The Environmental Sensitivity of Cold-water Corals Lophelia pertusa

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The Environmental Sensitivity of Cold-water Corals: 
*Lophelia pertusa*

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Abstract

This study examined the occurrence of *Lophelia pertusa* on North Sea oil infrastructure and its environmental sensitivity to oil and gas activities. Underwater videos from industry platform surveys were examined to identify *L. pertusa*, detail its occurrence at two sites (Heather and North Alwyn A (NAA)), and to look for evidence of exposure to drilling muds and cuttings (discharges). In addition, live corals were exposed to 4-h sedimentation events of increasing rates and polyp behaviour analysed. Sediment removal mechanisms were also examined. Finally, skeletal characteristics and trace metal concentrations were measured in skeletons sampled from platform sites exposed to drilling discharges and control sites.

The results showed a newly established sub-population of *L. pertusa* in the northern North Sea. *L. pertusa* was identified on 14 platforms and 947 colonies were recorded on Heather and NAA between 59 to 132 m depth coinciding with the presence of year round Atlantic water. Original recruits were likely from the northeast Atlantic and are now annually self-recruiting to the platforms. Additional video from Tern in 1993, 1994, 1998, and 2002 provided the first *in situ* colony growth rate (26 ± 5 mm yr⁻¹) for *L. pertusa*. Visual evidence of contamination from drilling discharges was limited to colonies close to drilling discharge points where partial and complete colonies were dead.

Polyp behaviour was negatively affected only at the highest sedimentation rates (12-19 mg cm⁻² min⁻¹), which are likely to be significantly higher than *in situ* rates, and polyps cleared sediment with ciliary currents and ingestion, which may be an indiscriminate feeding response. Corals exposed to discharges had shorter and narrower corallites compared to controls but other causal factors merit consideration such as genetics and hydrography. Further results showed that polyps bud annually and reach their maximum height in their first year, while the theca thickens at a constant rate, thus implying that the innermost growth band likely represents the first year of growth. Relatively depleted δ¹³C and δ¹⁸O along the inner growth band, which indicates fast calcification, supported this result.

Copper and barium in coral skeletons including visible detrital inclusions were significantly higher in exposed versus control colonies. Chromium and barium along the growth axis, avoiding detrital inclusions, showed one exposed polyp from a colony living two meters above the cuttings pile on North West Hutton (NWH) with higher barium compared to control colonies. Short-lived barium spikes were observed in two polyps from a control colony sampled from North Cormorant. It is hypothesised that the NWH coral may have been exposed to dissolved barium released during cuttings resuspension, while barium spikes in the control colony may result from natural fluctuations in seawater barium, thus advocating that *L. pertusa* can act as an archive of the marine environment.
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For Craig

“When I go down deep in the ocean, there may be a lot of unknowns, but I feel comfortable about the risks” Sylvia Earle
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Chapter 1  

Introduction

1.1 Cold-water corals

Cold-water corals, unlike their tropical relatives, are not restricted to shallow, warm-water marine environments. Many tropical corals are found at depths where light can penetrate because they contain photosynthetic dinoflagellate algae (zooxanthellae). These zooxanthellae symbionts can provide 100% of the coral’s carbon requirement, and in return, their hosts provide phosphorus and nitrogen, which may otherwise be limited in tropical seawater. The relationship is thought to enhance coral growth in oligotrophic tropical waters (Falkowski et al. 1993; Muller-Parker and D’Elia 1997). Cold-water corals are azooxanthellate. Their distribution is not limited by depth and they rely on suspension feeding to extract food particles from the water column. As a result, they are found in environments with accelerated currents, which are believed to provide a reliable food supply (Frederiksen et al. 1992; Freiwald 2002; White et al. 2005). Cnidarians, including tropical corals and likely cold-water corals, can also take up dissolved organic compounds from seawater through the extremely high surface area to volume ratio of corals (Muller-Parker and D’Elia 1997).

Cold-water corals encompass stony corals (Scleractinia), soft corals (Octocorallia), black corals (Antipatharia) and hydrocorals (Stylasteridae) (Roberts et al. 2006). Of the 1334 stony corals species described, 672 species are azooxanthellate (Cairns 2001). The majority of the azooxanthellate stony corals are solitary cup corals; only six species are reef-forming (Cairns 2001). This is in contrast to around 800 tropical reef-forming
species (Freiwald et al. 2004). Of interest in the present study is the reef framework-forming scleractinian cold-water coral *Lophelia pertusa*, belonging to the class Anthozoa and subclass Hexacorallia.

### 1.2 *Lophelia pertusa*

#### 1.2.1 Distribution and habitat requirements

Cold-water corals are less accessible for scientific research compared to tropical corals. Consequently, they are less studied and not as well understood. However, of the cold-water corals, *L. pertusa* is the best known. Research on cold-water corals dates back to the nineteenth century (Challenger Expedition 1873-1876; Fleming 1846; Gosse 1860) and much of the information available today about *L. pertusa* relates to its distribution (e.g. Dons 1944; Wilson 1979a; Roberts et al. 2003). *Lophelia pertusa* is a cosmopolitan species, one of the most widespread cold-water corals, and appears most abundant on the continental margins in the Atlantic Ocean with highest concentrations in the Northeast Atlantic (Freiwald et al. 2004). This perhaps reflects the intense level of benthic survey activity in this region but may also relate to the depth of the aragonite saturation horizon, which is below 2000 m in the northeast Atlantic and as shallow as 50-600 m in the north Pacific where octocorals and stylasterids dominate (Guinotte et al. 2006). The largest known cold-water coral reef complexes are dominated by *L. pertusa* and are found off the coast Norway (Fosså et al. 2000; Mortensen et al. 2001). Other occurrences vary between small, scattered colonies west of Shetland (Wilson 1979a; Roberts et al. 2003), to larger coral patches on Rockall Bank (Wilson 1979b) and in the northern Rockall Trough (Masson et al. 2003), to carbonate mounds in the Porcupine Seabight (Hovland et al. 1994; De Mol et al. 2002; Van Rooij et al. 2003) and Rockall Trough (Kenyon et al. 2003).
Within the environments described above, *L. pertusa* has its main bathymetric distribution at depths between 200-1000 m (Zibrowius 1980) but shallower records from just 39 m exist from Norwegian fjords with a direct deep-water connection (Dons 1944; Fosså et al. 2002). *Lophelia pertusa* requires oceanic water with temperatures of 4-12 °C and salinities between 35 PSU and 37 PSU (Dons 1944; Freiwald 2002; Roberts et al. 2003). It requires hard substrata on which to settle (Wilson 1979a), and as with most cold-water coral species fast currents for food supply and to keep the corals and substrata free of sediment (Grigg 1974; Genin et al. 1986; Rogers 1999; Freiwald 2002).

1.2.2 Growth

1.2.2.1 Coral calcification

The current knowledge of coral calcification has been primarily based on tropical coral research. Coral colonies are made up of individual cup-like calices connected by the formation of a hard skeleton made of aragonite fibrous crystals (Wainwright 1964). Each calyx contains a single polyp composed of two cell layers: the epidermis (or ectoderm) and the gastrodermis (or endoderm), which are separated by the mesoglea (Goreau 1959; Muller-Parker and D’Elia 1997). The gastrodermis is in contact with seawater in the coelenteron, and the site of calcification is below the epidermis called the calicoblastic epidermis (Goreau 1959; Muller-Parker and D’Elia 1997). Based on tropical coral research, Goreau (1959) proposed that Ca\(^{++}\) has an active transport across the cell layers and is absorbed onto the calicoblastic epidermis; this has since been determined to be an energy requiring transcellular transport process from seawater (Tambutté et al. 1996). Goreau (1959) proposed the following equation for coral calcification:
\[
\text{Ca}^{++} + 2\text{HCO}_3^- \leftrightarrow \text{Ca}\text{(HCO}_3\text{)}_2
\]

The product from this reaction is unstable and breaks down:

\[
\text{Ca}\text{(HCO}_3\text{)}_2 \leftrightarrow \text{CaCO}_3 + \text{H}_2\text{CO}_3
\]

Sources of skeletal carbon come from both metabolic CO\textsubscript{2} and dissolved inorganic carbon (DIC) in seawater (Goreau 1961; Pearse 1970). Goreau (1961) tested the calcification process using both radioactive carbonate from seawater and radioactive calcium and consistently found higher rates of calcification by measuring the radioactive calcium. He concluded that this was because the radioactive carbonate from seawater was being diluted with metabolic CO\textsubscript{2}. The relative amounts of DIC and carbon contributions from metabolic CO\textsubscript{2} remain in question. Furla et al. (2000) estimated contributions of metabolic carbon of between 70-80 % in a tropical coral, whereas Adkins et al. (2003) and Griffin and Druffel (1989) predicted a metabolic carbon contribution of between 5-10 % to skeletal carbon in some cold-water corals. However, Adkins et al. (2003) concluded that \textit{L. pertusa} has a greater contribution of metabolic carbon based on the greater offset of skeletal $\delta^{13}$C from seawater equilibrium.

For all corals, the skeleton surrounding an individual polyp is referred to as a corallite and consists of four major elements: the basal plate, the septa, the epitheca (or theca) and the dissepiments (Barnes 1970) (Figure 1.1). The calyx is the cup-like depression in the corallite and the septa are radially arranged and project into the calyx. The septa can be divided into primary, secondary, and tertiary structures. The theca is the polyp wall connecting the septa and enclosing the polyp. The dissepiments are partitions laid down within the coelenteron, which is the gastrovascular cavity within the corallite. \textit{Lophelia pertusa} skeletons contain the same skeletal components as described above, except the basal plates are not always clearly distinguishable. It is likely that the first polyp to
grow from the colony’s original attachment point will have a clearly defined basal plate, but it is not clear whether subsequently budded polyps also lay down a basal plate.

A. Cross section from above  B. Transverse section through major septa

Figure 1.1 Coral structures

All corals grow from their basal plate where aragonite needles form in clusters called sclerodermites (Wainwright 1964; Barnes 1970; Sorauf 1972). All parts of a coral skeleton are made up of these sclerodermites where crystals are arranged in three-dimensional fans (Barnes 1970). Barnes (1970) examined crystal growth by staining live corals with sodium alizarinesulfonate for different time periods; microscopic examinations of the skeletons revealed crystals growing out from the centres of calcification until they encountered crystals growing from neighbouring centres of calcification and growth was stopped. Sorauf (1972) described the epitheca as the septotheca which is formed from the lateral extension of adjacent septa and joins two septa together.

Coral skeletons contain approximately 1 % organic material associated with approximately 4 % tightly bound water (Wainwright 1964; Cohen and McConnaughey 2003; Cuif and Dauphin 2005). A closer examination of associated organic material led
to a new model of coral calcification (Cuif et al. 1999; Cuif et al. 2003; Cuif and Dauphin 2005). The new model proposed that coral crystal fibres are not monocrystalline units but they are biologically driven and an organic matrix sheet guides cyclical formation of organo-mineral increments. The model agrees with previous declarations that centres of calcification are where calcification first occurs, referred to as “early mineralising zones”, but that they are distinct units containing higher organic content compared to surrounding fibres and that they do not act as seeds for crystal growth (Cuif and Dauphin 1998, 2005). Rather, the surrounding fibres are independently produced and permanently controlled by the coral calicoblastic ectodermis (Cuif et al. 1999). Cohen and McConnaughey (2003) offer an alternative to the role of organic matrix sheets: that they act as inhibitors of calcification rather than seeding calcification. They suggested that corals can quickly build up the rate of calcification, but that preventing the coral from calcifying beyond the overlying tissue could be an important requirement.

1.2.2.2 Morphological variation

Skeletal features described in the previous section vary greatly between coral species, but intraspecific morphological variations are also common for scleractinian corals including *L. pertusa* (Zibrowius 1984). Freiwald et al. (1997) described three morphotypes of *L. pertusa* from the Stjernsund reef (Norway) based on corallite characteristics such as polyp height and shape, thecal wall thickness, and budding rate. The first morphotype, *tubular*, was based on dead colonies from the reef and was characterised by deep calices, thin thecal walls, long trumpet-like corallites between 4-5 cm in height, and a maximum of six buds per polyp. The second type, *stereome-thickened*, was the main framework constructor of the reef. Thick thecal walls with clearly visible dark and light growth bands, and just one to two buds per polyp
characterized both the dead and living colonies. The final morphotype, *stout and crowded*, was characterised by densely packed polyps all dividing in one plane and with a budding rate related to corallite length.

Kaszemeik and Freiwald (unpublished report) also described morphotypes for specimens of *L. pertusa*, which were sampled from a range of localities in the North Atlantic. Their morphotypes were based on the appearance of septa, the width to length ratio of the corallite, and thecal thickness. These included *extrovert* colonies with exserted septa, wide diameter calices, and clear annual growth bands, and comprised colonies from the Sula Reef (Norway) and Rockall Bank (UK). *Introvert* colonies were characterized by septa that did not extend beyond the top of the calyx, strong thecal walls, a high number of dissepiments, and were found in the Stjernsund Reef (Norway), Kosterfjord (Sweden), and the Propeller Mound (west of Ireland). Finally, *elongate* colonies, similar to the *tubular* form described by Freiwald et al. (1997), were characterized by elongate and slender corallites measuring up to 5 cm in height, thin thecal walls, sunken and exserted septa, and were commonly found in the Sula Reef.

1.2.2.3 Growth rates

Linear extension rates of *L. pertusa* have been estimated between 4.1 to 25 mm yr$^{-1}$. Estimates of minimum extension rates can be made when corals colonise an artificial substrates of a known age. Such estimates for *L. pertusa* range between 5-8 mm yr$^{-1}$ (Duncan 1877; Wilson 1979b; Roberts 2002) and 26 mm yr$^{-1}$ (Bell and Smith 1999). Estimates based on seasonal trends in stable isotopes of oxygen and carbon along the polyp skeletal wall reflected the upper end of the above-mentioned range: 25 mm yr$^{-1}$ (Mikkelsen et al. 1982) and 20 mm yr$^{-1}$ (Freiwald et al. 1997). Methods based on growth lines observed in septa were just 5.5 mm yr$^{-1}$ (Mortensen and Rapp 1998).
Finally, direct observation of growth in aquaria resulted in average growth rates of 9.4 \( \text{mm yr}^{-1} \) (Mortensen 2001), which may reflect stressed conditions for the coral and/or different feeding regimes. Growth rates for \( L. \text{pertusa} \) from different regions may vary, but the large discrepancies described above are more likely a reflection of the different means used to measure linear extension. For example, estimates from corals growing on artificial substrates are most probably underestimates of coral extension rates unless the largest colony is measured, such as in the case of the estimates put forward by Bell and Smith (1999) from a North Sea offshore oil and gas platform, and recruitment occurred as soon as the substrate was installed. Stable isotope estimates from the thecal wall are close to those of Bell and Smith from the North Sea. However, estimates based on growth lines in septa were much lower (Mortensen and Rap 1998). This coral had been growing in a Norwegian fjord, which may explain the difference in growth rates.

\subsection*{1.2.3 Food sources}

\( Lophelia \text{pertusa} \) is limited to environments with a source of nutrient-rich water and its occurrence generally coincides with regions of high seasonal surface productivity (Frederiksen et al. 1992; Freiwald 2002; White et al. 2005). Direct in situ observations from a submersible on the Sula Reef, revealed \( L. \text{pertusa} \) polyps preying on zooplankton such as copepods and cumacean crustaceans (Freiwald 2002). Additional results based on lipid and \( \delta^{15}\text{N} \) analyses of coral tissue taken from a number of sites along the western European margin also suggested that they feed largely on zooplankton (Kiriakoulakis et al. 2005). Further results based on the \( \delta^{15}\text{N} \) in potential food sources (collected in sediment traps) and coral tissue from Galicia Bank (NW Spain), suggested that \( L. \text{pertusa} \) feeds on a mixture of phytodetritus and zooplankton (Duineveld et al. 2004).
1.2.4 Reproduction

Compared with tropical corals, less information is available on the reproduction of cold-water corals; however, it is assumed that they will follow similar reproductive modes to tropical corals. Research to date has looked at the reproductive ecology of 15 species of scleractinian cold-water corals, including nine species of cup corals and six species of reef-forming corals (summarised by Waller 2005). Fifteen species, including *L. pertusa*, were gonochoric (separate sexes), and three solitary species, all *Caryophyllia*, were hermaphrodites. Egg sizes and fecundity varied between species, for example, *L. pertusa* produced small eggs (140 µm) with a high fecundity (3327 oocytes per cm$^2$) and *Madrepora oculata*, which often occurs with *L. pertusa*, produced large eggs (350 µm) with a low fecundity (256 oocytes per cm$^2$) (Waller and Tyler 2005). Twelve species including *L. pertusa* were spawners and three species of the solitary coral *Flabellum* from the Antarctic were brooders (Waller 2005).

Waller and Tyler (2005) found that *L. pertusa* in the Porcupine Seabight exhibited seasonal reproduction producing a single cohort of gametes. Samples taken in August 2000, September 2002, and October 2002, showed increasing egg size to a maximum egg size of 139 µm. Based on these findings, they concluded that oogenesis is initiated in the late summer with spawning of lecithotrophic larvae likely to occur in January/February. Furthermore, one male sample from October 2002 contained spermacysts (scleractinians do not have gonads but sperm develop in cysts held together by a mesogleal envelop (Waller 2005)) all at the same developmental stage (Waller and Tyler 2005). Waller and Tyler (2005) suggested that the onset of the gametogenic cycle in late summer may relate to the seasonal plankton blooms in the Porcupine Seabight, which occur in July and could provide a significant input of particulate organic matter,
thus fulfilling the coral’s nutritional requirements before reproduction begins (Waller and Tyler 2005).

In contrast to the Porcupine Seabight corals, no reproductive colonies were found in samples of *L. pertusa* from the Darwin Mounds (west of Scotland) (Waller and Tyler 2005). The authors speculated that this was related to the heavy evidence of trawling seen in this region. They suggested that trawling breaks up the colonies keeping them too small to become reproductive (Waller and Tyler 2005). Preliminary results from Brooke et al. (2005) confirm a similar reproductive cycle for *L. pertusa* from Trondheimsfjord (Norway), with the exception that the gametogenic cycle began in early spring rather than late summer as described by Waller and Tyler (2005), and Brooke et al. (2005) reported a maximum oocyte diameter from samples taken the following February. The northeast Atlantic examples are in contrast to samples of *L. pertusa* from the northern Gulf of Mexico. Corals in July showed small vitellogenic oocytes and corals from late September were non-reproductive suggesting that *L. pertusa* in the Gulf of Mexico probably spawns in late August or early September. Future work will examine environmental data to investigate factors regulating reproduction in this region (Brooke et al. 2005). No other research to date has examined the reproductive ecology of *L. pertusa*.

### 1.2.5 *Lophelia pertusa* as habitat

*Lophelia pertusa* can form large reefs, the largest of which, the Røst Reef, stretches an impressive 35-40 km along the Norwegian continental shelf break (Fosså et al. 2005). These reefs are important habitat and support diverse species assemblages similar in certain respects to tropical coral reefs. (Jensen and Frederiksen 1992; Freiwald 1998; Rogers 1999; Fosså et al. 2000). The most recent estimate of the number of species
living on or in association with *L. pertusa* reefs is 1317, which is considered an on-going estimate as many groups remain to be examined by relevant experts (Freiwald et al. 2004; Roberts et al. 2006). Little evidence exists of obligate associations on *L. pertusa* reefs as is often seen with associated fauna on tropical coral reefs (Rogers 1999). Rogers (1999) pointed out, however, that the importance of the reef’s ecological role in the life cycles of many species should not be ignored despite the facultative associations of species to the reefs. Some species may be found more abundantly on *L. pertusa* reefs than in any other habitat (Rogers 1999). Additionally, species may use *L. pertusa* reefs as a nursery area as many species are only found as juveniles on the reefs (Jensen and Frederiksen 1992). Finally, fish species richness and abundance, including 17 commercially important species, increased on *L. pertusa* reefs compared to the surrounding seabed (Costello et al. 2005).

### 1.3 Threats to *Lophelia pertusa*

Increased pressure on exploited natural resources on land and in coastal areas has led to an expansion of human activities into deeper water that has had, and will continue to have, direct and indirect environmental impacts (Glover and Smith 2003). Activities of greatest concern for present and future impacts on deep-water benthic environments are bottom fishing, oil and gas extraction, disposal of wastes to the seabed, marine mineral extraction and climate change (Gage and Tyler 1996; Glover and Smith 2003; Smith et al. in press).

Currently, the most immediate and widespread threat to cold-water corals including *L. pertusa* is damage from bottom trawling. As research progresses and the current understanding of these coral ecosystems grows, so does the evidence of trawling impacts (Freiwald et al. 2004). Massive damage to cold-water corals, including many
cases of damage to *L. pertusa*, has been examined on the European continental margin, on seamounts off Tasmania, and from the east and west coasts of North America (Koslow et al. 2001; Krieger 2001; Fosså et al. 2002; Hall-Spencer et al. 2002; Mortensen et al. 2005). The protruding structure of *L. pertusa* colonies makes them especially vulnerable to physical damage. *Lophelia pertusa* reefs are believed to be long-lived; reefs off Norway have been aged at 9000 yr (Hovland and Mortensen 1999), hence they may take thousands of years to recover if damaged by human activities.

1.3.1.1 Oil and gas exploitation

Less explored, but of interest for the present study, are the potential impacts on cold-water corals from offshore oil and gas exploitation. Such activities are increasingly moving into deeper areas, and concerns have been raised regarding the negative effects this may have on deep-water species including cold-water corals (Cimberg et al. 1981; Rogers 1999; Butler and Gass 2001; Hartley 2005; Skadsheim et al. 2005). The UK oil and gas industry has expanded beyond the North Sea into the deep-waters west of Shetland, an area with numerous reported occurrences of *L. pertusa* (Bett 2001; Gage 2001; Roberts et al. 2003). In 1999, Greenpeace took the UK government to court arguing, amongst other issues, that by opening this region, known as the Atlantic Frontier, to exploration for oil and gas it was not applying the EC Habitats Directives (92/43/EEC) out to the UK 200 mile EEZ and insufficient environmental assessments were being carried out prior to opening new areas for licensing. The argument was made based on the case of protecting cetaceans and reef-forming habitats such as cold-water corals; it brought much debate about the potential deleterious impacts of new oil and gas activities on *L. pertusa* (e.g. Roberts 1997; Gage 2001). The English High Court eventually ruled in favour of Greenpeace and the UK must now apply the EC
Habitats Directive out to the 200-mile EEZ\(^1\). In addition, the European Strategic Environmental Assessment Directive 2001/42/EC, incorporated into UK Law as of 2004, requires offshore areas of interest for future oil and gas developments to undergo a strategic environmental assessment (DTI 2006b).

It is not only in the UK where oil and gas development and cold-water corals are converging. The Brazilian oil company Petrobras is drilling at water depths greater than 2000 m. Surveys carried out by Petrobras led to the discovery of *L. pertusa* reefs at 800-1000 m in the Campos Basin (Fernandez et al. 2005). Additionally, oil and gas drilling and production is already under way in deep areas of the Gulf of Mexico, but only recently has information about the distribution and abundance of *L. pertusa* and other cold-water corals in these areas become available (Schroeder 2005). This has created new interest in the potential impacts on these coral occurrences near deep-water drilling in the Gulf of Mexico (Ahlfeld and Boland 2005). Finally, exploration licences off Nova Scotia now cover deep-water areas along the continental shelf and slope which coincide with documented occurrences of cold-water corals (Gass 2002).

Several aspects of offshore oil and gas activities could have deleterious impacts on cold-water corals, including *L. pertusa*. These include the emplacement and removal of platforms and pipelines in the vicinity of coral colonies, and the discharge of produced water and drilling muds and cuttings from platforms into the marine environment. The potential impacts of platforms, pipelines, and produced water are thought to be localized and relatively minor for benthic species and these are discussed briefly below. The potential impacts from drilling muds and cuttings are of greater concern, and

\(^{1}\) *Regina v Secretary of State for Trade and Industry, ex parte Greenpeace (No.2)*
experimental evaluation of the response of benthic species to drilling muds and cuttings has recently been highlighted as a research priority with respect to the influence of human activities on deep-water benthic environments (Smith et al. in press).

a) Platform Emplacement and Removal

Platform emplacement and pipeline laying could impact corals by directly crushing them, by increasing sedimentation levels in the water column as a result of the resuspension of naturally occurring bottom sediments, and by altering essential currents and nutrient flows, all reducing habitat suitability (Cimberg et al. 1981). Pipelines are thought to have limited impacts on benthic ecosystems (de Groot 1996). Platforms can act as artificial reefs for local benthos including *L. pertusa*; however, the current European legislation requires that all platforms must eventually be removed once production stops (OSPAR Decision 98/3 on the Disposal of Disused Offshore Installations). The removal of the platforms will cause the same potential damage as during its emplacement with the additional risks of contamination from disturbing cuttings piles (if they exist) and the direct removal of fouling organisms.

b) Produced Water

Produced water originates from the oil reservoir where it occurs intermixed with oil (Strømgren et al. 1995). The oil is separated from the water on the platform and several chemicals may be added during different stages of the separation process. Once separated and treated, the water is discharged into the marine environment as produced water. At the beginning of production, the amount of water produced with the oil is low, however, as the field ages the amount of water produced can be several times that of oil produced due to breakthrough of water from outside the reservoir and water injection to improve oil recovery (Strømgren et al. 1995). For example, the amount of produced
water discharged into the North Sea has recently increased with many fields slowing production and reaching completion (Henderson et al. 1999). Produced water is unlikely to be a concern for benthic species such as corals because it is usually discharged near the surface and will be highly diluted before reaching the seafloor (Strømgren et al. 1995; Holdway 2002; Berry and Wells 2004).

c) Drilling muds and cuttings

Drill cuttings are small pieces of rock that are created from drilling a well. They can vary in size and texture from fine silt to gravel (UKOOA 2001) and can contain naturally occurring heavy metals (GESAMP 1993; Breuer et al. 2004). Drilling muds are essential to drilling operations. They are used to remove cuttings from beneath the drill bit and bring them to the surface where they can be separated from the drilling mud for disposal. Their other functions are to lubricate and cool the drill bit and maintain hydrostatic pressure in the well hole to prevent blow-outs (GESAMP 1993). The three major types of drilling muds are water-based, oil-based, or synthetic oil-based muds depending on the principle liquid phase component (NRC 1983). Synthetic-oil based muds have recently replaced oil-based muds as they perform as well but are less toxic (Gray et al. 1999), particularly compared to oil-based mud containing diesel (Holdway 2002).

The constituents of drilling muds vary from well to well, depending on drilling conditions, and the management philosophy of the operator (Gettleson 1980; Steinhauer et al. 1992; Holdway 2002). There are over 100 products available (Holdway 2002). However, standard ingredients include a weighting agent such as barite (BaSO₄), viscosity agents such as bentonitic clays, surfactants and detergents, corrosion inhibitors, lubricants, biocides, and a variety of specialty additives for particular drilling
problems (GESAMP 1993). Heavy metals including mercury, chromium, zinc, cadmium, copper, lead and nickel are also present in components of drilling mud (GESAMP 1993).

The muds and cuttings are mixed during drilling activities. In order to separate the cuttings and reuse the muds, the fluid and cuttings mixture is passed through a mechanism that separates the two and the fluid is then returned to the tanks for recirculation (NRC 1983). As much of the mud as possible is recycled while, in the OSPAR region (the northeast Atlantic), the cuttings are either discharged to the seabed, taken ashore for treatment, or re-injected into wells depending on their oil content (UKOOA 2001). OSPAR Decision 2000/3 currently prohibits the discharge of cuttings containing more than 1 % oil, which is only achievable with the use of water-based muds. If cuttings are discharged to the seabed, they will be discharged continuously during drilling operations (Gettleson 1980; Steinhauer et al. 1992), drilling actually occurs approximately 30-50 % of the time during the drilling of a well (Gettleson 1980). The number of wells drilled per platform varies, for example, in the North Sea, 48 wells were drilled at North West Hutton (personal communication Catherine Denny, BP) and 27 wells at Tern Alpha (personal communication Ali Onder, Shell UK Ltd.). Drilling muds are discharged more sporadically. The solids control mechanism cannot remove the fine clay and colloidal particles that are generated during drilling through flocculation. As the fluid is recirculated the concentration of these fine particles continues to increase and eventually the fluid becomes too viscous for further use. At this time, a portion of the mud is discharged and the discharged volume is replaced with the principle liquid phase being used to bring the level of fine solids back to a workable level, then appropriate quantities of additives are added. The entire fluid system is discharged once drilling has been terminated (NRC 1983), and can be up to 200-500 m$^3$. 


in volume and the discharge can last between one and three hours (Gettleson 1980; Steinhauer et al. 1992; Neff 2005).

Brandsma et al. (1980) have created a model that demonstrates the fate of drilling discharges in the marine environment (Figure 1.2). The model shows the formation of two plumes; the lower plume contains the larger particulates from the drilling fluid, which are always denser than seawater and therefore sink to the bottom through a process of convective descent (less dense seawater moves upwards replacing the denser sinking drilling fluid). This lower plume accounts for 90% of the total drilling mud discharged (NRC 1983). The site depth, water currents, depth of discharge, and particle size and density of the original material will determine how quickly the lower plume will settle on the ocean floor, and the distance it will travel from the discharge site. In deeper water, the particles will travel greater distances as they take longer to reach the bottom (NRC 1983).

There is still the possibility that the particles from the lower plume will be resuspended and carried further away from the platform after they have settled on the seafloor. The length of time that settled material remains at the well site depends on environmental factors that govern sediment resuspension, transport and dispersion, such as water depth and energy regime (NRC 1983). In deeper water, the particles will tend to stay where they settle because there is less wave action (MacLaren Plansearch Ltd. 1997), and they can accumulate under the platform to form piles such as those in the northern North Sea (UKOOA 2001).
The upper plume, which consists of 10% of the original solids discharged, remains in the water column (NRC 1983). This upper plume is transported away from the well by the ambient currents. It has the potential to affect the biota in the water column, but it will experience large dilutions and will probably not adversely affect benthic environments (NRC 1983).

Adverse ecological impacts on benthic communities, including reduced community species diversity as a result of drill cuttings discharge have been documented and the most severe effects are observed close to the drilling sites (Davies et al. 1984; Daan et al. 1992; Olsgard and Gray 1995; Daan and Mulder 1996; Montagna and Harper Jr 1996; Grant and Briggs 2002). However, benthic contamination from drill cuttings has been observed up to 6 km from a platform (Olsgard and Gray 1995).

Both physical smothering from the rock cuttings and toxicity from heavy metals, hydrocarbons and/or organic enrichment contribute to observed negative effects from drilling muds and cuttings, but it is difficult to separate the degree to which each causes...
harm (Olsgard and Gray 1995). Tropical coral reefs are sensitive to increased sedimentation in general; excessive sedimentation can adversely affect physical and biological processes associated with reef ecosystems (Rogers 1990). Corals themselves use a number of passive and active mechanisms to deal with sediment influx, and threshold levels are species-specific (Bak and Elgershuizen 1976; Lasker 1980; Rogers 1990; Stafford-Smith and Ormond 1992). This is discussed further in Chapter 3. With the exception of Raimondi et al. (1997), no research has been done to look specifically at the above-mentioned effects on cold-water corals. However, research primarily from tropical corals has shown that drilling mud can affect polyp behaviour (Thompson et al. 1980), feeding (Szmant-Froelich et al. 1981), growth (Dodge 1982), tissue loss (Raimondi et al. 1997) and at certain concentrations can be lethal (Thompson and Bright 1980; Raimondi et al. 1997). It is not clear whether these effects from drilling discharges are a result of the physical smothering or toxic components, or synergistic effects of both. Table 1.1 presents the results from published studies on the effects of drilling muds on corals and demonstrates the range of responses observed to varying exposure levels.

The effects of hydrocarbons and heavy metals on coral reproduction

Several researchers have recognized the importance of reproductive biology and larval recruitment in any assessment of toxicological effects on corals (Loya and Rinkevich 1980; Dodge and Szmant-Froelich 1985). As drilling discharges contain hydrocarbons and heavy metals, the following studies are relevant to the potential effects of such discharges on corals.
<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Drilling fluid and length of exposure</th>
<th>Conc. (ppm)</th>
<th>Reaction at time x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thompson Jr. et al. (1980)</td>
<td><em>Porites divaricata</em></td>
<td>Freshwater ferrochrome lignosulfonate 96 h</td>
<td>100</td>
<td>No reaction 96 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>316</td>
<td>Completely retracted polyps and thin layer of mucus 24 h</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>Mucus 0.5 mm 48 h</td>
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<td></td>
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<td></td>
<td>Polyps normal expansion 72 h</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Same as above except no recovery at 96 h</td>
</tr>
<tr>
<td></td>
<td><em>Porites furcata</em></td>
<td>Freshwater ferrochrome lignosulfonate 96 h</td>
<td>100</td>
<td>Polyp retraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>316</td>
<td>Retraction and mucus 24 h</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>Showed signs of shedding mucus nearing 96 h</td>
</tr>
<tr>
<td></td>
<td><em>Porites astreoides</em></td>
<td>Freshwater ferrochrome lignosulfonate 96 h</td>
<td>100</td>
<td>Retraction and mucus (thicker) no signs of recovery</td>
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<td></td>
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<td>316</td>
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<td></td>
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<td></td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Montastrea annularis</em></td>
<td>Freshwater ferrochrome lignosulfonate 96 h</td>
<td>100</td>
<td>Complete retraction 17 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>316</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Agarica agaricites</em></td>
<td>Freshwater ferrochrome lignosulfonate 96 h</td>
<td>100</td>
<td>Mucus production</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>316</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>Dead 24 h</td>
</tr>
<tr>
<td></td>
<td><em>Dichocoenia stokesii</em></td>
<td>Freshwater ferrochrome lignosulfonate 96 h</td>
<td>100</td>
<td>No reaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>316</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acropora cervicornis</em></td>
<td>Freshwater ferrochrome lignosulfonate 96 h</td>
<td>100</td>
<td>Polyp retraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krone and Biggs (1980)</td>
<td><em>Madracis decacits</em></td>
<td>Drilling mud with ferrochrome lignosulfonate Mobile Bay, Alabama 17 d exposure</td>
<td>100</td>
<td>Increased ammonia excretion</td>
</tr>
<tr>
<td>Szmant-Froelich et al. (1981)</td>
<td><em>Monstastrea annularis</em></td>
<td>Drilling fluid, 6 week exposure</td>
<td>1</td>
<td>Normal feeding response</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>Normal feeding response</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>No feeding response</td>
</tr>
<tr>
<td>Dodge (1982)</td>
<td><em>Monstastrea annularis</em></td>
<td>Ferrochrome-lignosulfonate, 6 wk applied 4 times at 2.5 h intervals</td>
<td>2-4 mm layer</td>
<td>Decreased growth rate over 6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Raimondi et al. (1997)</td>
<td><em>Paracyathus stearnsii</em></td>
<td>Water-based drilling fluid Santa Maria Basin, California, 10 d exposure</td>
<td>0.02</td>
<td>No data for survivorship</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40 % tissue loss 8 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60 % drop in viability 8 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Survivorship was 90 % 10 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90 % tissue loss 8 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Viability decreased to 1 % 8 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Survivorship was 60 % 8 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100 % tissue loss 6 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Viability was 0 % 6 d</td>
</tr>
</tbody>
</table>
The effects of oil pollution on different phases of coral reproduction have shown a range of negative effects. Effects of chronic oil pollution on the tropical coral *Stylophora pistillata* from a reef near Eilat, Red Sea (Israel) showed smaller numbers of breeding colonies, a decrease in fecundity, and a lower settlement rate of planulae (Rinkevich and Loya 1977). Loya and Rinkevich (1979) carried out experiments in the laboratory exposing colonies of *S. pistillata* removed from the reefs during its peak reproductive period and exposed them to a range of concentrations of Iranian crude oil (0.1 to 10 ml l\(^{-1}\)). The results showed that all concentrations of oil initiated higher extrusion of planulae than the control corals, and the response was always immediate. Loya and Rinkevich (1980) argued that shedding planulae during an oil spill would significantly reduce their survival because direct exposure to oil can cause morphological damage (planulae can develop incomplete mesenteries), and changes to the physical properties of the reef flat may inhibit their settlement. This could explain why chronically oil-polluted reefs, such as the reefs in the Eilat nature reserve, are not experiencing colonization, whereas high colonization rates are observed on control reefs (Loya and Rinkevich 1980).

Guzman and Holst (1993) examined the long term effects of an oil spill on the reproduction of *Siderastrea siderea* four to five years after a spill in the Caribbean Sea off the coast of Panama. No significant differences were found between the percent of reproductive male and female colonies on oiled reefs compared with unoiled reefs; neither was there a significant difference between the number of female gonads per polyp between the exposed and control reefs. However, gonad size was larger in corals at unoiled reefs compared to oiled reefs, which Guzman and Holst (1993) concluded indicated lower fecundity due to stress.
Heavy metals from oil and gas discharges can also have adverse effects on coral reproduction and recruitment. Increased copper concentrations (42 to 200 µg l\(^{-1}\)) reduced and/or inhibited larval settlement of *Acropora tenuis* on the Great Barrier Reef, Australia (Reichelt-Brushett and Harrison 2000). Concentrations of copper (20 to 200 µg l\(^{-1}\)) also reduced fertilization success up to 41 % for *Goniastrea aspera*, whereas similar concentrations of zinc and cadmium had no effect (Reichelt-Brushett and Harrison 1999).

### 1.4 Corals and bio-indicators

Oil and gas developments in deep-water areas have potential to increase contaminant heavy metals in the marine environment. Deep-water dumping of sewage sludge and dredge spoil can also contribute to anthropogenic sources of heavy metals to the deep sea (Glover and Smith 2003). Moreover, the deep sea and its inhabitants are ultimately sinks for persistent and toxic human pollutants (Smith et al. in press). Human activities on land can also eventually lead to increased metals in deep-water environments via atmosphere-ocean interactions (Clark 2003).

Tropical corals have been used to look investigate past and present levels of contaminants in coastal environments. Corals accrete their calcium carbonate skeletons in relation to the chemical and physical state of their ambient environment; they are sessile and long-lived, so they can act as bio-indicators of past and present levels of metals in seawater (Hanna and Muir 1990; Tudhope et al. 1996; Scott and Davies 1997; Esslemont 2000). For the purpose of this thesis, a bio-indicator is defined as a biological organism that can be used to monitor the condition of a marine environment through time. Corals have provided historical information on the rise and fall of metals in the marine environment, e.g. anthropogenic sources of lead (Dodge and Gilbert 1984; Shen
and Boyle 1987; Medina-Elizalde et al. 2002), and they can monitor increases in heavy metals from contaminated sites (Guzman and Jimenez 1992; Esslemont 2000; Fallon et al. 2002; David 2003; Runnalls and Coleman 2003; Ramos et al. 2004)

Cold-water corals could provide marine bio-indicators with a much wider global distribution than tropical corals and they offer potential as archives of deep environments that are difficult and expensive to access. They could also be used to monitor specific sites of industrial development, such as oil and gas extraction activities, or long-term increases in heavy metal concentrations in deep-water from non-point source pollutants. Cold-water coral skeletons have been studied for their potential as climate recorders using stable isotopes of carbon and oxygen, and temperature-induced variations in Sr/Ca and Mg/Ca (Montagna et al. 2005; Sherwood et al. 2005a; Shirai et al. 2005; Sinclair et al. 2005), and by using $^{14}$C and uranium-thorium dating (Adkins et al. 1998; Goldstein et al. 2001). But this study is the first attempt to explore their potential as bio-indicators of pollution in the marine environment. The level of contaminants found within coral skeletons may also be directly linked to physiological changes in the organisms themselves. For example, coral skeletons recording high levels of contaminant metals may also show slower growth rates or weaker skeletal structures.

1.5 *Lophelia pertusa* in the North Sea

For over 40 years, the North Sea has been a well-known region for oil and gas development, and recently, *L. pertusa* has been documented growing on several of the older oil and gas structures found there. The first record of *L. pertusa* in the North Sea described a dead specimen caught in a bottom trawl at a depth of 56 m (Wilson 1979a). No other records were made until 1999 when *L. pertusa* was discovered colonising the
Brent Spar oil storage buoy during its controversial decommissioning (Bell and Smith 1999). Further investigations found *L. pertusa* living on the Beryl single point mooring (Pearce 1999; Roberts 2002) and on an actively drilling Brent Alpha platform where colonies were observed partially covered in drilling muds/cuttings (Roberts 2000b). These findings lead to questions about the actual sensitivity and potential impacts of oil and gas drilling on *L. pertusa* (Edwards 1997; Roberts 1997; Rogers 1997; Bell and Smith 1999; Roberts 2000a).

Finding *L. pertusa* on oil and gas platforms in the North Sea provides a unique opportunity to investigate questions about its sensitivity to oil and gas activities and creates a 20+ year natural experiment in an accessible environment with visual data available from annual remotely operated vehicle (ROV) inspections. These inspections also provide an excellent means for collecting samples of *L. pertusa* from the platforms.

This study sets out to examine the environmental sensitivity of *L. pertusa*. Environmental sensitivity for the purpose of this thesis is defined as the ability of an organism to deal with changing environmental conditions. The present study is specifically examining how *L. pertusa* responds to changing conditions directly caused by oil and gas drilling discharges. In addition, the presence of *L. pertusa* on oil and gas platforms offered several opportunities to further the current understanding of environmental controls on its distribution, growth rates, skeletal patterns, recruitment to the North Sea, and its reproductive biology.

### 1.6 Thesis Structure

*Lophelia pertusa* had been reported from oil and gas infrastructure in the North Sea, but the extent of its occurrences on North Sea structures was unknown. Chapter 2 presents a
detailed description of its occurrence in the North Sea based on its presence in industry-run platform survey videos and photographs. Its vertical distribution and size class distribution were analysed from the Heather and North Alwyn A platforms. Coral colonies growing on Tern Alpha were analysed for in situ colony growth rates, and samples of corals were taken from a selection of platforms, including colonies exposed to drilling discharges, were examined for reproductive structures. Finally, videos were examined for visual evidence of coral colony exposure to drilling muds and cuttings. These results are also presented in Chapter 2.

Partial and full colony mortality was observed as a result of exposure to drilling muds and cuttings in some areas of the platforms. The level of exposure was impossible to deduce from videos, so experiments using living polyps of *L. pertusa* were designed to investigate polyp behavioural responses and tolerance limits to sedimentation events, and to examine polyp active sediment clearing mechanisms. Chapter 3 presents the results from these investigations.

The second half of the thesis deals primarily with skeletons of *L. pertusa* sampled from sites on North Sea platforms exposed to drilling muds and cuttings and potential contamination from cuttings piles, and skeletons sampled from control sites located away from drilling discharges and cuttings piles. Chapter 4 presents results based on measurements of skeletal features including morphological variations between control and exposed colonies from the North Sea platforms. Growth patterns including thecal banding are presented, and coral budding in relation to growth rates is discussed. Chapter 5 presents results from a stable isotope analysis of carbon and oxygen skeletal signatures using both bulk sub-sampling and higher resolution sub-sampling within thecal growth bands. The results from Chapters 4 and 5 propose a skeletal chronology
for *L. pertusa* from the North Sea, which is directly applied to the methods developed in
Chapter 6.

Chapter 6 presents the final set of thesis results derived from analyses assessing the
trace metal content of coral skeletons from exposed and control sites on North Sea oil
platforms. *Lophelia pertusa* was also sampled from the Sea of Hebrides near the island
of Mingulay, Scotland for an additional control site and natural reef comparison. These
analyses measured concentrations of heavy metals in coral skeletons to test for exposure
to drilling muds and cuttings. The results are discussed in relation to cold-water corals
as bio-indicators of marine pollution in deep-water environments. Finally, Chapter 7
provides a final overview and discussion of the results.

1.7 Thesis Aims

1. To assess and describe the extent of the distribution of *L. pertusa* in the North
Sea.
2. To examine the vertical zonation of *L. pertusa* in relation to hydrography and
habitat requirements.
3. To examine recruitment and growth rates of *L. pertusa*.
4. To visually assess the extent of exposure to drilling muds and cuttings of the
coral colonies on the platforms.
5. To assess if coral samples are reproductive despite their close proximity to
drilling discharges.
6. To examine if *L. pertusa* is following the previously documented reproductive
periodicity.
7. To investigate the behavioural response of *L. pertusa* to influxes of clean sediment as a first step towards understanding its ability to cope with drilling muds and cuttings.

8. To examine skeletal growth patterns of *L. pertusa*.

9. To examine whether skeletal morphology differs between colonies sampled from sites exposed to drilling discharges and control sites.

10. To investigate seasonal patterns in stable carbon and oxygen isotopes along the growth axis of *L. pertusa* and to examine stable isotope signatures from thecal growth bands.

11. To examine the potential of *L. pertusa* as a bio-indicator of marine pollution by measuring trace metals in coral skeletons sampled from exposed and control sites.

