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Effect of the temperature on cold-water coral holobiont in the North-East Atlantic Ocean

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Sorbonne Université

École doctorale des Sciences de l'Environnement d'Ile de France (ED 129)

Laboratoire d'Ecogéochimie des Environnements Benthiques (UMR 8222)

Effect of the temperature on cold-water coral holobiont in the North-East Atlantic Ocean

Thèse préparée et présentée par

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Abstract

Cold-water corals such as *Lophelia pertusa*, a key reef-building species distributed worldwide, provide highly valuable habitats for diverse biological communities. They thrive in water characterized by year-round temperatures $\leq 14^{\circ}\text{C}$, and are therefore thought to be particularly threatened by ocean warming. Recent studies have characterised cold-water coral ecology and showed degraded health status in response to temperature changes. However, it is still not clear if populations from different oceanic regions, living under different temperature conditions (i.e., 10°C in the Atlantic and 13°C in the Mediterranean Sea), respond in similar ways to temperature changes. The general objective of this thesis was to investigate the effects of temperature on NE Atlantic populations of *L. pertusa* and to test if they share the same thermal ecological niche as Mediterranean populations. In the first section of the thesis (Chapters 2 and 3) we conducted experiments to study the response of *L. pertusa* holobiont to temperature changes at different time scales. We determined the holobiont's response by describing the microbiome's diversity and function and the coral's physiology (survival, growth, nutrition, gene expression). These experiments showed that NE Atlantic *L. pertusa* exhibited a large mortality when temperature increased beyond $+3^{\circ}\text{C}$. Before death, the corals showed clear signs of stress, and their microbiome changed (dysbiosis) with the invasion of pathogenic bacteria. Inversely, lower water temperature did not affect *L. pertusa* health conditions. In the second part of the thesis, we conducted *in situ* studies aiming at increasing our understanding of the reproduction (Chapter 4), the microbiomes (Chapter 5) and the growth (Chapter 6) of cold-water corals from the NE Atlantic Ocean and the Mediterranean Sea, to ultimately find out if climate change would impact differently these two populations. The study of the coral's reproductive biology in the Mediterranean Sea suggested that *L. pertusa* spawns seasonally from autumn to early winter, when episodes of dense water cascading are more likely, while *Madrepora oculata* showed continuous reproduction, as earlier identified for other populations. Another difference between the two coral species is that *L. pertusa* had a more variable microbiome in both the NE Atlantic and the Mediterranean Sea, with high spatial variability within canyons. Inversely, *M. oculata* exhibited stable microbiomes across the different regions characterized by a strong association with the bacteria *Endozoicomonas*. Finally, our first *in situ* estimations of *L. pertusa* growth in a canyon of the Bay of Biscay showed that they grow slower than in the Mediterranean Sea. Overall our findings show a very high sensitivity of Atlantic *L. pertusa* to $+3/+4^{\circ}\text{C}$ increase in temperature, suggesting that they have a lower upper thermal limit than their Mediterranean counterpart. Considering the high sensitivity of *L. pertusa* from both populations, they appear to have a low resilience and limited long-term capacity for adaptation to increasing temperature in the context of global climate change.

Keywords: *Lophelia pertusa*, Ocean Warming, Microbiome, Physiology, Thermal niche, Atlantic Ocean and Mediterranean Sea

Résumé

Les coraux d'eau froide tels que *Lophelia pertusa*, une espèce clé constructrice de récifs, constituent des habitats importants pour diverses communautés biologiques. Ils se développent dans des eaux $\leq 14^{\circ}\text{C}$, et sont donc considérés comme particulièrement menacés par le réchauffement des océans. Des études récentes ont caractérisé l'écologie des coraux froids et ont montré une dégradation de leur état de santé en réponse aux changements de température. Cependant, il n'est toujours pas clair si les populations de différentes régions océaniques, vivant dans des conditions de température différentes (10°C en Atlantique et 13°C en Méditerranée), répondent de manière similaire aux changements de température. L'objectif général de cette thèse était d'étudier les effets de la température sur les populations de *L. pertusa* de l'Atlantique Nord-Est et de vérifier si elles partagent la même niche écologique thermique que la population méditerranéenne. Dans la première partie de la thèse (Chapitres 2 et 3), nous avons mené des expériences pour étudier la réponse de l'holobionte *L. pertusa* aux changements de température à différentes échelles de temps. Nous avons déterminé la réponse de l'holobionte, au niveau de la diversité et des fonctions du microbiome, et de la physiologie du corail (survie, croissance, nutrition, expression des gènes). Ces expériences ont montré que *L. pertusa* de l'Atlantique Nord présentait une forte mortalité lorsque la température augmentait au delà de $+3^{\circ}\text{C}$. Avant de mourir, les coraux ont montré des signes évidents de stress, et leur microbiome a changé (dysbiose) avec l'invasion de bactéries pathogènes. Inversement, la baisse de la température de l'eau n'a pas affecté leur état de santé. Dans la deuxième partie de la thèse, nous avons mené des études *in situ* visant à améliorer notre compréhension de la reproduction (Chapitre 4), du microbiome (Chapitre 5) et de la croissance (Chapitre 6) des coraux d'eau froide de l'océan Atlantique Nord-Est et de la mer Méditerranée, afin de déterminer si le changement climatique aurait un impact différent sur ces deux populations. L'étude de la biologie reproductive en mer Méditerranée suggère que *L. pertusa* se reproduit de manière saisonnière entre l'automne et le début de l'hiver, lorsque les épisodes de cascades d'eau dense sont plus probables, alors que *Madrepora oculata* présente une reproduction continue, comme cela a déjà été identifié pour d'autres populations. Une autre différence entre les deux espèces est que *L. pertusa* avait un microbiome plus variable à la fois dans l'Atlantique Nord-Est et dans la mer Méditerranée, avec une grande variabilité spatiale au sein des canyons. Inversement, *M. oculata* présente des microbiomes stables dans les différentes régions, caractérisés par une forte association avec la bactérie *Endozoicomonas*. Enfin, nos premières estimations *in situ* de la croissance de *L. pertusa* dans un canyon du golfe de Gascogne ont montré que leur croissance est plus lente que dans la mer Méditerranée. Dans l'ensemble, nos résultats montrent une très grande sensibilité de *L. pertusa* de l'Atlantique à une augmentation de $+3/+4^{\circ}\text{C}$ de la température, ce qui suggère qu'ils ont une limite thermique supérieure plus basse que leurs homologues méditerranéens. Compte tenu de la grande sensibilité des deux populations, elles semblent avoir une faible résilience et une capacité limitée d'adaptation à long terme au réchauffement dans le contexte du changement climatique.

Mots clés : *Lophelia pertusa*, Réchauffement, Microbiome, Physiologie, Niche thermique, Océan Atlantique et mer Méditerranée

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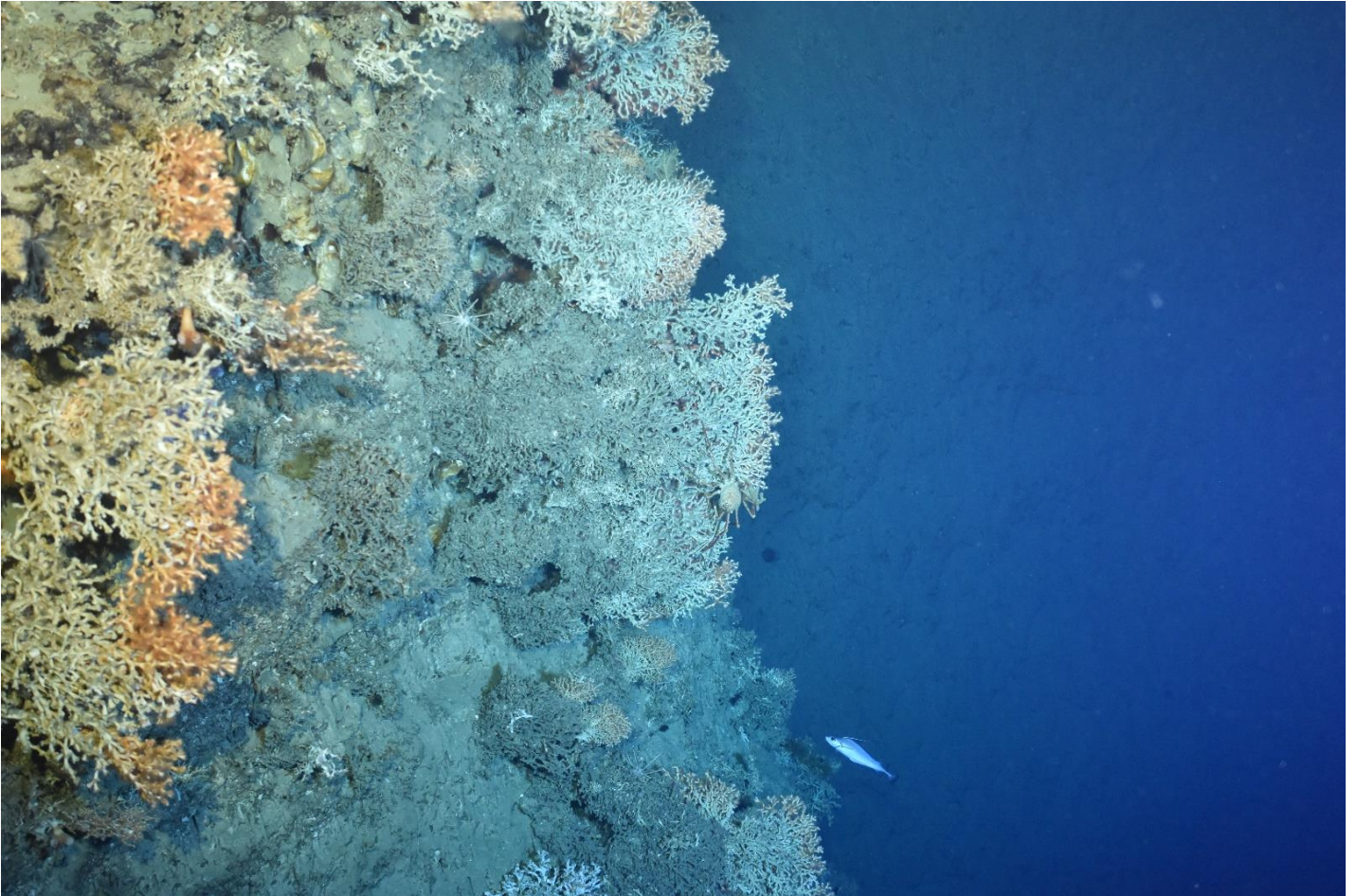
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List of abbreviations

AA - arachidonic acid
ALOX12B_11 - Arachidonate 5-lipoxygenase
AMEX - Laboratory of Adaptations to Extreme Environments
ANOSIM - Analysis of similarities
ASH - Aragonite saturation horizon
ASV - Amplicon sequence variants
AUV - Autonomous underwater vehicle
BCO1 - Beta,beta-carotene 15,15'-monooxygenase
CA - Carbonic anhydrase
CA10 - Carbonic anhydrase- protein 10
Ca²⁺ - Calcium ions
CaCO₃ - Calcium carbonate
CO₃²⁻ - Carbonate ions
CASP9 - Caspase interleukin-1 beta converting enzyme (ICE) ues
CHEK1_2 - Serine/threonine-protein kinase Chk
CO₂ - Carbon dioxide
CWC - Cold-water corals
DNA - Deoxyribonucleic acid
EBSD - Electron Back-Scatter Diffraction
EDTA - Ethylenediaminetetraacetic
ENACW - Eastern North Atlantic Central Water
H₂O₂ - hydrogen peroxide solution
HCO₃⁻ - bicarbonate ions
HMCN1_47 - Hemicentin-1
HSBP1 - Heat shock factor-binding protein 1
IPCC - The Intergovernmental Panel on Climate Change
ISOW - Iceland–Scotland Overflow Water
KEGG - Kyoto Encyclopedia of Genes and Genomes
K-W - Kruskal-Wallis
LBBM - Laboratoire de Biodiversité et Biotechnologies Microbiennes
LECOB - Laboratoire d'Ecogéochimie des Environnements Benthiques
LSW - Labrador Sea Water
Lfc - Log fold change
MOW - Mediterranean Outflow Water
NE - North-east
NGS - Next generation sequencing
nMDS - Non-metric multidimensional scaling
NADW - North Atlantic Deep Water
NOS1 - Nitric oxide synthase
PCoA - Principal Coordinates Analysis
PCR - Polymerase chain reaction

Permanova - Permutational multivariate Anova
pH - potential hydrogen
pval - p-value
POC - Particulate organic carbon
RAD54B_19 - DNA repair and recombination protein rad54b
RCP - Representative Concentration Pathways
RNA - Ribonucleic acid
ROS - Reactive Oxygen species
ROV - Remotely operated vehicle
R/V - Research vessel
SCTLD - Stony coral tissue loss disease
SEM - Scanning electron microscope
Sp. - Species
SPTLC2_5 - Serine palmitoyltransferase 2
SSP - Shared Socio-Economic Pathways
T1SS - Type I Secretion System
T3SS - Type III Secretion System
TLR - Toll-like receptor
TLR2 - Toll-like receptor 2
TNF - Tumor necrosis factor
TNFAIP6 - Tumor necrosis factor-inducible protein 6 protein
UK - United Kingdom
uvrD2 - ATP-dependent exoDNAse (exonuclease V) alpha subunit

Chapter one: Introduction



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1.1 From exploration to exploitation: human impacts on the deep ocean

The deep ocean defined as being below 200 m depth bridges the continental shelf and slope (Tyler, 2003). It is composed of different ecosystems such as continental margins (which host submarine canyons and cold seeps), abyssal plains, seamounts and mid-ocean ridges where hydrothermal vents are found (Ramirez-Llodra et al., 2010). The deep ocean covers 95 % of the total volume of the oceans and yet is considered the least explored biome (Tyler, 2003). However, deep oceans play an essential role in Earth's climate regulation, and they are a significant sink for atmospheric carbon dioxide (CO₂) since they have absorbed nearly 50 % of the anthropogenic CO₂ (Sabine et al., 2004) and stored more than 35 % of the Earth's uptake of heat since 1970 (Gleckler et al., 2016). Furthermore, the deep ocean enables the proper functioning of marine ecosystems, which rely on nutrient cycling, carbon sequestration, productivity, habitat support and nursery grounds, trophic support, and it provides many ecosystem and ecological services (Thurber et al., 2014).

Unfortunately, the deep ocean is under several threats due to anthropogenic activities, including exploitation, disposal, and climate changes (reviewed in Ramirez-Llodra et al., 2011), which are increasingly affecting physical, chemical, and biological conditions in the deep ocean, harming deep-sea communities (Cooley et al., 2022).

1.1.1 Impacts of human activities on the deep ocean

Amongst the threats to the deep ocean, bottom trawling and bottom longlines are the most destructive forms of deep-sea fishing in the world, causing substantial ecological impacts throughout the world's oceans (Clark et al., 2016). Since the collapse of shelf fishes' stocks due to overexploitation, deep-sea fisheries have been increasingly expanding (Morato et al., 2006). With the emergence of more powerful engines, robust materials, and precise mapping, deep-sea trawlers can now operate in submarine canyons over rough seafloor (Roberts et al., 2004). There is evidence that deep-sea trawling causes considerable damage to the seabed *via* direct changes of the substrate and topography of the seabed as well as resuspension of sediment (Clark & Koslow, 2007). In addition to damages to fish populations and by-catch species, it also causes rapid and persistent destruction of deep-sea benthic communities, especially sessile communities associated with biogenic habitats formed by cold-water corals (CWCs) and sponges. Extensive damage on CWC reefs has been reported worldwide off North America,

Australia and New Zealand and off European coasts from Norway to Spain (Roberts et al., 2004). Thus, between one-third and one-half of deep-sea reefs have been damaged or destroyed by trawling in Norwegian waters (Fosså et al., 2002). Accordingly, deep-sea fishing below 800 m was banned in 2016 in all European waters, and legislation has just been strengthened for the protection of 87 sensitive areas of vulnerable marine ecosystems below 400 m depth in the NE Atlantic Ocean. However, considering the deep-sea species' slow growth and long-life spans, the impacts of deep-sea fisheries are almost irreversible.

Marine litter, including illegal disposal, abandoned fishing gear, and more generally wastes, is estimated to represent 4.8 to 12.7 million tonnes dumped into the oceans yearly (Jambeck et al., 2015). Among marine litter, plastic pollution is one of the key challenges of our century as they are responsible for major environmental impacts worldwide from coastlines down to the deep ocean (Woodall et al., 2014), including in the submarine canyons (e.g., Schlining et al., 2013) and hadal trenches (Peng et al., 2020). Due to ocean circulation, vertical transport, and cascading processes, the deep ocean represents a significant depository and ultimate sink for macro and microplastics (Peng et al., 2020; Harris et al., 2021). Plastic pollution now exposes habitats and deep-sea faunal communities to chemical and ecotoxic risks as plastic may be a transport vector for pollutants (Abd-Aziz et al., 2019) or pathogenic agents (Amaral-Zettler et al., 2020). Plastics also constitute physical hazards *via* direct ingestion, covering and entanglement, and they can alter feeding behaviour (Mason et al., 2022) and bioaccumulate within the food web.

In addition, oil and gas extraction have extended to deeper environments, increasing the risks of accidental oil spillage, which dramatically threatened communities (e.g., Deepwater Horizon accidental spillage) (Ramirez-Llodra et al., 2011). Drilling activities threatened deep-sea benthic communities from the installation of the infrastructure by sediment resuspension plumes, anchors deployment, and laying pipelines to discharges of disposal waste and oil-based drilling muds and fluids, including produced and sewage waters and chemicals (Cordes et al., 2016). Impacts on deep-sea communities can result from physical and chemical disturbances, and can persist in time and space, particularly affecting vulnerable ecosystems, such as CWCs (Purser & Thomsen, 2012; Larsson et al., 2013).

Concurrently, during the last decades, deep-sea mineral resources (manganese nodules, cobalt-rich crusts, and polymetallic sulphide deposits) have been investigated for commercial exploitation (Glasby, 2000), although the scientific community agrees that deep-sea mining could ultimately be the most critical and large-scale anthropogenic threat to the deep-sea (Ramirez-Llodra et al., 2011). Among the impacts of deep-sea mining, the removal of the near-

surface sediments will lead to the death of all organisms living on the mined surface of the seabed and the burying of the communities in the vicinity of the mined area caused by sediment plumes (Miller et al., 2018). In addition, Levin et al. (2016) suggest that cobalt-rich crust mining on seamounts may disrupt pelagic species aggregations due to the removal of the benthic communities they rely on. The complete removal of substrate from seamounts will also comprise the removal of sessile species, such as CWC reefs that can concentrate on seamounts and form habitats and refuges for many associated species (Freiwald, 2002).

Last but not least, the deep ocean is particularly threatened by ongoing global changes (Levin & Le Bris, 2015). The deep ocean has already absorbed a quarter of the carbon released from anthropogenic activities (Sabine and Feely, 2007). The ongoing warming has penetrated at least down to 700 m depth over the last 40 years, and one third of the observed warming occurred in the ocean's 700-2000 m layer (Levitus et al., 2012). Since the 1970s, global warming has resulted in a temperature increase of 0.11°C *per* decade in the surface layer (0-75 m), while at 700 m, the increase was about 0.015°C (Levitus et al., 2012). Even though Brito-Morales *et al.* (2020) showed that in the last 50 years, mesopelagic layers (200-1000 m) were warming slower than bathypelagic layers (1000-4000 m), they reported that projected climate velocities would be even faster in deep environments than at the surface. Since the deeper ocean is considerably contributing to heat sequestration (Talley et al., 2016), these results suggest that deep ecosystems and communities, which are already threatened by global warming, will be even more exposed in the future decades. Sweetman et al. (2017) thus modelled increased temperatures of up to 4.4°C and lowered pH between -0.01 to -0.37 in bathyal waters (200-3000 m) from the Atlantic Ocean by 2100 (Figure 1.1). Concurrently, the increase in CO_2 , responsible for ocean acidification, is also responsible for the shoaling of the aragonite saturation horizon (ASH) (Doney et al., 2009). The ASH corresponds to the depth at which aragonite, a polymorph of calcium carbonate (CaCO_3), is in thermodynamic equilibrium (saturation state of aragonite $\Omega_{\text{arg}} = 1$). When $\Omega < 1$, water is undersaturated, and carbonate dissolution is favoured over calcification (Gattuso et al., 1998), likely affecting calcification processes and capacities of calcifying organisms (Orr et al., 2005). Since the saturation state is already naturally lower in deep waters and cold high-latitude regions, deep-sea communities, already experiencing a lower saturation state, are susceptible to live in aragonite undersaturated waters by the end of the century. Finally, deoxygenation is predicted to have a greater effect in intermediate waters from 100 to 1000 m by 2100 (Stramma et al., 2012; Bopp et al., 2013).

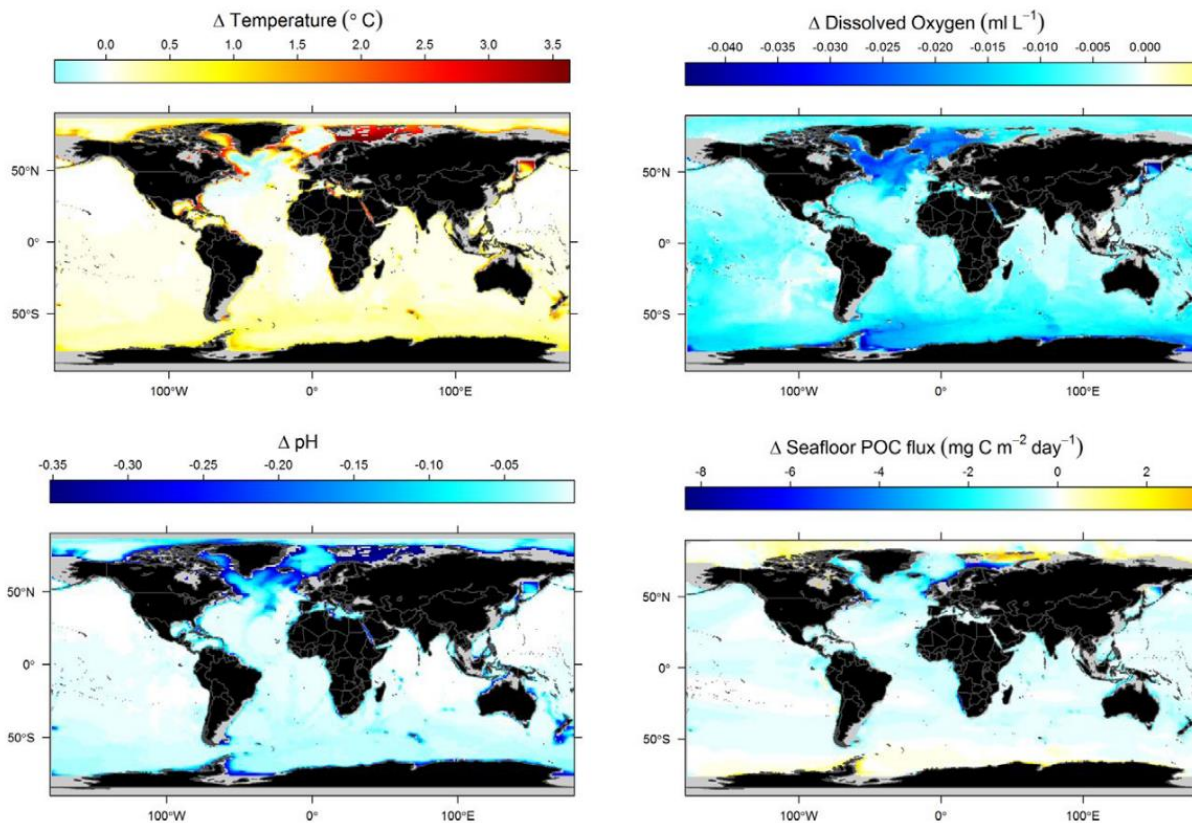


Figure 1.1. Modelled environmental changes (temperature, dissolved oxygen, pH and seafloor particulate organic carbon (POC) flux) at the deep seafloor at the end of the century relative to present-day conditions (based on data generated by Earth Systems Models as part of the Coupled Model Inter-comparison Project Phase 5 (CMIP5, Tyler et al., 2012). Figure from Sweetman et al. (2017).

1.1.2 Submarine canyons under threat

Within deep-sea habitats, submarine canyons are “keystone structures” playing major ecological roles (reviewed in Fernandez-Arcaya et al., 2017). They are hotspots of benthic biomass, offering privileged habitats for numerous reef-forming species, such as CWCs, bryozoans, sponges and oyster banks. Indeed, submarine canyons present complex and heterogeneous topography, creating specific hydrological processes, such as intensified mixing and advection of water masses, internal waves, and dense shelf water cascading, which favour particle transport from the productive coastal zone down to continental slopes and deep waters. These hydrodynamic features enhance transport of particulate organic matter, marine litter, and coarse material from the shelf to deeper waters (Canals et al., 2006), benefiting benthic communities. However, submarine canyons are particularly exposed to global changes. As climate changes may affect and modify the intensity of currents (Canals et al., 2009), decrease cascading events (Canals et al., 2006), and reduce surface productivity (Steinacher et al., 2010),

this could impair nutrient supply through POC, to deep benthic communities. Predictions suggest that a reduction in organic matter supply in the deep sea will result in a reduction of the benthic biomass worldwide and important deep-sea habitat, including CWC reefs (Jones et al., 2014). For example, 85 % of submarine canyons and over 90 % of regions with reef-forming CWCs are likely to experience declines in benthic biomass in the next century (Jones et al., 2014).

All these human-related threats are already co-occurring, if not, they will be in deep environments in the near future (Figure 1.2). Deepening acidification, warming and expanding deoxygenation are likely to interact, influence each other and have antagonistic, additive or synergistic effects on living organisms, potentially altering their physiology (Pörtner & Langenbuch, 2005) and ecosystem functioning. Ongoing warming and deoxygenation may lead species to seek deeper refugia, while shallowing of ASH may lead them to seek shallower habitats. Considering the unprecedented speed of current environmental changes, we enter unknown territories regarding the evolution of future changes, including biodiversity loss.

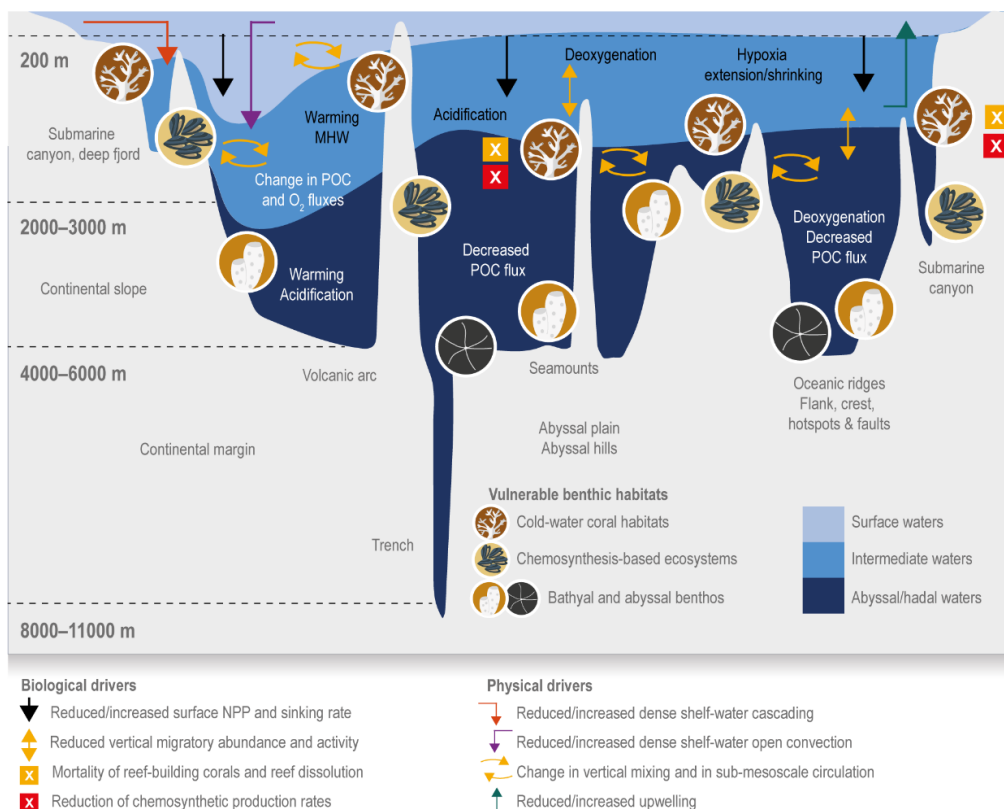


Figure 1.2. Schematic representation of the combination of climate-induced drivers in different deep-ocean ecosystems, inducing changes in key physical and biological processes in the deep-sea and benthic habitats with specific vulnerabilities. Figure from Cooley et al. (2022).

1.2 Cold-water corals

1.2.1 Anatomy, taxonomy & diversity of cold-water corals

Corals belong to the class Anthozoa of the phylum Cnidaria, defined by the presence of cnidocysts (i.e., stinging cells), used for predation, but also to protect the organism from predators. Colonial corals are composed of an assembly of similar units called polyps. A coral polyp is organized by only two cell layers, an outer ectoderm (epiderm) and an inner endoderm (gastroderm), separated by an acellular matrix, the mesoglea. The coelenteron is the gastrovascular opening to seawater connected to a mouth surrounded by tentacles by the actinopharynx. Cells are not organized into organs but are rather specialized and clustered in the epiderm or gastroderm according to their function. For example, calicoblastic cells form the epithelial layer of cells, which will excrete the aragonitic skeleton of hard corals (scleractinians). At the same time, unicellular glands in the gastroderm are responsible for enzyme and mucus excretion to allow digestion. Colonial polyps are connected by the coenosarc, composed of an oral epithelium, within which the cnidocysts are concentrated in the oral ectoderm, and an aboral epithelium, where calicoblastic cells concentrate in the aboral ectoderm (Allemand et al., 2004).

Over half of the approximately 5100 known coral species described in the world's ocean lives in the deep ocean (Roberts et al. 2009). Cold-water corals include scleractinia (reef-forming, stony corals), antipatharia (black corals), zoanthidae (encrusting or button polyps), pennatulacea (sea pens), alcyonace (octocorals, i.e., soft corals and gorgonians). The diversity of scleractinian CWCs is great and encompasses a majority of solitary CWCs, such as *Caryophyllia huinayensis*, *Tethocyathus endesa*, *Javania cailleti* or the best known cosmopolitan *Desmophyllum dianthus*, but also some colonial reef-building species, such as *Dendrophyllia cornigera*, *Lophelia pertusa* (now named *Desmophyllum pertusum*, Addamo et al., 2016), *Madrepora oculata*, *Solenosmilia variabilis*, *Enallopsammia rostrata*, *Oculina varicosa* or *Goniocorella dumosa* (Freiwald et al., 2004). Among scleractinian CWCs, *L. pertusa* and *M. oculata* are the most emblematic hermatypic species distributed worldwide (Figure 1.3). The complex 3D structures of *L. pertusa* colonies may reach 2 m high and 1.5 m large (Shroeder, 2002; Freiwald, 2002), and are formed by large polyps (~9 mm high and ~15 mm diameter). *Madrepora oculata* forms typical and fragile zig-zag shaped branches of smaller polyps (~6 mm high and 3 mm diameter). Both decalcification and micro CT scans have revealed that *L. pertusa* polyps are connected by the coenosarc and by a thin canal of

mesenterial filaments, while in *M. oculata*, polyps are only connected through the coenosarc (Figure 1.3, Lartaud et al., 2019; Fabri et al., 2022). These two species often live in close association with each other, growing in the same regions and sometimes forming chimera-like colonies (Corbera et al., 2019; Lartaud et al., 2019). Although *M. oculata* was thought to form secondary reef frameworks compared to *L. pertusa* (Freiwald, 2002), the dominant presence of one species can alternate depending on geographical location across oceans and within specific areas (Arnaud-Haond et al., 2015), depending on physical, geochemical, and biological factors, determining their distribution.

