Cruise report for Lophelia 2015

Operating authority:
Sven Lovén Centre for Marine Sciences, Tjärnö, University of Gothenburg, Sweden

Owner: University of Gothenburg, Sweden

Name of master: Roger Johansson

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Activity during 2015

May 5'th: Retrieval of deployed current meter using ROV

Scientific Publications stemming from the activities

Strömberg SM (2016) Early life history of the cold-water coral *Lophelia pertusa* –with implications for dispersal. Ph.D. thesis at the University of Gothenburg

The publication is attached

Thesis for the Degree of Doctor of Philosophy

EARLY LIFE HISTORY OF -WATER CORAL

Lophelia pertusa

– WITH

Susanna M Strömberg

2016

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EPARTMENT OF MARINE SCIENCES

Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap med inriktning

Fakultetsopponent: Associate Professor Rhian G. Waller,

Early Life History of the cold

BSTRACT

OPULÄRVETENSKAPLIG S

vi nu är på väg mot samma kemiska sammansättning i haven som tidigare har lett till

massutdöenden.

ELATED WORK

LIST OF PAPERS

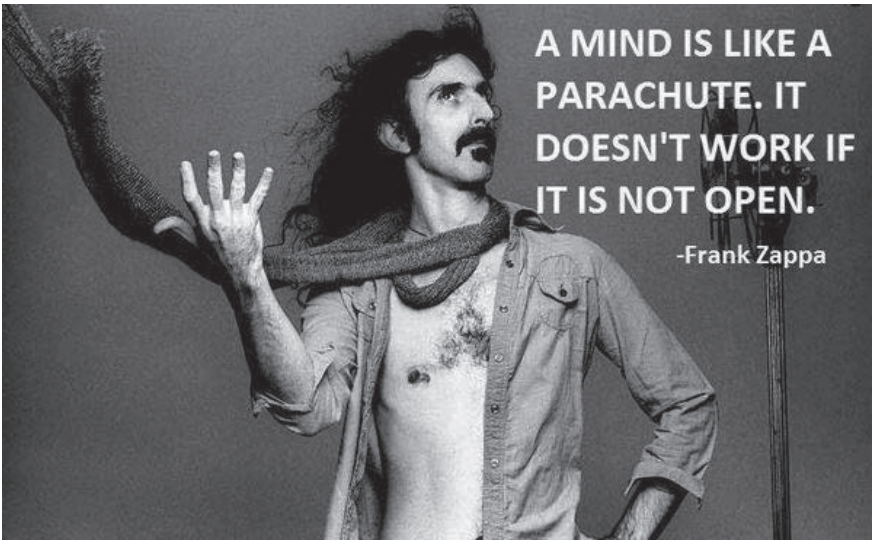
This thesis is a summary of the following papers:

PAPER I. Larsson, A. I., Järnegren, J., Strömberg, S. M., Dahl, M. P., Lundälv, T. and Brooke, S. 2014. Embryogenesis and larval biology of the cold-water coral *Lophelia pertusa*. – *PLoS One* **9:** e102222.

PAPER II. Strömberg, S. M., Östman, C. 2016. The cnidome and internal morphology of *Lophelia pertusa* (Linnaeus, 1758) (Cnidaria, Anthozoa). – *Acta Zoologica* In press

PAPER III. Strömberg, S. M., Östman, C., Larsson, A. I. The cnidome and external morphology of late planulae in *Lophelia pertusa* (Linneaus, 1758) – with implications for settling competency. Manuscript

PAPER IV. Strömberg, S. M., Larsson, A. I. Larval behavior and longevity in the cold-water coral *Lophelia pertusa* indicate potential for long distance dispersal. Manuscript


A MIND IS LIKE A PARACHUTE. IT DOESN'T WORK IF IT IS NOT OPEN.
-Frank Zappa

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INTRODUCTION

Thinking of corals brings to mind colorful tropical corals in luscious reefs, conveniently situated at diving depth. But surprisingly, over half of the approximately 5100 known coral species lives in the deep (Roberts *et al*. 2009). Deep-sea, or cold-water corals, span from the Barents Sea in the Arctic to the Antarctic shelf, from 40 m depth in areas where deep oceanic water is forced up to shallower depths by bottom topography and currents, while the deepest known coral species is found below 6000 m. There are solitary species and species capable of building continuous reefs covering areas up to 100 km^2. Most deep-sea corals thrive at temperatures of 4–13°C, while some corals in the Polar Regions tolerate near-zero, or even periods of sub-zero temperatures (Bett 2000; Freiwald *et al.* 2004; Waller and Feehan 2013).

The main difference between tropical and deep-sea corals is that the tropical corals rely to a large extent on photosynthesizing symbionts (zooxanthellae) for nutrients, while deep-sea corals are predators and opportunistic heterotrophs, i.e. feeding foremost on zooplankton, but also on particulate organic matter, pico- and nanoplankton (Houlbrèque *et al.* 2004; Dodds *et al* 2009; Mueller *et al*. 2014; Naumann *et al*. 2015). And while the corals themselves contribute to a large amount of the biodiversity in tropical reefs, by the assemblage of many different coral species, deep-sea coral reefs usually is made up by only one, or a few species. Nonetheless, deep-sea coral reefs are diversity hot spots, fully comparable to, or even exceeding, tropical reefs in species richness (Jensen and Frederiksen 1992; Freiwald *et al.* 2004).

A brief history on deep-sea scleractinian research

The very first to take interest in cold-water corals were the Danish author, bishop, and historian Erik Pontoppidan (1698–1764) during his years as Bishop of Bergen in 1747–1754. Chapter 6 of his book *The natural History of Norway* (1755) was dedicated to "Sea-vegetables", and he described a specimen of what three years later would be described by Carl von Linné as *Madrepora pertusa* (syn. *Lophelia pertusa*) with the words: "*entirely white, the flowers much larger than the former, some of them even exceeding a shilling; and likewise expanded like a flower in full bloom, for which singular beauty I caused a draught of it to be taken*" (Roberts *et al*. 2009). The confusion on what kingdom corals belong to was understandable, whether plants or animals. Aristotle (384–322 BC), the great Greek philosopher and father of natural philosophy, classified corals as zoophyta (animal-plants) recognizing their dual characteristics in his *Scala Naturae*. Not until the eighteenth century, with the help of the microscope, William Herschel, a British astronomer and constructor of telescopes, established that coral cells had the characteristics of animal cells (Wikipedia). About this time, the "father of biology", Abraham Trembley (1710–1784) had made the entire Europe perplexed with his and the Bentinck boy's descriptions of another cnidarian, the tiny pond creature, *Hydra*, a freshwater cnidarian polyp that intrigued by its confusing attributes of both plant and animal, and capacity for regeneration. The enigmatic *Hydra* was first recognized as a plant, due to its green color derived from their photosynthesizing symbionts, but then it was observed "walking". Trembley's nephew Bennet wrote in an excited letter: "*One can say that you have discovered the point of passage from the Vegetable to the Animal*" (Stott 2012).

Deep-sea scleractinian coral taxonomy has mostly been based on skeletal characteristics. Much of the sampled materials came from dredging expeditions and

soft tissues were probably not in a condition for description. The first to take interest in the biology, rather than skeleton morphology, were the British naturalist Philip Henry Gosse (1810–1888). Gosse was also the one bringing the deep sea to the public, by equipping London Zoo with the first aquarium in 1853, and at this point coined the term "aquarium". Gosse provided detailed descriptions of the soft tissues, for instance in his generously illustrated work *The British sea anemones and corals* (1860). Cairns (2001) has provided a historic overview of the taxonomic research. Some of the earliest scleractinian taxonomists worth mentioning are Henri Milne Edwards, Jules Haime, Louis François de Pourtalès, and later Helmut Zibrowius. Stephen Cairns himself has made great contributions to scleractinian taxonomy.

Beginning in 1868, a series of deep-sea dredging expeditions went out to explore the deep sea to challenge the British naturalist Edward Forbes' "azoic theory". Forbes' had broad interests covering geology, paleontology, and marine biology, and he believed that below 600 m depth (300 fathoms) the environmental conditions were not suitable for animal life, and the deep seafloor was expected to be a barren landscape. However, the Scotsman Charles Wyville Thomson, natural historian and marine zoologist, had seen what Michael Sars (theologian and biologist) had recovered in his dredge hauls in Norway from even greater depths. Thomson led the first expedition on the *HMS Lightning* covering the waters between the Faeroe Islands and Scotland, followed by expeditions with *HMS Porcupine* (1869), and the first great global expedition with the *HMS Challenger* during the years 1872–1876 (Roberts *et al*. 2009). The *HMS Challenger* journey covered 68000 nautical miles (c. 126 thousand km) and sounded the depth of a sampling station in the Mariana Trench to 8184 m (4475 fathoms), very close to the now deepest known place in Earth's seabed of almost 11000 m. Thomson found life, covering all marine invertebrate groups, as deep as 1200 m (650 fathoms), and thereby proved Forbes wrong.

Since then great technical progress has been made, with sonar and multibeam echosounders for seabed mapping, manned submarines, and smaller unmanned submersibles—so called remotely operated vehicles (ROV) connected to the vessel with cables—and autonomous underwater vehicles (AUV) programmed onboard to follow a specific route and return to the vessel or surface after completed mission. These technological advances has made deep-sea habitat mapping and monitoring possible, and has revealed diverse habitat types and life forms in the oceans deepest abysses proving Forbes wrong many times over (Roberts *et al.* 2009).

In the Skagerrak area where the present work was conducted, Tomas Lundälv and Lisbeth Jonsson started mapping the cold-water coral reefs and other deep habitats, especially in the Koster-Hvaler area, beginning in the late 1990's. First with rented ROVs, and later with ROV's purchased for the Gothenburg University field station at Tjärnö, using the dedicated research vessel R/V Lophelia for their surveys. The Norwegian Trench and the bathymetry of the Koster-Hvaler area funnels deep oceanic water from the Atlantic to relatively shallow depths in the Koster-Hvaler archipelagos, creating suitable conditions for deep-sea fauna (Wisshak *et al*. 2005), resulting in a uniquely accessible area for deep-sea research. The presence of the University of Gothenburg's field station on Tjärnö, adjacent to the Koster Fjord, and the ROV surveys has lead to that a 450-km^2 area was made the first marine National Park in Sweden in 2009. An equal sized area in Hvaler, on the Norwegian side, was simultaneously made a marine National Park in Norway.

Ecological importance of cold-water coral reefs

Reef building deep-sea scleractinian corals are constructing three-dimensional frameworks, creating habitats for other organisms. They change the environment they inhabit by their own growth, modulating the availability of resources. Their three-dimensional skeleton matrix slows down near-bed currents and induces the precipitation of particles ("marine snow", i.e. particulate organic material, POM) and pelagic larvae within the matrix, thus at the same time enhancing nutrient availability and concentrating life to the reef habitat. This kind of influence on the environment is defined as "autogenic engineering" (Lawton and Jones 1995). Most of the associated fauna found within cold-water coral reefs are the same fauna found in the geographic area in general, although highly concentrated. There are a few exceptions of specialized inhabitants that are found solely in reef habitats, sometimes in species-specific symbiotic associations (Jensen and Fredriksen 1992). Examples of organisms found almost uniquely in for example *Lophelia pertusa* reefs are the large polychaete *Eunice norvegica,* the squat lobster *Munidopsis serricornis*, and the hemichordate *Rhabdopleura normani*.

This habitat engineering quality of some of the deep-sea scleractinians is making them profoundly important for the associated fauna, and for the ecosystem functioning at a scale we do not fully comprehend yet. They have been suggested to be important nursing or feeding grounds for some commercially important fishes, such as rockfish (*Sebastes* sp.), cod (*Gadhus morhua*), and pollock (*Polachius virens*). Costello *et al.* (2005) found that 80% of individual fishes and 92% of species were associated to *Lophelia pertusa* reef habitat. The reef habitat commonly attract abundant zooplankton that hide amongst the coral branches, besides the many polychaetes and other invertebrate fauna accumulating within the coral matrix. Many of which are recovered in the stomach contents of associated fishes.

Threats

Natural resource extractions

This important ecosystem service that deep-sea scleractinians are providing us is threatened in several ways. The most devastating and direct destruction is caused by deep-sea trawling; in heavily trawled areas large reefs can soon be completely leveled to the seafloor, leaving unconsolidated coral rubble with no three-dimensional structure left intact and no recovery observed even decades later (Althaus *et al*. 2009). Up to 95–98% reduction of coral cover has been observed in some trawled areas, leading to a subsequent depletion of the targeted fish stocks (Gianni 2004). Norse and Watling (1999) compared the impact of mobile fishing gear with forest clear-cutting, and alerted to the potential harm trawling could do to biodiversity. In Norway, long-line and gillnet fishermen contacted the Institute of Marine Research (IMR) in the 1990s, due to their concerns of the impacts that trawling fisheries seemed to cause to their fishing grounds (Fosså 2002). The long-line and gillnet fishermen commonly target coral reef areas due to the rich catches they usually get there. When these fishing grounds also had been targeted for trawling, catches went down. A series of ROV surveys conducted during 1998–1999 (Fosså 2002) in response to these

concerns revealed that the coral cover had been reduced by 30–50% of the estimated original total cover, with some of the reef sites inspected completely leveled.