12. To test for exposure of *L. pertusa* to biologically available heavy metals from drilling discharges.
Chapter 2 –

The Occurrence of the Cold-water Coral *Lophelia pertusa* (Scleractinia) on Oil and Gas Platforms in the North Sea: Colony Growth, Recruitment, Reproduction and Environmental Controls on Distribution

2.1 Introduction

The following chapter is an extended version of the paper Gass and Roberts (2006) “The occurrence of the cold-water coral *Lophelia pertusa* (Scleractinia) on oil and gas platforms in the North Sea: Colony growth, recruitment and environmental controls on distribution” published in Marine Pollution Bulletin (Appendix 1).

2.1.1 Distribution

This chapter considers the occurrence of *L. pertusa* on oil platforms in the North Sea where, until recently, *L. pertusa* had not been recorded live. The known distribution of *L. pertusa* is described in Chapter 1. The only published record of this species from the UK sector of the North Sea described a dead specimen brought up in a trawl northeast of Buckie, Scotland (Wilson 1979a). The North Sea is a well-developed region of oil and gas production. The first well was drilled in the UK sector in 1964 (UKOOA 2005) and in 2003, when this study began, there were 240 fields in production and over 2000 production wells, 1500 exploration wells, and 1000 appraisal wells had been drilled (de Groot 1996; DTI 2006a). The first reports of live *L. pertusa* from the North Sea came in the late 1990s when it was found living on oil and gas infrastructures. It was first reported during the decommissioning of the Brent Spar oil storage buoy (Bell and Smith 1999), followed by its identification during visual surveys of infrastructure in the Beryl
field (Pearce 1999; Roberts 2002), and in 2000, it was found on the actively drilling Brent Alpha platform (Roberts 2000b). The full extent of the occurrence of *L. pertusa* in the North Sea has not yet been assessed. Therefore, the first aim of this chapter is to assess and describe the extent of the distribution of *L. pertusa* on oil and gas infrastructure in the UK Sector of the North Sea, both to investigate controls on coral distribution and to provide study sites for the work described in subsequent chapters.

### 2.1.2 Environmental Controls

In addition to assessing the broad distribution of *L. pertusa* on platforms in the North Sea, its presence on the platforms offers a unique vertical zonation from the surface to the seabed to examine some of the proposed environmental controls on its distribution, which are described in Chapter 1. Briefly, habitat requirements for cold water corals including hard substrata for larval settlement, regions with enhanced currents which keep substrata clean of sediment, and nutrient-rich waters that provide a reliable food supply (Dons 1944; Grigg 1974; Wilson 1979b; Genin et al. 1986; Frederiksen et al. 1992; Freiwald 2002). The distribution of *L. pertusa* appears limited to oceanic water masses with a temperature and salinity range of 4 to 12°C and 33-37 PSU respectively (Freiwald 2002), and the vertical force of wave action could also control its minimum depth limit (Frederiksen et al. 1992). There is also a suggested link with hydrocarbon seepage (Hovland et al. 1997), a hypothesis that remains controversial and with little reported evidence (Rogers 1999; Roberts et al. 2006). Therefore, the second aim of this chapter is to examine the vertical zonation of *L. pertusa* in relation to hydrography and habitat requirements.
2.1.3 Growth

In addition to providing a good opportunity for examining the vertical zonation of *L. pertusa*, the platforms also act as a 20-30 yr settlement experiment from which to investigate coral growth and recruitment. Little is known about the growth of *L. pertusa* and *in situ* growth rates have never been recorded through time. Several estimates of linear growth rates ranging from 5 to 26 mm yr\(^{-1}\) have been made based on settlement on artificial substrates (Duncan 1877; Wilson 1979b; Bell and Smith 1999; Roberts 2002; Roberts et al. 2003). Seasonal signals from stable isotopes of carbon and oxygen (Mikkelsen et al. 1982; Freiwald et al. 1997; Mortensen and Rapp 1998) and aquarium observations (Mortensen 2001) have also been used to derive linear growth rates. The third aim of this chapter is for the first time to examine recruitment and growth rates of *L. pertusa* in the field.

2.1.4 Environmental sensitivity and reproduction

The presence of *L. pertusa* on platforms, including observations of drill cuttings on living coral colonies (Roberts 2000b), leads to questions about its environmental sensitivity. Rogers (1999) outlined potential impacts on *L. pertusa* from oil and gas exploitation including the physical and toxic effects of discharged drill cuttings to the seabed and the toxic effects of oil contamination. Further details on the potential negative impacts from oil and gas exploitation on corals are outlined in Chapter 1. Additionally, several researchers have recognized the importance of reproductive biology for the assessment of potential toxicological effects on corals (Loya and Rinkevich 1980; Dodge and Szmant-Froelich 1985). Pollution in the form of oil and heavy metals has been shown to negatively affect coral reproduction (Loya and Rinkevich 1979; Loya and Rinkevich 1980; Guzman and Holst 1993; Reichelt-Brushett and Harrison 1999, 2000).
Waller and Tyler (2005) have examined the reproductive biology of *L. pertusa* in the North Atlantic. Their results suggested that *L. pertusa* spawns in the winter months; however, due to the difficulty of collecting samples in the winter, data are limited to the spring, summer and autumn months (Waller and Tyler 2005). Because samples collected over winter were available from the North Sea, the final aim of this chapter is to examine if *L. pertusa* in the North Sea is following the predicted reproductive periodicity documented by Waller and Tyler (2005).

The presence of *L. pertusa* on the legs of oil and gas platforms in light of the potential negative impacts may be an indication that it has a certain level of tolerance to these potential sources of contamination. However, the extent to which corals on oil and gas platforms are actually exposed to drilling discharges is unclear (Roberts 2000a).

Summary of chapter aims:

1. To assess and describe the extent of the distribution of *L. pertusa* in the North Sea.
2. To examine the vertical zonation of *L. pertusa* in relation to hydrography and habitat requirements.
3. To examine recruitment and growth rates of *L. pertusa*.
4. To visually assess the extent of exposure to drilling muds and cuttings of the coral colonies on the platforms.
5. To assess if coral samples are reproductive despite their close proximity to drilling discharges.
6. To examine if *L. pertusa* is following the previously documented reproductive periodicity.
2.2 Methods

2.2.1 Study Area – The North Sea

The North Sea is loosely divided into the shallow southern North Sea (0-50 m depth), the central North Sea (50-100 m), deeper northern North Sea (100-200 out to the edge of the continental shelf), and the deep Norwegian Trench and Skagerrak (up to 700 m) (OSPAR 2000). The circulation in the North Sea, showing the North Atlantic inflows has been described by Turrell (1992b) (Figure 2.1). The southern North Sea is a mixture of North Atlantic and fresh water inputs and surface temperatures fluctuate between 4 – 14°C. It remains well mixed by tidal action throughout the year. The deeper regions of the North Sea contain primarily pure North Atlantic water and surface temperatures range between 5-16 °C. The deeper areas remain well mixed in winter but there is a marked stratification during the summer as surface waters warm but depths below 50-60 m remain cold. The North Sea has a spring plankton bloom followed by a smaller bloom in the autumn as early winter mixing which brings nutrients from depth to the surface. The surficial geology of the North Sea consists primarily of soft bottom types with a mixture of mud and sandy mud in the deeper depressions and the remaining seabed consisting of sand or coarse sand and gravel (Eisma 1981).
2.2.2 Video Analysis

Videotapes and still images recorded by remotely operated vehicles (ROVs) during oil industry platform inspections were provided following a request for sightings of *L. pertusa* sent to all hydrocarbon companies working in the UK sector of the North Sea. Existing information indicated that *L. pertusa* was most commonly found on platforms in the northern region of the North Sea, rather than the central or southern regions, so the present investigation concentrated on this area (Bell and Smith 1999; Roberts 2002; personal communication D. McKay, Subsea 7).

Two platforms, Heather Alpha (Heather) and North Alwyn Alpha (NAA), were chosen to examine the depth distribution and colony size of *L. pertusa* based on video footage of conductors. Conductor surveys were not available from other platforms. Conductors are wide-diameter pipes that run from the drilling platform to the seabed to guide drilling and transport drilling fluid or oil. Conductors are the simplest component of the
platform to survey as they are straight pipes of known diameters that run from the topsides of the platform at the surface to the seabed. As the videos were reviewed, the water depth of each colony was recorded and its diameter estimated from the widest portion of the colony on the video screen in relation to the diameter of the conductors provided by the oil company.

Fifty percent of the Heather conductors’ surface area was examined because the ROV only surveyed one side of the conductors from whichever angle was most accessible to the ROV. The NAA platform conductors were surveyed in a spiralling fashion to maximize the area viewed during the survey. It is estimated that this spiralling survey allowed approximately 75% of the conductor to be viewed (personal communication N. Duncan, Total). Colony density on each conductor was standardized to the area viewed and was recorded as the number of colonies per square metre. The area of the conductors was calculated as $2\pi rh$ (where $r =$ radius of conductor and $h =$ height of conductor; the conductor radii were provided by the oil companies and height was taken from ROV depth readings). Coral colony densities were compared between the two platforms using two-sample t-tests where the null hypothesis is that the two sample means come from a population with the same mean (Dytham 2003).

2.2.2.1 Growth Rates

Growth rates of *L. pertusa* colonies were estimated from colonies on the Tern Alpha platform in the northern North Sea. Videotapes of inspection surveys during Sept-1993, Jan-1994, June-1998, and May-2002, were reviewed and the same 15 individual colonies were identified over time using depth and easily identifiable structural components of the platform, such as sacrificial anodes, to locate the colonies. The 15 colonies were the only colonies which could be identified with certainty from more than
one inspection survey. Again, colony diameter was estimated relative to the known
diameter of the platform members, and colony extension rates were calculated as the
extension of the colony radius over the relevant number of months (to the nearest half
month), and then normalised to a yearly extension rate. Settlement dates were estimated
by dividing the size of the colonies in 2002 by their annual extension rates.

Samples were taken from the platforms to confirm species identification. Historical
hydrographical data for the northern North Sea, chosen from locations within 50 km of
the platforms being analysed, were obtained from the British Oceanographic Data
Centre (BODC). The Fisheries Resource Services Marine Laboratory, Aberdeen,
collected the hydrographical data from 1988-1999.

2.2.3 Coral Sampling

Coral colonies growing on oil and gas platforms in the northern North Sea were
sampled opportunistically in collaboration with oil and gas companies and their
associated ROV survey companies. Sampling details are presented in Table 2.1.

<table>
<thead>
<tr>
<th>Operator</th>
<th>Platform</th>
<th>Survey company/Ship</th>
<th>Survey Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell UK Ltd.</td>
<td>Tern Alpha</td>
<td>Subsea 7/MSV</td>
<td>May-June 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seisranger</td>
<td></td>
</tr>
<tr>
<td></td>
<td>North Cormorant</td>
<td>Subsea 7/MSV</td>
<td>May-June 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seisranger</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brent Delta</td>
<td>Subsea 7/MSV</td>
<td>May 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seisranger</td>
<td></td>
</tr>
<tr>
<td>Lundin Britain Ltd.</td>
<td>Thistle</td>
<td>Rovtech Ltd./N/A</td>
<td>May 2003</td>
</tr>
<tr>
<td>BP</td>
<td>North West Hutton</td>
<td>Subsea 7/N/A</td>
<td>December 2003</td>
</tr>
<tr>
<td></td>
<td>(NWH)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In all cases, except for sampling from NWH, written instructions were provided along
with a briefing to ROV pilots detailing the sampling protocol (Appendix 2). I conducted
the coral sampling from NWH following the same guidelines as supplied for the other
cases. Colonies of *L. pertusa* were sampled from sites exposed to drilling muds and cuttings and from control sites free from drilling muds and cuttings. Control sites were chosen as areas on the platform away from drilling discharge points, for example, corals growing on non-drilling structures such as buoys or moorings, corals located far from cuttings piles, or corals located far from and upstream of discharge pipes. Exposed sites were areas close to or below drilling discharge points where there was visual evidence of drilling muds and cuttings, or close to drill cuttings piles. Twenty-five colonies (11 control and 13 exposed) were sampled. Four control colonies were sampled from North Cormorant, four control from Thistle, and three control from NWH. Four exposed colonies were sampled from Tern Alpha, two exposed from Brent Delta, two exposed from Thistle, and six exposed from NWH. Table 2.2 summarizes the coral colonies that were sampled and the criteria used for their selection.

The majority of colonies were sampled using a net and scraper attached to an ROV (Figure 2.2). The colonies were scraped from the platform structures, caught in the net, and brought to the surface. One white and one orange colony were sampled with each dive. The two exceptions were colonies NWH1 and NWH9, which were sampled during decommissioning of the conductors on NWH. As the conductors were lifted to the surface and pulled through the guide frames, the colonies were scrapped off and subsequently caught in the ROV net as they fell through the water column.
Table 2.2 Samples of *L. pertusa* from five platforms in the North Sea

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample ID</th>
<th>Platform</th>
<th>Collection Date</th>
<th>Depth (m)</th>
<th>Polyp Colour</th>
<th>Exposed/Control</th>
<th>Sample Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NC1</td>
<td>North Cormorant</td>
<td>16 June 2003</td>
<td>109</td>
<td>White</td>
<td>Control</td>
<td>Depths and location away from discharges</td>
</tr>
<tr>
<td>2</td>
<td>NC2</td>
<td>North Cormorant</td>
<td>15 June 2003</td>
<td>102</td>
<td>Orange</td>
<td>Control</td>
<td>Depths and location away from discharges</td>
</tr>
<tr>
<td>3</td>
<td>NC3</td>
<td>North Cormorant</td>
<td>16 June 2003</td>
<td>85</td>
<td>Orange</td>
<td>Control</td>
<td>Depths and location away from discharges</td>
</tr>
<tr>
<td>4</td>
<td>NC4</td>
<td>North Cormorant</td>
<td>18 June 2003</td>
<td>102</td>
<td>White</td>
<td>Control</td>
<td>Depths and location away from discharges</td>
</tr>
<tr>
<td>5</td>
<td>TA1</td>
<td>Tern Alpha</td>
<td>23 June 2003</td>
<td>107</td>
<td>Orange</td>
<td>Exposed</td>
<td>Sample approx. 3-4 m below and east of the termination of the Hazardous Drains Caisson 43-010. Visual signs of contamination</td>
</tr>
<tr>
<td>6</td>
<td>TA2</td>
<td>Tern Alpha</td>
<td>23 June 2003</td>
<td>153</td>
<td>White</td>
<td>Exposed</td>
<td>Depth below discharge. The drill cuttings chute terminates at 116 m. Specimen taken from –153 m. Visual signs of contamination.</td>
</tr>
<tr>
<td>7</td>
<td>TA3</td>
<td>Tern Alpha</td>
<td>23 June 2003</td>
<td>151</td>
<td>Orange</td>
<td>Exposed</td>
<td>In vicinity of the drill cuttings chute 43-001, visual signs of contamination</td>
</tr>
<tr>
<td>8</td>
<td>TA4</td>
<td>Tern Alpha</td>
<td>23 June 2003</td>
<td>155</td>
<td>White</td>
<td>Exposed</td>
<td>In vicinity of the drill cuttings chute 43-001</td>
</tr>
<tr>
<td>9</td>
<td>BD1</td>
<td>Brent Delta</td>
<td>15 May 2004</td>
<td>83</td>
<td>White</td>
<td>Exposed</td>
<td>Muds visible in area, 20 m below cuttings chute, most likely areas where corals would be exposed to muds</td>
</tr>
<tr>
<td>10</td>
<td>BD2</td>
<td>Brent Delta</td>
<td>15 May 2004</td>
<td>83</td>
<td>Orange</td>
<td>Exposed</td>
<td>Muds visible in area, 20 m below cuttings chute, most likely areas where corals would be exposed to muds</td>
</tr>
<tr>
<td>#</td>
<td>Location</td>
<td>Site</td>
<td>Date</td>
<td>Depth below discharge</td>
<td>Notes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----------</td>
<td>------</td>
<td>------------</td>
<td>-----------------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>TH1</td>
<td>Thistle</td>
<td>24 May 2003</td>
<td>145 Orange Exposed</td>
<td>Depth below discharge which terminated at 60 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>TH2</td>
<td>Thistle</td>
<td>24 May 2003</td>
<td>154 Orange Exposed</td>
<td>Depth below discharge which terminated at 60 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>TH3</td>
<td>Thistle</td>
<td>24 May 2003</td>
<td>84 Orange Control</td>
<td>Depth above discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>TH4</td>
<td>Thistle</td>
<td>24 May 2003</td>
<td>87 White Control</td>
<td>Depth above discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>TH5</td>
<td>Thistle</td>
<td>25 May 2003</td>
<td>93 Orange Control</td>
<td>Depth above discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>TH6</td>
<td>Thistle</td>
<td>25 May 2003</td>
<td>93 White Control</td>
<td>Depth above discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>NWH1</td>
<td>NWHutton</td>
<td>06 December 2003</td>
<td>100 Orange Exposed</td>
<td>Depth below discharge, sampled from conductor closest to drilling discharge chute which terminated at 60 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>NWH2</td>
<td>NWHutton</td>
<td>08 December 2003</td>
<td>136 White Exposed</td>
<td>2 m above cuttings pile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>NWH3</td>
<td>NWHutton</td>
<td>08 December 2003</td>
<td>132 Orange Exposed</td>
<td>6 m above cuttings pile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>NWH4</td>
<td>NWHutton</td>
<td>08 December 2003</td>
<td>82 Orange Exposed</td>
<td>Depth below discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>NWH5</td>
<td>NWHutton</td>
<td>08 December 2003</td>
<td>48 White Control</td>
<td>Depth above discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>NWH6</td>
<td>NWHutton</td>
<td>08 December 2003</td>
<td>47 Orange Control</td>
<td>Depth above discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>NWH7</td>
<td>NWHutton</td>
<td>08 December 2003</td>
<td>78 White Exposed</td>
<td>Depth below discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>NWH8</td>
<td>NWHutton</td>
<td>08 December 2003</td>
<td>47 White Control</td>
<td>Depth above discharge. Samples may be mixed up with samples from 114 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>NWH9</td>
<td>NWHutton</td>
<td>06 December 2003</td>
<td>100 Orange Exposed</td>
<td>Depth below discharge</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2.4 *Histological protocol and reproductive structures*

Sub-samples of coral colonies sampled from the platforms were fixed and stored in a buffered 4% formaldehyde solution for histological investigations of the reproductive status of the coral samples. A selection of colonies were analysed for the presence of reproductive structures, including the majority of colonies sampled from the Shell platforms and all colonies sampled from the NWH platform. Training in the histological techniques was provided by Dr. Rhian Waller (Woods Hole Oceanographic Institute) who carried out initial histological investigations on the coral samples from the Tern Alpha, North Cormorant, and NWH platforms. Subsequently, I completed a replicate investigation on the NWH colonies. Initially, five polyps were examined from each colony; however, due to time constraints it was decided that only two polyps would be examined. Two to five coral polyps per colony were removed from formalin and rinsed in alcohol to remove all traces of formalin. Polyps were then soaked in a rapid decalcifier solution until the calcium carbonate skeleton was completely dissolved. The coral polyp tissue was removed from the decalcifier solution, rinsed in fresh water, and placed in 50% propan-2-ol. The tissue was dehydrated using an automatic embedder involving a series of submersions in 50 to 100% propan-2-ol for three to four hours each, cleared with xylene for 12 h, followed by submersion in paraffin wax kept at 65
°C for 6 to 12 h. The polyp was then poured into a standard mould and placed in the freezer to set.

To test for the presence of reproductive structures, three sections were taken from each polyp: 5 µm sections of the wax blocks were sliced to make up histological slides leaving 400 µm between each to examine tissue from the middle and each side of the polyp. Sections were stained with Masson’s Trichrome stain. Sections of each individual were examined under a Zeiss Axioskop 2 Plus microscope for the presence of gametes. When a female colony was found, ten additional polyps were sectioned for visual examination, and three of these were serially sliced (5 µm sections) every 50 µm to obtain egg size measurements. Dr. Rhian Waller provided data on the Feret diameter (the diameter of the egg if it were spherical) of 100 eggs for each of these three female polyps. Photographs taken under the Zeiss Axioskop 2 Plus microscope were imported into Sigma Scan Pro Version 4 to measure oocyte Feret diameter.

2.3 Results

2.3.1 Distribution and colony description

Fourteen of the 15 platforms examined in the northern North Sea were colonised by L. pertusa; all colonised platforms were installed between 1975 and 1988 and located in the region of the North Sea with depths greater than 100 m (Figure 2.3). Lophelia pertusa was not identified on the Scott platform installed in 1993. Records to date of living L. pertusa in the North Sea are presented in Table 2.3. Anecdotal reports from ROV personnel carrying out platform inspection surveys throughout the North Sea note that L. pertusa is less abundant on platforms in the central North Sea and is not seen on platforms in the southern North Sea (personal communication D. McKay, Subsea 7).
Samples taken from six platforms confirmed the species identification as *L. pertusa*. If unobstructed, this coral generally formed distinctive hemispherical-shaped colonies and grew around any obstructions. Two colour varieties of polyps were observed, orange and white, which occasionally overlapped where two colonies merged, or where one colony apparently settled on the other (Figure 2.4). The coral skeletons had protruding septa and some visible growth banding in the thecal wall and septa. Polyp shape and size varied between colonies with some long, slender corallites with thin thecal walls and others with shorter corallites and thicker theca. Colony morphology is explored further in Chapter 4. Coral budding was intra-tentacular. Polyp density appeared relatively high compared to samples from natural reefs (personal observation), and neighbouring offspring often fused together forming complex structural branching patterns (Figure 2.5).
Figure 2.3 Oil and gas platforms in the UK sector of the North Sea. Those with confirmed presence of *L. pertusa* are in the northern North Sea.
Table 2.3 Oil and gas infrastructure in the North Sea where *L. pertusa* has been recorded

<table>
<thead>
<tr>
<th>Platform</th>
<th>Operator</th>
<th>Installation Date</th>
<th>Platform Type</th>
<th>Type of Fluid</th>
<th>Field Depth (m)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alwyn North</td>
<td>Total</td>
<td>May-85</td>
<td>Steel</td>
<td>Oil/Gas</td>
<td>125</td>
<td>Present Study</td>
</tr>
<tr>
<td>Beryl Alpha</td>
<td>Exxon Mobil</td>
<td>July-75</td>
<td>Steel</td>
<td>Oil/Gas</td>
<td>119</td>
<td>Roberts, 2002</td>
</tr>
<tr>
<td>Flare Support Tower</td>
<td>Exxon Mobil</td>
<td>July-75</td>
<td>Steel</td>
<td>Oil/Gas</td>
<td>119</td>
<td>Roberts, 2002</td>
</tr>
<tr>
<td>Beryl SPM 2</td>
<td>Exxon Mobil</td>
<td>1976</td>
<td>SPM</td>
<td>-</td>
<td>119</td>
<td>Roberts, 2002</td>
</tr>
<tr>
<td>Beryl SPM 3</td>
<td>Exxon Mobil</td>
<td>1976</td>
<td>SPM</td>
<td>-</td>
<td>119</td>
<td>Roberts, 2002</td>
</tr>
<tr>
<td>Brae Bravo</td>
<td>Marathon</td>
<td>May-87</td>
<td>Steel</td>
<td>Oil</td>
<td>102</td>
<td>Present Study</td>
</tr>
<tr>
<td>Brent Alpha</td>
<td>Shell UK</td>
<td>May-76</td>
<td>Steel</td>
<td>Oil/Gas</td>
<td>142</td>
<td>Roberts, 2000</td>
</tr>
<tr>
<td>Brent Bravo</td>
<td>Shell UK</td>
<td>August-75</td>
<td>CGBS</td>
<td>Oil/Gas</td>
<td>140</td>
<td>Present Study</td>
</tr>
<tr>
<td>Brent Delta</td>
<td>Shell</td>
<td>July-76</td>
<td>CGBS</td>
<td>Oil/Gas</td>
<td>~140</td>
<td>Present Study</td>
</tr>
<tr>
<td>Brent Spar</td>
<td>Shell UK</td>
<td>1976</td>
<td>Steel</td>
<td>-</td>
<td>-</td>
<td>Bell and Smith, 1999</td>
</tr>
<tr>
<td>Claymore Talisman</td>
<td>June-76</td>
<td>Steel Oil</td>
<td>111</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dunlin Shell UK</td>
<td>June-77</td>
<td>CGBS Oil</td>
<td>151</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eider Shell UK</td>
<td>May-88</td>
<td>Steel Oil</td>
<td>159</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heather Lundin Britain</td>
<td>May-77</td>
<td>Steel Oil</td>
<td>144</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnus BP</td>
<td>April-82</td>
<td>Steel</td>
<td>Oil</td>
<td>190</td>
<td>Present Study</td>
<td></td>
</tr>
<tr>
<td>North Cormorant Shell UK</td>
<td>May-81</td>
<td>Steel Oil</td>
<td>160</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NW Hutton BP</td>
<td>September-81</td>
<td>Steel Oil</td>
<td>146</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tartan Talisman</td>
<td>June-79</td>
<td>Steel Oil</td>
<td>141</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tern Alpha Shell UK</td>
<td>May-88</td>
<td>Steel Oil</td>
<td>167</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thistle Lundin Britain</td>
<td>August-76</td>
<td>Steel Oil</td>
<td>160</td>
<td>Present Study</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CGBS, Concrete Gravity Based Structure; SPM, Single point mooring

Figure 2.4 Domed-shaped orange and white colonies of *L. pertusa* on the Heather platform taken from > 60 m depth (photo courtesy of Lundin Britain Ltd.).
2.3.2 Vertical zonation

The depth distributions of the colonies on the Heather and NAA showed that most of the colonies were found between 80 and 120 m (Figure 2.6). The minimum and maximum depths for the Heather conductors were 59 m and 132 m with a mean depth of 106 m. The minimum and maximum depths of colonies on NAA conductors were 62 m and 118 m with a mean depth of 95 m. The shallowest record of *L. pertusa* during the study was from 47 m on the NWH platform.

2.3.3 Hydrographical parameters in the northern North Sea

BODC temperature and salinity profiles taken from within 50 km of a platform identified with *L. pertusa* (Figure 2.7) show well-mixed winter conditions and stratified summer conditions. *Lophelia pertusa* colonies were found below 60 m depth reflecting temperatures between seven and 11 °C and salinities between 35 and 35.3 PSU.
Figure 2.6 The depth distribution of *L. pertusa* colonies on conductors from (A) the Heather conductors and (B) the North Alwyn Alpha (NAA) platforms. Field depth at NAA is 125 m and at Heather is 144 m.
Figure 2.7 (A) Temperature and (B) salinity profiles from six stations in the northern North Sea within 50 km of NAA and Heather platforms. Straight lines represent winter CTDs and non straight lines represent summer CTDs. The dashed line represents the upper depth limit of *L. pertusa* on NAA and Heather.
2.3.4 Growth and recruitment

Nineteen conductors of varying ages were examined from NAA and five from Heather (Table 2.4). The number of colonies of sufficient size to be visible on video varied between two and 22 on NAA, and between 98 and 224 on Heather. No coral colonies were visible on two of the NAA conductors installed in 1995 and 1999. Coral colony density varied by an order of magnitude (0.02 to 0.39 per m$^2$) between conductors on NAA, and ranged between 1.23 and 2.8 per m$^2$ on Heather conductors (Table 2.4). There is a significant difference between the mean densities from the full range of depths from the two platforms based on results from a two-sample t-test (p<0.05) (data normally distributed with homogeneous means). The maximum colony diameter varied widely (SD = 34 %) for the colonies on the NAA conductors and less so for the colonies on the Heather conductors (SD = 16 %). There was also a significant difference between the largest colonies on NAA and Heather (two-sample t-test p<0.001). The size distribution of colonies from the NAA and Heather platforms was unimodal left skewed with several exceedingly large colonies and a lack of very small colonies (Figure 2.8). There was no relationship found between the depth of occurrence and the size of colonies. The largest colonies were re-examined to see whether two or more colonies could have merged to form what might have appeared as one single colony. No obvious visual evidence for this was found as the colonies formed smooth uniform domed-shapes, the largest of which was 132 cm in diameter from the Heather platform (Figure 2.9). There was no relationship between colony size and depth (linear regression $r^2 = 0.125$; p>0.05).

2.3.4.1 Growth rates

Assuming that the largest colony was the first to colonize the conductor, minimum colony extension rates were calculated based on the conductor installation date.
Extension rates based on these estimates were between 6 and 26 mm yr\(^{-1}\) from NAA colonies, and between 24 to 33 mm yr\(^{-1}\) for Heather colonies (Table 2.4).

Table 2.4 The number, size, estimated extension rate, and density of \textit{L. pertusa} colonies on conductors examined from 2002 inspection surveys from NAA and Heather

<table>
<thead>
<tr>
<th>Platform</th>
<th>Conductor no.</th>
<th>Conductor installation date</th>
<th>No. of colonies</th>
<th>Max. diameter (cm)</th>
<th>Min extension rate (cm yr(^{-1}))</th>
<th>Density (no. colonies m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA 4</td>
<td>1986</td>
<td>5</td>
<td>39</td>
<td>1.2</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>NAA 5</td>
<td>1986</td>
<td>2</td>
<td>38</td>
<td>1.2</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>NAA 7</td>
<td>1986-87</td>
<td>7</td>
<td>52</td>
<td>1.8</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>NAA 8</td>
<td>1986-87</td>
<td>14</td>
<td>41</td>
<td>1.4</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>NAA 11</td>
<td>1986-87</td>
<td>38</td>
<td>44</td>
<td>1.5</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>NAA 14</td>
<td>1986</td>
<td>2</td>
<td>18</td>
<td>0.6</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>NAA 16</td>
<td>1986-87</td>
<td>12</td>
<td>66</td>
<td>2.3</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>NAA 22</td>
<td>1986-87</td>
<td>18</td>
<td>40</td>
<td>1.4</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>NAA 23</td>
<td>1986</td>
<td>6</td>
<td>42</td>
<td>1.3</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>NAA 25</td>
<td>1989</td>
<td>12</td>
<td>51</td>
<td>2.0</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>NAA 26</td>
<td>1986</td>
<td>7</td>
<td>77</td>
<td>2.6</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>NAA 27</td>
<td>1986-87</td>
<td>14</td>
<td>51</td>
<td>1.8</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>NAA 28</td>
<td>1986</td>
<td>22</td>
<td>52</td>
<td>1.7</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>NAA 30</td>
<td>1987</td>
<td>16</td>
<td>11</td>
<td>0.4</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>NAA 32</td>
<td>1986-87</td>
<td>30</td>
<td>54</td>
<td>1.9</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>NAA 34</td>
<td>1987</td>
<td>15</td>
<td>58</td>
<td>2.0</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>NAA 35</td>
<td>1987</td>
<td>19</td>
<td>46</td>
<td>1.6</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>14 ± 10</td>
<td>46 ± 16</td>
<td>1.6 ± 0.6</td>
<td>0.14 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Heather 18</td>
<td>1981</td>
<td>177</td>
<td>118</td>
<td>2.8</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>Heather 25</td>
<td>1982</td>
<td>212</td>
<td>103</td>
<td>2.6</td>
<td>2.65</td>
<td></td>
</tr>
<tr>
<td>Heather 36</td>
<td>1982</td>
<td>224</td>
<td>132</td>
<td>3.3</td>
<td>2.80</td>
<td></td>
</tr>
<tr>
<td>Heather 38</td>
<td>1982</td>
<td>137</td>
<td>94</td>
<td>2.4</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>Heather 39.5</td>
<td>1988</td>
<td>98</td>
<td>93</td>
<td>3.3</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>170 ± 52</td>
<td>108 ± 17</td>
<td>2.9 ± 0.4</td>
<td>2.12 ± 0.65</td>
<td></td>
</tr>
</tbody>
</table>

Survey inspection videos were provided from the Tern Alpha platform with video surveys from 1993, 1994, 1998, and 2002. Colony extension rates were assessed for 15 specimens of \textit{L. pertusa} identified in either 1993 or 1998 and again in 2002 (Table 2.5).

An example of a colony seen in 1993 and then again in 2002 is shown in Figure 2.10.

Settlement dates were estimated by dividing the size of the colonies in 2002 by their annual extension rates. This showed initial settlement from 1988, when the platform
was installed, and annual colonization events until 1995. It is likely that corals that settled after 1995 were not yet clearly identifiable on the videos.

Figure 2.8 The size class distribution of *L. pertusa* on (A) Heather Alpha and (B) North Alwyn A.
Figure 2.9 Video frame grab of the largest colony recorded measuring 132 cm in diameter and located at 94 m depth on the Heather platform.

Table 2.5 Growth rates of 15 L. pertusa colonies between 1993, 1998, and 2002 from Tern Alpha

<table>
<thead>
<tr>
<th>Colony</th>
<th>Depth (m)</th>
<th>No. of months between measurements</th>
<th>Extension rate (mm yr⁻¹)</th>
<th>Estimated settlement date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>115</td>
<td>105</td>
<td>19</td>
<td>1988</td>
</tr>
<tr>
<td>2</td>
<td>115</td>
<td>104.5</td>
<td>24</td>
<td>1989</td>
</tr>
<tr>
<td>3</td>
<td>115</td>
<td>104.5</td>
<td>21</td>
<td>1991</td>
</tr>
<tr>
<td>4</td>
<td>115</td>
<td>104.5</td>
<td>33</td>
<td>1992</td>
</tr>
<tr>
<td>5</td>
<td>115</td>
<td>104.5</td>
<td>24</td>
<td>1991</td>
</tr>
<tr>
<td>6</td>
<td>115</td>
<td>104.5</td>
<td>34</td>
<td>1991</td>
</tr>
<tr>
<td>7</td>
<td>94</td>
<td>100.5</td>
<td>22</td>
<td>1988</td>
</tr>
<tr>
<td>8</td>
<td>96</td>
<td>47</td>
<td>26</td>
<td>1989</td>
</tr>
<tr>
<td>9</td>
<td>108</td>
<td>47</td>
<td>19</td>
<td>1991</td>
</tr>
<tr>
<td>10</td>
<td>112</td>
<td>47</td>
<td>27</td>
<td>1993</td>
</tr>
<tr>
<td>11</td>
<td>112</td>
<td>47</td>
<td>34</td>
<td>1994</td>
</tr>
<tr>
<td>12</td>
<td>106</td>
<td>47.5</td>
<td>25</td>
<td>1990</td>
</tr>
<tr>
<td>13</td>
<td>110</td>
<td>47.5</td>
<td>32</td>
<td>1995</td>
</tr>
<tr>
<td>14</td>
<td>110</td>
<td>47.5</td>
<td>31</td>
<td>1989</td>
</tr>
<tr>
<td>15</td>
<td>115</td>
<td>47.5</td>
<td>21</td>
<td>1992</td>
</tr>
</tbody>
</table>

Mean ± SD  

26 ± 5
2.3.5 Reproduction

Sixteen colonies, four from the North Cormorant, two from Tern Alpha, two from Brent Delta, and eight from NWH, were investigated for the presence of reproductive structures. Only two colonies, both sampled from NWH in December 2003, were reproductive: one male and one female (Table 2.6). Oocytes were measured in three of the female polyps and varied between 68-150 µm, mean Feret diameter of the oocytes for the three polyps were 108 ± 14(SD), 96 ± 15(SD) and 110 ± 16(SD) µm. The eggs were of a size expected in the later stages of development: Stage III Vitellogenic > 30 µm to Stage IV Late Vitellogenic > 140 µm, based on the staging scale described by Waller and Tyler (2005). Figure 2.11 shows images of the female oocytes and male spermacysts from colonies NWH4 and NWH6.
Table 2.6 The reproductive status of 16 colonies of *L. pertusa* sampled from the North Sea

<table>
<thead>
<tr>
<th>Colony ID</th>
<th>No. of polyps</th>
<th>Date sampled</th>
<th>Polyp wet weight (g)</th>
<th>Reproductive Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC1</td>
<td>5</td>
<td>June 2003</td>
<td>0.11, 0.13, 0.07, 0.14, 0.04</td>
<td>NR</td>
</tr>
<tr>
<td>NC2</td>
<td>5</td>
<td>June 2003</td>
<td>0.24, 0.37, 0.24, 0.05, 0.22</td>
<td>NR</td>
</tr>
<tr>
<td>NC3</td>
<td>5</td>
<td>June 2003</td>
<td>0.35, 0.19, 0.11, 0.29, 0.30</td>
<td>NR</td>
</tr>
<tr>
<td>NC4</td>
<td>5</td>
<td>June 2003</td>
<td>0.28, 0.18, 0.21, 0.14, 0.09</td>
<td>NR</td>
</tr>
<tr>
<td>TA1</td>
<td>5</td>
<td>June 2003</td>
<td>0.05, 0.06, 0.04, 0.03, 0.02</td>
<td>NR</td>
</tr>
<tr>
<td>TA2</td>
<td>5</td>
<td>June 2003</td>
<td>0.19, 0.19, 0.17, 0.06, 0.27</td>
<td>NR</td>
</tr>
<tr>
<td>NWH1</td>
<td>2</td>
<td>Dec 2003</td>
<td>0.18, 0.08</td>
<td>NR</td>
</tr>
<tr>
<td>NWH2</td>
<td>2</td>
<td>Dec 2003</td>
<td>0.15, 0.11</td>
<td>NR</td>
</tr>
<tr>
<td>NWH3</td>
<td>2</td>
<td>Dec 2003</td>
<td>0.17, 0.12</td>
<td>NR</td>
</tr>
<tr>
<td>NWH4</td>
<td>12</td>
<td>Dec 2003</td>
<td>0.34, 0.2, 0.01, 0.21, 0.13, 0.28, 0.43, 0.31, 0.06, 0.16, 0.20, 0.28</td>
<td>8-Female; 4 NR</td>
</tr>
<tr>
<td>NWH5</td>
<td>2</td>
<td>Dec 2003</td>
<td>0.36, 0.06</td>
<td>NR</td>
</tr>
<tr>
<td>NWH6</td>
<td>3</td>
<td>Dec 2003</td>
<td>1.05, 0.22, 0.38</td>
<td>3-Male</td>
</tr>
<tr>
<td>NWH7</td>
<td>2</td>
<td>Dec 2003</td>
<td>0.26, 0.03</td>
<td>NR</td>
</tr>
<tr>
<td>NWH8</td>
<td>2</td>
<td>Dec 2003</td>
<td>0.22, 0.12</td>
<td>NR</td>
</tr>
<tr>
<td>BD1</td>
<td>2</td>
<td>May 2004</td>
<td>0.15, 0.17</td>
<td>NR</td>
</tr>
<tr>
<td>BD2</td>
<td>2</td>
<td>May 2004</td>
<td>0.03, 0.03</td>
<td>NR</td>
</tr>
</tbody>
</table>

Figure 2.11 Reproductive structures found in two colonies of two *L. pertusa* colonies sampled from North West Hutton in December 2003.
2.3.6 Evidence of contamination

In this study, *L. pertusa* colonies appeared in good condition with polyps fully expanded for feeding in the majority of videos and video frame grabs examined. However, colonies contaminated by drilling muds and cuttings were seen on both Tern Alpha and Heather. Tern Alpha colonies were sampled from exposed sites and exposure to drilling muds/cuttings was evident from discoloration of colonies, accumulated sediment on coral skeletons and up to 30% polyp mortality was estimated visually. This contamination was limited to colonies growing close to drilling muds/cuttings discharge chutes. Visual signs of contamination were also observed on coral colonies on Heather conductors. These conductors were grouped together 1.5 m apart with the drilling cuttings discharge chute located within the conductor group and discharging at 107 m depth. Five conductors, three located directly next to the cuttings chute and two located two conductors away from the chute, were examined from video recorded in 2002. All coral colonies observed on these conductors between 107 m and 122 m (after which no colonies were found) were partially or completely covered in drill cuttings/muds. Colonies located shallower than 107 m appeared clean, except for colonies on the conductor to the southwest where 100% of the colonies examined between 107 m and 85 m showed visual signs of contamination on the lower half of the colonies. This indicates some upward movement of discharges in addition to the downward settlement that produces the large cuttings pile that had accumulated under the platform.

2.4 Discussion

2.4.1 Distribution of *Lophelia pertusa* in the North Sea

Before the present study, there were two published records of *L. pertusa* on oil and gas infrastructure in the North Sea but it was unclear whether these were exceptional occurrences. The results from the present study revealed *L. pertusa* on 14 more
platforms in the northern North Sea. Platforms in the central and southern North Sea were not examined; however, anecdotal observations by sub-sea survey engineers suggest *L. pertusa* is also found in the central North Sea but at lower densities, and not in the southern North Sea (personal communication D. McKay, Subsea 7). The lower densities in the central North Sea may result from larvae having further to travel (see section 2.4.2). Depth, influence from fresh water river inputs, and both the high and variable temperatures in the southern North Sea are not suitable for *L. pertusa* and therefore it is unlikely that it will ever colonize structures located in this region.

Several environmental controls on the distribution and growth of *L. pertusa* have been proposed including its requirement for hard substrate for larval settlement (Freiwald 2002). Although the northern North Sea satisfies the majority of the required criteria, the surficial geology in the North Sea consists of mud, sand and some coarse sand and gravel (Eisma 1987) and so lacks significant-areas of hard substrata. It was not until the installation of the oil and gas platforms in the 1970s that hard substrata became readily available and this may explain the lack of records of *L. pertusa* in the North Sea prior to the development of the North Sea oil industry.

2.4.1.1 Hydrographical controls on coral distribution

Temperature exerts a strong control on the distribution of *L. pertusa* which is related to the presence of oceanic water between certain depths (Dons 1944; Freiwald 2002; Roberts et al. 2003). Seawater in the northern North Sea is of suitable oceanic origin (northeast Atlantic) and enters from the north flowing south to the central North Sea (Turrell 1992a). An upper limit (60 m) on the vertical distribution of *L. pertusa* was observed on the NAA and Heather platforms that coincides with the well-documented summer stratification in the northern North Sea (Turrell 1992b). Oceanic seawater in the
northern North Sea is altered as spring and summer conditions warm surface waters, creating a distinct thermocline at approximately 50 m water depth. Roberts (2002) made the same interpretation to explain the vertical distribution of *L. pertusa* within the Beryl field in the northern North Sea, and Dons (Dons 1944) reported that the minimum depth of occurrence for corals off Norway was limited by the thermocline.

In the summer, surface waters in the North Sea can reach between 13 and 16 °C (Otto et al. 1990; OSPAR 2000; BODC data this study). These temperatures are not only above the highest known temperature for *L. pertusa* of 12 °C (Freiwald 2002), but they also show up to an 8 °C fluctuation between summer and winter temperatures. *Lophelia pertusa* is most commonly found at depths greater than 200 m where it will only experience changes in temperature of 1 or 2 °C. For example, results of a lander deployment by a *L. pertusa* reef off Norway showed temperature fluctuations between 7.33 and 8.35 °C over a three week deployment (Roberts et al. 2005b). The force of wave action and related current speeds may be a significant control on in the vertical distribution of *L. pertusa* on the platforms, as has been suggested for corals in the northeast Atlantic (Frederiksen et al. 1992). Increased turbulence from wave action in the northern North Sea may dislodge recently recruited corals at the shallower depths most affected by wave action. In the northern North Sea, severe storms are intense enough to resuspended sediments on the seabed to depths of 100 m (Tyler et al. 1999; OSPAR 2000).

The BODC data showed salinity levels varying between 35.1 and 35.3 PSU, increasing with depth as less dense water gathers near the surface. Salinities around 35 PSU are suitable for *L. pertusa*, and thus salinity is unlikely to vary significantly to act as a significant factor controlling depth distribution in the northern North Sea.
2.4.2 Growth and recruitment

The colony size distribution from both Heather and NAA shows a continuous distribution of size classes with few exceedingly large colonies and few very small colonies. The lack of small colonies probably corresponds to the difficulty of identifying with certainty such colonies on the survey video. *Lophelia pertusa* was not seen on two NAA conductors installed in 1995 and 1999 and it therefore seems unlikely that colonies younger than six or seven years can be clearly identified on videos. This is in accordance with the results from the Tern Alpha growth study where the most recent colonization was also reported from 1995.

Large colonies from the size class distributions on Heather, one of the oldest platforms in the northern North Sea first installed in 1977, probably represent the first coral recruits to the platforms and possibly early recruits to the North Sea. The first explanation for the low number of large colonies is that many could have already become detached from the platform for two possible reasons: 1) the physical characteristics of the platforms, i.e. the rate of decay of paint coatings and corrosion of the steel platforms; and 2) colony size and weight relative to the strength of the colony attachment point(s). It is unlikely that corals have been physically removed by cleaning operations to remove marine fouling as this has not been routinely practiced on either Heather (personal communication Aly Lyon, Lundin Britain Ltd.) or NAA (personal communication Neil Duncan, Total). The paint used to protect the underlying steel will eventually degrade and the steel will begin to corrode making colony attachment unstable. In addition, if colonies have started surpassing the maximum size and weight that the attachment point(s) can support, then colonies will be lost from the platform. The remaining large colonies observed in the size class distributions, therefore, likely represent those with the strongest attachment point(s) and/or those attached to the best-
preserved parts of the conductors. A large detached colony seen in a still photograph lying on top of the Heather cuttings pile provides some evidence to support this interpretation.