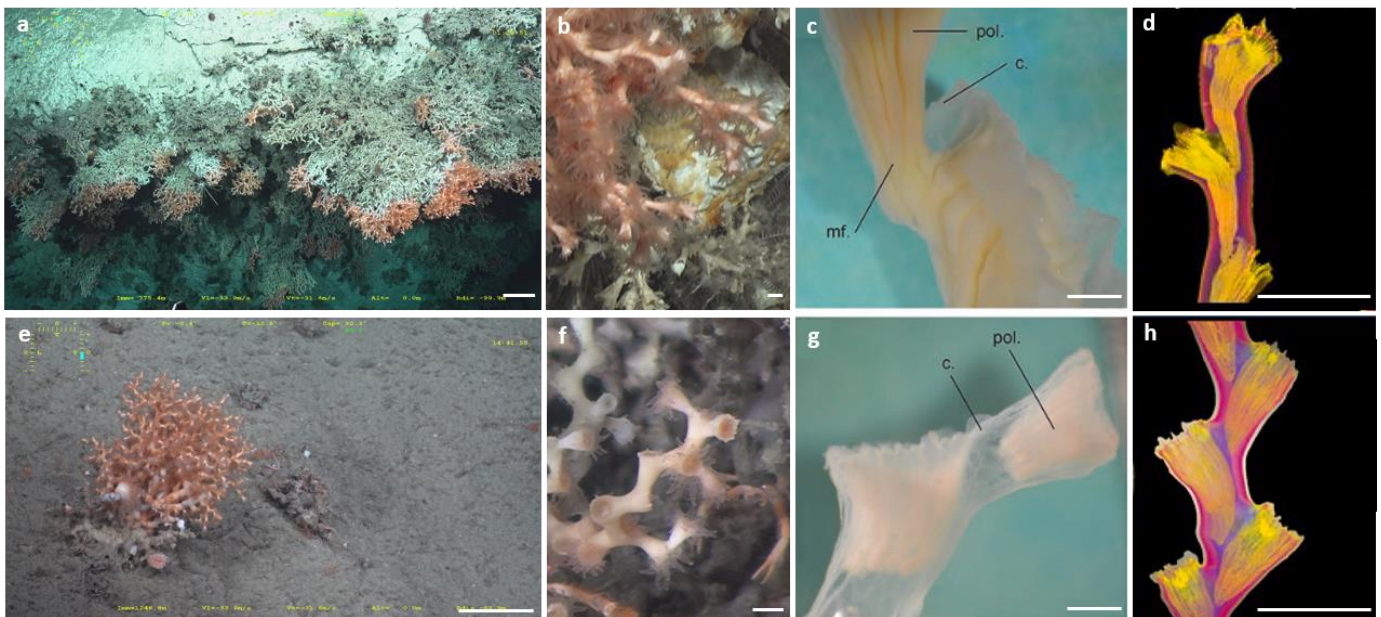


Figure 1.3. Picture representing *L. pertusa* reef (a), polyps (b), decalcified tissues (c) and micro CT scan showing connection between polyps (d); and *M. oculata* colony (e), polyps (f), decalcified tissues (g) and micro CT scan showing individual polyps (h). Scale bars for *L. pertusa* are 1 cm and 6 mm for *M. oculata*, except for (a and e) where scale is 10 cm. Credit for photos of living colonies ©CheReef cruise/ Ifremer in the Lampaul canyon, NE Atlantic (Ifremer) (a to f), of decalcified tissues from Lartaud et al. (2019) (c and g), and micro CT scan from Fabri et al. (2022) (d and h).

1.2.2 Distribution

Scleractinian CWCs are azooxanthellate, which means they don't rely on photosynthesizing symbionts as tropical corals (Freiwald et al., 2004). This has allowed a species distribution outside the photic zone (Zibrowius, 1988) down to the deep ocean. They are found in all of the world's oceans, in the Mediterranean Sea and the Red Sea (Figure 1.4), mainly between 200 and 1000 m depth (Mortensen & Fosså, 2006), but also in shallower waters in fjords, while

some species were recorded down to 6000 m (Keller, 1976). They occur in diverse habitats, particularly in fjords, along the continental margins, in submarine canyons, on seamounts as well as on mid-oceanic ridges, where physical, geochemical, and biological factors enable the formation of deep-coral reefs (Flögel et al., 2014). Among the factors determining CWC reefs formation and distribution, temperature rather than depth controls CWC distribution (Wayland Vaughan, 1940 in Roberts et al., 2009), and CWCs thus mainly occur between 4 and 14°C (Roberts et al., 2009). Salinity, oxygen concentration, carbonate chemistry, density gradients, current speed, turbulence and its control over food availability and quality, are also important factors determining CWC distribution (Mienis et al., 2007; Dullo et al., 2008; Davies & Guinotte, 2011; Maier et al., 2023). The most abundant reef-forming species in the northeast Atlantic are *L. pertusa* and *M. oculata* (Davies et al. 2008). The highest densities of *L. pertusa* have been observed along continental margins in the North Atlantic Ocean and have meaningfully contributed to our current knowledge of CWC's biology and ecology (Roberts et al., 2009).

In recent years, predictive models and maps of suitable habitats at both regional and global scales have been used to predict habitat and better understand the factors controlling the occurrence of CWC reefs (Davies et al., 2008; Davies & Guinotte, 2011), but also the potential effects of global changes on CWC distribution (Morato et al., 2020).

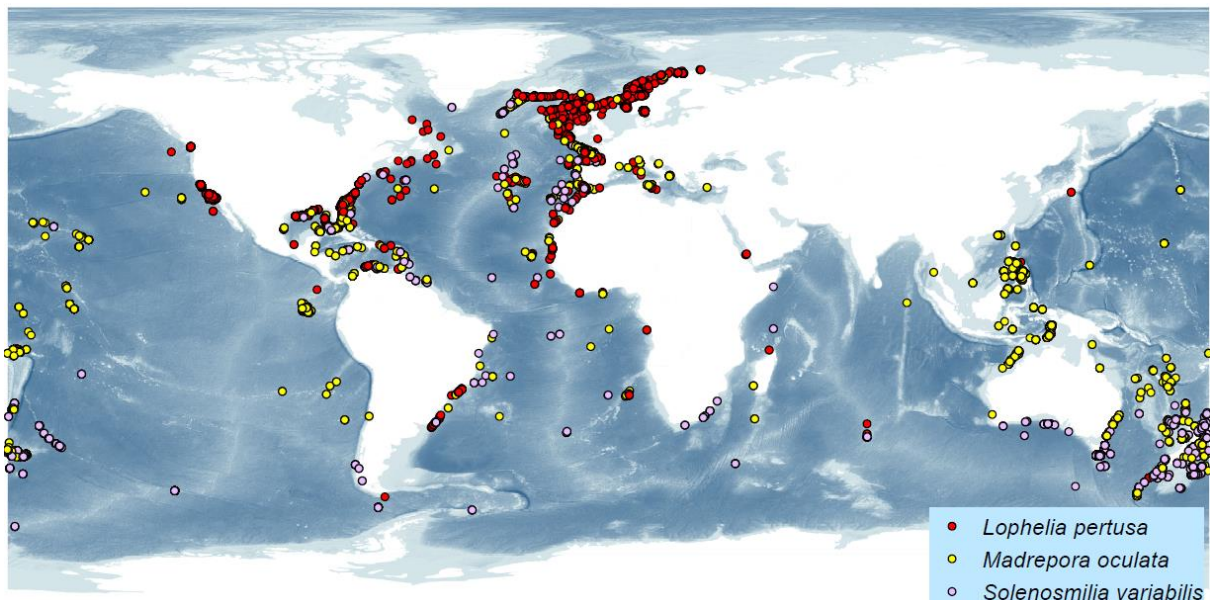


Figure 1.4. Global distribution of the main reef-forming scleractinian cold-water corals *Lophelia pertusa*, *Madrepora oculata* and *Solenosmilia variabilis* (Freiwald et al., 2021).

1.2.3 Ecological importance

CWC constitute ecosystems of high ecological values and play a major role in ecosystem functioning because they contribute to fix CO₂ into their skeleton, may be paleoclimate indicators, are hotspot of metabolic activity, and carbon and nitrogen turnover in the deep sea (Armstrong et al., 2014; Maier et al., 2023).

Furthermore, among CWCs, 26 % are colonial (Cairns, 2007) and form complex large, and long-lasting carbonate frameworks. They are considered engineer species, as they shape their environment by their three-dimensional growth, modifying the hydrodynamic conditions (e.g., turbidity regimes and flow patterns), facilitating the deposition of particulate matter, feeding numerous associated species and providing habitat and refuge against strong currents, as well as spawning and nursery sites. Thus, CWC reefs represent marine biodiversity hotspots, equivalent if not even exceeding tropical reefs species richness (Freiwald et al. 2004), with more than thousands of transient or obligated species associated (Roberts et al., 2006; 2014) and some symbionts, such as the polychaete *Eunice norvegica* (Buhl-Mortensen & Buhl-Mortensen, 2004).

The complex structure of CWCs offers heterogeneous microhabitats, providing substrata of different ages, different stages of colonization, and different supply of food for the associated fauna: the living part of the colony, dead corals below, and rubbles of dead corals on the seafloor (Figure 1.5, Buhl-mortensen et al., 2016). Live polyps are restricted to the apical outer part of the colonies and fringes of the reef, which are the most regularly exposed areas to freshly transported nutrients in suspension. Fish, shrimp, other arthropods, and mobile filter feeders, which require exposure to elevated currents, may occupy this live coral zone to reach higher current flow conditions. Below the living fringe, older polyps at the base of the colonies become hidden from food-delivering currents and die (Wilson, 1979; Mortensen & Fossa, 2006). From the « coral forest », this is the most diverse microhabitats for associated fauna. As the coenosarc disappears, the naked skeleton becomes a substrate for the establishment of gorgonian corals and sessile suspension feeders, such as bivalves, sponges, anemones, and hydrozoans, which may spread and colonize living polyp fringes (Henry & Roberts, 2007; Buhl-mortensen et al., 2016). It also provides structural refuges, spawning and nursery grounds for fish and other mobile fauna hiding within the coral (Buhl-mortensen et al., 2016). Following death, the carbonate skeleton becomes exposed to physical and biological erosion and breaks into rubbles. Rubbles are often colonized by filter feeders, such as soft corals, sponges, anemones, and a high density of ophiuroids. Trapping of mobile sediment leads to the

framework cementation and the formation of CWC reefs (Roberts et al., 2006). Over time, the carbonate structures formed can reach several dozen meters high, forming mounds (e.g., Rockall Bank in the NE Atlantic or giant mound barrier reef off Mauritania, Ramos et al., 2017) and they may extend for several kilometers, as seen in Norway (e.g., Rost and Sula reefs), reaching thousands to millions of years old (Roberts et al., 2006). The largest mound province in the Atlantic Ocean exists along the Mauritanian margin, where up to 100 m high mounds extend over ~400 km (Wienberg et al., 2018).

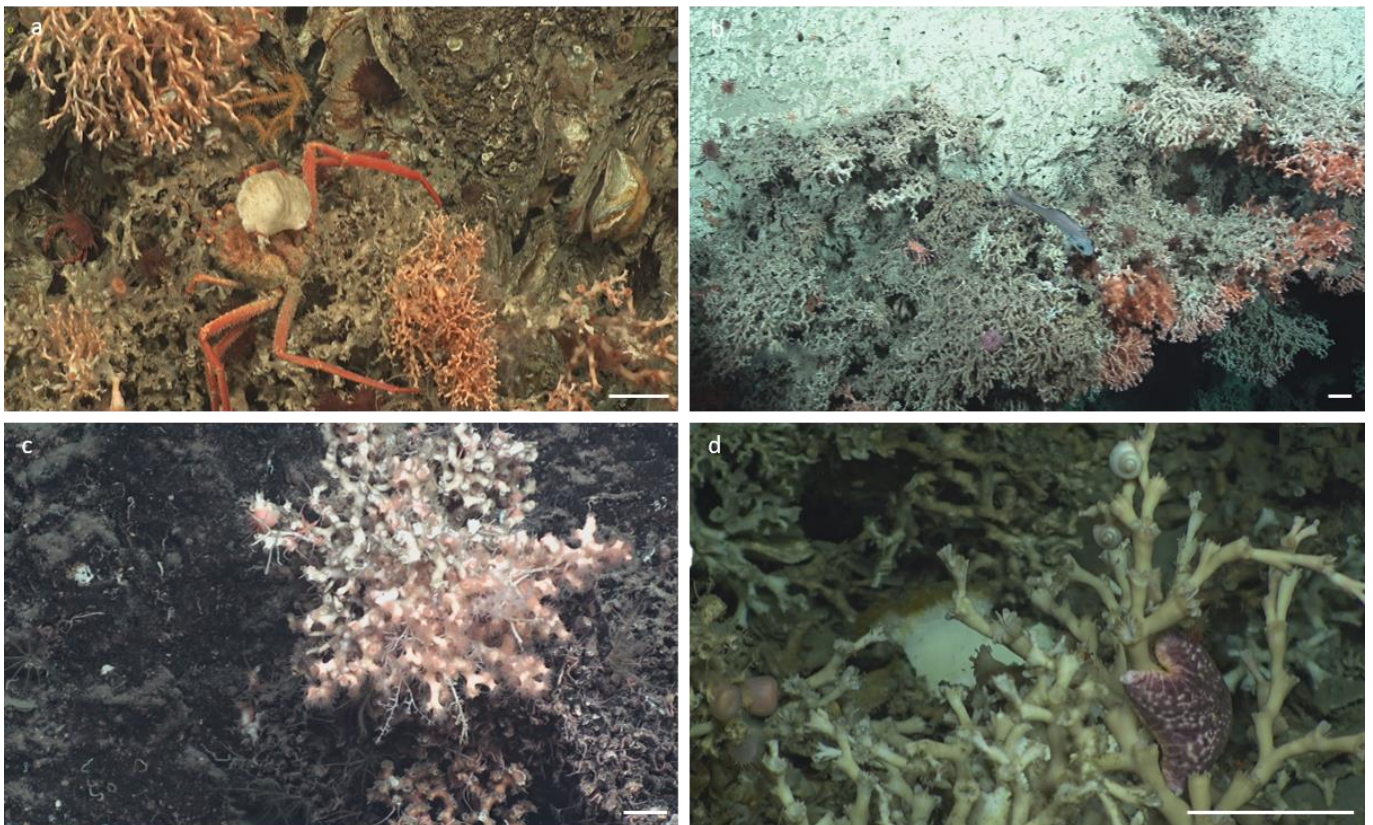


Figure 1.5. CWC reefs as hotspot of biodiversity. Crinoids, anemone, crabs living in the vicinity of *L. pertusa* and *M. oculata* growing on oyster banks (a). Fringes of *L. pertusa* on cliffs with crabs and sea star living on the dead parts of the colonies, with a grenadier (b). Living colony of *S. variabilis* growing on dead framework, with ophiuroids, anemone and soft coral living within it (c). Recently dead *L. pertusa* colony, colonized by sea star, shrimp and gastropods and rubbles in background with anemones (d). ©CheReef cruise/ Ifremer in the Lampaul canyon. Scale bars are 5 cm.

1.2.4 Biology

While the first descriptions of ‘white corals’ were done in Norway by Pontoppidan (1755), and the first description and illustration of *Madrepora pertusa* (i.e., *Lophelia pertusa*) was reported

by Carl von Linné (1758), we had to wait until the end of the 19th and the 20th, with the first dredging expeditions (e.g., HMS Challenger between 1872-1876, and the French expeditions of the Travailleur in 1880 or the Talisman in 1883), and then sonar surveys (in the late 1940s), for a description of deep habitats and a mapping of coral distribution (Roberts et al., 2009). In the last decades, the advent of new technologies, particularly submersible vehicles such as ROV (remotely operated vehicle) or AUV (autonomous underwater vehicle) and acoustic tools such as multibeam echosounder, has allowed to map and observe these species in their natural habitats, and more importantly, to collect corals. This has allowed to conduct the first *in situ* and controlled experiments in aquaria to study the ecology and the biology of these species.

a. CWCs as holobionts

All corals are associated with complex microbial communities that are drivers of biogeochemical cycling and contribute significantly to coral health, including nutrition, growth, immunity, and energy flow within the ecosystem (Bourne et al., 2016; van Oppen & Blackall, 2019). Corals harbour a large diversity of microorganisms, including eukaryotes (e.g., fungi), prokaryotes (archaea and bacteria), and viruses, which form together with the host, the holobiont (Bourne et al., 2016; Stévenne et al., 2021). The coral holobiont was first studied in tropical reefs, and the majority of the results on the topic and conceptual theories originate from these environments. For instance, it has been shown that bacteria are found in different compartments of the coral, including the mucus, coral tissue (including the gastrodermal cavity), and the skeleton, with distinctive populations of bacteria according to the compartment (Bourne & Munn, 2005; Koren & Rosenberg, 2006) and so associated to different function in coral physiology (Rosenberg et al., 2007). However, little is known about the localization of specific bacteria within different coral compartments. Researches on tropical corals have also highlighted that the microbiome is not only crucial for the coral's fitness (Rosenberg et al., 2007), but it is also involved in reef resilience, as it may offer the flexibility to adapt to local environmental variations (Reshef et al. 2006) and buffer or exacerbate cumulative impacts of environmental drivers (Bourne et al., 2016). Microbiome may thus contribute to tropical coral's stress tolerance (Bénard et al., 2020), including heat tolerance (Ziegler et al., 2017). Microbial communities have been suggested as indicators of environmental stress and tropical coral health (Glasl et al., 2017). While some microorganisms benefit their host, others opportunistic or pathogenic bacteria can cause diseases (Rosenberg et al., 2007; Munn, 2015; Vega Thurber et al., 2020). A shift in the bacterial community (i.e., dysbiosis) together with disruption of interaction with some bacteria or symbionts may cause the death of the organism (Vega Thurber

et al., 2020). Bacterial communities may be influenced by environmental changes and the interaction of opportunistic and pathogenic bacteria that may invade the host.

In deep environment, the first studies based on culture-independent approaches focused on characterising microbial community of black and bamboo corals in the Gulf of Alaska (Penn et al. 2006), and of *L. pertusa* corals from the central Mediterranean Sea (Yakimov et al. 2006) and Norwegian fjords (Neulinger et al., 2008; 2009). Further studies showed that *L. pertusa* from NE Atlantic Ocean were dominated by *Spongiibacteraceae* (Van Bleijswijk et al. 2015), while *Anaplasmataceae* and more broadly *Rickettsiales* and *Sphingomonadales*, respectively, dominated the bacterial community in the Mediterranean Sea (Meistertzheim et al. 2016; Galand et al., 2018) and the Gulf of Mexico (Kellogg et al., 2017). Differently, bacterial community of *M. oculata* were dominated by *Gammaproteobacteria* in NE Atlantic Ocean (Hansson et al., 2009) and more specifically by *Endozoicomonas* in the Mediterranean Sea samples (Meistertzheim et al. 2016; Galand et al., 2018), while *Flaviobacteria* were dominant in Norwegian samples (Jensen et al., 2019). Most of these studies have focused on total bacterial community, considering the bacterial diversity in the mucus, coral tissues (including the gastrodermal cavity), and the skeleton. However, Van Bleijswijk et al (2015) showed distinct bacterial populations in the mucus and the skeleton of *L. pertusa*, and *M. oculata* harboured different bacteria between the mucus and polyp tissues (Hansson et al., 2009). In addition, *L. pertusa* exhibited more variable and diverse microbiome, with high spatial variability (across different regions, latitudinal gradient or between colonies within a region) (Schöttner et al., 2012; Meistertzheim et al. 2016; Kellogg et al., 2017) and temporal variability (e.g., seasonal) (Chapron et al., 2020a). Furthermore, aquaria studies showed that microbial community associated to wild *L. pertusa* changed within a day of captivity (Galand et al., 2018). On the contrary, *M. oculata* showed more stable and specific microbiome across spatial and temporal scales (Meistertzheim et al. 2016; Chapron et al., 2020a). Thus, *M. oculata* maintained its associated community for 6 months in aquaria with the remarkable resilience of *Endozoicomonas* under rearing conditions (Galand et al., 2018). The dominance of *Endozoicomonas* both *in situ* and in aquaria, as well as across large geographic scale (Meistertzheim et al., 2016; Galand et al., 2018; Kellogg & Pratte, 2021) suggest true host-associated bacteria. This versatile or conserved diversity of microbiome has been suggested to be linked, among other factors, to different ecological strategies between species (Meistertzheim et al., 2016). For example, since diet is a major factor influencing bacterial communities, including in cold-water corals (Galand et al., 2020), different feeding strategies may reflect differences in associated bacteria. As a more opportunistic species, *L. pertusa* may

adapt its nutritional strategy, while *M. oculata* is less tolerant to environmental changes and so may be less resilient to changes in its specific diet. This may partially explain its more conserved microbiome. However, *L. pertusa* and *M. oculata*, fed on a common diet and reared in the same conditions, exhibited different microbiomes, clearly showing that corals' microbial communities are also defined by coral phenotype (Galand et al., 2020).

Microbial communities of other cold-water corals have been described, including *Acanthogorgia* species, *D. dianthus* (Kellog & Pratte, 2021), *Eguchipsammia fistula* (Röthig et al., 2017). These studies have shown very diverse microbiomes in cold-water corals, but also conserved host-specific associations, such as *Endozoicomonas* (Kellog & Pratte, 2021), and even core microbiomes conserved across the class Anthozoa (Kellog, 2019). However, the role of these conserved bacteria in CWC health and their potential metabolisms are only in the early stages of description and understanding (Yakiminov et al., 2006; Neulinger et al., 2008; Röthig et al., 2017). Recently chemoautotrophic and nitrogen-fixing symbiotic bacteria were identified as organic nitrogen providers in *L. pertusa* (Middelburg et al., 2015).

b. Calcification and growth of corals

Calcification is a key physiological parameter of organism fitness and health, which provides support, structure, and protection for internal soft tissues against predators. Calcifying organisms form their skeleton by periodically adding CaCO_3 , which can exist in various forms, such as calcite, aragonite, or amorphous calcium carbonate, and assembled following different microstructures (prismatic, fibro-prismatic, nacreous, crossed-lamellar).

Coral skeleton is extracellular and biomineralization is endogenous, taking place in extracellular calcifying medium. The assemblage of the organic matrix (mixture of proteins, glycoproteins, and polysaccharides) with calcium Ca^{2+} and carbonate CO_3^{2-} enables the calcification reaction and formation of a first initial thermodynamically unstable amorphous calcium carbonate prior to its conversion into aragonite (Allemand et al., 2004). During the calcification process, carbonic anhydrase (CA), a key enzyme in calcification process, is involved in supplying dissolved inorganic carbon originating from metabolic CO_2 , produced by calcicoblastic cell respiration, or seawater absorbed $\text{CO}_2/\text{HCO}_3^-$, to the calcification site and removing carbonic acid H^+ from the calcification site (Figure 1.6, Bertucci et al., 2013).

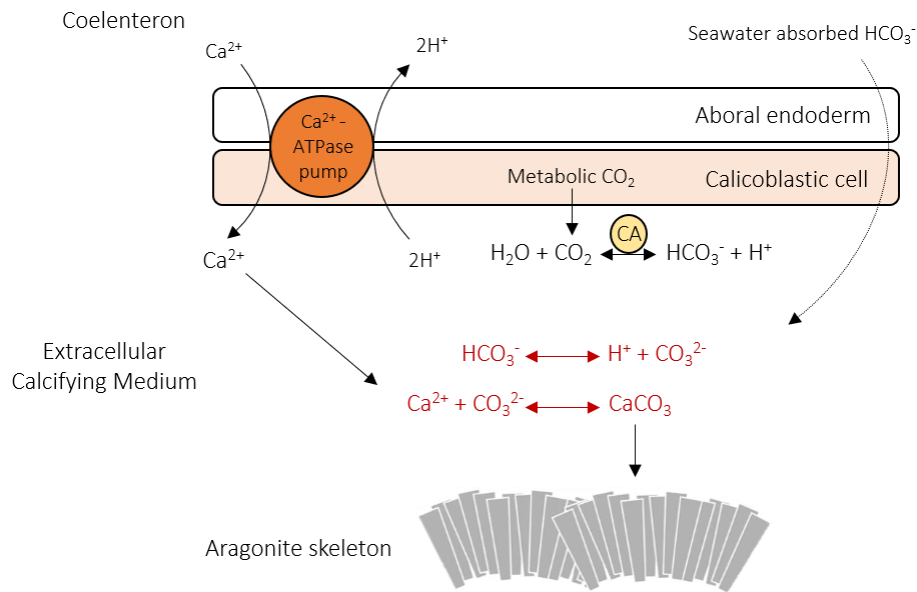


Figure 1.6. Simplified schema of coral calcification occurring in the extracellular calcifying medium. Carbonate anhydrase (CA) catalyse the forward reaction converting CO_2 into bicarbonate HCO_3^- ions, which then are used to produce carbonate CO_3^{2-} ions, to ultimately form calcium carbonate CaCO_3 . Source of dissolved inorganic carbon may occur *via* diffusion of CO_2 and/or HCO_3^- or metabolic CO_2 . Figure is modified from McCulloch et al. (2012).

Branching CWCs, such as *L. pertusa* and *M. oculata*, grow by budding, corresponding to the successive addition of polyps, growing directly on top of previous polyps. This allows colonies to grow and extend both linearly and radially, thus forming three-dimensional structures. Strong regional variability in growth patterns was observed between populations coming from different regions (e.g., Gulf of Mexico, Mediterranean Sea, NE Atlantic Ocean) (reviewed in Lartaud et al., 2019). If part of differences could be due to the various measurement methods used between studies, the environmental conditions and local regional circulation may be major factors controlling growth. Hydrological conditions and food supply control growth patterns (Chapron et al., 2020b). In the Mediterranean Sea, strong water plume events are associated with high budding rates, but low colony linear extension, suggesting that corals may favour the thickening of the colony as a protection from high current velocities. Differently, medium intensity water plume events caused high budding rates and linear extension, as organic matter supply was high and corals could capture food. Finally, in the absence of water plume events, low budding rates, low linear extension, and increased mortality were observed mainly due to high sedimentation rates, leading to the burial of the corals (Chapron et al., 2020). Sediment particles limit scleractinian corals' tissues and skeleton growth (Anthony et al., 2002).

c. Nutrition

As sessile suspension feeders, CWCs rely on organic matter produced at the ocean's surface, such as phytodetritus and zooplankton (living and dead zooplankton as well as faecal pellet), transported by ocean currents to the deep ocean. Phytodetritus and fecal pellets aggregate to form POM, also called 'marine snow', which sinks into the deep ocean and makes food particles available for deep-sea organisms (Turner, 2015). CWCs are predators and opportunistic heterotrophs using various organic resources, capturing living preys and POM with their tentacles and mucus nets (Murray et al., 2019). Lipid and stable isotope analyses revealed that they mainly feed on zooplankton but also on particulate and dissolved organic matter, as well as pico- and nanoplankton (Kiriakoulakis et al., 2005; Maier et al., 2023).

Food supply in CWC habitats varies at small spatial and temporal scales, with more periodic food pulses rather than constant food supply (Maier et al., 2023), particularly in submarine canyons where hydrodynamic events bring food into the deep, often seasonally (Canals et al., 2006). Therefore, CWCs usually store excess resources acquired during high food availability periods as tissue reserves (Maier et al., 2019), and mainly as short-term and long-term stored lipids (Maier et al., 2023). The preferred strategy for lipid storage varies from each species' food supply and strategy. For example, *L. pertusa* prefers zooplankton while *M. oculata* uses phytodetritus or mixed phytodetritus–zooplankton diet to build up storage lipids (Galand et al., 2020). However, the use of energy reserves during a low-food period has not been clearly established in *L. pertusa*, but has been confirmed to overcome the costly gametogenesis and potentially to synchronise the spawning timing (Brooke & Järnegren, 2013; Maier et al., 2020).

d. Reproductive biology

Although reproductive biology is a fundamental ecological process undergone by every species on Earth, less than 4 % of all known CWC species reproduction has been reported. Generally, we lack a description of fecundity, gametogenesis, spawning period and the environmental factors controlling it, larval development, recruitment strategy, and more comprehensively individual- and population-level variation in reproductive output (Waller et al., 2023). Colonial CWCs are the result of both sexual and asexual reproduction. A colony involves the settlement of one sexually produced larva, which then asexually grows by budding of polyps formed at the edge of the branch. Some colonies may originate from several larvae that have fused during the growing process. Sometimes, those fused larvae are from different species, forming

chimera-like colonies, as observed with *L. pertusa* and *M. oculata* (Corbera et al., 2019; Lartaud et al., 2019).

Lophelia pertusa and *M. oculata* have been described as gonochoristic broadcast spawners, releasing gametes in the surrounding water (Waller and Tyler, 2005). Colonies produce spermatozoa and oocytes, directly developing in the mesenteries, and once spawned into the water column, spermatozoa will fertilize an oocyte, which then will develop into a planula larva that will settle and become a primary polyp. In *L. pertusa*, oocyte production takes almost a year, while spermatocytes develop faster (Waller & Tyler, 2005). Spawning of large amounts of small oocytes may occur during 6-8 weeks (Brooke & Järnegren, 2013) and occurs between January and March in Norway (Larsson et al., 2014), between September and November in the South US and the Gulf of Mexico (Brooke and Shroeder, 2007) and between May and July off Brazil (Pires et al., 2014). Differently, *M. oculata* shows continuous reproduction or even multiple spawning events over the year (Pires et al. 2014), with spawning of few but large oocytes. While reproductive strategies are very different between these two species, food availability generally govern the reproductive timing of broadcast-spawning species. Spawning is very energetically costly, and synchronisation with food supply allows to restock the reserves of the adult colony (Maier et al., 2020), but also the feeding of the coral larvae, which start feeding 3 weeks after fertilization (Strömberg & Larsson, 2017). Food supply may also trigger periodic spawners, such as *M. oculata*, to produce, initiate vitellogenesis, and spawn several times a year (Waller & Tyler, 2005). Moreover, strong current events (e.g., dense water cascading in submarine canyons), which may supply food in the deep, may trigger spawning and are likely to promote larval dispersal of these sessile species, for which the only chance of dispersal occurs during the pelagic larval stage.

Reproductive processes have been less described in some regions, with substantially more North Atlantic Ocean studies (Waller et al., 2023). Since population-level differences in reproductive processes have been observed in different species, including *L. pertusa* (Waller & Tyler, 2005; Brooke & Järnegren, 2013; Pires et al., 2014), we need more studies from regions never described before, including the Mediterranean Sea, as well as studies on early life stages. This knowledge is of particular interest to understand and predict the maintenance of a population and its resilience to environmental changes and anthropogenic pressures (reviewed in Richmond et al., 2018).

1.3 Response of CWC to ocean warming

Although many environmental changes have occurred in geological times, organisms are now facing unprecedented, faster, more intense environmental changes than the ones they experienced in their evolutionary history (Bindoff et al., 2019).