The importance of three-dimensional structures were evident when a revisit at a reef site at Väderöarna, in the very southern part of the Koster Fjord, suddenly displayed dense mats of very young coral colonies, or single polyp juveniles, seemingly recently settled (Tomas Lundälv and Lisbeth Jonsson, personal comments). This reef site had been observed to no longer host any live corals during previous visits. The newly settled corals had used old lumps of dead coral skeleton as substrate. This is in contrast to other old reef sites in the area that consist of unconsolidated coral rubble with no three-dimensional structures left, and where no signs of new recruitment and recovery have been observed. Samples for DNA analysis to investigate the origin of these newly settled have been taken, but a full analysis is not yet done.

Even if not the entire reef is leveled, partial destruction of a reef could seriously hamper the reproductive success. Since *L. pertusa* and most other deep-sea scleractinians are gonochoric (separate sexes, see Waller 2005), one colony usually is the result of one settled planula and therefore is of one sex. The density and distribution of colonies of different sexes are crucial for reproductive success, and furthermore, not all parental crosses are compatible (Baums *et al*. 2013), thus the unfortunate destruction of specific coral colonies during a sweep with a trawl could easily leave the reef reproductively dead.

Other threats to these ecosystems are the extraction of oil and gas (Fosså 2002; Gianni 2004; Davies *et al.* 2007). The installations of offshore platforms for oil and gas extraction has ambiguous effects on cold-water corals, on one hand, platforms usually harbor healthy colonies of both *L. pertusa* and other fauna; however, colonies found close to the discharge chutes have been observed to have discoloration and 30% polyp mortality (Gass and Roberts 2006). Bakke *et al*. (2013) did a review on the present knowledge of potential effects of operational discharge from oil and gas platforms. There is evidence of fish being attracted to the structures, as well as negative physiological effects in fish sampled in areas with extensive production.

Besides the direct effects of increased sedimentation load by the released drill cuttings, and possible direct toxicity of petroleum products, there are also endocrine disruptive components in the discharge products. This has the potential of affecting reproduction and growth of exposed fauna. Fish are mobile and might be able to avoid the highest concentrations of discharge fluids, in contrast, the sessile invertebrate fauna has little chance of avoidance. The endocrine disruptive components of drill discharge have the potential to disturb coral reproduction since gametogenesis and reproduction is controlled by estradiol and testosterone, just as in vertebrates (Atkinson and Atkinson 1992; Twan et al. 2006). In a study by Negri and Heyward (2000) fertilization success was observed to decrease by exposure to petroleum products, especially crude oil in combination with dispersant chemicals. The most sensitive stage was metamorphosis—the transition from larva to juvenile—that was almost completely inhibited by the crude oil and dispersant combination. These effects were probably caused by other than endocrine disruptive properties of the dispersant chemicals, and no direct studies of reproductive effects on coral populations in the vicinity of platforms has been made.

Studies of the direct smothering or toxic effects of discharged sediments from oil extraction have been undertaken. For example, Larsson and Purser (2011) found partial tissue loss in *L. pertusa* exposed to realistic sedimentation loads of drill cuttings, but with an overall efficiency of sediment shedding and only slight decrease

of calcification rates. While normal operational running of platforms may have limited negative effects on surrounding fauna —with the reservation of disturbances in hormone regulation and reproduction that is not yet fully investigated—there is always the risk of a blowout. After the Deepwater Horizon disastrous oil spill in the Gulf of Mexico in 2010, affected coral communities was observed up to 11 km from the site, with tissue loss, and other stress responses in corals in the visual consensus made by White *et al.* (2012).

There is also an emerging threat of deep-sea mining that has caused researchers to lift a warning finger, and stress the importance of assessing the potential impacts on benthic fauna (Boschen *et al*. 2013). Deep-sea mining has been an issue since the1970's, with a patent for an apparatus for harvesting mineral nodules filed already in 1977 (Diggs 1977). Many more patents have been registered since, but falling prizes of minerals has delayed harvesting (Glasby 2000), and deep-sea researchers such as Van Dover (2011) are calling out for tight regulations before any large-scale harvesting projects are launched.

Ocean acidification

While the above anthropogenic impacts are fairly localized and easy to address through management and regulations—and an increasing amount of marine protected areas are established—the increasing carbon dioxide concentration in the atmosphere with decreasing ocean pH as a consequence, i.e. ocean acidification, is a far more challenging threat.

Several studies have been done examining the effects of ocean acidification on different marine organisms, including scleractinian corals. There have been variable results considering the sensitivity of coral calcification rates to ocean acidification. The study of Maier *et al.* (2009) found a positive net calcification in corals exposed to predicted future values of oceanic pH and aragonite saturation state (Ω_a), although there was a 40–59% reduction. The fastest growing apical polyps showed the largest reduction. Hennige *et al.* (2014) found decreasing respiration rates with maintained calcification rates in response to lowered pH correlating to a CO_2 partial pressure of 750 ppm, and this maintenance of calcification was attributed to the utilization of lipid reserves. The experiment was running for 21 days and was performed on freshly collected coral material, and thus the corals may not have depleted their energy reserves during the experimental period. A study by Tambutté *et al.* (2015) revealed that the skeleton of corals exposed to acidified conditions were more porous than the skeletons in control conditions. Linear growth apparently does not reveal the full story considering calcification rates in corals.

Calcification is a blunt instrument for measuring the effects of ocean acidification, and a study by Kaniewska *et al*. (2012) on the tropical coral *Acropora millepora* revealed that before effects on calcification were observed, there were changes in gene expression consistent with metabolic suppression, evidence of oxidative stress, and upregulation of genes involved in cytoskeletal remodeling. There was a down-regulation of genes involved in the mitochondrial electron transport chain, indicating a reduced capacity of generating ATP and NADPH. A similar study was done on the cold-water coral *Desmophyllum dianthus* by Carreiro-Silva *et al*. (2014), in which they found no significant differences in calcification or respiration. Contrary to this, they found significant effects in gene expression, with upregulation of genes involved in stress responses and immune defense. The corals also

upregulated genes involved in skeleton synthesis; apparently compensating for the less favorable conditions for calcification. It is obvious that calcification rates are kept up to a cost, and that this is depending on ample food availability.

While there is evidence for some ability of corals to compensate for acidified conditions (Form and Riebesell 2011; McCulloch *et al.* 2012; Carreiro-Silva *et al*. 2014), this is usually tested on pieces of coral with a full cover of live tissue on the skeleton. The calcifying compartments are isolated from the surrounding water and their environment is strictly regulated with alkalinity upregulated through ion pumping to create conditions favorable for calcification. The coral itself provides the nucleation sites for aragonite crystallization through an organic matrix laid down within the calcifying compartment, and through proton and calcium ion pumping the pH is elevated to allow for spontaneous precipitation of crystals (Allemand *et al.* 2004). This gives corals some leverage to counteract acidification. It is the naked skeleton of the dead parts at the base of, and in the center of the reefs, that are in more immediate danger, and there is a risk of reef collapse and loss of three-dimensional complexity. There have even been observations of ability of corals to survive complete decalcification as soft-bodied polyps that after return to normal conditions picked up on calcification and rebuilt colonies (Fine and Tchernov 2007). As soft-bodied polyps they are, however, vulnerable to predation and does not provide the large three-dimensional habitat for other organisms to utilize. The problems with acidification might therefore lie more in the ecosystem services coral reefs provide for other organisms, rather than a threat of extinction of the corals themselves. There is, however, emerging evidence that the early life history of corals is more sensitive. For example, a study of Albright *et al.* (2010) has shown that ocean acidification compromised fertilization success and thereby reduced larval availability in the studied species, *Acropora palmata*. Furthermore, ocean acidification reduced settling success and postsettlement growth.

In a study by Morabito *et al*. (2013) the effect of lower pH on cnida discharge capability in the jellyfish *Pelagia noctiluca* was tested, and low pH was observed to reduce the discharge response. The tested pH values were 6.5 and 4.5 (control pH 7.65), and thus lower than what is ecologically relevant in comparison to plausible scenarios of ocean acidification. Nonetheless, it is a very important aspect to investigate. If pH values within the plausible ranges for future ocean acidification could hamper cnidae discharge, it could hamper coral feeding, and thus the ability of the corals to compensate for the increased energy demand for maintaining calcification rates in a more acidic environment.

The second aspect of climate change due to rising carbon dioxide is the ongoing warming of the oceans. Embryo and larval development rates are temperature dependent, i.e. in cold waters development is slow, while a rise in temperature speeds up development. A rise in the temperature of the oceans will reduce pelagic larval durations (PLD), resulting in shorter dispersal distances (Hoegh-Guldberg and Pearse 1995; O'Connor *et al.* 2007). So, not only do the environmental conditions deteriorate due to ocean acidification, it will also be harder for most larvae to disperse long enough distances to escape into more favorable conditions.

The full consequences of ocean acidification are difficult to anticipate, and any recovery back to the pre-industrial baseline will take centuries or millennia, if at all possible. These are large-scale processes and once the wheels have turned there is no turning back, just the slow route of change, working at geological timescales.

A historic perspective of ocean acidification

The potential problem with ocean acidification is not a novel insight to the research community; already in 1951 William W. Rubey stated in his *Geologic history of seawater—An attempt to state the problem*, and I quote: "*Carbon plays a significant part in the chemistry of sea water and in the realm of living matter. The amount now buried as carbonates and organic carbon in sedimentary rocks is about 600 times as great as that in today's atmosphere, hydrosphere, and biosphere. If only 1/100 of this buried carbon were suddenly added to the present atmosphere and ocean, many species of marine organisms would probably be exterminated*".

Rubey referred back to a range of studies made on the physiological effects of different levels of pH that had been undertaken. Gattuso and Hansson (2011) have synthesized the history of ocean acidification in their book *Ocean acidification: background and history,* most certainly hair-rising reading for those who dare.

Another notable early alarm signal is from an educational documentary produced in 1958 by Frank Capra for Bells Labs called *Unchained Goddess* (available on YouTube at https://youtu.be/0lgzz-L7GFg), in which Richard Carlson discussed the potential effects of human induced increase of atmospheric carbon dioxide concentrations: global warming, melting polar ice caps, and sea level rise, were all mentioned in the video. Despite the early rise of awareness of the potential problem, the inertia of the human population to respond to these warnings has led to that we now, in the eleventh-hour, stand at the verge of the tipping-point when our last-minute attempts to mitigate our influence on the planet just might not make the difference we need. The only thing exceeding the inertia of the human population is the inertia of the planet itself, to our demise—a painful proof of Newton's first law.

Distribution and physical environment

The distribution of deep-sea scleractinian corals (fig. 1) is largely explained by four environmental variables: temperature, aragonite saturation, depth, and salinity (Davies and Guinotte 2011). They are restricted to oceanic conditions (4–12°C, 34–36 psu) and topographic highs with enhanced current velocities on continental slopes or seamounts (Freiwald *et al*. 2004; Roberts *et al*. 2009). In addition, their global distribution follows the distribution of water masses supersaturated with respect to aragonite (Guinotte *et al*. 2006; Davies and Guinotte 2011). This is probably the reason why deep-sea scleractinian corals barely exist in the Pacific Ocean while they thrive in abundance in the North Atlantic and other ocean basins. The aragonite saturation horizon (ASH[1]) is at a mere 140 m in the Pacific, compared to 2000 m in the North Atlantic, due to large-scale oceanic processes. These ASH depths will change as the effects of the increasing atmospheric carbon dioxide concentrations progress, with increasing ocean acidification as a result. The ASH is largely determined by pH and pressure, the lower the pH, the less pressure needed to flip the

[1] The aragonite saturation horizon (ASH) is the depth at which there is a shift in the occurence of dissolution or precipitation of the aragonite form of calcium carbonate. Above the ASH, aragonite will precipitate, i.e. the chemical equilibrium reactions will favor the solid phase, while below the ASH the equilibrium will favor dissolution into ions. Aragonite is more readily dissolved than calcite due to the orthorombic crystal structure, in contrast to the more stable tetragonal structure of calcite. Calcite thus has a saturation horizon that lies deeper than the ASH. *Lophelia pertusa* produces a skeleton of aragonite, but other invertebrates can have shells or skeletons made of calcite.