A second explanation for the lower number of large colonies relates to the likely northeast Atlantic origin of the early recruits to the platforms. In comparison with shallow-water corals, little is known about the reproductive biology of *L. pertusa* and no larvae have ever been sampled. However, these occurrences in the North Sea provide good evidence for a planktonic larval stage (Roberts 2002). As there was no evidence for living *L. pertusa* in the North Sea before the development of the offshore oil and gas industry, the recruits to the North Sea platforms have probably come from larvae transported in the northeast Atlantic water entering the North Sea. The possible pathway for coral larvae to reach the northern North Sea is through the substantial inflow of Atlantic water flowing southwards east of Shetland from the Atlantic shelf edge current and the Fair Isle Current (Roberts 2002). These currents and circulation patterns have also been used to explain the dispersal of fish larvae such as Atlantic herring (*Clupea harengus*) that hatch from spawning grounds to the north and west of Scotland (Heath 1990) and must cross the northern North Sea to reach nursery grounds in the Skagerrak and southern North Sea (Bartsch et al. 1989).

Populations of *L. pertusa* are found west of Scotland (Roberts et al. 2003) and could be source populations for recruits to the North Sea. It is possible that the largest colonies seen on Heather were early recruits from west of Scotland but only a small percentage of larvae remained competent to settle. As more corals colonised the platform and became reproductive, it is possible that because there are spawning corals present in the North Sea that more larvae will be present resulting in a steady input of recruits as seen
in the colony size distribution. This is supported by the histological studies that showed two reproductive *L. pertusa* colonies, one male and one female, among samples taken from the NWH platform in the northern North Sea in December 2003 (see reproduction discussion below).

The NAA size class distribution shows a similar trend to that of Heather; however, there are fewer colonies (densities on NAA are 0.14 versus 2.12 colonies m\(^{-2}\) on Heather) and the colonies are smaller. This difference cannot be explained solely by the difference in conductor age (NAA conductors are approximately five years younger than Heather conductors, and the NAA platform is seven years younger than Heather). Other possible explanations for NAA having fewer colonies relate to platform coatings and colony recruitment. Heather conductors are painted but not with anti-fouling paint. The NAA conductors are painted and, according to the Total Alwyn Area ROV Inspection Manual, some older conductors have additional unspecified coatings, possibly with anti-fouling properties. Therefore, potential anti-fouling coatings could have deterred the corals from settling on the platform.

Moreover, the location of the Heather platform may provide a higher chance of larval encounter and settlement compared to NAA. The major currents entering the North Sea are from the northwest, north of Shetland, and travel southwards into the central North Sea (Turrell 1992b). Within the group of oil and gas platforms in the northern North Sea, Heather is located the furthest to the west and NAA to the southeast with several other platforms located between NAA and Heather, and to the north of NAA. Coral larvae from the North Atlantic will have had further to travel and more opportunities to settle on suitable substrata prior to reaching NAA as compared to Heather. The situation is similar to a study that examined fouling on the Montrose and Forties platforms in the...
central North Sea, and reported significantly higher concentrations of *Mytilus edulis* on Forties as compared to Montrose (Forteath et al. 1982). It was suggested that, although similar currents carry *M. edulis* larvae passed these two platforms, the length of time it takes to reach the platforms and, the three to four week competency of the larvae, may explain the lack of *M. edulis* on Montrose, which is located approximately 40 km southeast of Forties (Forteath et al. 1982).

The smaller colonies on NAA may also reflect the anti-fouling properties of the paint. Not only will this affect the overall number of colonies, but also there will be a delay in settlement affecting the age and size of the colonies present. Another factor to consider is that the growth rates of colonies on NAA are slower than colonies on Heather; however, there is no reason to assume that the different location of Heather and NAA would result in more or less nutrient supply to the corals.

The size distribution of the corals on NAA shows a similar trend to Heather. The difference lies in that the rise in number of colonies occurs at a smaller size class (approximately 50 cm diameter compared with 70 cm diameter for Heather). The explanation given for Heather was that the rise in colonies related to the onset of self-recruitment occurring on Heather, but taking into account the points discussed above regarding the smaller colonies on NAA, this rise in numbers of colonies could reflect a wider-spread self-recruitment occurring in the northern North Sea.

**2.4.3 Growth rates**

*In situ* growth rates of *L. pertusa* have not been previously examined because of the inherent difficulties associated with studying these animals at great depths in their natural habitats. The visual records of 15 colonies through time on Tern Alpha
presented in this study provided the first *in situ* *L. pertusa* growth rates and revealed annual recruitment events on the platforms. Other growth estimates of between 5 and 26 mm yr\(^{-1}\) have been made based on colonization of artificial substrata such as submarine cables (Duncan 1877; Wilson 1979b), shipwrecks (Roberts et al. 2003) as well as the Brent Spar oil storage buoy (Bell and Smith 1999) and the Beryl single point mooring (Roberts 2002). Since the colonization events must have happened after the hard substrata was introduced, the maximum time that coral growth could have taken place is known and thus the minimum possible growth rates estimated. Additional growth rate estimates for *L. pertusa* are based on skeletal carbon and oxygen stable isotopes and vary from 5 mm yr\(^{-1}\) (Mortensen and Rapp 1998) to 25 mm yr\(^{-1}\) (Mikkelsen et al. 1982; Freiwald et al. 1997; Spiro et al. 2000), and fall within the similar range of growth estimates from the artificial substrata.

The results in the present study showed a linear extension estimate of 26 mm yr\(^{-1}\) from the Tern Alpha growth study and up to 33 mm yr\(^{-1}\) from the largest colonies on the Heather platform. These extension rates are similar to the rate suggested by Bell and Smith (1999) based on colonies from Brent Spar in the northern North Sea. Linear extension rates reported by Roberts (2002) of 5 mm yr\(^{-1}\) from the Beryl single point mooring in the North Sea were based on a sample taken from the structure that was not selected to represent the largest colony on the structure and could have settled well after the mooring had been installed. It is possible that other lower growth rates of 8 mm yr\(^{-1}\) (Duncan 1877), 6 mm yr\(^{-1}\) (Wilson 1979b) and 12 mm yr\(^{-1}\) (Roberts et al. 2003) also represent underestimates of the true growth rates. If growth rates of *L. pertusa* are higher in the North Sea, this could be related to colonies living higher in the water column where more surface organic primary production will be available, and above any deleterious effects caused by sediment resuspension. Furthermore, competition for
space on the platforms may drive corals to extend more quickly while compromising the skeletal wall thickness. There was no visual evidence that colonies used in this growth study had been exposed to drill muds or cuttings. It is not known whether growth rates of colonies living near drilling discharge points are affected by the increased sediment and possible toxicological stress.

### 2.4.4 Reproduction

Platform structural inspection surveys using ROVs are carried out in the North Sea during spring and summer months. As a result, the majority of the coral colonies sampled during this study were also sampled at this time. There was one exceptional opportunity where coral colonies from the NWH platform were sampled in December 2003 as it was being decommissioned with an ROV available on the platform. According to Waller and Tyler (2005), *L. pertusa* in the North Atlantic has a seasonal reproductive cycle producing a single cohort where oogenesis is initiated in the late summer and *L. pertusa* likely spawns in January/February. Waller and Tyler (2005) came to these conclusions based on corals collected from the Porcupine Seabight west of Ireland over a period of four months. The first samples collected in March had no gametes present, followed by evidence of increasing egg size from coral fragments sampled in August, September, and October. Male gametes were only seen from fragments sampled in October (Waller and Tyler 2005). If *L. pertusa* colonies in the North Sea follow the same reproductive cycle as those from the Porcupine Seabight, then this could explain the lack of reproductive activity in coral colonies sampled in June 2003. The two reproductive colonies sampled in December 2003 from the present study provide further evidence that colonies in the North Sea are following the same seasonal cycle as those in the Porcupine Seabight. The corals showed an egg size (between 68-150 µm) equivalent to that of later egg development stages (Stage III to
Stage IV as per staging scale by Waller and Tyler (2005)), which were the expected size if spawning were to occur in January/February.

Not all colonies sampled in December were reproductive and only eight of 12 polyps examined were reproductive from the female colony. This may be a result of natural variation, for example, the reproductive status of polyps in scleractinian corals is influenced by colony size, polyp size and the position of the polyps within the colony (Rinkevich and Loya 1987; Hall and Hughes 1996; Sakai 1998; Goffredo et al. 2002). Although exact colony sizes are unknown, colony size is not likely a controlling factor for the NWH colonies that were examined in this study. The colonies sampled were not particularly small compared to average colony size on NWH. Furthermore, non-reproductive colonies were larger than the reproductive colony. Polyps also need to reach a certain size and maturity before they become reproductive and this size varies from species to species (Hall and Hughes 1996). Waller and Tyler (2005) found that all *L. pertusa* polyps weighing less than 0.08 g showed no evidence of gametogenesis. This could explain why two of the NWH polyps, which weighed 0.01 and 0.06 g, were non-reproductive. NWH1, NWH7, and NWH5 also each had one small polyp weighing less than 0.08 g (wet weight). However, this still leaves two other polyps that were non-reproductive from NWH4 although above the weight threshold (0.13 g and 0.28 g).

Evidence exists showing negative impacts on scleractinian coral reproduction because of exposure to oil pollution and trace metals (see Chapter 1 for more details). Rinkevich and Loya (1977) found a smaller number of breeding colonies on oil polluted reefs compared to on a clean control reef. Drilling has not occurred on the NWH platform since 1992 and production stopped in January 2003. Therefore, the colonies that would be most likely affected by toxic stress would be those living close to the cuttings pile.
where they could be exposed to contaminants leaching from the sediments or from sediment resuspension events. Both colonies NWH2 and NWH3 were situated 2 m and 6 m from the cuttings pile and were non-reproductive. However, further research on a larger sample size of polyps and colonies is needed to distinguish between the natural variation between polyps and colonies and their and negative effects caused by exposure to drilling discharges.

### 2.4.5 Evidence of contamination

Concerns over the deleterious human impacts on *L. pertusa* have to date concentrated on the impacts from bottom fishing (Dons 1944; Fosså et al. 2002; Hall-Spencer et al. 2002). However, concerns about the impacts from petroleum exploitation on cold water corals, including *L. pertusa*, have also been raised (Cimberg et al. 1981; Roberts 1997; Rogers 1997; Rogers 1999; Butler and Gass 2001). A major concern relating to oil and gas exploitation is the increased levels of sedimentation caused either by physical disturbances from anchoring, laying pipelines and oil rig emplacement and/or from drill muds and cuttings discharged close to coral colonies (Cimberg et al. 1981; Dodge and Szmant-Froelich 1985). Thus, it is surprising to find numerous colonies of *L. pertusa* growing directly on oil and gas platforms in the North Sea. Scleractinian coral species have also been reported on oil and gas platforms in the Gulf of Mexico (Sammarco et al. 2004).

*Lophelia pertusa* appears to be able to grow on platforms in areas above and away from muds and cuttings discharge pipes. However, evidence of contamination and polyp mortality were observed from colonies living in the vicinity of discharge points. Partial colonies are able to exist in these environments where polyps on the tops or bottoms of colonies are fully expanded while the other half of the colony is dead. It is likely that
physical smothering by drilling muds and cuttings caused the lethal effects, but it remains unknown if, and to what degree, coral colonies were affected by the toxicity of hydrocarbons, metals, or other potentially toxic additives found in drill muds.

There are many factors affecting the fate of drilling muds and cuttings discharged into the marine environment. It appears that the depth of the discharge pipe dictates which colonies will be most affected on the Heather platform. Other contributory factors include the speed and direction of currents at the time of discharge, wave regime, eddies caused by water flow around the platform itself, depth of the thermocline, rate of discharge, and duration of discharge (Thompson et al. 1980). In situ measurements of drilling mud concentrations at varying distances from the discharge point on a oil platform in the Gulf of Mexico showed a maximum dilution rate to occur within 6 m, after which the dilution factor decreases slowly with increasing distance from the platform (Shinn et al. 1980). This could explain the heightened contamination observed within close proximity to the discharge source if a similar magnitude of dilution was also occurring in the North Sea.

2.4.6 Study limitations

The study was limited by whether a company responded to information requests and whether they were willing to co-operate by supplying survey videos. As a result, several platforms in the northern region of the North Sea were not investigated for the presence of L. pertusa. In addition, platforms from the Norwegian sector of the North Sea were not considered. Furthermore, video quality may have limited identification of small colonies of L. pertusa, and sometimes only certain components of the platforms are included in each survey. Despite these limitations, it has become clear that L. pertusa is
growing on the majority of older platforms in the northern North Sea and it has probably colonised younger platforms.

The presence of colonies was documented on 14 platforms in the northern North Sea, and their vertical distribution was examined in more detail on Heather and NAA. In all cases, the area of the platforms where *L. pertusa* was present was examined, but its absence from other areas was not documented. Future work involving a complete assessment of a platform for the presence and absence of *L. pertusa* may elucidate a pattern as to where corals are and are not growing in relation to point-source drilling discharges and height above cuttings piles. As not all parts of the platform are surveyed annually, several years of video surveys would have to be made accessible to be able to view a large enough surface area of the platform to carry out such an analysis. Alternatively, a specific survey would be needed, but with ROV vessel survey costs of up to £150 k a day, this is not likely.

Sampling *L. pertusa* in collaboration with oil and gas companies during platform inspection surveys resulted in high quality samples which have also been used for the studies presented in Chapters 4, 5, and 6. The number of samples taken from the NWH platform, which were likely to be useful for the reproduction study, was limited by the available ROV time and winter weather conditions. There were several days when the swell at the sea surface was too great to risk deploying the ROV. This is a limitation that will exist every winter in the North Sea; success at overcoming this limitation is directly related to the number days the researcher can allocate to sampling, and the number of days the ROV is scheduled to work.
A more detailed study looking at the reproductive status, including fecundity and egg size, of a greater number of polyps from each colony, and comparing more colonies from sites exposed to drilling discharges to colonies from clean control sites would help further the understanding on the effects of oil and gas related pollution on the reproduction of *L. pertusa*. This may be possible if more sampling time is allocated for winter sampling and/or if a multi-year study is initiated to gather samples from several consecutive years. However, it will remain difficult to find such opportunities where ROVs will be available on platforms in the winter, as this is a rare occurrence, only available to this study because NWH was being decommissioned. Despite the sample size limitations, the results from the reproductive study have shown that the population of *L. pertusa* on oil and gas platforms in the North Sea has the potential to reproduce sexually, and this helps in the understanding of potential population growth and with interpreting the importance of this new population in a wider context. See Chapter 7 for a further discussion on these points.

In several instances coral colonies showed exposure to drilling muds and cuttings and polyp mortality. In some cases, the colonies appeared to be able to withstand such influxes of sediments, and in other cases large portions of the colonies were dead. The sedimentation rates around different corals on the platforms are unknown. There are many difficulties associated with attempting to measure such rates *in situ*, although it could be considered for future work. The next chapter uses laboratory studies to uncover further information on the limits of *L. pertusa*’s ability to cope with known rates of sediment influx, and active mechanisms, if any, it uses to cope with heightened sedimentation, such as it might experience from discharges of drill cuttings.
Chapter 3  –

The Effects of Sedimentation on the Polyp Behaviour of *Lophelia pertusa*: Laboratory Experiments

3.1 Introduction

Tropical coral reefs are experiencing increased sedimentation caused by human activities including dredging, increased runoff, mining, and discharged waste from hydrocarbon exploitation (Dodge and Sz mant-Froelich 1985; Rogers 1990; Fallon et al. 2002; Fabricius 2005). Deleterious effects on coral reefs as a result of increased sedimentation have been documented from all major tropical reefs worldwide (Rogers 1990; Stafford-Smith and Ormond 1992; Fabricius 2005). Sedimentation is a controlling factor in reef development including coral abundance and cover, coral species distribution (Brown et al. 1990; McClanahan and Obura 1997; Gleason 1998), and colony size structure (Wittenberg and Hunte 1992). High species diversity is normally limited to areas with low sedimentation rates, i.e. < 10-20 mg cm$^{-2}$ d$^{-1}$ (Rogers 1990; Stafford-Smith 1990). However, Stafford-Smith (1990) suggested that there are many very diverse reefs with high coral cover on the fringing reefs of the Great Barrier Reef (Australia) where sedimentation rates are reported to be much higher, i.e. up to 250 mg cm$^{-2}$ d$^{-1}$. Not all coral species are sensitive to high sedimentation environments, and both naturally turbid tropical environments and some that experience high levels of sedimentation may host a suite of sediment-tolerant species (McClanahan and Obura 1997; Gleason 1998).

Although some species show a higher tolerance to heightened levels of sedimentation, corals are ultimately sessile animals, unable to move out of harms way and, as
suspension feeders, require clear polyps which can expand to capture food. Consequently, individual corals will suffer deleterious effects from a certain level of sedimentation. The amount of sedimentation required to cause such effects is species-specific (Bak and Elgershuizen 1976; Lasker 1980; Thompson et al. 1980; Stafford-Smith and Ormond 1992; Stafford-Smith 1993). Observed effects include polyp death and tissue necrosis (Thompson 1980; Rogers 1983; Wesseling et al. 1999; Wesseling et al. 2001; Fabricius 2005) and changes in a coral’s energy budget including increased respiration relative to photosynthetic production by algal symbionts (Dallmeyer et al. 1982; Riegl and Branch 1995; Fabricius 2005). Changes in metabolic costs will depend on the coral’s response to sediment influx and the required actions to clear sediment (Stafford-Smith and Ormond 1992). If more energy is given to keeping a polyp free from sediment, then other processes such as growth and reproduction may ultimately be affected (Stafford-Smith and Ormond 1992).

Little is known about the behaviour and habitat limitations of *Lophelia pertusa*, i.e. its ability to cope in high sedimentation environments. Results from Chapter 2 showed *L. pertusa* living on oil and gas platforms in the North Sea, a high sedimentation environment if living close to discharges of drilling muds and cuttings, where partial and full colony mortality were observed for those coral colonies living closest to drilling discharges. These observations suggested that *L. pertusa* has some capability to cope with sediment deposition but the mechanisms it uses to do so are unknown. Clearing mechanisms have been studied in some tropical scleractinian corals (see below) but how these relate to the behaviour of *L. pertusa* is also unknown. Other sub-lethal effects are suspected but could not be determined from video records used in Chapter 2. Recent research from the Porcupine Seabight, west of Ireland, revealed that *L. pertusa* exists in an active sediment transport environment and is an integral component of carbonate
mound formation by trapping sand and creating positive features for further coral development (Wheeler et al. 2005; Roberts et al. 2006). This provides further evidence that *L. pertusa* has some ability to cope in high sedimentation environments.

Responses and capabilities to react and cope with increased sediment vary between scleractinian coral species (Bak and Elgershuizen 1976; Stafford-Smith and Ormond 1992; Stafford-Smith 1993). Corals can cope with increased sediment by both active and passive sediment removal mechanisms: active mechanisms are related to coral behaviour and passive mechanisms are related to colony morphology, fine skeletal architecture, gravity and environmental factors such as local currents that allow corals to passively shed sediment (Lasker 1980; Stafford-Smith 1993). Results from coral behaviour studies on a wide range of scleractinian species revealed several active sediment removal mechanisms. These include ciliary movements, mucus production followed by sloughing off of sediment, tissue inflation leading to enhanced sediment runoff, tentacular movements (Bak and Elgershuizen 1976; Thompson 1980; Stafford-Smith and Ormond 1992; Riegl 1995), tissue pulsing, and sediment ingestion (Stafford-Smith and Ormond 1992).

The degree of sensitivity of *L. pertusa* to high sedimentation environments will affect the extent to which it is negatively impacted by offshore oil and gas drilling discharges and may also help explain its role in carbonate mound formation (as discussed above). Discharges of drilling muds and cuttings around coral reefs can increase local sedimentation and include a potentially toxic component from chemical additives and oil (see Chapter 1). A review by Dodge and Szmant-Froelich (1985) summarized the early studies on the effects of drilling muds on reef corals. In brief, several studies have looked at the effects of drilling muds and cuttings on tropical corals from the Gulf of
Mexico (Hudson and Robbin 1980; Krone and Biggs 1980; Thompson et al. 1980; Szmant-Froelich et al. 1981; Dodge 1982), California (Raimondi et al. 1997), and the Philippines (Hudson et al. 1982). Observed effects are similar to effects observed from clean sediment, including decreased growth rate (Dodge 1982), reduced coral cover (Hudson et al. 1982), reduced survivorship and viability (Raimondi et al. 1997), changes in metabolism (Krone and Biggs 1980) and feeding behaviour (Szmant-Froelich et al. 1981), polyp retraction, tissue damage, and death (Thompson 1980). The extent to which the toxicity of the muds and cuttings, or the physical effects of increased sedimentation, or the synergistic effects of the two are causing the negative impacts remains unclear (Dodge and Szmant-Froelich 1985).

### 3.1.1 Chapter Aims

This chapter aims to investigate the reaction and potential coping mechanisms of *L. pertusa* to clean sediment deposition as a first step towards understanding its ability to cope with drilling muds and cuttings and its ability to contribute in carbonate mound formation. As *L. pertusa* is an azooxanthellate coral, it relies primarily on suspension feeding for food and thus requires expanded polyps for food capture. Therefore, the objectives of the chapter are to 1) examine the polyp tentacle expansion and retraction behaviour of *L. pertusa* in response to brief sedimentation events of increasing intensity, and 2) to examine in more detail the clearing rates and mechanisms used by *L. pertusa* to remove sediment from its surface.
3.2 Methods

3.2.1 Coral sampling and husbandry

Live samples of *L. pertusa* were collected using the Greenpeace vessel *MY Esperanza* in 2005 (Roberts et al. 2005a) from the Sea of Hebrides east of Mingulay (56° 49N and 07° 23W) using a vanVeen grab with a camera and light providing a live image of the seafloor to guide sampling (as described by Mortensen et al. 2000). The samples were brought on board and kept alive in a portable temperature-controlled recirculating aquarium until they were brought to the laboratory at SAMS and transferred to the permanent aquaria. The living conditions in the aquaria were kept as close as possible to the natural conditions experienced by *L. pertusa*. The corals were kept in a holding system, which consisted of recirculating aquaria equipped with a UV sterilizer, protein skimmer and biological filter and partial water changes were carried out weekly. Offshore seawater of the correct salinity was collected and stored for use in the aquaria. The whole system was kept in a temperature-controlled room where the seawater was kept at 8°C. The temperature and salinity of the system were monitored daily and adjusted as required, and the nutrient levels of the seawater were monitored weekly. Corals were fed the krill *Eupasia pacifica* (Tropical Marine Centre Ltd.) twice a week with a directed feeding method that ensured each polyp received food. Although recent information speculates that corals probably have a mixed diet of phytoplankton and zooplankton (Duineveld et al. 2004), it is certain that they feed on zooplankton (Freiwald 2002) and *L. pertusa* has been shown to feed on krill in previous aquarium studies (Mortensen 2001). The seawater was pumped through the system using a submersible pump and flow rates were evenly distributed in each tank using spray bars. The flow of seawater in each tank was kept at a high rate that encouraged polyp expansion (approximately 300 ml sec⁻¹). The room was kept in darkness at all times when researchers were not attending to the aquaria.
3.2.2 Experiment 1 - Four-hour sedimentation experiments

The sedimentation experiments were carried out in a recirculating system located in the same temperature-controlled room as the holding system and used the same source of seawater. The system included two identical tanks; one was used for the control experiments and one for the sedimentation experiments. Seawater was pumped from a sump tank and split between the control and experimental tanks. The seawater was then passed through two settlement tanks, which ensured that any sediment that escaped from the sedimentation tank settled out and was not carried through the rest of the system. The system also contained a protein skimmer, UV sterilizer, and biological filter. The system was checked and monitored jointly with the holding system.

Silica sand with a grain size ranging from 122 to 948 µm, and mean grain size of 317 µm was used in these experiments. The sedimentation chamber was based on a design by Riegl (1995). The system uses an airlift to bring sand from the source up a tube and is then deposited over an inverted perforated funnel, which allows the sand to rain down evenly over the coral fragment. The sand was collected in Petri dish and the sedimentation rate was measured as the weight of dry sand per surface area of the Petri dish over time (mg cm\(^{-2}\) min\(^{-1}\)). The airflow was controlled by a rotameter connected to a controllable valve. A rotameter consists of a tapered tube of glass with a float inside that is pushed up by airflow and pulled down by gravity. At a higher flow rate, more area (between the float and the tube) is needed to accommodate the flow, so the float rises. See Figure 3.1 for a diagram of the experimental set up.
Figure 3.1 Diagram of experimental apparatus used during the four hour sedimentation experiments
Coral fragments were placed in a vertical position in the control and experimental tanks to acclimatize for 12 h before the polyp expansion was recorded for 12 h before the sedimentation event, for 4 h during the sedimentation event, and for a 12 h recovery period. Therefore, polyp activity was recorded for 28 h. To measure polyp activity, a silhouette was created and recorded using time lapse video as described by Roberts and Anderson (2002). A video camera linked to a time-lapse recorder was set up facing the coral fragment. An infrared diode shone through a Fresnel lens facing opposite to the camera creating a silhouette of the coral fragment (Figure 3.2) where polyp activity was recorded. Before the beginning of the experiment, the polyps were lightly tapped until they had fully retracted into their calices and the outline of the fully retracted polyps was recorded. The outline was used as the zero-baseline for polyp expansion and expansion was measured as the maximum polyp expansion in mm. The calyx diameter was also measured at this time as the width viewed on the video and all polyp expansion measurements were standardized to calyx diameter so the final units were mm polyp expansion/mm calyx diameter. This takes into account changes in the distances and zoom of the cameras, and accounts for larger polyps being capable of greater expansion.

Figure 3.2 Silhouettes of contracted (left) and expanded (right) coral polyps. The same coral polyps are shown in both images.
Polyp expansion was measured at 20 min intervals from the recorded video. At least three polyps were measured per control and sedimentation experiment depending on the number of polyps that were clearly visible from the silhouette. One experiment (2.0 mg cm\(^{-2}\) min\(^{-1}\)) had only one experimental polyp because the airflow line shifted in front of the image during the experiment leaving only one polyp visible for the full length of the experiment. The results were compared using pair-wise statistical comparisons of polyp expansion before and after the sedimentation event. Experiments were grouped into low (1-4 mg cm\(^{-2}\) min\(^{-1}\)), medium (6-10 mg cm\(^{-2}\) min\(^{-1}\)) and high (12-19 mg cm\(^{-2}\) min\(^{-1}\)) sedimentation rates. Data from each individual polyp were included in the pair-wise statistical comparisons.

Initial results showed that both the control and experimental polyps retracted at the beginning of the experiment probably due to mechanical disturbance to the control tank when starting sediment deposition. Thus, the rate of polyp expansion recovery of both the control and experimental polyps were measured. The recovery time was determined as the time it took for the polyp to expand to the mean expansion from the 12 h prior to the sedimentation event after the end of the 4 h sedimentation event. The rates were statistically compared using two-sample t-test for the high sedimentation rate and the non-parametric Mann Whitney-U test for the low and medium rates because the data were not normally distributed.

### 3.2.3 Experiment 2 - Clearing rates and mechanisms

Three instantaneous doses of sediment were applied to coral polyps: 30 mg cm\(^{-2}\), 60 mg cm\(^{-2}\) and 120 mg cm\(^{-2}\). These are comparable levels to those used in other experiments assessing the effects of sedimentation on tropical corals (Lasker 1980; Stafford-Smith 1993). The lowest dose of 30 mg cm\(^{-2}\) provided a light covering of sediment over a
coral polyp leaving some tissue exposed and the highest dose of 120 mg cm$^{-2}$ provided a complete covering of the polyp surface with a thicker layer of sediment. It was not feasible to use the silica sand from the previous sedimentation experiments described above because it was not visible against the white tissue of the coral polyps. Therefore, dyed blue sand (from www.homecrafts.co.uk) with a mean grain size of 274 µm (range of 92 to 494 µm) was used.

Each dose (30, 60 and 120 mg cm$^{-2}$) was applied to six different polyps from different colonies (replicates A-F). All polyps were positioned with the calyx facing directly upwards to standardise the polyp position across all experiments. Polyps were placed in an experimental chamber, which consisted of a 1.8 l Perspex tank filled with seawater from the same source as that used in the holding aquaria. The chamber was kept at a temperature of between 8.3 and 8.9 °C, in a water bath cooled with a water chiller. The Perspex tank was insulated with polystyrene to keep the temperature and salinity constant. The salinity was checked after the initial six experiments to ensure that there was no evaporation and that the salinity remained constant throughout the experiment. A small viewing window was created with a one centimetre thick piece of glass positioned below the water surface at the end of a round plastic tube. This prevented evaporation and condensation on the microscope lens, water movement disturbance in the photographs, and provided insulation. Water movement was created using an air stone attached to a portable air pump. This apparatus is illustrated in Figure 3.3.

Coral polyps were left to acclimatize in the chamber for a two-hour minimum or until polyp tentacles became visible. A microscope equipped with a digital camera (Minolta Dimage 5, 3.3. mega pixels) with a repeat timer setting was positioned above the coral polyp. Sediment was deposited over the polyp using a guide frame to distribute the sand
evenly over a predefined area (1 cm², 1.3 cm² or 1.5 cm²), which was always greater than the surface of the polyp. Photographs were taken at five-minute intervals for the first hour followed by 30 min intervals until the end of the experiment. The experiments lasted 360 min for the 30 and 60 mg cm⁻² dose experiments and 1440 min for the 120 mg cm⁻² experiments. The polyps were visually checked at the end of each experiment for evidence of blue sediment remaining within the coral gut.

Images were analysed for percent area covered by sediment from all photographs and then results from 0, 10, 30, 60 and 360 min for the 30 and 60 mg cm⁻² dose experiments, and from 720 and 1440 min from the 120 mg cm⁻² dose experiments were compared statistically using a one-way ANOVA. The percent cover cleared at each time interval was measured using image analysis software Image-J (software developed at the National Institutes of Health, US, downloaded from http://rsb.info.nih.gov/ij/download.html). To normalize the percent cover for comparisons between experiments, the percent area cleared relative to the initial area covered immediately after the sediment was deposited was calculated. Finally, photographs were viewed and notes were taken on polyp behaviour and the sequence of events which occurred as the coral polyp cleared the sediment.
3.3 Results

3.3.1 Experiment 1 - Four-hour sedimentation experiments

Twelve experimental runs were carried out with varying rates of sedimentation ranging from 1 to 19 mg cm\(^{-2}\) min\(^{-1}\). The runs were grouped into low, medium, and high rates and the mean polyp expansions from each set of control and experimental polyps are presented in Table 3.1. Polyp activity before the sedimentation event showed some polyp movement but generally remained expanded throughout the pre-sedimentation event period. In many cases, including the control animals, polyps retracted at the beginning of the sedimentation events (see example of polyp behaviour in Figure 3.4). The reason for this is likely to be mechanical disturbance to the control tank when
starting sediment deposition. The control polyps and the low sedimentation runs showed a relatively steep recovery during the sedimentation event, whereas the medium and high sedimentation runs showed a more gradual recovery; this was especially evident for the high sedimentation runs.

Table 3.1 Mean polyp expansion values for low, medium, and high sedimentation rates

<table>
<thead>
<tr>
<th>Category</th>
<th>Rate mg cm⁻² min⁻¹</th>
<th>No. of Polyps</th>
<th>Mean Expansion mm/mm calyx diameter ± SD</th>
<th>No. of Polyps</th>
<th>Mean Expansion mm/mm calyx diameter ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>2</td>
<td>6</td>
<td>0.40 ± 0.1</td>
<td>1</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>0.32 ± 0.07</td>
<td>4</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>0.35 ± 0.08</td>
<td>5</td>
<td>0.22 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>0.37 ± 0.07</td>
<td>3</td>
<td>0.27 ± 0.07</td>
</tr>
<tr>
<td>Medium</td>
<td>9</td>
<td>3</td>
<td>0.34 ± 0.1</td>
<td>3</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>3</td>
<td>0.27 ± 0.03</td>
<td>5</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3</td>
<td>0.34 ± 0.06</td>
<td>5</td>
<td>0.27 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3</td>
<td>0.22 ± 0.05</td>
<td>5</td>
<td>0.28 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>0.53 ± 0.06</td>
<td>4</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>High</td>
<td>19</td>
<td>3</td>
<td>0.41 ± 0.02</td>
<td>4</td>
<td>0.29 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3</td>
<td>0.25 ± 0.08</td>
<td>5</td>
<td>0.31 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>4</td>
<td>0.35 ± 0.1</td>
<td>5</td>
<td>0.23 ± 0.02</td>
</tr>
</tbody>
</table>

Figure 3.4 An example of a control polyp retracting at the beginning of the sedimentation event followed by a fast recovery to full polyp expansion during a low sedimentation rate (2 mg cm⁻² min⁻¹). The hatched bar indicates the sedimentation event.
To assess statistically the effects of the sedimentation events on polyp behaviour, paired t-tests were carried out on the mean polyp expansion before and after the sedimentation events for the control and experimental corals (Table 3.2). The data were not normally distributed for the before- low control polyps and the before- high polyps and therefore the non-parametric equivalent of a paired t test was carried out: Wilcoxon signed ranks test. Although the control polyps did retract during the sedimentation events, the results from the statistical tests showed that this had no effect on before and after polyp expansion for all control polyps. The sedimentation events had no significant effect on polyp expansion for the low dose runs. The medium dose runs showed a significant increase in polyp expansion after the sedimentation event. Finally, the high dose runs showed a significant decrease in polyp expansion after the sedimentation event (Figure 3.5).

Table 3.2 Results from statistical paired t-tests and Wilcoxon signed ranks test to examine differences between polyp expansion before and after sediment influx

<table>
<thead>
<tr>
<th></th>
<th>Control Before vs After</th>
<th>Experimental Before vs After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>p=0.501*</td>
<td>p=0.549</td>
</tr>
<tr>
<td>Medium</td>
<td>p=0.113</td>
<td>p=0.003</td>
</tr>
<tr>
<td>High</td>
<td>p=0.290</td>
<td>p=0.023*</td>
</tr>
</tbody>
</table>

*Indicates results from a non-parametric Wilcoxon signed ranks test

One polyp exposed to the 19 mg cm\(^{-2}\) min\(^{-1}\) rate was buried in sediment. At the end of the sedimentation event, the polyp was removed from the sediment pile, and its recovery was observed. The polyp did eventually recover within the time frame of the experimental run, although at a slower rate compared to the other polyps in the same run which had not been buried (Figure 3.6).
Figure 3.5 Two examples (A & B) of slow recovery of polyp expansion and a reduced polyp expansion after exposure to a high sedimentation rate (19 mg cm\(^{-2}\) min\(^{-1}\)). The hatched bar indicates sedimentation event.
Figure 3.6 One of the experimental polyps became buried in sediment during a high sedimentation rate event (19 mg cm\(^2\) min\(^{-1}\)). Polyp expansion recovery began near the end of the run. The hatched bar represents the sedimentation event.

Since the control polyps also retracted at the beginning of the sedimentation events, a recovery time was calculated and mean recovery times from the control versus experimental coral polyps are presented in Figure 3.7. Recovery rates were tested for differences using Mann-Whitney U tests for low and medium rates and two-sample t-tests for high rates. Significant differences were only observed for recovery rates between the experimental and control coral polyps from the high sedimentation rates.
Figure 3.7 Mean recovery times for control and experimental polyps exposed to (A) low, (B) medium, and (C) high levels of sedimentation. Error bars represent 95% confidence interval. Only the high sedimentation rates showed significant differences p<0.05. Different letters indicate significant differences.
3.3.2 Experiment 2 - Clearing mechanisms and rates

Six experimental runs (replicates A-F) were carried out each for a deposition of sediment of 30 mg cm$^{-2}$, 60 mg cm$^{-2}$ and 120 mg cm$^{-2}$. A general trend of initial fast clearing within the first few minutes followed by slower clearing was observed (Figure 3.8). During several runs, a slight increase in percent cover was observed after an initial decrease. This is explained by sediment that was initially ingested by the coral polyp and then subsequently expelled. Slight changes in percent area covered occurred when sediment was moved by the coral from one area to another before being removed from the polyp.

There were several behavioural mechanisms observed during the clearing of sediment from the polyp surface and these are outlined in Table 3.3. Some of the polyps retracted their tentacles upon deposition of sediment. In the remainder of the runs, especially if the polyp was fully expanded, the sediment covered the polyp surface and the central tentacles while leaving the outer tentacles expanded. Sediment that landed close to the edges of the polyp was cleared first by most polyps. Although coral cilia could not be seen under a stereo microscope with 50 times magnification, it is assumed that these are responsible for moving individual grains and aggregated clusters of sediment as no other possible mechanism was observed similar to observations made by Stafford (1990) and Lasker (1980). The sediment was cleared off between septa and there was no evidence that tentacle movements aided in the process. In four runs (30A, 30B, 60-B, 120-A), it was not possible to distinguish whether the edges cleared first as the coral cleared the sediment before the first photograph reflecting the fast initial clearing previously documented. In one case (120D), mesenteries were visible on an outer calyx edge and appeared to clear the sediment from this edge (Figure 3.9).
Figure 3.8 Results for replicate polyps (A-F) for each level of sediment deposition (30, 60 and 120 mg cm\(^{-2}\)) as the percent polyp surface area cleared over time.
Table 3.3 Polyp behaviour during sediment clearing (sequence of behaviour runs from left to right)

<table>
<thead>
<tr>
<th>Experimental run number (sediment weight + replicate)</th>
<th>Initial expansion state</th>
<th>Initial retraction</th>
<th>Edges cleared first</th>
<th>Sediment cleared in &gt; 3 directions</th>
<th>Sediment clears off in 1 direction</th>
<th>Sediment left in centre</th>
<th>Sediment moves into centre</th>
<th>Sediment moves off in clusters, no. of directions</th>
<th>Sediment in centre moves off in several directions, no. bundles</th>
<th>Sediment Ingested</th>
<th>Number of expulsions</th>
<th>No. of clusters, no. of directions</th>
<th>Final retraction state</th>
<th>Other observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-A Full</td>
<td>Un</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>1 2, 2</td>
<td>Full</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-B Partial</td>
<td>Un</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Full Cleared sediment quickly difficult to see what occurred</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-C Partial</td>
<td>Partial</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>3 (clusters), 3 (directions)</td>
<td>-</td>
<td>1 3, 3 Full A few grains move off on their own not part of the bundles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-D Full</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>1 1, 1</td>
<td>Full</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-E Partial</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Full Mouth open at end of run</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-F Full</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>1 1, 1</td>
<td>Full</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-A Full</td>
<td>-</td>
<td>✓</td>
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<td>-</td>
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<td>-</td>
<td>1 1, 1</td>
<td>Full</td>
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</tr>
<tr>
<td>60-B Full</td>
<td>-</td>
<td>Un</td>
<td>✓</td>
<td>-</td>
<td>-</td>
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<td>✓</td>
<td>1 1, 1</td>
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<td>60-C Full</td>
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<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓ 0 -</td>
<td>Full Mesenteries prevented expulsion</td>
<td>-</td>
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<tr>
<td>60-D Full</td>
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<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>7 7, 1</td>
<td>Partial</td>
<td>-</td>
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<td>60-E Partial</td>
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<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>2 2 Un</td>
<td>Partial</td>
<td>-</td>
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</tr>
<tr>
<td>60-F Partial</td>
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<td>✓</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>Un</td>
<td>Partial</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>120-A Partial</td>
<td>Partial</td>
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<td>Un</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>Partial Brown filaments appear near end of run</td>
<td>-</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>120-B Full</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>1, 1</td>
<td>-</td>
<td>-</td>
<td>Partial Prior to clearing off middle bundle appears trapped by tentacles</td>
<td>-</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>120-C Partial</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>2 0, &gt; 3 1 1</td>
<td>Partial Initially ingested all sediment, sediment visible in calyx at end</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120-D Partial</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>2</td>
<td>0 Un</td>
<td>✓</td>
<td>Partial</td>
<td>Mesentaries clear one edge of calyx, sediment visible inside calyx at end</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120-E Partial</td>
<td>✓ ✓</td>
<td>-</td>
<td>✓</td>
<td>1, 1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>5 3, 1 2 Un</td>
<td>Partial Brown filaments visible at end</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120-F Partial</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ 5</td>
<td>Partial</td>
<td>-</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Un = Unknown and behaviour not directly observed because edges had been cleared by the second photograph
Sediment that was not initially removed from the edges was either completely cleared off in multiple directions or it all followed the same path along the polyp surface. However, in several cases the sediment was moved towards the mouth, again by ciliary actions, and was ingested by the coral. Eventually, ingested sediment was expelled, either all at once or in multiple expulsions and was cleared from the coral surface as described above.

The final state of the polyp was noted as expanded, partially expanded or retracted. There were no runs where the coral was completely retracted once it had cleared the sediment. Near the end of two runs (120-A: 960 min; 120-E: 900 min) what looked like brown mesenteries came out of the coral’s mouth but their function was not clear.

Figure 3.10 shows the percent area cleared at 10, 30, 60 360, 720 and 1440 min. These results confirmed that the 30 and 60 mg cm\(^{-2}\) runs showed similar clearing rates but that
the clearing rates from the 120 mg cm$^{-2}$ runs were generally lower. However, if the differences are statistically tested (one-way ANOVA) it is only after 360 min that the 30 & 60 mg cm$^{-2}$ results differ significantly from the 120 mg cm$^{-2}$ results.

The corallites were assessed at the end of each run for any remaining blue sediment visible through the skeleton. Two of the six 120 mg cm$^{-2}$ dose runs had sediment visible in the corallite at the end of the run and after a subsequent 24 h.

Figure 3.10 Percent area cleared after 10, 30, 60, and 360 min for 30 and 60 mg cm$^{-2}$, in addition to 720 and 1440 min for 120 mg cm$^{-2}$. Error bars represent 95% confidence intervals and a significant difference was observed only after 360 min for 120 mg cm$^{-2}$ compared to other levels as indicated by the different letters.