1.3.1 General physiological response of ectotherm to temperature

Environmental variations in temperature are likely to have greater effects on marine ectotherms' physiology, as they do not maintain a stable internal temperature regardless of environmental variations. Temperature is a key factor controlling metabolism, influencing the kinetics of cellular reactions, protein and lipid synthesis, acid-base status, and metabolic rate (Pörtner, 2002; Somero, 2010). Thus, the presence and success of an organism will depend on its window of thermal preference (Figure 1.7, Shelford, 1911), which we will refer as the thermal niche hereafter. The extent of this thermal niche, within which organism' metabolic performance is maximised, is specific to each individual and depends on the organism' phenotypic plasticity (Angiletta, 2009; Magozzi & Calosi, 2015). Thermal tolerance is linked to the adjustment of the aerobic capacity of the whole organism, in addition to parallel adjustments at the molecular and membrane level (Pörtner, 2002). Metabolism increases exponentially with increasing temperature until it reaches a maximum, representing the maximum capacity of an individual to supply its tissues with O₂ (Claireaux & Chabot, 2016) and to use it in the mitochondria to produce energy *via* the aerobic pathway. Above this critical threshold temperature, aerobic scope disappears and transition to an anaerobic mode of mitochondrial metabolism occurs, while cellular energy levels may become insufficient: the organism can no longer provide sufficient energy to maintain its metabolism. Above the optimum temperature, at the cellular level, the synthesis of proteins and lipids is greatly affected since their structure and production are dependent on a particular set of temperatures (e.g., Carter & Houlihan, 2001).

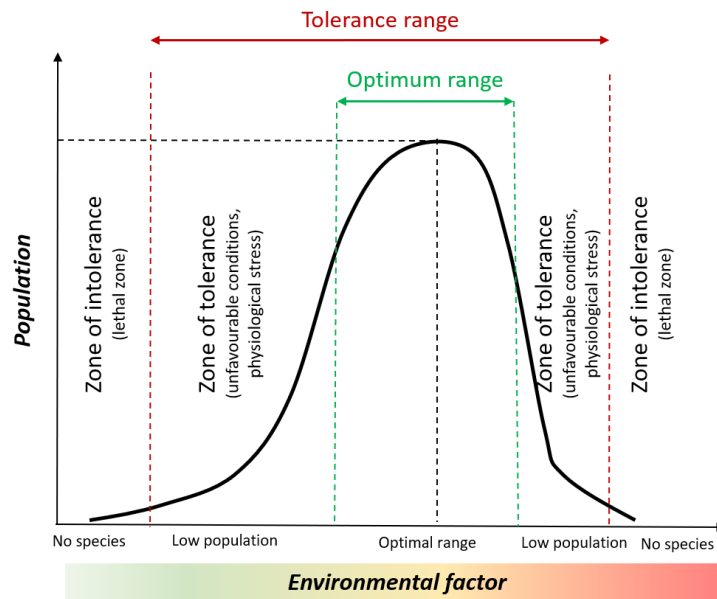


Figure 1.7. Graphical representation of Shelford's law of tolerance

Within the context of climate changes, this limited performance of the organism due to thermal stress may directly impact the organisms' growth, reproduction, foraging or predator/capturing behaviour, immune capacities, and competitiveness (Pörtner & Farrell, 2008). Organisms might be able to maintain their performance and fitness in a warming ocean through acclimation or adaptation. Acclimation involves phenotypically plastic responses in physiology, including the whole metabolism, morphological traits, energy allocation or behaviour that can help maintain the organisms' performance in a changing environment, without involving any underlying change in the organisms' genetic composition. As a result of phenotypic plasticity, no change might be observed in a particular response (e.g., growth), despite underlying changes in gene expression or organismal behaviour. Conversely, adaptation involves selection on genetic variation that shifts the average phenotype or the change of genetic composition of a population over time (Sunday et al., 2013). Acclimation may interact with genetic adaptation and thus promote or constrain evolution (Sandford & Kelly, 2011).

1.3.2 What are the effects of temperature on CWCs?

Several studies have investigated the biological response of different CWCs, mainly *M. oculata*, *L. pertusa*, and *D. cornigera*, to climate change in short and long-term controlled conditions experiments (reviewed in Maier et al., 2019). However, only a third of these studies have investigated the effect of changing seawater temperature on CWCs, mainly in the Mediterranean Sea (Figure 1.8).

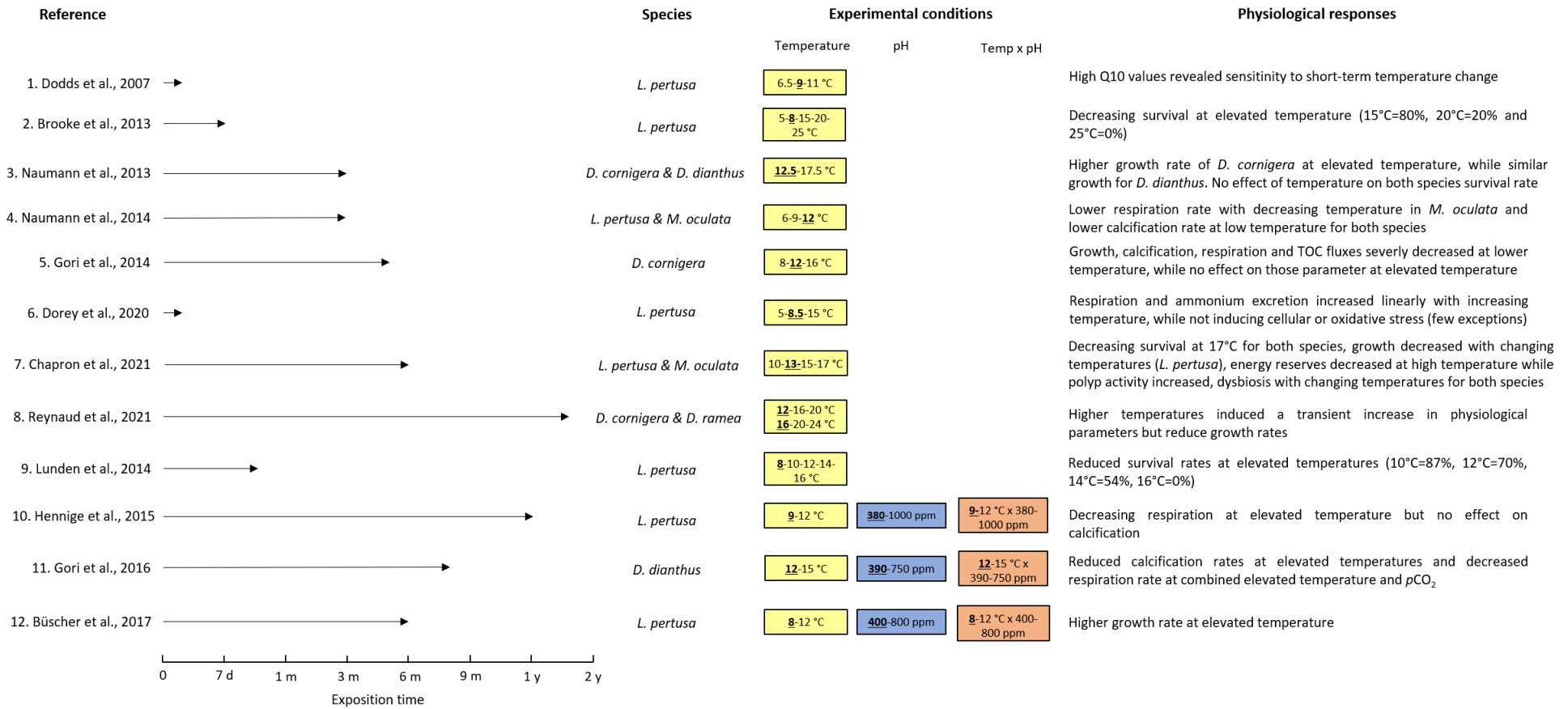
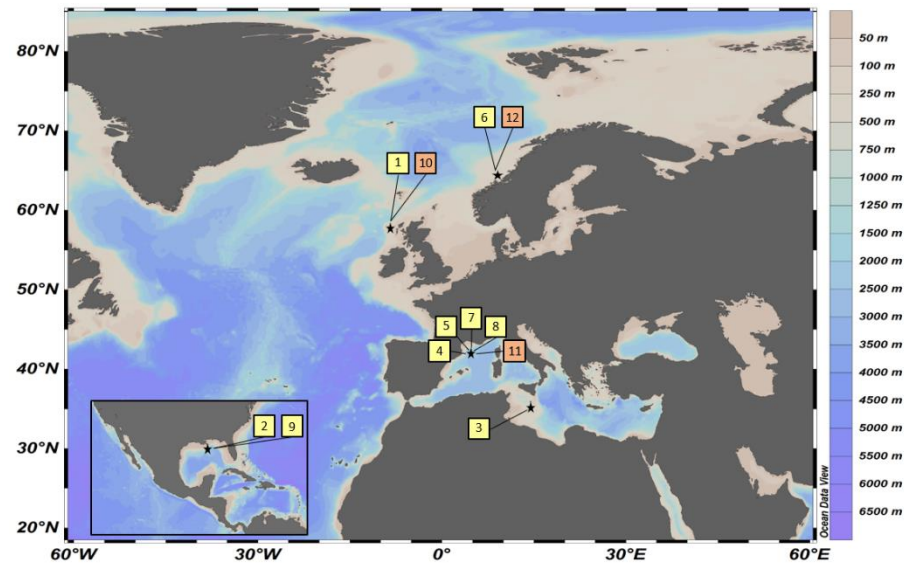


Figure 1.8. Chart summarizing results from research investigating the effect of temperature, and combined effect of temperature and pH on *L. pertusa* (a). Map showing the origin of the corals used in the studies (b). Modified from Hennige et al., (2015).



a. *Metabolic rate and respiration*

As presented before, metabolism generally increases with increasing temperature until it reaches the organism's maximum capacity. Respiration rate of *L. pertusa* increased by 35 % when temperature was increased from 9 to 11°C in the NE Atlantic Ocean (Dodds et al., 2007). Similarly, acute and stepwise increase in temperature from 5 to 15°C during 58 h showed linear increase in Norwegian *L. pertusa* metabolic rates (Dorey et al., 2020). On the contrary, lower temperatures generally cause lower metabolic activities, and *L. pertusa*'s respiration decreased by 45 % after rapid decrease from 9 to 7.5°C (Dodds et al., 2007). Differently, during gradual decreasing temperature over three months from 12 to 6°C, Naumann et al. (2014) found no difference in respiration rates, suggesting a possible sign of long-term acclimation of *L. pertusa*. Substantial results were observed after 3 to 6 months acclimation, showing no significant changes in respiration in Mediterranean and North Atlantic *L. pertusa* in response to lower or higher temperature (Naumann et al. 2014; Hennige et al. 2015).

Mediterranean *M. oculata* seemed to acclimate at a much slower rate to changing temperature than *L. pertusa*, as significantly lower respiration was reported after 3 months when temperature decreased from 12 to 9 and down to 6°C (Naumann et al. 2014).

b. *Growth and calcification*

Dendrophyllia cornigera exhibited higher growth and calcification at +5°C temperature increase in the Mediterranean Sea (Naumann et al., 2013; Gori et al., 2014), while *D. dianthus* showed similar growth at elevated temperature (Naumann et al., 2013). These results suggest very high thermal tolerance of these two Mediterranean species.

In the Mediterranean Sea, *M. oculata* maintained its growth regardless of the temperature during a 6 months experiment (Chapron et al., 2021), but its calcification significantly decreased when temperature was decreased by 3 and 6°C after 3 months (Naumann et al., 2014). Growth rates and calcification of *L. pertusa* were decreased when temperature was increased from 13 to 15°C and lowered from 13 to 10°C (Chapron et al., 2021), and from 12 to 6°C (Naumann et al., 2014). The reduced growth of Mediterranean *L. pertusa* may be due to alteration of the metabolism at higher temperature, as seen by the reduction of energy reserves (Chapron et al., 2021).

In the North Atlantic Ocean, calcification of *L. pertusa* was slightly lower at 12°C when compared at 9°C (i.e., control) after 3 and 6 months in aquaria, while after 12 months, calcification was 1.5 greater at elevated temperature than at ambient temperature (Hennige et

al., 2015). Similarly, growth rates of Norwegian *L. pertusa* increased when temperature increased by 4°C (Büscher et al., 2017). In both studies, the maintenance of growth observed at higher temperature might be at the detriment of other physiological processes, as a result of trade-off and re-allocation of energy within the organism (Hennige et al., 2015; Büscher et al., 2017).

c. Impact of temperature on the microbiome dynamics

Recent work on *L. pertusa* and *M. oculata* has shown that the microbiome composition and diversity changed in response to *in situ* variation in environmental conditions at temporal and spatial scale (e.g., Kellog et al., 2009; Meistertzheim et al., 2016; Chapron et al., 2020a), as well as in response to maintenance in aquaria (Galand et al., 2018) or different diet (Galand et al., 2020). *Ex situ* exposure to lower and elevated temperatures caused a shift in microbial communities in *L. pertusa* after 2 months at 10, 15 and 17°C (13°C, ambient temperature, Chapron et al., 2021). The increase in abundance of opportunistic and potentially pathogenic bacteria, such as Rhodobacterales earlier associated with stressed corals (e.g., Mouchka et al., 2010), suggest a limited capacity of *L. pertusa* to maintain or regulate its microbiome under elevated temperature. By contrast, *M. oculata* did preserve its microbiome at elevated temperatures, suggesting higher tolerance to environmental changes (Chapron et al., 2021).

d. Mortality and thermal limits

Dendrophyllia cornigera and *D. dianthus* showed very effective thermal acclimation to elevated temperatures, with a great tolerance for a 4-5°C increase with no mortality after 3 months at 17.5°C (Naumann et al., 2013). *Dendrophyllia cornigera* showed even higher performance at higher temperatures than at lower temperatures (Gori et al., 2014). The thermal tolerance of Mediterranean *D. cornigera* suggest that they are not currently living at their upper thermal limit (Gori et al., 2014). However, longer exposure may cause alterations of physiological parameters, although survival is not affected (Reynaud et al., 2021).

The survival of *L. pertusa* from the Gulf of Mexico was tested by exposing *L. pertusa* from 1 to 8 days at 8 (i.e., control), 15, 20 and 25°C (Brooke et al. 2013) or for 15 days to changing temperature in consecutive stepwise increase from 8, 10, 12, 14 to 16°C (Lunden et al. 2014). Both studies showed severe mortality when temperature exceeds 15°C, with 0 % of survival after 15 days at 16°C (Lunden et al. 2014) and after 24h at 25°C (Brooke et al. 2013). In the Mediterranean Sea, *L. pertusa* were strongly affected by exposure to water temperatures of 17°C with only 50 % survival after 2 months, and only 20 % after 6 months, whereas no

mortality was reported at 15°C (Chapron et al., 2021). As for *L. pertusa*, Mediterranean *M. oculata* showed similar survival at 13 (i.e., control) and 15°C but only 30 % polyps survived after one month of exposure to 17°C and 0 % after 6 months (Chapron et al., 2021).

Considering the impacts on physiological parameters and the survival of *L. pertusa* to increasing temperature, the upper thermal limit is suggested at 15°C in the Gulf of Mexico (Brooke et al., 2013; Lunden et al., 2014) and close to 13°C in the Mediterranean Sea (Chapron et al., 2021). In the Gulf of Mexico, CWCs can experience seasonal flushing warm-water currents, resulting in a rapid significant increase in seawater temperature (Brooke et al. 2013). Differently, Mediterranean CWCs are not subject to such abrupt temperature increase, but rather to episodic decrease in seawater temperature during cascading events occurring in the submarine canyons of the Gulf of Lion (Canals et al., 2006). They nevertheless live at the extreme temperatures reported for all CWCs (i.e., 13-14°C). Considering the reduced survival at higher temperatures (Chapron et al., 2021), it has been suggested that Mediterranean CWCs are actually living close to their upper thermal limit (13°C). Any prolonged further increase in temperature might be detrimental for them. These difference in thermal tolerance may be attributed to different environmental conditions in CWC habitats, imposing contrasting selective pressures for physiological, behavioural or life-history traits and responses. This may promote local adaptation and phenotypic diversity within a local environment and illustrate differential responses among populations. Here we consider the term ‘population’ as biogeographically separated populations, with no specific reference to genetic variability.

In Norway, exposure to acute, stepwise increase in temperature (5–15°C in 58 h) showed linear increase in respiration and ammonium excretion with increasing temperature (Dorey et al., 2020). The authors suggest that *L. pertusa* from the Norwegian continental shelf and fjords is tolerant to a broad thermal range of temperatures (5– 15°C), as a result of exposition to high thermal variability in their natural environment. Tolerance to short-term exposure to warmer temperatures, as seen in the Gulf of Mexico, the Mediterranean Sea, and in Norway may be beneficial if not necessary to cope with natural variable environments (e.g., seasonality). However, considering the very short time of exposure in Dorey et al. (2020) study, it is impossible to draw any conclusions about the actual thermal limit of *L. pertusa* in this region. Thus, no study has yet determined the thermal limit of *L. pertusa* in the NE Atlantic Ocean and more generally the response to changing temperatures across different CWC populations has not been determined.

1.4 Aim of the thesis

Recent studies have characterised CWC ecology and biology of several distinct populations, both *in situ* and through aquaria experiments. However, it is still not known whether populations from different oceanic regions will respond similarly to ocean warming. Since distinct populations can differ in their tolerance to environmental changes, information on population structure, reproductive output, and responses to changing environmental conditions is essential for conservation strategies and the establishment of protected sites. Moreover, the molecular mechanisms underlying their response to temperature changes at the holobiont level (i.e., the coral host and its associated microbiome) are still poorly known. This raises several questions:

- What are the effects of temperature on *L. pertusa* holobiont from the NE Atlantic Ocean? Do NE Atlantic *L. pertusa* share the same thermal niche as their Mediterranean counterparts?
- Do *L. pertusa* from the Atlantic and the Mediterranean Sea exhibit similar patterns of reproductive strategies, microbiome diversity and growth dynamics? Do they share some of these patterns with *M. oculata*?

To address these questions, we used an integrative approach combining *in situ* and experimental studies, considering different levels of biological organization of the coral holobiont (i.e., reproduction, growth, feeding, gene expression, and microbiome) to better understand the sensitivity of species across different populations.

1.5 Study sites and methods

1.5.1 Study sites

a. The Lampaul canyon in the Bay of Biscay

The Bay of Biscay is part of the North-East Atlantic Ocean, located west of France and north of Spain, from Brittany to the Cantabria coast. Geologically, the Bay of Biscay can be divided into the Celtic, Armorican and Aquitaine margins. These margins are incised by more than a hundred canyons (Bourillet et al., 2012, Figure 1.9a). Lampaul canyon is part of the Chapelle drainage basin in the northern Armorican margin (Bourillet & Lericolais, 2003).

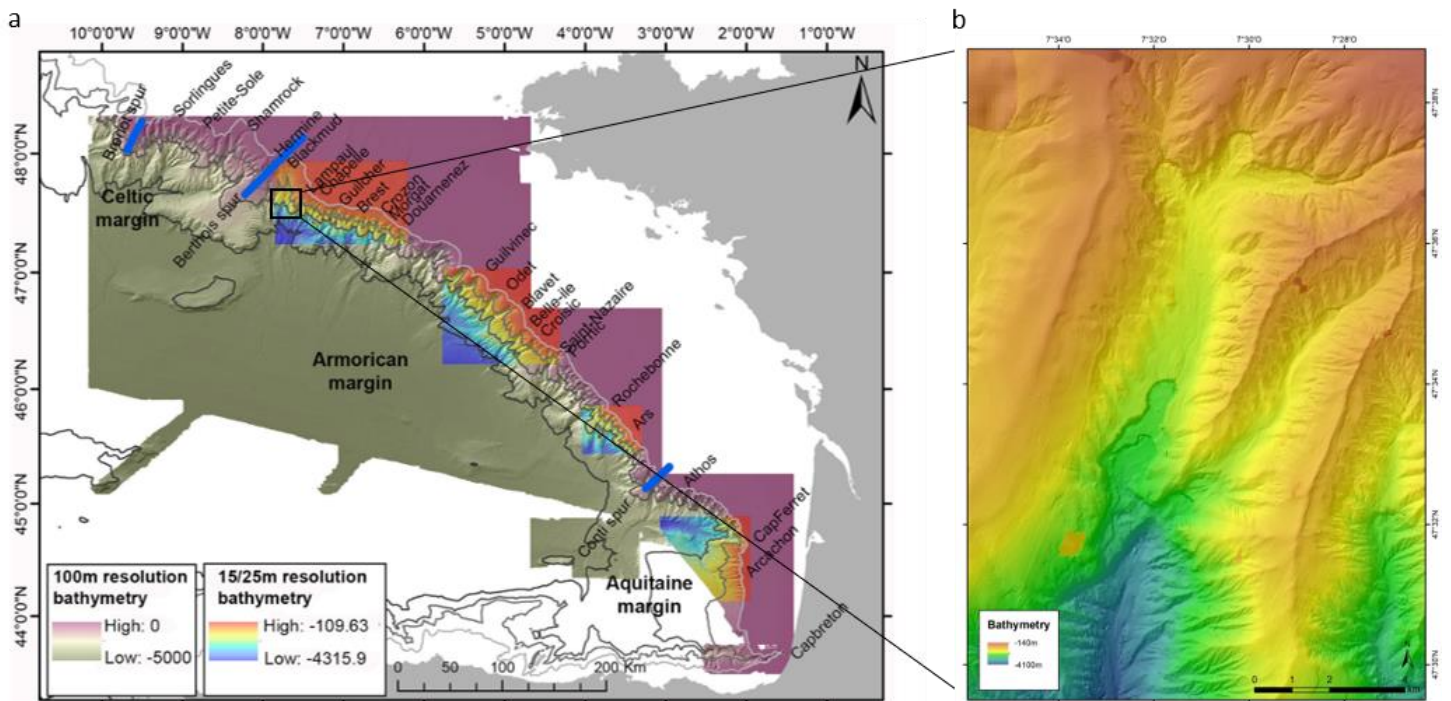


Figure 1.9. Map of the submarine canyons incising the continental slope of the Bay of Biscay. Bathymetry is indicated both at a 100 m resolution for the whole Bay of Biscay (green-purple) (Van den Beld et al., 2017), and at a 15/25 m resolution for four boxes representing each zone (rainbow-colored) (Bourillet et al., 2012) (a), and a 15 m resolution for the study site, the Lampaul canyon (b).

The Bay of Biscay is characterized by different water masses that vary spatially in their properties and depth distribution (Durrieu de Madron et al., 1999; Van Aken, 2000). The uppermost water mass is the Eastern North Atlantic Central Water (ENACW). Below, the Mediterranean Outflow Water (MOW), a more saline and denser water mass, generally flows

down to 1500 m water depth. Its salinity maximum occurs at around 1000 m depth, with for example, a salinity maximum of 35.76 psu at 1000 m in the Guilvinec Canyon, in the Bay of Biscay (De Mol et al., 2011). Between 1500 and 3000 m, the North Atlantic Deep Water (NADW) is observed, including Labrador Sea Water (LSW) and Iceland–Scotland Overflow Water (ISOW) (Van Aken, 2000). In the Guilvinec canyon, the mixing the ENACW and the MOW results in enhanced suspended materials, supplying food to scleractinians in canyons (de Stigter et al., 2007). CWCs have been reported at the physical boundary of these two water masses (de Mol et al., 2011).

The occurrence of CWC has been known in the Bay of Biscay since the end of the 19th century, and Joubin published a map of the distribution of deep-sea corals from Ireland to southern Spain in 1922. Le Danois (1948) updated the distribution map of coral reefs from the Bay of Biscay and proposed a zonation scheme between 400 and 2000 m depth. He described the Banc de la Chapelle reef: " il occupe une surface de 2.600 milles carrés et élève une muraille en forme de croissant entre 170 et 1000 mètres ". In the 2010s, Ifremer undertook the mapping of 24 canyons in the Bay of Biscay (CoralFish project) using modern technology. These data have allowed defining seven Natura 2000 sites to preserve these reef habitats in the Bay of Biscay. The present thesis was conducted within the frame of the ChEReef project (habitat Characterisation and Ecology of cold-water coral Reef, part of Life Marha project) that aims at determining the health and conservation status of these CWC reefs in the Bay of Biscay.

Among all the Bay of Biscay seafronts, the Lampaul canyon off the North-West coast of France (Figure 1.9b) has been selected as a study site of interest for different reasons. First, this canyon is interesting from a historical point of view, since Le Danois described the Banc de la Chapelle reef as early as in the 1950s. Furthermore, this canyon is of interest for monitoring the impact of fishing activities, as the slopes and the interfluves have been intensely trawled in the 20th and 21st centuries. The canyon presents a geomorphology of particular interest, with a succession of cliffs, hosting a great diversity of coral habitats (van den Beld, 2017), partially described during previous oceanographic campaigns, and is therefore included in a Natura 2000 network, aiming at preserving deep-sea reef habitats in the Bay of Biscay.

b. Lacaze-Duthiers and Blanes canyons in the Mediterranean Sea

The Lacaze-Duthiers canyon is 23 km long and located in the north-western part of the Mediterranean Sea, in the Gulf of Lion Marine Nature Park. It harbors one of the most abundant population of CWCs in the Mediterranean Sea, particularly of *L. pertusa* and *M. oculata* between 300 and 600 m depth (Gori et al., 2013). This canyon is subject to strong episodic

currents, called cascading events (Canals et al., 2006), resulting from severe coastal storms or the formation and sinking of dense water masses (Durrieu de Madron et al., 2005; Heussner et al., 2006; Palanques et al., 2006). Dense water shelf cascading increases sediment transport down the shelf and slopes (Palanques et al., 2006) and organic matter transport down to deeper environments (Heussner et al., 2006). These cascading events impact fauna biodiversity and function in canyon ecosystems (Pusceddu et al., 2013), including CWC reef functioning, survival and biology. The biology of *L. pertusa* and *M. oculata* have been described in this canyon, including growth dynamics (Lartaud et al., 2013, 2014, 2017; Chapron et al., 2020b), microbiome (Meistertzheim et al., 2016; Galand et al., 2018; Chapron et al., 2020a), the coral response to temperature (Chapron et al., 2021) and plastic pollution (Chapron et al., 2018; Mouchi et al., 2019).

In the North Catalan margin of the northwestern Mediterranean Sea, the Blanes canyon is 180 km long and is also subject to dense water shelf cascading as other canyons in the area (Canals et al., 2006), creating a favorable habitat for CWCs. The presence of large communities of reef-building species, including *L. pertusa* and *M. oculata* and dense associated fauna, have been described recently in this canyon (Aymà et al., 2019). The ABRIC scientific cruise (Institute of Marine Sciences CSIC, 2020) has also revealed dense colonies at depths up to 1100 meters. However, the biology of corals in this canyon has never been described yet.

c. *Connectivity between Mediterranean and Atlantic populations*

Connectivity, genetics, and phylogeography studies have suggested a link between Mediterranean and Atlantic populations of CWC, the Mediterranean population likely representing a glacial refuge (Henry et al., 2014; Boavida et al., 2019a). However, contemporary gene flow is very limited between the two genetic backgrounds (Boavida et al., 2019b) and *L. pertusa* and *M. oculata* show very different genetic structures between the two populations, suggesting two different stories since the last glacial event. Genetic data showed that Mediterranean *L. pertusa* recolonised the Atlantic at the end of the last glacial episode (Boavida et al., 2020a), which would have connected northern populations *via* the MOW (Henry et al., 2014). *Lophelia pertusa* today appeared as a large panmictic unit in the Atlantic (i.e., no clear genetic differences across the Bay of Biscay, off Ireland and South Iceland). By contrast, *M. oculata* exhibits a more complex pattern with several different historical and present-day connectivity as well as several genetic backgrounds (Boavida et al., 2019b).

1.5.2 Methods

An overview of the methods used in the different studies is given below. The precise approaches and protocols are detailed in each chapter.

a. Description and determination of reproductive biology and cycle

The reproductive biology of Mediterranean *L. pertusa* and *M. oculata* was investigated using histological techniques to describe the gametogenic cycle. The fecundity index and the identification of the different stages of maturation were determined by respectively counting oocytes on mesenteries sections and oocyte diameter measurements for both species.

b. Skeletal growth

Skeletal growth of *L. pertusa* from the Lampaul canyon was determined using fluorescent calcein staining and by measuring polyp linear extension *via* a sclerochronological approach, but also by using a novel protocol of structured-light 3D scanning.

Before the start of the experiment or redeployment of nubbins *in situ*, coral fragments were stained, and at the end of the exposure period, they were cleaned, and individualized polyps were observed under fluorescence microscope with excitation at 495 nm. Growth was assessed by measuring the distance between the calcein label and the outer edge of the septum of the calyx on images taken from the microscope.

For 3D growth, coral fragments were scanned at the start, and the end of the aquaria experiment using AutoScan Inspec (Shining 3D, China), and surfaces and volumes were compared.

Finally, for *in situ* experiment we also considered breakage, survival, and budding rate (i.e., percentage of new polyp grown *per* polyp initially present *per* year).

c. Microbiome

The diversity and community composition of the microbiome was described by targeting the 16S rRNA gene by metabarcoding. Briefly, DNA from individual polyps (including skeleton, tissues, and mucus) was extracted, the V1–V3 region of the bacterial 16S rRNA genes was amplified, and samples were sequenced. For 16S rRNA sequence analysis, a table of abundance of Amplicon Sequence Variant (ASV) was built using DADA2, and diversity analyses were then performed.

The function of the microbial communities was studied by sequencing all the DNA through a metagenomics approach. After DNA extraction and sequencing, genes were annotated and a

table of gene abundance was constructed to identify the potential microbial metabolisms and their distribution.

d. Capture rate

During aquaria experiment, coral prey capture rates were measured by determining *Artemia salina* nauplii concentrations in the seawater before and after feeding, and normalized to the number of polyps over a 4-hour period. To do so, triplicate water samples (100 mL) were taken every hour after feeding and filtered on 55 μm mesh.

e. Gene expression

The molecular changes at subcellular level in *L. pertusa* in response to temperature changes were studied by metatranscriptomics. Briefly, RNA from individual polyps (including skeleton, tissues and mucus) was extracted and sequenced. Transcripts were assembled and annotated against the first published *L. pertusa* genome (Herrera & Cordes, 2023). A table of transcript abundance was built, and differential expression between conditions was determined (up and downregulation of genes). A set of genes, considered as appropriate molecular biomarkers, was selected to determine and describe molecular changes at the subcellular level in response to temperature.

PART I: THE EFFECTS OF TEMPERATURE ON *L. PERTUSA* HOLOBIONT FROM THE NE ATLANTIC OCEAN



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The first section of the manuscript is dedicated to results from aquaria experiments testing the response of NE Atlantic *L. pertusa* holobiont to temperature changes. We first investigated the effects of elevated temperatures through physiological measurements and by revealing the diversity and the function of the microbiome through metabarcoding and metagenomics approaches (Chapter 2). Then, a long-term experiment was conducted to investigate the response of *L. pertusa* to temperature change from the cellular level of the coral gene expression and microbial diversity, to the organism level of feeding, growth and survival (Chapter 3).

Chapter two: *Lophelia pertusa* mortality under ocean warming is associated with pathogenic bacteria

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Abstract

Cold-water corals form vast reefs that are highly valuable habitats for diverse biological communities in the deep. The deep ocean is, however, increasingly warming, and models predict that temperature will further increase by up to 3°C in the Atlantic Ocean before the end of the century. Understanding the response of cold-water corals to ocean warming is therefore essential to evaluate their resilience to future water temperatures. Here, we investigate at the holobiont level (the coral host and its associated microbiome) the effects of elevated temperatures on the reef-forming cold-water coral *Lophelia pertusa* (now named *Desmophyllum pertusum*) from the north east Atlantic Ocean. We showed that at temperature increase of +3 and +5°C, *L. pertusa* exhibited a significant mortality concomitant with changes in its microbiome. A metagenomic approach reveals the presence of genes markers for virulence factors suggesting that coral death was due to infection by pathogenic bacteria. We also detected colony-specific microbiome signatures and survival patterns indicating strong inter colony variability in *L. pertusa* in response to warming waters. We hypothesize that *L. pertusa*, wherever their ocean of origin, can only survive a temperature increase < 3°C over a long period of time. Regional variations in deep sea water temperature rise should therefore be taken into account before estimating global distributions of cold-water corals.

