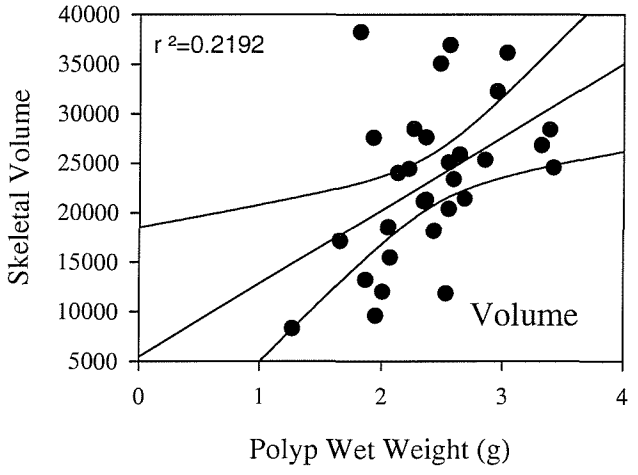
C. cornuformis – Polyp Wet Weight vs Skeletal Dimensions
95 % Confidence Intervals; n=31; p=>0.01; f=y0+a*x



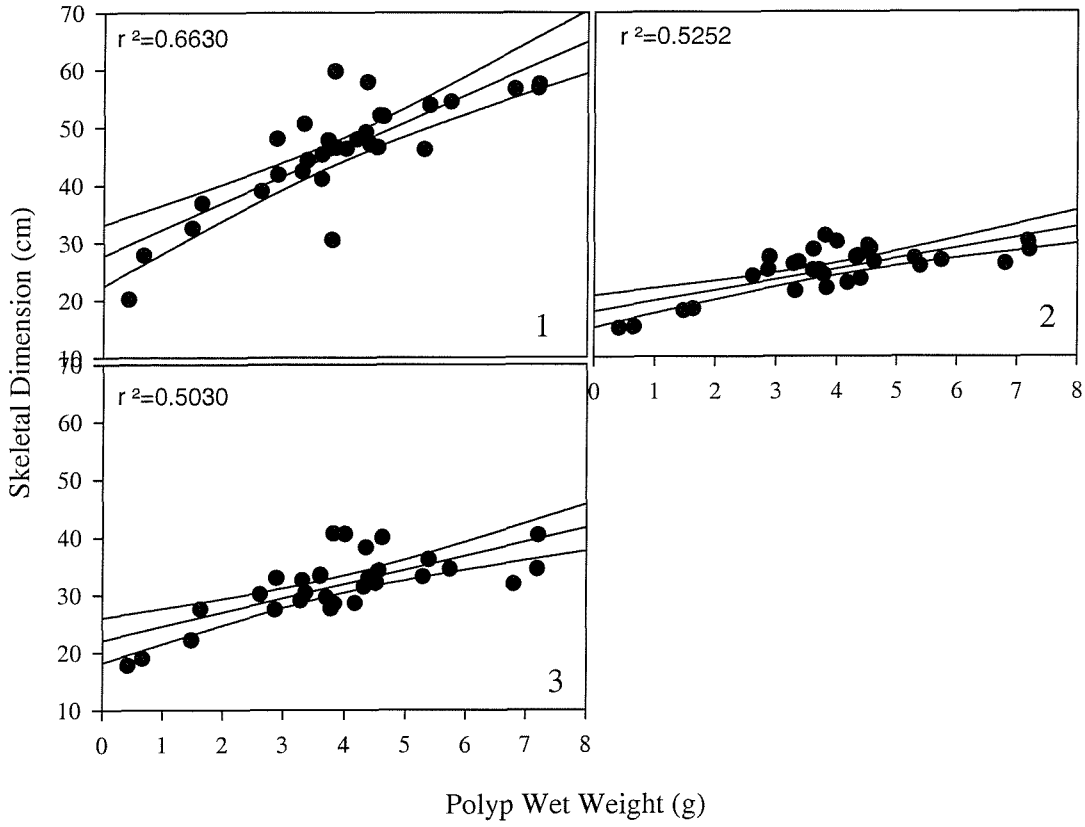


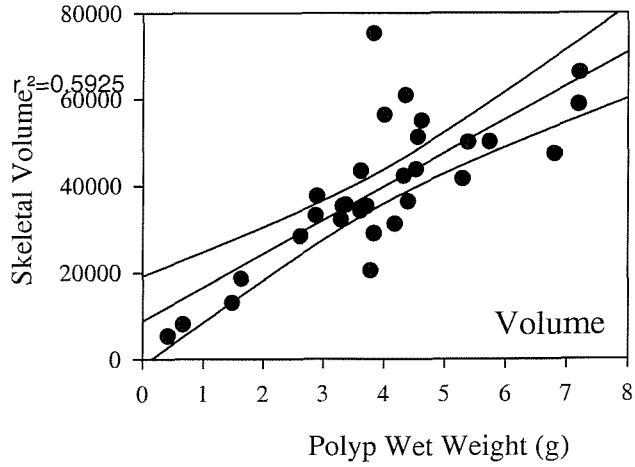
C. seguenzae – Polyp Wet Weight vs Skeletal Dimensions
95 % Confidence Intervals; n=45; p=>0.01; f=y0+a*x





F. angulare – Polyp Wet Weight vs Skeletal Dimensions
95 % Confidence Intervals; n=30; p=ns; f=y0+a*x





F. alabastrum – Polyp Wet Weight vs Skeletal Dimensions
95 % Confidence Intervals; n=30; p=>0.01; f=y0+a*x