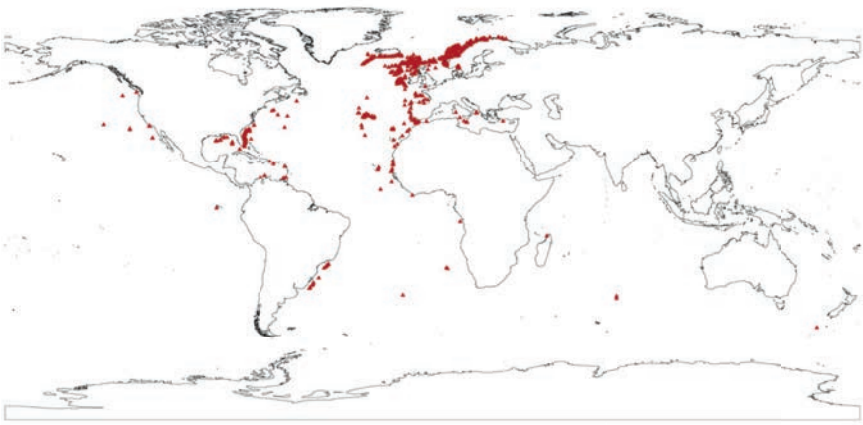

Fig. 1 – The global distribution of *Lophelia pertusa* shown with red triangles. Other species of cold-water corals is distributed more in the southern hemisphere, e.g. in the Indian and Pacific Oceans, and around Australia and New Zeeland. Map from Davies *et al.* 2008.

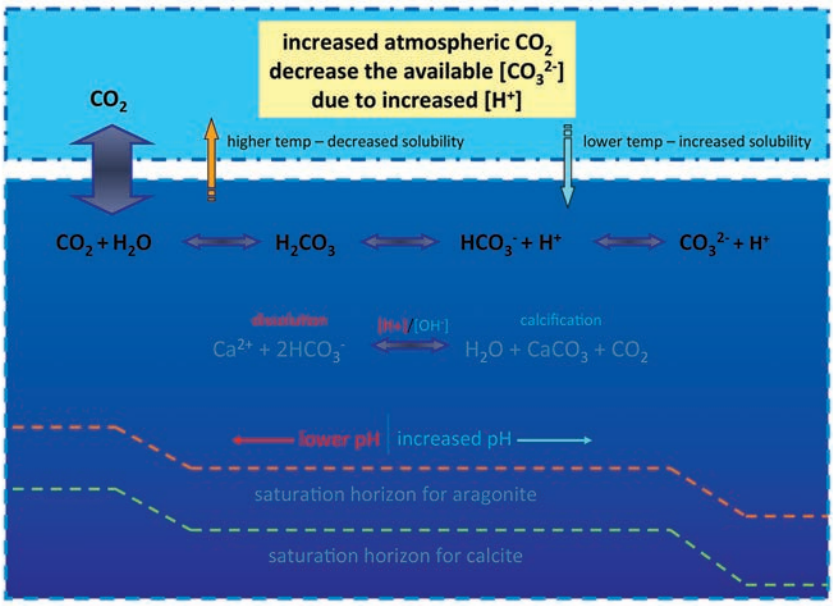

Fig. 2 – Water chemistry under ocean acidification: the increase in atmospheric carbon dioxide leads to more free hydrogen, and thus a lowering of the pH of the ocean. The equilibrium reactions of dissolution and precipitation (calcification) of aragonite is depending on pH and pressure, and thus, with lower pH follows that less pressure is needed to flip the equilibrium towards dissolution, and the aragonite saturation horizon will shoal to lesser depth. Gasses are more readily dissolved in colder water, thus uptake of atmospheric carbon dioxide increase at higher latitudes.

chemistry over to dissolution (fig. 2). Orr *et al.* (2005) estimated that the ASH depth in the Pacific will shoal from the present day 140 m to 70 m by 2100. For the North Atlantic the rise will be from 2000 m to 610 m. These figures were based on their "conservative scenario". In the study by Guinotte *et al.* (2006), more than 95% of deep-sea corals were found in locations that were saturated during pre-industrial times; locations of which 70% will be undersaturated by 2100 if the projections of Orr *et al.* (2005) will prove correct. The distribution of deep-sea corals will surely change dramatically the coming centuries.

Deep-sea hard substrate habitats are naturally patchy, separated by vast plains of siliceous soft sediments. Available surface for settling is scarce, and the competition is furious. The period after the last glaciation during the Pleistocene–Holocene transition (12 ka BP) was a splendid example of the contrary. As the ice retreated from Scandinavia it left clean hard surfaces in surplus, and as soon as oceanographic conditions allowed, cold-water corals established in the area (Correa *et al.* 2012). Over a period of 400 years *Lophelia pertusa* made a 7500 km range-expansion from deglacial refugia, according to the tracing of the genetic origin of the present *L. pertusa* populations in the North Sea done by Henry *et al*. (2014). In present days we see *L. pertusa* establishing on man-made structures such as offshore oil platforms. On North Sea oil rigs the depth distribution is observed to be 60–130 m, with peak density between 90–110 m, corresponding to depths around or below the summer stratification level and temperatures between 7° and 11°C (Roberts 2002; Gass and Roberts 2006). The potential for spread is enormous in this species, as long as the conditions are right.

Dispersal and population connectivity

For sessile marine benthos such as *Lophelia pertusa,* and other sessile invertebrates, the only chance of dispersal is during the pelagic larval stage. Once settled and metamorphosed into their sessile adult form, they are stuck. The pelagic larval stage is also the means of connecting different populations. Larvae are transported by ocean currents, and since the major oceanic currents usually are uni-directional and stable, this results in one-way transport of larvae from source to sink populations[2]. Henry *et al.* (2014; see also Dahl *et al*. 2013) traced the origins of the northeastern Atlantic populations of *L. pertusa* to Mediterranean populations, that through the Strait of Gibraltar connected northern populations via the Mediterranean outflow water (MOW), with populations at Galicia Banks, Aviles Canyon, and British Isles as stepping stones for further dispersal to the Norwegian shelf and Barents Sea. Most populations in the northeast Atlantic enjoy high gene flow, while populations in Skagerrak and the Norwegian Fjords show high clonality in the most hydrodynamically isolated locations (Le Goff-Vitry *et al.* 2004; Dahl *et al.* 2012; Flot *et al*. 2013).

At smaller scales, neighboring populations could potentially have multi-lateral connectivity through alternating current directions, such as tidal currents or regular

[2] A source population is defined as a population "*in which birth rates are greater than death rates, and emigration rates are greater than immigration rates*", thus a net contributer to neighbouring meta-populations. A sink population is defined by the opposite: i.e. "*birth rates are less than death rates and emigration is less than immigration*", and thus a net receiver of propagules (Crowder *et al*. 2000). A theory originally formalized by Pulliam (1988).

upwelling and downwelling events, depending on the main current regimes working during spawning season and larval drift periods. Although genetic studies like the above can trace genetic origins and establish the connectivity between metapopulations, they cannot resolve the question of how far larvae can be transported in one step. This is confounded by the contributions of larvae from populations acting as stepping-stones, in which the reproducing individuals have the same genetic origin as an upstream source population.

Previously, marine invertebrate larvae were conceived to be dispersed more or less like passive particles, with no means of affecting their dispersal routes. Recent studies have, however, shown that larvae can exert an influence on their vertical depth distribution and that this affects dispersal directions and distance. Only a slight capacity for vertical positioning potentially has profound effects on dispersal. By vertical migration the larvae have the potential of catching counter-directional currents at different depths and thereby change the direction of dispersal (Fiksen *et al*. 2007; Metaxas and Saunders 2009; Shanks 2009; Corell *et al*. 2012; Drake 2013). While larval behavior is increasingly recognized as influential during dispersal, and biophysical dispersal models are developed to include these biological drivers, very little is yet known about the swimming abilities and ontogenic shifts in the properties and behavior of deep-sea invertebrate embryos and larvae.

Morphology and biology

Lophelia pertusa belongs to the stony corals, i.e. scleractinians, and build large arborescent colonies that can extend continuously over hundreds of square kilometers. Usually, the upper one-meter of the colonies contain live polyps, while older polyps below dies off due to age, reduced water circulation and food availability (Wilson 1979; Cairns and Kitahara 2012). One colony is the result of the asexual growth of one settling sexually produced larva, with budding occurring from the rim of the older polyp. It is possible that colonies can originate from several larvae that have anastomosed (fused) during growth, since gregarious settling is common in corals. However, while chimerism and fusing of closely related juveniles is a common phenomenon; as the colonies grows this self-recognition of siblings and acceptance of each others tissues can be reversed, and later one individual will take over (Rinkevich 2004). Pires *et al*. (2014) have found gametes of both sexes from different polyps in the same colony of *L. pertusa*, so there are some evidence that this species could have anastomosed colonies, although it seem rare. Each polyp resides in a skeletal cup, i.e. a *corallite*, up to 20 mm long and 10 mm wide (Gass and Roberts 2010).

Shick (1991) aptly described anthozoans as being at "*the origami level of construction*", as their bauplan basically consist of epithelial sheets of either epidermis and gastrodermis, or two layers of gastrodermis (as in the mesenteries), with a layer of acellular mesoglea in between. This play on word is derived from the terminology *tissue* or *organ grade of construction*. Anthozoans do not possess organs, and thus are at the tissue grade of construction. Each epithelial layer consists of single cells, sometimes elongate with differentiation between distal and basal part of cells, thereby giving the epithelia a pseudostratified appearance. These sheets are folded into the few tissue types and appendages that make up the animal, such as the tentacles, mesenteries, and the actinopharynx, i.e. the passage from the mouth into the gastrovascular cavity (Fautin and Mariscal 1991; Fautin 2009). Rather than having cells organized into organs, there are specialized cells carrying out the necessary

functions, and these cells are clustered in the epidermis or gastrodermis according to their function. For instance, calicoblastic cells are the ones excreting the aragonitic skeleton of scleractinians. These constitute the epithelial layer of cells aligning the skeleton both on the outside and the inside of the corallite. The gastrodermis lining the mesenteries contain unicellular gland cells excreting enzymes and mucus for digestion. Simple gonads, without oviducts or sperm ducts, are produced as outpocketings of the mesenteries. All epidermal and gastrodermal layers contain ciliated supporting cells that govern cross surface transport: on the external surfaces they help shedding sediment off the living tissues, and in the actinopharynx they help in food transport into the gastrovascular cavity and general water circulation. There are also neurons, epitheliomuscular cells, and sensory cells with mechano- and chemoreceptors.

Lophelia pertusa are predators and opportunistic heterotrophs, as most deep-sea scleractinians. They feed foremost on zooplankton, but also on particulate and dissolved organic matter, pico- and nanoplankton (Houlbrèque *et al.* 2004; Dodds *et al* 2009; Gori *et al*. 2014; Mueller *et al*. 2014; Naumann *et al*. 2015), and recently it has been discovered that they possibly also house chemoautotrophic sulfur oxidizing, and nitrogen fixing symbiotic bacteria (Middelburg *et al*. 2015).

Fig. 3 – Egg release in *Lophelia pertusa.* Female polyps forcefully eject thousands of eggs in one big squirt, followed by a couple of smaller squirts with 15–20 minutes in between. Male polyps let their sperm slowly ooze out, like a trail of smoke.

Reproduction in *L. pertusa* is by broadcast spawning, i.e. release of gametes to the surrounding water, and subsequent external fertilization (fig. 3). Gonochoric broadcast spawning is the dominating sexual system and reproductive mode in deep-sea reef-building scleractinians, while hermaphroditic spawning dominates in tropical corals. Brooding of embryos and larvae are common in solitary species, and more prevalent in temperate and cold-water Atlantic species than in other ocean basins (Waller 2005; Kerr *et al.* 2010).

Gametogenesis and spawning is probably regulated by estradiol and testosterone, as in vertebrates. Peak levels of glucuronided testosterone and 17β-estradiol have been measured during spawning over reefs, with a 100-fold increase in glucuronided estrogen at spawning compared to one month prior to spawning (Atkinson and Atkinson 1992; Twan et al. 2006). In *L. pertusa*, gametogenesis takes a full year, or more, for the oocytes (eggs), while spermatocytes developed faster (Waller and Tyler 2005; Brooke and Järnegren 2012). Spawning occurs in January-February in Skagerrak, and February-March in the Trondheim Fjord populations (Larsson *et al.* 2014), while populations in the Gulf of Mexico and off Brazil is estimated to spawn during September-November and May-July, respectively (Waller and Tyler 2005; Pires *et al*. 2014). The annual spawning of *L. pertusa* is in contrast to other reef-forming deep-sea scleractinians that shows continuous or multiple spawning over the year (Pires *et al*. 2014).

The synchronization of spawning is most likely regulated by hormones; however, this has not yet been fully investigated. Most research on spawning periodicity has been connecting spawning with lunar cycles (Babcock *et al.* 1986; Harrison et al 1984; Hayashibara *et al*. 1993), with lunar periodicity in spawning synchrony of other invertebrates extending even into the deep-sea (Mercier *et al*. 2011). Solar insolation (Penland *et al*. 2004) and calm periods with a reduction in wind driven currents (van Woesik 2010) has also been linked to spawning periodicity in tropical corals. The calm periods could potentially induce spawning due to that concentrations of water soluble hormonal signals builds up in the more still water; however, this possibility was not investigated.