Keywords: Cold-water corals, North Atlantic Ocean, Bacteria, Holobiont, Metagenomic, Climate change

2.1 Introduction

In the deep sea, cold-water corals form extensive reefs that represent highly valuable habitats as nursery grounds, and a source of food for numerous marine species (Buhl-mortensen *et al.*, 2016). These key ecosystem engineers thus act as local biodiversity enhancer and provide many ecosystem and ecological services (Armstrong *et al.*, 2014). Nevertheless, these corals now face serious anthropogenic threats, particularly in submarine canyons, including unsustainable fishing activities, pollution and global warming (Morato *et al.*, 2020). Sea water is warming down to the deep ocean (Barnett *et al.*, 2005), and projected climate scenarios indicate that warming is likely to be faster in deep environments than at the surface of the oceans in the future (Brito-Morales *et al.*, 2020). In the Atlantic Ocean, mesopelagic and bathypelagic water temperatures are predicted to rise by up to 3°C before the end of the century (reviewed by Sweetman *et al.*, 2017).

Temperature changes are known to impact cold-water corals' health by changing their associated microbiome (i.e., associated microbial communities), organic carbon fluxes, respiration, feeding and skeletal biomineralization, which could lead to coral death in some cases (Dodds *et al.*, 2007; Brooke *et al.*, 2013; Gori *et al.*, 2014; Naumann *et al.*, 2014; Chapron *et al.*, 2021). In the Mediterranean Sea, where corals live at 13°C to 14°C, a recent study showed that *Lophelia pertusa* (now synonymized as *Desmophyllum pertusum*, Addamo *et al.*, 2016), the main reef-building and most widespread cold-water coral species, showed reduced physiological functions when exposed to warmer waters, and appeared to be less tolerant to future warming than *Madrepora oculata*, another common coral of the deep-sea reefs (Chapron *et al.*, 2021). The study conducted at the holobiont level, considering both the coral host and its microbiome, showed that at warmer temperature, physiological activity, growth rates and energy reserves were reduced, and behavior was altered with increased polyp activity. At the same time, the coral microbiome changed in composition, with the appearance of potential opportunistic bacteria (Chapron *et al.*, 2021).

The coral microbiome is closely related to tropical coral health and growth as it contributes substantially to the metabolisms of the host (Bourne *et al.*, 2016), and may contribute to stress tolerance (Bénard *et al.*, 2020). However, while certain microorganisms are beneficial to their host, others can cause coral diseases (Rosenberg *et al.*, 2007; Vega Thurber *et al.*, 2020). Thus, recent studies have suggested that coral associated microbial communities might be good indicators of tropical coral health (Glasl *et al.*, 2017). In cold-water corals, a number of studies have now demonstrated that the microbiome is impacted in its composition and diversity in

response to changing environment (Kellog et al., 2009; Meistertzheim et al., 2016; Galand et al., 2018, 2020; Chapron et al., 2020a, 2021), but the function of the associated bacteria and their potential metabolisms have never been described.

In the north east Atlantic Ocean, cold-water corals populations live at temperatures below those of the Mediterranean Sea (i.e., 8 to 12°C in the gulf of Biscay, van den Beld et al., 2017). Although deep sea temperatures are now rising, knowledge on the impacts of warming on Atlantic *L. pertusa* remains limited to respiratory physiology (Dodds et al., 2007; Hennige et al., 2015) and growth (Büscher et al., 2017). It is thus not known whether Atlantic *L. pertusa* are already living at their thermal optimum, which exposes them strongly to global warming, or whether they can thrive in waters as warm as their Mediterranean counterparts, and would therefore be less affected by future thermal changes in this area.

In this context, the aim of the present study was to determine, under laboratory conditions, the effects of elevated temperatures on the reef-forming cold-water coral, *Lophelia pertusa*, from a submarine canyon in the Bay of Biscay (north-east Atlantic Ocean). We conducted two-months aquaria experiment in which corals were exposed to three different temperature conditions: the *in situ* temperature (10°C), and temperatures corresponding to two different warming scenarios (13°C and 15°C). We investigated the coral response at the holobiont level by measuring coral survival and growth, and by describing the diversity of microbiome by metabarcoding, and its functions by metagenomics.

2.2 Material and methods

2.2.1 Specimen collection, maintenance on board and acclimation

Corals were sampled in the Lampaul canyon in the Bay of Biscay, North-east Atlantic Ocean (N.47°36.703, W07°32.192). Five distinct colonies of *L. pertusa* (Linnaeus 1758) (orange specimens) were collected at 800 m depth, within a water mass corresponding to the Mediterranean Outflow Water (De Mol et al., 2011), using the remotely operated vehicle (ROV) Ariane from the R/V Thalassa, during the research cruise ChEReef (habitat Characterization & Ecology of cold-water coral Reefs) in august 2021. On board, corals were maintained in oxygenated seawater at ambient seabed temperature of 10°C using a cooling unit (ICE400, Aquavie, Connaux, France). Once at the laboratory (Banyuls Oceanological Observatory), coral colonies were kept for 5 months at their *in situ* temperature to acclimate to laboratory conditions in thermoregulated room in the dark, in aerated 80 L tanks, continuously supplied (>1 renewal day⁻¹) with filtered (5 µm) seawater pumped from 10 m depth. Corals were fed three times a

week alternately with freshly hatched *Artemia salina nauplii* (350 L⁻¹) and 5 mL of marine snow plankton diet (Two Little Fishies Inc, Miami Gardens, Florida, USA), to provide a complete and diverse nutrient supply (Galand et al., 2020). After this period of acclimatization, *L. pertusa* colonies were cut into nubbins that contained 3–13 living polyps. The nubbins were glued onto PVC blocks using an aquatic epoxy resin (Hold Fast Sand, Aquarium System, Sarrebourg, France), and were transferred to experimental tanks.

2.2.2 Experimental design

In order to investigate the impacts of warming on *L. pertusa* coral holobionts, colonies were exposed to three different temperature conditions: 10, 13 and 15°C, where 10°C represented the *in situ* temperature (control), 13°C (+3°C increase) represented the *in situ* temperature in the Mediterranean Sea, and 15°C (+5°C) represented a severe warming near the presumed upper thermal tolerance limit for this species (Brooke et al., 2013). Experiments were conducted following the protocol detailed in Chapron *et al.* (2021). Between 22 to 24 nubbins were randomly distributed in each of the three 36L experimental tanks (376 polyps in total) with enough distance between nubbins to avoid any contact among the different colonies and polyps (Orejas et al., 2019). Four different colonies were present in each experimental condition. For each colony, 1 to 2 nubbins were used for growth measurements, and 1 nubbin for microbiome analyses at each sampling time.

Each experimental tank was equipped with a small water pump (NJ400, Newa Jet, Loreggia, Italy) that maintained a constant flow (3 cm s⁻¹) and allowed water mixing. Each coral tank was placed in a larger water bath tank to ensure stable temperature regulation. Each coral tank was also equipped with a temperature probe connected to a temperature controller (Biotherm Eco, Hobby Aquaristik, Gelsdor, Germany, precise at 0.1°C), coupled with cooling unit (ICE400, Aquavie, Connaux, France) in the water bath. The temperature in each tank was monitored every 30 min using an autonomous IBUTTON probe and manually checked twice a day using a digital thermometer (Checktemp thermometer, Hanna Instrument, Woonsocket, USA). The pH, oxygen concentrations and salinity were measured manually twice a week using probes (Supplementary table 1) (C3010 Multi-parameter analysers, Consort). Nubbins were acclimated for two weeks in the experimental aquaria prior to temperature changes. Then, the water temperature of the experimental tanks was gradually adjusted over 10 days until the targeted temperatures were reached, following the protocol described by Naumann et al (2014). Feeding routine was maintained during the entire experiment.

2.2.3 Coral survival and skeletal growth

Survival was monitored every day and dead polyps were identified. Dead nubbins were removed from the aquarium. Survival rate was assessed as the percentage of surviving polyps at each sampling time relative to the initial number of polyps *per* temperature condition.

Skeletal growth was measured using fluorescent calcein staining (Lartaud et al., 2013) and by measuring polyp linear extension, but also by using a novel protocol of structured-light 3D scanning. At the beginning of the experiment, one to two nubbins *per* colony (i.e., 6 to 8 nubbins with 5 to 13 polyps for each temperature) were stained with calcein fluorescein at 150 mg L⁻¹ following the same protocol as described in Chapron *et al.* (2018). They were then scanned using AutoScan Inspec (Shining 3D, China) before the start of the experiment (Supplementary Fig. 1).

At the end of the two-months experiment, nubbins were scanned again, using the same protocol as at the start of the experiment. They were then cleaned in a hydrogen peroxide solution (H₂O₂, 4 %) at 60°C for 12 hours to remove all organic tissues, and rinsed with demineralised water. Then, each polyp calyx was individualised and placed on a slide, and glued with Patafix© in order to identify polyp's septa under microscope. The calcein labelling was observed under fluorescence microscope (Olympus IX51, Olympus, Tokyo, Japan) with excitation at 495 nm. Images were taken using a camera and image analysis was carried out using Image J software. Growth was assessed by measuring the distance between the calcein label and the outer edge of the septum of the calyx (repeated 10 times). Apical and subapical polyps were identified and their growth was compared (Supplementary Fig. 2.2).

2.2.4 Bacterial communities sampling and DNA extraction

For each experimental condition, three polyps *per* colony of *L. pertusa* were sampled at the start of the experiment (T0), and after 2, 4, 6 and 8 weeks of the experiment. In addition, at each sampling time, one liter of aquarium seawater was sampled and filtered sequentially through a 3 µm pore-size polycarbonate filter (Millipore, Darmstadt, Germany) followed by a 0.22 µm filter. Coral samples and the filters were flash frozen in liquid nitrogen and then stored at -80°C until nucleic acid extractions. For DNA extraction, individual polyps (including skeleton, tissues and mucus) were first crushed using a sterile hammer, then grounded in tubes containing a garnet matrix, and lysed mechanically using a FastPrep Instrument (MP, Biomedical, Illkirch-Graffenstaden, France). DNA extraction was performed using the Maxwell Blood DNA Purification Kit LEV and the Maxwell 16 MDx Instrument (Promega, Madison, WI, United

States) following the manufacturer's instructions. The FastPrep grinding and lysing protocol was also applied for the seawater samples after cutting the 0.22 μm filters in small fragments.

2.2.5 16S rRNA amplicon sequencing and data analysis

The V1–V3 region of the bacterial 16S rRNA gene was amplified by PCR using the primers 27F-AGRGTTCGATCMTGGCTCAG and 519R-GTNTTACNGCGGCKGCTG with the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, United States) and the high-fidelity Phusion polymerase under the following conditions: 30 s at 98°C, 16 cycles of 98°C for 10 s, 60°C for 30 s, 72°C for 80 s and final extension for 5 min at 72°C. Following the PCR, all the amplicon products from the different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, United States). The DNA library was prepared using the purified PCR products following the Illumina TruSeq DNA library preparation protocol. All the samples were sequenced on the same Miseq Illumina sequencer run using Miseq reagent kit V3 (Illumina, CA, United States) producing 2×300 -bp long reads. PCR and sequencing were conducted in a commercial laboratory (Integrated Microbiome Resource, Halifax, Canada).

Sequence analysis was performed with DADA2 in R (Callahan *et al.*, 2016, v 1.26.0). We applied the standard pipeline with the following parameters: trimLeft = 20, truncLen = c(290, 270), maxN = 0, maxEE = c(2,5), truncQ = 2. The sequences were filtered, dereplicated, and chimeras removed, to obtain amplicon sequence variants (ASVs). ASVs were classified against the SILVA v. 128 database (Quast *et al.*, 2012) for taxonomic assignment. An additional BLAST (Altschul *et al.*, 1990) search was performed on the ASVs selected by SIMPER analysis with the vegan package (Oksanen *et al.*, 2013).

2.2.6 Metagenomic sequencing and data analysis

A total of 12 samples (two per temperature conditions at T0 and 8 weeks) were used for sequencing metagenomes generated with Truseq DNA Nano and sequenced on Illumina Novaseq (2x150 bp) with 80 Gb/sample as target. After quality filtering, sequences were assembled with MegaHIT (Li *et al.*, 2015). Subsequently, a separate assembly was conducted on an individual sample using EukRep to differentiate and segregate eukaryotic-assembled contigs from prokaryotic ones (West *et al.*, 2018), with a stipulated minimum contig size of 2 Kbp. The Eukaryotic genes (coral host) were then forecasted using MetaEuk (Levy *et al.*, 2020) with the following parameters: metaeuk-val= 0.0001, metaeuk-tcov= 0.6, maximal E-Value=

100, min-length= 40, min-exon-aa= 20. Eukaryotic sequences (coral host) were then removed from the dataset and prokaryotic genes were predicted on prokaryotic contigs using two distinct tools: MetaGeneMark, a metagenomic gene discovery tool (Zhu et al., 2010), and Prodigal, a software designed for the prediction of proteins within prokaryotic genomes (Hyatt et al., 2010). While MetaGeneMark had the capability to predict both complete and partial genes, Prodigal focused exclusively on complete genes. To ensure downstream analysis quality, only genes with a length of 250 bp or more were retained.

To build a gene catalog, the predicted genes were clustered with the 'linclust' gene clustering algorithm, integrated within MMseqs2 (Steinegger et al., 2017), and the number of sequences falling in each cluster was counted. The count value was normalized by dividing the corresponding gene length in kilobases. Prokaryotic genes were taxonomically annotated with MMseqs2 against the Genome Taxonomy Database (GTDB) (Parks et al., 2020), and functionally annotated using blastp with Diamond [v0.9.22] against the KEGG (Kyoto Encyclopedia of Genes and Genomes) database.

2.2.7 Statistical analysis

Tests for normality of variance were performed using the Shapiro–Wilk test with the R software (v. 4.2.2). Distribution of survival was normal allowing a multiple factors ANOVA analysis. HSD post hoc tests were performed to determine differences among temperatures. As distribution was not normal for growth rates ($p < 0.05$), a non-parametric multiple comparison Kruskal–Wallis (K–W) test was used to test possible statistical differences between thermal conditions and sample times.

The vegan package (Oksanen *et al.*, 2013) was used for the following computations. Diversity was assessed by calculating the Shannon diversity index (Shannon, 1948). A nMDS based on Bray–Curtis similarity was constructed using the Hellinger transformed ASV table (Legendre and Gallagher, 2001). Significant differences between community composition were tested with PERMANOVA with the *adonis* function. Homogeneity of variances was tested with the function *betadisper* followed by *permutest*. The assumption of homogeneity was respected for the comparison of the temperature conditions through time. A simpler test was then performed (Wickham, 2011) to identify the ASVs that contribute the most to the differences between temperature conditions.

DESeq2 (Love et al., 2014) was used on the gene abundance table to identify the genes that varied the most between temperature conditions. A nMDS based on Bray–Curtis similarity was

constructed using the genes that vary the most and the significant difference between groups was tested with PERMANOVA with the *adonis* function in *vegan*.

2.3 Results

2.3.1 Survival and skeletal growth

Survival rates differed significantly between temperature conditions ($F_{2,3} = 110.266$, $p < 0.001$) (Figure 2.1a). More precisely, survival rates were significantly lower at 15°C, with high polyp mortality after 15 days (survival of 52 ± 17 %, HSD post hoc test), and then a continuous decrease in survival until the end of the experiment (survival of 31 ± 13 % at eight weeks). Overall, survival rates also differed significantly between sampling times ($F_{4,5} = 91.416$, $p < 0.001$). At 10°C, the survival rates showed the strongest decrease during the first two weeks of experiment, but did not decrease much after that with 73 ± 9 % of the colonies being alive at the end of the experiment. Finally, survival rates differed significantly between colonies ($F_{4,5} = 82.874$, $p\text{-value} < 0.001$). Some colonies displayed lower survival rates than others (e.g., colonies 5 and 7, HSD post hoc test $p\text{-value} < 0.01$, Supplementary Fig. 2.3).

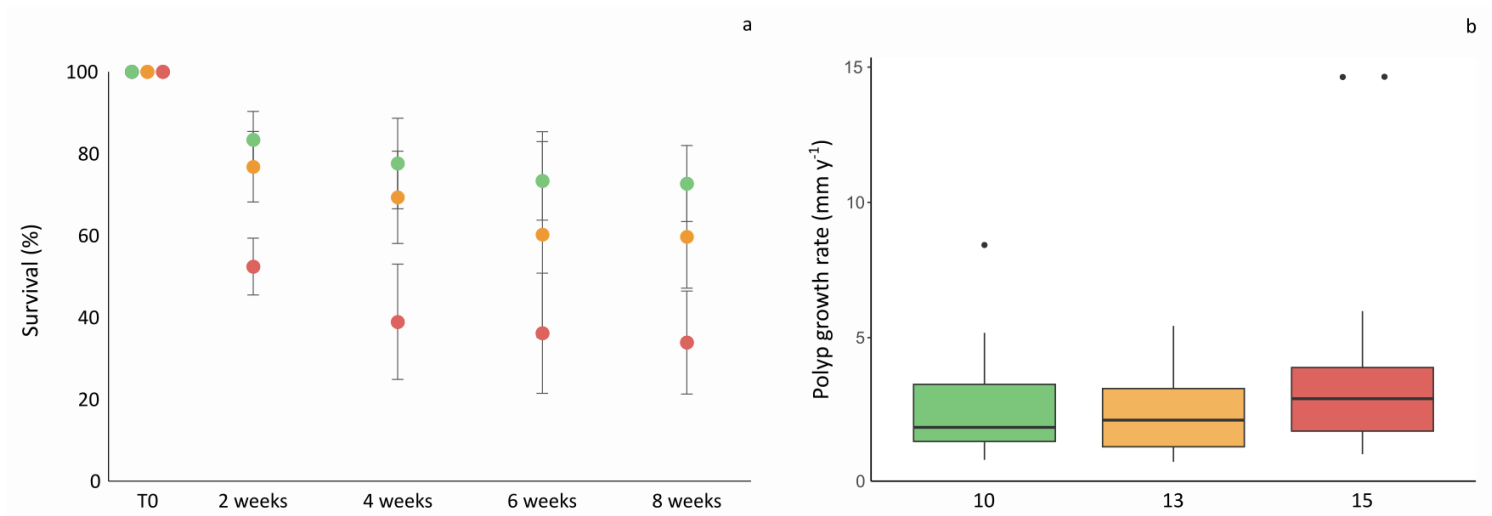


Figure 2.1. Polyp survival rate (mean values from four colony replicates per time point and error type are presented) (a), and polyp growth rate (b) of *L. pertusa* at 10°C (in green), 13°C (in orange) and 15°C (in red) during 8 weeks of experiment. Box plots plain lines indicate the median and quartiles, and points represent extreme values exceeding the 95 % quantile for each treatment.

The average polyp linear growth rate was not significantly different between corals exposed to water at 10°C (2.6 ± 2.3 mm y⁻¹), 13°C (2.2 ± 1.7 mm y⁻¹) and 15°C (3.5 ± 2.9 mm y⁻¹) (K–

W, $n = 44$, $p > 0.05$) (Figure 2.1b). Apical (i.e., the younger polyps at the summit of the branch that drive the linear extension) and subapical polyps showed no significant differences in linear growth rate (Kruskall-Wallis, $n = 44$, $p > 0.05$, Supplementary Table 2.2). No budding (i.e., formation of new polyps) was observed during the experiment.

The growth rates (biovolumes and surfaces) measured with the 3D scanner could not be used because values were mostly negative (Supplementary Table 2.3). The accuracy of the scanner (max 0.01 mm) was likely too low to measure growth of nubbins during a short-term experiment on slow growing cold-water corals.

2.3.2 Coral bacterial communities

The non-metric multidimensional scaling ordination (nMDS) showed that at the start of the experiment (T0) the samples from the different temperature conditions grouped together, indicating similar microbial community composition (Figure 2.2). After 2 weeks, *L. pertusa* samples at 15°C started separating from the 10 and 13°C samples. After 4 weeks, samples from the different temperatures separated from each other, and the separation was maintained after 6 weeks. After 8 weeks, samples from the 13 and 15°C were more spread and still separated from the 10°C samples, which were more grouped. A non-parametric multivariate analysis of variance (PERMANOVA) showed significant differences between temperatures at all experimental times. However, toward the end of the experiment, there were larger pseudo F -ratios values, indicating more pronounced separation between temperature groups, and higher R^2 , showing that temperature better explained variation in the model at the end of the experiment than at the beginning (Supplementary Table 2.4).

The bacterial community composition of the water was different from the coral bacterial communities (Supplementary Fig. 2.4, Adonis, $R^2 = 0.31$, $p < 0.001$), and was similar between temperature conditions during the experiment.

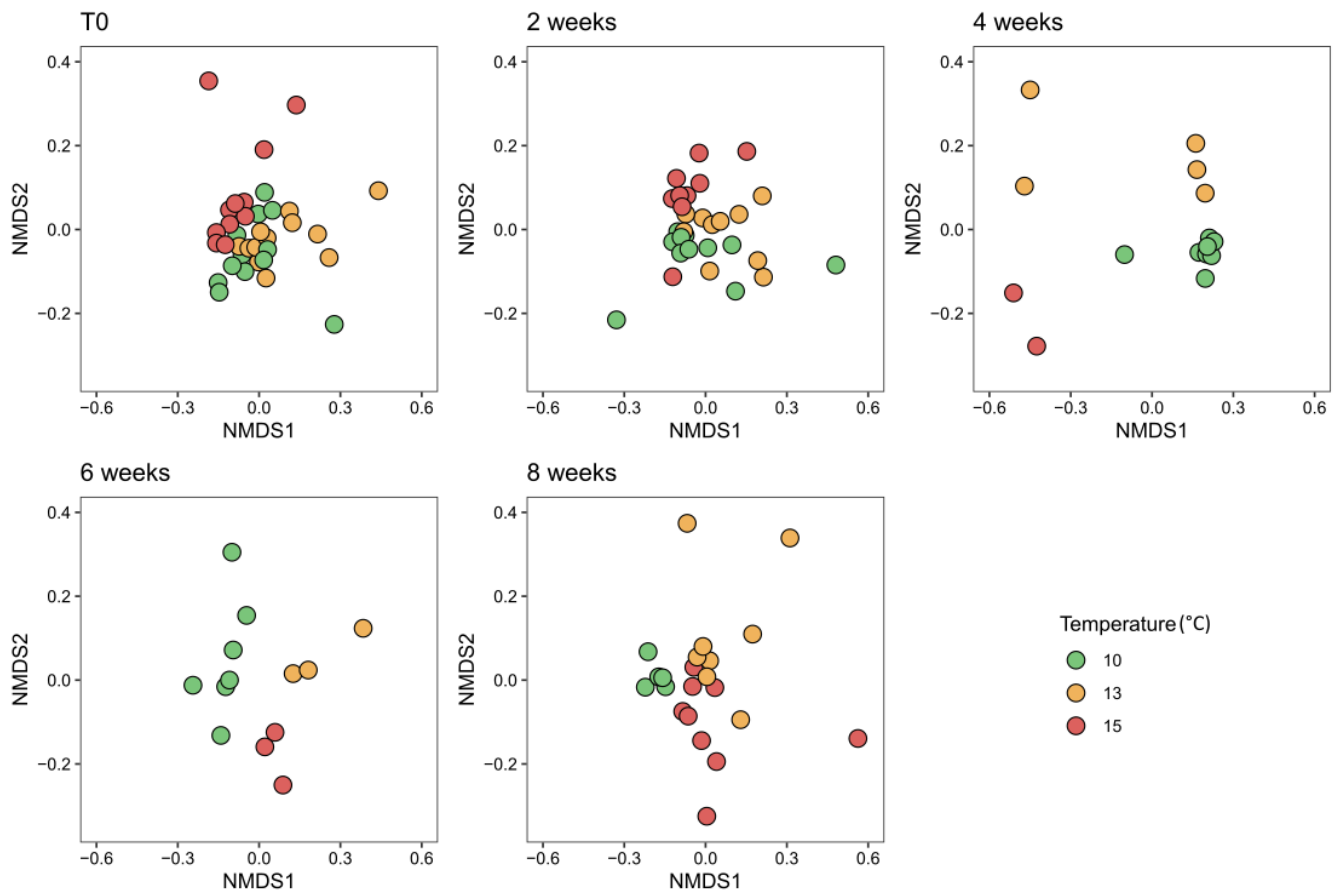


Figure 2.2. Non-metric multi-dimensional scaling plot (nMDS) based on the Bray-Curtis similarity index showing similarity between bacterial community compositions of *L. pertusa* at the start of the experiment (T0), and after two, four, six and eight weeks at 10°C, 13°C and 15°C.

Regarding the bacterial community composition at the class level, *L. pertusa* bacterial communities were dominated by ASVs belonging to Alphaproteobacteria, which represented respectively in average along the experiment 37.5, 38 and 34 % of the sequences at 10, 13 and 15°C (Figure 2.3). Gammaproteobacteria were also one of the main class characterizing the bacterial communities, but tended to decrease slightly at the end of the experiment in all temperature conditions. *Desulfobacteria* and *Clostridia* both had highest abundance after 4 weeks at 13 and 15°C (Figure 2.3). Acidimicrobiia had highest relative abundance after 8 weeks.

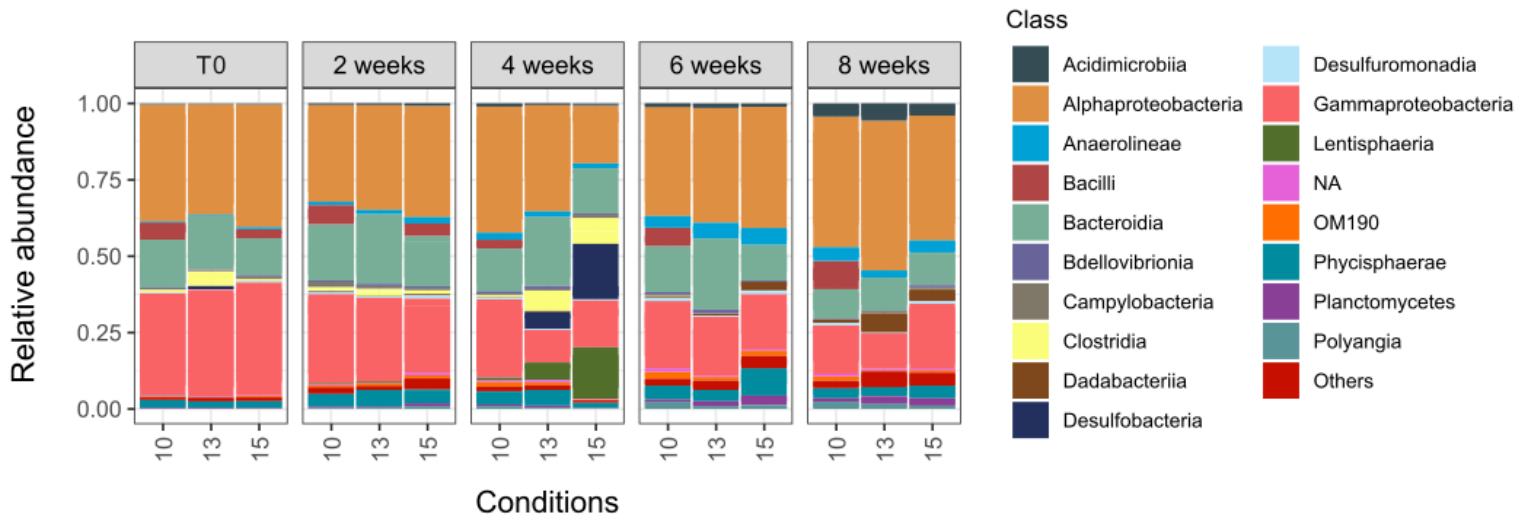


Figure 2.3. Relative proportion of bacterial sequences at the class level (18 most abundant classes) in *L. pertusa*. The composition is based on average values over the triplicate of each colony at each temperature condition (10°C, 13°C and 15°C) at the start of the experiment T0, and after two, four, six and eight weeks of experiment.

The bacterial community diversity did not differ overall between temperature conditions (Shannon diversity index, K-W $p=0.61$), but differed through time (K-W $p<0.01$). Community diversity was lower from 4 weeks to the experiment for all experimental conditions (Dunn test, $p<0.01$) (Supplementary Fig. 2.5).

We sequenced metagenomes from samples taken at the beginning and at the end of the experiment for all temperatures to identify bacterial genes that could vary in relative abundance between experimental conditions. The nMDS based on the genes that varied the most showed that at T0, there was no significant differences in gene composition between temperatures (PERMANOVA, $p = 0.14$), but that after 8 weeks, the gene composition differed between coral microbiomes incubated at different temperatures (PERMANOVA, $p = 0.04$) (Figure 2.4a). There were no patterns of sample separation when looking at all genes together (Supplementary Fig. 2.6).

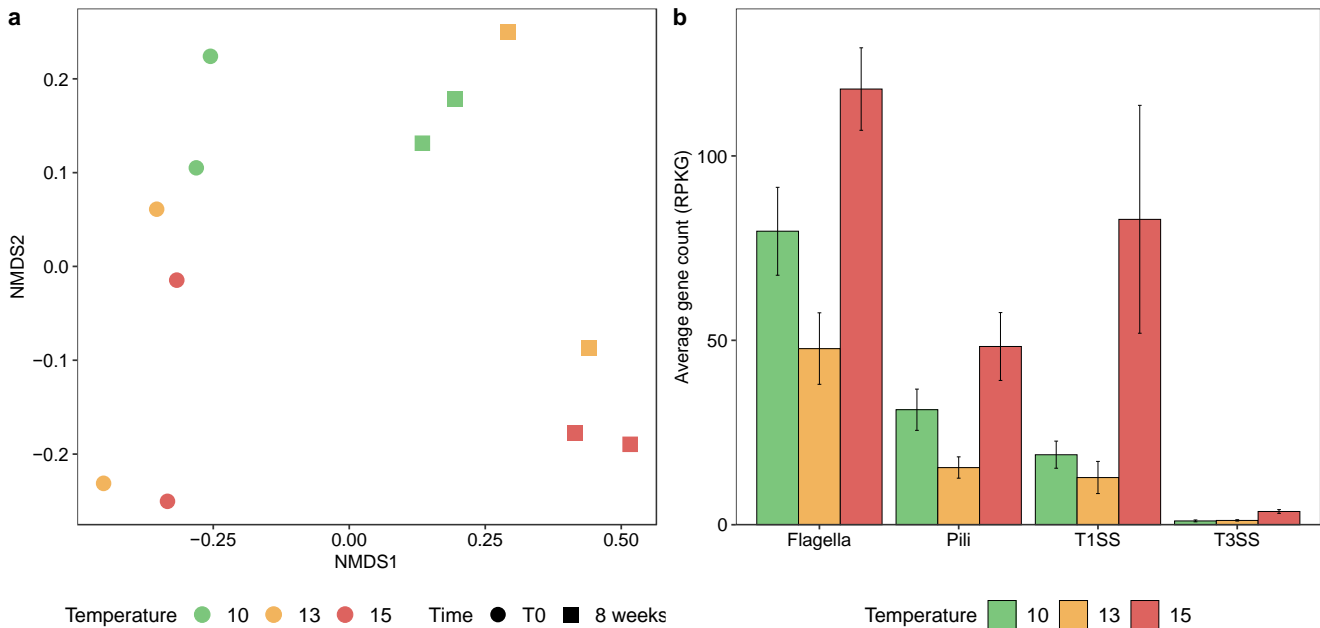


Figure 2.4. Gene composition of the microbial communities. Non-metric multi-dimensional scaling plot (nMDS) based on the abundance of genes that vary the most between conditions ($n = 5018$) at the beginning and at the end of the experiment as identified with DESeq2 (a). Abundance of genes annotated as belonging to flagella and pili formation, and involved in type 1 and 3 secretion systems (T1SS and T3SS) at the end of the experiment under incubation at 10°C, 13°C and 15°C (b).