### 3.4 Discussion

Several relevant studies have examined the clearing rates and behavioural responses of corals to the influx of sediment; these are summarized in Table 3.4 and will be referred to throughout the discussion.
<table>
<thead>
<tr>
<th>Location</th>
<th>Dose</th>
<th>Species</th>
<th>Clearing Rates</th>
<th>Behavioural Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caribbean</td>
<td>0.75, 1.5, &amp; 3 g over 3 cm diameter circle</td>
<td>19 scleractinian species</td>
<td>Clearing times increase with size and density of particles</td>
<td>Polyp expansion active clearing mechanism</td>
<td>Bak and Elgershuizen (1979)</td>
</tr>
<tr>
<td></td>
<td>Grain size: 1200 µm; carborundum powder</td>
<td>Laboratory</td>
<td>19 scleractinian species</td>
<td>Clearing times increase with size and density of particles</td>
<td>Polyp expansion active clearing mechanism</td>
</tr>
<tr>
<td>Great Barrier Reef, Australia</td>
<td>Single dose 200 mg cm$^{-2}$</td>
<td>18 scleractinian species</td>
<td>Species specific clearing rates</td>
<td>Fine sediment rejected faster than coarse sediment</td>
<td>Stafford-Smith (1993)</td>
</tr>
<tr>
<td></td>
<td>Grain size fine 62-250 µm coarse 500 µm - 1mm</td>
<td>In situ</td>
<td>18 scleractinian species</td>
<td>Species specific clearing rates</td>
<td>Fine sediment rejected faster than coarse sediment</td>
</tr>
<tr>
<td>Panama</td>
<td>Single dose 18.5 &amp; 74.4 mg cm$^{-2}$</td>
<td>Montastraea cavernosa</td>
<td>Initial fast clearing rates</td>
<td>None reported</td>
<td>Lasker (1980)</td>
</tr>
<tr>
<td></td>
<td>Grain size 500-1000 µm 60-250 µm</td>
<td>Laboratory</td>
<td>Montastraea cavernosa</td>
<td>Initial fast clearing rates</td>
<td>None reported</td>
</tr>
</tbody>
</table>
Table 3.4 continued

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Species</th>
<th>Response</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Barrier Reef, Australia</td>
<td>Single dose 50 mg cm⁻²</td>
<td>42 scleractinian species</td>
<td>None reported</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grain size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 63 µm</td>
<td></td>
<td>Ciliary movements</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63-250 µm</td>
<td></td>
<td>Polyp retraction following sediment influx</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 µm - 1 mm</td>
<td></td>
<td>Tentacle movement assisted clearing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-3 mm</td>
<td></td>
<td>Sediment ingestion of clean and food-coated particles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In situ &amp; Laboratory</td>
<td></td>
<td>Extrusion of Mesenteries</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mucus production</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tissue expansion</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stafford-Smith and Ormond (1992)</td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>Single dose 200 mg cm⁻²</td>
<td>8 scleractinian species</td>
<td>Corals cleared single dose</td>
<td>Polyp inflation</td>
</tr>
<tr>
<td></td>
<td>Grain size</td>
<td></td>
<td>No overall difference between morphologies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 0.125 mm</td>
<td></td>
<td>All colonies cleared over 50% of sand within 1000 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.125-0.250 mm</td>
<td></td>
<td>No difference in clearing efficiency between grain sizes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 0.5 mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Laboratory</td>
<td></td>
<td>Riegl (1995)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyp inflation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tentacular action</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ciliary activity inferred</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 mg cm⁻² for 6 wk</td>
<td>8 scleractinian species</td>
<td>Tissue necroses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grain size</td>
<td></td>
<td>No mortality observed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 0.25 mm</td>
<td></td>
<td>Polyp inflation</td>
<td></td>
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<tr>
<td></td>
<td>Laboratory</td>
<td></td>
<td>Extrusion of mesenteries</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mucus production</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tissue expansion</td>
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Table 3.4 Continued

<table>
<thead>
<tr>
<th>Location</th>
<th>Burial depth</th>
<th>Species</th>
<th>Observations</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW Philippines</td>
<td>Burial under 1-5 cm sand</td>
<td><em>Porites</em></td>
<td>No effect after 6 h. Significant effect on tissue health after 20 and 68h. Increasing stress from 0 to 68h burial. Recovery within 4 wk from 68h burial. <em>Acropora</em> death after 20h.</td>
<td>None reported. Wesseling et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>0, 6, 20, 68 h</td>
<td><em>Galaxea</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Heliopora</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Acropora</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>In situ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida Keys</td>
<td>Burial under 10-12 cm sand</td>
<td>9 scleractinian species</td>
<td>12.5h some tissue loss. 24h significant tissue loss. 72h 70 - 100% tissue loss and no signs of recovery after 41h. Extrusion of mesenteries. Mucus production. Tissue expansion.</td>
<td>Thompson (1980)</td>
</tr>
<tr>
<td></td>
<td>12.5, 24, 72 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>In situ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>Single dose</td>
<td><em>Montastraea annularis</em></td>
<td>200 mg cm(^{-2}) <em>A. plamata</em> no signs of recovery after 6d. 400 mg cm(^{-2}) - <em>M. annularis, A. cervicornis</em> some bleaching. 800 mg cm(^{-2}) <em>M. annularis</em> dead tissue and no sign of recovery.</td>
<td>Mucus production. Rogers (1983)</td>
</tr>
<tr>
<td></td>
<td>200, 400, 800 mg cm(^{-2})</td>
<td><em>Acropora cervicornis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean grain size: 0.5 mm</td>
<td><em>Diploria strigosa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Acropora plamata</em></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>In situ</td>
<td></td>
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</table>
### 3.4.1 Experiment 1 - Four-hour sedimentation experiments

The four-hour sedimentation experiment examined how *L. pertusa* responded to continuous sediment deposition over time. Control and experimental corals responded by retracting their polyps at the beginning of the sedimentation events, likely as a consequence of movements and vibrations in the temperature-controlled room as a result of the researcher entering to start the airflow rather than the initial influx of sediment. However, all control polyps recovered quickly from this initial retraction with no significant differences between before and after polyp expansion. This demonstrated that this initial reaction did not have a significant effect on the polyp behaviour during the last 12 h of the runs. Therefore, the comparisons made between the polyp behaviour before and after the sedimentation events remained valid.

Only the high sedimentation rates (12-19 mg cm\(^{-2}\) min\(^{-1}\)) showed a significant decrease in polyp expansion and a significantly slower polyp expansion recovery. These rates are likely to be far higher than levels *L. pertusa* would experience from natural sedimentation *in situ* and are higher than it would experience from the oil and gas drilling discharges (see Section 3.4.5 for further discussion on sedimentation rates).

The high sedimentation rates were high enough for one polyp to become completely buried for just over two hours, and after the sediment was removed, it had partially extended its tentacles by the end of the 12 h recovery period. Other scleractinian species have also shown resilience to burial for 12 and 20 h before they began to show signs of stress (Thompson 1980; Wesseling et al. 1999).

No significant difference in polyp expansion was observed before and after the low sedimentation rates, and a significant increase in polyp expansion was observed after
the medium rates. It is possible that maximum tentacle expansion and potential tentacle movement is a means of preventing sediment accumulation on polyp tissue (Bak and Elgershuizen 1976; Stafford-Smith and Ormond 1992), but that when the sedimentation rates become too high polyp retraction is used as a defence mechanism (such as in the high sedimentation runs). Polyp retraction can make sediment particles unstable which, when followed by polyp expansion, can assist in sediment removal (Bak and Elgershuizen 1976).

3.4.2 Experiment 2 - Clearing rates and mechanisms

Results from the clearing rate experiment showed that all coral polyps have an initial fast clearing rate where 50 % of the area covered was cleared in the first 10 min, at least for the two lower sediment loads of 30 and 60 mg cm\(^{-2}\), and almost 40 % of the area cleared for the highest sediment load (120 mg cm\(^{-2}\)). A much slower clearing rate was observed after the first 60 min. Lasker (1980) found a similar trend of initial fast clearing while Stafford-Smith (1993) and Rogers (1983) found more variation in responses and clearing rates which were species-specific (see Table 3.4).

The results also showed that the coral polyps rapidly cleared at least 90 % of the lower sediment loads of 30 and 60 mg cm\(^{-2}\) within six hours but took longer to clear the highest sediment load of 120 mg cm\(^{-2}\) within 12 h, or even 24 h in three cases. Referring to results from the literature presented in Table 3.4, Stafford-Smith (1993), who used a sediment load higher than in the present study, found that some corals still had 90 % sediment cover after 24 h, whereas three species only had 10 % left after four hours. She also found that branching corals cleared sediment faster than non-branching corals. Lasker (1980) used 18.5-74.4 mg cm\(^{-2}\) and tested two morphologies of *Montastrea cavernosa*. The nocturnal morph was able to clear the sediment in 8 h, whereas diurnal
morph still had 20-30 % cover after 8 h. The difference could be accounted for by less initial sediment cover on the nocturnal colonies due to its morphology.

Not all coral polyps responded to sediment application in the same way and there was no clear sequence of actions carried out by the polyps. However, in all cases, ciliary currents were the main mechanism used by polyps to shift sediment and sediment was moved in aggregated clusters, which could be a more energy efficient way to clear sediment. Tentacle movement was observed, as is normal for coral polyps, but this did not appear to aid with sediment clearing. In one case, it appeared to hinder sediment removal. Bak and Elgershuizen (1976) found a similar result when fully expanded polyps of Diploria strigosa and Meandrina meandrites obstructed the clearing of carborundum powder, whereas, Stafford-Smith and Ormond (1992) found that tentacle movement was used by many species to assist in sediment removal. They suggested that, although not well coordinated, tentacle movements were effective at mobilizing sediment.

Eleven of the 18 polyps examined here moved sediment by ciliary currents to the centre of the polyp and ingested sediment. This behaviour could represent a clearing mechanism used by the polyp, or it could be an indiscriminate feeding response. Lewis and Price (1976) examined the ciliary currents of Atlantic and Pacific tropical reef corals and concluded that there was a basic pattern to ciliary currents common to all reefs corals, and that “currents flow away from the mouth towards the edge of the polyp and up or out between the tentacles” (Lewis and Price 1976). The exception was for the central region of the oral disk where currents moved towards the mouth (Lewis and Price 1976). The movements away from the mouth and off the calyx are a “cleansing” behaviour whereas the movements in the central region towards the mouth are a feeding
behaviour. Ciliary current reversal was not observed in Atlantic corals, but the direction of flow can change depending on the state of expansion of the polyps, and flow inwards towards the mouth when polyps are contracted (Lewis and Price 1976). This is a possible explanation for the results of the present study where inward movement of sediment was only observed in corals that were partially expanded and never for those that were fully expanded.

Stafford-Smith and Ormond (1992) also observed that almost all species ingested both clean and food-coated particles. They concluded that indiscriminate ingestion of food particles was part of normal feeding behaviour. Rosenfeld et al. (1999) showed that the tropical solitary coral *Fungia horrida* from the Red Sea feeds on organic material associated with fresh sediments, and therefore they suggested that sediment may be a food source for tropical corals living in oligotrophic waters. Finally, Anthony and Frabricius (2000) also showed that tropical corals were able to shift feeding strategies when exposed to turbid environments and were able to use suspended particulate matter as a significant food source. Suspended particulate matter associated with sediment resuspension events could also be a food source for *L. pertusa*, which lives in deep ocean environments where food is limited and seasonal. Thus, it could be normal behaviour for *L. pertusa* to feed on organic material associated with resuspended sediments to supplement its diet (Duineveld et al. 2004; Kiriakoulakis et al. 2004). If resuspended particles are an important feeding source for *L. pertusa*, then this could imply that *L. pertusa* is well adapted to ingesting sediment.

Mesenteries are part of a coral’s digestive system and are extruded in response to feeding and extracoelenteric digestion, or as an aggressive manoeuvre on a neighbouring coral (Barnes and Hughes 1999). Mesentery extrusion was observed
during two runs (60-C and 120-D) in the present study as well as during other sedimentation studies (Thompson et al. 1980; Stafford-Smith and Ormond 1992; Riegl 1995). They have been observed extruded as a feeding response (Stafford-Smith and Ormond 1992) or possibly as a clearing response in the present study where they were observed directly involved in clearing sediment grains from the edge of a polyp.

Mucus production and complete polyp tissue inflation (where the whole polyp, not just tentacles, expand) are recognized as sediment removal mechanisms for tropical reef corals (Bak and Elgershuizen 1976; Thompson et al. 1980; Rogers 1983; Stafford-Smith and Ormond 1992; Riegl 1995), but these responses were not observed in the present study. Mucus secretion has been observed in *L. pertusa*, as a stress response during sampling of fresh colonies (personal observation). It is possible that mucus is not an important mechanism for sediment removal for *L. pertusa*, or the lack of mucus could be a consequence of *L. pertusa* living in aquaria. Riegl (1995) reported that although mucus sheets formed during sedimentation experiments in the laboratory, they were never comparable to those observed *in situ* under natural conditions.

3.4.3 *Potential factors affecting the clearing capability of Lophelia pertusa*

Water movement and sediment grain size were kept constant in the present studies; however, these two factors have been shown to affect coral sediment clearing. Fine particles are less likely to settle on coral surfaces in more turbulent environments (Lasker 1980), and *in situ* turbulent water movements increase the sediment removal efficiency for corals (Bak and Elgershuizen 1976), although are not sufficient to eliminate the need for some active clearing (Stafford-Smith 1993). Stafford-Smith and Ormond (1992) and Lasker (1980) found that corals were able to clear fine sediment faster than coarse sediment. Riegl (1995) found no significant difference between
clearing rates and grain size, and Bak and Elgershuizen (1976) found that clearance rate of carborundum powder or sand varied by species. Water movement in the experiments presented in this chapter was kept constant, and thus the effects of slower or faster water movements were not tested.

3.4.4 Effects of drilling muds and cuttings on corals

The present set of experiments assessed the effects of clean sediment on polyp behaviour of *L. pertusa*, which was the first step to understanding the effects of drilling muds and cuttings on corals. When examining the effects of only drilling muds and cuttings it can be difficult to separate the effects of drilling muds that are caused by the smothering of sediment, and those that are caused by the toxic component of the muds (Szmant-Froelich et al. 1981). Several other studies have also looked at the effects of drilling muds on several species of tropical corals. The results from the present study offer some indication of the effects of clean sediment on *L. pertusa*, but the effects from drilling muds and cuttings may be more severe. Furthermore, generalizing about these effects is difficult because the constituents of drilling muds are constantly changing based on the depth of wells and different recipes for drilling muds which are used by different companies. Furthermore, difficulties exist because the chemical properties of muds change over time and fresh muds must therefore be obtained on a regular basis.

Studies directly comparing the effects of drilling muds versus clean sediment on tropical corals have shown lower clearing rates of drilling muds compared with clean sediment (Thompson 1980). In addition, more severe effects, including lethal effects, were observed for corals buried in drilling muds compared with the same burial in clean carbonate sediment which Thomson (1980) attributed to the toxic soluble additives in the drilling muds. Studies examining the effects of drilling muds on coral polyp
behaviour and coral survivorship have shown significant effects at even the lowest levels tested (Thompson et al. 1980; Raimondi et al. 1997). This indicates that caution must be employed when applying results from clean sediment experiments to what corals might experience when exposed to drilling discharges.

3.4.5 Sedimentation rates

The sedimentation rates used in the present experiments are high compared to what is known about natural sedimentation rates on a tropical coral reef, which are usually limited to reefs which experience approximately 20 mg cm\(^{-2}\) day\(^{-1}\) (Stafford-Smith 1990). Stafford-Smith (1990) argued that some fringing reefs in the Great Barrier Reef experienced sedimentation rates up to 250 mg cm\(^{-2}\) day\(^{-1}\). Both these rates are much lower than the rates used in both the four-hour sedimentation experiment and single application of sediment experiment.

Very little is known about sedimentation rates on L. pertusa reefs, but offshore sedimentation rates on the continental slope are several orders of magnitude less than coastal environments (20 cm kyr\(^{-1}\) for the continental slope compared with >2 cm yr\(^{-1}\) for coastal environments, see Gage and Tyler 1996). However, particle resuspension measured during a lander deployment on a cold-water coral reef on the Galway Mound in the Porcupine Seabight showed evidence of sediment resuspension events occurring in bi-weekly phases likely following the tidal cycle (unpublished data J.M. Roberts and S.E. Gass). In addition Frederiksen et al. (1992) discusses the importance of internal tidal waves along the shelf break (around the Faroe Islands), which lead to increased phytoplankton production, and a greater flux of detritus to L. pertusa. Therefore, although sediment accretion is minimal in deep-water areas, internal tidal waves may be important with respect to the extent of exposure of deep-water organisms to suspended
particular matter and resuspended sediment. Furthermore, *L. pertusa* has been described as playing an integral part in carbonate mound formation by acting as a sediment trap and building positive features for enhanced coral development (Wheeler et al. 2005). These examples would suggest that *L. pertusa* is well adapted to living in regions of active sand transport, which could explain its ability to cope with relatively high sedimentation.

Drill cuttings are discharged continually during drilling, although drilling occurs approximately 30-50% of the time during the drilling of a well, with more short-term episodic discharges of drilling muds lasting from minutes to several hours (Gettleson 1980) (see Chapter 1 for more details on drilling discharges). Thus, the four-hour experiments in this study are more analogous to drilling mud discharges, but are much shorter than drill cuttings discharges. Computer simulation models exist to predict concentrations of drilling muds and cuttings at various distances from drilling platforms based on pre-defined environmental parameters such as the vertical distribution of temperature and salinity, depth, ocean currents, as well as information on the discharge depth and release rate of discharges (Rye et al. 2004). Furthermore, the type of muds being used must be considered because oil-based and water-based muds will each behave differently when released into the marine environment (Rye et al. 2006).

Two such predictive models include the US-based Offshore Operators’ Committee (OOC) model (Brandsma and Smith 1999) and the Norwegian designed ParTrack (particle tracking) model (Rye et al. 1998). Results from model predictions based on deep-water drilling are summarised in Table 3.5. The discharge is assumed continuous over a number of days and the resulting rates are up to several magnitudes smaller than the highest sedimentation rates used in the present study.
### Table 3.5 A summary of drilling discharge concentrations from the literature

<table>
<thead>
<tr>
<th>Location</th>
<th>Method</th>
<th>Distance from source</th>
<th>Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norne Field, Norway</td>
<td>Partrack model</td>
<td>0.5 km</td>
<td>100-300 mg cm⁻²</td>
<td>Rye et al. 2004</td>
</tr>
<tr>
<td>Same as above</td>
<td>Partrack model</td>
<td>2 km</td>
<td>0.1-0.3 mg cm⁻²</td>
<td>Rye et al. 2004</td>
</tr>
<tr>
<td>Northern North Sea</td>
<td>OCC model</td>
<td>2 km</td>
<td>0.05 mg cm⁻² min⁻¹ 0.3 cm thickness</td>
<td>Brandsma 1996</td>
</tr>
<tr>
<td>Haltenbanken</td>
<td>OCC model</td>
<td>2 km</td>
<td>3412.5 mg cm⁻²; 0.42 mg cm⁻² min⁻¹ 2.3 cm thickness</td>
<td>Brandsma 1996</td>
</tr>
<tr>
<td>Offshore California</td>
<td>Sediment traps, Ba as tracer</td>
<td>0.5 km</td>
<td>0.05 mg cm⁻² d⁻¹</td>
<td>Coats 1994</td>
</tr>
<tr>
<td>Offshore California</td>
<td>Sediment traps, Ba as tracer</td>
<td>6.4 km</td>
<td>0.0074 mg cm⁻² d⁻¹</td>
<td>Coats 1994</td>
</tr>
<tr>
<td>Offshore California</td>
<td>Plume trajectory model</td>
<td>1.5 km</td>
<td>0.04-0.05 mg cm⁻² d⁻¹</td>
<td>Hyland et al. 1994</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td>12-19 mg cm⁻² min⁻¹ showed significant effects</td>
<td>Present study 4-h experiment</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td>Coral began to struggle clearing sediment at 120 mg cm⁻²</td>
<td>Present study clearing rates experiment</td>
</tr>
</tbody>
</table>

One field study off the coast of California has measured the estimated flux of drilling particles deposited in sediment traps based on estimates of elevated barium concentrations in the trapped sediments (Coats 1994) (Table 3.5). Sediment traps were placed at varying distances (0.5 km to 6.5 km) from three drilling platforms during 1987 and 1988 and the material collected analysed for barium content (Coats 1994). However, Hartley (1996) has highlighted inconsistencies associated with using barium as a tracer for drilling muds when different extraction methods are used. A second study
in the same region also examined the flux of drilling particles to the seafloor in addition to investigating changes in biota on hard bottom substrates (Hyland et al. 1994). The study found that the cold-water coral *Caryophyllia* sp. decreased in abundance at high flux stations after drilling began. The authors attributed this to the physical effects of drilling discharges and non-toxic effects which suggests that these rates of drilling discharges were high enough to significantly reduce coral abundance (Hyland et al. 1994).

The sedimentation rates reported by Coats (1994) and Hyland et al. (1994) are well below what was observed to cause a significant effect on the polyp behaviour of *L. pertusa* in the present study. However, the daily rates were calculated based on sediment traps left *in situ* for six-month periods. Supplemental data provided on the daily discharge of drilling muds from each of the three platforms showed large variations in the day-to-day volume discharged with maximum discharges reaching 500 m³ d⁻¹ (Hyland et al. 1994). Therefore, some days would have had significantly higher sediment deposition rates compared with the mean rate over a six-month period. Consequently, daily sedimentation rates during certain periods of drilling will have been much higher than 0.05 mg cm⁻² d⁻¹, but will still not reach the rates used in the present study which were magnitudes greater. Significant effects on *Caryophyllia* sp. may be indicative of negative effects that can occur as a result of chronic exposure to influx of drilling particles rather than a one time four-hour event as in the present study.

It is difficult to predict how the aforementioned studies would relate to a situation where drilling for oil and gas would occur near *L. pertusa* occurrences in the northeast Atlantic. Although the examples discussed above all took place in deep-water environments, slight changes in depth, currents, water temperature, and salinity can all
affect the fate of the drilling muds and cuttings once they are released. Furthermore, exploration drilling, production drilling, the number of wells per field, well depths, and the company drilling the well, will all affect the drilling fluid ingredients and the volume of drilling muds discharged. Additionally, one needs to consider the cumulative effects of having numerous exploration wells drilled in the same area and/or having numerous production fields concentrated in the same region. For these reasons, it is almost impossible to predict the concentrations of muds and cuttings that would reach a \textit{L. pertusa} reef in its natural habitat without a detailed study using relevant parameters from such an environment.

### 3.4.6 Potential physiological effects of changes in polyp behaviour

Coral polyp tentacles can alternate between an expanded and retracted state. \textit{Lophelia pertusa} must rely solely on its own feeding ability to obtain nutrition, as it is an azooxanthellate coral. Therefore, polyp retraction, as seen in the present study’s high sedimentation rate experimental runs, can have negative effects on the physiology of a cold-water coral by disrupting its ability to feed.

The energy budget of a coral will be further disrupted as energy is consumed during sediment clearing activities (Dellmeyer et al. 1982; Stafford-Smith and Ormond 1992). Long-term displacement of energy into sediment clearing could ultimately affect a coral’s ability to reproduce (Stafford-Smith and Ormond 1992) and can result in decreased growth (Krone and Biggs 1980; Dodge 1982).

The present chapter has shown that high levels of sedimentation affect \textit{L. pertusa}’s polyp expansion behaviour, which in turn could have longer-term effects on its physiology. But, the experiment represents short-term intense exposure events, rather
than the more realistic longer-term exposure to lower sedimentation rates which are expected in the field. The next chapter will examine the growth patterns of *L. pertusa* living on oil and gas platforms in the North Sea, and how long-term exposure in an environment with increased sedimentation may affect its skeletal morphology.
Chapter 4  –
Growth Patterns of *Lophelia pertusa*: Skeletal Features

4.1 Introduction

4.1.1 Coral growth patterns

Coral growth occurs as the skeleton accretes more calcium carbonate and can be measured in a number of ways by: 1) by direct measurements of colony size, including branch length, diameter or height of a colony; 2) by surface area, volume, or weight of a colony; 3) by calcification measurements; 4) by examining changes in water chemistry including stable isotopes of oxygen in the coral skeleton; or 5) by retrospectively making measurements from coral attached to substrata of a known age, or by skeletal growth patterns such as by counting annual density bands in tropical massive corals (Buddemeier and Kinzie III 1976). Buddemeier and Kinzie III (1976) reviewed coral growth and stated that, “it has long been recognized that corals contain regular growth patterns in their skeleton”. In recent years, much research has focused on density bands observed in a variety of species of massive zooxanthellate corals from different oceans around the world. These bands have been used to provide a chronology for retrospective analyses of environmental data recorded in coral skeletons (Barnes and Devereux 1988). Dense and less dense band couplings usually correspond to annual events, resulting from seasonal changes in the coral’s environment (Knutson et al. 1972; Barnes and Devereux 1988); however, inconsistency in results has created debate regarding the reliability of density band-based chronology (Barnes and Lough 1993). The cause behind density banding in corals is also much discussed and is thought to result primarily from changes in light (Knutson et al. 1972) and/or temperature (Dodge and Vaisnys 1975; Weber et al. 1975), as well as from secondary factors such as nutrient
availability or a coral’s reproductive cycle (Highsmith 1979; Wellington and Glynn 1983). Finer banding patterns have also been observed at a higher resolution and were speculated to represent lunar cycles, but this has yet to be confirmed (Barnes and Lough 1989). There has also been some debate about whether the difference in skeletal density between bands is a result of differences in the microstructure (the aragonite crystal packing), as was suggested by Buddemeier et al. (1974) or the meso-structure (the arrangement of skeletal elements within and between corallites), as suggested by Barnes and Devereux (1988) and Risk and Sammarco (1991).

### 4.1.2 Skeletal variations in *Lophelia pertusa*

Colony morphology of *Lophelia pertusa* differs from tropical coral colonies and its growth patterns are poorly understood. *Lophelia pertusa* is a branching scleractinian coral and colonies from the North Sea oil and gas platforms are dome shaped with densely packed polyps as described in Chapter 2. Corallites of *L. pertusa* are cylindrical to conical-shaped with septa arranged in 3 cycles (Mortensen and Rapp 1998). The colonies are dendroid and branches extend by asexual intratentacular polyp budding. Each corallite contains the basic skeletal components that have been described in Chapter 1; coral polyps are continuous from one to the other.

Zibrowius (1984) described intraspecific morphological variations for several branching forms of cold-water corals including *L. pertusa*. He stated that variation is most evident in differences between the calices themselves, as well as in their arrangement, which alters the overall shape of the colonies. Intraspecific morphological differences are common among scleractinian corals and may be caused by genetic differences or phenotypic plasticity in response to environmental factors including increased sedimentation (Foster 1979; Chappell 1980; Foster 1980; Riegl et al. 1996; Bruno and
Edmunds 1997; Todd et al. 2001). Freiwald et al. (1997) described three morphotypes of *L. pertusa*, tubular, stereome-thickened and stout and crowded, from the Stjernsund reef (Norway) based on corallite characteristics such as polyp height, shape, thecal thickness, growth banding, and budding rate. Kaszemeik and Freiwald (unpublished report) also divided specimens of *L. pertusa* sampled from a range of localities in the North Atlantic into three morphotypes, extrovert, introvert and elongate, based on the appearance of septa, the width to length ratio of the corallite, and thecal thickness. For more information on these morphotypes see Chapter 1.

The first aim of this chapter is to examine skeletal characteristics from sequential polyps along branches of *L. pertusa*. This will be a retrospective analysis of growth patterns based on sequential polyp budding. The skeletal characteristics will be examined to determine if intercolony skeletal morphological differences exist between control and exposed colonies from the North Sea.

**4.1.3 Banding patterns in Lophelia pertusa**

Longitudinal white (optically dense) and grey (optically less dense) bands in the thecal wall and skeletal patterns along the growth direction in septa have been documented in polyps of *L. pertusa* (Wainwright 1964; Freiwald et al. 1997; Mortensen and Rapp 1998; Rollion-Bard et al. 2003; Blamart et al. 2005; Risk et al. 2005). Although skeletal patterns such as skeletal density banding in many massive corals is generally accepted as annual (see earlier section), growth rhythmicities in azooxanthellate corals, such as *L. pertusa*, which live in deep water with minimal temperature and salinity changes, are poorly understood (Freiwald et al. 1997; Nagelkerken et al. 1997; Mortensen and Rapp 1998; Risk et al. 2005). Hence, although skeletal banding has been observed in *L.*
pertusa, little is known about its timing, consistency across different colonies, and environmental influences.

Interspecific differences in the microarchitecture of coral skeletons exist and there are no consistent differences between cold-water corals and tropical zooxanthellate corals (Wainwright 1964). The underlying structural causes of skeletal banding patterns observed in thecal walls of L. pertusa are not well understood. They may be caused by changes in organic content and/or altered crystal orientation, size, or packing. Lophelia pertusa has been described to have a continuous line made up of numerous centres of calcification, which cover extensive areas of the skeleton, and indicate that growth in L. pertusa occurs at the crests of the septa and calyx walls (Wainwright 1964). The second aim of this chapter is to examine polyps of L. pertusa from the North Sea for the presence of growth bands both at a micro- and macro-scale and examine any relationship between the number of bands and the placement of the polyp along a coral branch. Thecal banding of optically dense and less dense bands might represent annual couplets, a hypothesis put forward by Risk et al. (2005), and if polyp budding is a yearly event then it is anticipated that the number of couplets should increase by one with every sequential polyp.

4.1.4 Summary of chapter aims

Results from Chapter 2 provided a mean linear extension rate of 25 mm yr\(^{-1}\) for colonies of L. pertusa sampled from the Tern Alpha platform in the North Sea. The aim of the present chapter is to further the understanding of skeletal growth and potential variations in skeletal characteristics of L. pertusa. A retrospective analysis of growth will be carried out based on the knowledge that L. pertusa buds new polyps sequentially, and the following hypotheses will be tested:
1. Skeletal characteristics of *L. pertusa* colonies from the exposed sites on North Sea platforms differ from colonies from control platforms sites (see Chapter 2 section 2.2.3).

2. *Lophelia pertusa* growth is based on predictable patterns.

3. Coral polyp banding across the theca is directly related to polyp number along a branch.

4. Coral banding is related to crystal structure.

### 4.2 Methods

#### 4.2.1 Skeletal morphological variability and growth patterns

To investigate the growth patterns of *L. pertusa*, coral branches with at least three consecutive polyps were randomly selected from all control and exposed colonies from the North Sea presented in Chapter 2 Table 2.2. The branches, which had been frozen after sampling, were soaked in bleach (<5% sodium hypochlorite) for 24-48 h, rinsed with water to remove any remaining tissue, and dried at 60°C. Polyps were assigned a number starting from one for the outermost polyp and counting back following along the branch towards the centre of the colony (Figure 4.1). Using this approach, Polyp 1 (P1) always corresponds to the youngest generation of polyps and subsequent polyps are labelled P2, P3 etc. This method of polyp numbering is also used in Chapters 5 and 6. Digital callipers were used to make the following measurements for each polyp:

- calyx diameter at the top of the polyp (from outer edges),
- calyx diameter at the base of the polyp (from outer edges),
- height of the polyp corallite from its budding point to the top of the calyx, and
- thickness of the thecal wall at the rim of the calyx.

The number of buds from each polyp was also counted.
Two-sample t-tests were used to test for differences between the various dimensions of the control and exposed colonies where data was normally distributed with equal means (calyx diameter top P2, P4; calyx diameter bottom P1, P3, P4; polyp height P4). Mann-Whitney U tests which is the non-parametric equivalent of the two-sample t-tests and compares medians in unmatched samples (Fowler et al. 1998) were used to test for differences between data sets that were not normally distributed (calyx diameter top P1, P3; calyx diameter bottom P2; polyp height P1, P2, P3; thecal width P1, P2, P3, P4).

Skeletal characteristics were compared between polyp generations using one-way ANOVA (analysis of variance) where the null hypothesis is that samples are drawn from normally distributed populations with equal means and variances (Fowler et al. 1998). Significant results from ANOVA were followed by the Fisher least significant difference (LSD) post hoc tests (Dytham 2003). The non-parametric equivalent of a one-way ANOVA, the Kruskal-Wallis test, was carried out on data that were not normally distributed and without homogenous variances. The Kruskal-Wallis test has the null hypothesis that all samples are taken from populations with the same median (Dytham 2003). Significant results were followed by the non-parametric Mann-Whitney U test. Data from the control colonies were used in this analysis because in some cases significant differences were observed between the control and exposed colonies suggesting that extraneous factors may be influencing growth of the exposed colonies.
4.2.2 Skeletal growth bands

A selection of coral branches prepared as previously described were sliced longitudinally, ground to an even surface on both sides and polished using 400 -1200 μm carborundum paper. One half of each polyp was examined under a Zeiss stereomicroscope using reflected light and the white and grey band couplets in the thecal wall were counted. A band couplet is defined as an area where there is a clear alteration between grey and white skeletal material in the coral thecal wall. Figure 4.2 shows a polyp section under reflected light. Results from band counting were plotted against polyp number using linear regression.
A selection of coral polyps, including samples from North Cormorant (NC3) and North West Hutton (NWH1) that previously demonstrated clear growth banding were examined under a scanning electron microscope (SEM). Samples were investigated for skeletal microarchitecture, including evidence of banding patterns and skeletal growth characteristics. Coral sections, which had already been prepared as described above, were etched in a weak nitric acid solution (1 %) for 30 sec, rinsed in deionised water and dried at 60°C. The samples were then mounted on a sample holder and sputtered with gold under high vacuum. The samples were viewed using a Cambridge S90 Scanning Electron Microscope.

4.3 Results

4.3.1 Skeletal morphological variation and growth patterns

There was a consistent trend of narrower shorter polyps with thinner thecal walls for exposed colonies compared with controls (Figure 4.3).
Figure 4.3 Skeletal characteristics (A) calyx diameter at the top of the corallite, (B) calyx diameter at the bottom, (C) polyp height, and (D) thecal width grouped by polyp number from control and exposed site polyps. Error bars represent the 95% confidence interval. * indicates significant differences between control and exposed polyps where p < 0.05.
Figure 4.4 The results from the skeletal measurements from all control data (A) calyx diameter across the top of the polyp, (B) calyx diameter at the base of the polyp, (C) polyp height, and (D) thecal width as measured at the top of the calyx. * indicates significant differences between polyp numbers where p < 0.05. Error bars represent 95% confidence intervals.

Results showing skeletal characteristics from control North Sea corals are presented in Figure 4.4 A-D. The data sets were not all normally distributed; therefore, the results were tested for significant differences using Kruskal-Wallis tests, followed by Mann-Whitney U tests when the Kruskal-Wallis test gave a significant result.

Figure 4.4A and B show the calyx diameter at the top and bottom of the corallite increasing in size from Polyp 1 to Polyp 3, but with only significant change is between Polyps 1 and 2. Similar to the top of the calyx, the largest increase in growth of the calyx base was between Polyp 1 and 2. Figure 4.4C shows a significant increase in polyp height between Polyp 1 and 2 but not thereafter for Polyps 2, 3 and 4. The
average polyp height of Polyps 2, 3 and 4 was 26.8 ± 5 mm. Finally, Figure 4.4D shows growth that is more consistent with thecal width significantly widening at an almost even rate from Polyp 1 through to Polyp 3 and slows down for Polyp 4. The mean number of buds for Polyps 2, 3, and 4 was approximately two buds per polyp ± 1.

4.3.2 Banding patterns in Lophelia pertusa

Figure 4.5 shows an example of corals with different degrees of thecal banding. For example, a coral colony from Tern (TA1) had lesser banding than a coral colony from North Cormorant (NC3). Corals with thicker thecal walls had more banding. Dark and light bands were not always present along the full length of the thecal wall and the number of bands reported is the greatest number of bands that were visible.
Linear regressions were significant for polyp number versus band couplet for individual branches (Figure 4.6). Only one branch (NC3a) showed a slope of one indicating an increase in one band pair with each polyp number, which was the original hypothesis. Other results show variable rates of banding never exceeding one band pair per polyp number.
Figure 4.6 Linear regression analysis for band pairs versus polyp number for individual branches of *L. pertusa* from (A) North West Hutton (NWH), (B) Tern Alpha (TA), and (C, D, E and F) North Cormorant (NC) and platforms in the North Sea. All regression analyses were significant (p<0.05).

4.3.2.1 Micro-scale skeletal growth patterns of *Lophelia pertusa*

Images from the SEM of transverse and longitudinal sections of *L. pertusa* revealed the microstructure in the theca and septa. Centres of calcification were evident in the theca and septa in all coral polyps examined (Figure 4.7). Cross sections revealed centres of calcification coming down the septa and running into the theca and then dividing to run along the inner portion of the thecal wall (Figure 4.7A). Longitudinal sections revealed lines of centres of calcification running along the inner portion of the thecal wall (Figure 4.7C). In several cases, observations from longitudinal sections revealed two centres of calcification which merged/separated (Figure 4.7E). Crystals were seen radiating away from the centres of calcification in high resolution images (Figure 4.7D). Bundles of
crystal fibres were also observed radiating in the direction away from the centre of calcification (Figure 4.7F).

SEM images of a colony from North Cormorant (NC3 P3) revealed thecal banding. Figure 4.8 shows a series of images running across its thecal wall. It is possible that as fibre bundles grow away from the centres of calcification, a distinct line forms where the bundles stop growing and new ones begin. Evidence for this is especially apparent in Figure 4.8 B, E, and F.

A second type of banding was observed for a colony from North West Hutton (NWH1 P6) where the solubility in weak acid of fibre bundles appeared to alternate creating a banding pattern along the thecal wall. The bands alternated between textured regions where the bundles were more soluble and smooth regions where bundles were less soluble (Figure 4.9).

Images shown in Figure 4.10 revealed a correspondence between the white and grey bands observed under reflected light and thin bands observed in the coral micro-structural under SEM. It may be that the lines observed under SEM corresponded to the end of the fibre bundles because they appear similar to those observed in Figure 4.8.
Figure 4.7 Scanning electron images from a selection of acid etched coral polyps from the North Sea. Centres of calcification and crystal fibre bundles (sclerodermites) as well as some banding are evident.
Figure 4.8 A series of images from a longitudinal cross section of the thecal wall from a colony from North Cormorant (NC3 P3) etched in weak acid. (A) Full view of coral theca. (B-F) series of images crossing the thecal wall starting with the inner theca and moving outwards. Arrows indicate visual signs of longitudinal banding where fibre bundles potentially end and new ones begin.
Figure 4.9 A polyp from a colony from North West Hutton (NWH1 P6) showing thecal bands. (A) the overall image of the thecal wall. (B) the centre of calcification (arrowed). (C) the transition from the first smooth band to the first textured band. (D) the transition from the first textured to the second smooth band. (E) the second smooth to second textured band. (F) the second textured band. (G) the outer edge of the second textured band and thecal wall.
4.4 Discussion

4.4.1 Skeletal variations of Lophelia pertusa

The results in the present study revealed that there was a general trend of narrower, shorter polyps with thinner thecal walls for exposed corals but the differences were not always significant, notably for Polyps 3 and 4, and for thecal wall thickness. Morphological variation within species of scleractinian coral species is well documented, including variation in *L. pertusa* (Zibrowius 1984; Freiwald et al. 1997; Kaszemeid and Freiwald unpublished report), which is either a plastic response to environmental factors, including sedimentation in the present case for example, or a genetic differentiation between different morphotypes (Foster 1979). However, in addition to sedimentation rates (Foster 1979; Chappell 1980; Foster 1980; Rogers 1990; Riegl et al. 1996; Bruno and Edmunds 1997; Carricart-Ganivet and Merino 2001; Todd et al. 2001), environmental factors such as increased hydrodynamic stress, (Foster 1979; Chappell 1980; Kaandorp 1999), light intensity (in zooxanthellate corals) (Foster 1979,
1980; Muko et al. 2000), and food supply (Foster 1979, 1980) can also contribute to morphological variation in scleractinian corals. In the present study, exposed colonies were living in high sedimentation environments where they were exposed to discharges of drilling muds and cuttings. However, other causal factors merit consideration, such as different platform locations and location on the platform in relation to local hydrodynamics.

Coral colonies were selected from several platforms located in the northern North Sea. The detailed sampling procedure supplied to oil companies responsible for collecting coral samples for this analysis requested that equal numbers of control and exposed coral colonies be sampled from each platform. This would have created a balanced nested experimental design. However, in three cases, oil companies sampled all control colonies from one platform and all exposed colonies from another. This eliminates the possibility of testing for the effect of platform location as a factor influencing skeletal morphology. Colonies from individual platforms may be more genetically similar and may be exposed to different hydrographical conditions, although the latter seems less likely as all platforms were located within the same region of the northern North Sea.

In addition, variability on a very local scale such as competition for space and food may affect coral morphology (Freiwald et al. 1997). Being confined to platform structures may create an environment where colonies are competing with other coral colonies and other fouling organisms for space. The direction in which the coral colony was facing in relation to currents may also influence skeletal morphology. To test this hypothesis, further details on the local environments of coral colonies from platforms would be required. It is possible that the platform structure creates changes in water flow on a very local scale. All colonies in the present study were sampled from round cylindrical
structures, with the exception of Brent Delta colonies which were sitting on the top of a cell top, a wide flat structure. Colonies from Brent Delta may therefore have been exposed to a different flow around the colonies, which may account for the smaller polyps. For the remainder of the colonies, a slight difference in the direction they are facing with respect to local currents may affect water movement around them. However, looking at one example based on data collected in the present study provides some evidence against such environmental factors influencing skeletal morphology. Polyp height of the first two Tern colonies sampled, TA1 and TA2 (23 cm), was different from the second two colonies, TA3 and TA4 (30-31 cm), even though they were sampled from the same region of the platform and were facing in a southwest direction according to the ROV heading recorded on the video during sampling (see colony descriptions in Chapter 2).

4.4.2 Skeletal growth patterns of Lophelia pertusa

The overall morphology of coral polyps from North Sea corals is most similar to the tubular morphotype describe by Freiwald et al. (1997) because of the long trumpet shape with relatively thin thecal walls and a lack of well defined growth bands (see Chapter 1 for a detailed description of the tubular morphotype). Furthermore, the mean budding rate per polyp generation was two for North Sea corals with a maximum of six and a maximum of six buds was also reported for the tubular morphotype. However, they differ in their polyp height and North Sea corals are shorter than the tubular morphotype (2-3 cm versus 4-5 cm). A comparative study of several morphotypes spanning the cosmopolitan distribution of L. pertusa may reveal that the North Sea corals are atypical and form their own morphotype because of their unique circumstances growing on vertical platforms above the seabed.
One of the more important findings in this chapter, which will help with the interpretation of the remainder of the results, is related to the mean polyp height of North Sea coral polyp numbers 2, 3, and 4, which was 24.5 ± 6 (SD) mm. This falls within the 26 ± 5 mm annual growth rate calculated in Chapter 2 based on 15 colonies from the Tern platform. This link between the two studies suggests that colonies of *L. pertusa* growing on platforms in the North Sea bud and extend the full length of one polyp per year. This has not been previously documented for *L. pertusa*.

Several growth patterns emerged based on the analysis of skeletal characteristics between sequential polyps along branches of *L. pertusa*. The calyx diameter at the top and bottom of the polyp behaved somewhat differently. As the corals grew upwards, they grew in a trumpet shape and the calyx diameters at the top got wider, at least for the first three polyps or first three years. The calyx continued to widen but not significantly between Polyps 3 and 4. The calyx diameter at the base of the polyp showed continuous expansion for all four sequential polyps. In both cases, the growth between Polyps 1 and 2 was much greater than between Polyps 2 and 3, and 3 and 4.

The polyp height was significantly different between Polyps 1 and 2 and not between Polyps 2 and 3, and Polyps 3 and 4, indicating that polyps reach their maximum height in their first year. Mortensen and Rapp (1998) also suggested that coral polyps from a fjord in mid-Norway initially grew at a relatively fast rate reaching approximately a third of its total height in its first year, but that it then continued to increase in height at a slower rate during subsequent years. This difference suggests that growth patterns of *L. pertusa* in the North Sea may vary from coral colonies from a Norwegian fjord used by Mortensen and Rapp (1998). In the present study, the measurements of thecal width showed a steady increase from Polyps 1 to 2, Polyps 2 to 3, and then only a slight
decrease in the thickening rate between Polyps 3 to 4. So it would appear that the increase in thecal width is steady throughout at least the first four years of the polyp’s life.

In summary, the majority of polyp growth occurs in the first year; the polyp reaches its maximum height and creates the characteristic trumpet shape by initial fast outward growth at the top of the calyx. The calyx continues to widen over subsequent years at both the top and base of the corallite. In contrast, the thecal wall thickens at a steady rate for at least the first four years of the coral polyp’s life.

4.4.3 Banding patterns of Lophelia pertusa

A significant relationship was found between polyp number and the number of growth bands when polyps were analysed individually. Rates of band formation in relation to polyp number, as measured by the slope of the linear relationship, were variable between samples indicating that individual polyps create growth bands at different rates. Counting the bands was difficult as not all bands were continuous along the full length of the polyp, and different values would be obtained from different regions of the theca. In the present study, the region with the greatest number of visible bands was analysed. Corallites may not always be completely covered by tissue. It remains unknown whether tissue or mucus covers the outside of coral skeletons because no histological studies have looked at this. However, Freiwald (1998) reported that tissue rarely extends over the growing edge and that it has never been reported covering larger portions of the skeleton. As a result, he suggested that coral thecal thickening is a result of mucus mediated calcification. However, tissue covering large areas of the skeleton has been observed for several colonies sampled from the North Sea, but the tissue
retracts soon after sampling (personal observation). It may be possible that tissue and/or mucus retract and extend in situ resulting in uneven banding patterns.

The innermost band (white and optically dense) was the only band that was consistently present in all polyps examined, and it ran the full length of the polyp. This is in contrast to findings of Mortensen and Rapp (1998) who found that density bands in the theca ran up the polyp with the innermost band being the shortest and the second innermost band being the next longest etc.

In future work, a larger sample size of polyps from each colony may help establish consistent banding rates within colonies. The polyps with the highest number of density bands also had thick thecal walls. Freiwald et al. (1997) described morphotypes showing variation in the presence of density bands, i.e. the tubular morphotype had thin thecal walls and did not show banding, while the stereome-thickened morphotype had thick thecal walls and showed clear banding. Coral skeletons that are completely covered with tissue or mucus may result in clear consistent banding patterns, whereas corals whose tissue or mucus does not extend beyond the growing edge will have less banding.

SEM images of *L. pertusa* clearly show centres of calcification for each coral polyp. These centres made up a continuous line and were up to 50 μm in width which is large compared to previous reports centres of calcification from tropical corals which varying from 3 to 40 μm (Perrin 2003). The centres of calcification were observed in cross sections running down septa and intercepting with centres of calcification running along the inner half of the thecal wall. These likely represent the centres of calcification from the major septa (Sorauf and Freiwald 2002). Growth in *L. pertusa* is believed to be
initiated in the major septa followed by growth in the thecal wall (Wainwright 1964). In longitudinal sections, the centres of calcification ran parallel along the axis of growth, as is reported to occur in all Scleractinia (Perrin 2003), separating the inner theca from the outer theca. Furthermore, several cases were observed where two lines of centres of calcification either merge or separate. It was likely that one was from a major septum and the other the theca. In all cases, the centres of calcifications could be followed along the inner thecal wall from the base of the polyp to the distal end. Hence, the thickening of the thecal wall documented earlier in the chapter, occurs on the outer edge of the polyp wall. Thus, the innermost growth band, optically dense and white in colour, and present in all polyps observed in this study, likely represents the earliest skeletal material accreted by each polyp. It also probably includes the centres of calcification which have been reported as optically dense regions in thecal walls (Adkins et al. 2003; Blamart et al. 2005; Lutringer et al. 2005).