While annual cycles in environmental conditions can govern the onset of gametogenesis and the general annual cyclicity of reproduction, the final maturation and release of gametes should be governed by hormones. Atkinson and Atkinson (1992) suggested that, as gametes have matured, the release of conjugated hormones will give a negative feedback on gonadotropin to decrease hormone production, and thus affect the mesogleal lining of the oocyte and spermatocyte pockets so that they disintegrate, and gametes can be released into the gastrovascular cavity and subsequently expelled by the polyp. The hormone system of cnidarians is not fully understood yet (Tarrant 2005), but teleost fish use conjugated hormones as exogenous signals, i.e. pheromones, to synchronize gamete maturation and spawning in conspecific mates (Kobayashi *et al*. 2003; Stacey 2003), and it is thus likely that there are similar functions in corals.

Cnidae

One cannot discuss the morphology and biology of cnidarians without the mentioning of the defining character of the entire phylum, i.e. cnidae[3]. The name Cnidaria is derived from the Greek word κνίδη (*knídē*), meaning nettle, based on the burning sensation received by the encounter with certain jellyfish (e.g. Lion's mane jellyfish, *Cyanea capillata*). It is apparent while looking through a microscope at a coral tentacle that cnidae is of profound importance, since cnidocysts are just about everything you see in the tentacle epidermis as well as in other tissues. The density of

[3] Cnidae is the umbrella term for a range of different types of cnidae, and different levels of cnidae terminology. Cnido*cyte* is the cell bearing a cnido*cyst*, i.e. a stinging organell with a capsule and a tubule, sometimes equipped with a shaft. A cnido*blast* is a cell containing a developing cnido*cyst*.

these stinging organelles is impressive. Cnidae are used by corals to catch prey and to defend themselves from aggressors or predators.

Cnidocysts are considered the most complex organelles in the animal kingdom (Shick 1991; Beckmann and Özbek 2012), a complexity that is in stark contrast to the animal's structural simplicity at the tissue level and number of cell types present. Although as many as 25 to 30, or more, morphological types of cnidocysts have been described (reviewed in: Östman 2000; Kass-Simon and Scappaticci 2002; Fautin 2009), there are only a few functional types: i.e. penetrating, ensnaring, and glutinant.

Penetrating cnidae usually has a distinct shaft that pierce through the epithelia or cuticle of the prey (or aggressor), however, some cnidae types without shaft can also penetrate if the trajectory of the everting tubule is straight and the discharge kinetics is sufficient (Östman *et al.* 1997; Colin and Costello 2007).

The everting tubule of *ensnaring* cnidae has a sweeping discharge trajectory and small spines along the entire tubule, effectively entangling the prey (Colin and Costello 2007). A special case of the ensnaring type is desmonemes: their spring-like thread has so much spring tension that they can crush the cuticle of copepods (Östman *et al.* 1991).

Glutinant cnidae secrete a sticky substance, and/or, are equipped with adhesive microfibrillae along the everted tubule, as in spirocysts (Mariscal *et al.* 1977a) and ptychocysts (Mariscal *et al.* 1977b). While spirocysts is present in all anthozoans (concentrated to the tentacle epidermis), ptychocysts are highly specialized and only occurring in burrowing cerianthid anemones. They are used for building the framework of their dwelling-tube, in which particles adhere to add to the construction.

Certain types of cnidae (i.e. atrichous isorhiza, without spines on the tubule), is used both by adult polyps of *Hydra sp.* (Ewer 1947, in Kass-Simon and Scappaticci 2002), and the actinula larvae of the hydrozoan *Tubularia mesembryanthemum*, as a means of temporary attachment during locomotion or settling (Yamashita *et al.* 2003). These cnidae types have yet to be functionally described to be able to classify them as either of the above functional types, although it seems from manuscript III in this thesis that they should be grouped with the glutinant types, which explains their function in temporary attachment; a function that was in no sense obvious from the smooth appearance of the tubule.

Cnidocysts are also grouped into three categories based on morphological characters: nematocysts, spirocysts, and ptychocysts. The highest diversity is found amongst nematocysts, the other two being basically monomorphic. Within Anthozoa the diversity of nematocysts is lower than in Hydrozoa (aprox. 10 of 30 types); however, the spirocysts and ptychocysts are specific for Anthozoa (Fautin and Mariscal 1991).

Cnidogenesis has been described by Slautterback and Fawcett (1959), Skaer and Picken (1966), Westfall (1966), Skaer (1973), Holstein (1981), Tardent and Holstein (1982), amongst others, reviewed by Kass-Simon and Scappaticci (2002). These early works have described the ultrastructure of the developing cnidae, and identified the role of the Golgi apparatus in the excretion of capsule and tubule. The capsule is excreted first, and the tubule is added externally to the capsule in the form of a cylindrical tube. This process is orchestrated by centrioles and microtubuli, also responsible for the correct split of the chromosomes during cell division. The microtubuli forms a funnel around the progressing tubule, and the number of microtubuli involved decides the width of the tubule produced. If the cnidocyst in the making is equipped with a shaft, the proximal part of the tubule will be made wider,

i.e. more microtubuli involved, and then the number of microtubuli will be reduced when the remaining tubule is built. Building blocks of sub-units of the proteins and mini-collagens involved gets lined up inside the microtubuli and bonds are formed. The tubule is made up of two or three layers with different properties. If spines are in line for production, the capsule is filled up with sub-units for the specific proteins making up the spines, and as soon as the external tubule has been invaginated into the capsule, the spine proteins will neatly fit in where they belong along the inverted tubule. A spine protein is identified for *Hydra*, and aptly named spinalin by Hellstern *et al*. (2006). Detailed presentations of this intricate process of invagination, folding, and packaging of the external tubule, to produce the mature, and ready to fire, stinging organelle with internalized tubule has been given by the work of e.g. Skaer and Picken (1966), and Tardent and Holstein (1982). More recent work has described the process of cnidogenesis in more molecular detail, identifying different mini-collagens as the building blocks of capsule and tubule walls (e.g. Engel *et al.* 2002; Adamczyk *et al.* 2007; David *et al.* 2008).

Documentation of the morphology of cnidoblasts in different stages of maturation has been done for the anthozoans *Metridium senile* and *Sagartiogeton viduatus* by Östman *et al.* (2010a-b; 2013). Already Möbius (1866) suggested that cnidoblasts differentiate beneath the mature cnidocytes (commented in Robson 2004), and Slautterback and Fawcett (1959) traced the origin of cnidoblasts from interstitial cells at the base of the epithelium. The maturing cnidae then migrates from the basal to the distal epithelium where final maturation takes place (Skaer 1973; Tardent 1995).

Trigger mechanisms

As if the cnidogenesis, and the resulting cnidocysts, was not exquisite enough, their mode of action brings it to a complete new level. The firing mechanisms for cnidocysts are exquisite in an insidious way, and some the most interesting studies on the subject have been done by Watson and Hessinger (1994), and Watson and Roberts (1994). Cnidae discharge is triggered by mechano- and chemoreceptors working in concert. These receptors are situated on supporting cells adjacent to the cnidocyte itself. The mechanoreceptors consist of stereocilia, or “hair bundles”, that are sensitive to vibrations of specific frequencies, tuned to trigger cnidae discharge when a suitable prey gets close to the cnidae laden tentacles. As the first round of fire has been executed, the prey finds itself pierced with penetrating nematocysts, and entangled in a sticky web of spirocysts. The more it fights to get free, the more it gets entangled with the sticky web. And soon the toxins and enzymes delivered by the nematocysts will subdue the prey that subsequently will be less active, i.e. its frequency of muscle activity will go down. This is when things get insidious. The pierced prey is now leaking body fluids, for instance the amino acid proline. The chemoreceptors are binding sites for proline, and what happens when proline binds in is that, the hair bundles will grow a little bit longer, and thereby become sensitive to lower frequencies. The next round of fire is executed. Game over. Now, the polyp can safely move the prey into the gut and enjoy the meal.

A coral polyp is an extremely delicate creature, with only two cell layers and the acellular mesoglea to protect them. Still, they take on prey with exoskeletons and sharp appendages, and they have evolved the perfect armory to do so.

Taxonomic classification

Phylum:	CNIDARIA
Class:	ANTHOZOA
Subclass:	Hexacorallia
Order:	Scleractinia
Family:	Caryophylliidae
Genus:	*Lophelia* H. Milne Edwards & Haime, 1849
	Syn. *Lophohelia* Milne Edwards & Haime, 1857 (misspelling)
Species:	*Lophelia pertusa* (Linnaeus, 1758)

Type species: *Madrepora prolifera* Pallas, 1766
Original name: *Madrepora pertusa* Linnaeus, 1758

Synonyms: *Madrepora prolifera* Pallas, 1766; *Lophelia californica* Durham, 1947 (junior synonym representing the *gracilis* variation sensu Duncan, 1873); *Dendrosmilia nomlandi* Durham and Barnard, 1952 (junior synonym representing the more robust *brachycephala* variation sensu Moseley, 1881); *Lophohelia oculifera* Whiteaves, 1901 (*nomen nudum*)
Referred to as *Lophohelia prolifera* by Gosse (1860) and Carlgren (1940), and *Lophelia prolifera* by Cairns (1979); finally receiving its now accepted name *Lophelia pertusa* in a revision by Zibrowius (1980).

Etymology

Gosse (1860) provided an etymology of the name *Lophohelia* as derived from the Greek words *lophos* (a tuft), and *helios* (the sun). *Lophos* refer to the arborescent branching (like a bouquet, a tuft). *Helios* refer to the radiating skeletal lamellae (septa) as seen when viewing the calyx from above, a characteristic feature of *Lophelia pertusa* (Cairns and Kitahara 2012). Further, *pertusus* means perforated and could refer to fine perforations of the skeleton.

Remarks on phylogeny and history

Milne Edwards and Haime (1849) first put the genus *Lophelia* within the scleractinian family Oculinidae but later it has been assigned to the family Caryophylliidae Dana, 1846. Caryophyllidae presently comprise 89 valid genera of which 38 are extinct, known only from fossil records Kitahara *et al.* (2010a). The Caryophylliidae is, however, not a monophyletic group, and a revision is likely to reduce the number of genera assigned to the family (Romano and Cairns 2000; Kitahara *et al*. 2010b).

The oldest known fossil assigned to the Caryophylliidae is from the Jurassic era (180 Mya), while some of the earliest fossil records of "scleractiniamorph" corals with characteristics indistinguishable from those of modern corals dates all the way back to the Ordovician 450 Mya (Stolarski *et al.* 2011).

The 51 extant genera of Caryophylliidae are found in all oceans, at all depths, including both zooxanthellate (with photosynthesizing symbionts) and azooxanthellate species (without symbionts). Two genera within Caryophylliidae with exclusively zooxanthellate and reef-building taxa group together in phylogenetic trees and has been gathered in the subfamily Eusmiliinae (Romano *et al.* 2000), thus it is

possible that the diversity of caryophylliids regarding symbiosis and growth pattern will be altered after a revision.

It has been suggested that the solitary azooxanthellate corals, possibly with a deep-sea origin, are the ancestral forms (Kitahara *et al*. 2010a; Stolarski *et al*. 2011), while shallow-water tropical corals seems to have invaded shallow waters at several occasions (Lindner *et al*. 2008).

While the majority of deep-sea scleractinians are solitary there are six prominent reef framework-building representatives: *L. pertusa*, *Solenosmilia variabilis, Goniocorella dumosa* (Fam. Caryophylliidae), *Oculina varicosa*, *Madrepora oculata* (Fam. Oculinidae), and *Enallopsammia rostrata* (Fam. Dendrophylliidae) (Freiwald *et al.* 2004; Davies *et al.* 2011). All with a similarly wide cosmopolitan distribution as *L. pertusa,* except for *G. dumosa* and *O. varicosa* that has a more geographically restricted distribution. However, *L. pertusa* is standing out as the species producing by far the most extensive reefs (Cairns 2007; Kitahara *et al*. 2010b).

AIM OF THE THESIS

For sessile marine organisms the pelagic larval phase is what connect populations, maintain genetic diversity, and provide a security against extinctions due to changing environmental conditions by letting the organisms spread to potentially more suitable habitats. Knowing the prerequisites for successful reproduction and the biological drivers for dispersal is of paramount importance in the work of finding sustainable management strategies so that we can mitigate the anthropogenic impacts imposed on these ecosystems, and hopefully help increase their resilience. Knowledge of the biological aspects that influence dispersal is very limited considering deep-sea benthos (Cowen *et al.* 2007; Cowen and Sponaugle 2009; Davies and Guinotte 2011; Hilario *et al.* 2015; Treml *et al.* 2015), and the aim for this thesis was to provide biophysical modelers with the biological data needed to improve the accuracy of the predictions from dispersal models for one of the major deep-sea habitat engineers, the cold-water scleractinian *Lophelia pertusa*.