We then compared gene composition specifically between 10°C and 15°C after 8 weeks of incubation with DESeq2. Among the 69 genes that varied the most in relative abundance, only 5 could be annotated, and only one showed consistent variations between replicates (KEGG K11004). The gene was annotated as coding for hemolysin A secretion system involved in pathogenicity. It prompted us to search for other potential markers potentially indicative of the presence of pathogenic bacteria. We therefore targeted genes involved in type 1 and 3 secretion systems (T1SS and T3SS), and flagella and pili construction (Supplementary Table 2.5). In average, the relative abundance of genes associated to T3SS, T1SS, flagella and pili was significantly higher in the microbiome of the corals incubated at 15°C compared to 10°C and 13°C (Figure 2.4b, t-test, $p < 0.01$).

The taxonomic annotation of the functional genes allowed us to find some ASVs with corresponding taxonomy within the 16 rRNA dataset. Among the genes coding for flagella, we identified 3 ASVs annotated at the genus level as *Vibrio* (*Vibrionaceae*) and uncultured *P3OB-42* (*Myxococcaceae*) (Figure 2.5). These ASVs (ASV212 and ASV763) had highest relative abundance at 15°C at the end of the experiment. Among genes coding for the T1SS, we

identified one *Puniceispirillales* (*Alphaproteobacteria*) ASV (ASV1130) that also had highest abundance after 8 weeks at 15°C, and one *UBA4486* (*Gammaproteobacteria*) (ASV213) and one *Alteromonadaceae* (ASV29) that were abundant in at both 13°C and 15°C at the end of the experiment (Figure 2.5).

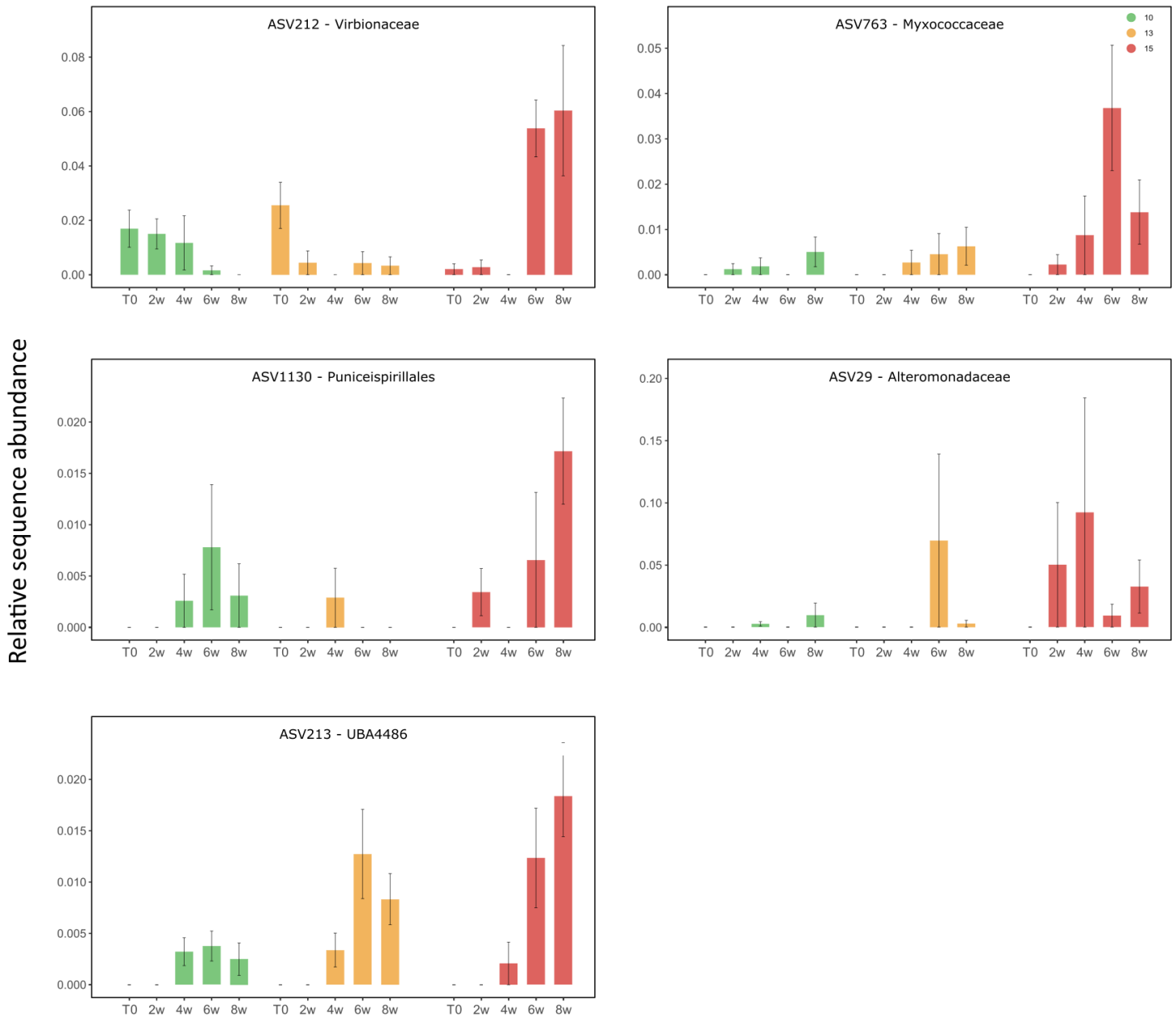


Figure 2.5. Relative sequence abundance of ASVs of interest characterizing *L. pertusa* bacterial communities under different experimental conditions (10°C, 13°C and 15°C) at the start of the experiment (T0), and after 2 weeks (2w), 4 weeks (4w), 6 weeks (6w) and 8 weeks (8w) of experiment. Mean values and standard deviations are presented.

Independently of the taxa linked to functional genes, we identified additional ASVs that became more abundant at 15°C. ASV150, order *Saprospiraceae*, and ASV446, order *Moritellaceae*, were not present at 10°C or 13°C but appeared at 15°C (Supplementary Fig. 2.7). Inversely, some ASVs were present at 10°C throughout the whole experiment, but had lower abundance at both 13 and 15°C at the end of the experiment (ASV20, order *Alteromonadaceae* and ASV65, order *Rhodobacteraceae*, Supplementary Fig. 2.7).

Finally, some colonies harboured specific ASVs that were not found at all in any other colony (i.e., ASV235, order *Thermoanaerobaculaceae*, in colony L9, Supplementary Fig. 8), or found only at very low abundance in other (i.e., ASV4, family *Spiroplasmataceae*, in colony L8, Supplementary Fig. 2.8). Within the colony that they characterized, these ASVs were present under different temperature conditions and along the different times of the experiment (Supplementary Fig. 2.8).

2.4 Discussion

In the present study, we show that warming had a strong negative effect on the *Lophelia pertusa* holobiont from the north-east Atlantic Ocean. Only 33 % of the corals survived after 8 weeks at 15°C and 60 % survived at 13°C. Several lines of evidence indicate that coral mortality could be due to the action of pathogenic bacteria that invaded the host during the course of the experiment. Metagenomics analysis showed that bacterial gene composition differed between coral microbiomes incubated at different temperatures, and that among the genes that were more abundant at higher temperatures, several were coding for secretion systems (T3SS and T1SS), flagella and pili. Swimming motility is an important factor in bacterial colonization and infection (Ushijima & Häse, 2018). Indeed, flagellum has been identified as critical for chemotaxis and for adhesion to the coral during the infection by *Vibrio* species in tropical reefs (Meron et al., 2009). In turn, secretion systems allow the direct injection into the extracellular medium (T1SS), or into the host targeted cells (T3SS), of effector proteins contributing to pathogen infections (Cornelis, 2006; Bleves et al., 2010). Increased abundance of microbial genes involved in virulence, motility and chemotaxis have earlier been observed in response to stress, including increased temperature, in the tropical coral *Porites compressa* (Thurber et al., 2009). In our study, the genes potentially indicator of virulence were in particular associated to a *Vibrionaceae*, a *Myxococcaceae*, a *Puniceispirillales* and a *Gammaproteobacteria* that were more abundant at higher temperatures. These bacteria may be part of the pathogens invading the stressed corals.

Other potential pathogens were detected by 16S rRNA metabarcoding. Several bacteria from the *Saprospiraceae* order were only present at 15°C. This order was earlier detected in tissues of the tropical coral *Acropora muricata* affected by White Syndrome (Sweet & Bythell, 2015) and heat stressed *Stylophora pistillata* (Savary et al., 2021), suggesting their potential implication as opportunistic pathogens. Similarly, *Clostridia* earlier identified as potential pathogens in tropical coral (Meyer et al., 2019) were present at 13 and 15°C. In a meta-analysis, Moucka et al. (2010), showed that *Clostridia*, together with *Rhodobacter* and *Cyanobacteria*, appeared to increase in abundance in the majority of diseased tropical corals. Concurrently, they observed that bleached corals had a higher proportion of opportunist bacteria such as *Vibrio* sp. than healthy colonies. Similarly, Thurber et al. (2009) showed that thermally stressed tropical corals exhibited disease-associated microbiome with low abundance of *Vibrio* sp., nevertheless significantly altering microbiome metabolism and functioning. Our results show for the first time that stressed cold-water corals are subject to invasions by pathogenic bacteria in the same way as tropical corals.

More generally, bacterial communities of *L. pertusa* changed at elevated temperatures (i.e., dysbiosis). Some variability of community diversity was observed at both 13°C and 15°C, which suggests that warming represented a stressing conditions that decreased the microbiome stability (McDevitt-Irwin et al., 2017). Changes in composition were due to the appearance of opportunistic and potentially pathogenetic bacteria as detailed above, but also to the concomitant disappearance of bacteria present in control conditions. These changes appeared early in the experiment (after 2 weeks), which suggests a rapid stress-induced dysbiosis under warming conditions. Altogether, our results reflect a limited capacity of the coral to maintain or regulate its microbiome under elevated temperature, which results in the invasion of the host by pathogenic bacteria, especially for a 5°C increase.

Mortality under elevated temperature has been reported earlier in *L. pertusa* from different regions during short- and long-term experiments, and 14-15°C is generally considered the upper limit of thermal tolerance for this species (Brooke et al., 2013). Previous experiments showed that Mediterranean *L. pertusa* living at 13°C were strongly affected by water temperatures at 17°C with only 50 % survival after 2 months of experimentation, and only 20 % after 6 months, whereas no mortality occurred at 15°C (Chapron et al., 2021). In the Gulf of Mexico, where corals live between 7.0°C and 9.5°C, Lunden et al. (2014) reported 54 % and 0 % of survival after 15 days respectively at 14°C and 16°C, while Brooke et al. (2013) reported a complete mortality of corals at 25°C after 24 h of experiment, and 80 % of survival after 7 days at 15°C. Taken together, our results and the variations seen between earlier studies, suggest that the level

of temperature increase relative to the natural conditions (e.g., +5°C), rather than a fixed arbitrary value (e.g., 15°C), should be taken into account when predicting coral survival in different habitats. We hypothesize that *L. pertusa*, wherever they come from, can only survive a temperature increase <3°C over a long period of time. Regional variations in deep-sea water temperature increase should therefore be taken into account before estimating the future global distributions of cold-water corals.

We observed differences in survival between colonies, suggesting an intra-species variability with the probable presence of genotypes that are more sensitive or more resilient to changing environment than others. Interestingly, we also observed colony specific differences at the microbiome level. Some colony had unique bacteria that were almost or totally absent in others (e.g., *Spiroplasmataceae* (class *Mollicutes*) and *Thermoanaerobaculaceae*). Variation between colonies have been shown earlier for different *L. pertusa* physiological parameters (Form & Riebesell, 2012; Lunden et al., 2014; Hennige et al., 2015; Georgian et al., 2016; Büscher et al., 2017; Kurman et al., 2017), but never before for their microbiome. The *Thermoanaerobaculaceae* had only 97 % similar to the closest hit in the databases (a sequence found in the sponge *Halicona tubifera* (Erwin et al., 2011), and the *Spiroplasmataceae* had only 91% similarity to reference sequences, so we could not directly relate our data to existing literature. Interestingly, the colony L8 harboring the *Spiroplasmataceae* exhibited one of the highest survival rates at 10°C. Although our experimental design did not allow to infer a direct relationship between colony specific microbiome and survival, we can hypothesis that the microbiome could play a role. Future investigations should consider the microbiome when exploring inter-individual variations and their possible role in the resilience of specific genotypes within a reef or a population. It is of paramount importance for predicting potential population adaptation within the context of the global change.

We reported here the first time growth rate estimations for *L. pertusa* from the Bay of Biscay, and we observed that they are in the lower range compared to values published for this species in other geographical areas, both in aquaria and *in situ*. *In situ* measurements showed *L. pertusa* growth rates ranging from 2.44 to 32 mm y⁻¹ in the Gulf of Mexico (Brooke & Young, 2009; Larcom et al., 2014), 1 to 40 mm y⁻¹ in the Mediterranean Sea (Lartaud et al., 2017; Chapron et al., 2020b), from 1 to 26 mm y⁻¹ in Norway (Mikkelsen et al., 1982; Büscher et al., 2019) and up to 26 mm y⁻¹ in the North Sea (Gass and Roberts, 2006). Growth rates are usually lower in aquaria experiments where they range from 1 to 17 mm y⁻¹ for Mediterranean *L. pertusa* (Orejas et al., 2008; Lartaud et al., 2013), and are up to 9.4 mm y⁻¹ for corals from

Norway (Mortensen et al., 2001). The values measured in our study never reach these maxima. However, considering that no budding (i.e., new polyp formation) occurred during the 2 months experiment, and that the growth rates of old polyps is significantly lower than that of new ones (Lartaud et al., 2013), it is not surprising to observe such low values in aquaria. The growth rates measured here are close to those found by Chapron et al. (2021) using a similar experimental setup. Earlier studies conducted in aquaria collected corals originating from shallow depth in Norwegian fjords (Orejas et al., 2019), to 690 meters depth in the Mediterranean Sea at the deepest (Lartaud et al., 2014; Naumann et al., 2014). Here, the specimens collected in the Lampaul canyon came from a depth of 800 m, which, to our knowledge, corresponds, to the deepest corals maintained in aquaria for such medium-term experiments. The lower growth rate may therefore also be explained by the fact that our corals came from deeper waters, since maintaining such a deep population in aquariums at atmospheric pressure could be detrimental to their health. This hypothesis is supported by the lack of difference in growth rates between subapical and apical polyps, which normally grow faster (Chapron et al., 2021). The fact that there was some mortality in the control conditions (72 % survival at 10°C after 2 month), although physico-chemical conditions remain stable, is a further indication that aquaria conditions may not be optimal for these corals. Alternatively, the lower growth rate may simply reflect different ecological properties of the Lampaul canyon corals. A better characterization of *in situ* coral biology is thus required.

In the current experiment, temperature had no significant effect on skeletal growth rates, which contrasts with results from previous studies. Based on similar temperature values for Mediterranean *L. pertusa* (i.e., 10, 13 and 15°C), Chapron et al., (2021) observed the highest growth rates at 13°C, in the *in situ* conditions, and a lower both at 10 and 15°C. Decrease of calcification rates with lower temperatures was described by Naumann et al. (2014) on Mediterranean *L. pertusa* when exposed at 12°C and 6°C, but these corals were placed in lower temperatures compared to *in situ* conditions (i.e., ~13°C at 300m depth in the Cap de Creus canyon, Ulses et al., 2010). A warming experiment on *L. pertusa* from a norwegian fjord revealed higher calcification rates for corals exposed to 12°C rather than 8°C, their natural habitat conditions (Büscher et al., 2017). Corals from different regions thus seem to respond differently to a changing environment and do not exhibit the same sensitivity. An increase in growth rates is not necessarily associated to good health status conditions, but the fact that temperature had no effect on polyp's growth rate during our two-months experiment, indicates that corals had likely maintained sufficient reserves and/or metabolism that could be invested to sustain growth. However, as cold-water corals are known as slow-growing species compared

to tropical corals, a longer-term experiment could have allowed to detect more precise differences in growth response (Mouchi et al., 2019; Chapron et al., 2021).

2.5 Conclusion

In the present study we showed that an increase of water temperatures of +3°C and +5°C was responsible for dramatic mortality in *L. pertusa*. Mortality appears to be associated to bacterial pathogens as indicated by the strong increase in the number of genes coding for virulence factors such as motility and secretion systems. In the more resilient individuals, the polyps survived, but elevated temperatures caused rapid changes of bacterial community composition (dysbiosis). Interestingly, some specific colonies had specific microbiomes. Such colony specific features, may explain that some individuals are more resilient to a changing environment. Finally, our results suggest that NE Atlantic *L. pertusa* are as sensitive to warming as Mediterranean or Gulf of Mexico populations. Although the NE Atlantic *L. pertusa* have a lower upper thermal limit than other *L. pertusa* populations (<13°C vs <15°C), it appears that all *L. pertusa*, regardless of the region they originate from and the water temperature in which they live, will be strongly impacted by a +3°C increase. Future works on the thermal tolerance of cold-water corals should therefore consider the level of temperature increase in an ecological context, rather than an arbitrarily fixed value.

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Supplementary material

Supplementary Table 2.1. Mean (mean \pm SD) physico-chemical parameters of the seawater from different treatments measured temperature ($^{\circ}\text{C}$), pH, salinity (psu), and oxygen concentration (% saturation).

Treatment	Temperature ($^{\circ}\text{C}$)	pH	Salinity (psu)	Oxygen (% sat.)
10 $^{\circ}\text{C}$	10.1 \pm 0.2	8.16 \pm 0.05	37.9 \pm 0.5	96.4 \pm 3.3
13 $^{\circ}\text{C}$	12.9 \pm 0.2	8.17 \pm 0.05	37.9 \pm 0.5	96.2 \pm 3.2
15 $^{\circ}\text{C}$	14.9 \pm 0.2	8.20 \pm 0.07	37.8 \pm 0.6	97.4 \pm 3.1

Supplementary Table 2.2. Average polyp linear growth rates \pm s.d. (mm yr^{-1}) of *L. pertusa* for all, apical and subapical polyps, under different temperature conditions after 8 weeks of experiment. The number in brackets represent the number of replicates.

	all polyps (mm y^{-1})	apical polyps (mm y^{-1})	subapical polyps (mm y^{-1})
all	2.9 \pm 2.5 (44)	2.5 \pm 1.9 (9)	3.0 \pm 2.7 (35)
10 $^{\circ}\text{C}$	2.6 \pm 2.3 (12)	1.2 \pm 0.0 (2)	2.8 \pm 2.4 (10)
13 $^{\circ}\text{C}$	2.2 \pm 1.7 (11)	2.5 \pm 0.0 (1)	2.2 \pm 1.8 (10)
15 $^{\circ}\text{C}$	3.5 \pm 2.9 (21)	3.0 \pm 2.2 (6)	3.7 \pm 3.2 (15)

Supplementary Table 2.3. Biovolumes (mm³) and surface (mm²) of coral nubbins obtained by 3D scanner at the start of the experiment (T0) and after 8 weeks (T4), and the calculated growth in biovolume and surface.

Analyzed nubbin	Colony	Temperature	Scan biovolume T0 (mm ³)	Scan biovolume T4 (mm ³)	Growth in biovolume (mm ³)	Scan surface T0 (mm ²)	Scan surface T4 (mm ²)	Growth in surface (mm ²)
L5_C10_1	L5	10	Unusable scan	3097.44	/	Unusable scan	3153.9	/
L6_C10_1	L6	10	435.59	427.496	-8.094	872.041	1198.48	326.439
L6_C10_2	L6	10	232.307	226.794	-5.513	495.062	789.429	294.367
L7_C10_1	L7	10	1301.99	1137.29	-164.7	1701.3	1856.36	155.06
L8_C10_1	L8	10	1242.86	1187.94	-54.92	2306.7	1655.74	-650.96
L8_C10_2	L8	10	Unusable scan	Unusable scan	/	Unusable scan	Unusable scan	/
L5_C13_1	L5	13	596.595	509.332	-87.263	806.664	912.827	106.163
L5_C13_2	L5	13	1406.36	1274.08	-132.28	2152.07	3267.67	1115.6
L6_C13_1	L6	13	750.804	760.317	9.513	1001.04	1046.28	45.24
L6_C13_2	L6	13	804.916	760.804	-44.112	972.527	1000.49	27.963
L7_C13_1	L7	13	974.569	865.162	-109.407	1166.31	1189.25	22.94
L7_C13_2	L7	13	587.249	593.805	6.556	863.841	958.735	94.894
L9_C13_1	L9	13	1073.19	1107.9	34.71	1321.01	1337.54	16.53
L5_C15_1	L5	15	1044.78	966.255	-78.525	1486.05	1608.76	122.71
L5_C15_2	L5	15	1344.75	1259.4	-85.35	1300.63	1493.75	193.12
L7_C15_1	L7	15	1318.9	1488.13	169.23	2061.5	1857.44	-204.06
L7_C15_2	L7	15	1966.33	Unusable scan	/	3279.25	Unusable scan	/
L8_C15_1	L8	15	1103.86	1028.35	-75.51	1574.44	1617.65	43.21
L8_C15_2	L8	15	718.696	661.919	-56.777	990.956	1110.2	119.244
L9_C15_1	L9	15	1183.17	1072.79	-110.38	1433.94	1483.06	49.12
L9_C15_2	L9	15	883.06	788.079	-94.981	1330.94	2088.23	757.29

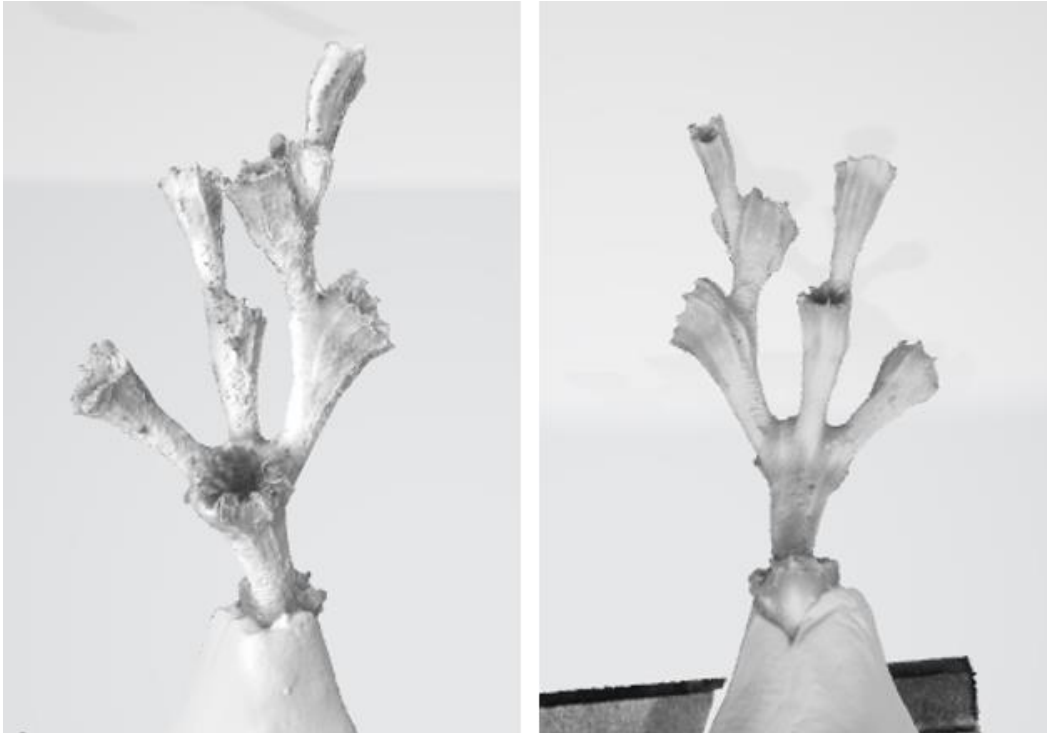
Supplementary Table 2.4. Summary statistics of the pairwise PERMANOVA testing the effect of temperature and time on microbial community composition.

Time	Comparisons	R	F	p-value
T0	10 vs 13	0.100	2.450	0.001
	10 vs 15	0.119	2.826	0.001
	13 vs 15	0.155	3.853	0.001
2 weeks	10 vs 13	0.063	1.414	0.084
	10 vs 15	0.126	2.601	0.001
	13 vs 15	0.149	2.973	0.002
4 weeks	10 vs 13	0.223	3.223	0.005
	10 vs 15	0.447	6.460	0.026
	13 vs 15	0.288	2.020	0.097
6 weeks	10 vs 13	0.254	2.717	0.013
	10 vs 15	0.244	2.582	0.010
	13 vs 15	0.406	2.738	0.100
8 weeks	10 vs 13	0.229	3.273	0.003
	10 vs 15	0.260	3.162	0.003
	13 vs 15	0.148	2.098	0.002

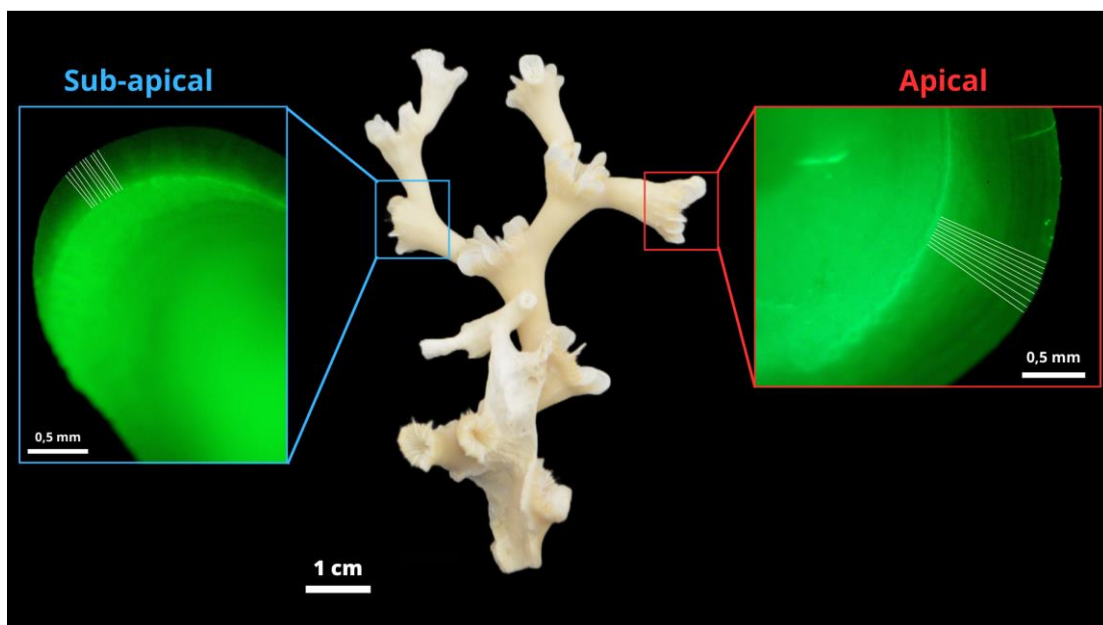
Supplementary Table 2.5. Relative abundance of targeted genes involved in type 1 and 3 secretion systems (T1SS and T3SS), and flagella and pili construction in the microbiome of the corals incubated at 10°C, 13°C, and 15°C.