Different types of banding patterns were seen under the SEM. Fine lines were observed running along the longitudinal sections of the thecal wall which corresponded with grey bands observed under reflected light. These bands appeared to form at the edges or ends of fibre bundles. Additional images illustrated wider banding of what appeared to be alternating fibres which were more and less weak-acid soluble. It was not clear whether these corresponded to the white and grey bands. It could be hypothesized that these bands are related to differences in organic content. Bundles visible as depressions or textured regions in the thecal wall could represent the more soluble organo-mineral increments, and the more uniform fibre bundle might represent the less soluble fibres with less organic material. Similar patterns have been observed in other scleractinian corals but at a much smaller banding scale with bands two orders of magnitude smaller than those observed in the present study (Cuif et al. 1999; Perrin 2003). Cuif et al. 2003
used X-ray absorption to examine the organic content in coral skeletons from centres of calcification and surrounding fibres and found that sulphur bearing amino acids and sugars were more abundant in centres of calcification. Subtle changes in micro-porosity and crystal size may also contribute visual white and grey bands, although this was not obvious from the SEM images.

4.5 Conclusions

The results from this chapter have revealed some aspects of the sequence of polyp skeletal growth of *L. pertusa* from North Sea oil and gas platforms. In particular, when tied with results from Chapter 2, it showed that colonies extend the length of one polyp per year. Furthermore, colonies living close to oil and gas discharges had consistently shorter and more narrow polyps compared to control colonies, but that these differences in skeletal morphology may also be governed by additional local environmental factors or genetic influences.

The results have also shown that centres of calcification are present in all polyps in the inner thecal wall and that banding patterns observed in thecal walls are not consistent between colonies. Hence, the use of outer thecal growth bands as a chronology for environmental tracers will not be effective until a better understanding of the root cause behind banding patterns and their inconsistencies between colonies is determined. SEM images revealed some banding in etched corals which appeared to correspond to banding observed under reflected light and may result from boundaries between crystal fibre bundles or differing organic content of crystal fibre bundles.
Chapter 5 –

Growth Patterns of *Lophelia pertusa*: Stable Isotopes of Carbon and Oxygen

5.1 Introduction

Annual density banding in massive corals, as described in Chapter 4, has provided a chronology vital to scientists examining geochemical parameters such as stable isotopes and trace elements in relation to paleo-temperatures and specific long-term climate events (Swart 1983; Cole and Fairbanks 1990; Beck et al. 1992; McCulloch et al. 1994; Tudhope et al. 2001). Corals can provide an unaltered record of the chemical and physical properties of ambient seawater at the time they laid down their skeletons (Druffle 1997). The examination of carbon and oxygen stable isotopes is the oldest method used to reconstruct paleo-oceanographic histories in coral skeletons, and in particular $\delta^{18}O$ varies in relation to seawater temperature and isotopic composition (e.g. Weber and Woodhead 1970; Goreau 1977; Emiliani et al. 1978; Erez 1978; Swart 1983; Leder et al. 1996 with continued research to the present day). More recently, research has also focused on the relationship of Sr/Ca and Mg/Ca with seawater temperatures (Mitsuguchi et al. 1996; Cohen and Hart 1997; Mitsuguchi et al. 2001; Cohen et al. 2002).

Similar paleo-climate studies have also focused on cold-water corals as they offer the potential for use over a wider area (Emiliani et al. 1978; Swart 1983; Smith et al. 1997; Smith et al. 2000; Adkins et al. 2003; Sherwood et al. 2005a), including carbon and oxygen isotopic studies of *Lophelia pertusa* skeletons (Weber 1973; Mikkelsen et al. 1982; Freiwald et al. 1997; Mortensen and Rapp 1998; Spiro et al. 2000; Freiwald 2002;
Rollion-Bard et al. 2003; Blamart et al. 2005; Lutringer et al. 2005; Risk et al. 2005). Although the isotopic signatures in cold-water corals are simplified by the lack of zooxanthellae and influences from photosynthesis (see below), lower seasonal temperature variations in deep-water are easily masked by contributions from metabolic CO₂ (see below). Furthermore, a lack of understanding of growth rates and banding patterns in cold-water corals make it difficult to link isotopic signatures to oceanographic histories. Recent research, however, has started to provide detailed growth rates and further insights into banding patterns for several cold-water species (Lazier et al. 1999; Adkins et al. 2004; Sherwood et al. 2005b). In particular, clear tree-ring-type banding has proven useful in gorgonians (Sherwood et al. 2005a; Sherwood et al. 2005b)) but further research is required to complete a growth chronology for *L. pertusa* (Freiwald et al. 1997; Mortensen and Rapp 1998).

One way to investigate banding patterns and growth rates of coral skeletons is to use the same theories that enable isotopic studies to reveal oceanographic histories. Isotopic signatures from skeletal material sampled along a growth axis should reflect seasonal changes in seawater temperature (Epstein et al. 1953; Weber and Woodhead 1972) and seasonal influences such as food supply which result in faster calcification (see below).

**5.1.1 δ¹³C and δ¹⁸O: vital effects**

Corals, unlike some other calcifying organisms, do not secrete their skeleton in isotopic equilibrium with seawater. The offset from equilibrium is attributed to “vital effects” (Weber and Woodhead 1972). Vital effects simultaneously influence δ¹³C and δ¹⁸O fractionation during calcification in the absence of other factors, such as zooxanthellae and changing seawater temperatures and isotopic composition, and results in a strong
relationship between $\delta^{13}C$ and $\delta^{18}O$ in azooxanthellate corals. The causes behind the vital effects are still debated but several theories have been proposed including two models proposed by McConnaughey (1989a; 1989b) and Adkins (2003). Both models show that increased calcification rates lead to greater offsets of $\delta^{13}C$ and $\delta^{18}O$ from equilibrium although different mechanisms are used to explain the models.

Goreau (1959) proposed the following calcification reaction:

$$\text{Ca}^{2+} + 2\text{HCO}_3^- \rightleftharpoons \text{Ca}((\text{HCO}_3)_2 \rightleftharpoons \text{CaCO}_3 + \text{H}_2\text{CO}_3$$

The following reactions are also relevant to the vital effects models:

$$\text{CO}_2(aq) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \quad \text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}$$

McConnaughey (1989a; 1989b; 2003) discussed kinetic and metabolic effects on the rate of fractionation of oxygen and carbon between seawater and calcium carbonate. He proposed that kinetic fractionation occurs during the hydration and hydroxylation of CO$_2$ during coral calcification. As calcification rates increase, the selectivity of the lighter isotopes of carbon and oxygen during the hydration and hydroxylation of CO$_2$ and H$_2$O increases which results in lower $\delta^{13}C$ and $\delta^{18}O$ in the coral skeleton. Kinetic behaviour tends to result in depletion from seawater equilibrium of up to 10-15 ‰ for $^{13}C$ and 4 ‰ for $^{18}O$ (McConnaughey 1989a). Metabolic effects can offset the kinetic effects and are caused by both photosynthesis (in zooxanthellate corals) and coral respiration. Photosynthesis will preferentially use the lighter isotopes of carbon enriching the calcifying fluid in the heavy isotopes, while coral-respired CO$_2$ is isotopically lighter, and has an opposite but lesser effect. As a result, zooxanthellate corals have higher $\delta^{13}C$ compared to azooxanthellate corals and deviate further from expected kinetic effects (McConnaughey 1989a).
Adkins et al. (2003) proposed that, at least in azooxanthellate corals, the fractionation of carbon and oxygen isotopes is controlled by a pH gradient created at the site of calcification, the extra-cellular calcifying fluid (ECF), which increases with faster calcification rates. Skeletal carbon can come from two sources: from leakage of dissolved inorganic carbon from the surrounding seawater or from $^{13}$C depleted CO$_2$(aq) across the cell membrane. As calcification increases, the pH gradient drives passive diffusion of CO$_2$(aq) and the pCO$_2$ gradient also increases until it reaches a maximum gradient at very low CO$_2$ levels in the ECF. At this point, the CO$_2$(aq) will be a larger component of carbon in the calcifying fluid and will thus be the major component of carbon in the skeleton.

Adkins et al. (2003) proposed a different mechanism to explain the $\delta^{18}$O in coral skeletons. Oxygen in inorganic carbon species is more enriched in $^{18}$O compared to seawater. Further, carbonic acid is more enriched in $^{18}$O than carbonate, therefore increases in pH (which results in a higher proportion of CO$_3^{2-}$) leads to lighter $\delta^{18}$O compared to more acidic waters. There are a finite number of $^{13}$C atoms from inorganic species in the ECF, whereas oxygen has an effectively infinite amount of $^{18}$O in seawater. Therefore, the skeletal $\delta^{13}$C stabilizes once the pCO$_2$ gradient is large enough and CO$_2$ crossing the cell membrane reaches a maximum, but $\delta^{18}$O will continue to be depleted offsetting the well-documented linear relationship between $\delta^{13}$C and $\delta^{18}$O in cold-water corals.

### 5.1.2 Isotopic signatures and growth rates of Lophelia pertusa

Oxygen and carbon isotopic signatures in skeletons of *L. pertusa* have been used to estimate growth rates for *L. pertusa* of between 13- 25 mm yr$^{-1}$ based on isotopic
patterns attributed to seasonal changes in temperature and/or food availability (Mikkelsen et al. 1982; Freiwald et al. 1997; Spiro et al. 2000). These rates are comparable to those measured by colonization of corals on artificial substrates of a known age, which range between 5-26 mm yr\(^{-1}\) (Duncan 1877; Wilson 1979b; Bell and Smith 1999; Roberts 2002; this study Chapters 2 and 3). Sub-samples for isotopic analyses were taken along thecal walls, and corresponding patterns of temperature and \(\delta^{18}O\) (Mikkelsen et al. 1982), or observed cyclical patterns in both \(\delta^{18}O\) and \(\delta^{13}C\) equated to one year were used to estimate growth rates (Freiwald et al. 1997; Spiro et al. 2000). In all three studies, single polyps were used to demonstrate growth rates.

Banding patterns in \(L.\ pertusa\) have also recently been investigated using stable isotope of carbon and oxygen and have revealed that optically dense bands are consistently depleted in \(\delta^{18}O\) and \(\delta^{13}C\) (Adkins et al. 2003; Lutringer et al. 2005; Risk et al. 2005). According to consequences of vital effects described above this should indicate faster growth for optically dense bands (Adkins et al. 2003).

The aims of this chapter are to examine \(\delta^{18}O\) and \(\delta^{13}C\) signatures along branches of \(L.\ pertusa\) and across polyp thecal walls to look for seasonal patterns that can be used to determine growth rates and patterns. Results from Chapter 4 suggested significant differences in growth morphology of coral polyps from control and exposed sites; therefore, growth rates may also differ between control and exposed colonies. In addition, if growth rates are determined using this method, it could be possible to compare growth rates in relation to the heavy metal content of the skeletons which could be subsequently analysed using techniques described in Chapter 6.
The following hypotheses will be tested in this chapter:

1. $\delta^{18}O$ and $\delta^{13}C$ will follow a seasonal pattern along the growth axis of *L. pertusa* from which a growth timeline can be deciphered.

2. The inner most optically dense growth band will represent relative fast skeletal accretion based on vital effects theories proposed by Adkins et al. (2003) and McConnaughey (1989a; 1989b).

3. Optically dense and less-dense bands across the thecal wall of *L. pertusa* will have different isotopic signatures.

### 5.2 Methods

Sub-sampling for the stable isotope analyses of carbon and oxygen was carried out using two different methods, bulk sub-sampling of the thecal wall and higher resolution sub-sampling along the innermost growth band of the thecal wall. In both cases, selected branches of *L. pertusa* were soaked in bleach (<5 % sodium hypochlorite) for 24-48 h, rinsed in deionised water to remove any remaining tissue, and dried at 60°C.

Bulk sampling was used to examine one branch of *L. pertusa* from a coral colony sampled by Dr. Murray Roberts from a Beryl Single Point Mooring (SPM) at 100 m depth in the northern North Sea in October 1999 (Roberts 2002). The branch was sectioned into individual polyps, which were sliced longitudinally, and the septa scraped out of the calices. The polyps were sub-sectioned at 1 mm intervals using a Dremmel tool with a dental cutting disc. Individual sub-samples were then crushed into a powder using an agate pestle and mortar. Oxygen and carbon stable isotope analyses were performed on sub-samples of carbonate powder (0.66-1.0 mg).
Single branches, each containing five polyps, were chosen from a control colony from North Cormorant (NC3) and exposed colony from Tern Alpha (TA1) for the second stable isotope analysis. See Chapter 2 for further information on these samples. The branches were sectioned into individual polyps, each was embedded in resin, and a 3 mm thick section from the middle of each polyp was cut and polished (Figure 5.1). The longitudinal section was taken from to the extent possible from the centre of each polyp and avoided areas where new polyps had budded. A computer-controlled micro drill (New Wave Research Leica GZ6 MicroMill at the University of Edinburgh) with a sampling width of 200 µm was used to sample the coral skeleton at high resolution. Results from Chapter 4 suggested that the innermost growth band in sequential polyps represents early polyp growth. Therefore, this band was used in the high-resolution analyses. Pre-designated lines along the innermost growth band of the thecal wall were identified using a computer and software system connected to the micro drill, and they were drilled to a depth of 200 or 400 µm (Figure 5.2).

Figure 5.1 Longitudinal sections were sliced from the centre of each polyp.
Figure 5.2 High resolution sampling along the inner-growth band of a coral from North Cormorant (NC3 P5). (A) Before sampling and (B) after sampling where lines 200 µm wide and 2500 µm long were drilled along the inner optically dense (white) growth band.

The surface area and depth drilled for sub-samples was designed to obtain between 0.1 and 0.4 mg carbonate material for the analysis. Lines 5000 µm long and 200 µm deep were initially drilled followed by lines 2500 µm long and 400 µm deep once it had been visually checked that drilling to this depth did not penetrate beyond the designated growth band. This allowed for three to six sub-samples per polyp. Longer lines covering the entire growth band were also drilled from outer grey and white bands. Data was combined from the North Cormorant and Tern Alpha grey and white skeletal samples to increase the data set to examine the differences in isotopic signatures from grey (optically less dense) versus white (optically dense) skeletal material. Compressed air was used to clean polyp sections between drilling of sub-samples and prevent cross-contamination.

Oxygen and carbon stable isotope analyses were performed on crushed and drilled powder sub-samples of skeletal material. The carbonate powder was reacted with 100 %
orthophosphoric acid at 90 °C in an Isocarb automatic carbonate preparation system. The resulting CO₂ was then analysed on a VG Isogas Prism III stable isotope ratio mass spectrometer in the Wolfson Laboratory, University of Edinburgh for both δ¹³C and δ¹⁸O from the same sub-sample. The standard deviations for eight analyses of a standard marble powder (MAB2B) run as a sample on the same days as the bulk skeletal samples (3-5-Mar-03) were ± 0.07 ‰ for δ¹³C and ± 0.09 ‰ for δ¹⁸O. The standard deviations for eight analyses run on the same day as the micro-drilled samples (1-3-Dec-04) were ± 0.06 ‰ for δ¹³C and ± 0.08 ‰ for δ¹⁸O. All carbonate isotopic values are reported in the conventional δ notation relative to the Pee Dee belemnite (PDB) reference standard:

\[
\delta = \frac{(R_{\text{sample}} - R_{\text{standard}}) \times 1000}{R_{\text{standard}}} \ (\text{‰})
\]

Where R is the ratio of ¹⁸O:¹⁶O or ¹³C:¹²C in the sample or standard.

5.3 Results

Results for δ¹³C and δ¹⁸O from the bulk sampling of skeletal material from a branch of L. pertusa from the Beryl SPM are presented in Figure 5.3. Values for δ¹³C ranged from -9.69 to -4.25 ‰ and δ¹⁸O values ranged from -2.88 to 1.44 ‰. Both stable isotopes follow a similar trend along the branch. Previous studies have reported similar trends using regression lines (McConnaughey 1989a; 1989b; Spiro et al. 2000; Smith et al. 2000; Blamart et al. 2005; Lutringer et al. 2005). A linear regression of the present results is shown in Figure 5.4 where a strong relationship was observed between δ¹³C and δ¹⁸O. A cyclical trend of δ¹³C and/or δ¹⁸O would be expected if one or both were reacting to environmental cues and/or changes in skeletal growth rates as a result of environmental cues. The cycle would then be assumed to represent a time line if the source of the signal could be deciphered. This was not observed for either element.
Variation observed along Polyp 4 showed a gradual increase and then decrease in the values for δ\textsuperscript{13}C, and there was a large increase in both δ\textsuperscript{13}C and δ\textsuperscript{18}O along the first half of Polyp 2.

Micro sub-sampling results of skeletal material from the North Cormorant (NC) colony (NC3) are shown in Figure 5.5. Polyp 4 originally had three sub-samples but one was lost during the weighing process. δ\textsuperscript{13}C values ranged from -7.69 to -10.34 ‰, and values for δ\textsuperscript{18}O ranged from -0.74 to -2.47 ‰. The results for δ\textsuperscript{13}C showed five peaks along the branch, one for each polyp, and generally in the middle of the polyp. The results for δ\textsuperscript{18}O did not always follow the same trend as δ\textsuperscript{13}C and the results from a linear regression analysis had an r\textsuperscript{2} of less than 50 % but the p-value remained significant (Figure 5.6).

The results from the micro-sampled analysis of the Tern Alpha (Tern) colony (TA1) are shown in Figure 5.7. The values for δ\textsuperscript{13}C ranged from -7.69 to -9.33 ‰, and for δ\textsuperscript{18}O ranged from -0.73 to -1.71 ‰. There was no clear cyclical pattern observed for either δ\textsuperscript{13}C or δ\textsuperscript{18}O along the branch, although troughs and peaks were present which roughly equated to one peak per polyp. δ\textsuperscript{13}C and δ\textsuperscript{18}O did not appear to be following the same trend and the results from a linear regression had a p value of 0.051 just above the significance level of 0.05, and the r\textsuperscript{2} value indicated that variation in δ\textsuperscript{18}O could only explain 21 % of the variation in δ\textsuperscript{13}C (Figure 5.8).
Figure 5.3 The stable (A) carbon and (B) oxygen isotopic compositions of a branch of *L. pertusa* sampled from the Beryl Single Point Mooring and analysed using bulk samples.

Figure 5.4 The relationship between the stable oxygen and carbon isotopic compositions of a branch of *L. pertusa* from the Beryl Single Point Mooring.
Figure 5.5 Stable (A) carbon and (B) oxygen isotopic compositions of a branch of *L. pertusa* sampled from North Cormorant using high resolution microdrill sub-sampling.

Figure 5.6 The relationship between the stable oxygen and carbon isotopic compositions of a branch of *L. pertusa* from North Cormorant.
Figure 5.7 The stable (A) carbon and (B) oxygen isotopic compositions of a branch of *L. pertusa* sampled from Tern Alpha using high resolution microdrill sub-sampling.

Figure 5.8 The relationship between the stable oxygen and carbon isotopic compositions of a branch of *L. pertusa* from Tern Alpha.
Data from all three stable isotopic experiments generally fell on the same trend line (Figure 5.9). However, the Tern and NC colonies fell at the lower end of the values compared to Beryl, and there were values from all three experiments that fell below the trend line, particularly at the lightest end of the isotopic values.

![Figure 5.9 Stable isotopic data for the three analyses. All data appear to follow the same trend except for several values from each of the three analyses which fall below the line of best fit at the lighter end of the isotopes.](image)

White (optically dense) and grey (optically less dense) growth bands were visible on polyps from both NC and Tern branches. Grey bands were less depleted in $\delta^{13}$C and $\delta^{18}$O compared to white bands (Figure 5.10).
5.4 Discussion

5.4.1 Bulk versus micro-sampling

Results from the bulk analysis of the Beryl SPM, and micro-drill analyses of the NC and Tern samples gave values for $\delta^{13}$C and $\delta^{18}$O within the lower range of what has been previously published for *L. pertusa* (Table 5.1). Bulk sub-sampling of the thecal wall from the Beryl sample showed a variation of up to 5.3 ‰ for $\delta^{13}$C and 4.3 ‰ for $\delta^{18}$O, while the micro-skeletal analysis along the inner growth bands of the NC and Tern branches resulted in a smaller range of 2.6 and 1.6 ‰ for $\delta^{13}$C and 1.7 and 0.98 ‰ for $\delta^{18}$O.
Table 5.1 $\delta^{13}$C and $\delta^{18}$O for L. pertusa

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Carbonate Sampling Method</th>
<th>Seawater Temp. $^\circ$C</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{18}$O</th>
<th>Slope of $\delta^{13}$C vs $\delta^{18}$O</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various locations off coast of Norway</td>
<td>Bulk sampling</td>
<td>7-10</td>
<td>-6.99 to -5.9</td>
<td>-1.18 to -0.39</td>
<td>0.38</td>
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<td>Norwegian Shelf</td>
<td>~ 1 mm drill (longitudinally along theca)</td>
<td>-</td>
<td>-8.56 to -1.30</td>
<td>-1.65 to 2.53</td>
<td>0.67</td>
<td>Mikkelsen (1982)</td>
</tr>
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<td>Trondheimsfjord Norway</td>
<td>~ 1 mm drill (longitudinally along theca)</td>
<td>7-9</td>
<td>-9.61 to -7.64</td>
<td>-2.00 to 1.13</td>
<td>1.25</td>
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</tr>
<tr>
<td>Oslofjord, Norway</td>
<td>~ 1 mm drill (longitudinally along theca)</td>
<td>-</td>
<td>-6.20 to 3.08</td>
<td>0.49 to 1.30</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Stjernsund Sill, Norway</td>
<td>~ 1 mm drill (longitudinally along theca)</td>
<td>6-7</td>
<td>-9.25 to -5.76</td>
<td>-0.20 to 2.97</td>
<td>0.35</td>
<td>Freiwald et al. (1997)</td>
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<tr>
<td>Norwegian Shelf</td>
<td>Grinding horizontally across theca</td>
<td>-</td>
<td>-7.58 to -4.33</td>
<td>-0.89 to 1.33</td>
<td>~0.6</td>
<td>Mortensen and Rapp (1998)</td>
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<tr>
<td>Trondheimsfjord</td>
<td>Grinding along septa</td>
<td>7-8</td>
<td>-7.95 to -5.05</td>
<td>-1.93 to 0.73</td>
<td>~0.7</td>
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<td>1 mm drill (longitudinally along theca)</td>
<td>&lt;8 to 10.8</td>
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<td>-2.4 to 1.6</td>
<td>0.5</td>
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<td>Numerous locations</td>
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<td>0.0 to 2.5</td>
<td>0.23-0.67</td>
<td>Smith et al. (2000)</td>
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<td>-4.50 to 4.40</td>
<td>0.54</td>
<td>Rollion-Bard et al. (2003) / Blamart et al. (2005)</td>
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<td>0.8 mm drill</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Ion microprobe from septa across theca</td>
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<td>-15.3 to -0.7</td>
<td>-4.50 to 4.40</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>horizontally within centre of calcification</td>
<td>-</td>
<td>-14.3 to -10.9</td>
<td>-3.2 to -2.1</td>
<td>0.54</td>
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<tr>
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<td>Ion microprobe from septa across theca</td>
<td>-</td>
<td>-15.3 to -0.7</td>
<td>-4.50 to 4.40</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>horizontally outside of centre of calcification</td>
<td>-</td>
<td>-14.3 to -10.9</td>
<td>-3.2 to -2.1</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>
The results from the Beryl sample showed a strong relationship between $\delta^{13}C$ and $\delta^{18}O$ which has been consistently reported for cold-water corals (McConnaughey 1989a, 1989b; Smith et al. 2000; Adkins et al. 2003) including *L. pertusa* (Mortensen and Rapp 1998; Spiro et al. 2000; Lutringer et al. 2005; Risk et al. 2005); the results from the NC and Tern colonies showed weaker relationships. The bulk sampling of the Beryl sample meant that the full range of skeletal material (optically dense and less-dense bands) was represented in the sub-samples, whereas material from NC and Tern was selected from within the innermost optically dense growth band. The results for the NC and Tern colonies fell at the isotopically lightest end of previously reported data. This supports the theory that the innermost growth band is accreted at a relatively fast rate. Skeletal material formed through gradual thickening (including optically less-dense bands) of the skeleton was avoided by sub-sampling along this band. Therefore, the spread observed in the linear regression analyses of the Tern and NC colonies only represents a subset of possible calcification rates, and this could partly explain the weak relationship that was observed.

Adkins et al. (2003) analysed the cold-water coral *Desmophyllum cristgalli* and found that at the fastest calcification rates $\delta^{18}O$ continues to become depleted while $\delta^{13}C$ reaches a limit, thus offsetting the linear trend between $\delta^{13}C$ and $\delta^{18}O$ at the lightest values. Their results were based on calcium carbonate sampled from within the centre of calcification. This phenomenon might also be expected from the results of sampling from the inner growth band of *L. pertusa*, which contains the centre of calcification. At least one data point from the NC colony could represent this phenomenon (see Figure 5.8 and data points with lowest $\delta^{18}O$ values), but it is not observed from the Tern data, and thus cannot be used to explain the weak linear relationship between $\delta^{13}C$ and $\delta^{18}O$. In fact, several data points from the Beryl sample also fall into this category even
though it was bulk sub-sampled. These light values came from the base of Polyp 6 that may have contained faster growing material. Other studies also report most depleted values for $\delta^{13}C$ and $\delta^{18}O$ from the base of polyps, where polyps are believed to start accreting skeleton at a fast rate (Emiliani et al. 1978; Swart 1983; Mortensen and Rapp 1998; Spiro et al. 2000).

5.4.2 Cyclical variations of $\delta^{13}C$ and $\delta^{18}O$

No clear cyclical pattern was observed in the Beryl sample because bulk sampling integrates skeletal material accreted over different times into one sub-sample. Beryl Polyps 3 and 5 had constant values for $\delta^{13}C$ and $\delta^{18}O$ as would be expected from integrated skeletal material. However, Polyps 2, 4 and 6 showed some changes in $\delta^{13}C$ and $\delta^{18}O$ over the length of the polyp. Polyp 2, in particular, showed a sharpened increase in $\delta^{13}C$ and $\delta^{18}O$. Based on images of the coral branch and where the polyps were sliced, when Polyp 2 budded, skeletal material from Polyp 3 began to swell out and created what could be considered the base of Polyp 2. The high $\delta^{13}C$ and $\delta^{18}O$ values correspond to this part of the skeleton (Figure 5.11). This phenomenon would therefore probably not have been observed if Polyp 2 had been sliced directly from the top of Polyp 3. It is not clear why this region of Polyp 2 resulted in higher $\delta^{13}C$ and $\delta^{18}O$, but it is possible that the majority of skeleton sampled from here is from the outside bands that are believed to include skeleton that was accreted at slower growth rates. Polyps along a branch of *L. pertusa* are continuous from one to the next, and the division between polyps is not always clear. Therefore, there is a certain amount of subjectivity involved when slicing polyps along a branch.
Figure 5.11 Polyps 1-4 from the Beryl SPM coral branch. The area between the arrows corresponds to the regions of high values for $\delta^{13}C$ and $\delta^{18}O$ for Polyp 2. The black line represents where the polyp was sliced from the branch.

Results from the NC and Tern colonies showed cyclical variations (smaller variations than reported from previous studies), which were most conspicuous for the NC branch $\delta^{13}C$, and showed one peak per polyp. Results from Chapter 2 and Chapter 4 together provided evidence that coral colonies were extending the length of one polyp per year. These results both point towards the potential for a seasonal trigger. $\delta^{13}C$ and $\delta^{18}O$ variations along the Tern branch were less regular. The Tern sample was an exposed colony living 3-4 m below a discharge chute. The variability observed from the Tern branch may be a result from periodic exposure to discharges that could have slowed calcification. Both $\delta^{13}C$ and $\delta^{18}O$ for the Tern branch were approximately 0.05 ‰ greater than for the NC branch indicating possible slower calcification. A greater difference might be expected if calcification had been altered considerably. Alternatively, the innermost bands on the Tern branch were narrower than those of the NC branch offering more potential to sample outside this band into carbonate material that was accreted at a slower rate.
Cyclical increases in $\delta^{13}C$ and $\delta^{18}O$ observed along the NC branch potentially reflect slower calcification. Coral calcification rates are influenced by environmental factors such as seawater temperature, increased light in the case of zooxanthellate corals, food availability, and the onset of reproduction (Weber and Woodhead 1970; Land et al. 1975; Emiliani et al. 1978; Erez 1978; Swart 1983; McConnaughey 1989a; Freiwald et al. 1997; Smith et al. 2000; Tudhope et al. 2001; Adkins et al. 2003). Factors that are most likely to contribute to changes in cold-water coral growth rates are seasonal availability of food and the onset of the reproductive cycle (Freiwald et al. 1997; Spiro et al. 2000; Risk et al. 2005). However, changes in seawater temperature may also be a contributing factor for corals living on North Sea platforms, which are found at shallower depths (85-107 m) where temperatures between the warmest and coldest season vary by 2 °C (IRI LDEO Climate Data Library Levitus94 2006). Although tropical corals experience temperature changes over 10 °C (Wolanski 1994) a change of 2 °C can affect skeletal $\delta^{18}O$ by 0.44 ‰ and $\delta^{13}C$ by 0.07‰.

Reproduction, nutrient inputs, and changes in seawater temperature are seasonal occurrences. The reproductive cycle of *L. pertusa* is an annual event (Waller and Tyler 2005), but not all polyps necessarily become reproductive (see Chapter 2). Reduced growth rates caused by energy allocation toward reproduction may increase both $\delta^{13}C$ and $\delta^{18}O$ by slowing calcification. However, polyps must reach a certain size before they become reproductive and this probably does not occur in their first year. Limited information exists on the diet of *L. pertusa*; however, recent studies have suggested that cold-water corals, including *L. pertusa*, feed on zooplankton (Kiriakoulakis et al. 2005) as well as on algae and dissolved organic matter (Duineveld et al. 2004). Phytoplankton blooms are seasonal in the North Sea occurring in spring with often a second smaller bloom in the autumn. Zooplankton abundance follows a similar seasonal trend to
phytoplankton. Metabolic activity affects the contributions of isotopically light carbon from metabolic CO$_2$ to accreted skeleton by not more than 1-2 ‰ at least in tropical corals (McConnaughey 1989a; McConnaughey 2003). The resulting increase in skeletal growth rates will simultaneously affect $\delta^{13}$C and $\delta^{18}$O though kinetic effects and should also correspond to skeletal material more depleted in $\delta^{13}$C and $\delta^{18}$O. Therefore, excursions in a negative direction would be expected if the above-mentioned seasonal factors are contributing to the observed trends, rather than the short-lived positive peaks observed in the present study.

Temperature changes are also seasonal and stable isotope data for carbon and oxygen reported from Mortensen and Rapp (1998), Mikkelsen et al. (1982) and Weber (1973) have shown close relationships between $\delta^{18}$O in *L. pertusa* and local temperature changes of just 1 °C. Research on molluscs has shown that an increase of 2 °C, as recorded from the North Sea, corresponds to a decrease in $\delta^{18}$O of 0.44 ‰ (Epstein et al. 1953) but does not affect $\delta^{13}$C to the same degree (Grossman and Ku 1986). It is possible that there are more than one seasonal factor, e.g. both temperature and metabolic effects, acting on $\delta^{13}$C and $\delta^{18}$O, which could contribute to lack of clear seasonality and the weak relationship observed between $\delta^{13}$C and $\delta^{18}$O from both the Tern and NC colonies.

It is difficult to conclude which factors or combinations thereof are causing the changes in $\delta^{13}$C and $\delta^{18}$O based on only two branches each from different colonies. Furthermore, the variation observed in the Tern and NC colonies is small compared to the bulk sampling and other previous studies (Table 5.1) and the variation may be caused by more subtle environmental changes or slight differences in the rate of skeletal accretion among coral polyps. Therefore, the peaks were not explicit enough to predict polyp
growth rates as has been done in previous isotopic studies of *L. pertusa* (Mikkelsen et al. 1982; Freiwald et al. 1997; Spiro et al. 2000).

It is possible that a more distinct seasonal signal would be expressed if not for the lack of high-resolution sampling along the direction of growth. The micro-drill facilitated accurate sampling along the inner growth band and avoided sampling skeletal material from surrounding bands. However, there was a resolution trade-off involved in this sub-sampling strategy. The widths of thecal bands in *L. pertusa* are narrow (<250 µm); therefore, to obtain enough carbonate material for the analysis, it was necessary to sample in long thin lines. The long thin lines maintained the resolution within the growth band but lost resolution along the growth axis.

Recent advances mean that mass spectrometry analyses now have the capacity to run even smaller sample sizes than used in the present study. Future research could link this with the micro-drill sampling technique to carry out high-resolution sampling in all directions along the coral polyp’s inner growth band. If seasonal signals are influencing the skeletal growth of *L. pertusa* and δ_{13}C and δ_{18}O skeletal signatures, then replicate branches from the same colony analysed as described above should generate reproducible δ_{13}C and δ_{18}O trends.

5.4.2.1 Growth banding

White (optically dense) and grey (optically less-dense) bands have been analysed for stable isotopes of carbon and oxygen in *Desmophyllum cristagalli* (Adkins et al. 2003) and *L. pertusa* (Blamart et al. 2005; Luttringer et al. 2005; Risk et al. 2005). The bands were investigated at different micro scales, from sampling directly on centres of
calcification at 50 - 100 µm (Adkins et al. 2003; Rollion-Bard et al. 2003; Blamart et al. 2005) to sampling across thecal banding patterns of a transverse section at 200 µm + (Lutringer et al. 2005; Risk et al. 2005). Centres of calcification, which are optically dense, and other optically dense bands, consistently had depleted values of δ¹³C and δ¹⁸O. This is in agreement with the results from skeletal material from both NC and Tern branches taken from the optically dense inner band, which may also contain the centre of calcification, and with results from the sub-samples taken along both the white and grey bands. Furthermore, Lutringer et al. (2005) stated that there is a general decreasing trend in δ¹³C and δ¹⁸O from the exterior to the interior of the thecal wall suggesting fastest growth at the interior of the growth bands, again similar to the fast growing innermost bands analysed in the present study.

5.5 Conclusions

The results from the stable isotope analyses of δ¹³C and δ¹⁸O along the innermost optically dense band, the bulk sampled theca and the samples from the different growth bands confirm that the innermost band represents fast skeletal calcification. This is in agreement with the results from Chapter 4 where data suggested that coral polyps extend quickly in their first year and then continue thickening at a much slower rate. Potentially, annual peaks in δ¹³C and δ¹⁸O along the innermost bands also suggest a seasonal influence on growth that might also be linked to polyp budding. However, the peaks were not consistent enough to extract a growth rate based on seasonal influences as previously hypothesised. Thus, this analysis was not carried further.

The results in this chapter have assisted with the interpretation and method development for the analysis of trace metals in skeletons of *L. pertusa* presented in the following chapter. High-resolution trace metal analyses of polyp skeletons focused on sub-
sampling skeletal material from the innermost growth band along sequential polyps of coral branches. This will create a skeletal chronology from which to investigate trace metals associated with oil and gas drilling discharges.
Chapter 6  –  

*Lophelia pertusa* as an Archive of Marine Pollution

6.1 Introduction

6.1.1 Corals as bio-indicators

The previous chapter introduced corals as paleo-climate recorders based on skeletal trends in stable isotopes of carbon and oxygen. Corals also incorporate other chemical elements present in ambient seawater into their skeleton at the time of accretion. Tropical corals have been investigated as bio-indicators of environmental pollution by measuring the abundance of heavy metals in skeletal material. The rise and fall of industrial lead has been documented, as well as other anthropogenic inputs of heavy metals from coastal industrial activities such as mining, dredging, and refineries (Dodge and Gilbert 1984; Shen and Boyle 1987; Scott 1990; Guzman and Jimenez 1992; Guzman and Jarvis 1996; Esslemont 1999; Fallon et al. 2002; David 2003; Runnalls and Coleman 2003; Inoue et al. 2004). In addition, elements such as strontium, magnesium, and barium preserved in coral skeletons can be used to trace past sea surface temperatures, rainfall, land runoff, and upwelling events (Lea et al. 1989; Linn et al. 1990; Beck et al. 1992; Hart and Cohen 1996; Mitsuguchi et al. 1996; Shen et al. 1996; Bastidas and Garcia 1999; Fallon et al. 1999; Tudhope et al. 2001; Cohen et al. 2002; Alibert et al. 2003; Sinclair and McCulloch 2004).

Cold-water corals offer potential as marine bio-indicators with the advantage of a much wider global distribution compared to tropical corals. They provide a means of
monitoring deep marine environments that are difficult and expensive to access because they can be used to extract a time series of data from a single sample. While recent studies have investigated their potential as temperature recorders using trends in skeletal strontium and magnesium (Montagna et al. 2005; Sherwood et al. 2005a; Shirai et al. 2005; Sinclair et al. 2005), the present study is the first to investigate a cold-water coral for traces of marine pollution and to assess its value as a bio-indicator of marine pollution.

Ideal bio-indicators should be able to provide a reliable skeletal chronology. As discussed in Chapter 4, *Lophelia pertusa* and the majority of cold-water scleractinian corals do not always generate clearly defined growth bands and the timing of such patterns is not yet well understood. However, results from Chapters 2 and 4 show that *L. pertusa* from oil and gas platforms in the North Sea bud and extend one full corallite length each year, followed by thickening of the thecal wall. Moreover, coral skeletal growth is believed to originate from the centres of calcification (Barnes 1970; Cuif and Dauphin 1998), and the $\delta^{13}$C and $\delta^{18}$O results presented in Chapter 5 confirm that the innermost optically dense growth band next to and/or including the centre of calcification is accreted relatively fast. This information will be applied to high resolution sub-sampling of *L. pertusa* skeletons in this chapter.

### 6.1.2 Lophelia pertusa in the North Sea

*Lophelia pertusa* growing on oil and gas platforms in the northern North Sea provides an opportunity to investigate the potential of this species as a bio-indicator of marine pollution. Colonies were found living close to point-source discharges of drilling muds and cuttings, as well as growing just above cuttings piles that have accumulated on the seabed (Chapter 2). Drilling muds are used during drilling to cool and lubricate the drill
bit, maintain hydrostatic pressure in the well, and remove cuttings from the well (Reis 1992), see Chapter 1 for a detailed description of drilling muds and cuttings. Three primary concerns of releasing drilling discharges into the marine environment are the physical smothering of benthic organisms, and their hydrocarbon and heavy metal content (GESAMP 1993). The present study is concerned with the presence of heavy metals. Barite (BaSO₄) is a major constituent of drilling muds and thus barium is often used as a tracer of drilling muds and cuttings in marine sediments (Hyland et al. 1994; Olsgard and Gray 1995; Hartley 1996; Rye et al. 2004). Other metals including mercury, chromium, zinc, cadmium, copper, lead, and nickel are also present, in smaller quantities, as a result of direct associations with barite, the addition of specialty chemicals, and from natural sediment contained in cuttings (Breuer et al. 2004; Neff 2005).

In the central and northern region of the North Sea, drill cuttings have accumulated forming large piles below drilling platforms (Bell et al. 1998; UKOOA 2001; Eames et al. 2002; Marsh 2003; Breuer et al. 2004) and levels of barium, cadmium, chromium, copper, nickel, lead, vanadium and zinc in the piles are well above background levels for North Sea sediments (Breuer et al. 2004). However, the bioavailability of metals present in cuttings piles is generally thought to be negligible because they are primarily associated as insoluble inclusions in drilling muds and cuttings particles (UKOOA 2001). The majority of available exchangeable metals will be released as the muds and cuttings travel through the water column before settling on the seabed (Neff 1991). However, lead is the metal of greatest concern with respect to North Sea water-based muds because barite can contain high concentrations of leachable lead (Neff 2005). Furthermore, lead was the only metal to show significant bio-accumulation in the turbot Scophthalmus maximus during laboratory tests (UKOOA 2002). In situ measurements
of metal concentrations in the water column just above the Beryl Alpha cuttings pile showed leaching of arsenic and nickel (Edwards 1998), and research from Beryl Alpha shows that other dissolved metals in sediment pore water will be released to the overlying water if cuttings piles are significantly disturbed (Shimmield et al. 2000). However, further field data are required to confirm actual leaching levels from cuttings piles (Gerrard et al. 1999; Det Norske Veritas 2000; Breuer et al. 2004). Furthermore, the potential for natural disturbance is not well documented, although modelling predicts significant cuttings piles erosion during severe storm weather conditions (Tyler et al. 1999).

6.1.3 Uptake of metals by corals

Metals can be incorporated into coral skeletons through several pathways (Figure 6.1) which result in different metal-skeleton associations (Livingston and Thompson 1971; Amiel et al. 1973; Howard and Brown 1984; Shen and Boyle 1988). The uptake of metals into coral skeletons can result from dissolved metals in seawater, from contaminated food (e.g. plankton), or by directly ingesting contaminated sediment (Howard and Brown 1984). Sediment ingestion has been observed by Stafford-Smith and Ormond (1992) and Rosenfeld et al. (1999), and in the present study (Chapter 3). Consequently, metals can be associated with coral skeleton by direct replacement of calcium in the aragonite lattice, depending on the relative ion size compared to calcium (Howard and Brown 1984), or metals can also be associated as inclusions of particulate material into skeletal cavities, through adsorption on exposed areas of dead skeleton, and/or associated with organic material within skeletons (Howard and Brown 1984).

Research examining trace metals in coral skeletons has primarily focused on isolating metals which have replaced calcium in the calcium carbonate lattice. It has been
suggested that this is the only way to isolate metals which have been incorporated into skeletons in a predictable manner and which reflect the coral’s ambient environment at the time of skeletal accretion (Shen and Boyle 1988). Similar arguments have been made for paleo-environmental studies using benthic Foraminifera (Lea and Boyle 1993). Shen and Boyle (1988) proposed an extensive step-wise cleaning method that eliminates adsorbed metals, metals associated with organic material and incorporated particulate matter, and isolates only the lattice bound metals in coral skeletons.

Figure 6.1 Pathways and associations of metal incorporation and associations with zooxanthellate scleractinian corals (redrawn from Howard and Brown 1984).
Although many researchers have followed or adapted this cleaning method, several recent studies have considered both lattice-bound and extra-lattice metals and have shown that extra-lattice bound metals can also be used as a reliable tracer for marine pollution. David (2003) and Inoue et al. (2004) found reproducible results using both extensive cleaning methods to isolated lattice-bound metals and using simplified cleaning methods that did not eliminate extra-lattice metals. Both studies found that extra-lattice bound metals were at higher concentrations but showed the same trends as lattice-bound metals. Furthermore, isolated lattice-bound elements were sometimes below detection limits, while extra-lattice elements were found at detectable levels and were able to provide relevant environmental information (Inoue et al. 2004).

Runnals and Coleman (2003) chose to analyse all associated phases of metals for two reasons: 1) they could not be sure that all relevant elements will substitute for calcium in the lattice, and 2) they believed that metals present in particulate matter associations were relevant with respect to potential effects on the organism and wider community. In addition, several other studies have used pre-treatments with just deionised water to successfully isolate skeletal traces of localised pollution in several marine areas such as the Mexican Caribbean (Medina-Elizalde et al. 2002), Great Barrier Reef (Esslemont 2000), Hong Kong (Scott 1990) and the Red Sea (Hanna and Muir 1990).

Recent studies have taken advantage of micro-beam techniques including laser-ablation inductively coupled plasma-mass spectrometry (ICP-MS) to analyse trace metals in coral skeletons (Sinclair et al. 1998; Fallon et al. 1999; Fallon et al. 2002; Runnalls and Coleman 2003; Sinclair and McCulloch 2004; Wyndham et al. 2004; Montagna et al. 2005; Sinclair 2005; Buster and Holmes 2006). As well as assessing skeletal sampling to below 20 µm resolution (Sinclair et al. 1998), this method removes the need for the
time-consuming digestion protocols because the solid skeletal material is introduced by laser-ablation directly into the ICP-MS (Durrant 1999). In addition, fresh coral skeleton can be analysed from sections within the skeleton reducing concern over contamination from storage and handling and adsorbed metals onto the outside of the skeleton.