The dispersive phase of marine organisms can be divided into several stages. Treml *et al*. (2015) provided a conceptual framework of these stages, and the intrinsic and extrinsic drivers affecting dispersal during each stage. The initiation—*stage 1*—of dispersal is affected by the abundance and density of the reproducing population, with reproductive mode (brooding or broadcast spawning), and fecundity (number of gametes per individual) as key intrinsic factors. The habitat quality and structure, as well as depth and timing will affect the outcome of the reproductive effort. *Stage 2*—the actual dispersal stage—is affected by mortality rates, intrinsic properties of the propagule such as morphology, buoyancy, and behavior, and extrinsic factors such as water quality, hydrodynamics at different scales, and predator-prey dynamics. The settlement stage of dispersal—*stage 3*—is determined by when and how metamorphic and settling competency is achieved by the larva, and for how long this competency window lasts; thus setting the limits for the pelagic larval duration (PLD). The availability of suitable substrate and habitat will influence the outcome. The final step—*step 4*—is the actual recruitment, which is depending on the condition of the larva at the time when it finds suitable substrate and its capacity to cope with the competition with other organisms, and the environmental qualities of the chosen habitat.

As noted by Hilàrio *et al*. (2015) the dispersal stage (stage 2) can be further broken down into sub-stages depending on ontogenic shifts, i.e. during embryo and larval development the propagules can alter between passive and active stages. The term PLD is often used independent of whether the embryogenesis is included in the pelagic phase or not—as is the case in broadcast spawning—leading to the suggestion by Hilàrio *et al*. (2015) of changing PLD to PPD—pelagic propagule duration—to get a terminology that encompass the embryogenesis.

With the framework of Treml *et al.* (2015) in mind, and with the additional aspects presented by Hilàrio *et al*. (2015), we aimed at mapping the early life history of *L. pertusa*, from spawning to settling.

Results in Summary

Timeline – from spawning to settling competency

In summary, we have established that after gamete release and fertilization, the zygotes and developing embryos are transported as passive particles until day five, when they become swimming blastulae (fig. 4). During this period they are positively buoyant, slowly ascending, and most probably transported by advection away from the reef (paper I). Day 6–9 they are alternating between modest swimming and being passive during the process of gastrulation. From day 10 to 20 they are in the pre-competency phase, actively swimming upwards in the water column with a swimming speed of 0.44 ±0.16 mm s^{-1} (mean ±SD, measured day 14).

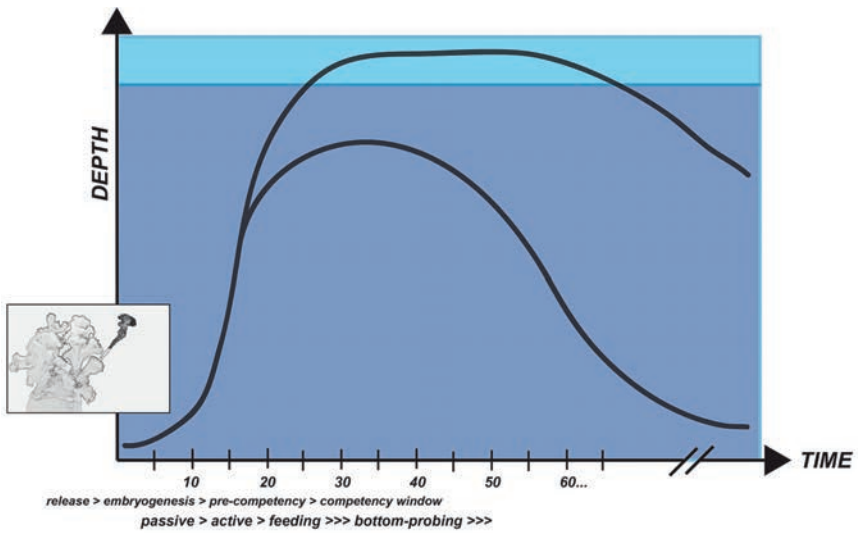

Fig. 4 – Timeline for the pelagic phase of *Lophelia pertusa* embryos and larvae, indicating the ontogenic shifts and behavior of the planulae that affect dispersal. Until day 5 embryos are positively buoyant and transported by advection as passive particles. Between day 5 and 9 they are capable of moderate swimming, but remain still while they undergo gastrulation. From day 10 they are actively swimming upwards. They enter their settling competency period after day 20 and starts feeding. After aproximately 30–40 days they start their search for suitable habitat for settling, and can probably spend several months searching. Potentially they can enter the photic zone.

After this pre-competency period they simultaneously develop a flexible mouth and starts foraging actively, as well as developing functional cnidocysts, and start actively swimming downward and displaying bottom-probing behavior (paper I and IV). They have now entered their competency window, in which they are capable of metamorphosis—possibly only after successful foraging to gain necessary nutrients—and ready to settle. Although the bottom-probing behavior started day 21, not all larvae started swimming downward immediately. The downward swimming became increasingly common over a period of two weeks (day 21–35), after which

almost all larvae displayed the same behavior. At the same time, vertical upward swimming was still observed in 36 days old planulae, measured to 0.74 ±0.20 mm s^{-1} (mean ±SD), corresponding to a potential vertical migration of 64 m per 24 hours. It is plausible that planulae will alter between downward and upward swimming during a period expanding beyond 35 days. And foraging will influence their swimming.

The duration of the competency window is yet to be resolved, but a few of the larvae from the spawning season 2015 have survived in lab for a full year, although in very poor condition by the end. The interesting aspect is that they seem to spend almost all of their competency window period further down in the water column since they start downward swimming simultaneously with foraging and gaining functional cnidae. And although planulae in lab was showing interest in small size microalgae their main diet seemed to be of animal origin, or picoplankton, similar to the diet of the adults. Potentially they are capable of spending long periods near-bottom, after the initial 20–30 days of dispersal higher up, searching for suitable habitat and foraging.

The above described developmental rates was observed in planulae reared at a temperature of 7–8°C, and are likely to be affected by *in situ* temperatures during the dispersal period of naturally dispersing larvae. For example, in the Koster-Hvaler area the spawning season coincide with dropping temperatures, from c. 8°C in January to c. 6°C in March-April (fig. 5), measurements taken in 2015 with a Aanderaa RCM9 acoustic Doppler current profiler, ADCP, with turbidity meter). These are the temperatures at the reef site, at 120 m depth, and do not necessarily reflect the temperature at the depth occupied by the larvae. During the corresponding period, the water temperature of the deep-water from the Koster Fjord, taken from 45 m depth and continuously measured in lab (with a few gaps in the data due to technical failure), dropped from c. 9°C in December down to c. 5°C in February-March, but were then rising to 8°C by April (data from the Sven Lovén Centre water inlet loggers). We have yet to resolve the question of how high up in the water column the planulae ascend during the pelagic phase; however, since they did show interest in small size microalgae and passed through haloclines with no hesitation (paper IV), they can potentially ascend to the photic zone. The depth distribution of *L. pertusa* on oil rigs (60–130 m, Gass and Roberts 2006) gives at least an indication of the depth distribution at settling competency. It is important to consider the ambient temperatures and the effect it has on the development rates of larvae when modeling, and allow for variation. These data will, nevertheless, greatly enhance the accuracy of predictions of larval dispersal for *L. pertusa*.

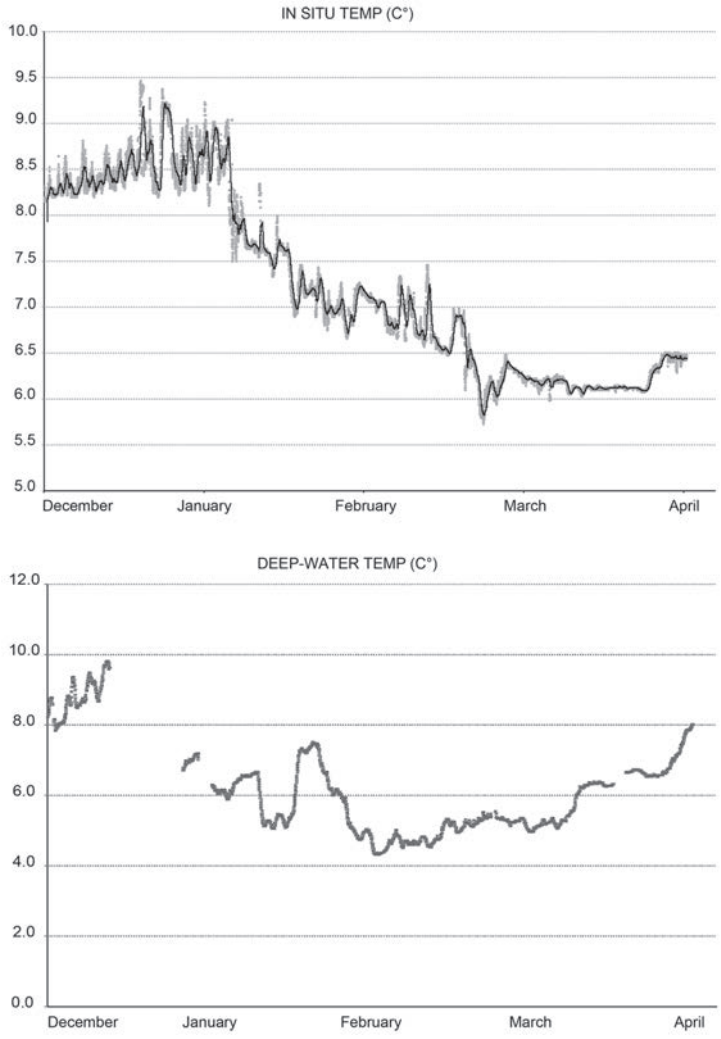

Fig. 5 – *In situ* water temperature (°C) at 120 m depth at the Tisler reef during December 9, 2014 to April 27, 2015 in the top graph. The bottom graph shows the temperature of the deep-water of the flow-through system at the laboratory (water inlet at 45 m depth in the Koster Fjord) for the corresponding period. The *in situ* measurements were taken with an ADCP (Aanderaa RCM9), and the data from the deep-water system in the lab were extracted from the Sven Lovén Centre web page. The water temperature and salinity is continuously logged. Note the different scales of the y-axes.

Timing of spawning

In the two most successful years of spawning, 2013 and 2015, the timing of spawning varied between years. In 2013 the first spawning event occurred in early January, starting the 9th and 10th, followed by a quiet period until January 20, when an intense spawning period started. This period lasted a full week, until January 27, which happened to also be the time for full moon. Two more occasions with minor gamete release was observed at February 14–15 and 28, which was the last observed spawning this year. The February full moon was the 25th.

In 2015 there was no intense week of spawning. The first event was observed in January 22, followed by release on January 25, 27*, 29*, and in February 2*, 4, 13, 18*, 19*, 20, 23**, and finally the 25th. Those marked with an asterisk (*) were major releases, with February 23rd being the major event. Full moon occurred January 5 and February 4, and thus no apparent connection could be found to the moon cycle this year.

In an attempt of resolving the question if spawning in lab coincided with spawning in the field, we made a long-term deployment of an ADCP (Aanderaa RCM9) to measure *in situ* turbidity (NTU) and current directions and speed (cm s^{-1}) at the Tisler reef, where the parental colonies had been collected. The equipment was deployed over a period between December 9 (2014) and April 27 (2015), to cover the spawning season with good margins. To our surprise the equipment came back with a single peak in turbidity registered at January 18, lasting for one hour at 06:10–07:10, and slightly elevated turbidity values following after that for almost the entire remaining period (fig. 6). This is too early to coincide with early spring phytoplankton blooms, and no dredging activity was reported during this time. The gametes of *L. pertusa* are highly reflective and should be easily detectable with an NTU-type (Nephelometric Turbidity Units) of turbidity meter that measures light backscatter. The single peak reached almost 6.0 NTU, while the background turbidity was relatively low at 0.2–1.2, with occasional smaller peaks <1.8 NTU. The ADCP was standing at the edge of a larger reef patch, unfortunately in a slope and in a depression between *L. pertusa* colonies. The position was not optimal, but promising. In hindsight it is apparent that to be able to accurately pick up peaks in turbidity caused by spawning we need to put out several measuring devices coupled to time-lapse cameras to verify that the peaks are indeed caused by spawning products. This single peak could be caused by a dense aggregation of zooplankton or what not, and further deployments needs to be done to resolve the question of the timing of spawning in the field.