KEGG	Gene	L7_M10_1_B	L8_M10_1_B	L6_M13_1_B	L9_M13_1_C	L7_M15_1_C	L9_M15_1_B	L7_M10_5_C	L8_M10_5_B	L6_M13_5_B	L9_M13_5_C	L7_M15_5_C	L9_M15_5_B
Type 3 secretion system													
K04054	yscK, sctK; type III secretion protein K	0,006035	0,087521	0,105143	0,09932	0,126018	0	0,019731	0	1,437995	0,195678	0,602849	2,780785
K04056	yscO, sctO; type III secretion protein O	0	0	0	0	0	0	0	0	1,328473	0,108132	0,709154	2,682928
K04057	yscP, sctP; type III secretion protein P	0,078578	0	0	0	0,04372	0	0	0,033271	1,560497	0,080915	1,359817	3,836513
K04058	yscW, sctW; type III secretion protein W	0,338501	0,124512	0,384671	0,165989	0,124794	0,386769	0,599632	0,189934	1,546715	0,294235	2,350168	5,454316
K04059	yscX, sctX; type III secretion protein X	0,045298	0	0	0,125144	0	0	0	0	1,239428	0,139935	0,57358	2,187851
K04060	yscY, sctY; type III secretion protein Y	0,14625	0,040923	0,169057	0,108543	0,103519	0,941097	0,17665	0,011856	0,289336	0,077379	0,806641	0,362697
K03219	yscC, sctC, ssaC; type III secretion protein C	2,458578	1,044205	1,162734	1,126955	1,793622	2,426361	2,983507	1,230679	3,352953	0,943602	6,162918	7,537436
K03220	yscD, sctD, ssaD; type III secretion protein D	0,250538	0,065423	0,222559	0,083429	0	0,887134	0,714763	0,35122	1,356055	0,154806	3,543556	2,663407
K03221	yscF, sctF, ssaG, prgl; type III secretion protein F	0	0	0	0	0	0	0	0	0,094401	0	0,120381	0,037433
K03222	yscJ, sctJ, hrcJ, ssaJ; type III secretion protein J	0,425372	0,15514	0,803114	0,594675	0,427957	1,199572	2,111744	0,622868	2,18596	0,669376	3,109072	3,087045
K03223	yscL, sctL; type III secretion protein L	0,322355	0,230925	1,047255	0,571176	0,30398	2,460047	1,894796	0,768505	1,579715	0,387111	3,723233	3,96313
K03224	yscN, sctN, hrcN, ssaN; ATP synthase in type III secretion protein N [EC:7.4.2.8]	1,281483	0,096757	2,039901	1,035079	1,172424	4,267782	3,898487	1,007501	3,370369	1,995269	8,903193	8,566855
K03225	yscQ, sctQ, hrcQ, ssaQ, spaO; type III secretion protein Q	0,565994	0,038628	1,733908	0,204815	0,419702	4,3629	1,62221	0,266495	1,933302	0,283707	2,492169	3,169049
K03226	yscR, sctR, hrcR, ssaR; type III secretion protein R	0,939339	0,061717	1,427603	0,518603	0,312162	1,566869	2,002232	0,485067	1,773426	0,498042	3,916835	2,745344
K03227	yscS, sctS, hrcS, ssaS; type III secretion protein S	0,210401	0,023526	0,726882	0,307312	0,285201	0,459989	1,351578	0,055856	1,357641	0,365503	2,256994	2,449365
K03228	yscT, sctT, hrcT, ssaT; type III secretion protein T	0,70799	0,077699	0,98325	0,379696	0,206238	1,139294	0,748676	0,311132	1,34325	0,153801	3,523127	1,372631
K03229	yscU, sctU, hrcU, ssaU; type III secretion protein U	1,25764	0,503166	1,567709	0,565943	0,366631	2,346792	2,111585	1,2407	2,124221	1,263517	4,745076	3,724908
K03230	yscV, sctV, hrcV, ssaV, invA; type III secretion protein V	2,594754	0,388669	4,717458	1,706763	1,898448	8,403984	8,055382	2,855723	5,185876	2,037306	13,323896	10,915568
Type 1 secretion system													
K11003	hlyD, cyaD; membrane fusion protein, hemolysin D	6,866158	0,860659	7,125296	4,242938	3,748039	8,663988	9,932998	16,377813	7,200507	4,823232	26,565677	48,031379
K11004	hlyB, cyaB; ATP-binding cassette, subfamily B, bacterial HlyB/CyaB	11,783166	4,116559	14,241261	11,559321	7,815448	22,855192	26,431717	23,302165	24,039311	15,194531	89,961046	166,706929
Flagella													
K02406	fliC, hag; flagellin	101,415891	43,202071	294,256314	109,686064	109,488947	296,751769	151,165165	93,173692	87,697942	58,127556	251,111882	196,129793
K02396	flgK; flagellar hook-associated protein 1	74,443565	22,088667	223,067482	63,255538	75,819683	215,009221	101,784839	69,140746	62,381997	37,349039	161,645976	151,774769
K02397	flgL; flagellar hook-associated protein 3 FlgL	41,165377	14,224118	130,391641	37,036911	48,687758	127,202132	59,330007	43,88014	32,542259	19,301779	99,519651	81,388091
K02390	flgE; flagellar hook protein FlgE	78,895606	25,05897	202,798047	74,628882	84,813502	213,831953	111,635134	67,070033	58,584434	42,424267	161,59418	165,150086
K02387	flgB; flagellar basal-body rod protein FlgB	33,50302	9,089745	88,144539	26,81897	29,842943	102,279181	48,901723	33,435343	28,55581	14,070545	75,875809	53,76976
K02388	flgC; flagellar basal-body rod protein FlgC	34,39394	9,20949	94,199126	31,068568	30,968459	104,019579	50,46657	30,783843	26,10818	15,117662	70,245986	53,715751
K02391	flgF; flagellar basal-body rod protein FlgF	37,64856	11,833159	110,415368	30,884484	32,266766	103,136252	44,248398	31,8412	29,221094	17,551753	66,043084	74,303811
K02392	flgG; flagellar basal-body rod protein FlgG	48,671746	12,291868	132,090868	45,818348	39,393342	123,977472	62,281844	48,687293	33,64144	24,460571	97,213287	106,143153
K02409	fliF; flagellar M-ring protein FliF	79,327353	28,75105	232,336569	118,068892	72,596937	229,250089	114,796418	72,131167	74,02402	43,107605	175,442218	137,984984
K02410	fliG; flagellar motor switch protein FliG	53,693871	17,974567	184,790813	52,935292	61,918594	186,361909	80,246582	42,926553	42,263304	22,983924	120,329255	83,232259
K02416	fliM; flagellar motor switch protein FliM	31,305145	11,136864	89,886661	35,83712	28,885133	89,31109	64,820635	37,237526	26,507778	17,152073	85,335425	72,210073
K02417	fliN; flagellar motor switch protein FliN	35,55309	9,787905	91,203989	37,181788	32,848055	102,544896	66,216553	27,609389	29,366325	18,215462	82,425286	63,343127
K02556	motA; chemotaxis protein MotA	58,758032	15,899565	151,772029	46,990054	59,505706	182,715986	76,766687	57,926515	37,746874	26,62088	106,681272	100,336563
K02557	motB; chemotaxis protein MotB	111,933217	33,677803	305,790854	105,250483	111,269448	329,578348	141,147695	74,028963	68,730117	44,425968	168,531978	161,815805
K02400	fliA; flagellar biosynthesis protein FliA	283,256874	209,174577	357,59085	360,870177	298,223041	343,956377	411,33656	340,909317	321,008591	278,302394	402,766179	283,264707
K02401	fliB; flagellar biosynthesis protein FliB	48,811228	18,714915	129,978809	42,236211	44,406286	136,246526	82,943512	53,61282	47,423702	26,552384	115,153715	93,610782
K02419	fliP; flagellar biosynthesis protein FliP	44,440546	12,400646	130,412853	35,348936	37,257423	123,434173	58,332873	35,632716	33,218993	17,442606	85,631639	63,74004
K02420	fliQ; flagellar biosynthesis protein FliQ	21,81774	6,028362	68,985747	19,510602	20,918918	70,731247	33,966972	22,993354	20,510642	10,202296	54,096475	35,398495
K02421	fliR; flagellar biosynthesis protein FliR	34,981599	9,316351	101,342217	34,528233	30,738902	109,999179	60,305303	35,995686	32,120866	19,537589	91,582192	68,185453
K02412	fliI; flagellum-specific ATP synthase [EC:7.4.2.8]	59,208204	20,520063	186,702041	62,437482	59,398373	183,519581	92,652148	51,033315	38,979825	26,99835	112,437496	96,270789

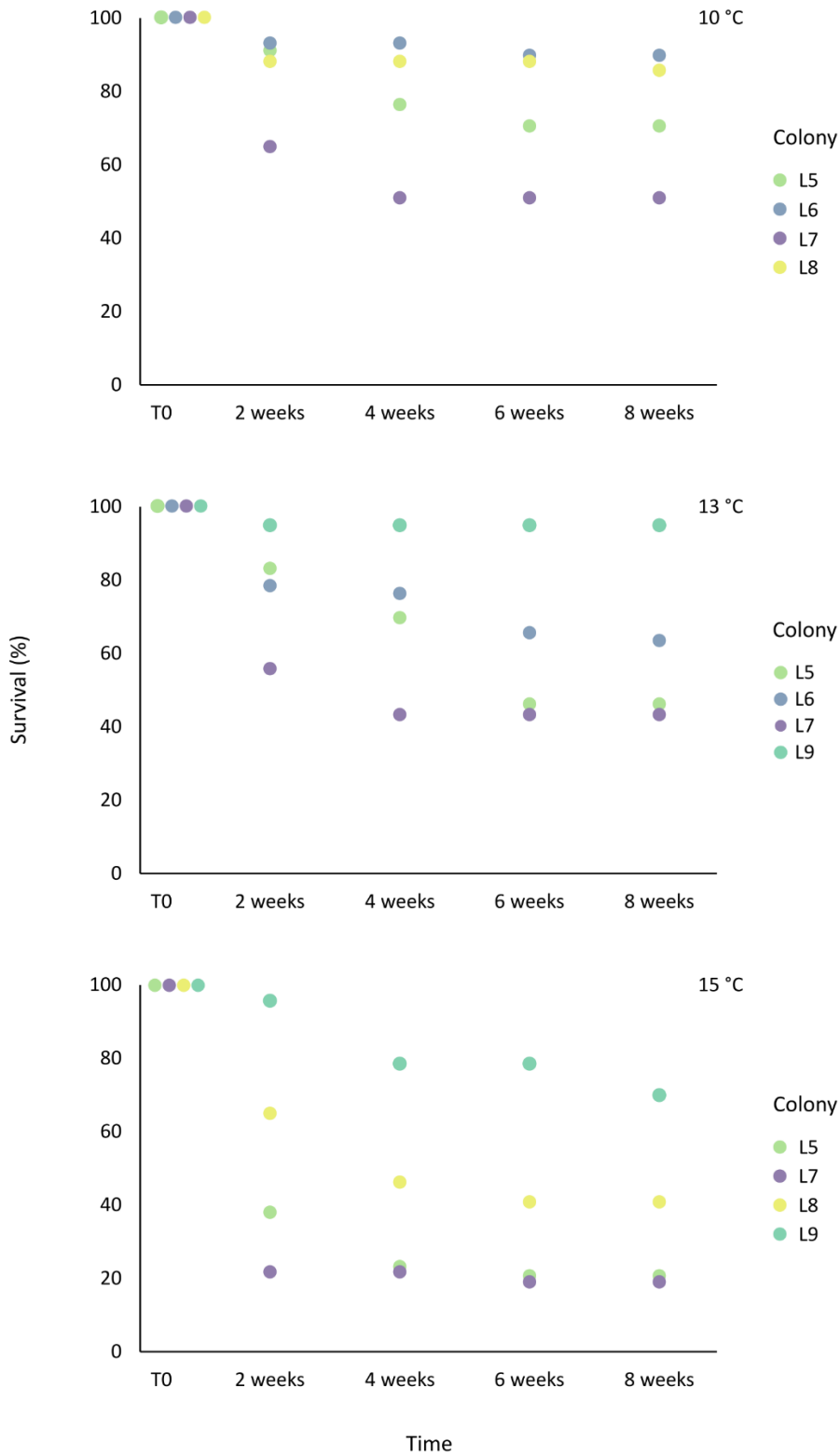
KEGG	Gene	L7_M10_1_B	L8_M10_1_B	L6_M13_1_B	L9_M13_1_C	L7_M15_1_C	L9_M15_1_B	L7_M10_5_C	L8_M10_5_B	L6_M13_5_B	L9_M13_5_C	L7_M15_5_C	L9_M15_5_B
Pili													
K02650	pilA; type IV pilus assembly protein PilA	20,895395	4,653353	54,52995	14,832253	14,471252	58,242239	25,263512	24,31083	13,873075	9,204673	38,551669	32,212639
K02655	pilE; type IV pilus assembly protein PilE	15,134035	5,812062	48,536359	12,292412	14,960094	41,644395	15,574052	13,303096	7,033185	5,670071	19,931173	17,783
K02671	pilV; type IV pilus assembly protein PilV	13,517385	5,992183	39,056706	10,375296	15,966709	36,90708	13,506069	15,043151	7,244848	3,923507	18,286317	16,008743
K02672	pilW; type IV pilus assembly protein PilW	14,694378	6,062598	42,26226	10,669448	19,025779	35,671673	24,20475	16,080928	8,429888	6,570338	32,566119	24,70914
K02673	pilX; type IV pilus assembly protein PilX	10,514032	3,786031	32,434321	8,068122	15,101802	34,427807	7,909915	6,423868	4,241586	2,682428	11,390082	12,422126
K02658	pilH; twitching motility two-component system response regulator PilH	7,878582	1,63627	11,654122	5,284194	6,578955	16,582981	10,059125	7,447571	3,639879	5,383195	8,485222	11,139496
K02659	pilJ; twitching motility protein PilJ	3,903328	1,777687	11,060377	3,548484	5,587552	9,392384	9,620699	11,986518	3,770364	3,941518	5,258617	10,446773
K02660	pilI; twitching motility protein PilI	18,78987	6,021698	33,358058	12,531063	17,404813	37,35698	23,251912	26,915074	14,842042	10,983499	34,061268	46,900285
K02661	pilK; type IV pilus assembly protein PilK	1,058352	0,516164	3,067625	1,208742	1,972516	3,442374	1,558428	0,512002	0,055954	0,027156	0,541142	1,755196
K02662	pilM; type IV pilus assembly protein PilM	41,214915	11,7722	95,837172	35,991915	41,140718	102,982661	56,622181	39,720392	26,096689	27,877989	85,307244	83,467362
K02663	pilN; type IV pilus assembly protein PilN	14,262622	4,175809	35,106234	8,774806	15,160172	33,956271	15,424309	14,362188	8,495841	7,320589	26,119431	20,510297
K02664	pilO; type IV pilus assembly protein PilO	18,627864	7,205273	45,129313	11,648749	19,479678	44,905826	21,047225	18,612832	7,982125	9,087606	34,550093	27,958044
K02665	pilP; type IV pilus assembly protein PilP	15,28842	3,596687	30,661118	10,24574	13,509293	43,30838	13,755616	12,990812	5,531417	4,526438	15,500881	14,664939
K02653	pilC; type IV pilus assembly protein PilC	83,897023	22,966374	174,302722	63,743672	64,296268	184,01495	105,57527	71,987136	46,780266	42,871639	151,117907	135,740961
K02657	pilG; twitching motility two-component system response regulator PilG	7,609875	1,776618	11,71168	5,029468	4,449101	12,473628	15,020598	10,377989	3,705799	2,279142	14,132616	10,905672
K02669	pilT; twitching motility protein PilT	56,151747	17,655728	120,146397	62,539029	54,80553	143,318093	101,3237	67,205994	37,244341	59,445141	141,745313	138,813102
K02652	pilB; type IV pilus assembly protein PilB	88,579347	25,32318	185,797905	92,481002	94,941371	217,150703	137,939898	104,150842	55,281908	57,045278	194,228121	197,982642
K02656	pilF; type IV pilus assembly protein PilF	35,043591	19,528524	68,963044	29,988159	33,489744	67,037158	34,174634	29,878647	21,046812	24,589284	54,939775	50,394411



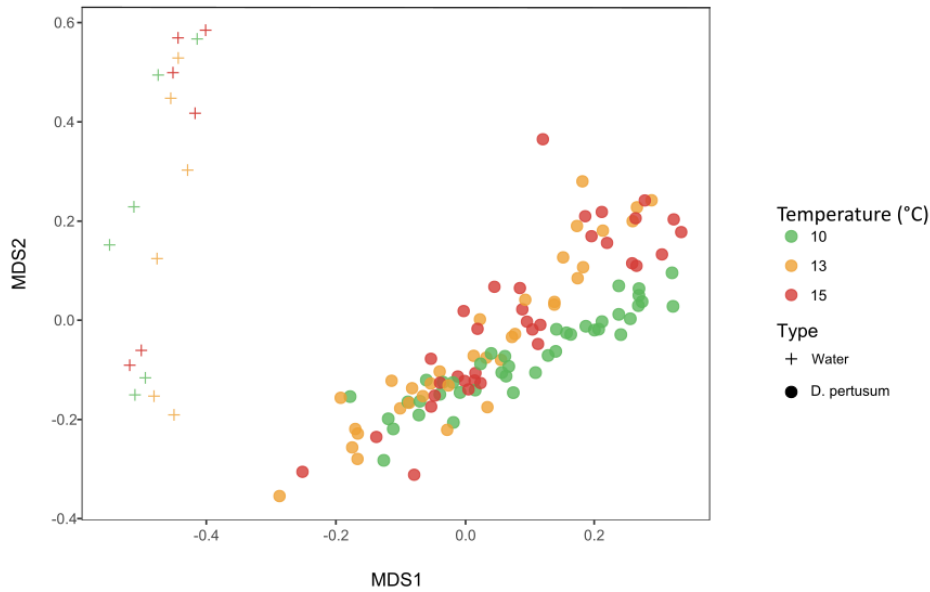
Supplementary Fig. 2.1. Pictures of a scanned nubbins used for growth measurement, using AutoScan Inspec 3D scanner.



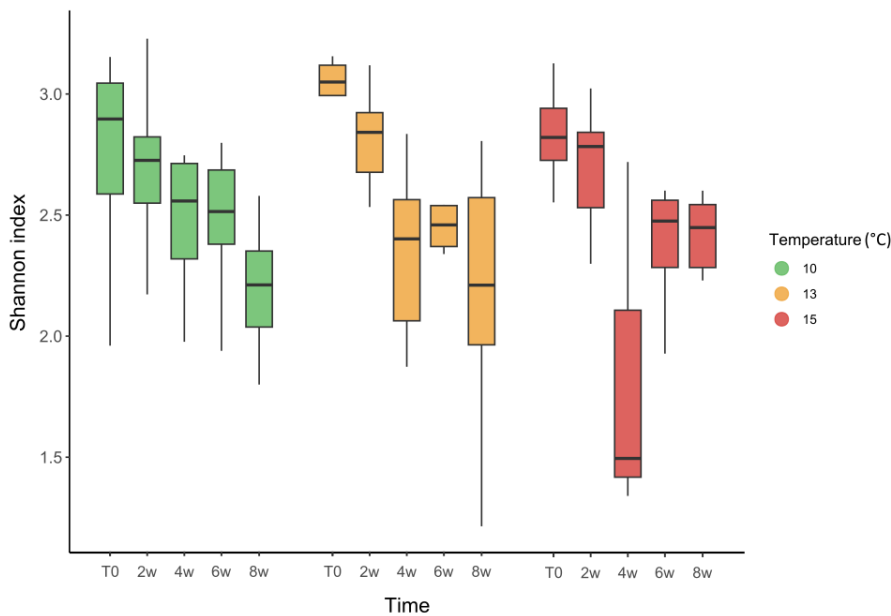
Supplementary Fig. 2.2. Nubbins of *L. pertusa* with apical (red square) and subapical (blue squares) polyps with the major septum observed under fluorescence revealing the calcein staining (green line). The white lines correspond to linear extension measurements taken on Image J.



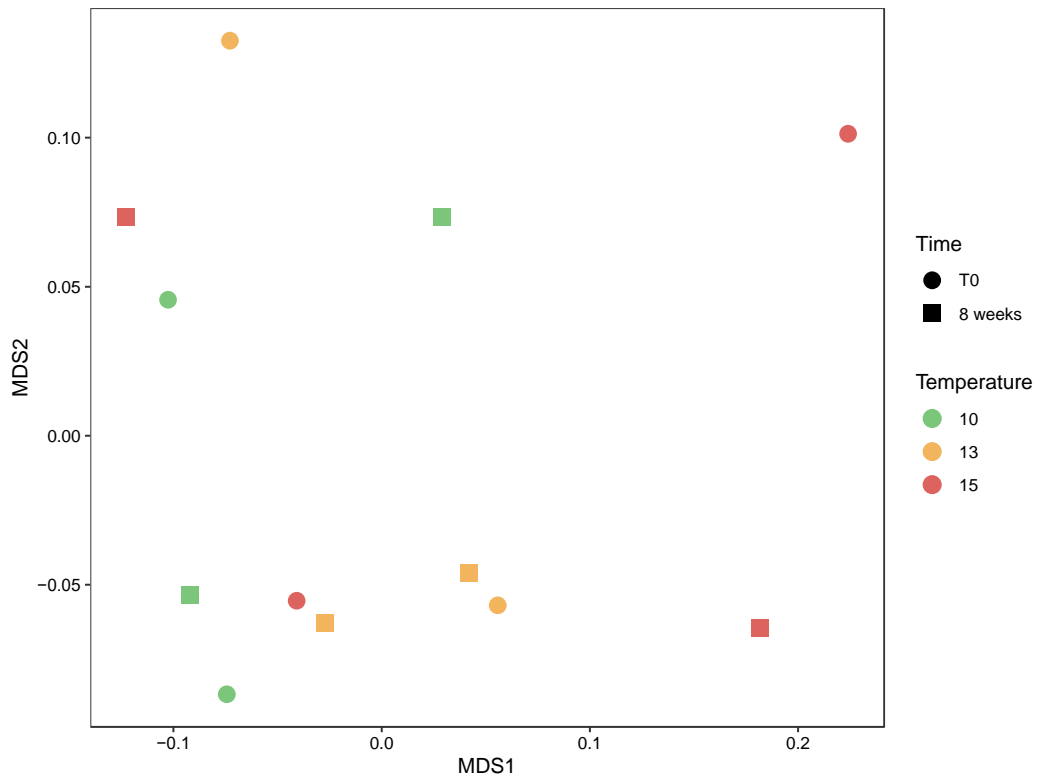
Supplementary Fig. 2.3. Polyp survival rate (%) of *L. pertusa* for each colony in each temperature condition (10°C, 13°C and 15°C) during eight weeks of experiment.



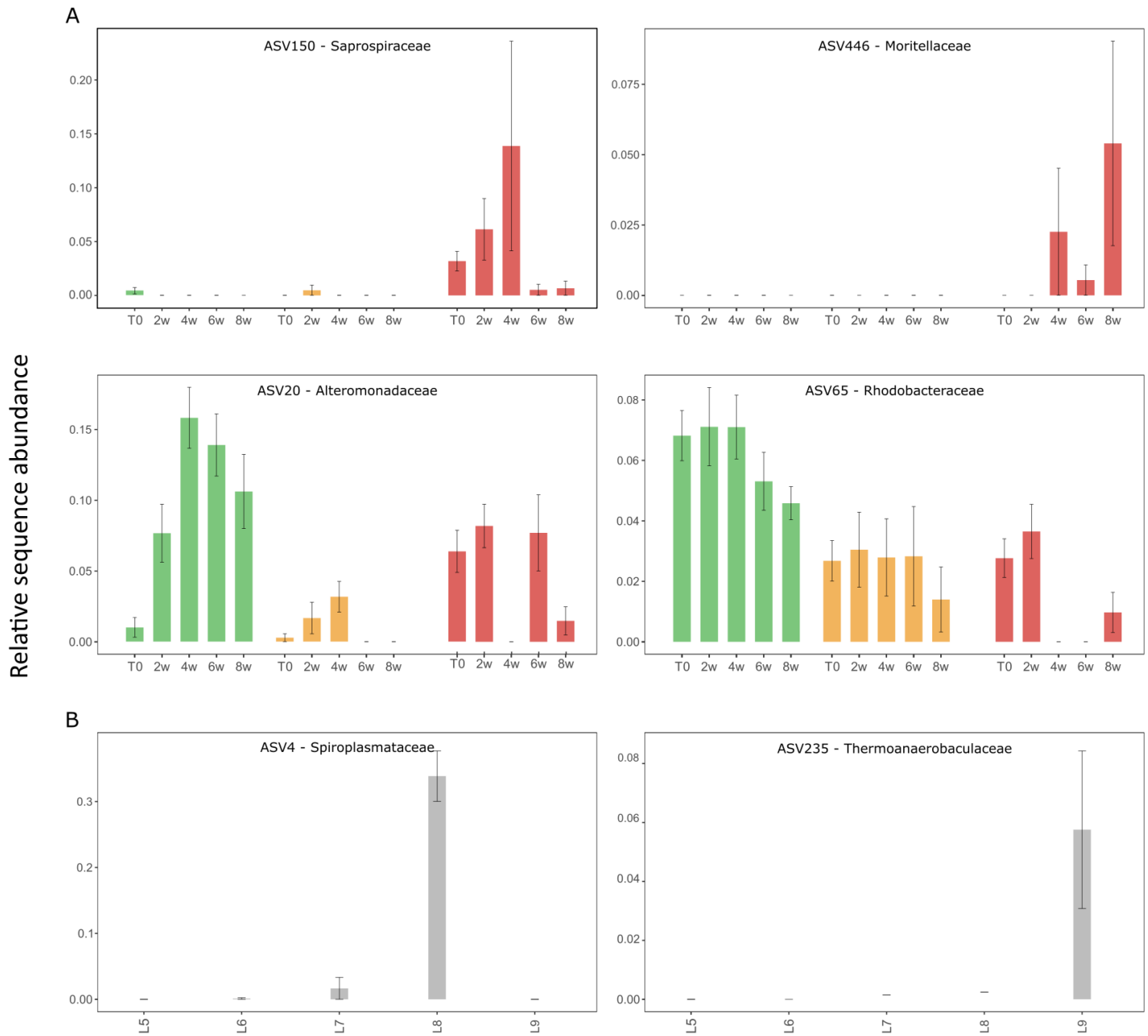
Supplementary Fig. 2.4. Multi-dimensional scaling plot (nMDS) based on the Bray-Curtis similarity index showing the similarity between bacterial community compositions of *L. pertusa* and seawater during the eight weeks experiment at 10°C, 13°C and 15°C.



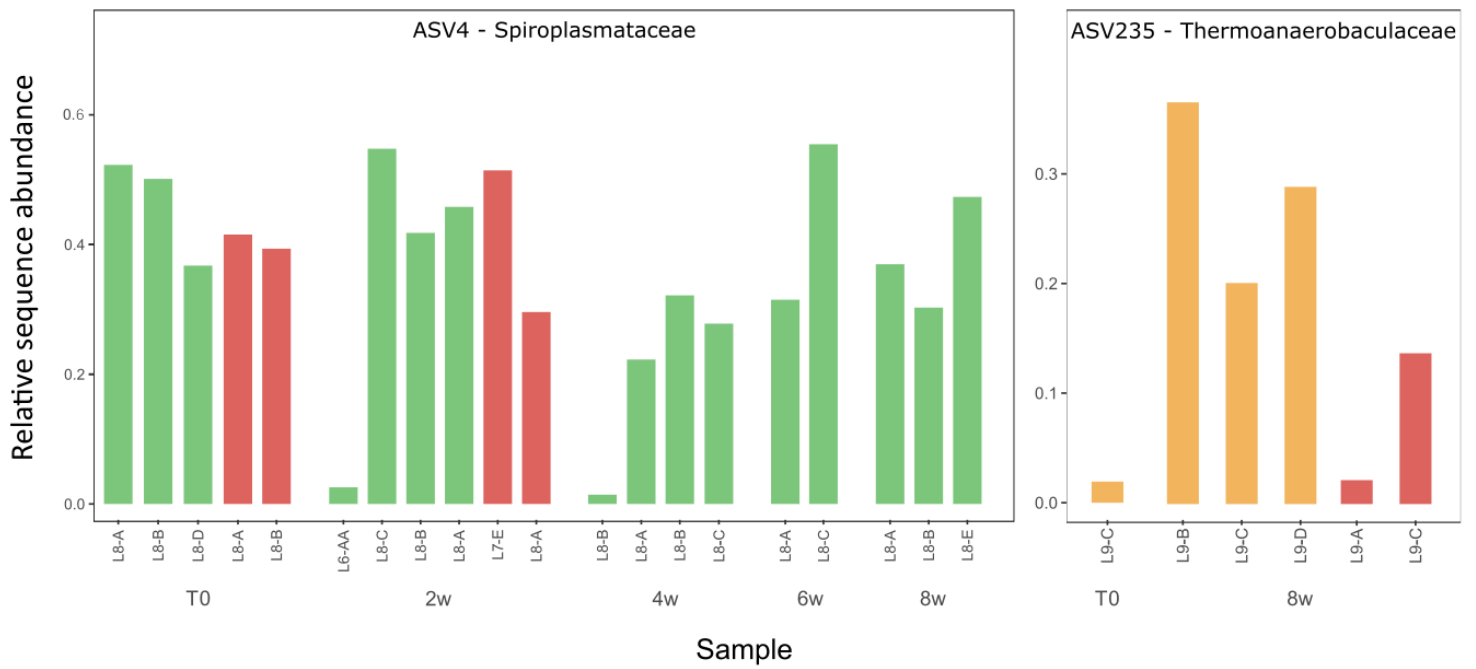
Supplementary Fig. 2.5. Shannon diversity index of *L. pertusa* bacterial communities under experimental conditions at 10°C, 13°C and 15°C at the start of the experiment (T0) and after 2 weeks (2w), 4 weeks (4w), 6 weeks (6w) and 8 weeks (8w) of experiment. Values presented in the box plot correspond to the median and quartile.



Supplementary Fig. 2.6. Multi-dimensional scaling plot (nMDS) based on the relative abundance of all bacterial genes at the beginning of the experiment (T0) and after 8 weeks for corals incubated at 10°C, 13°C and 15°C.



Supplementary Fig. 2.7. Relative sequence abundance of ASVs of interest characterizing *L. pertusa* bacterial communities under different experimental conditions (10°C, 13°C and 15°C) at the start of the experiment (T0), and after 2 weeks (2w), 4 weeks (4w), 6 weeks (6w) and 8 weeks (8w) of experiment. (A) and unique ASVs that characterize specific colonies of *L. pertusa* during the experiment (B). The affiliation of the ASVs at the family level is given, except for ASV4 for which the affiliation at the phylum level is available. Mean values and standard deviations are presented.



Supplementary Fig. 2.8. Detailed abundance of unique ASVs that characterize specific colony of *L. pertusa* in the different temperature conditions at the start of the experiment (T0), and after 2 weeks (2w), 4 weeks (4w), 6 weeks (6w) and 8 weeks (8w) of experiment.

Chapter three: Rising water temperature induces stress and dysbiosis in the Atlantic deep-sea coral *Lophelia pertusa*

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Abstract

Cold-water corals form large reef structures that act as hotspots of marine biodiversity in the deep ocean. They are found throughout the world's oceans over a wide range of temperatures. However, cold-water corals are particularly threatened by rising temperatures in the deep sea, caused by climate change. While degraded health in response to both lower and higher temperatures has been observed in several scleractinian species, the underlying molecular mechanisms of their thermal response remain poorly understood. Here we report the results of a long-term, four-month experiment to determine the response of the holobiont cold-water coral *Lophelia pertusa* (now named *Desmophyllum pertusum*) to temperature at both the organismal and gene expression levels. Our results showed that while a 4°C decrease in temperature did not affect the physiology and microbiome of *L. pertusa*, a 4°C increase in temperature resulted in massive mortality. This mortality appears to be associated with high levels of stress in the coral, as evidenced by the upregulation of the number of genes related to immune, inflammatory and antioxidant responses, cell death and apoptosis, DNA repair and maintenance, but also the shift in the coral bacterial community towards pathogens and opportunistic bacteria. Understanding the response of cold-water corals to ocean warming using an integrative approach at the holobiont level is of paramount importance to assess their resilience and adaptive capacity in response to current and intensifying future global changes.

Keywords: Cold-water coral, Holobiont, Transcriptomics, Gene expression, Pathogens, Climate change

3.1 Introduction

Scleractinian cold-water corals are principally found in cold-water deep-sea environments (Freiwald et al., 2011), where they form large three-dimensional reef frameworks that act as marine biodiversity hot spots (Roberts et al., 2009). These ecosystem engineers shape highly valuable habitats, providing ecological niches, nursery grounds, and food for thousands of associated species (Roberts and Cairns, 2014; Buhl-mortensen et al., 2016). Among cold-water scleractinians, some species such as the reef forming corals *Lophelia pertusa* (now synonymized as *Desmophyllum pertusum*, Addamo et al., 2016), *Madrepora oculata* and *Solenosmilia variabilis*, and the solitary coral *Desmophyllum dianthus* show a widespread distribution throughout the world's oceans. They can live under a wide temperature range and are supposed to have a broad thermal tolerance (Naumann et al., 2013; Dorey et al., 2020). *L. pertusa* is the most common reef-building cold-water coral species, distributed worldwide at depths of 40–3300 m, and in water temperatures between 4 and 15°C (Roberts et al., 2009).

Temperature has been proposed as one of the key factors structuring the habitat of cold-water corals, and thus determining their distribution around the world (Davies & Guinotte, 2011). However, due to climate change, temperature is rapidly rising including in the deep ocean (Sweetman et al., 2017). In the deep waters of the Atlantic Ocean, temperatures have increased by 0.2–0.3°C since 2000 (Chen and Tung, 2014), and could further rise by as much as 3°C before the end of the century (Sweetman et al., 2017). It could threaten cold-water corals, as ectotherms are particularly sensitive to temperature changes due to their limited ability to actively regulate their own temperature (Angilletta, 2009). Recent studies have therefore recently focused on the effect of temperature on the health of cold-water corals. Degraded health has been documented in experiments with corals from the Gulf of Mexico (Brooke et al., 2013), the Mediterranean Sea (Gori et al., 2014; Naumann et al., 2014; Chapron et al., 2021), and the NE Atlantic Ocean (Dodds et al., 2007; Büscher et al., 2017; Manuscript's chapter 2). In general, elevated temperatures affected *L. pertusa* physiology by decreasing their survival (Brooke et al., 2013; Lunden et al., 2014; Chapron et al., 2021; Manuscript's chapter 2), increasing polyp activity, but simultaneously lowering their energy reserves (Chapron et al., 2021), changing growth (Büscher et al., 2017), and changing their microbiome (Chapron et al., 2021; Manuscript's chapter 2), with the appearance of gene markers for pathogenic bacteria (Manuscript's chapter 2). Conversely, several studies considered the impact of lower temperatures, and showed lower calcification rate when temperature decreased (Naumann et al., 2014; Chapron et al., 2021). However, while all populations seem to be sensitive to long-

term warming (from + 3°C), they did not all respond in the same way and may not have the same upper thermal limit. In the Mediterranean Sea, *L. pertusa* live at 13-14°C (Tursi et al., 2004; Freiwald et al., 2009), which is now considered to be close to the upper limit of thermal tolerance known for this species (Brooke et al., 2013; Lunden et al., 2014; Chapron et al., 2021). In the Bay of Biscay in the NE Atlantic, a temperature of 13-14°C would represent an increase of +3-4°C of the *in situ* temperature. No study to date has investigated the impact of such temperature changes on the biology of *L. pertusa* at the holobiont scale over several months.