6.1.4 Chapter aims

By sampling colonies of *L. pertusa* from sites close to point-source discharges of drilling muds and cuttings as well as just above cuttings piles, it is hypothesised that these coral skeletons will contain higher concentrations of heavy metals compared to colonies sampled from cleaner regions on the platform well away and upstream from discharges. In addition, cleaning protocols are tested to investigate the association of metals with skeletons from exposed and control sites. A coral colony sampled from the Sea of Hebrides was also analysed as a natural reef comparison. It is further hypothesised that corals growing close to discharge points and cuttings piles record discrete discharging and/or sediment resuspension events.

6.2 Coral samples and study sites

Coral colonies were either exposed or control samples as described in previous chapters. Descriptions of colonies, their sample locations, and their exposed or control status are given in Table 6.1. Further details on the platforms from the northern North Sea (Brent Delta, North Cormorant, North West Hutton, and Tern Alpha) are given in (Table 6.2).
The remainder of this chapter is divided into two parts based on the type of sub-sampling and sample introduction into the ICP-MS. Initial analyses used a bulk sub-
PART ONE: Solution nebulisation Inductively Coupled Mass Spectrometry (ICP-MS)

6.3 Methods

The analyses carried out in this chapter used ICP-MS to measure trace metal concentrations in coral skeletons. The basic functioning of ICP-MS is as follows (see series of papers by R. Thomas in Spectroscopy vol. 16, 2001 and 17, 2002). The liquid analyte enters the ICP-MS either as a fine aerosol or directly as a solid through laser ablation. A plasma is formed from argon gas which is used to atomise and ionise the elements in the analyte at high temperatures (6000-7000 K). The resulting ions are extracted from the plasma through the interface, a series of two metal cones, and pass through an ion focusing system that electrostatically steers the ions into a high vacuum mass analyser. The mass analyser separates the ions according to their mass-to-charge ratio. The detector in the mass spectrometer then counts the number of ions from the mass analyser by converting the ions into electrical pulses. The magnitude of the pulses is proportional to the amount of the element in the original analyte. The concentrations of trace elements in the analyte are determined by comparing the ion signal with that of a known calibration or reference standards run under the same conditions as the analyte.

6.3.1 Sample preparation

Coral branches from the control colony NC2 and the exposed colony TA2 were used to compare concentrations of trace elements in coral skeletons from control and exposed
sites on drilling/production platforms in the North Sea. Samples from the Beryl SPM were used as a secondary reference material.

6.3.1.1 Sub-sampling and Pre-cleaning treatments

Samples frozen after collection were removed from the freezer and placed in <5 % sodium hypochlorite for 24 h. The samples were then rinsed with deionised (18.2Ω) water to remove any remaining tissue. Single branches with at least three polyps were chosen from exposed (TA2) and control (NC2) colonies. The branches were sliced at 5 mm increments using a diamond-coated dental cutting disc attached to a Dremmel tool. Sub-samples were then rinsed three times in deionised water in an ultrasonic bath for 10 min, decanting and refilling with fresh water each time. The samples were placed in acid-washed glass vials, partially covered, and dried at 60 °C. Each 5 mm section was ground with an agate mortar and pestle and the powder kept in acid-washed glass vials. Some coral polyps contained visible detrital inclusions. These inclusions were not always removed during the cleaning process as they were trapped within the coral skeleton. One polyp from a colony sampled from the Beryl SPM was also cleaned as described above, dried, and crushed using an agate mortar and pestle.

6.3.2 Sample digestions

Approximately 2 mg of Beryl SPM coral powder and 0.05 mg of the other coral sub-samples were weighed to the fourth decimal place and the weights recorded. Each sample was digested in 10 ml (Beryl SPM) or 2.5 ml (NC2 and TA2) ROMIL-UpA™ HNO₃ (very pure with trace element impurities in parts per trillion) in an acid-washed Teflon beaker for one hour. Each digested coral sample was transferred to a 200 ml
(Beryl SPM) or 50 ml (NC2 and TA2) volumetric flask and filled to the mark with deionised water. All apparatus had been acid washed and rinsed in deionised water.

6.3.3 Reference materials

There are no suitable certified standard reference materials which are matrix-matched to calcium carbonate samples. The uncertified reference material Macs-1 was used in the analyses in addition to the secondary reference (coral powder from Beryl SPM). Macs-1 is a synthetic calcium carbonate material that was prepared in the laboratory at the United States Geological Survey (USGS) (contact person Dr. Stephen A. Wilson, USGS) by combining solutions of CaCl₂ and K₂CO₃. The solutions were spiked with a series of trace elements at a 100 ppm level and then combined producing a CaCO₃ precipitate. The trace metals were co-precipitated, and the final solid material was dried and ground to a powder. Three sub-samples of the Macs-1 powder were digested in ROMIL-UpA™ HNO₃ and analysed for the elements of interest on a VG PlasmaQuad 3 (S-Option) ICP-MS at the Scottish Association for Marine Science (SAMS), Dunstaffnage Marine Laboratory. Calcium was measured using an ICP-optical emission spectrometer (also located at SAMS) as the concentration was too high for the ICP-MS. The results are used as the reference concentrations throughout this chapter (Table 6.3).

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotope measured</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>⁵²Cr</td>
<td>122.2 ± 5.1</td>
</tr>
<tr>
<td>Ni</td>
<td>⁶⁶Ni</td>
<td>137.6 ± 4.0</td>
</tr>
<tr>
<td>Cu</td>
<td>⁶⁵Cu</td>
<td>118.0 ± 4.2</td>
</tr>
<tr>
<td>Zn</td>
<td>⁶⁶Zn</td>
<td>133.3 ± 10.4</td>
</tr>
<tr>
<td>Cd</td>
<td>¹¹¹Cd</td>
<td>116.4 ± 3.1</td>
</tr>
<tr>
<td>Ba</td>
<td>¹³⁷Ba</td>
<td>116.1 ± 1.9</td>
</tr>
<tr>
<td>Pb</td>
<td>²⁰⁸Pb</td>
<td>120.2 ± 2.5</td>
</tr>
<tr>
<td>Ca</td>
<td>⁴⁳Ca</td>
<td>587200</td>
</tr>
</tbody>
</table>
6.3.4 Standard addition

A multi-element standard solution was used to calibrate concentrations of trace metals in the analytes. However, because of matrix effects, the instrument response for an analyte in a complex sample, or a sample with high concentrations of certain elements (e.g. calcium) may not be the same as for an analyte in a standard solution. Consequently, standard addition, where standard solutions are added to the analyte and allows matrix-matched calibration, was used for all the solution nebulisation analyses.

6.3.5 Instrument specifications

Solution analyses were carried out using the ICP-MS located at SAMS. This was operated in a scanning mode with an acquisition time of 60 sec. The carrier gas was argon at a flow rate of 0.9 l min⁻¹. A mass range of 6-240 atomic mass units was scanned and values were obtained from an average of three readings. The elements measured in the analyses were chromium, nickel, copper, zinc, cadmium, barium, and lead. These are all of interest with respect to the oil and gas discharges and are also present in the Macs-1 reference material.

Before each analysis, the ICP-MS technician optimised the instrument sensitivity to ensure consistency between analyses. The technician also operated the instrument for each analysis. Internal standards indium and bismuth at 10 ppb were added to all solutions (blanks, standards, and samples) to monitor the sensitivity of the instrument response throughout each analysis. The following list shows the order of standards and samples run for ICPMS analysis:

1. a set of normal standards (not standard addition)
2. a set of standard addition standards at 0, 1, 3, 8, 20, 40 and 100 ppm
3. six coral samples
4. two standard addition checks at 0.5 and 1 ppm
5. three coral samples
6. Macs-1
7. secondary reference sample (Beryl SPM)
8. procedural blank

Acid blanks were run before and after every set of standards and samples, and procedural blanks were run to check for contamination during sample digestion and dilution.

6.3.6 Data processing

The concentrations of the procedural blanks were checked against the limit of detection (LOD), which is derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure and is considered as three times the standard deviation of the acid blanks (IUPAC 1997). The procedural blanks were then subtracted from the analyte data. Next, the analyte data were checked and rejected if below the LOD. The data were also checked against the limit of quantification (LOQ) which is the smallest concentration that can be adequately measured or quantified and is equal to 3.3 times the LOD (Inczydy et al. 1997). Data which fell below the LOQ were noted. The data were then multiplied by the dilution factor using the known weights of the digested samples and elemental concentrations were calculated to give a final concentration in parts per million (ppm).
6.3.7 Solution nebulisation ICP-MS: Coral cleaning experiments

Protocols described above were applied to pre-treatment cleaning experiments. The aim of the cleaning experiments was to examine the associations of metals with coral skeleton. This experiment examined the difference between adsorbed metals and metals associated with organic material. Furthermore, it examined the ability of deionised water versus weak nitric acid to remove adsorbed metals. Three different cleaning methods were applied to five samples from the control colony (NC2) and five samples from the exposed colony (TA2) based on protocols outlined by (Shen and Boyle 1988) (Table 6.4). A sample was either one large polyp or two small polyps. All polyps were taken from the same region of the coral colony and were all living when originally sampled.

The following cleaning treatments used ROMIL-UpA™ HNO₃, ROMIL-UpA™ H₂O₂ and purified NaOH. NaOH was purified by La-hydroxide co-precipitation and centrifugation. 40 ml of 2.0N NaOH was added to 8 ml of 1.0N LaOH, forming a white precipitate. Following centrifugation and dilution to 0.2N, the NaOH solution was added directly to 30 % H₂O₂ to make a 50:50 oxidizing mixture of H₂O₂ and NaOH. The samples were then digested and run on the ICP-MS as described in the previous section.
Table 6.4 Three cleaning protocols

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionised water</td>
<td>Deionised water &amp; weak nitric acid</td>
<td>Deionised water, weak nitric acid, &amp; oxidising treatment</td>
</tr>
<tr>
<td>1. Samples rinsed with deionised water and placed in 50 ml vials.</td>
<td>1. Treatment 1 repeated up to and including step 3.</td>
<td>1. Treatment 2 repeated up to and including step 11.</td>
</tr>
<tr>
<td>2. Samples covered with deionised water and placed in ultrasonic bath for 10 min.</td>
<td>2. 5 min of ultrasonic cleaning with 0.2 N HNO₃</td>
<td>2. 20 min of ultrasonic cleaning in a 50:50 oxidizing mixture of 30 % H₂O₂ and 0.2 N NaOH with alternating heating in a water-bath T&gt;90°C.</td>
</tr>
<tr>
<td>3. Above step repeated three times, each time with fresh deionised water.</td>
<td>3. Above step repeated three times, each time with fresh HNO₃</td>
<td>3. Samples rinsed with deionised water.</td>
</tr>
<tr>
<td>4. Skeleton coarsely crushed (~5 mm) using agate mortar and pestle.</td>
<td>4. Samples rinsed with deionised water to remove HNO₃</td>
<td>4. 3 min of ultrasonic cleaning in 0.15 N HNO₃.</td>
</tr>
<tr>
<td>5. Coral fragments placed into 15 ml vials placed in an ultrasonic bath.</td>
<td>5. 10 min of ultrasonic cleaning in deionised water.</td>
<td>5. Samples rinsed with deionised water.</td>
</tr>
<tr>
<td>6. 10 min of ultrasonic cleaning in deionised water.</td>
<td>6. Skeleton coarsely crushed (~5 mm) using agate mortar and pestle.</td>
<td>6. Samples dried at 60°C.</td>
</tr>
<tr>
<td>7. Samples dried at 60°C.</td>
<td>7. Coral fragments placed into 15 ml vials and placed in an ultrasonic bath.</td>
<td>7. Skeletal material ground to a powder in an agate mortar and pestle.</td>
</tr>
<tr>
<td>8. Skeletal material ground to a powder in an agate mortal and pestle</td>
<td>8. 3 min of ultrasonic cleaning in 0.15 N HNO₃.</td>
<td></td>
</tr>
<tr>
<td>9. 10 min of ultrasonic cleaning in deionised water.</td>
<td>9. 10 min of ultrasonic cleaning in deionised water.</td>
<td></td>
</tr>
<tr>
<td>10. Samples dried at 60°C.</td>
<td>10. Samples dried at 60°C.</td>
<td></td>
</tr>
<tr>
<td>11. Skeletal material ground to a powder in an agate mortar and pestle.</td>
<td>11. Skeletal material ground to a powder in an agate mortar and pestle.</td>
<td></td>
</tr>
</tbody>
</table>

6.4 Results

The results are presented for the three solution nebulisation analyses: exposed coral (TA2) run on 19-Feb-2005, control coral (NC2) run on 22-Feb-2005 and the cleaning experiment run on 18-Feb-2005.
6.4.1 Analysis errors, accuracy and precision

The analytical error for Macs-1 was below 5%. The accuracy of the results checked against Macs-1 varied for different elements (Table 6.5). Macs-1 results were precise to 3 - 15% for the elements of interest across all three analyses (run on 18, 19, and 22 February, 2005). Precision within each analysis was variable and in particular the standard deviation for lead concentrations was high for the exposed coral analysis, and for chromium concentrations for both the exposed and control analyses.

Table 6.5 Results for Macs-1 reference material from the three solution nebulisation analyses. Concentrations are reported in ppm ± SD

<table>
<thead>
<tr>
<th>Elements</th>
<th>Cleaning</th>
<th></th>
<th></th>
<th></th>
<th>Precision across analyses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 18-Feb-2005 (n=2)</td>
<td>Exposed 19-Feb-2005 (n=2)</td>
<td>Control 22-Feb-2005 (n=2)</td>
<td>Recorded values (n=3)</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>147.6 ± 4.5</td>
<td>137.1 ± 25.6</td>
<td>124.3 ± 23.9</td>
<td>122.2 ± 5.1</td>
<td>15</td>
</tr>
<tr>
<td>Ni</td>
<td>163.0 ± 4.5</td>
<td>144.7 ± 2.8</td>
<td>159.9 ± 14.5</td>
<td>137.6 ± 4.0</td>
<td>7</td>
</tr>
<tr>
<td>Cu</td>
<td>135.2 ± 7.8</td>
<td>137.6 ± 3.0</td>
<td>127.8 ± 3.5</td>
<td>118.0 ± 4.2</td>
<td>5</td>
</tr>
<tr>
<td>Zn</td>
<td>124.9 ± 8.6</td>
<td>124.8 ± 1.9</td>
<td>122.4 ± 2.8</td>
<td>133.3 ± 10.4</td>
<td>3</td>
</tr>
<tr>
<td>Cd</td>
<td>111.9 ± 3.1</td>
<td>106.2 ± 2.5</td>
<td>111.8 ± 7.5</td>
<td>116.4 ± 3.1</td>
<td>4</td>
</tr>
<tr>
<td>Ba</td>
<td>117.1 ± 11.0</td>
<td>121.9 ± 0.7</td>
<td>107.3 ± 3.7</td>
<td>116.1 ± 1.9</td>
<td>9</td>
</tr>
<tr>
<td>Pb</td>
<td>116.5 ± 1.7</td>
<td>133.6 ± 22.8</td>
<td>116.0 ± 2.4</td>
<td>120.2 ± 2.5</td>
<td>10</td>
</tr>
</tbody>
</table>

The analytical error for the secondary reference material was less than 5% except for cadmium and lead which were below 10%. The secondary reference material was a coral from a control site in the northern North Sea and hence was a good representative of the coral skeletons being analysed. It cannot be used to test for accuracy because the concentrations are unknown, but it was used to test for precision across the three analyses. Elements which were close to the LOD: chromium, cadmium, and lead, showed poor precision over of the three analyses (18, 19 and 22 February, 2005), while copper and nickel showed variable responses (19 and 24%) and barium and zinc were precise to 10% (Table 6.6).
Table 6.6 Results for the secondary reference material run with the three solution nebulisation analyses. Concentrations are reported in ppm ± SD

<table>
<thead>
<tr>
<th>Element</th>
<th>Cleaning Exp. 18-Feb-2005 (n=3)</th>
<th>TA2 Analysis 19-Jan-2005 (n=2)</th>
<th>NC2 Analysis 22-Feb-2005 (n=2)</th>
<th>Precision across analyses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>Below LOD</td>
<td>2.9 ± 0.7</td>
<td>1.9 ± 0.1</td>
<td>29</td>
</tr>
<tr>
<td>Ni</td>
<td>21.4 ± 3.6</td>
<td>23.9 ± 9.4</td>
<td>30.1 ± 2.4</td>
<td>24</td>
</tr>
<tr>
<td>Cu</td>
<td>3.5 ± 0.5</td>
<td>4.6 ± 1.1</td>
<td>4.5 ± 0.6</td>
<td>19</td>
</tr>
<tr>
<td>Zn</td>
<td>11.1 ± 1.4</td>
<td>11.4 ± 0.1</td>
<td>10.7 ± 1.8</td>
<td>10</td>
</tr>
<tr>
<td>Cd</td>
<td>0.26 ± 0.06</td>
<td>0.7 ± 0.004*</td>
<td>0.42 ± 0.03*</td>
<td>47</td>
</tr>
<tr>
<td>Ba</td>
<td>10.7 ± 0.9</td>
<td>11.2 ± 0.8</td>
<td>9.3 ± 0.1</td>
<td>10</td>
</tr>
<tr>
<td>Pb</td>
<td>0.16 ± 0.05</td>
<td>0.47 (n=1)</td>
<td>0.36 ± 0.02*</td>
<td>51</td>
</tr>
</tbody>
</table>

* indicates the values were above the LOD but below the LOQ

6.4.2 Incremental analysis of control and exposed branches

Trends along branches from the exposed (TA2) and control (NC2) corals are presented in Figure 6.2. Analytical error in the two analyses was less than 8 % except for the NC2 analysis where cadmium was < 15 %. Nickel showed constant values along both exposed and control branches, and the concentrations are similar for both. Zinc showed high variation for both the exposed and control corals; several subsections of the exposed branch were below the LOD. Copper, barium, and lead showed correlating spikes in the exposed coral that corresponded to discolorations on the skeleton. Background levels (i.e. concentrations from coral skeleton without discolorations) of copper for the exposed coral were similar to the control, whereas background levels of lead were below the LOD for the exposed coral. The spikes in barium were three orders of magnitude greater than the background levels (see Barium Figure 6.2 and note different scales for exposed and control corals). Exposed coral background levels of barium ranged from ~13 – 27 ppm, which is higher than the background concentrations found in the control (~9-10 ppm) and the secondary reference material (~9-11 ppm). The control coral showed constant concentrations of copper and lead along the branch and a background level for barium of approximately 10 ppm similar to the secondary
reference material, but with increases up to 17 ppm. No discolouration was observed on the control branches.

Figure 6.2 The results for the 5 mm increment solution nebulisation ICP-MS analysis of an exposed (TA2) and control colony (NC2). (A) Nickel, (B) Copper, (C) Zinc, (D) Barium, and (E) Lead

6.4.3 Cleaning Experiment

The cleaning treatments were compared using one-way ANOVA for data which were normally distributed with equal means: copper, cadmium, barium and nickel in the control coral, and cadmium, zinc, and nickel in the exposed coral. The remainder of the metals were tested with the non-parametric equivalent, Kruskall-Wallis test, because the data were not normally distributed. The results showed no significant effects on the concentrations of associated metals for the control corals sampled (Figure 6.3). There
was a general trend of decreasing concentrations for the exposed coral samples after a weak acid wash, but this was only statistically significant for zinc (Figure 6.4). Standard deviations for the exposed coral samples for copper, barium, and lead were greater than those for the control coral probably because of the uneven distribution of detrital inclusions associated with drilling muds and cuttings in the exposed samples, which were not present in the control samples (see Discussion).

Figure 6.3 Results from three cleaning treatments for the control colony (NC2). (A) Concentrations greater than 1 ppm and (B) concentrations less than 1 ppm. No significant differences were found (p>0.05). Error bars represent 95% confidence intervals.
Figure 6.4 Results from three cleaning treatments for the exposed colony (TA2). (A) Elements with concentrations greater than 1 ppm, (B) elements with concentrations less than 1 ppm, and (C) elements with concentrations greater than 30 ppm. The only significant difference was between Treatment 1 and 2 for zinc as indicated by *. Error bars represent 95% confidence intervals.

6.4.4 Summary of results

The trace metal concentrations from the control and exposed corals from the incremental sections and the overall mean concentrations from the cleaning experiment are presented in Table 6.7. Nickel concentrations did not vary greatly between colonies across analyses, and are similar to the secondary reference material. Chromium was below the LOD; cadmium was mostly below the LOD or very close to it. The results for zinc were variable and close to the LOD. Barium concentrations for the control coral
were similar to the secondary reference material. Barium, copper, and lead all showed peaks along the exposed coral branch which were not observed in the control colony in the incremental sampling analysis.

Table 6.7 Summary of concentrations of NC2 and TA2 (ppm ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Exposed (TA2) (n=18)</th>
<th>Control (NC2) (n=13)</th>
<th>Exposed (TA2) Cleaning Exp. (n=14)</th>
<th>Control (NC2) Cleaning Exp. (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>Below LOD</td>
<td>Below LOD</td>
<td>Below LOD</td>
<td>Below LOD</td>
</tr>
<tr>
<td>Ni</td>
<td>29.2 ± 2.2</td>
<td>27.2 ± 3.7</td>
<td>16.4 ± 3.6</td>
<td>22.8 ± 2.9</td>
</tr>
<tr>
<td>Cu</td>
<td>4.6 ± 1.6</td>
<td>2.8 ± 0.4</td>
<td>2.3 ± 1.9</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Zn</td>
<td>2.5 ± 3.5*</td>
<td>7.2 ± 8.1*</td>
<td>4.8 ± 1.5</td>
<td>4.5 ± 3.0</td>
</tr>
<tr>
<td>Cd</td>
<td>Below LOD</td>
<td>0.6 ± 0.3*</td>
<td>Below LOD</td>
<td>Below LOD</td>
</tr>
<tr>
<td>Ba</td>
<td>156.1 ± 289.2</td>
<td>11.5 ± 2.2</td>
<td>67.0 ± 85.5</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td>Pb</td>
<td>4.3 ± 3.8</td>
<td>0.3 ± 0.1*</td>
<td>1.8 ± 3.5</td>
<td>0.1 ± 0.2*</td>
</tr>
</tbody>
</table>

*above LOD but below LOQ

6.5 Discussion

The accuracy of the results for concentrations of zinc, cadmium, barium and lead fell within 10% of the mean of the recorded values for Macs-1, while chromium, nickel and copper were less accurate. As a result, some caution should be applied to actual concentrations of those analyses where elements in Mac-1 were less accurately measured.

The secondary reference material was taken from coral growing on the Beryl SPM, which is located 1.5-2.0 km from the Beryl drilling platform. This, in addition to the control colony sampled from the North Cormorant platform, can provide background concentrations for metals associated with *L. pertusa* growing in the North Sea, and these can be compared to results from previous studies reported in the literature.
Zinc concentrations were consistent in the secondary reference, but were variable for the exposed and control samples. Inconsistently high background levels from procedural blank samples meant that some values fell below the LOD. Although precautions were taken by acid-washing sample tubes before use, this could still be a source of zinc contamination as it is used in tube manufacturing. The differences in precision between the Macs-1 reference and the coral secondary reference material may be accounted for by the lower concentrations of metals in the coral material, and the homogeneity of the carbonate material in the coral is unknown, thus may be more variable compared to Macs-1.

Concentrations of trace metals from coral skeletons reported in the literature vary greatly and the results from the background concentrations in the present study fall within published ranges (Table 6.8). Concentrations of nickel found in the present study are relatively high and correspond to levels found in tropical corals from polluted sites such as close to ore refinement and shipping activities (Esslemont 2000) and near an open-cut gold mine in Papua New Guinea (Fallon et al. 1999). Nickel and zinc are found in northern North Sea seawater at similar concentrations, 0.247 ± 0.11 ppb and 0.250 ± 0.07 ppb respectively (Nolting et al. 1999), but nickel was twice as high as zinc in coral skeletons. The high levels of nickel in corals on the platforms may relate to the combustion of oil which can release nickel, or from the steel platforms as nickel is often used in steel and other alloys (Clark 2003). Alternatively, the higher concentrations of nickel may reflect a higher biological discrimination against zinc compared to nickel. Varying degrees of biological discrimination across a range of coral species for both zinc and nickel have been previously reported (Livingston and Thompson 1971; Howard and Brown 1984) making it difficult to know if, and to what degree L. pertusa may discriminate against one over the other. This would have to be tested and until then
it is only possible to look for trends over time assuming that all *L. pertusa* discriminate equally.

Background copper concentrations from corals in the present study (2.5-4.6 ppm) are approximately in the middle of the range of concentrations reported from the literature. Corals from polluted regions contained copper concentrations of 0.8-36.8 ppm. In addition to drilling discharges, potential anthropogenic sources of copper to coral skeletons in the North Sea are from steel platforms and copper used in anti-fouling paint (Clark 2003). Concentrations of zinc from the present study are similar to other studies with the exception of Esslemont (2000) who reported concentrations up to 900 ppm from corals growing close to a zinc shipment port. Zinc from paint primers used on industrial structures, and potentially platforms, could be an additional source of zinc to North Sea corals (Scott and Davies 1997). Concentrations of cadmium and lead also fall within those reported from the literature.

The concentrations of background barium in the present study were consistently above the LOD, were precise to 10% in the secondary reference material (coral from Beryl SPM), and background concentrations from the secondary reference material and the control coral NC2 were consistent; the concentrations ranged between 9.3-11.5 ppm. These levels agree with the two published sources which present fully quantitative data for barium concentrations in coral skeletons (Livingston and Thompson 1971; Howard and Brown 1987). Other studies have examined barium in coral skeletons normalised to calcium and will be discussed in Part Two of this chapter.

Concentrations of copper, barium, and lead were higher in the exposed coral compared with the control, and these three metals showed correlated peaks along the exposed branch which corresponded to discolorations on the coral skeleton. The peak
concentrations were 12 ppm for copper and 11 ppm for lead, which were on the high end of previous reports. Barium peaked at 1212 ppm, a concentration two orders of magnitude greater than previous reports (Table 6.8).

The results from the cleaning experiments showed that adsorbed metals and those associated with organic material did not significantly affect the concentration of metals in coral skeletons. However, the cleaning methods were not able to remove all detrital inclusions for the exposed coral, which could be masking small decreases after adsorbed or organic-bound metals were removed. The metal concentrations from the exposed coral were lower in the cleaning experiments compared to the 5 mm incremental analyses from the same coral colony, which is likely a result of the coarse crushing of the sample during cleaning which could have allowed some detrital inclusions to be released. Exposed corals may become covered in sediment from drill cuttings and will likely ingest sediment as a means of clearing their polyp surface or as an indiscriminate feeding response as was observed in Chapter 3, which can lead to the inclusion of detrital matter in the skeleton (Howard and Brown 1984; Guzman and Jimenez 1992). Furthermore, corals can calcify over large particles that have landed on their skeleton (Freiwald 1998). These particles become difficult to eliminate from the coral skeleton once they are incorporated. In conclusion, it is believed that high concentrations of metals reported from the exposed coral were a direct consequence of measuring detrital inclusions, likely to be drill cuttings, and did not reflect metals which had replaced calcium in the skeletal aragonite lattice.
Table 6.8 Summary of metal concentrations from coral skeletons (ppm) reported from previous studies. A wide range of digestion and analytical techniques have been used which limit direct comparisons, but results provide a general overview.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Location</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
<th>Cd</th>
<th>Ba</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livingston &amp; Thompson</td>
<td>Stained corals*</td>
<td>Mid-Atlantic Ridge; Rekjanes Ridge; equatorial Atlantic; all below 1000m</td>
<td>&lt;2-23</td>
<td>&lt;2-14</td>
<td>&lt;2-9</td>
<td>-</td>
<td>20-40</td>
<td>&lt;2-42</td>
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<tr>
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<tr>
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<td>Desmophyllum crustogalli</td>
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<tr>
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<td>Caryophyllia calvus</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>C. communis</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Trochocyathus sp.</td>
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<tr>
<td></td>
<td>S. variabilis (Unstained)</td>
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<td>Cladocroa patriarcha</td>
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<td>Bathycyathus maculatus</td>
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<tr>
<td></td>
<td>Meandrina areolata</td>
<td>Florida Bay; Florida Keys; Brazil coast; Discovery Bay, Jamaica;</td>
<td>&lt;2-4</td>
<td>1-20</td>
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<tr>
<td>St. John (1974)</td>
<td>Acropora</td>
<td>Heron Island, Australia</td>
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</tr>
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<tr>
<td></td>
<td>Poritidae</td>
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</tr>
<tr>
<td></td>
<td>Montipora</td>
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<td></td>
<td></td>
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<tr>
<td>Howard &amp; Brown (1987)</td>
<td>Pocillopora damicornis</td>
<td>Phuket, Thailand, Near tin smelter</td>
<td>-</td>
<td>11.6</td>
<td>2.7</td>
<td>8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phuket, Thailand, Away from tin smelter</td>
<td>-</td>
<td>10.5</td>
<td>0.1</td>
<td>8.7-9.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dudge & Gilbert (1984)
### Table 6.8 continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Location</th>
<th>Polluted Site</th>
<th>Unpolluted Site</th>
<th>Value Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanna &amp; Muir (1990)</td>
<td><em>Porites lutea</em></td>
<td>Jeddah, Saudi Arabia</td>
<td>0.15-0.21</td>
<td>0.83-1.94</td>
<td>2.85-9.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Goniastrea retiformis</em></td>
<td>Jeddah, Saudi Arabia Unpolluted site</td>
<td>0.07-0.11</td>
<td>0.78-1.32</td>
<td>1.0-259</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Porites verrucosa</em></td>
<td>Jeddah, Saudi Arabia Unpolluted site</td>
<td></td>
<td></td>
<td>47-55</td>
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</tr>
<tr>
<td>Scott (1990)</td>
<td><em>Platygyra</em></td>
<td>Hong Kong, Tolo Harbour and Channel</td>
<td>-</td>
<td>8.87-8.57</td>
<td>0.06-2.62</td>
<td>0.21-0.79</td>
</tr>
<tr>
<td>Guzman &amp; Jimenez (1992)</td>
<td><em>Siderastrea sidera</em></td>
<td>Costa Rica, Caribbean Sea</td>
<td>91.6</td>
<td>2.0</td>
<td>10.2</td>
<td>31.0</td>
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<td></td>
<td></td>
<td>Panama, Caribbean Sea</td>
<td>93.7</td>
<td>3.8</td>
<td>8.9</td>
<td>7.6</td>
</tr>
<tr>
<td>McConchie &amp; Harriot (1992)</td>
<td><em>Goniastrea favulus</em></td>
<td>Wistari Reef and Heron Island Harbour, Australia</td>
<td>-</td>
<td>&lt;0.05-0.13</td>
<td>1.5-2.7</td>
<td>0.05-0.13</td>
</tr>
<tr>
<td></td>
<td><em>Acropora aspera</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acropora valida</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><em>Pocillopora damicornis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bastidas &amp; Garcia (1999)</td>
<td><em>Montastrea annularis</em></td>
<td>Punta Brava, Bajo Caiman, Venezuela</td>
<td>-</td>
<td>1.5-5.2</td>
<td>8.0-9.3</td>
<td>1.7-1.16</td>
</tr>
<tr>
<td>Esslemont et al. (2000)</td>
<td><em>Pocillopora damicornis</em></td>
<td>Townsville, Australia</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Esslemont (1999)</td>
<td><em>Acropora nobilis</em></td>
<td>Heron Island, Australia</td>
<td>0.45-1.62</td>
<td>0.23-0.32</td>
<td>0.86-1.87</td>
<td>0.04-0.09</td>
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<tr>
<td></td>
<td><em>Goniastrea aspera</em></td>
<td>Darwin Harbour, Australia</td>
<td>0.35-1.97</td>
<td>0.36-0.76</td>
<td>1.20-5.03</td>
<td>0.05-0.08</td>
</tr>
<tr>
<td>Esslemont (2000)</td>
<td><em>Goniastrea aspera</em></td>
<td>Magnetic Island, Australia</td>
<td>&lt;62-≤249</td>
<td>10.4-11.6</td>
<td>128.9-236.7</td>
<td>7.4-≤16.2</td>
</tr>
<tr>
<td></td>
<td><em>Pocillopora damicornis</em></td>
<td></td>
<td></td>
<td></td>
<td>2.8-4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acropora formosa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Townsville Harbour, Australia</td>
<td>20.9</td>
<td>36.8</td>
<td>899.7</td>
<td>10.2</td>
</tr>
</tbody>
</table>
Table 6.8 continued

<table>
<thead>
<tr>
<th>Author</th>
<th>Species</th>
<th>Location</th>
<th>pH</th>
<th>Calcium</th>
<th>Magnesium</th>
<th>Iron</th>
<th>Mn</th>
<th>Other Metals</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallon et al. (2002)</td>
<td><em>Porites</em></td>
<td>Misima Island, PNG Close to mining</td>
<td>19.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.468</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control site away from mining</td>
<td>0.393</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.053</td>
</tr>
<tr>
<td>David (2003)</td>
<td><em>Porites</em></td>
<td>Ihatub reef; Ulan reef, Philippines Exposed to mine tailings</td>
<td>-</td>
<td>0.06-4.0</td>
<td>0.7-2.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caganhao reef, Philippines Control site away from mine tailings</td>
<td>-</td>
<td>0.7</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Stained corals described by Livingston and Thompson (1971) represented hydrated oxides of iron and manganese deposits on skeleton
The results from the present study consistently showed higher concentrations of barium, lead, and copper in corals growing in an environment exposed to high influxes of drilling discharges. These results showed that metals associated with corals were a direct reflection on its ambient environment. The results are not likely useful as a time series recorder because thecal walls showed gradual thickening over time in Chapter 4, but are rather more useful for confirming the exposure of *L. pertusa* to drilling muds and cuttings. By using a higher resolution sub-sampling technique, as described in the next section, it is possible to investigate changes in metals along the skeletal chronology.
PART TWO: Laser ablation ICP-MS (LA-ICP-MS)

6.6 Methods

6.6.1 Sample preparation

As per the solution nebulisation ICP-MS, colonies of *L. pertusa* sampled from sites exposed to drilling discharges and sites away from drilling discharges were used in the LA-ICP-MS analyses. Exposed colonies were BD, TA2, NWH1 and NWH2; control colonies were NC2, NWH5 and MING1 (Table 6.2). MING1 was sampled from the Sea of Hebrides for a natural reef comparison.

Branches with at least three polyps starting from the outermost polyp were cut from each colony. All polyps were living when originally sampled. The branches were sliced into individual polyps using a diamond-coated dental cutting disc attached to a Dremmel tool. Polyps were sectioned at the sectioning laboratory (School for Geosciences, University of Edinburgh). They were embedded in Beuhler Epo-Thin resin under vacuum, and then further sectioned longitudinally from the centre of the polyp into 1.5 mm flat sections using a circular saw with a continuous rim diamond blade at 300 rpm. Each section was rinsed in deionised water (18.2 Ω) and sonicated three times for 10 min decanting and replenishing with clean distilled water each time. The sections were then placed in acid-washed glass vials, partially covered, and dried at 60 °C.

6.6.2 Instrument specifications

The laser ablation analysis was carried out with the ICP-MS described in Part One coupled to a VG/New Wave Microprobe II pulsed Nd:YAG 266 nm wavelength laser. The standard operating conditions of the instrument are presented in Table 6.9.
Table 6.9 Instrument specifications for laser-ablation ICP-MS

<table>
<thead>
<tr>
<th>Laser Ablation ICP-MS</th>
<th>VG PlasmaQuad 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition mode</td>
<td>Peak jumping</td>
</tr>
<tr>
<td>Acquisition time</td>
<td>60 sec (blanks) 35 sec (spots)</td>
</tr>
<tr>
<td>RF power</td>
<td>1350 W</td>
</tr>
<tr>
<td>Cool gas flow rate</td>
<td>0.9 l min⁻¹</td>
</tr>
<tr>
<td>Auxiliary gas</td>
<td>0.85 l min⁻¹</td>
</tr>
<tr>
<td>Nebuliser gas</td>
<td>1.03 l min⁻¹</td>
</tr>
<tr>
<td>Gas type</td>
<td>Argon</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laser</th>
<th>VG Microprobe II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser type</td>
<td>Nd:YAG wave length 266 nm</td>
</tr>
<tr>
<td>Laser mode</td>
<td>Q-switched, time resolved mode</td>
</tr>
<tr>
<td>Laser energy</td>
<td>0.4 mJ</td>
</tr>
<tr>
<td>Ablation type</td>
<td>Spot</td>
</tr>
<tr>
<td>Spot size diameter</td>
<td>75 µm</td>
</tr>
<tr>
<td>Spot depth</td>
<td>15 µm</td>
</tr>
<tr>
<td>Scan speed</td>
<td>10 µm sec⁻¹</td>
</tr>
<tr>
<td>Repetition rate</td>
<td>10 Hz</td>
</tr>
<tr>
<td>Output</td>
<td>70 %</td>
</tr>
</tbody>
</table>

Nd:YAG: Neodymium-doped yttrium aluminum garnet

6.6.3 Standards

Two reference materials were run with each analysis: the certified standard glass reference material NIST-612 and the uncertified pressed calcium carbonate pellet Macs-1 supplied by the USGS (see section 6.3.3). One of the major difficulties in carrying out analyses of major and minor elements in calcium carbonate material using LA-ICP-MS is the lack of commercially produced homogenous solid state matrix-matched certified standard reference materials (Hart and Cohen 1996; Sinclair et al. 1998; Durrant 1999). The NIST-612 is a basalt composition glass (72 % SiO₂, 12 % CaO, 14 % Na₂O, and 2 % Al₂O₃) and has the advantage that it is a certified reference material and is known to be more than 95 % homogenous. However, the disadvantage is that it is a poor chemical matrix match for calcium carbonate coral skeletons. Material of different chemical matrices will fractionate differently during ablation and the transport and ionisation processes can also differ resulting in differences in the analyte signal responses (Jarvis and Williams 1993; Morrison et al. 1995; Sylvester and Ghaderi 1997). Therefore, non-
matrix-matched standards can lead to inaccurate standardization. NIST-612 was used to calibrate Macs-1 and check for accuracy and precision across analyses.

The synthetic calcium carbonate reference material Macs-1 was pressed into pellets at 15000 psi at the USGS. The concentrations for Macs-1 are given in section 6.3.3. The matrix of Macs-1 more closely reflects that of a coral calcium carbonate skeleton minimizing potential matrix effects. However, it is important to note that the homogeneity of the pellet has not been thoroughly tested and pressed pellets may have different ablation efficiencies compared to a more solid coral skeleton (Vanheuzen and Morsink 1991; Sinclair et al. 1998).

The laser was scanned over the coral skeleton for approximately 60 sec at the beginning of each analysis to condition the instrument to calcium carbonate material. Argon gas blanks were run at the beginning, middle and end of each analysis to record gas contamination and machine noise. The blanks were run by using the same settings as for spot ablation but with the laser turned off. The NIST-612 and Macs-1 were both run at the beginning and end of each analysis to monitor any changes in instrument sensitivity.

6.6.4 Coral samples

Single spots provide better accuracy and precision compared to continual scanning as a sampling strategy for laser ablation (Gonzalez et al. 2004). Fifteen to 32 spots spaced approximately 600 µm apart were laid out in a line along the innermost growth band of each coral polyp using the integrated computer software (New Wave Research Ltd.) (. On several occasions, the outer growth bands were analysed, as it was difficult to distinguish the inner from the outer bands (NC2 P3, TA2 P1, NWH1 P10). For the remainder of the polyps where spots were ablated along the innermost growth band,
each line of spots along subsequent polyps represents successive years. As in previous chapters, polyp 1 (P1) represent the most recent generation. Coral samples were collected between 2003 and 2005 (Table 6.1). For each analysis, spots were pre-ablated to expose fresh material for analysis and remove any traces of contamination which may have occurred during storage and handling. The settings used for the pre-ablation covered large shallow spots (200 µm diameter, 5 µm deep) at a low laser repetition rate (5 Hz).

Data were collected for the following elements calcium, vanadium, chromium, iron, nickel, copper, zinc, barium, and lead. As before, the LOD was calculated based on three times the standard deviation of the blank counts. Only calcium, chromium, zinc, barium, and lead were consistently above the LOD. The mean percent relative standard deviation (RSD) of integrated data for all spots over all analyses for zinc and lead were the highest at 26 % and 28 % respectively.

Figure 6.5 Laser spots along the inner growth band of a coral polyp from a colony sampled from near Mingulay.
6.6.5 Data processing

Time resolved analysis was used to record signal intensity data. The integration intervals were selected once output readings stabilised. The mean counts per second (CPS) and % RSD over the integration intervals for each spot were calculated. The mean CPS of the blanks were subtracted from the elemental CPS.

Next, the CPS of the elements were normalized to the CPS of calcium, which is assumed constant in coral skeletons and has been shown to be an appropriate internal standard for calcium carbonate material (Craig et al. 2000). An internal standard in laser ablation is essential to account for potential variation in the amount of ablated material and the effect of changing plasma conditions. It also helps identify and correct for potential decreases in signal sensitivity during individual analyses. The least abundant isotope, $^{43}$Ca, was chosen so the CPS were as close as possible to those of the elements of interest.

The calcium-normalized signal for the elements was then related to the calcium-normalized signal of the Macs-1 standard. Macs-1 was run at the beginning and end of each analysis to monitor instrument sensitivity as this can change over time, and by different amounts across the mass spectrum. The sensitivity drift can be approximated to a linear function (Sinclair et al. 1998); therefore, the standard values from the beginning and end of the analysis were interpolated using a linear equation to correct for each spot. In many cases, very little change was recorded. The calcium-normalized signal for each element was multiplied by the known element/calcium ratio of Macs-1 and divided by the linear equation using time as the independent factor.
\[ E_{s(t_i)} = S \times E_{(t_i)} \div S_{(t_i)} \]  
(eq. 6-1)

\( E_{s(t_i)} \) = Standardized ratio (Element/Ca) at time i

\( S \) = Known mole ratio of Macs-1 (calculated from solution nebulisation)

\( E_{(t_i)} \) = Ca normalised ratio at time i

\( S_{(t_i)} \) = Standard at time i based on linear equation taking into account any change in Macs-1 between the beginning and end of the analysis.

6.7 Results

6.7.1 Accuracy and Precision

The NIST-612 counts were standardized to Macs-1 to check for accuracy and precision of using Macs-1 as a calibration standard (Table 6.10). There were two days of analyses (19-Jul-2005 and 20-Jul-2005) where values for NIST-1 were 2.0-2.2 times less than the mean of the other analyses. The results for the coral values between 19-Jul and 20-Jul appear consistent with other results with the exception of those run on 20-Jul-2005 (see below for more details); therefore, NIST-612 results from these two days were excluded from the precision and accuracy calculations. NIST-612 was precise to < 14 % for all elements. The accuracy of the results obtained when NIST-612 was calibrated to Macs-1 was between 23-45 % for chromium, nickel, copper, and zinc but fell within 10 % of the known concentrations for barium, and within 2 % for cadmium and lead.

<table>
<thead>
<tr>
<th></th>
<th>NIST</th>
<th>This study</th>
<th>± %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr/Ca</td>
<td>462.8</td>
<td>347.8 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>Ni/Ca</td>
<td>283.3</td>
<td>389.2 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>Cu/Ca</td>
<td>259.5</td>
<td>319.0 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>Zn/Ca</td>
<td>259.5</td>
<td>376 ± 9.5</td>
<td></td>
</tr>
<tr>
<td>Cd/Ca</td>
<td>130.1</td>
<td>127 ± 12.0</td>
<td></td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>129.3</td>
<td>116 ± 13.5</td>
<td></td>
</tr>
<tr>
<td>Pb/Ca</td>
<td>89.9</td>
<td>88.7 ± 10.4</td>
<td></td>
</tr>
</tbody>
</table>
Mean ratios for all elements are presented in Table 6.11. Nickel, copper, zinc, and cadmium often fell below the LOD and showed a high variation within and between analyses, and thus these were not investigated further. Cr/Ca, Ba/Ca and Pb/Ca, however, were consistently above the LOQ and Cr/Ca and Ba/Ca showed less variation between analyses (generally below 10 % relative standard deviations (RSD) with a maximum of 12 %) whereas Pb/Ca still showed high mean RSDs and less consistent results and thus Pb/Ca was not considered further. Calcium was the internal standard and had a mean concentration of 334,522 ppm ± 23 %. Calcium results were checked for all analyses, and on 20-Jul two samples (TA2-P3 and TA2P3Bot) showed a significant drop in calcium at several points throughout the analysis and therefore when the elements were normalised to calcium, this created considerable increases in the element/Ca ratios. These results were thus removed from the analysis. Chromium and Barium are both typically found at significant concentrations in drilling discharges and the results for these two elements showed the most reliable results as discussed above. Thus, these ratios were examined in more detail, and ratios for each spot along the polyps were plotted (Figure 6.6-Figure 6.9). Results from previous chapters suggest that an annual growth chronology for L. pertusa from the North Sea can be followed along the innermost growth band of subsequent polyps. Therefore, each polyp in Figures 6.5-6.9 represent one year, with Polyp 1 (P1) representing the most recent generation.