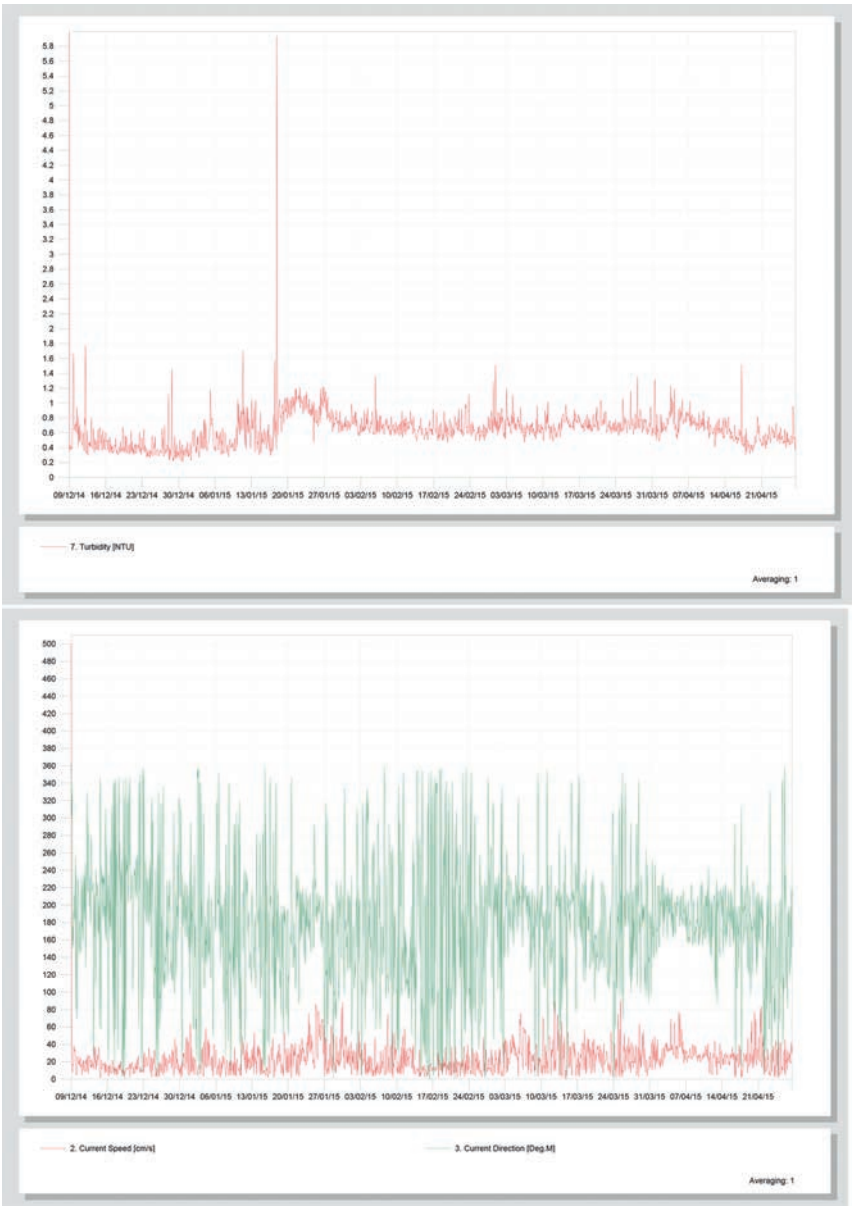

Fig. 6 – *In situ* measurements of turbidity (NTU) in the top graph, and current speed (cm s^{-1}, red) and direction (green) in the bottom graph. Measurements were taken at 120 m depth at the Tisler reef during the period December 9, 2014, to April 27, 2015. The turbidity shows a single peak at January 18, for one hour between 06:10–07:10 hours. The single peak was an unexpected outcome, since the corals in lab were spawning on several occasions and we expected to see several peaks in January-February caused by spawning products. Further field measurements are needed, in combination with cameras, to verify that any peaks are indeed spawning events and not caused by something else. Measurements were taken with an Aanderaa RCM9 acoustic Doppler current profiler (ADCP) with turbidity meter.

Embryogenesis and early planulae

In paper I (Larsson *et al*. 2014) we established the timeline for embryogenesis and larval development, with focus mostly on the embryogenesis. The embryo development of *L. pertusa* is slow, compared to development times in temperate and tropical corals. At 7–8°C it took 48 hours to reach the 64-cell stage, and 5 days to become ciliated blastulae. They did not develop into planulae until after accomplished gastrulation day 9–10, and starting the onset of settling competency after three weeks. As seen in paper IV, the development rate doubled in embryos reared at 11–12°C, i.e. what normally took six days, was achieved in only three days at the higher temperature. A full developmental series of scanning electron micrographs (SEM) from non-hydrated egg to mature planula can be seen in fig. 7.

These development rates can be compared to those of the temperate cup coral *Caryophyllia smithii* that become fully developed feeding planulae after only 48 hours and ready to settle after 8–10 weeks, reared at 15°C (Tranter *et al*. 1982). In broadcasting tropical corals planulae are commonly formed 24–36 hours after spawning, with feeding starting day two or three, and settling from day five, or even as early as 2.5 days post-spawning (Krupp 1983; Hayashibara *et al.* 1997; Schwarz *et al*. 1999; Miller and Mundy 2003; Chui *et al.* 2014), with competency periods and longevity spanning over periods of 10 days to over 200 days (Wilson and Harrison 1998; Graham *et al.* 2008).

The only deep-sea corals followed from release to settling are brooding soft coral species (Octocorallia), and thus release is of fully developed planulae. These have been observed to be either demersal larvae, crawling over the substrate, or swimming close to the bottom, with settling and metamorphosis occurring from day 1–30 in two species (Sun *et al*. 2010a,b) and day 3–70 after release in two other species (Sun *et al*. 2011). Planula release is probably continuous over the year in these species, and furthermore, planulae can be produced via parthenogenesis, i.e. without fertilization. One interesting aspect with the studies on these octocorals was that larvae with an origin from deeper (1200 m) populations seemed less selective considering substrates than those from the same species collected at more shallow depths (500 m, Sun et al. 2010a).

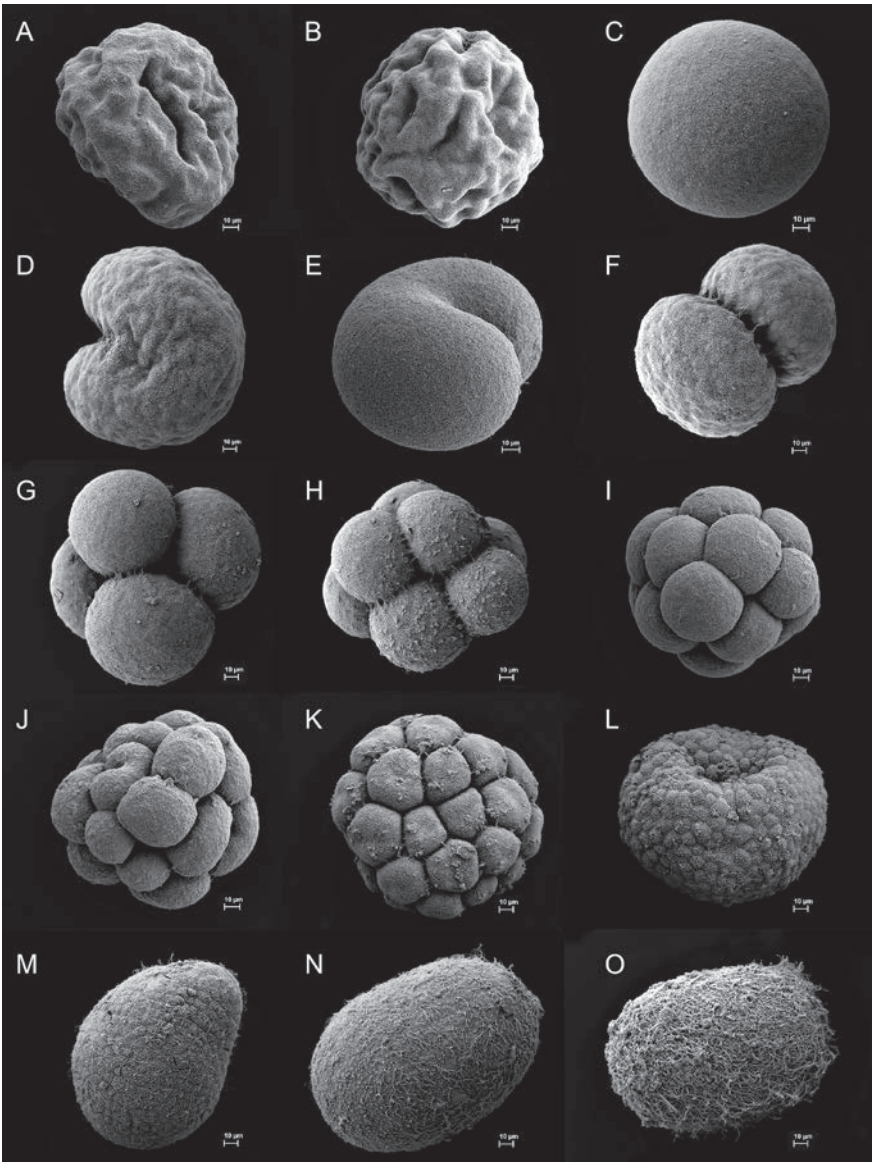

Fig 7 – A development panel for *Lophelia pertusa*, from recently released egg (A-B), not yet fully hydrated, to a planula (O). The 64-cell stage (L) was reached after 48 hours and ciliation occurred day 3–5 (M) followed by gastrulation. N represent a 14 days old early planula with an oral pore, and O is a fully developed planula. Scanning electron micrographs.

Comparison of adult and larval cnidome

In paper II we present the internal morphology of *L. pertusa*, with focus on the cnidome (i.e. the cnidae complement) of the adult polyps. This study was done to be able to compare the adult and larval cnidome and from this derive differences in cnidae function between adults and larvae, in combination with observations of larval cnidae usage. The cnidome of *L. pertusa* had previously been described by Carlgren (1940); however, to make a proper analysis of the differences we needed more detail in the cnidocyst morphology. Very few studies have been done comparing adult and larval cnidae (e.g. Yamashita *et al.* 2003; Holst *et al*. 2007; Zenkert *et al*. 2011), but those done have shown that the planulae have a unique cnidae complement. We suspected that cnidae are used by planulae for preliminary anchoring before attaching more permanently, and therefore a sign of settling competency. In paper III we concluded that the larval cnidome indeed is very different from the adult's, and could confirm that planulae use them for temporary anchoring. The evidence is still not completely to satisfaction and more studies needs to be done to verify cnidae function in planulae. For instance, we wanted to fix settled planulae for scanning electron microscopy (SEM), so that both planulae and their attachments by cnidae tubules were visible. Unfortunately only one planula was observed to settle on one of the substrates adapted for fixation, and subsequently fixed for SEM, but the planula itself was lost in the process, leaving only cnidae tubules. The types of tubules present on the substrate where the planula had attached had adhesive microfibrillae or other adhesive strands; however, they were not verified on the surface of planulae in SEM preparations and there is therefore still an uncertainty about these results. Observations under dissection microscope and light microscope confirmed attachment by cnidae tubules, although not the exact type of cnidae or mode of attachment.

From the observations made during this study (paper III), it is also apparent that the planulae could use cnidae as a defense, to evade predators. The atrichous isorhizas (i.e. nematocysts without shafts, and with smooth tubules) had rigid tubules that could hold off potential predators, or rather, holding the planulae off the predators. Cnidae also fired in response to water turbulence which can be an effect of a predator closing in; however, potentially this could also be a mechanism for planulae to avoid being swept out of a reef matrix by eddies. If they have managed to find a suitable reef habitat for settling they will need to have a means of staying there, despite small-scale hydrodynamic forces that could sweep them away. These cnidae functions need further investigation, but are an interesting avenue for further research.

The investigation of the internal morphology in paper II also resulted in some controversy considering the free extensions of the mesenterial filaments, what we chose to call acontia, as in sea anemone anatomy. The focus on skeletal characters in scleractinian taxonomy has probably led to some confusion considering the naming of soft tissues in scleractinians. Since the early taxonomist had not provided names for soft tissue homologs to other anthozoans, such as sea anemones that has been generously described according to their soft tissue anatomy since they lack a skeleton, it seems as if later researchers has been shy to apply names of anthozoan soft tissue anatomy to scleractinians. The basic bauplan of scleractinians is homologous to that of sea anemones, both groups belonging to the subclass Hexacorallia. In the synthesis of anatomical and molecular taxonomic evidence made by Daly *et al.* (2003) this controversy is discussed, and their conclusion is that Actiniaria (sea anemones), Antipatharia (black corals), Ceriantharia (tube-dwelling anemones), Corallimorpharia, Zoanthidea, and Scleractinia are a monophyletic group. In addition, Actiniaria,

Corallimorpharia, and Scleractinia share the paired hexamerously arrangement of the mesenteries, i.e. the soft tissue compartments of the polyp. The soft tissue anatomy is complicated by the high variation within groups, and that different features are shared across different pairs of groups. Daly *et al*. (2003) also discuss the complications that arise from differences in terminology between groups of hexacorallians. The free extensions of the mesenterial filaments in *L. pertusa* are fitting the descriptions of acontia for sea anemones, as we discuss in paper II.