Holobiont studies of cold-water corals are now combining knowledge of coral physiology and behaviour with descriptions of the diversity of their microbiome (Chapron et al., 2021). Recently, metagenomics approaches have also revealed the functional role of the microbiome and in particular the presence of bacterial genes associated to virulence (Manuscript's chapter 2). The molecular response of the coral itself remains, however, poorly known. Genomics are emerging as promising diagnostic tools for characterizing and understanding coral stress response (Louis et al., 2017). Among them, transcriptomics allow the detection of the expressed genes used as indicators of thermal stress in corals (Desalvo et al., 2010; Barshis et al., 2013; Ricci et al., 2019). In tropical corals, significant upregulation of genes involved in heat shock, immune and inflammatory responses and oxidative stress are thus often registered. While transcriptomics and proteomics are often used for temperate and tropical corals to detect and quantify coral stress in relation to disease infection (e.g., Libro et al., 2013; Ricci et al., 2019), bleaching events and more generally climate change (e.g., Desalvo et al., 2008; 2010; Seneca et al., 2015), these tools have been rarely used for cold-water corals in general (Carreiro-Silva et al., 2014; 2022), and only once in *L. pertusa* to study of the effect of pH (Glazier et al., 2020). This study showed the upregulation of ion transport related genes, concomitant with lowered expression of genes involved in metabolic processes in response to low pH. The effect of temperature on *L. pertusa* gene expression is not yet known.

In this context, the aim of the present study was to improve our knowledge and understanding of the molecular mechanisms underlying the thermal response of the holobiont *L. pertusa* from a submarine canyon in the Bay of Biscay (NE Atlantic Ocean). We conducted a long-term experiment (four-months) in aquaria, in which corals were exposed to three different temperature conditions, close to the thermal limits of their distribution range: *in situ* temperature (10°C), and temperatures corresponding to a colder and a warmer scenarios (6°C and 14°C). We studied the response of the coral at the holobiont level by measuring coral gene expression, survival and growth, prey capture rate, and by describing the diversity of microbiome by metabarcoding.

3.2 Material and methods

3.2.1 Specimen collection, maintenance on board and in the laboratory

Specimen of *Lophelia pertusa* (Linnaeus, 1758) were collected at 800 m depth from the Lampaul canyon, in the Bay of Biscay, North-east Atlantic Ocean (N.47°36.703, W07°32.192), using the remotely operated vehicle (ROV) Victor from the R/V Thalassa, during the research cruise ChEReef (habitat Characterization & Ecology of cold-water coral Reefs) in august 2022. Five distinct colonies (pink specimens) were sampled within the same area. On board, corals were maintained in the dark, in oxygenated seawater, at ambient seabed temperature (10°C) using a cooling unit (ICE400, Aquavie, Connaux, France). Once at the laboratory (Banyuls Oceanological Observatory), coral colonies were kept for 3 weeks to acclimate to laboratory conditions in thermoregulated room in the dark in aerated 80 L tanks, continuously supplied (>1 renewal day⁻¹) with filtered (5 µm) seawater pumped from 10 m depth. The corals were fed three times a week alternately with freshly hatched *Artemia salina nauplii* (1000 L⁻¹) and with 5 mL of marine snow plankton diet (Two Little Fishies Inc, Miami Gardens, Florida, USA), to provide a complete and diverse nutrient supply (Galand et al., 2020). After acclimatization to laboratory conditions, *L. pertusa* colonies were cut into nubbins that contained 5–21 living polyps. The nubbins were glued onto cement blocks using an aquatic epoxy resin (Hold Fast Sand, Aquarium System, Sarrebourg, France) and were transferred to experimental tanks.

3.2.2 Experimental design, set up and sampling protocol

L. pertusa colonies were exposed to three different temperatures: 6, 10 and 14°C. The temperature of 10°C is the *in situ* temperature in the north east Atlantic Ocean, 14°C represents an elevated temperature at the upper limit of *L. pertusa* current thermal range (i.e., colonies in the Mediterranean Sea live at 13-14°C, Tursi et al., 2004; Freiwald et al., 2009), and 6°C is close to the minimum temperature tested to measure thermal tolerance of this (Dorey et al., 2020).

Experiments were conducted following the protocol detailed in Chapron *et al.* (2021). Briefly, each colony was fragmented into nubbins that contained 5 to 21 living polyps. For each colony, 2 nubbins were used for growth measurements, and 1 nubbin for transcriptomics and microbiome analyses. 36 nubbins (9 nubbins for each colony) were randomly distributed in each of the three 36L experimental tanks (974 polyps in total) with enough distance between

nubbins to avoid any contact among the different colonies and polyps (Orejas et al., 2019). Four different colonies were present in each experimental condition.

Each experimental tank was equipped with a small water pump (NJ400, Nawa Jet, Loreggia, Italy) to allow water mixing with a constant flow (3 cm s^{-1}). Each tank was placed in a larger water bath tank to ensure stable temperature regulation. Each tank was also equipped with a temperature probe connected to a temperature controller (Biotherm Eco, Hobby Aquaristik, Gelsdor, Germany, precise at 0.1°C), coupled with cooling unit (ICE400, Aquavie, Connaux, France) in the water bath. The temperature in each tank was monitored every 30 min using an autonomous IBUTTON probe and manually checked twice a day using a digital thermometer (Checktemp thermometer, Hanna Instrument, Woonsocket, USA). The pH, oxygen concentrations and salinity were measured manually twice a week using probes (C3010 Multi-parameter analysers, Consort).

Nubbins were acclimated for two weeks in the experimental aquaria prior to temperature changes. Then, the water temperature of the experimental tanks was gradually adjusted over 10 days until the targeted temperatures were reached, following the protocol described by Naumann et al. (2014). During the experiment, feeding routine was maintained as detailed above. Survival was monitored every day and dead polyps were identified. Dead nubbins were removed from the aquarium.

3.2.3 Prey capture rate

Coral prey capture rates were measured as described in Chapron et al. (2018) for each thermal condition at the start of the experiment, and then every two weeks until the end of the experiment. *Artemia salina* nauplii concentrations were counted in the seawater before and after feeding, and normalized to the number of polyps over a 4 hours period. To do so, triplicate water samples (100 mL) were taken every hour after feeding and filtered on $55 \mu\text{m}$ mesh. Control measurements were conducted in tanks without corals to quantify sedimentation.

3.2.4 Skeletal growth

Skeletal growth was measured using fluorescent calcein staining (Lartaud et al., 2013). At the beginning of the experiment, one to two nubbins *per* colony (i.e., six to eight nubbins of five to 13 polyps for all temperatures) were stained with calcein fluorescein at 150 mg L^{-1} following the same protocol as described in Chapron *et al.* (2018).

At the end of the four-months experiment, nubbins were cleaned in a hydrogen peroxide solution (H_2O_2 , 4 %) at 60°C for 12 hours to remove all organic tissues and rinsed with demineralised water. Then, each polyp calyx was individualised and placed on a slide, glued with Patafix©, in order to identify the polyp septa under microscope. The calcein labelling was observed under fluorescence microscope (Olympus IX51, Olympus, Tokyo, Japan), with excitation at 495 nm. Images were taken using a camera and image analysis was carried out using Image J software. Growth was assessed by measuring the distance between the calcein label and the outer edge of the septum of the calyx (repeated 10 times). Apical and subapical polyps were identified and their growth was compared.

3.2.5 Sampling for transcriptomics and metabarcoding, and RNA/DNA extractions

For each temperature conditions and each sampling time, three polyps *per* colony of *L. pertusa* (3x4=12 polyps) were sampled on the nubbin intended for molecular analyses. Sampling times were at the start of the experiment (T0), and after 8 days (T1), 15 days (T2), 30 days (T3), 60 days (T4), 90 days (T5) and 120 days (T6). In addition, at each sampling time, one litre of aquarium seawater was sampled and filtered sequentially through a 3 µm pore-size polycarbonate filter (Millipore, Darmstadt, Germany) followed by a 0.22 µm filter. Coral samples and the filters were flash frozen in liquid nitrogen and then stored at -80°C until nucleic acid extractions, for both transcriptomics and metabarcoding analyses.

For RNA/DNA extraction, individual polyps (including skeleton, tissues and mucus) were first crushed using a sterile hammer, then ground in tubes containing a garnet matrix, and lysed mechanically using a FastPrep Instrument (MP, Biomedical, Illkirch-Graffenstaden, France). RNA extraction was performed using the Maxwell RSC SimplyRNA Blood Kit and the Maxwell 48 MDx Instrument (Promega, WI, United States) following the manufacturer's instructions. RNA concentrations and quality were measured respectively by spectrophotometry (Quantus Fluorometer, Promega, Madison, WI, United States) and high-resolution automated electrophoresis (2100 Bioanalyzer, Agilent, CA, United States). DNA extraction was performed on the RNA lysate using the Maxwell RSC Blood DNA Kit LEV and the Maxwell 48 MDx Instrument (Promega, Madison, WI, United States) following the manufacturer's instructions. The FastPrep grinding and lysing protocol was also applied for the seawater bacteria community after cutting the 0.22 µm filters in small fragments. All DNA

samples analysed for microbiome determination were subjected to RNase A (4 mg mL⁻¹) treatment for 30 min at 37°C.

3.2.6 Transcriptomic

The RNA sequencing was performed on polyps from three different colonies *per* temperature condition (biological triplicates) sampled at the start of the experiment (T0) and after 30 days, last date for which there were still live corals at 14°C. Three *in situ* colonies were also analysed. RNA samples that met quality control standards, as detailed above, were sent to MacroGen Europe Center (Amsterdam, Netherlands) to proceed with RNA-seq library preparation and single-end sequencing using the kit TruSeq Stranded mRNA LT Sample Prep (Illumina, Inc, San Diego, California, USA), and the Illumina Hi-Seq2000 sequencer (2x150 pb).

Data analysis was performed using two pipelines: nf-core/rnaseq 3.11.2 and nf-core/differentialabundance 1.2.0. Reads were first clipped and trimmed to eliminate adapters and low-quality regions using TrimGalore 0.6.7, and then mapped onto the reference genome assembly of *L. pertusa* available under the BioProject accession PRJNA922177 (Herrera & Cordes, 2023), using STAR 2.7.10a (Dobin et al., 2013). Raw abundances were quantified using Salmon 1.10.1 (Patro et al., 2017). Gene expression analysis was performed using the R packages DESeq2 1.36 (Love et al., 2014) and vegan 2.6–2 (Oksanen et al., 2022). Transcripts were considered upregulated if they had a logfold change (lfc) > 1 and an adjusted p-value < 0.05, and downregulated if they had a lfc < -1 and an adjusted p-value of < 0.05.

Based on previous studies carried out on tropical and cold-water corals, a set of genes considered as appropriate molecular biomarkers for stress was selected to determine and describe molecular changes at the subcellular level in response to temperature: genes involved in immune, inflammatory and stress responses, cell death and apoptosis, DNA repair and maintenance, cell structure and integrity and antioxidant.

Raw reads will be deposited in the NCBI Sequence Read Archive.

3.2.7 16S rRNA metabarcoding sequencing and data analysis

For metabarcoding, the V1–V3 region of the bacterial 16S rRNA genes were amplified using the primers 27F AGRGTTT GATCMTGGCTCAG (Lane, 1991) and 519R GTNTTACNGCGGCKGCTG (Turner et al., 1999). Amplicon fragments were first PCR-amplified using the high-fidelity Advantage2 polymerase (Takara Holdings, Kusatsu, Japan) under conditions of 30 s at 94°C, 40 s at 56°C, 1 min at 68°C for 35 cycles.

PCR products were reamplified using the same primers primers 27F/519R, with the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, United States) and the high-fidelity Phusion polymerase under the following conditions: 30 s at 98°C, 16 cycles of 98°C for 10 s, 60°C for 30 s, 72°C for 80 s and final extension for 5 min at 72°C. Following the PCR, all the amplicon products from the different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, United States). The DNA library was prepared using the purified PCR products following the Illumina TruSeq DNA library preparation protocol. All the samples were sequenced on the same Miseq Illumina sequencer run using Miseq reagent kit V3 (Illumina, CA, United States) producing 2×300 -bp long reads. PCR reamplification and sequencing were conducted in a commercial laboratory (Integrated Microbiome Resource, Halifax, Canada). All sequences will be deposited in GenBank.

Sequence analysis was performed with DADA2 in R (Callahan et al., 2016, v 1.26.0). We applied the standard pipeline with the following parameters: `trimLeft = 20`, `truncLen = c(290, 270)`, `maxN = 0`, `maxEE = c(2,5)`, `truncQ = 2`. The sequences were filtered, dereplicated, and chimeras removed, to obtain amplicon sequence variants (ASVs). ASVs were classified against the SILVA v. 128 database (Quast et al., 2012) for taxonomic assignment. An additional BLAST (Altschul et al., 1990) search was performed on the ASVs selected by SIMPER analysis from the vegan package.

3.2.8 Statistical analysis

For gene expression, a principal coordinate analysis (PCoA) was computed using a gene count matrix normalized with the variance stabilizing transformation as implemented in DESeq2 using a design that included temperature and time. Significant differences in gene expression patterns were tested with PERMANOVA with the *adonis* function of the vegan package. DESeq2 was also used for pairwise comparisons on the normalized gene expression table between T0 and T4 to identify the genes that were differentially expressed (up or down regulated) in the different temperature conditions: 6, 10 and 14°C, based on $lfc > 1$ and $p < 0.05$.

For metabarcoding, to enable comparison of the bacterial community composition and diversity, the sequences abundance table was normalized with Hellinger transformation (Legendre and Gallagher, 2001). The Shannon diversity index (Shannon, 1948) was computed between and within samples and a nMDS based on Bray–Curtis similarity was conducted to

visualize similarities in community composition between samples with the *vegan* package in R (Oksanen et al., 2022). Significant differences between community composition were tested with PERMANOVA with the *adonis* function of the *vegan* package. Homogeneity of variances was tested with the function *betadisper* followed by *permutest* of the *vegan* package. The assumption of homogeneity was respected for the comparison of the temperature conditions through time. A simpler test was then performed with the *plyr* package (Wickham, 2011) to identify the ASVs that contribute the most to the differences between temperature conditions.

Tests for normality of variance were performed using the Shapiro–Wilk test with the R software 4.2.2. Distribution of survival and growth were normal but variances were not homogenous, so non-parametric Kruskal Wallis tests were performed to test possible statistical differences among thermal conditions, sampling times and colonies. Non-parametric relative contrast effect tests were then performed to determine which conditions and times differed using R package *nparcomp* 3.0 (Konietschke et al., 2015).

3.3 Results

3.3.1 Polyp survival and prey capture rate

Polyp survival rates differed significantly between temperature conditions (Chi squared = 20.079, df = 2, p-value < 0.001) and sampling times (Chi squared = 50.818, df = 6, p-value < 0.001) (Figure 3.1). Survival rates were significantly lower at 14°C (Tukey tests, p < 0.01), with a strong mortality after 15 days (survival of 60 ± 16 %), and continued to decrease down to 26 ± 9 % after 30 days, and no polyps survived after that. Survival was significantly lower at 14°C compared to 6°C after 15 days, and compared to 10°C after 30 days (Supplementary table 3.1).

Survival differed significantly between 6 and 10°C after 30 days only (Tukey test, p = 0.01). No differences between colonies were observed during the experiment (Chi squared = 59.252, df = 48, p-value = 0.128).

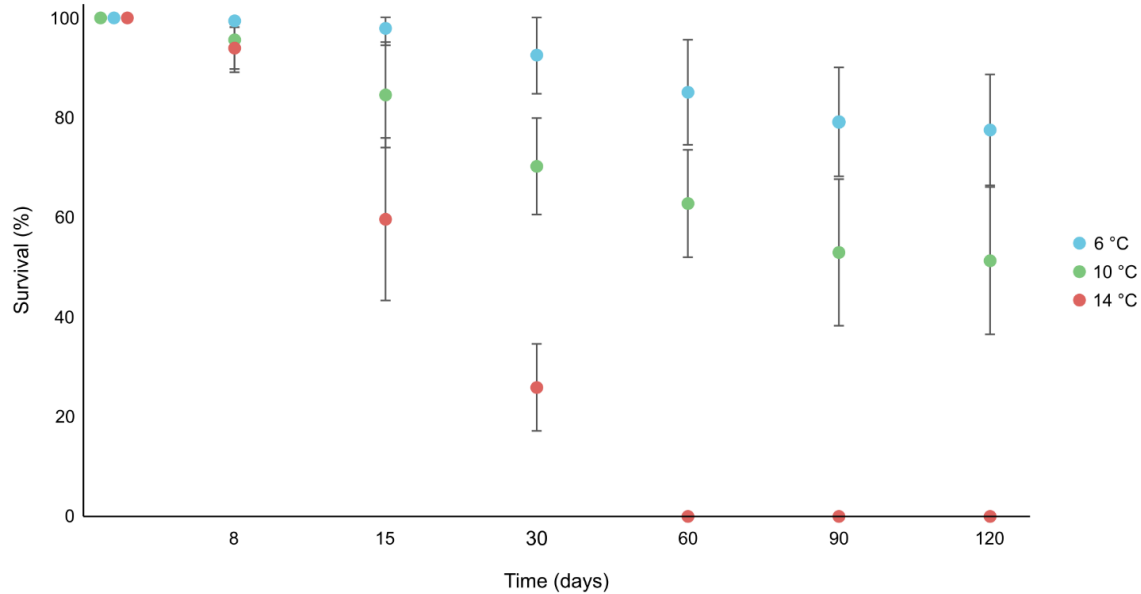


Figure 3.1. *Lophelia pertusa* polyp survival rate (%) at 6°C, 10°C and 14°C during 120 days of experiment. Mean values from four colony replicates *per* time point and standard error are presented.

The polyp average prey capture rates were below 10 artemia *per* polyp *per* hour for each sampling time along the experiment, with a very high variability within 4 hours of measurements (Supplementary Fig. 3.1). Temperature had no effect on the average prey capture rate (K–W, $p > 0.05$).

3.3.2 Skeletal growth

Polyp linear growth rate was significantly different between temperatures (Chi squared = 31.53, $df = 2$, p -value < 0.01). Corals maintained at 14°C showed faster growth than at the other temperatures (Tukey test, p -value < 0.01, Supplementary table 3.2), with respectively 1.89 ± 0.95 , 1.58 ± 1.13 and 5.33 ± 2.14 mm y^{-1} at 6, 10 and 14°C (Figure 3.2). There were no significant differences in linear growth rates between apical (i.e., younger polyps at the summit of the branch, driving the linear extension) and subapical polyps (K-W Chi squared = 1.031, $df = 1$, p -value = 0.310, Supplementary table 3.3).

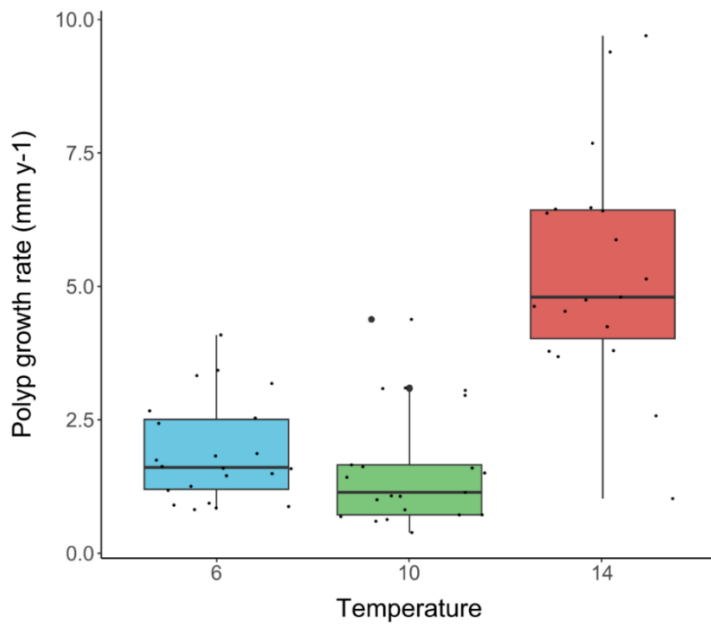


Figure 3.2. Polyp growth rate (mm y⁻¹) of *L. pertusa* at 6°C, 10°C and 14°C after 120 days of experiment.

3.3.3 Coral gene expression

The gene expression analysis against a reference genome of *L. pertusa* produced a table of 38,843 transcripts. nMDS based on the abundance of these transcripts in the different samples showed that at the start of the experiment (T0) the samples from the different temperature conditions grouped together separated from the *in situ* samples (Figure 3.3). At 10°C, samples from T0 and T3 (30 days) grouped together, except for an outlier T3 sample that grouped with an outlier T3 sample at 14°C. At 14°C, T3 samples were spread and separated from the T0 samples. At 6°C, T3 samples grouped together with the samples from T0. A non-parametric multivariate analysis of variance (PERMANOVA) test showed significant difference between temperatures both at T0 (Adonis, $R^2 = 0.534$, $p < 0.001$) and T3 (Adonis, $R^2 = 0.547$, $p = 0.01$).

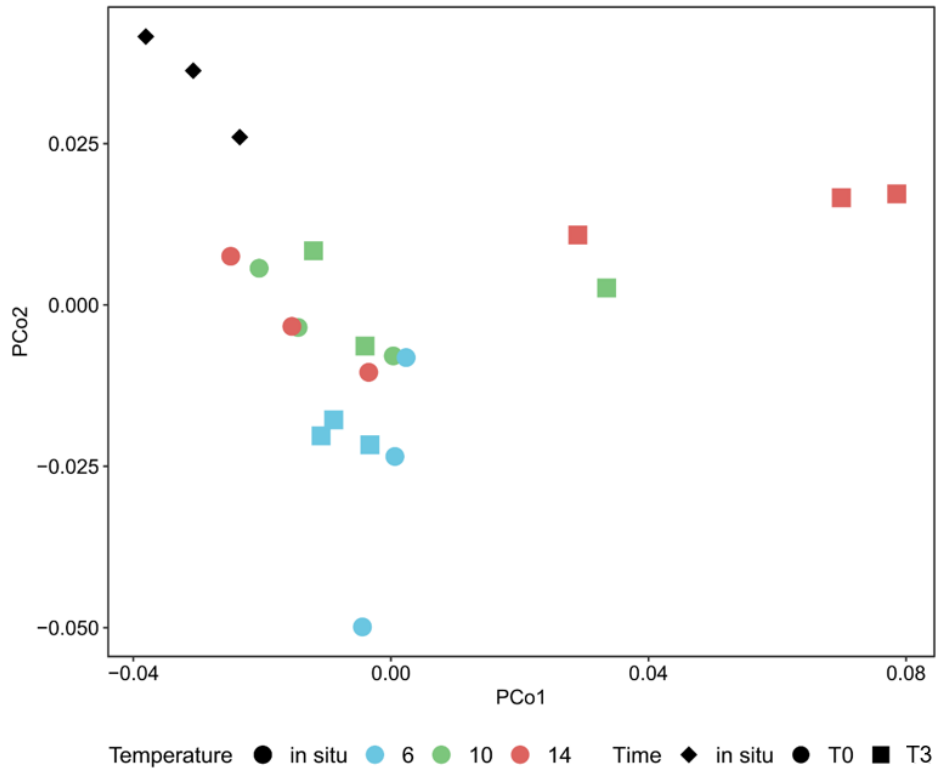


Figure 3.3. Principal coordinate analysis plot of gene expression for all 38,843 transcripts. Colors indicate temperature conditions (6°C, 10°C and 14°C) and shapes represent sampling times (*in situ*, start of the experiment (T0) and after 30 days (T3)).

Among the 38,843 transcripts, we identified 8,774 that were differentially expressed at between T0 and T4 at 14°C. This is almost 119 times more than at 10°C, where only 74 genes were differentially expressed (Figure 3.4). At 6°C, 369 genes were differentially expressed, so 5 times more than at 10°C (Figure 3.4). Moreover, there were more differentially expressed genes between corals at 6°C and 14°C (146) than between corals at 10°C and 6°C or 14°C (Figure 3.5).

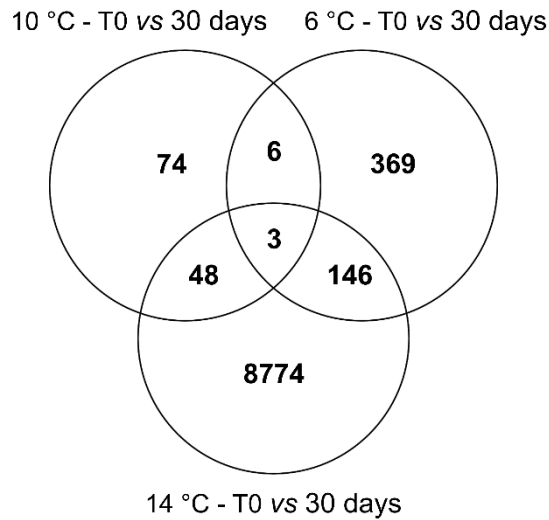


Figure 3.4. Venn diagram showing the number of differentially expressed genes shared or unique to the corals at each temperature conditions (6°C, 10°C and 14°C).

To investigate the effects of temperature in more detail at the gene level, we identified the functions of the genes that were differentially expressed. The annotation showed that genes coding for immune, inflammatory and stress responses, cell death and apoptosis, DNA repair and maintenance, cell structure and integrity and antioxidant were systematically upregulated at 14°C (Figure 3.5). All those genes were significantly upregulated with 1.9 to 9 log fold changes (Figure 3.5, Supplementary table 3.4). Only two genes (SPTLC2_5 and CA10) were not significantly upregulated. None of these genes were significantly upregulated at 6 and 10°C (Figure 3.6, Supplementary table 3.4).

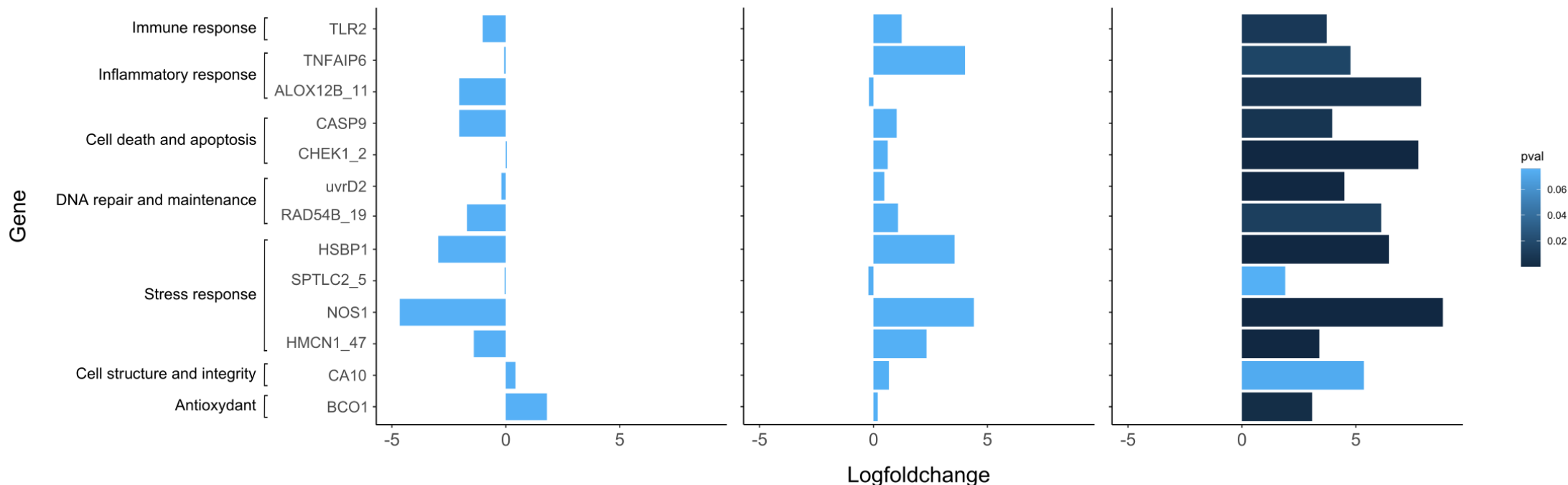


Figure 3.5. Logarithmic expression levels (Logfold change) for the different target genes represented according to the significant change of p-value. Gene abbreviations are given: Toll-like receptor 2 (TLR2), Tumor necrosis factor-inducible protein 6 protein (TNFAIP6), Arachidonate 5-lipoxygenase (ALOX12B_11), Caspase interleukin-1 beta converting enzyme (ICE) ues (CASP9), Serine/threonine-protein kinase Chk (CHEK1_2), ATP-dependent exoDNAse (exonuclease V) alpha subunit (uvrD2), DNA repair and recombination protein rad54b (RAD54B_19), Heat shock factor-binding protein 1 (HSBP1), Serine palmitoyltransferase 2 (SPTLC2_5), Nitric oxide synthase (NOS1), Hemicentin-1 (HMCN1_47), Carbonic anhydrase- protein 10 (CA10), Beta,beta-carotene 15,15'-monooxygenase (BCO1). Detailed abundance of gene expression and Logfold change are given in Supplementary tables 3.4 and 3.5.

3.3.4 Coral bacterial communities

The non-metric multidimensional scaling ordination (nMDS) plot based on bacterial community composition showed that at the start of the experiment (T0) the samples from the different temperature conditions grouped together (Figure 3.6). After 8 days, at 14°C some *L. pertusa* samples started separating from the control and the 6°C samples. After 15 days, samples from the 3 different temperatures separated from each other. After 30 days, samples from the 14°C condition were more spread and still separated from the 6 and 10°C samples. After 60 days, all corals had died at 14°C and the 6°C and 10°C samples remained separated from each other until the end of the experiment, time at which the 6°C samples were more dispersed (Figure 3.6). A non-parametric multivariate analysis of variance test showed significant difference between temperatures at all experimental times (ANOSIM, $R = 0.752$, $p < 0.001$). However, toward the end of the experiment, and after 15 days at 14°C, there were larger pseudo *F*-ratios, indicating more pronounced separation between temperature groups, and higher R^2 , indicating that temperature better explained variation in the model along the experiment (Supplementary Table 3.6).

The bacterial community composition of the water was similar between temperature conditions during the experiment, and was different from the coral bacterial communities (Supplementary Figure 3.2, Adonis, $F = 7.587$, $p < 0.001$).