6.7.2 Control colonies

Results from control samples revealed some variability in the background values (Figure 6.6 and Figure 6.7) as is reflected in the means and standard deviations. Overall, Cr/Ca varied between approximately 1 µmol/mol to 6 µmol/mol, and Ba/Ca showed a larger variation of between approximately 4.5 to 21 µmol/mol. Within individual polyps, Cr/Ca varied by approximately 3 µmol/mol and Ba/Ca varied by approximately
2 μmol/mol with the exception of polyps from control colony NC2. NC2 P1 and P4 each have one spike reaching 15 and 20 μmol/mol respectively. There was no obvious difference between the concentrations from the Sea of Hebrides coral and the North Sea corals.

6.7.3 Exposed colonies

The results from branches taken from exposed colonies are presented in Figure 6.8, Figure 6.9, and Figure 6.10. Variation in the background values for Cr/Ca was particularly evident in branches from BD and NWH1. Variability within polyps was evident from NWH2-P2. Results for Ba/Ca showed more consistent background levels and barium spikes were evident in NWH2-P3. Polyp 1 was missing from the NWH2-P3 analysis because the thecal walls were too narrow for laser sub-sampling.
Table 6.11 Mean ratios (µmol/mol ± %RSD) for all polyps. Blank cells represent concentrations below the LOD.

<table>
<thead>
<tr>
<th>Colony - Polyp No.</th>
<th>Control/Exposed</th>
<th>Date</th>
<th>Ca (ppm)</th>
<th>Cr/Ca</th>
<th>Ni/Ca</th>
<th>Cu/Ca</th>
<th>Zn/Ca</th>
<th>Cd/Ca</th>
<th>Ba/Ca</th>
<th>Pb/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHW1-P2C</td>
<td>Exposed</td>
<td>29-Jul-05</td>
<td>319200 ± 8</td>
<td>2.69 ± 4</td>
<td>-</td>
<td>0.326* ± 26</td>
<td>0.58 ± 46</td>
<td>0.05 ± 32</td>
<td>5.44 ± 4</td>
<td>0.0180 ± 22</td>
</tr>
<tr>
<td>NHW1-P10</td>
<td>Exposed</td>
<td>28-Jul-05</td>
<td>263200 ± 18</td>
<td>2.86 ± 11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.83 ± 9</td>
<td>0.0522 ± 41</td>
</tr>
<tr>
<td>NHW1-P2A</td>
<td>Exposed</td>
<td>28-Jul-05</td>
<td>294300 ± 12</td>
<td>2.76 ± 14</td>
<td>-</td>
<td>0.298* ± 29</td>
<td>0.66 ± 60</td>
<td>-</td>
<td>5.7 ± 4</td>
<td>0.0177 ± 34</td>
</tr>
<tr>
<td>NHW1-P5</td>
<td>Exposed</td>
<td>27-May-05</td>
<td>331900 ± 8</td>
<td>4.11 ± 10</td>
<td>-</td>
<td>-</td>
<td>1.43* ± 76</td>
<td>-</td>
<td>7.68 ± 6</td>
<td>0.0255 ± 36</td>
</tr>
<tr>
<td>NHW1-P6</td>
<td>Exposed</td>
<td>19-Aug-05</td>
<td>562700 ± 4</td>
<td>1.83 ± 12</td>
<td>0.43 ± 126</td>
<td>0.44 ± 32</td>
<td>3.27 ± 50</td>
<td>-</td>
<td>6.51 ± 9</td>
<td>0.0248 ± 30</td>
</tr>
<tr>
<td>NHW1-P8</td>
<td>Exposed</td>
<td>26-May-05</td>
<td>384500 ± 9</td>
<td>4.37 ± 8</td>
<td>-</td>
<td>-</td>
<td>0.45* ± 38</td>
<td>-</td>
<td>6.79 ± 6</td>
<td>0.0282 ± 37</td>
</tr>
<tr>
<td>NHW1-P9</td>
<td>Exposed</td>
<td>26-May-05</td>
<td>387200 ± 11</td>
<td>3.94 ± 8</td>
<td>-</td>
<td>-</td>
<td>0.59* ± 70</td>
<td>-</td>
<td>6.87 ± 6</td>
<td>0.0605 ± 95</td>
</tr>
<tr>
<td>NHW1-P2B</td>
<td>Exposed</td>
<td>29-Jul-05</td>
<td>312900 ± 12</td>
<td>2.64 ± 7</td>
<td>-</td>
<td>-</td>
<td>0.4 ± 34</td>
<td>-</td>
<td>5.54 ± 4</td>
<td>0.0181 ± 20</td>
</tr>
<tr>
<td>NHW1-P7</td>
<td>Exposed</td>
<td>26-May-05</td>
<td>303100 ± 19</td>
<td>5 ± 8</td>
<td>2.59* ± 21</td>
<td>0.413* ± 57</td>
<td>0.8* ± 64</td>
<td>-</td>
<td>5.96 ± 11</td>
<td>0.0426 ± 104</td>
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<tr>
<td>NHW2-P2</td>
<td>Exposed</td>
<td>01-Jul-05</td>
<td>342500 ± 19</td>
<td>3.29 ± 77</td>
<td>-</td>
<td>0.337* ± 33</td>
<td>1.45 ± 131</td>
<td>0.11 ± 40</td>
<td>8.64 ± 10</td>
<td>0.0169 ± 32</td>
</tr>
<tr>
<td>NHW2-P3A</td>
<td>Exposed</td>
<td>01-Jul-05</td>
<td>322200 ± 11</td>
<td>2.51 ± 22</td>
<td>-</td>
<td>0.463* ± 31</td>
<td>1.04 ± 58</td>
<td>0.18 ± 52</td>
<td>11.89 ± 36</td>
<td>0.0265 ± 89</td>
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<tr>
<td>NHW2-P3B</td>
<td>Exposed</td>
<td>18-Aug-05</td>
<td>461000 ± 6</td>
<td>1.33 ± 11</td>
<td>-</td>
<td>-</td>
<td>0.09* ± 48</td>
<td>-</td>
<td>9.16 ± 35</td>
<td>0.0279* ± 91</td>
</tr>
<tr>
<td>NHW2-P4</td>
<td>Exposed</td>
<td>27-Jul-05</td>
<td>267700 ± 15</td>
<td>2.53 ± 7</td>
<td>-</td>
<td>0.607* ± 63</td>
<td>2.16 ± 58</td>
<td>0.10 ± 60</td>
<td>6.32 ± 5</td>
<td>0.0242* ± 29</td>
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<tr>
<td>TA2-P1</td>
<td>Exposed</td>
<td>27-Jul-05</td>
<td>274000 ± 14</td>
<td>2.86 ± 23</td>
<td>-</td>
<td>-</td>
<td>3.19 ± 35</td>
<td>-</td>
<td>6.15 ± 8</td>
<td>0.0310 ± 91</td>
</tr>
<tr>
<td>TA2-P2BOT</td>
<td>Exposed</td>
<td>20-Jul-05</td>
<td>197100 ± 40</td>
<td>4.91 ± 64</td>
<td>-</td>
<td>0.534* ± 69</td>
<td>5.47 ± 69</td>
<td>-</td>
<td>10.85 ± 56</td>
<td>0.1118 ± 106</td>
</tr>
<tr>
<td>TA2-P2</td>
<td>Exposed</td>
<td>27-Jul-05</td>
<td>286500 ± 4</td>
<td>2.53 ± 5</td>
<td>-</td>
<td>0.188* ± 22</td>
<td>1.06 ± 33</td>
<td>-</td>
<td>6.44 ± 3</td>
<td>0.0270 ± 21</td>
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<td>TA2-P3</td>
<td>Exposed</td>
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<td>185600 ± 34</td>
<td>3.11 ± 54</td>
<td>-</td>
<td>1.64* ± 72</td>
<td>-</td>
<td>7.99 ± 48</td>
<td>0.0400* ± 75</td>
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<tr>
<td>TA2-P3BOT</td>
<td>Exposed</td>
<td>19-Jul-05</td>
<td>295400 ± 14</td>
<td>2.94 ± 7</td>
<td>-</td>
<td>0.283* ± 92</td>
<td>1.01 ± 56</td>
<td>0.10 ± 39</td>
<td>7.29 ± 14</td>
<td>0.1069 ± 247</td>
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<tr>
<td>BD-P1</td>
<td>Exposed</td>
<td>25-May-05</td>
<td>330900 ± 9</td>
<td>4.77 ± 5</td>
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<td>1.3* ± 96</td>
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<td>6.45 ± 6</td>
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<tr>
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<td>24-May-05</td>
<td>299900 ± 11</td>
<td>4.2 ± 9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.13 ± 5</td>
<td>0.019* ± 30</td>
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<tr>
<td>BD-P3</td>
<td>Exposed</td>
<td>27-Jul-05</td>
<td>295400 ± 7</td>
<td>2.74 ± 8</td>
<td>-</td>
<td>-</td>
<td>1.88 ± 73</td>
<td>-</td>
<td>6.12 ± 11</td>
<td>0.0330 ± 53</td>
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<tr>
<td>NC2-P2</td>
<td>Control</td>
<td>19-Aug-05</td>
<td>484600 ± 19</td>
<td>1.49 ± 17</td>
<td>-</td>
<td>0.381* ± 36</td>
<td>1.65 ± 51</td>
<td>-</td>
<td>5.75 ± 14</td>
<td>0.0196 ± 30</td>
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<td>NC2-P3</td>
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<td>28-Jul-05</td>
<td>324700 ± 13</td>
<td>2.85 ± 5</td>
<td>1.34* ± 16</td>
<td>0.427* ± 52</td>
<td>1.63 ± 146</td>
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<td>0.0258 ± 25</td>
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<td>4.4 ± 17</td>
<td>-</td>
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<td>1.65* ± 96</td>
<td>-</td>
<td>9.22 ± 18</td>
<td>0.027 ± 28</td>
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<tr>
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<td>19-Aug-05</td>
<td>372900 ± 8</td>
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<td>-</td>
<td>-</td>
<td>3.79 ± 29</td>
<td>-</td>
<td>7.97 ± 45</td>
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<td>287600 ± 6</td>
<td>3.56 ± 7</td>
<td>-</td>
<td>0.352 ± 74</td>
<td>1.52* ± 155</td>
<td>-</td>
<td>6.5 ± 7</td>
<td>0.0155 ± 13</td>
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<td>Control</td>
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<td>452900 ± 20</td>
<td>1.57 ± 28</td>
<td>0.24* ± 22</td>
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<td>0.13 ± 152</td>
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<td>6.45 ± 6</td>
<td>0.0122 ± 67</td>
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<td>395200 ± 11</td>
<td>1.48 ± 12</td>
<td>-</td>
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<td>0.95 ± 81</td>
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Table 6.11 continued

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<th>Concentration (± SD)</th>
<th>Concentration (± SD)</th>
<th>Concentration (± SD)</th>
<th>Concentration (± SD)</th>
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<td>NWH5-P4</td>
<td>Control</td>
<td>20-Jul-05</td>
<td>294800 ± 4</td>
<td>3.63 ± 4</td>
<td>-</td>
<td>-</td>
<td>2.08* ± 68</td>
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<td>6.76 ± 5</td>
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<tr>
<td>MING1-P3</td>
<td>Control</td>
<td>31-May-05</td>
<td>306300 ± 11</td>
<td>3 ± 4</td>
<td>0.73* ± 20</td>
<td>0.532* ± 54</td>
<td>1.59* ± 85</td>
<td>0.17* ± 26</td>
<td>8.96 ± 4</td>
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<tr>
<td>MING1-P6</td>
<td>Control-SH</td>
<td>23-May-05</td>
<td>388200 ± 9</td>
<td>4.56 ± 7</td>
<td>-</td>
<td>-</td>
<td>0.81 ± 74</td>
<td>0.13 ± 44</td>
<td>6.86 ± 4</td>
</tr>
<tr>
<td>MING1-P2</td>
<td>Control-SH</td>
<td>31-May-05</td>
<td>317100 ± 9</td>
<td>3.28 ± 5</td>
<td>0.67 ± 15</td>
<td>0.291 ± 39</td>
<td>1.50 ± 58</td>
<td>0.15 ± 51</td>
<td>7.8 ± 10</td>
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<tr>
<td>MING1-P4</td>
<td>Control-SH</td>
<td>25-May-05</td>
<td>368800 ± 17</td>
<td>4.34 ± 19</td>
<td>-</td>
<td>-</td>
<td>1.49* ± 139</td>
<td>-</td>
<td>5.79 ± 11</td>
</tr>
<tr>
<td>MING1-P5</td>
<td>Control-SH</td>
<td>24-May-05</td>
<td>350900 ± 8</td>
<td>3.96 ± 8</td>
<td>-</td>
<td>-</td>
<td>1.12* ± 73</td>
<td>0.16 ± 50</td>
<td>6.35 ± 5</td>
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* Concentrations below limit of quantification. Control-SH is coral from the Sea of Hebrides.
Figure 6.6 Cr/Ca µmol/mol measured from spots along branches from coral colonies sampled from control sites (A) North Cormorant and (B) North West Hutton in the North Sea, and from (C) near Mingulay in the Sea of Hebrides. Growth direction is from left to right.
Figure 6.7 Ba/Ca µmol/mol measured from spots along branches from coral colonies sampled from control sites (A) North Cormorant and (B) North West Hutton in the North Sea, and from (C) near Mingulay in the Sea of Hebrides. NC = North Cormorant; NWH = North West Hutton; MING = coral from Sea of Hebrides near Mingulay. Growth direction is from left to right.
Figure 6.8 Cr/Ca µmol/mol measured along branches of three of the four exposed colonies sampled from North Sea oil and gas platforms (A) = Brent Delta, (B) = North West Hutton, and (C) = Tern Alpha. Growth direction is from left to right.
Figure 6.9 Ba/Ca µmol/mol measured along branches of three of the four exposed colonies sampled from North Sea oil and gas platforms (A) = Brent Delta, (B) = North West Hutton, and (C) = Tern Alpha. Growth direction is from left to right.
Figure 6.10 (A) Cr/Ca and (B) Ba/Ca µmol/mol measured along branches of the fourth exposed colony sampled from North West Hutton (NWH). Growth direction is from left to right.
NWH1-P2 was reanalysed to check for reproducibility of the results. A second and third set of spots were ablated just above the original spots (Figure 6.11). Line 1 was run on 28-Jul-05 and lines 2 and 3 were run on 29-Jul-05 within the same analysis. The results showed consistent concentrations of barium along the coral thecal wall and between analyses, although they may indicate some internal heterogeneity of the calcium carbonate material as the spots were not reablated directly on the original spot because of the enhanced fractionation that would result from ablating deep spots. The increase in Cr/Ca on line A was caused by a drop in calcium near the end of the analysis.

Figure 6.11 The mid area of the coral polyp section (NWH1-P2) were groups of three spots representing the three repeat analyses are visible. 1 = analysis run on 28-Jul-05; 2 = analysis (a) run on 29-Jul-05; 3 = analysis (b) run on 29-Jul-05.
Figure 6.12 Results from three repeat analyses of (A) Cr/Ca and (B) Ba/Ca along North West Hutton coral 1 polyp 2 (NWH1 P2). 1 = analysis run on 28-Jul-05; 2 = analysis (a) run on 29-Jul-05; 3 = analysis (b) run on 29-Jul-05.

The results from NWH2-P3 which showed the most conspicuous increase in barium was reanalysed seven weeks later to examine the reproducibility of the heightened barium (Figure 6.13). A second set of spots was ablated just above each of the original spots the same as described above. The results were consistent in that they showed heightened barium along the second half of the polyp.
6.8 Discussion

To enhance sampling resolution and overcome problems of inconsistent results from exposed coral skeletons based on detrital inclusions, a second technique was used to sub-sample material from the coral skeletons: laser ablation ICP-MS. This technique proved advantageous as fresh skeleton was exposed by sectioning the coral polyps and pre-ablating the surface, and as a result, there were no problems with visible detrital inclusions. Results were shown to be reproducible when a second and third set of spots were ablated just below the original line of spots on NWH1-P2, which was not possible in the solution nebulisation ICP-MS. Furthermore, high-resolution sampling allowed for sampling along the inner growth band, which provided a yearly timeline (see Chapter 4). This strategy was similar to the high-resolution sampling of stable isotopes of carbon and oxygen in Chapter 5, but the laser offered a much higher resolution along the growth axis of the polyp.

Disadvantages of using LA-ICP-MS included the lack of matrix-matched certified standard reference materials to use for the fully quantitative analysis. Despite this, NIST-612 showed good precision for all elements analysed when calibrated against
Macs-1. However, NIST-612 was accurate to 23-45 % for metals at the lower end of the mass spectrum, but improved for barium (10 %), cadmium and lead (2 %) at the higher end of the spectrum. Theoretically, the accuracy should be even better for the coral samples as Macs-1 and the coral are both made of calcium carbonate material. The inconsistent results from 19-20- Jul-2005 might be explained by the instrument or laser responding differently to the glass matrix compared to the calcium carbonate matrix on those particular days because no obvious differences were observed in the results from the coral samples that were run in the same analysis.

Many of the elements measured in this analysis were found below the LOD, or close to it. However, although chromium and barium were consistently above the LOQ, there were still inconsistent results with respect to background concentrations. Although Macs-1 was a good matrix match to the coral samples, it was the only reference material used and therefore single point calibrations were carried out. Furthermore, concentrations of chromium and barium in Macs-1 are 120 and 116 ppm respectively, whereas chromium and barium in L. pertusa are 2-3 ppm and 9-10 ppm respectively (see Table 6.5 and Table 6.7). The discrepancy in the concentrations between the analyte and the standard, and the single point calibrations used may account for some of the variation observed in the background concentrations of chromium and barium. However, no other standard reference materials which more closely match the coral concentrations are currently available.

6.8.1 Chromium analysis

The results for chromium showed a shifting background. This is evident from the mean ratios presented in Table 6.7 where means are more easily grouped by date of analysis and not by colony, location, or exposure. In particular, samples run on 18- and 19-Aug
ranged between 1.33-1.83 µmol/mol which are lower concentrations than other analyses. The ICP-MS was optimised using the NIST-612, Macs-1, and a coral sample to ensure that the instrument sensitivity was consistent for each analysis. The laser energy was also recorded and was consistent throughout the analyses. Regardless of the instrument optimisation, on 18-19-Aug the CPS for chromium were 3 times lower than average, but not for barium. Even after calibration with Macs-1, the coral samples showed lower Cr/Ca ratios. Although laser-ablation minimises interferences, and in particular should reduce the level of polyatomic and oxide species (Jarvis et al. 2003), it is possible that chromium may still have interferences from the following: $^{40}\text{Ar} + {^{12}\text{C}}$ (98.5 %), $^{12}\text{C} + {^{40}\text{Ca}}$ (95.8 %), $^{13}\text{C} + {^{39}\text{K}}$ (1.0 %), $^{103}\text{Rh}^{2+}$ (100.0 %), $^{104}\text{Ru}^{2+}$ (18.3 %), $^{104}\text{Pd}^{2+}$ (9.3 %). Because of inconsistencies in the coral background values, it was not possible to statistically compare concentrations between colonies. However, it was possible to look at within polyp variation.

The majority of polyps showed consistent profiles of chromium with the exception of NWH2-P2, NC2-P4, NWH5-P1, and MING-P3, which showed variability of 2-3 µmol/mol. All polyps from NWH1 also showed variability of approximately 1.0 µmol/mol. Since the analytical percent residual standard deviations were less than 10 % for chromium, this variability does reflect heterogeneity along coral skeletons. Although there was some variation in background concentrations in chromium between polyps, the results from the Mingulay coral polyps fell within the range of concentrations from North Sea corals. Moreover, within polyp variation was also similar between the two sites. Therefore, it can be concluded that chromium from drilling muds and cuttings is not present in a biologically available form or L. pertusa has a mechanism to exclude it. Other potential causes of variation in chromium are unknown. Esslemont et al. (2000) also found within colony variability in chromium in Pocillopora damicornis where...
concentrations ranged from <1 to 7 ppm. Chromium has not been previously measured in coral skeletons relative to calcium.

6.8.2 Barium analysis

Background values for barium between polyps were less variable than those for chromium. This is possibly because the instrument response was more stable at the higher mass range. The small variability in barium levels between polyps is still more likely a result of slight changes in the instrument response than true differences between polyps. Regardless, the Sea of Hebrides coral polyps and North Sea coral polyps showed similar background levels of barium. Therefore, although not tested statistically because of the daily unaccountable changes in the instrument sensitivity as discussed in the results, it is possible to conclude that the northern North Sea seawater is not elevated in dissolved barium compared to the Sea of Hebrides; both locations ultimately have the same source of deep Atlantic seawater (Roberts et al. 2005a). Barium in seawater in the open Gulf of Mexico is twice that in the Atlantic or Pacific, but it is not known if this is solely due to natural sources or whether extensive oil and gas activities are also involved (Deslarzes et al. 1995).

Furthermore, it was possible to examine within polyp variation and look for discrete drilling muds and cuttings discharging events or cuttings pile resuspension events which may have led to increases in skeletal barium. The results for the majority of polyps showed consistent levels of barium throughout each polyp, or only small variations. This leads to the conclusion that the majority of coral colonies living below drilling discharges points are not exposed to heightened barium levels which are biologically available to *L. pertusa*. The exceptions were for polyps P1 and P4 from the control colony NC2, and polyp P3 from the exposed colony NWH2, which showed spikes in
barium. NWH2-P3 was reanalysed using a second set of spots located just blow the previous set and the results again showed a similar increase in barium along the top half of the polyp. The lack of 100 % replication is likely indicative of the small scale spatial variability which exists in coral skeletons (Sinclair et al. 1998). Both spikes in the exposed NWH2 and control NC2 colonies are of the same magnitude, but the spike from NWH2 is present over a greater distance along the coral polyp. These spikes are several magnitudes smaller than those observed in the solution nebulisation analysis and similar spikes were not observed for other metals analysed. This implies that the increases in barium are a result of more barium replacing calcium in the skeletal matrix or that barite particles have been incorporated into the skeleton in the absence of other drilling muds constituents. Barium concentrations in drilling muds and cuttings can be several orders of magnitude greater than chromium or other metals (GESAMP 1993; Breuer et al. 2004), which may explain its presence over other metals associated with drilling muds and cuttings. However, short term barium spikes, similar to those in the control NC2 polyps have also been observed in corals from areas not related to oil and gas activities (see discussion below).

Control colony NC2 was selected from a location on North Cormorant upstream and well away from point-source discharges of drilling muds/cuttings. It is possible that no areas on an offshore oil and gas platform are truly free from drilling discharges and that NC2 had been exposed; North Cormorant was still actively drilling in 2003 when the coral colonies were sampled. NC2 polyps analysed using solution nebulisation ICP-MS also showed a small increase in barium, but this was insignificant compared to the large increases observed in the exposed coral TA2. However, other possible explanations need to be considered in light of barium reports from tropical corals not associated with oil and gas exploitation.
Barium/calcium ratios in coral skeletons have been measured as a proxy for upwelling (Lea et al. 1989; Fallon et al. 1999; Reuer et al. 2003) and river inputs to coastal marine environments (Alibert et al. 2003; Sinclair and McCulloch 2004). Colonies growing on offshore platforms in the northern North Sea are not influenced by upwelling or fresh water river inputs from coastal areas. In other studies, seasonal short-lived barium spikes in *Porites* have been reported, but these have yet to be correlated with any causal environmental or biological event, and have no association with oil and gas activities (Hart and Cohen 1996; Tudhope et al. 1996; Sinclair 2005). Similar seasonal spikes in barium have also been reported from bivalves (Stetcher et al. 1996; Vander Putten et al. 2000; Gillikin et al. 2006).

Average background Ba/Ca mole ratios previously reported for corals ranged from 3-10 µmol/mol (Deslarzes et al. 1995; Fallon et al. 1999; Alibert et al. 2003; Reuer et al. 2003; Sinclair and McCulloch 2004; Sinclair 2005). This compares well with the results from the present study with background concentrations ranging between 5-10 µmol/mol. The amplitude of the unexplained barium spikes reported in the literature are three to 10 fold (Hart and Cohen 1996; Sinclair 2005). This is also comparable to the results from the present study where the three barium spikes correspond to a two, three, and 3.3 fold increases in barium.

Similar barium spikes reported from bivalves correlated with phytoplankton blooms (Stetcher et al. 1996; Vander Putten et al. 2000), and barium associations with phytoplankton have been documented (Dehairs et al. 1980; Dehairs et al. 1991). Phytoplankton blooms and the onset of reproduction have also been considered as explanations for anomalous barium spikes in corals, but these remain untested...
hypotheses (Hart and Cohen 1996; Tudhope et al. 1996; Sinclair 2005). Phytoplankton blooms are a possible seasonal food source for corals on the platforms, and corals on North Sea platforms have been shown to be reproductive (see Chapter 2). Therefore, these may be linked to the barium spikes. Furthermore, single spikes per polyp are reminiscent of the one peak per polyp observed in the $\delta^{13}$C along a coral branch from control colony NC3 and are consistent with the theory of a seasonal signal influencing coral growth. Although the barium spikes observed in the control colony NC2 show the same short bursts as observed in previous studies, the main difference between NC2 and reports in the literature is that the spikes were only observed in two of the four polyps analysed or in any of the other coral polyps, which had been analysed along the innermost growth band. Annual phytoplankton blooms would influence all corals growing in the North Sea.

Scanning electron microscope (SEM) images presented in Chapter 4 showed centres of calcification running along the inner theca. Furthermore, centres of calcification rarely ran in a straight line but rather followed a more undulating or zigzag pattern. In the present study, spots were ablated in a straight line along the inner growth band, but because of the undulating nature of the centre of calcification, spots would have been ablated at varying distances from the centre of calcification indicating that the sub-samples will not be evenly spaced in time. This could result in bypassing a seasonal signal of barium spikes along the polyps. Future research should focus on using a greater number of spots at varying distances from the inside of the thecal wall to explore the variability in this skeletal region. Similarly, continuous scanning of the coral skeleton could be analysed to explore the finer heterogeneity that exists within the coral skeleton.
Allison (1996) found barium enriched by a factor of 3.2-3.5 in centres of calcification of *Porites lutea* compared to surrounding fibres (from 4.07 µmol/mol in surrounding fibres to 13.79 µmol/mol in centres of calcification). The association of barium with centres of calcification may be a result of increased organic material in these areas (Wainwright 1964; Cuif et al. 2003) as coral tissue is enriched in metals compared to skeletons (Howard and Brown 1987; Esslemont 2000). However, SEM images taken of NWH2 P3 do show one ablation spot over the centre of calcification and this did not correspond to an increase in barium concentration.

The elevated barium observed in the third polyp from the exposed NWH colony was present along the top half of the polyp; therefore, it did not resemble the sharp short-lived spikes observed in the control polyps from NC2 and those described in the literature. In this case, there is a stronger possibility that the barium increase observed was a result of exposure to drilling muds and cuttings. Drilling on North West Hutton, and thus dumping of drilling muds and cuttings, ceased in 1992, over 10 yr prior to the coral sampling for this study, and the platform is now being decommissioned. Consequently, colonies growing on North West Hutton will not have been exposed to drilling muds and cuttings discharged from the platform since that time. However, North West Hutton has a large cuttings pile, over 25 000 m³ in volume (Marsh 2003), and colony NWH2 was growing just two metres above it.

Although there is a general lack of data pertaining to the leaching of metals from cuttings piles into the overlying water column (Gerrard et al. 1999; Det Norske Veritas 2000; Breuer et al. 2004), a study on the cuttings pile below Beryl Alpha in the North Sea found that barite dissolution was occurring in the sediment pore water as a result of sulphate reduction biogeochemical processes (Shimmield et al. 2000). Furthermore, it
was suggested that if the cuttings pile were disturbed and sediment resuspended in oxygenated seawater then substantial release of dissolved metals could occur. The potential for natural seabed disturbances is not well documented, but results using models predicted significant cuttings piles erosion during severe storm weather conditions (Tyler et al. 1999). Increased barium levels were not observed in other polyps along the same branch, which represent different years. This suggests that the incorporation of barium into NWH2-P3 may have arisen during a particularly stormy year in the northern North Sea.

6.9 Conclusions

It was evident from the solution nebulisation ICP-MS analyses that the TA2 coral trapped particles from drilling muds and cuttings that were discharged into its ambient environment. Therefore, *L. pertusa* skeletons were successfully used to distinguish the exposed environment from the control environment, but without linking to a highly resolved chronology. Results from Chapter 4 provide a better understanding of the skeletal growth patterns of *L. pertusa*. Based on these results, with further investigations into the relationship between time and skeletal banding in the thecal wall and careful dissection of coral skeletons which have been exposed to drilling discharges, it may be possible to use the trapped drilling discharge particles as a time recorder of cuttings exposure. However, it has not been shown whether *L. pertusa* is guaranteed to trap particles within its skeleton with every exposure event.

LA-ICP-MS techniques used in this chapter were successful in revealing discreet changes in barium and chromium along individual coral polyps, and in particular revealed two different trends in barium. However, background levels of trace metals in coral skeletons were not always consistent between polyps from the same colonies, but
were rather consistent by analysis date suggesting methodological problems related to changes in the instrument response. This prevented the statistical detection of small-scale differences in metal concentrations in coral skeletons from different locations in the North Sea and from the Sea of Hebrides. A reference material which more closely matches the concentrations found in the corals should help improve the technique. However, based on the results it was apparent that background levels of barium were consistent across all samples from the North Sea and the Sea of Hebrides.

The lack of high barium concentrations in the exposed corals analysed using LA-ICP-MS suggests that barium sources from drilling discharges are not highly soluble and thus not biologically available to *L. pertusa* when discharged from above. However, the results from the exposed North West Hutton colony living just above the cuttings piles suggests that cuttings piles do release dissolved barium when sediments are resuspended, and that *L. pertusa* acted as an archive of marine pollution in this particular instance. Further sampling opportunities could focus on collecting additional colonies living close to cuttings piles to test this hypothesis; however, further sampling was not feasible during the time-frame of this research project. A better understanding of natural sources of barium in coral skeletons will also help differentiate between anthropogenic and natural sources of barium around the platforms.
Chapter 7 - Final Overview and Discussion

7.1 Environmental Sensitivity

7.1.1 Research summary

Concerns have been raised in recent years about the impacts of deep-water oil and gas activities on cold-water corals as the offshore oil and gas industry moves into deeper water. In particular, concern is over the impacts from drilling discharges such as drilling muds and cuttings. Tropical corals are sensitive to the effects of increased sedimentation and are generally limited to regions of low sedimentation. Laboratory and field studies have examined the effects of drilling muds and cuttings on a range of tropical coral species and found responses to include changes to polyp behaviour, feeding, growth, tissue loss, and mortality at certain concentrations. The extent to which the toxicity of the muds and cuttings, or the physical effects of increased sedimentation, or the synergistic effects of the two are causing the negative impacts is not well understood.

Equivalent studies on cold-water corals had not been attempted until the present study and potential effects had only been hypothesised. The discovery of *Lophelia pertusa* on oil and gas infrastructure in the North Sea, including on a drilling platform and the controversial Brent Spar oil storage buoy, led to debate about potential negative effects and actual exposure levels of these corals to drilling discharges. These issues led to the present study, which used several approaches to investigate *L. pertusa* in the North Sea and its environmental sensitivity to increased sedimentation and oil and gas discharges.
Initial reports of *L. pertusa* in the North Sea were limited to several locations and provided few details. Therefore, the present study sought to describe the extent of its occurrence in the North Sea with details of its distribution at two study sites, the Heather and North Alwyn A platforms, using existing industry platform survey videos. During this initial investigation, evidence of visual contamination to coral colonies from drilling discharges was also noted. Further video surveys from the Tern Alpha platform were used to examine coral growth rates and recruitment. Results from video analyses revealed that some coral colonies had clearly been exposed to drilling discharges while others had not. Exposed and control (unexposed) corals were sampled opportunistically during industry ROV downtime from a selection of platforms in the northern North Sea and samples were used for further analysis in the laboratory.

Coral samples were investigated for signs of sexual reproduction, and skeletal characteristics were measured and compared between exposed and control colonies. In addition, the concentrations of relevant metals were analysed in skeletons of *L. pertusa* from North Sea platforms to assess heavy metal incorporation in connection with drilling muds and cuttings, and to investigate the potential of *L. pertusa* as a bio-indicator of marine pollution. Live samples of *L. pertusa* were collected from the Sea of Hebrides, kept in aquaria, and their polyp behaviour was examined in response to increasing levels of sedimentation. In addition, live corals were examined for active mechanisms used to cope with sediment influx. Throughout this work, several opportunities were presented to further the understanding of several basic biological processes of *L. pertusa* living in the North Sea. These were followed under the principle that a better understanding of the biology of *L. pertusa* is not only of interest to coral biologists, but will also assist with future planning and current interpretation of the effects on *L. pertusa* from oil and gas activities (see section 7.2).
7.1.2 Reproduction

This study indicated that \textit{L. pertusa} was resilient to increased sedimentation and to oil and gas drilling muds and cuttings, except in \textit{worst case} scenarios, particularly when there was potential for physical smothering. Colonies were notably abundant on Heather conductors and were present on at least 13 other platforms in the northern North Sea. However, corals living below the drilling discharge chute on Heather were observed to be smothered by drilling cuttings and were partially or completely dead. At least some corals present were reproductive and self-recruiting to the North Sea. However, not all polyps within a colony and not all colonies on North West Hutton were reproductive and further research with greater sample sizes is required to identify the cause(s) behind the non-reproductive polyps and colonies, which could potentially be a result of exposure to drilling discharges.

7.1.3 Polyp behaviour in response to sedimentation

Negative effects on polyp behaviour were only significant at the highest levels of sedimentation when tested in the laboratory. However, laboratory experiments do not always reflect \textit{in situ} situations. Natural sedimentation rates on \textit{L. pertusa} are yet unknown and thus not available for comparison but are likely orders of magnitude lower than the highest rates used in these experiments. However, \textit{L. pertusa} reefs in the Porcupine Seabight have been found to be an integral part of carbonate mound formation and actively trap mobile sand (Wheeler et al. 2005). This indicates some level of exposure to sediment resuspension on the mounds and potential for \textit{L. pertusa} to be naturally adapted to cope with sediment influx. The short-term nature of the experiments carried out in the present study does not reflect the actual chronic exposure corals might experience when exposed to drilling discharges during the drilling of multiple wells.
The experiments in the present study used clean sediment and did not test the effects of drilling muds and cuttings. Therefore, significant negative effects may have been observed at lower sedimentation rates with the additional stress from exposure to toxic components of muds and cuttings as observed in several studies on tropical corals (Thompson 1980; Thompson et al. 1980; Raimondi et al. 1997). It is difficult to separate the effects of drilling muds that are caused by the smothering of sediment, and those that are caused by the toxic ingredients in the muds (Szmant-Froelich et al. 1981), but apparent resilience to clean sand deposition suggests that toxic components may be critical. Furthermore, drill cuttings are continually discharged during drilling of a well (Gettleson 1980), and although drilling times are variable, it is likely that corals will experience exposure times greater than the four hour sand deposition used here. It is difficult to predict sedimentation rates that *L. pertusa* will experience if living close to a drilling site in deep water, such as beyond the continental shelf in the northeast Atlantic, as many factors, including local hydrography and location of corals relative to the discharge point, will affect the concentration of cuttings that actually reach the seabed.

*Lophelia pertusa* was observed under a binocular microscope following instantaneous deposition of sediment. Coral polyps removed half of the deposited sediment within 10 min, followed by slower clearing rates particularly after 60 min. Grains of sand were moved off the polyp surface using ciliary actions, and often sediment was moved in aggregations possibly to save energy. Finally, sediment was ingested and expelled, possibly as an active clearing mechanism or as an indiscriminate feeding mechanism. *L. pertusa* did not display complete polyp inflation and did not use mucus production as a means of clearing sediment, which are both active mechanisms that have been observed in tropical corals. But, *L. pertusa* clearly showed some ability to actively eliminate
sediment from its surface and expel ingested sediment up to the highest deposition of sediment (120 mg cm\(^{-2}\)) used in the experiment. While all five polyps cleared their surface within 24 h, two polyps had sediment remaining in their gut. If \(L.\ pertusa\) is continuously required to put energy towards active sediment clearing, this may have a secondary effects and reduce a coral’s ability to reproduce (Stafford-Smith and Ormond 1992) and can reduce growth (Krone and Biggs 1980; Dodge 1982) as discussed for tropical corals.

7.1.4 Skeletal characteristics

Skeletal characteristics were consistently different between exposed and control colonies, with shorter and narrower polyps with thinner thecal walls measured for exposed colonies. However, an unbalanced experimental design, where single platforms had been used as sample sites for only control or only exposed colonies, made it difficult to assign potential corresponding effects of platform location, such as genetic variation. Local hydrography around individual coral colonies may also have contributed to morphological variation, although further details of coral locations are needed to investigate this further.

7.1.5 Heavy metals

\(Lophelia\ pertusa\) skeletons analysed using solution nebulisation ICP-MS confirmed that an exposed colony from Tern had been exposed to drilling muds and cuttings compared to a control colony from North Cormorant. However, the results are not indicative of the bioavailability of metals as increased concentrations were direct results of visible detrital inclusions in the skeleton. Results from LA-ICP-MS showed that exposure to drilling muds and cuttings did not increase chromium and barium concentrations in
coral skeletons, with the exception of higher barium in one polyp from a North West Hutton colony living close to the cuttings pile. Pore water in the Beryl cuttings piles contained dissolved metals that could be released into the overlying water if cuttings were significantly disturbed (Shimmield et al. 2000). It is hypothesised that dissolved barium was released from the North West Hutton cuttings pile in the same way. Further investigations of more colonies living close to cuttings piles are needed to examine this further. These trace metal results suggest that *L. pertusa* could act as a bio-indicator of pollution, particularly in light of additional results from the present study, which has led to a skeletal chronology for *L. pertusa* from the North Sea (see next section).

The platforms in the North Sea provided an excellent opportunity to examine the effects of drilling discharges on corals. However, when these results are applied to corals naturally occurring on the seabed beyond the North Sea several factors should be considered. While North Sea corals living directly on platforms have the potential to experience the worst-case scenario of exposure to drilling discharges, this only holds true for those exposed colonies living close to discharge pipes and just above cuttings piles. Many of the colonies growing close to discharge points were partially or completely dead and coated in drilling muds and cuttings. The remaining colonies on the platforms were growing away from the points of discharge and did not appear to be at all exposed to drilling discharges, and hence it is not necessarily surprising that they are found on the platforms in abundance.

### 7.1.6 *Lophelia pertusa* from North Sea platforms and natural offshore occurrences

North Sea corals growing well above the seabed and suspended in the water column may have several advantages over *L. pertusa* colonies growing naturally on the seafloor. For those exposed corals on the platforms, being suspended above the seabed and above
the benthic boundary layer ensures good water flow around corals, and in addition, gravity will assist with passive clearing of falling particles. Furthermore, platform corals will not be affected by the physical build-up of drill cuttings on the seabed or by resuspension events of drilling muds and cuttings once the muds and cuttings have settled on the seabed (except for those living just above the cuttings piles). Finally, living at shallower depths on the platforms provides access to a higher quality food supply from surface productivity compared to the more highly degraded particulate matter with reduced organic content found in the deep-sea (Gage and Tyler 1996).

Natural occurrences of *L. pertusa* on the seabed near potential future oil and gas developments are generally reported from depths of 200-1000 m (Freiwald 2002). *Lophelia pertusa* colonies growing on natural seabed substrata will not likely ever experience the same worst case exposure levels to drilling muds and cuttings as the exposed corals on the platforms. At greater depths, muds and cuttings have further to travel to reach the seabed. This will result in reduced flux to the seabed, but covering a larger area, keeping in mind other factors such discharge rate, local currents, and stratification that will also contribute to the actual concentration that ultimately reaches the seabed (Brandsma 1996; Rye et al. 2004).

Coral colonies living on the seabed will also be affected by the build-up of muds and cuttings on the seabed, and the continual resuspension of muds and cuttings once settled on the seabed. For example, the low energy environment in the deeper northern North Sea (compared to the high energy environment in the shallower southern North Sea) has resulted in the build-up of cuttings piles (Eames et al. 2002). These are several meters high and certainly high enough to cover whole colonies of *L. pertusa*. Furthermore, coral recruitment to the area could be affected if sediment covers hard substrata (Rogers
In general, deep-water environments are predicted to have greater local environmental impacts from the discharge of drilling muds and cuttings because of their low energy status and low natural sedimentation rates (Glover and Smith 2003; Smith et al. in press). However, large cold-water coral occurrences generally occur in relatively high energy environments associated with high relief topography and strong bottom currents which specifically create an environment with exposed hard bottom substrata for coral planulae settlement (Grigg 1974; Genin et al. 1986; Rogers 1999; Freiwald et al. 2004). These areas would be less likely to experience sediment build-up, but this will depend on the quantity of discharged material. Less is known about the more patchy occurrences of *L. pertusa* that may have more potential to be impacted by drilling muds and cuttings. Furthermore, naturally occurring *L. pertusa* is an integral part of the benthic ecosystem; in the northeast Atlantic it provides habitat for over 1000 species (Freiwald et al. 2004; Roberts et al. 2006). Therefore, its sensitivity to anthropogenic influences is important for the wider goal of protecting marine biodiversity and the potential effects of drilling discharges on the associated fauna of *L. pertusa* should be considered.

In addition to the tests carried out in this thesis, other biological effects can be assessed to determine the impacts of pollution on organisms such as corals. These include assessing changes in the physiology (e.g. laboratory experiments examining changes in respiration), histopathology, and biochemical composition (e.g. lipid: protein ratios), as well as measuring mortality rates (Szmant-Froelich et al. 1981; Brown and Howard 1985; Dodge and Szmant-Froelich 1985; Moore 1985; Widdows 1985). Furthermore, trace metals in coral tissues could be investigated to provide further insight into the biological tolerances of living coral tissue to heavy metals. In addition to heavy metals and physical smothering, contamination from hydrocarbons is a major concern with...
respect to oil and gas discharges (GESAMP 1993; Grant and Briggs 2002) and should be examined in *L. pertusa*.

Despite the number of tests which are carried out in laboratories or on corals growing directly on platforms, when it comes to real *in situ* applications of the results, it is often difficult to differentiate between perceived and real risks from oil and gas activities because numerous site-specific environmental factors need to be considered (Gray 2002). As new deep water oil and gas developments begin, either in the northeast Atlantic west of Scotland, in the Campos Basin off Brazil, in the Gulf of Mexico, or along the Eastern Scotian Shelf off Nova Scotia, or anywhere else where cold-water corals and oil and gas activities coincide, a long-term environmental monitoring program should be established.

### 7.1.7 In situ environmental effects monitoring near an offshore oil and gas development

Environmental effects monitoring is already a key part of the offshore oil and gas regulatory regime in countries such as the USA, Canada, Norway and the UK (Gray et al. 1999; Crawford and Lee 2005; Hartley 2005; Wells 2005). *Lophelia pertusa* is a valued ecosystem component because it forms a long-lived habitat for a diverse associated fauna; therefore, a dedicated part of such a program should include monitoring the status of coral occurrences in the surrounding area. Repeated surveys on an annual or bi-annual basis should include a suite of biological and environmental assessments such as area covered by living coral colonies and changes in sediment build-up from discharges (similar to Brewer et al. 1991; Coats 1994). Corals could be integrated into the monitoring approach described by Hartley (2005) where fixed photographic quadrats on large boulders are periodically photographed by ROV, and
photos quantitatively analysed onshore. Large boulders are generally associated with other hard substrata suitable for *L. pertusa*, and therefore, it would be possible to identify the same *L. pertusa* reef during subsequent surveys based on its proximity to large boulders. Coral samples could also be collected and assessed for their associated fauna, reproductive status, histopathology, and biochemical composition.

In addition, skeletal samples could be used to monitor changes in trace metals present in seawater. Corals are not the only calcareous organisms which can be used as bio-indicators of marine environments, bivalves and molluscs have also shown potential as tracers of the marine environment (Stetcher et al. 1996; Toland et al. 2000; Richardson et al. 2001; Gillikin et al. 2005; Gillikin et al. 2006). Two bivalves commonly associated with living *L. pertusa* reefs are *Acesta excavate* and *Delectopecten vitreus* (Freiwald et al. 2004). These species may offer potential as bio-indicators to corroborate results from *L. pertusa* skeletons. Bivalves may offer the additional benefit of a more straightforward annual skeletal chronology. Such a long-term monitoring program will ultimately reveal actual impacts on *L. pertusa* and its associated community.