Larval feeding behavior

In paper IV we have confirmed that *L. pertusa* planulae are planktotrophic, feeding mainly on animal derivatives, but also showing interest in picoplankton and small size microalgae. It was apparent from our feeding trials that 14 days old planulae were not yet capable of feeding, while fully mature planula after developing the flexible mouth (>20 days old) responded strongly to the presence of food. The food choice is of interest, since different food types are distributed differently in the ocean; microalgae being present in the photic zone with a predictable depth distribution, while other food sources is expected to be more patchy and spread out. Knowing the preferred food of *L. pertusa* planulae could therefore give an indication of their vertical distribution during their pelagic period.

We found no limitations to the upward distribution of the planulae; they pass through haloclines and are interested in microalgae. Although, since foraging coincide with settling competency and onset of downward swimming, it is more likely that planulae will forage in random patches of animal derived detritus and picoplankton below the photic zone. Marine invertebrate larvae are known to be able to utilize dissolved organic matter and free amino acids, as well as bacteria and detritus (Manahan 1990; Boidron-Métairon 1995), and in a study by Ben-David-Zaslow and Benayahu (2000) it was estimated that uptake of free amino acids by coral planulae could cover 11% of the metabolic demand for the investigated species. Our planulae were enthusiastically feeding on crustacean homogenate, as been observed in other scleractinian and sea anemone planulae (Tranter et al. 1982; Schwarz *et al*. 1999; Schwarz *et al.* 2002). However, such high-quality food should be rather rare in the field.

Conclusions

From our results we can conclude that *Lophelia pertusa* embryos and planula ascend slowly the first ten days after release, and then show a more steep upward movement the following ten days. After this initial constant upward movement it is still unclear if they reside in the photic zone or stay at the depth indicated by settlement on the oilrigs at 60–130 m depth. The longest period of dispersal, beyond thirty-forty days, should be deeper down, actively searching for suitable habitat for settling. Planulae are potentially long-lived; a full year is not impossible, but perhaps unlikely. Setting a proper mortality rate for the modeling should give an end point sooner than one year.

Future perspectives

These studies has answered some of the questions regarding the dispersal potential and plausible routes for *L. pertusa* planulae and raised many more. The *in situ* timing for spawning to verify that spawning is prolonged over two months, or if concentrated to a peak spawning period still needs to be resolved. The timing for release is important for dispersal, and could also be valuable information for management. For example, one could regulate trawling activities, drilling or dredging, within a certain radius around cold-water coral reefs during spawning season to alleviate negative effects on reproduction from sedimentation and toxic substances.

Whether cnidae discharge is affected by ocean acidification is an important issue to investigate, since successful feeding is necessary for corals to maintain calcification rates under acidified conditions. And we now understand that cnidae plays an important role in larval settling; how this is affected by ocean acidification is also a very important question.

Now that we know how to rear *L. pertusa* larvae successfully (with a reservation for the parasites that needs to be managed) this opens up for running experiments on embryos and larvae, for instance, how these early stages are affected by ocean acidification, or even rearing larvae to juveniles for restoration purposes to replant *L. pertusa* in areas depleted of corals. More needs to be known of the *in situ* cyclicity of environmental conditions to be able to maintain reproductive periodicity in lab. Some of our parental colonies did spawn a second year in lab, but to keep the same corals fit to spawn in lab over several years we need to know more about what in the environmental conditions that maintain the reproductive periodicity.

Since we have not yet managed to rear larvae all the way to settling, there is still a question whether planulae when competent for settling are sensitive to pressure. Some deep-sea organisms are known to demand a certain hydrostatic pressure for successful embryonic development; this has been shown by Young and Tyler (1993) for the deep-sea echinoid *Echinus affinis*. Although *L. pertusa* embryos develop well in lab, and early planulae does not seem bothered by low pressure since they reside just below the surface in culture flasks, barotactic sensitivities could develop toward settling competency to assure that larvae will seek out deeper habitats. On the other hand, *L. pertusa* has such a vertiginous depth range—from 39 m to over 3000 m—that pressure does not seem to be the limiting factor. The poor results in settling probably are due to the late realization of the demand for cryptic habitat, with substrates offering minute crevices. This will be investigated further, if opportunity arises.

ACKNOWLEDGMENTS

First I would like to thank Tomas Lundälv and Lisbeth Jonsson for their work on mapping the *Lophelia pertusa* reefs starting in the late 1990's—without their work I would never have been able to do mine. When first contacting them I was wondering if I could do a Master project on the echiuran *Bonellia viridis*, I had seen that Tomas and Lisbeth had observed them with the ROV. No such projects were at hand, but they had some settling panels that needed attention. And so I began.
Secondly, I would like to thank Kerstin Johannesson for making it possible to get funding for this project. Without her name on the application I would still be sending in grant proposals.
I would also like to thank Roger Johansson and Thore Hilmersson for technical help with my rotating gadgets.
Talking about gadgets, I would also like to send a thanks to the company that helped me build them, the company with the fantastically swinglish name Plastic Produkter AB. Exellent work!

My cnidocyst friend and mentor, Carina Östman, deserves a big warm hug and many thanks for her generosity and enthusiasm letting all her knowledge spill over on me. After 40 years working with cnidocysts she is still childishly enthusiastic! I'm sorry you can't be with me the day of my defense. On the picture below Carina and a friend is standing on the Chinese wall. That's where she was while I was writing, and when it's time for the defense she'll be on her way to Åre to ski, as she does every year. She is the most energetic 77-year-old lady the world ever saw.

My friend and colleague and supervisor Ann Larsson is also worth a big thank you. We complement each other well, and I hope we will continue working together!

Further, I would like to thank Pink Floyd for making such excellent music. Whenever my mind strayed from the work on the thesis, I could turn on Pink Floyd and immediately I would feel just fine where I was. Sivert Høyem and Madrugada is not a bad company while writing either.

There are many more to thank at the lab at Tjärnö, thank you all!

THE COLD-WATER CORAL BREEDER'S HANDBOOK

The present work was depending on the successful rearing of *Lophelia pertusa* larvae, and when it came to keeping the parental colonies happy we were very successful. The corals were maintained in two polycarbonate chamber-sets, with each set divided into four 18 L chambers, equipped with Mississippi-type paddles providing that extra water movement to keep the corals in good shape (fig. 8A-B). It is apparent (from long hours of watching the corals while rearing them) that vigorous water movement is crucial to keeping the polyps fit. When corals have spent a long period in low water turbulence their tentacles become long slender and floppy. The muscles in the tentacles wither. Kept in high turbulence water flow the tentacles get stout and strong, and they get more control of their tentacle movements, which possibly affect their feeding capacity positively. It is difficult to advice on an exact rpm for the paddle rotation; the paddles were turned up to what seemed to be the maximum turbulence the polyps could manage without loosing control of tentacle movements. The suitable rpm is depending on both the condition of the corals, the exact dimensions of the paddles and tanks, and surely also on the specific set of corals in the tank, i.e. their morphology, mode of mounting, etc. factors affecting the hydrodynamics. This extra water movement also seemed to add the environmental factor that stimulated the corals to display what appeared to be a natural spawning behavior, and provided us with a plentitude of gametes.

The design of the chambers was inspired from an experimental set-up previously done by our colleagues from Jacobs University in Bremen (Annika Moje, Hannes Wagner, Majk Dressel and Torsten Behnke). The set-up was then designed to provide sealable chambers, where the Mississippi-paddles were to govern the internal water circulation while flow-through of water was turned off over-night, to be able to measure oxygen consumption in corals exposed to drill-cuttings from offshore oil extraction (Larsson *et al.* 2013). During that experiment, the first successful rearing of larvae was achieved in 2009, accidentally, as my supervisor Ann Larsson in the sealed-up chambers found gametes one morning while preparing for oxygen measurements. This encouraged us to apply for funding for the present PhD project and try to rear *L. pertusa* planulae to be able to conduct experimental work such as settling experiments, and general mapping of embryo and larval development. The tank design was modified to suit the new purposes, the chambers were made larger, and the back walls were made black to make it easier to see the small whitish eggs.

Experimental set-up for settling

The experimental set-up for the planned settling experiment consisted of 18 rotating 1 L beakers with submerged substrates on holders, originally designed by myself for this project. Small pieces (~10×10 mm) of four different substrates were glued onto glass slides and then conditioned in flow-through of seawater to get a cover of bacterial film (fig. 8C-E). There were double controls: control #1 with glass slides without substrates, and control #2 with substrates but no additional treatment. The treatments consisted of added food (fine fraction of centrifuged copepod homogenate) and/or adult signal (water from the tanks with adult corals). We wanted to know the prerequisites and cues for the planulae to achieve metamorphic and settling competency. This experimental set-up was not sufficiently tested to be able to evaluate the effectiveness of the set-up. And the rearing of *L. pertusa* larvae proved to be a bigger challenge than rearing the adult corals.

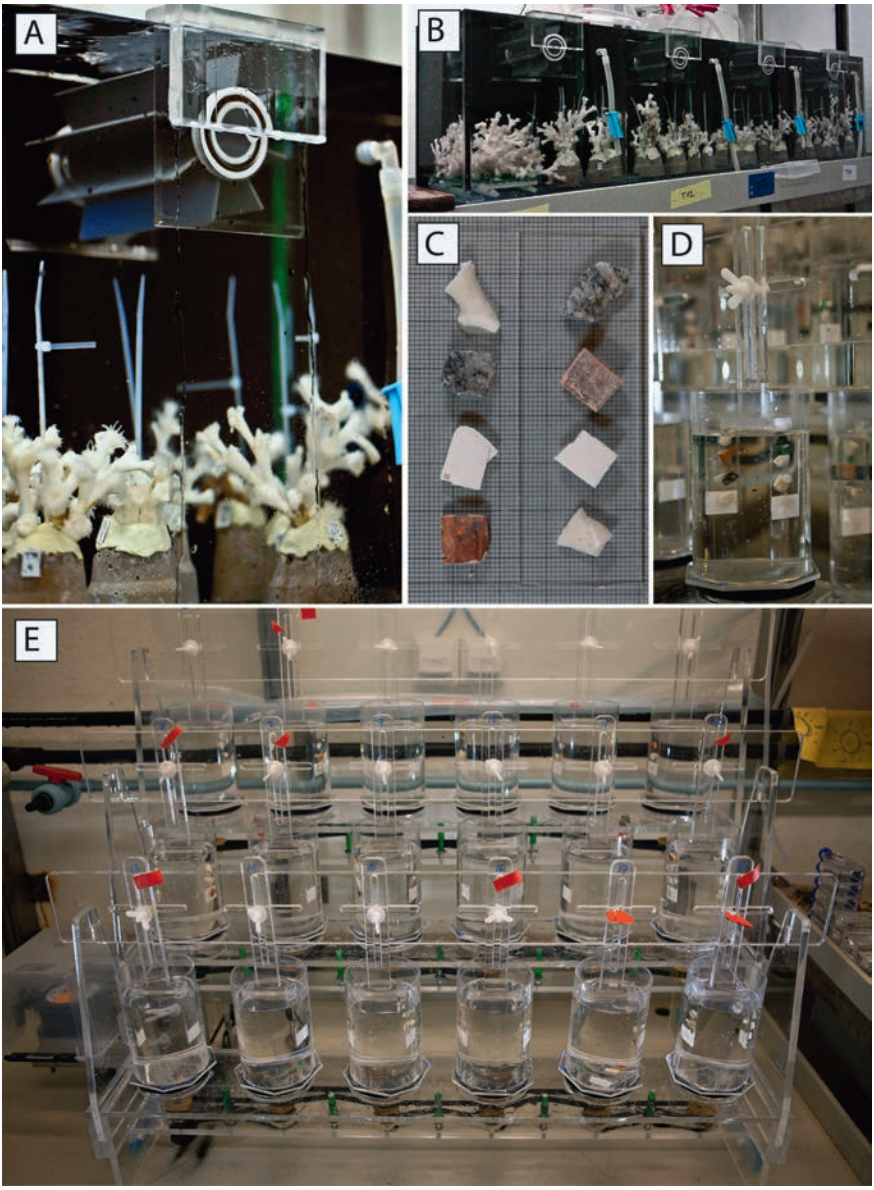

Fig. 8 – **A**. The much-appreciated (by the corals) Mississippi-type paddles to increase water turbulence in the aquaria. – **B**. The parental colonies were maintained in a chamber-set with four separated chambers (18 L), each equipped with a Mississippi-paddle to govern water movement. – **C**. The settling experiment aimed at testing four different substrates: coral skeleton, bivalve shell (*Arctica islandica*), stone, and a piece of ceramic tile. The glass slides with either plain glass, polylysine-coated glass, frosted area, and the thin space between the glass slides mounted in pairs, and the walls of the polycarbonate beakers provided additional substrates. – **D**. Two pairs of glass slides were submerged in the beakers. – **E**. The full set-up of the settling experiment consisted of 18 rotating 1 L beakers. Six treatments, three replicates each, were divided over the beakers.