### 7.2 The biology of *Lophelia pertusa*

The results in this thesis have contributed to the current understanding of the environmental distribution, reproduction, recruitment, and growth of *L. pertusa*. The vertical zonation of *L. pertusa* on Heather Alpha and North Alywn A platforms confirmed that *L. pertusa* is limited by the presence of oceanic water below 45-60 m which enters the North Sea from the North Atlantic and does not mix with overlying water (Roberts 2002). Two of eight colonies sampled from North West Hutton in December 2003 were reproductive with late vitellogenic oocytes which indicated that *L.*
pertusa in the North Sea was following a similar reproductive cycle to corals from Porcupine Seabight (west of Ireland) (Waller and Tyler 2005).

Growth rates and colony sizes from Tern Alpha suggested annual recruitment of corals to the platform. North Sea platforms offer further opportunities to investigate coral recruitment beyond what was achieved in the present study, including details of settlement and growth. Oil and gas platform structures offer parallels with benthic landers used to study benthic environments over timescales of days to several months (e.g. Duineveld et al. 2004; Roberts et al. 2005b), except the platforms are remarkably larger and offer a much longer study timescale of 30+ years. Furthermore, they are regularly surveyed by industry ROVs and divers. The platforms provide a structure to deploy settlement plates and examine recruitment from early larval settlement onwards; different substrate types could be tested. Settlement plates deployed by an ROV would be secured to an appropriate place on the platform, or an area of the platform could be scraped clean. During annual platform surveys, these substrata could be retrieved, videoed, and/or digitally photographed.

To complete the recruitment story, coral samples taken from a selection of platforms in the northern and central regions of the North Sea, and from potential source populations in the northeast Atlantic could be analysed for genetic similarity/differences. For example, work could build on methods developed by Le Goff-Vitry et al. (2004) and Le Goff-Vitry and Rogers (2005) used to study genetic diversity between L. pertusa populations from the European margin and Scandinavian fjords. This could reveal the larval dispersal capabilities between the North Atlantic and the North Sea and further explain recruitment between and within individual platforms.
The results in this thesis also provide evidence for a skeletal chronology for *L. pertusa* growing in the North Sea. Results in Chapter 2 presented the first *in situ* growth rates for *L. pertusa* and when coupled with skeletal characteristics analysed in Chapter 4, the annual colony extension rate was found to equal the mean polyp height of polyps which had already budded at least once. This suggested that coral polyps near the outside of the colony (i.e. those that will affect the colony linear extension) have one budding event (with a mean of two buds) per year. Further analyses revealed that *L. pertusa* polyps extend quickly to their full height during their first year, and then the thecal wall thickens at a slower consistent rate for at least the next four years and potentially for the remainder of the polyp’s life. If growth is assumed to originate from the centre of calcification then it is likely the innermost growth band represents skeleton accreted during the first year of polyp growth. Results from stable isotope analyses confirmed that the innermost growth band represents relatively fast skeleton accretion. Having such a skeletal chronology is ultimately essential if *L. pertusa* is to be considered an effective bio-indicator. In addition to extending the length of one polyp per year, two control colonies from North Cormorant both revealed potential seasonal signals within their innermost skeletal growth bands. Indeed, one sample (NC3) revealed one peak in δ¹³C per polyp and another sample (NC2) revealed one peak in barium per polyp. A better sub-sampling resolution for stable isotope analyses might reveal a more regular seasonal pattern than was possible in the present study.

Seasonal trends that were revealed along the growth axis need to be reproducible in coral polyps along replicate branches from a single colony to confirm the skeletal chronology for *L. pertusa* put forward in this thesis. Whereas this thesis focused on examining the variation between colonies of *L. pertusa* from different locations on
platforms (i.e. exposed and control), a similar study focusing on replicate branches from single colonies may corroborate the skeletal chronology.

Results on growth rates and skeletal patterns need to be applied with caution to *L. pertusa* colonies from areas outside the North Sea because of the differences observed between the North Sea and *L. pertusa* growth patterns reported in the literature (i.e. Freiwald et al. 1997; Mortensen and Rapp 1998). In particular, it cannot be assumed that all *L. pertusa* will grow and extend the length of one polyp per year. For example, Freiwald et al. (1997) described the polyp height of the tubular morphotype from Norway to be between 4-5 cm. It remains to be shown whether this then equates to colony growth of 4-5 cm yr\(^{-1}\). This will be a difficult hypothesis to test because North Atlantic corals lack the advantage of several years of visual time series that were available for Tern Alpha platform corals. Coral skeletons from regions outside the North Sea warrant further investigation to test growth characteristics and banding patterns for comparison with North Sea corals, and investigations into the influence of the polychaete *Eunice norvegica* on growth structure and form as it was not found associated with North Sea corals. A better understanding of the variability in skeletal patterns between regions (e.g. North Sea versus northeast Atlantic) will also influence *L. pertusa*’s utility as a bio-indicator.

7.3 Study limitations

Working with cold-water corals and other deep-water fauna from offshore environments presents several limitations to research. Offshore vessels and associated equipment, notably ROVs, are very costly to run and their use is constrained by bad weather conditions. Therefore, the number of sampling opportunities offshore is limited making it difficult or impossible to return and collect further samples after initial ones have been
analysed. By working with oil and gas companies, several opportunities were created to collect high quality samples for the present research project, but these limitations still applied. Furthermore, in the present study, sampling of coral colonies was carried out by employees of the offshore survey companies. For example, by sampling many control colonies from a single platform and many exposed from a different platform has limited the potential to examine the co-varying influences of platform location and exposure to drilling discharges. Despite the limitations discussed above, coral samples and analytical analyses have led to novel results including the first \textit{in situ} colony extension rates and the development of a skeletal chronology for \textit{L. pertusa} living in the North Sea, which can be applied to investigations of \textit{L. pertusa} as an environmental recorder.

Analytical analyses are also an expensive aspect of research. In the present study, the number of samples that were run on the ICP-MS was limited to the funding available for this PhD project. The initial objective of this study was to examine a range of control and exposed corals from different sites and to examine the potential of \textit{L. pertusa} as an archive of marine pollution. Despite the limited funds, results from trace metal analyses in the present study suggest that \textit{L. pertusa} can act as an archive of marine pollution and that cuttings piles below oil and gas platforms in the North Sea may be releasing dissolved barium. These results have created an excellent opportunity to continue this research further by directing sampling efforts to corals growing near cuttings piles.

\section*{7.4 Conclusions}

A new sub-population of \textit{L. pertusa} exists in the North Sea and coral colonies are found on at least 14 oil and gas platforms in the northern North Sea at depths from 47 m to the
top of the cuttings piles, which correlates with the boundary of the summer thermocline and the presence of North Atlantic water in the North Sea. *Lophelia pertusa* colonies in the North Sea have a linear extension rate of $26 \pm 5$ mm yr$^{-1}$ and have annual recruitment to the platforms. Whereas the majority of colonies growing on the platforms appear in good condition, colonies growing close to discharge points of drilling muds and cuttings can be smothered by discharges. At least some corals in the North Sea are reproductive and are following the same reproductive cycle as documented for *L. pertusa* in the Porcupine Seabight, but further research is required to assess the influence of drilling discharges on their reproductive status.

In the laboratory, *L. pertusa* polyps did not significantly retract when exposed to four-hour sedimentation events up to between 1-10 mg cm$^{-2}$ min$^{-1}$, but significant retraction is observed at levels between 12-19 mg cm$^{-2}$ min$^{-1}$. *Lophelia pertusa* primarily uses ciliary currents to move sediment off of its polyps. It takes advantage of gravity by removing sediment grains from its outermost edge first, and it moves sediment towards its mouth for ingestion potentially as an indiscriminate feeding response.

Upward polyp growth occurs in the first year of a polyp’s life followed by gradual thecal wall thickening over time, and a polyp’s maximum length is $24 \pm 6$ mm which is equivalent to the colony annual growth rate. The coral polyp’s innermost optically dense growth band is relatively depleted in $\delta^{13}$C and $\delta^{18}$O indicating fast skeletal accretion which corresponds to the initial fast linear extension of coral polyps.

Background concentrations of chromium, copper, zinc, cadmium, barium and lead from *L. pertusa* skeletons fall within previously recorded concentrations for corals, and nickel is within the range of published values of coral skeletons from polluted sites (16-29
ppm). Copper, lead, and barium concentrations peaked in relation to the incorporation of detrital inclusions from drilling muds and cuttings. Background concentrations of chromium and barium from exposed North Sea corals fall within variations found in the Sea of Hebrides coral skeleton and control North Sea. Short-lived barium spikes along a control coral from North Cormorant may be related to seasonal environmental signals. An exposed colony from North West Hutton sampled from just two meters above the cuttings pile shows increased barium along the top half of one polyp, which might be indicative of contaminants being released from the cuttings pile as a result of cuttings resuspension.

The oil and gas industry in the North Sea has much data to offer on the cold-water coral *L. pertusa*, which has been fouling platform structures in the northern North Sea since they were first installed. Decades of platform survey videos, and the presence of ROVs working at offshore platforms on an annual basis offer an excellent means of studying the environmental sensitivity and biological processes of *L. pertusa*. Continued industry-science collaborations with further well-adhered to sampling protocols have the potential to build-on the work carried out in this thesis.
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Appendix 1

The occurrence of the cold-water coral *Lophelia pertusa* (Scleractinia) on oil and gas platforms in the North Sea: Colony growth, recruitment and environmental controls on distribution

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Abstract

This study reports a newly established sub-population of *Lophelia pertusa*, the dominant reef-framework forming coral species in the north-east Atlantic, on oil and gas platforms in the northern North Sea. *L. pertusa* was positively identified on 13 of 14 platforms examined using existing oil and gas industry visual inspections. Two platforms were inspected in more detail to examine depth and colony size distributions. We recorded 987 colonies occurring between 59 and 132 m depth that coincided with cold Atlantic water at depths below the summer thermocline in the northern North Sea. We suggest that these colonies provide evidence for a planktonic larval stage of *L. pertusa* with recruits initially originating from populations in the north-east Atlantic and now self recruiting to the platforms. Size class distribution showed a continuous range of size classes, but with few outlying large colonies. The break between the largest colonies and the rest of the population is considered as the point when colonies began self recruiting to the platforms, resulting in greater colonization success. We present the first documented in situ colony growth rate estimate (26 ± 5 mm yr⁻¹) for *L. pertusa* based on 15 colonies from the Tern Alpha platform with evidence for yearly recruitment events starting the year the platform was installed. Evidence of contamination from drill muds and cuttings was observed on the Heather platform but appeared limited to regions close to drilling discharge points, where colonies experience partial as well as whole colony mortality.

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Keywords: Cold-water coral; *Lophelia pertusa*; North Sea; Environmental sensitivity; Oil and gas

1. Introduction

*Lophelia pertusa* is an azooxanthellate reef framework-forming coral which, unlike tropical shallow-water corals, is not restricted to warm waters or to depths within the euphotic zone. Although the full extent of its distribution remains unknown, *L. pertusa* is a cosmopolitan species (Zibrowius, 1983) that appears most abundant in the north-east Atlantic where its occurrence varies between small scattered colonies west of Shetland (Wilson, 1979a; Roberts et al., 2003), to larger coral patches on Rockall Bank (Wilson, 1979b) and in the northern Rockall Trough (Mason et al., 2002), to carbonate mounds in the Porcupine Seabight (Hovland et al., 1994; De Mol et al., 2002; Van Rooij et al., 2003) and Rockall Trough (Kenyon et al., 2003). The highest density and largest reefs of *L. pertusa* are reported from the Norwegian continental shelf and margin (Frelvold et al., 1997; Fossá et al., 2000, 2002). *L. pertusa* is predominantly found between depths of 200 and 1000 m (Zibrowius, 1983) but shallower records exist from Norwegian fjords where they have been observed at just 39 m (Dons, 1944; Fossá et al., 2002).

This study considers the occurrence of *L. pertusa* on oil platforms in the North Sea, where until recently, *L. pertusa* had not been recorded. The only published record of this species from the UK sector of the North Sea described a dead specimen brought up in a trawl north east of Buckie Scotland (Wilson, 1979a). The North Sea is a well
developed region of oil and gas production. The first well was drilled in the British North Sea in the 1960s and by 2003 there were 240 fields in production with over 2000 production wells. L. pertusa was reported on oil and gas infrastructure in the North Sea in the late 1990s with the decommissioning of the Brent Spar oil storage buoy (Bell and Smith, 1999) and visual surveys of live coral colonies on infrastructure in the Beryl field (Pearce, 1999; Roberts, 2002), and in 2000 from the actively drilling Brent Alpha platform (Roberts, 2000). The presence of L. pertusa on these structures, including observations of drill cuttings on living coral colonies (Roberts, 2000), leads to questions regarding the sensitivity of this species to oil and gas activities.

Concerns have been raised about the sensitivity of L. pertusa to oil and gas activities as the UK industry began to expand beyond the North Sea and new oil fields were developed west of Shetland in the north-east Atlantic in the late 1990s (Rogers, 1999). Of particular concern for L. pertusa was the exposure to drill cuttings and muds and associated oil and chemical additives (Rogers, 1999). Corals, as suspension feeders, are sensitive to the effects of sedimentation (Rogers, 1990). Studies on the effects of drill cuttings on tropical corals have shown direct mortality (Thompson et al., 1980), altered feeding behaviour (Somani-Froelich et al., 1981), altered polyp behaviour (Thompson and Bell, 1980), and effects on coral physiology (Krone and Biggs, 1980). A review of these effects concluded that no distinction could be made between the effects of sedimentation and potentially toxic effects of chemical additives or the synergistic effects of both (Dodge and Somani-Froelich, 1985).

The known occurrence of cold-water corals including L. pertusa falls within distinct environmental controls. Habitat requirements for cold-water corals include hard substrata for larval settlement and regions with enhanced currents which keep substrata clean of sediment and provide a reliable food supply (Dona, 1944; Grigg, 1974; Wilson, 1979b; Genin et al., 1986; Freiwald, 2002). The distribution of L. pertusa appears limited to within oceanic water with a temperature and salinity range of 4–12 °C and 33–37 PSU (Freiwald et al., 2002). The vertical force of wave action is also suggested to control the minimum depth limit (Frederiksen et al., 1992). Finding L. pertusa on oil and gas platforms in the North Sea offers a unique vertical zonation from the surface to the seabed to examine some of the proposed environmental controls. The platforms also act as a 20–30 year settlement experiment to investigate coral recruitment and growth.

The overall objective of this study was to describe the occurrence of L. pertusa on oil and gas platforms in the North Sea and interpret the results in context of current understanding of the coral’s growth, habitat, and environmental requirements.

Specific objectives were to (1) assess the distribution and describe the colonies of L. pertusa on oil and gas platforms in the North Sea using oil industry’s visual survey records from annual platform inspections; (2) examine recruitment and growth rates by measuring the size of colonies over time; (3) examine the vertical zonation of L. pertusa and assess the coral’s potential exposure to drill cuttings.

2. Materials and methods

Video tapes and still images recorded by remotely operated vehicles (ROVs) during oil industry routine platform inspections were provided following a request for sightings of L. pertusa sent to all hydrocarbon companies working in the British North Sea. Existing information indicated that L. pertusa was most commonly found on platforms in the northern region of the North Sea, rather than the central or southern regions, so our investigation concentrated on this area (Bell and Smith, 1999; Roberts, 2002; D. McKay, personal communication).

Two platforms, Heather Alpha (Heather) and North Alwyn Alpha (NAA), were chosen to examine the depth distribution and size of L. pertusa based on video footage of conductors, which are wide-diameter pipes that run from the drilling platform to the seabed to guide drilling and contain drilling fluid or oil (Fig. 1). The depth for each colony was recorded, and the colony diameter was assessed from the widest portion of the colony on the video screen and was calculated relative to the known diameter of the conductors provided by the respective oil company.

Fifty percent of the Heather conductors were surveyed as the ROV only surveyed one side of the conductors from whichever angle was most accessible to the ROV. The NAA conductors were surveyed in a spiralling fashion to maximize the area viewed during the survey. It is estimated that this spiralling survey allowed approximately 75% of the conductor to be viewed (Personal communication N. Duncan, Total). Colony density on each conductor was standardized to the area viewed and was recorded as the number of colonies per square metre. The area of the conductor was calculated as: 2πrh (where r is the radius of conductor and h is the height of conductor).
Growth rates of *L. pertusa* colonies were estimated from colonies on the Tern Alpha platform in the northern North Sea (Fig. 1). Videotapes of inspection surveys from 1993, 1998 and 2002 were reviewed and the same individual colonies were identified over time using depth and easily identifiable structural components such as calcified anodes to locate the colonies. The diameter of the colonies was estimated relative to the known diameter of the platform members as previously described, and colony extension rate was calculated as the extension of the colony radius over time.

Samples were taken from the platforms to confirm species identification. Historical hydrographical data for the northern North Sea were obtained from the British Oceanographic Data Centre (BODC).

3. Results

3.1. Distribution and colony description

Thirteen of 14 platforms examined in the northern North Sea were colonized by *L. pertusa*; all platforms were installed between 1975 and 1988 and located in the region of the North Sea with depths greater than 100 m (Fig. 1). *L. pertusa* was not identified on the Scott platform, installed in 1993. Records to date of living *L. pertusa* in the North Sea are presented in Table 1. Anecdotal reports from ROV personnel carrying out platform inspection surveys throughout the North Sea note that *L. pertusa* is less abundant on platforms in the central North Sea and is not seen on platforms in the southern North Sea (D. McKay, personal communication).

Samples taken from six platforms confirmed the species identification as *L. pertusa*. If unobstructed, this coral generally formed distinctive hemispherical-shaped colonies and grew around any obstructions. Two colour varieties of polyps were observed, orange and white, which occasionally overlapped where two colonies merged, or where one colony apparently settled on the other (Fig. 2). The coral skeletons had exserted septa and some visible growth banding in the thecal wall and septa. Polyp shape and size varied between colonies with some long, slender corallites with thin thecal walls and others with shorter corallites and thicker theca. Coral building was intruscentacular. Polyp density was relatively high and neighbouring offspring often fused together forming complex structural branching patterns. There was no evidence of the polychaete *Eunice norwegica* known to commonly occur with *L. pertusa* colonies in the Atlantic Ocean (Dans, 1944; Wilson, 1979b; Freiwald et al., 1997; Roberts, 2005).

3.2. Colony depth distribution

The depth distributions of the colonies on the NAA and Heacon conductors were skewed right with steep declines towards greater depths (Fig. 3). The minimum and maximum depths of colonies on NAA conductors were 62 m and 118 m with a mean depth of 95 m. The minimum and maximum depths for the Heacon conductors were 59 m and 132 m with a mean depth of 106 m. The shallowest record of *L. pertusa* during the study was from 47 m on the North West Hutton platform.

3.3. Hydrographical parameters in the northern North Sea

BODC temperature and salinity profiles taken from within 50 km of a platform identified with *L. pertusa* (Fig. 4A and B) show well-mixed winter conditions and stratified summer conditions. *L. pertusa* colonies were

<table>
<thead>
<tr>
<th>Platform</th>
<th>Operator</th>
<th>Installation date</th>
<th>Platform type</th>
<th>Field depth (m)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alwyn North</td>
<td>Total</td>
<td>May-1975</td>
<td>Shell</td>
<td>125</td>
<td>Present study</td>
</tr>
<tr>
<td>Beryl SP1 2</td>
<td>Exxon Mobil</td>
<td></td>
<td>SPM</td>
<td>119</td>
<td>Roberts (2002)</td>
</tr>
<tr>
<td>Beryl SP1 3</td>
<td>Exxon Mobil</td>
<td>1976</td>
<td>SPM</td>
<td>119</td>
<td>Roberts (2002)</td>
</tr>
<tr>
<td>Brent Bravo</td>
<td>Marathon</td>
<td>May-1977</td>
<td>CORBS</td>
<td>192</td>
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</tr>
<tr>
<td>Brent Alpha</td>
<td>Shell</td>
<td>May-1976</td>
<td>Shell</td>
<td>192</td>
<td>Roberts (2000)</td>
</tr>
<tr>
<td>Brent Bravo</td>
<td>Shell</td>
<td>August-1975</td>
<td>CORBS</td>
<td>190</td>
<td>Present study</td>
</tr>
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<td>Brent Delta</td>
<td>Shell</td>
<td>July-1979</td>
<td>CORBS</td>
<td>190</td>
<td>Present study</td>
</tr>
<tr>
<td>Brent Spar</td>
<td>Shell</td>
<td></td>
<td>Shell</td>
<td>190</td>
<td>Bell and Smith (1989)</td>
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<tr>
<td>Claymore</td>
<td>Totalman</td>
<td>June-1976</td>
<td>Shell</td>
<td>111</td>
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</tr>
<tr>
<td>Dana</td>
<td>Shell</td>
<td>June-1977</td>
<td>CORBS</td>
<td>131</td>
<td>Present study</td>
</tr>
<tr>
<td>Elder</td>
<td>Shell</td>
<td>May-1978</td>
<td>Shell</td>
<td>139</td>
<td>Present study</td>
</tr>
<tr>
<td>Heather</td>
<td>Lundin</td>
<td>May-1977</td>
<td>Shell</td>
<td>164</td>
<td>Present study</td>
</tr>
<tr>
<td>Magna</td>
<td>BP</td>
<td>April-1982</td>
<td>Shell</td>
<td>190</td>
<td>Present study</td>
</tr>
<tr>
<td>North Cormorant</td>
<td>Shell</td>
<td>May-1981</td>
<td>Shell</td>
<td>160</td>
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</tr>
<tr>
<td>NW Hutton</td>
<td>BP</td>
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<td>Shell</td>
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<td>May-1981</td>
<td>Shell</td>
<td>167</td>
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<tr>
<td>Thistle</td>
<td>Lundin</td>
<td>August-1976</td>
<td>Shell</td>
<td>160</td>
<td>Present study</td>
</tr>
</tbody>
</table>

CORBS, concrete gravity based structure; SPM, Single point mooring.
Fig. 2. Domed-shaped orange (shown as darker grey colonies above) and white colonies of *L. pertusa* on the Heather platform (photo courtesy of Lundin Britain).

![Graph A](image)

Fig. 3. The depth distribution of *L. pertusa* on (A) Heather conductors and (B) the North A Wyn Alpha conductors.

![Graph B](image)

found below 60 m depth reflecting temperatures between 7 and 11 °C and salinities between 35 and 35.3 PSU.

3.4. Recruitment and growth rates

Nineteen conductors of varying ages were examined from NAA and five from Heather (Table 2). The number of colonies of sufficient size to be visible on video varied between two and 22 on NAA, and between 98 and 224 on Heather. No coral colonies were visible on two NAA conductors installed in 1995 and 1999. Coral colony
density varied by an order of magnitude (0.02–0.39 m²) between conductors on NAA, and ranged between 1.23 and 2.8 m² on Heather conductors.

The maximum colony diameter varied widely (34%) for the colonies on the NAA conductors and less so for the colonies on the Heather conductors (16%). The size distribution of colonies from the NAA and Heather platforms was unimodal left skewed with several exceedingly large colonies and few small colonies (Fig. 5A and B). The outlying large colonies were re-examined to see whether two or more colonies could have merged to form what might have appeared as one single colony. No obvious visual evidence for this was found as the colonies formed smooth uniform domed-shapes, the largest of which was 132 cm in diameter from the Heather platform (Fig. 6). There was no relationship between colony size and depth.

3.5. Colony growth

Assuming that the largest colony was the first to colonize the conductor, minimum colony growth rates were calculated based on the conductor installation date (Table 2). Results show growth rates between 6 and 26 mm yr⁻¹ from NAA colonies, and between 24 to 33 mm yr⁻¹ for Heather colonies.

Survey inspection videos were provided from the Tern Alpha platform with video surveys from 1993, 1994, 1998 and 2002. Colony extension rates were assessed for fifteen specimens of L. pertusa identified in either 1993 or 1998 and again in 2002 (Table 3). An example of a colony seen in 1993 and then again in 2002 is shown in Fig. 7. Settlement dates estimated by dividing the size of the colonies

![Graph A](image1.png)  
![Graph B](image2.png)  

Fig. 5. Size class distribution of L. pertusa colonies from (A) Heather and (B) NAA.

<table>
<thead>
<tr>
<th>Platform</th>
<th>Conductor no.</th>
<th>Conductor installation date</th>
<th>No. of colonies</th>
<th>Max size (cm) diameter</th>
<th>Max growth rate (mm radial extension)</th>
<th>Density (No. colonies/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>4</td>
<td>1986</td>
<td>5</td>
<td>39</td>
<td>1.2</td>
<td>0.05</td>
</tr>
<tr>
<td>NAA</td>
<td>5</td>
<td>1986</td>
<td>2</td>
<td>38</td>
<td>1.2</td>
<td>0.02</td>
</tr>
<tr>
<td>NAA</td>
<td>7</td>
<td>1986 1987</td>
<td>7</td>
<td>52</td>
<td>1.8</td>
<td>0.07</td>
</tr>
<tr>
<td>NAA</td>
<td>8</td>
<td>1986 1987</td>
<td>14</td>
<td>41</td>
<td>1.4</td>
<td>0.15</td>
</tr>
<tr>
<td>NAA</td>
<td>11</td>
<td>1986 1987</td>
<td>38</td>
<td>44</td>
<td>1.5</td>
<td>0.39</td>
</tr>
<tr>
<td>NAA</td>
<td>14</td>
<td>1986</td>
<td>2</td>
<td>18</td>
<td>0.6</td>
<td>0.02</td>
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<tr>
<td>NAA</td>
<td>16</td>
<td>1986 1987</td>
<td>12</td>
<td>66</td>
<td>2.3</td>
<td>0.12</td>
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<tr>
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<td>22</td>
<td>1986 1987</td>
<td>18</td>
<td>40</td>
<td>1.4</td>
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<tr>
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<td>42</td>
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<tr>
<td>NAA</td>
<td>25</td>
<td>1989</td>
<td>12</td>
<td>51</td>
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<td>0.12</td>
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<tr>
<td>NAA</td>
<td>26</td>
<td>1986</td>
<td>7</td>
<td>77</td>
<td>2.6</td>
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<tr>
<td>NAA</td>
<td>27</td>
<td>1986 1987</td>
<td>14</td>
<td>51</td>
<td>1.8</td>
<td>0.15</td>
</tr>
<tr>
<td>NAA</td>
<td>28</td>
<td>1986</td>
<td>22</td>
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<td>1.7</td>
<td>0.24</td>
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<tr>
<td>NAA</td>
<td>30</td>
<td>1987</td>
<td>16</td>
<td>11</td>
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<td>0.04</td>
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<td>NAA</td>
<td>32</td>
<td>1986 1987</td>
<td>30</td>
<td>54</td>
<td>1.9</td>
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<td>NAA</td>
<td>34</td>
<td>1987</td>
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<td>58</td>
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<tr>
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<td>35</td>
<td>1987</td>
<td>19</td>
<td>46</td>
<td>1.6</td>
<td>0.32</td>
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<tr>
<td>Heather</td>
<td>38</td>
<td>1981</td>
<td>177</td>
<td>118</td>
<td>2.8</td>
<td>2.21</td>
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<td>Heather</td>
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<td>1982</td>
<td>212</td>
<td>103</td>
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<td>1982</td>
<td>221</td>
<td>132</td>
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<td>1982</td>
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<td>Heather</td>
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<td>1988</td>
<td>98</td>
<td>93</td>
<td>3.3</td>
<td>1.23</td>
</tr>
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</table>
Visual signs of contamination were also observed on Heather conductors. These were grouped together 1.5 m apart with the drill cuttings discharge chute located within the conductor group and discharging at 107 m depth. Five conductors, three located directly next to the cuttings chute and two located two conductors away from the chute, were examined from video recorded in 2002. All coral colonies observed on these conductors between 107 m and 122 m (after which no colonies were found) were completely or partially covered in drill cuttings/muds. Colonies located shallower than 107 m appeared clean, except for colonies on the conductor to the southwest where 100% of the colonies examined between 107 m and 85 m showed visual signs of contamination on the lower half of the colonies. This indicates some upward movement of discharges in addition to the downward settlement that produces the large cuttings pile that had accumulated under the platform.

4. Discussion

4.1. Distribution of *L. pertusa* in the North Sea and environmental controls

Before the present study there were two published records of *L. pertusa* on oil and gas infrastructure in the North Sea but it was unclear whether these were exceptional occurrences. The results from the present study revealed *L. pertusa* on 13 more platforms in the northern North Sea. Platforms in the central and southern North Sea were not examined; however, anecdotal observations by sub-sea survey engineers suggest *L. pertusa* is also found in the central North Sea but at lower densities, and not in the southern North Sea. This search was not exhaustive and was limited by survey results supplied by industry. Video quality may have limited identification of small colonies of *L. pertusa* and often platforms are not surveyed annually and only certain components of the platforms are included. In addition, not all companies operating in the northern North Sea participated in the study.

*L. pertusa* shows considerable interspecific variations in growth forms each with different skeletal characteristics (Friedwald et al., 1997). Colonies of *L. pertusa* from the platforms were similar to the *tabular* ecotype that has a characteristic trumpet-like tabular shaped corallites, deep calices and a thin stereome (Friedwald et al., 1997). There were several exceptions where stereome-thickened corallites were observed, which are reported as the typical reef-forming ecotype seen off Norway and are suggested to indicate a possible relationship with the presence of the polychaete *E. norvegicus* (Friedwald et al., 1997). *E. norvegicus* is commonly found associated with *L. pertusa* in the Atlantic Ocean, where its tubes are overgrown by the coral, which strengthens the overall reef framework (Dona, 1944; Wilson, 1979b; Friedwald et al., 1997; Mortensen, 2001; Roberts, 2005) and the polychaete forms an intimate symbiosis with the coral both apparently cleaning the skeleton (Mortensen, 2001).
and aggregating coral fragments (Roberts, 2003). However, to date *E. norvegica* has not been observed with coral colonies from North Sea platforms.

Several environmental controls on the distribution and growth of *L. pertusa* have been proposed, such as its requirement for hard substrata for larval settlement (Freiwald, 2002), a source of oceanic water of the correct temperature and salinity (Freiwald, 2002; Roberts et al., 2003), heightened currents (Frederiksen et al., 1992; Freiwald, 2002), nutrient-rich water for a reliable source of food (Frederiksen et al., 1992), and a suggested link with hydrocarbon seepage (Howland et al., 1997), a hypothesis that remains controversial (Rogers, 1999). Although the northern North Sea satisfies the majority of these criteria, the surficial geology in the North Sea consists of mud, sand and some coarse sand and gravel (Eisma, 1987) and so lacks hard substrata required for settlement. It was not until the installation of the oil and gas platforms in the 1970s that hard substrata became readily available and this may explain the lack of records of *L. pertusa* in the North Sea prior to the development of the North Sea oil industry.

4.2. Hydrographical controls on coral distribution

Temperature exerts a strong control on the distribution of *L. pertusa* (Doms, 1944; Freiwald, 2002; Roberts et al., 2003); however the origin of seawater has been suggested to be of higher significance (Frederiksen et al., 1992). Seawater in the northern North Sea is of suitable oceanic origin (north-east Atlantic) and enters from the north flowing south to the central North Sea (Turrell, 1992a). An upper limit (60 m) on the vertical distribution of *L. pertusa* was observed on the NAA and Heath platforms that coincides with the well documented summer stratification in the northern North Sea (Turrell, 1992b). Oceanic seawater in the northern North Sea is altered as spring and summer conditions warm surface waters, creating a distinct thermocline at approximately 50 m water depth. Roberts (2002) made the same interpretation for the vertical distribution of *L. pertusa* in the Beryl field in the northern North Sea, and Doms (1944) reported that the minimum depth of occurrence for corals off Norway was limited by the thermocline.

In the summer, surface waters in the North Sea can reach between 13 and 16 °C (BODC data this study, Otto et al., 1999; OSPAR, 2000). These temperatures are not only above the known range for *L. pertusa* of 12 °C (Freiwald, 2002), but they show up to an 8 °C fluctuation between summer and winter temperatures. *L. pertusa* is most commonly found at depths greater than 200 m where it will only experience changes in temperature of one or two degrees. The vertical force of wave action may be of equal importance in the vertical distribution of *L. pertusa* on the platforms, as it has been suggested for corals in the north-east Atlantic (Frederiksen et al., 1992). Increased turbulence from wave action in the northern North Sea may dislodge recently recrutaried corals in the upper depths of the North Sea most affected by wave action.

The BODC data showed salinity levels varying between 35.1 and 35.3 PSU, increasing with depth as less dense water6 gathers near the surface. Salinities around 33 PSU are suitable for *L. pertusa*, and thus salinity is unlikely to act as a single controlling factor of depth distribution of the *L. pertusa* in the northern North Sea.

4.3. Colony size and recruitment

The colony size distribution from both Heath and NAA shows a continuous distribution of size classes with few exceedingly large colonies and few very small colonies. The lack of small colonies probably corresponds to the difficulty of seeing such colonies on the survey video. *L. pertusa* was not seen on two NAA conductors that had been installed in 1995 and 1999 and it is unlikely that colonies younger than six or seven years can be seen on video. The latest colonization reported from the Tern Alpha coral colony growth results was also 1995.

Large colonies probably represent the first coral recruits to the platforms. Other large colonies could have already become detached from the platform for two main reasons:
the physical characteristics of the platforms, i.e. the rate of decay of paint coatings and corrosion of the steel platforms and (2) colony size and weight relative to the strength of the colony attachment point(s). The paint on the platform will eventually detach and the steel will begin to corrode making the colony's attachment point unstable. If colonies have started surpassing the maximum size and weight that the attachment point(s) can support or the paint has eroded, then the remaining large colonies represent those with the strongest attachment point(s) and/or those attached to the best preserved parts of the conductors. A large detached colony is seen in a still photograph lying on top of the Heather cuttings pile provides some evidence to support this theory. However, this does not explain the sparse large colonies on the NAA platform, which are smaller than those on the Heather platform. It is also unlikely that corals have been physically removed by cleaning operations to remove marine fouling as this is not routinely practiced on either NAA (Personal communication Neil Duncan, Total) or Heather (Personal communication Aly Lyon, Lundin Britain Ltd.).

A second explanation for the lower number of large colonies relates to the likely northeast Atlantic origin of the early recruits to the platforms. In comparison with shallow-water corals, little is known about the reproductive biology of L. pertusa and no larvae have ever been sampled. However, these occurrences in the North Sea provide evidence for a pelagic larval stage (Roberts, 2002). As there was no evidence for living L. pertusa in the North Sea before the development of the offshore oil and gas industry, the recruits to the North Sea platforms have probably come from larvae transported in the North Atlantic water mass entering the North Sea. The possible pathway for coral larvae to reach the northern North Sea is through the substantial inflow of Atlantic water flowing southwards east of Shetland from the Atlantic shelf edge current and the Fair Isle Current (Roberts, 2002). These currents and circulation patterns have also been used to explain the dispersal of fish larvae such as Atlantic herring (Clupea harengus) that hatch from spawning grounds to the north and west of Scotland (Heath, 1998) and must cross the northern North Sea to reach nursery grounds in the Skagerrak and southern North Sea (Bartsch et al., 1989).

Populations of L. pertusa are found west of Scotland (Roberts et al., 2003) and could be source populations for recruits to the North Sea. It is possible that the largest colonies seen on the North Sea platforms were early recruits from west of Scotland but only a small percentage of larvae remained competent to settle on North Sea platforms. As more corals colonized each platform and became reproductive, it is possible that these colonies began to self-recruit to the platforms resulting in a steady input of recruits as seen in the colony size distributions. This is supported by histological studies that showed two reproductive L. pertusa colonies, one male and one female, among samples taken from the North West Hutton platform in the northern North Sea in December 2003 (R. Walser and S. Gass, unpublished data).

Heather had significantly higher densities and larger colonies compared to NAA. Heather conductors are painted but not with anti-fouling paint. The NAA conductors are painted and according to the Total Alwyn Area ROV Inspection Manual some older conductors have additional unspecified coatings, possibly with anti-fouling properties. The differing paint properties could influence where L. pertusa is first likely to settle on a conductor, thus explaining the size difference and the variation observed within NAA conductors themselves, and the difference between colony size and density on Heather and NAA.

Colony size and density differences between NAA and Heather will partly reflect the installation of Heath conductors three and four years before NAA conductors, and that the Heath platform was installed eight years before NAA. Moreover, the location of the Heath platform may provide a higher chance of larval encounter and settlement. The major currents entering the North Sea are from the northwest, north of Shetland, and travel southwards into the central North Sea (Turrell, 1992b). Within the group of oil and gas platforms in the northern North Sea, Heather is located the furthest to the west and NAA to the southeast with a number of platforms in between the two and to the north of NAA. Coral larvae from the North Atlantic will have had further travel and more opportunities to settle on suitable substrata prior to reaching NAA as compared to Heather. The situation is similar to a study that examined fouling on the Montrose and Forties platforms in the central North Sea, and reported significantly higher concentrations of Mytilus edulis on Forties as compared to Montrose (Forteth et al., 1982). It was suggested that, although similar currents carry M. edulis larvae pass these two platforms, the length of time it takes to reach the platforms and the three to four week life span of the larvae may explain the lack of M. edulis on Montrose, which is located approximately 40 km southeast of Forties (Forteth et al., 1982).

4.4. Growth rates

In situ growth rates of L. pertusa have not been previously examined because of the inherent difficulties associated with studying these animals at great depths in their natural habitats. Our visual records of 15 colonies throughout time on Tern Alpha provide the first in situ L. pertusa growth rates and revealed annual recruitment events on the platforms. Other growth estimates of between 5 and 26 mm yr⁻¹ have been made based on colonization of artificial substrata such as submarine cables (Duncan, 1877; Wilson, 1979b), ship wrecks (Roberts et al., 2003) as well as the Brent Spar oil storage buoy (Bell and Smith, 1999) and the Beryl single point mooring (Roberts, 2002). Since the colonization events must have happened after the hard substrata was introduced, the maximum time that coral growth could have taken place is known. Additional
growth rate estimates for *L. pertusa* are based on skeletal carbon and oxygen stable isotopes and vary from 5 mm yr⁻¹ (Mortensen and Rapp, 1988) to 25 mm yr⁻¹ (Mikkelsen et al., 1987; Freiwald et al., 1997; Sprio et al., 2000), and fall within the similar range of growth estimates from the artificial substrata.

Our growth estimates of 26 mm yr⁻¹ from the Tern Alpha growth study and up to 33 mm yr⁻¹ from the largest colonies on the Heather platform fall slightly above these estimates but are similar to the rate suggested by Bell and Smith (1999) based on colonies from Brent Spar in the northern North Sea. Linear extension rates reported by Roberts (2002) of 5 mm yr⁻¹ from the Beryl single point mooring in the North Sea were based on a sample taken from the structure that was not selected to represent the largest colony on the structure and could have settled well after the mooring had been installed. Thus, higher growth rates reported from the North Sea may relate to colonies living higher in the water column above any deleterious effects of sediment resuspension and where more surface organic primary production will be available. Food supply may be further increased in the North Sea because in addition to a spring algal bloom, wind stress occurring in the autumn initiates vertical mixing bringing nutrients to the surface generating a second algal bloom. There was no visual evidence that colonies used in this growth study had been exposed to drill muds or cuttings. It is not known whether growth rates of colonies living in the vicinity of drilling discharge points are affected by the increased sediment and possible toxicological stress.

4.5. Contamination by drill muds and cuttings

Concerns over the deleterious human impacts on *L. pertusa* have to date concentrated on the impacts from bottom fishing (Dons, 1944; Fosså et al., 2002; Hall- Spencer et al., 2002). However, concerns about the impacts from petroleum exploitation on cold-water corals, including *L. pertusa*, have also been raised (Cimberg et al., 1981; Rogers, 1999; Butler and Coss, 2003). A major concern relating to oil and gas exploitation is the increased levels of sediment in the marine environment caused either by physical disturbances from anchoring, laying pipelines and oil rig emplacement and/or from drill muds and cuttings discharged close to a coral reef (Cimberg et al., 1981; Dodge and Smolntz-Foelich, 1985). Thus, it is surprising to find numerous colonies of *L. pertusa* growing directly on oil and gas platforms in the North Sea. However, other scleractinian coral species have been reported on oil and gas platforms in the Gulf of Mexico (Sammarco et al., 2004).

*L. pertusa* appears to be able to grow on platforms in areas above and away from muds and cuttings discharge pipes. However, evidence of contamination and polyp mortality were observed for colonies living in the vicinity immediately above and below such discharge points. It also appears that *L. pertusa* colonies are able to partially exist in these environments where polyps on the tops or bottoms of colonies are fully expanded while the other half of the colony is dead. It is likely that the lethal effects observed are a consequence of physical smothering by drill muds and cuttings, but it remains unknown if and to what degree coral colonies were affected by the toxicity of hydrocarbons, metals, or other potentially toxic additives found in drill muds.

There are many factors affecting the fate of drill muds discharged into the marine environment. In the case of the contaminated colonies observed on Heather, it is the depth of the discharge pipe that dictates which colonies will be most affected. Other contributing factors will include the speed and direction of currents at time of discharge, wave regime, eddies caused by water flow around the platform itself, depth of the thermocline, rate of discharge, and duration of discharge (Thompson et al., 1989). The concentration of drill muds at varying distances from the source of discharge with relation to the potential effects on tropical corals in the Gulf of Mexico showed the maximum dilution rate to occur within 6 m of the discharge source after which the dilution factor decreases slowly with increasing distance from the platform (Shinn et al., 1989). If a similar magnitude of dilution is occurring in the North Sea this could explain the heightened contamination observed within close proximity to the discharge source.

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Appendix 2 – Sampling Protocol

1. CHOOSING THE CORAL SAMPLING LOCATIONS

The goal of the project is to compare corals which have been exposed to drilling fluids/muds and/or cuttings and those that haven’t. Note that Lophelia coral almost never seen shallower than 60m.

We want corals from two separate locations:

1. Corals which have been exposed to drilling fluids and/or cuttings such as:
   a. Corals where muds/cuttings are visible on coral colonies
   b. Coral colonies which are close to the cuttings pile (located as close as possible to cuttings pile)
   c. Corals which are close to and downstream from discharge pipes

2. Corals which have not been exposed to any discharges (these will be the control corals) such as:
   a. Corals on non-drilling structures such as buoys or moorings
   b. Corals located far from cuttings pile
   c. Corals located far from and upstream of discharge pipes
2. SAMPLING PROCEDURE

I leave it up to your expertise to decide the best way of using the ROV to sample the coral. Below is an example of a net and scraper which has been used in the past for similar work. The scraper has been bent to the shape of the riser. The scraper is used to scrape the coral colony off the riser into the net and is brought to the surface.

I am requesting 2-4 coral colonies from each control and exposed site. 2 white and 2 orange ones. Whether you get 2 or 4 depends on the size of the colonies and the time available for the sampling. If the colonies are large and there is not a lot of time then 1 white and 1 orange from each site would suffice.

I’m requesting that the sampling be video recorded so I can see what the coral colonies looked like before they were taken.

Once you’ve chosen which corals you’re going to take note down the following details that will subsequently be used for labelling the corals when they are brought on board:

1. Platform name
2. Date
3. Approximate Time the corals were taken off the platform
4. Depth where the corals were taken from
5. Component number that the corals were taken from
6. Video tape number with recorded sampling of corals
7. Whether the corals are believed to be exposed to drilling muds and/or cuttings or whether they were the control corals which have not been exposed.
3. What to do with corals once they’ve been brought on board

For each coral that is brought to the surface please follow the directions below:

It is likely that some corals will break into a number of pieces as a result of sampling. If the coral comes on board in one piece break off appropriate sized pieces using the sizes of the buckets supplied as a reference.

1. The largest piece is to be kept alive in the coral hotel:
   a. Fill in the details on the live coral label with the marker provided
   b. Attach the label to the live coral around a branch where it will not come off.
   c. Place coral into one of the baskets of appropriate size in the coral hotel.

2. The next largest piece can be frozen:
   a. Find a white bucket of appropriate size labeled “Corals to be frozen”
   b. Label the bucket with appropriate details with marker provided
   c. Seal coral inside bucket
   d. Place the bucket in the freezer.

3. A relatively small piece can be put into alcohol:
   a. Find small buckets filled with alcohol
   b. Label bucket with appropriate details with marker provided
   c. Place coral into the bucket and tightly seal lid
   d. Replace bucket in safe location

4. A relatively small piece can be placed in formalin:
   a. Using gloves and safety glasses provided follow the same instructions as for alcohol (see 3. above)

*Note: corals piece put in alcohol and formalin should be small enough to be completely covered by these liquids
4. MONITOR CORAL HOTEL

Once all the corals have been collected the only thing left to do is to monitor the water temperature in the coral hotel. It should be steady around 8 °C. Please fill in the daily temperature in the chart provided located with the coral hotel.

It is important not to overload the baskets in the coral hotel. One piece of coral should be placed in the smaller baskets and up to 2 pieces in the larger baskets.

**It is very important that every piece of coral placed in the hotel has a label so we know where it came from.

Thanks so much for your assistance and have a good survey!

Feel free to contact me anytime:

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