The Cold-Water Coral Breeder's Nightmare

The gametes, embryos, and planulae were kept in filtered seawater (50 + 5 µm Ametek polypropylene cartridges) at 7–8°C, and a salinity of 33–35 psu. The water intake for the seawater flow-through system is situated at 45 m in the Koster Fjord, and thus sensitive to downwelling of less saline surface waters during hard weather. After a period of strong wind the salinity could drop to 28–29 psu. The water thus had to be spiked with additional salt occasionally to maintain suitable salinity. This water treatment was usually sufficient for keeping the planulae in good condition; however, over the years we had an increasing problem with parasites in our larval cultures. A shift to a 1-µm filter cartridge was tested, but the planulae did not develop well in that water. In addition, running a settling experiment it is crucial to condition the substrates to get a bacterial film, as proven by several studies on settling in tropical coral planulae.

Usually, in cultures of marine invertebrate larvae, it is ciliates that cause problems. But the two different parasites observed in these cultures were other types of protozoans. A flagellate was seen probing the planulae, using its flagella as a mosquito's feeding tube (proboscis), inserting it into the planulae. This parasite was rare, and did not seem to cause much damage. There was a more insidious parasite that proved to be able to cause considerable damage, wiping out culture flask after culture flask of planulae. This parasite had no visible cilia or flagella; it was a spherical entity with granular interior and a dented surface (fig. 9A-B). It was observed to move over the planula ectoderm, nibbling off the cilia, leaving the planulae incapacitated, with short and dysfunctional cilia. As a second step, the parasites were sinking into the larval ectoderm. The parasites then proliferated inside the planulae, sometimes causing abnormal growth (fig. 9C-F). Swarms of small parasites were later seen leaving the infested planulae. The parasites were captured in a SEM preparation (fig 9E-F), but the high-resolution SEM images did not reveal any cilia or other distinct characters that could give a clue of what kind of protozoan it was. These parasites were present already in 2013, but did not cause major problems until the spawning season in 2015. They probably thrive in the bends of the water pipes supplying the seawater flow-through, where organic matter and sediments builds up.

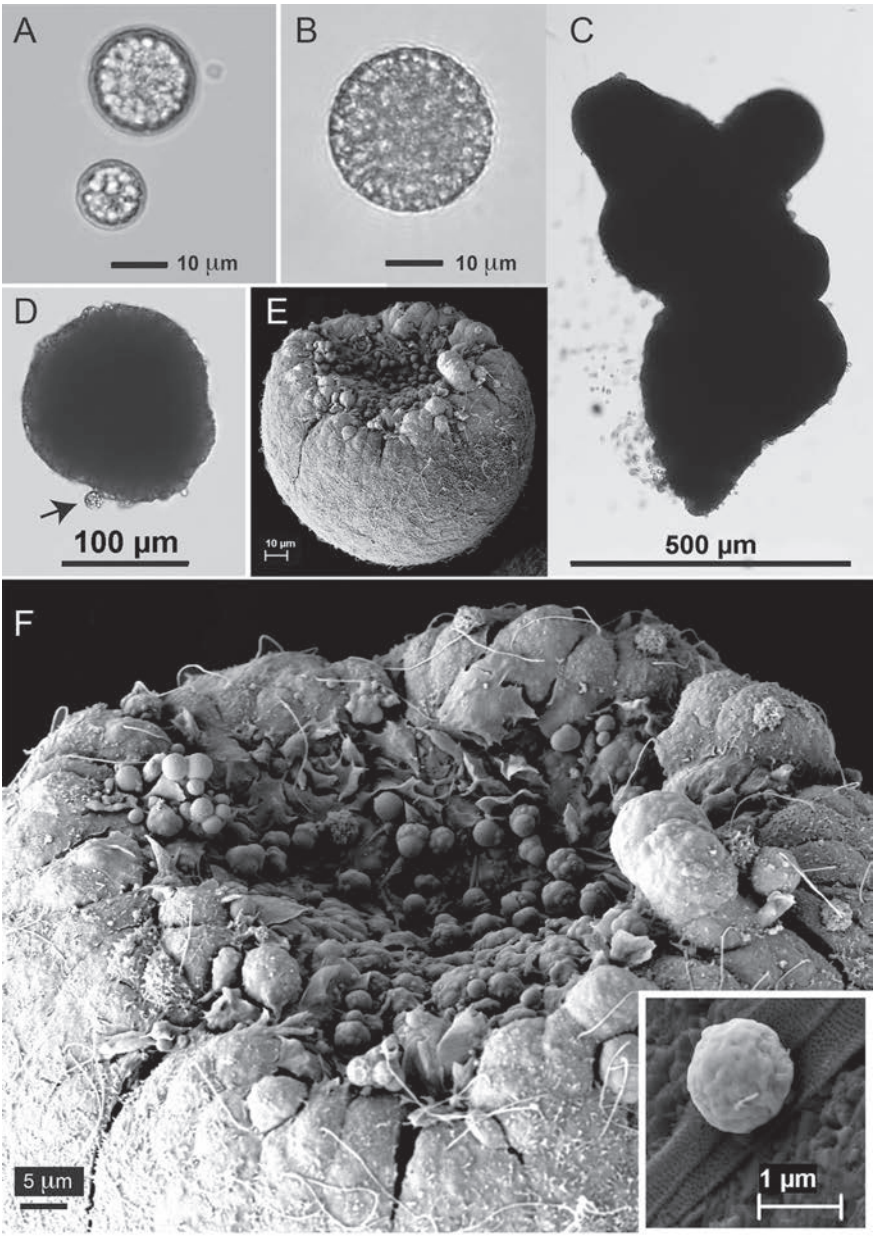

Fig 9 – **A-B.** The unidentified protozoan parasite under light microscopy. – **C.** An infested planula showing abnormal growth. – **D.** A planula in an early stage of infestation; a parasite is seen on the ectoderm, close to the oral pore (arrow). – **E-F.** A SEM preparation of an infested planula, the oral pore looks like a crater filled with small parasites. Two large parasites are seen at the edge of the crater to the right. The inset in the bottom right corner shows a parasite with what seems to be a cilia being phagocytized.

"If you build it they will come"

ECOLOGICAL RESTORATION

This doctoral project initially included a restoration experiment, and although initiated during the project period, it was not ready for evaluation within the project time frame. The aim for this part of the studies was to test if one can enhance settling rates of associated fauna by the design of the artificial reefs (AR), and/or the mode of deployment (i.e. solitary or clustered ARs). Three different designs of concrete AR units were built: 1) simple cubes; 2) irregular lumps; and 3) units with pinnacles (fig. 10). The simple cubes were controls, and the two following were to test the level of complexity necessary to enhance settling. Forty-five of these units were constructed, three of each type was deployed solitary, and three groups of four units deployed as clusters to see if nearness of units could enhance settling. The upstream unit were hypothesized to affect the downstream settling by creating eddies that would favor settling. The surfaces of the ARs were also enhanced by the inclusion of pebbles and shells to create many microhabitats.

On the ARs fragments of live corals were transplanted to test for coral growth on the different designs, and if growth were affected by transplanting corals in groups or as satellites (solitary fragments). Corals were stained with Alizarin that gets incorporated into the skeleton during incubations, and will be seen as pink bands on the fragments. New growth will be of normal coral color and can easily be measured from images.

The quote at the top of this page comes from the novel *Shoeless Joe* by the author W.P. Kinsella. The novel was adapted for the screen by Phil Alden Robinson in the 1989 drama *Field of Dreams*, starring Kevin Costner. The original quote was: "*If you build it, he will come*", alluding to the ghost of a famous baseball player that would come and play on a baseball field—if built by the farmer on his cornfield. The quote has later been adopted and adapted by researchers working in the field of restoration ecology, e.g. Palmer *et al.* (1997), to exemplify the *Field of Dreams hypothesis*. In essence the hypothesis is that if you get the conditions right, propagules will come and settle in the restored area.

With the complex design of the ARs I wanted to mimic the complexity of a reef, so that any transplanted corals would be sufficiently elevated from the boundary layer and that the AR would function as the dead parts of a reef, offering habitats for reef associated fauna until the coral transplants grows up to a functioning reef.

At revisits to check how the settling progressed, and document coral transplant survival and growth; it was obvious that the experiment would take longer than the project time frame due to the dense population of feather stars at the chosen site at Saekken (fig. 11). The feather stars *Hathrometra tenella* (Crinoidea, syn. *H. sarsii*) are mobile animals that settle on any protruding object on the seafloor. When other fauna finally manage to settle and establish themselves on the surfaces, the feather stars usually withdraw to peripheral spots on the objects (personal observation). At the time of revisits, however, they were still covering the entire AR units.

To be continued.

Fig 10 – Images of the artificial reefs (ARs): AR with pinnacles, simple cubes, and medium complex irregular lumps (thanks to Mårten Duvetorp for the fantastic design of this particular one). The casting of these concrete lumps were a big job, and I was grateful to get help from Mårten, Daniel Johansson, and my internship student Laurence de Clippele. Deployments were done from the R/V Nereus with the help from the skippers and technical staff at Sven Lovén Center. The pink coral transplants are stained with Alizarin.

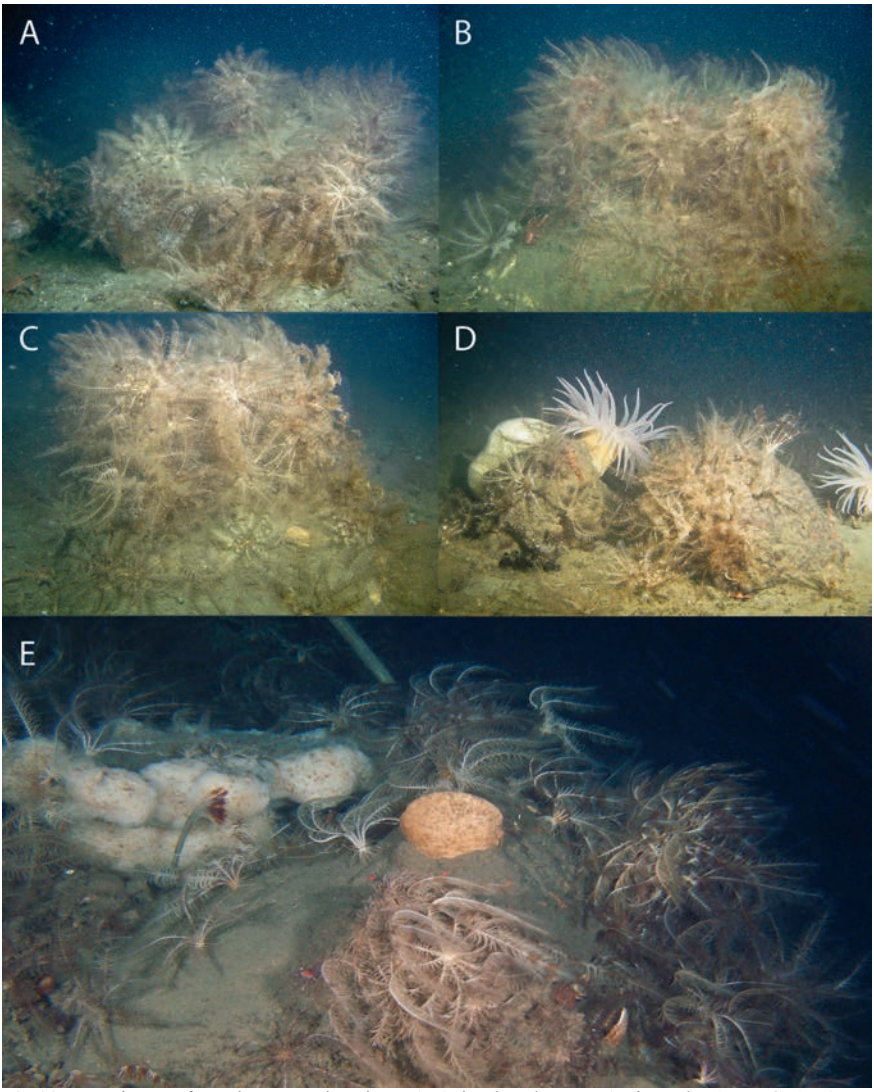

Fig 11 – UW-images from the restoration site. – A. Asimple cube. – B. An irregular AR. – C. An AR with pinnacles. – D. Natural rock habitat for comparison. – E. Close-up of an irregular AR with sponges, a *Sabella pavonina* (polychaete), plenty of crinoids (feather stars), and a small crustacean.

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