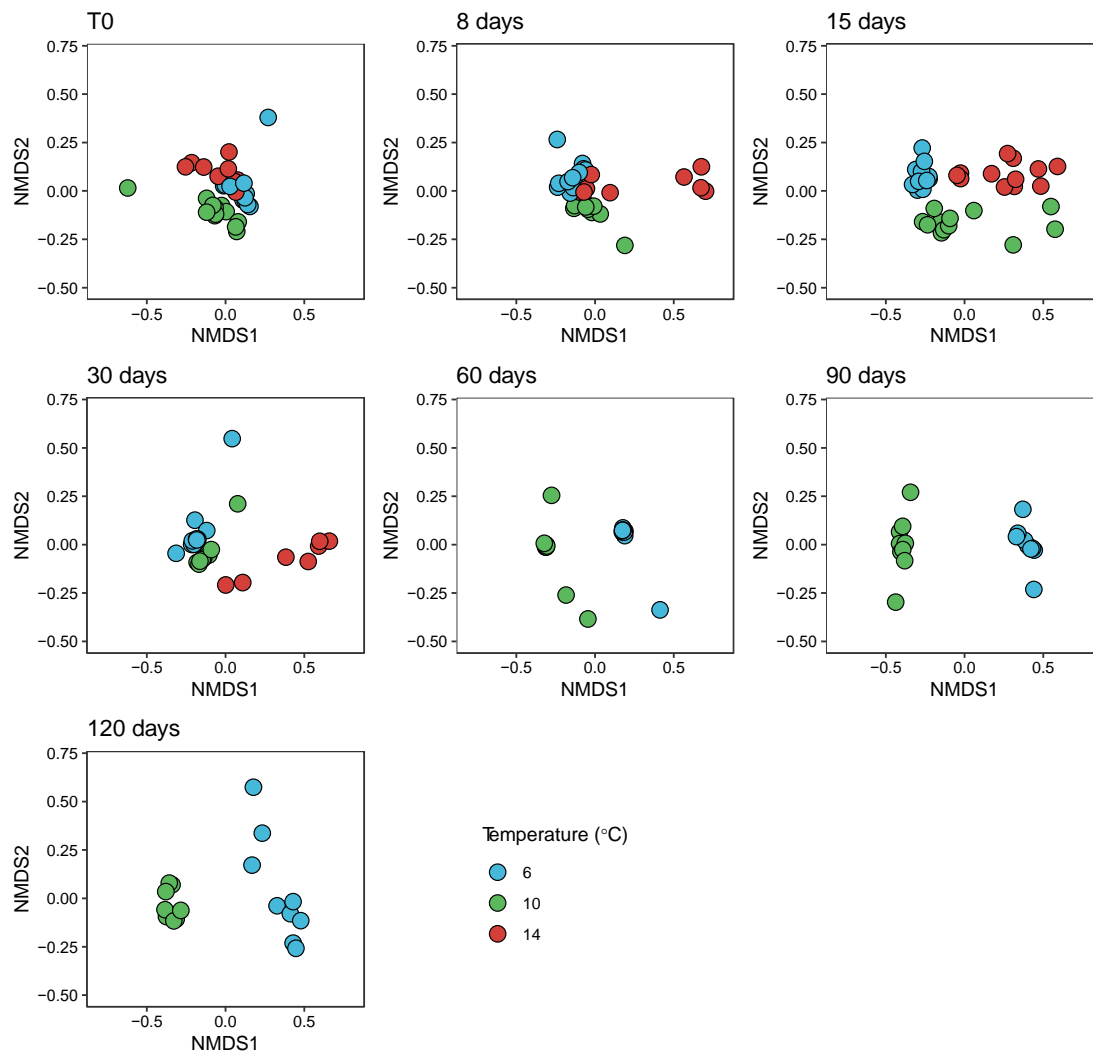


Figure 3.6. Non-metric multi-dimensional scaling plot (nMDS) based on the Bray-Curtis similarity index showing the similarity between bacterial community compositions of *L. pertusa* at the start of the experiment (T0), and after 8, 15, 30, 60, 90 and 120 days, at 6°C, 10°C and 14°C.

The bacterial community diversity differed significantly between temperature conditions, and through time (Figure 3.7, Kruskal-Wallis chi-squared = 78.065, df = 17, p-value < 0.01). Both at 6 and 10°C, community diversity decreased until T4 or T3 days respectively, before again increasing and stabilizing until the end of the experiment. At 14°C, diversity varied without marked trends. Post hoc test revealed that only the microbial community diversity at 10 and 14°C were different after 8 days (Dunn test, $p = 0.02$).

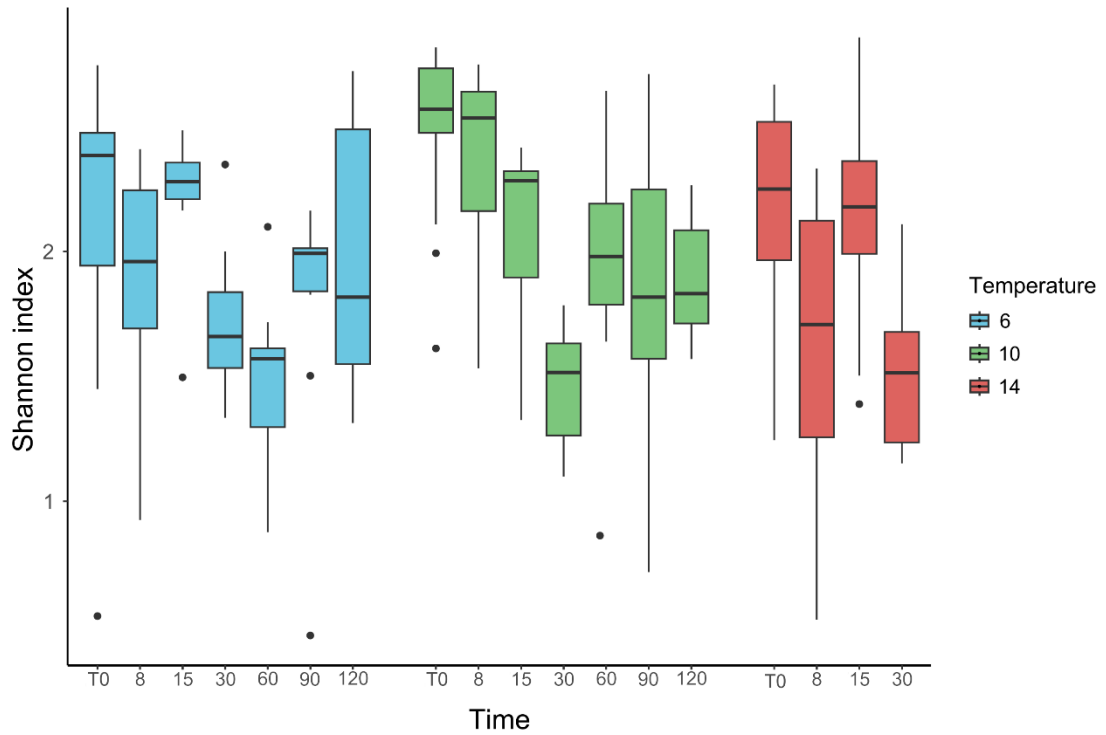


Figure 3.7. Shannon diversity index of *L. pertusa* bacterial communities under experimental conditions at 6°C, 10°C and 14°C along the experiment. Values correspond to the median and quartile, and points represent extreme values exceeding 95 % quantile.

We identified specific ASVs that became more abundant at the elevated temperature. ASV125, order *Rhodobacteraceae*, ASV1272, order *Flaviobacteraceae*, ASV31, order *Nitrincolaceae* and ASV274, order *Saprospiraceae*, were significantly more abundant at 14°C than at 6 and 10°C (Figure 3.8). ASV230, order *Fusibacteraceae*, was only present at 14°C where it increased in abundance with time (Figure 3.8).

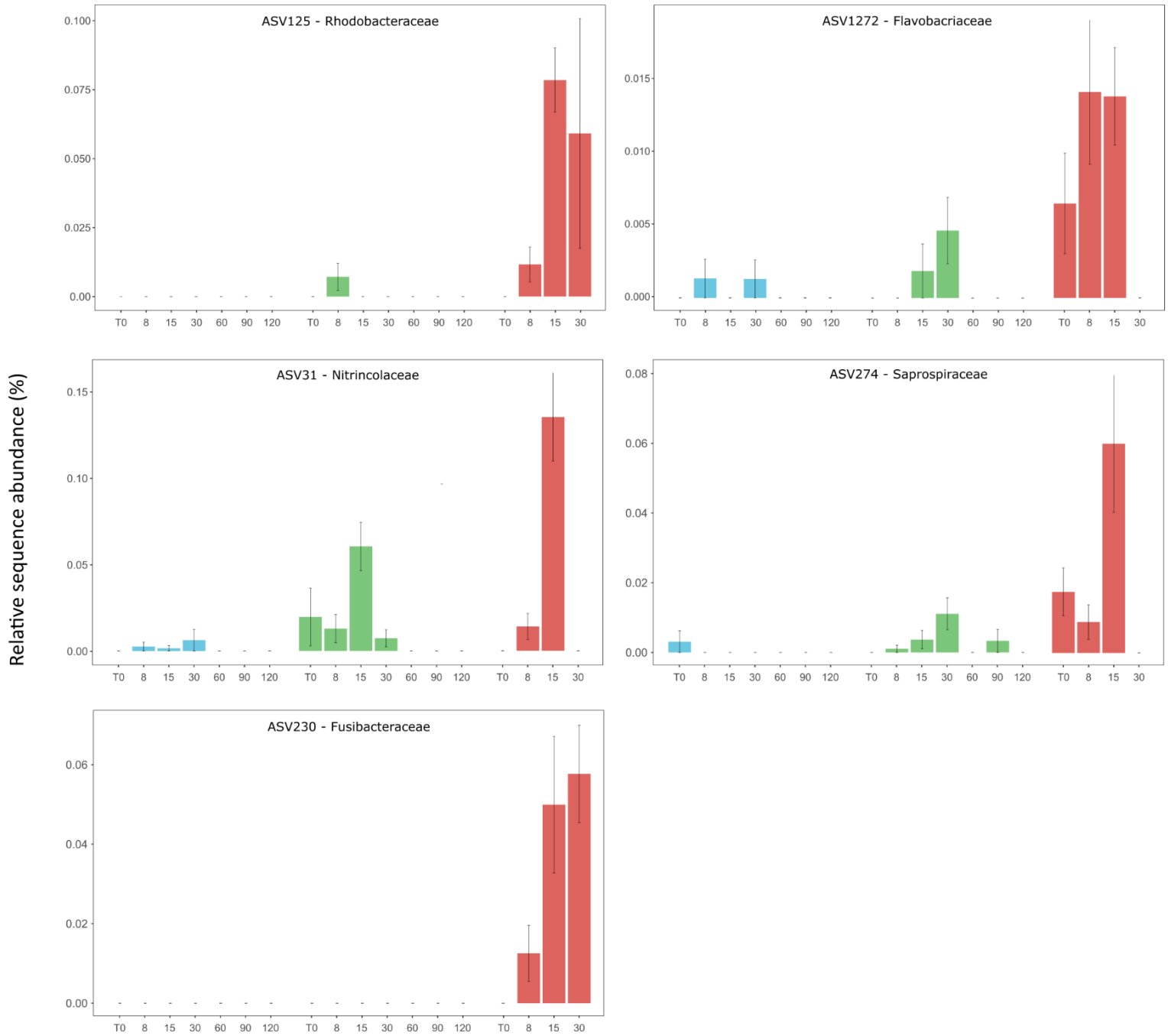


Figure 3.8. Relative sequence abundance of ASVs of interest characterizing *L. pertusa* bacterial communities under different experimental conditions (6, 10 and 14°C) during the experiment. Mean values and standard deviations are presented.

3.4 Discussion

The present study shows that *L. pertusa* holobionts from the NE Atlantic Ocean were strongly negatively affected by elevated sea water temperatures. After a month of experiment, all polyps

maintained at 14°C were dead. Our results suggest that the mortality was due to a combination of factors, in particular a high level of stress as evidenced by the transcriptomic results, a plausible depletion of energy reserves, but also a shift in the coral bacterial community towards pathogens. Transcriptomics analysis showed that gene expression differed between corals maintained at different temperatures, with particularly strong differences between the elevated temperature and the other two treatments. Genes associated with immune, inflammatory and stress responses, cell death and apoptosis, DNA repair and maintenance, cell structure and integrity, calcification and antioxidant response were all upregulated at elevated temperature. More precisely, the strong up-regulation of transcripts involved in arachidonic acid (AA) metabolism confirmed a strong inflammatory response in *L. pertusa* at 14°C. In metazoans, AA pathway and eicosanoids, biosynthesized by the arachidonate 5-lipoxygenase, are involved in inflammation regulation (Samuelsson, 1991; Dennis & Noris, 2015). Although, the role of AA metabolism has not been described in cnidarians, upregulation of transcripts related to AA metabolism has been demonstrated in the tropical coral *Acropora cervicornis* infected by white band disease (Libro et al., 2013), suggesting its implication in fighting disease. Similarly, the abundance of arachidonate 5-lipoxygenase proteins increased in the octocoral *Eunicea calyculata* infected by *Eunicea* black disease (Ricci et al., 2019). We also observed upregulation of tumor necrosis factors (TNFs), which are related to coral health and stress tolerance (Barshis et al., 2013). They are involved in the immune, inflammation and apoptosis responses, as they trigger signal transduction pathways that can lead to caspase activation (attested here by the upregulation of Casp9), and apoptosis (Barshis et al., 2013). It had never earlier been reported for cold water corals, but several studies have shown TNF upregulation in tropical corals in response to heat stress (Barshis et al., 2013; Palumbi et al., 2014; Seneca & Palumbi, 2015; Traylor-Knowles et al., 2017), and have suggested their involvement in the bleaching process (Traylor-Knowles & Palumbi 2014). Concurrently, the differential expression of RAD54 and uvrD2, which are proteins and helicases involved in DNA repair pathways, attests that elevated temperature treatment had structural effects on nucleic acids and proteins, as already observed in heat stressed embryos of the tropical coral *Montastraea faveolata* (Voolstra et al., 2009) and adults of *A. eurystoma* (Maor-Landaw & Levy, 2016). Other transcripts implicated in stress response, such as nitric oxide synthase (NOS1), serine palmitoyltransferase 2 (SPTLC2) and heat shock factor-binding protein 1 (HSBP1) were also upregulated. Increasing NOS activity in tropical coral symbionts has been reported in numerous studies (Trapido-Rosenthal et al., 2001, 2005; Bouchard & Yamasaki, 2008), as part of a cellular stress response related to coral bleaching (Trapido-Rosenthal et al., 2005; Perez & Weis, 2006). Concurrently, SPTLC2 is

implicated in cellular stress response and signalling, including heat shock (Perry, 2002). Finally, HSBP1 plays a critical role in the heat shock response and pathway, that have been particularly described in response to thermal stress in corals (Louis et al., 2017). In summary, our findings demonstrate that although cold water corals do not bleach, rising temperatures induce stress and a response similar to that seen in tropical corals.

Another key, but unexpected finding of this study is the upregulation of Beta,beta-carotene 15,15'-monooxygenase (BCO1). In metazoans, this enzyme catalyses the oxidative cleavage of provitamin A carotenoids *via* symmetrical cleavage of beta-carotene 15,15' (Lietz et al., 2010). Although the vitamin A pathway has been little studied in invertebrates and not in details in cnidarians, precursors such as carotenoids are known as high affinity electron acceptors that offer protection against oxidative damage on lipids, proteins, but also DNA, notably by intercepting reactive oxygen species (ROS) (Schneider et al., 2016). The accumulation of carotenoid pigments is responsible for coloration of certain corals (Cvejic et al., 2007), among which *L. pertusa* (Elde et al., 2012), in which they are hypothesised to protect the tissues from ROS (Cvejic et al., 2007). Here, we hypothesize that corals already had a stock of carotenoids in their tissues before the start of the experiment, since they fed very little during the experiment and were therefore unable to acquire them through their diet. Secondly, the upregulation of BCO1 suggests the preferential use of these carotenoids at 14°C, potentially to counter the oxidative stress experienced by corals at high temperatures. Considering this, we suggest that the pigmentation of coloured corals may be a potential indicator of stress, as the corals progressively changed colour over the course of the experiment, particularly at elevated temperatures (personal observations).

Under elevated temperature, *L. pertusa* showed higher growth rates, almost 3 times the ones measured at 6 and 10°C. Growth in corals directly results from the processes of calcification. An important step is the hydration/dehydration of CO₂ (Tambutté et al., 2007; Allemand et al., 2011) catalysed by carbonic anhydrase (CA), ultimately leading to carbonate production for calcification. In our study, CA gene transcripts were almost 5 times more expressed at 14°C than at 10°C indicating that corals were actively calcifying at higher water temperatures. In cold water corals, CA gene expression was up-regulated in *D. dianthus* to counter the negative effect of low pH on the biomineralization process (Carreiro-Silva et al., 2014). We also observed up-regulation of HMCN1 at elevated temperature, indicating a greater growth activity, as HMCN1 intervenes in the coral skeletal organic matrix (Ramos-Silva et al., 2013; Drake et al., 2013), but is also involved in wound healing in vertebrates (Welcker et al., 2021)

Corals maintained at 14°C had highest calcification rates as seen by growth measurement and gene expression although they did not feed during the first month of experiment, as shown by the absence of prey capture. Thus, the energy investment in growth could only last as long as the corals still had sufficient reserves. We can therefore hypothesise that the polyps started dying when the reserves became depleted as they were not renewed by feeding. In an experiment testing the effect of elevated temperature on the physiology of the temperate coral *Cladocora caespitosa* in the Mediterranean Sea, Rodolfo-Metalpa et al. (2006) showed a significant increase in growth for a 4 and 6°C increase during the first 3 weeks of experiment, after which a decrease in growth was monitored. They hypothesized that positive effect of higher temperature on growth was limited to the first weeks of experiment, after which energy allocation couldn't be sustain and didn't allow to maintain such rates of growth for a longer time. Similarly, Gori et al. (2014) and Naumann et al. (2013) showed that Mediterranean *Dendrophyllia cornigera* grew significantly faster for a 4°C and 5°C increase respectively. Similarly, *L. pertusa* growth was significantly higher for a 4°C increase in the Trondheim-Fjord (Büscher et al., 2017).

The microbiome analysis showed that the bacterial communities were different between corals incubated at different temperatures. At elevated temperature, the strong changes in bacterial communities (i.e., dysbiosis) were mainly due to the appearance of opportunistic and potentially pathogenetic bacteria. Several bacteria from the families *Fusibacteraceae*, *Rhodobacteraceae*, *Flavobacteriaceae*, *Saprospiraceae* and *Nitrincolaceae* appeared at 14°C after 8 days, and increased in abundance until 30 days when the polyps died. Some of these bacteria, which were totally absent from corals maintained at 6 and 10°C, could be pathogens since members of these families have been earlier associated to diseases in tropical corals (e.g., Jones et al., 2004; Sunagawa et al., 2009; Gignoux-Wolfsohn & Vollmer, 2015; Sweet & Bythell, 2015). The presence of potential pathogens in *L. pertusa* at 14°C is plausible since we recently found functional bacterial pathogenicity marker genes associated to rising temperature (Manuscript's chapter 2). Here, the coral transcriptome gave additional clues as coral genes coding for toll-like receptors (TLR) were up regulated at 14°C. TLR are known as primary pathogens detectors and are essential signalling pathway in the innate immune system (Silverman & Maniatis, 2001). In tropical corals, TLR2 homologs have been shown to be up-regulated in *A. cervicornis* with white band disease (Libro et al., 2013). Our results thus reflect the coral's limited capacity to maintain or regulate its microbiome at elevated temperatures, leading to invasion of the host by opportunistic or pathogenic bacteria as seen earlier in *L.*

pertusa from the Mediterranean (Chapron et al., 2021) and the Atlantic (Manuscript's chapter 2).

We observed similar health conditions between corals maintained at 6 and 10°C as seen by the metatranscriptomics analysis, although their microbiome differed. Furthermore, most of these genes related to stress response were downregulated at 6°C. This may partially explain the higher survival rate at low temperature compared to control along the experiment (82 % survival at 6°C vs 51 % at 10°C after 120 days). We hypothesize that the metabolism of corals maintained at 6°C was lowered and that they were therefore in a “dormant state”. Decreased metabolism in response to lower temperature has been observed in several species of cold-water corals. Gori et al. (2014) showed that in the Mediterranean Sea, *D. cornigera* lower growth, respiration and calcification rates when water temperature was lowered by 4°C. Similarly, calcification decreased by 58 % in Mediterranean *L. pertusa* when water temperature was lowered by 6°C (Naumann et al., 2014). Finally, a decrease of temperature from 13 to 10°C reduced *L. pertusa* prey capture rate, lipid content of tissues and growth rates (Chapron et al., 2021). Here, growth rates were similar between low temperature and control conditions, and was similar to the ones measured *in situ* (Manuscript's chapter 6) and in the first experiment (Manuscript's chapter 2). Growth was however in the lower range for this species compared to earlier reports (Brooke & Young, 2009; Orejas et al., 2011; Larcom et al., 2014; Lartaud et al., 2017; Büscher et al., 2017; Chapron et al., 2020; Chapron et al., 2021).

3.5 Conclusion

Our results show a massive mortality of *L. pertusa* when water temperature rises with +4°C. Mortality appears to be associated to a combination of factors including (i) elevated coral stress as revealed by the upregulation of transcripts related to general stress response (e.g., immune, inflammatory, antioxidant and stress responses, cell death and apoptosis, DNA repair and maintenance...), (ii) the energy investment in growth combined with the inability to feed, and (iii) a dysbiosis characterised by the development of potential pathogenic bacteria. This study described for the first time the molecular and holobiont responses of *L. pertusa* to higher temperature, and demonstrated that higher temperatures induce dysbiosis and stress in Atlantic *L. pertusa*, similar to the response reported in heat stressed tropical corals.

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Supplementary material

Supplementary table 3.1. Post-hoc Tukey HSD results assessing the significance of the effects of temperature on *L. pertusa* survival along the experiment.

Time	Comparisons	Estimator	Lower	Upper	Statistic	p-value
T1	10 vs 6	0.5	0.499	0.501	0	1
	10 vs 14	0.5	0.499	0.501	0	1
	6 vs 14	0.5	0.499	0.501	0	1
T2	10 vs 6	0.75	0.061	0.993	1.042	1
	10 vs 14	0.406	0.015	0.969	0.361	1
	6 vs 14	0.812	0.043	0.998	1.170	1
T3	10 vs 6	0.938	0.058	1	1.795	0.99999746754779
	10 vs 14	0.062	0	0.942	-1.795	0.999997478494881
	6 vs 14	0.999	0.996	1	20.0	0
T4	10 vs 6	0.999	0.996	1	20.0	0
	10 vs 14	0.001	0	0.004	-20.0	0
	6 vs 14	0.999	0.996	1	20.0	0
T5	10 vs 6	0.938	0.058	1	1.795	0.999997491323002
	10 vs 14	0.001	0	0.004	-20.0	0
	6 vs 14	0.999	0.996	1	20.0	0
T6	10 vs 6	0.938	0.058	1	1.795	0.999997506132194
	10 vs 14	0.001	0	0.004	-20.0	0
	6 vs 14	0.999	0.996	1	20.0	0
T7	10 vs 6	0.938	0.058	1	1.795	0.999997455928288
	10 vs 14	0.001	0	0.004	-20.0	0
	6 vs 14	0.999	0.996	1	20.0	0

Supplementary table 3.2. Post-hoc Tukey HSD results assessing the significance of the effects of temperature on *L. pertusa* growth rate.

Comparisons	Estimator	Lower	Upper	Statistic	p-value
10 vs 6	0.348	0.180	0.566	-1.632991	0.2300705904
10 vs 14	0.940	0.725	0.989	3.588322	0.0007699685
6 vs 14	0.940	0.758	0.987	3.979966	0.0002512938

Supplementary table 3.3. Average polyp linear growth rates \pm s.d. (mm yr^{-1}) of *L. pertusa* for all, apical and subapical polyps, under different temperature conditions at the end of eight weeks of experiment. The number in brackets represent the number of replicates.

	all polyps (mm yr^{-1})	apical polyps (mm yr^{-1})	subapical polyps (mm yr^{-1})
all	2.84 ± 2.21 (62)	2.44 ± 1.23 (19)	3.13 ± 1.39 (43)
6°C	1.89 ± 0.95 (22)	1.31 ± 0.40 (6)	2.11 ± 1.01 (16)
10°C	1.58 ± 1.13 (21)	1.06 ± 0.39 (6)	1.79 ± 1.27 (15)
14°C	5.33 ± 2.14 (19)	4.93 ± 2.91 (7)	5.48 ± 1.88 (12)

Supplementary table 3.4. Detailed logarithmic expression levels (Logfold change) for the different target genes and the p-value associated.

Gene	6°C		10°C		14°C	
	LFC	p-value	LFC	p-value	LFC	p-value
Toll-like receptor 2	-1.014	0.822	1.237	0.817	3.787	0.009
Tumor necrosis factor-inducible protein 6 protein	-0.067	0.994	4.025	0.391	4.852	0.016
Arachidonate 5-lipoxygenase	-2.036	1.000	-0.196	1.000	8.002	0.007
Caspase, interleukin-1 beta converting enzyme (ICE) ues	-2.038	0.400	1.020	0.829	4.033	0.008
Serine/threonine-protein kinase Chk1	0.046	0.995	0.629	1.000	7.877	0.0002
ATP-dependent exoDNAse (exonuclease V) alpha subunit	-0.186	0.960	0.480	0.962	4.576	0.0000004
DNA repair and recombination protein rad54b	-1.702	0.753	1.082	0.971	6.224	0.0136
Heat shock factor-binding protein 1	-2.957	0.363	3.563	0.265	6.571	0.000002
Serine palmitoyltransferase 2	-0.049	0.993	-0.221	0.987	1.936	0.0761
Nitric oxide synthase	-4.647	1.000	4.414	1.000	8.975	0.0001
Hemicentin-1	-1.396	0.503	2.331	0.651	3.458	0.0002
Carbonic anhydrase- protein 10	0.427	0.932	0.681	0.954	5.449	0.0738
Beta,beta-carotene 15,15'-monooxygenase	1.812	0.514	0.192	0.983	3.139	0.0027

Supplementary table 3.5. Normalized number of transcripts of targeted genes expressed in *L. pertusa* during the experiment at T0 and T4 at 6, 10 and 14°C.

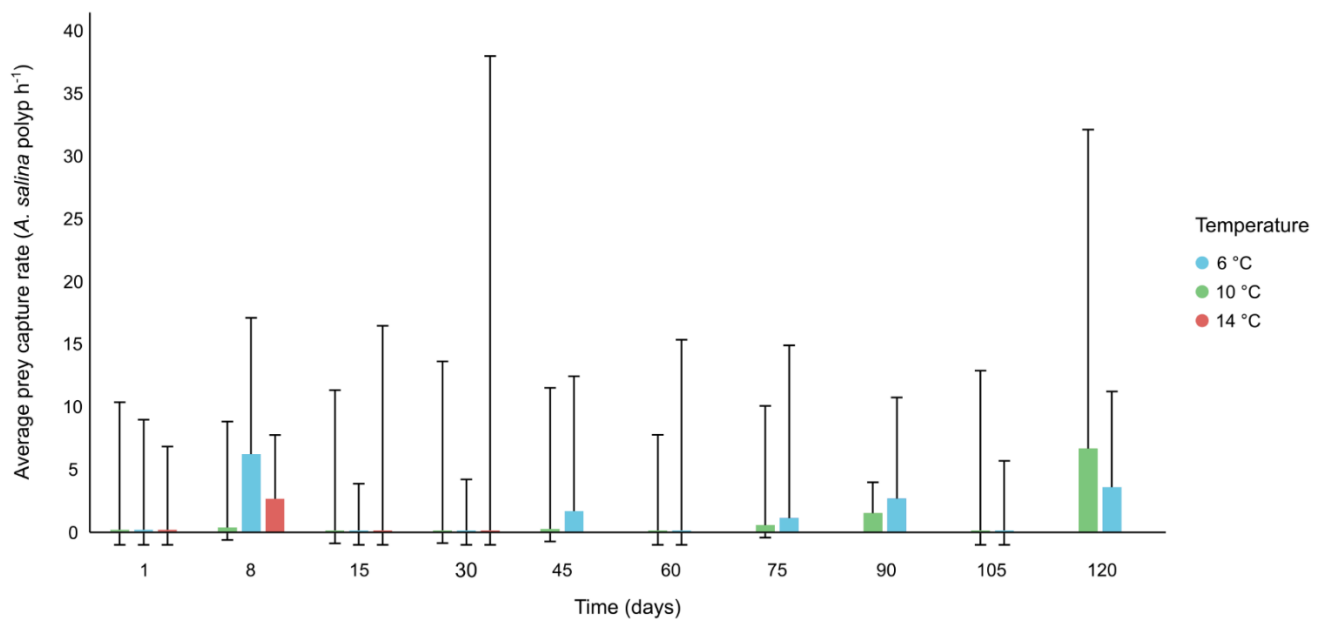
Gene ID	Gene	Metabolism	6°C - T0			6°C - T4			10°C - T0			10°C - T4			14°C - T0			14°C - T4		
			Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
014933	Toll-like receptor 2	Innate immune system	26.277	11.189	8.255	0.000	8.380	12.859	7.415	10.365	19.356	14.375	16.442	64.597	4.348	0.000	8.569	106.363	44.227	81.683
036245	Tumor necrosis factor-inducible protein 6	Inflammatory and immune responses	0.938	14.632	0.000	1.311	4.513	8.573	0.000	0.000	3.416	26.696	8.769	21.532	0.000	0.000	6.427	120.918	105.039	8.450
040654	Arachidonate 5-lipoxygenase	Inflammatory response	0.000	10.329	0.000	1.311	0.000	1.072	0.000	0.000	17.079	10.268	4.385	0.000	0.000	0.000	0.000	95.167	0.000	70.417
006210	Caspase, interleukin-1 beta converting enzyme (ICE) 1	Involved in cell death/apoptosis and inflammatory response	44.109	30.125	95.520	28.192	2.579	4.286	15.890	42.497	33.019	22.589	60.288	118.941	6.957	0.000	6.427	79.492	23.956	185.900
018226	Serine/threonine-protein kinase Chk1	Regulation of meiosis and apoptosis	6.569	5.164	7.076	1.967	2.579	13.931	3.178	0	5.693	10.268	2.192	2.051	0.000	0.000	0.000	62.698	26.720	58.587
019925	ATP-dependent exoDNase (exonuclease V) alpha subunit	Genetic recombination, maintenance of cell viability, and degradation of foreign or damaged DNA	60.063	50.783	28.302	25.569	7.736	82.513	16.949	190.720	121.828	61.606	269.650	148.676	5.217	39.533	44.272	1036.759	475.438	1223.560
040229	DNA repair and recombination protein rad54b	DNA repair and recombination	2.815	1.721	18.868	2.622	3.223	0.000	2.119	0.000	12.524	0.000	0.000	34.862	0.870	0.000	0.714	76.133	0.000	87.317
015742	Heat shock factor-binding protein 1	Involved in many pathways of the heat shock response	31.908	7.746	8.255	0.000	2.579	3.215	0.000	1.037	7.970	45.178	20.827	44.090	0.000	0.000	4.284	291.099	125.309	145.903
020820	Serine palmitoyltransferase 2	Stress response	19.708	117.919	47.170	0.656	88.316	76.083	3.178	119.200	154.847	18.482	116.191	115.865	13.044	24.850	11.425	113.081	46.991	81.120
004186	Nitric oxide synthase	Stress response (expressed by symbionts during bleaching)	0.000	10.329	1.179	0.000	0.000	0.000	0.000	0.000	0.000	12.321	0.000	1.025	0.000	0.000	0.000	180.257	0.921	143.650
036683	Hemicentin-1	Promoting extracellular adhesion during stress	39.416	74.883	63.680	11.146	9.670	42.864	13.771	1.037	40.989	36.964	27.403	246.085	7.826	9.036	34.989	294.457	190.728	240.543
023812	Carbonic anhydrase- protein 10	Cell structure and integrity, calcification metabolism	15.016	16.354	0.000	11.146	19.339	7.501	5.297	23.840	12.524	2.054	18.634	53.318	0.000	0.000	40.702	821.794	144.659	1337.916
034228	Beta,beta-carotene 15,15'-monooxygenase	Oxidative conversion of vitamin A and its derivatives	25.339	0.861	5.896	41.960	23.207	35.363	32.839	8.292	60.345	28.749	42.749	50.242	25.218	67.772	71.407	993.094	78.318	828.663

Supplementary table 3.6. Summary of the pairwise Adonis testing the effect of temperature and time on microbial community composition.

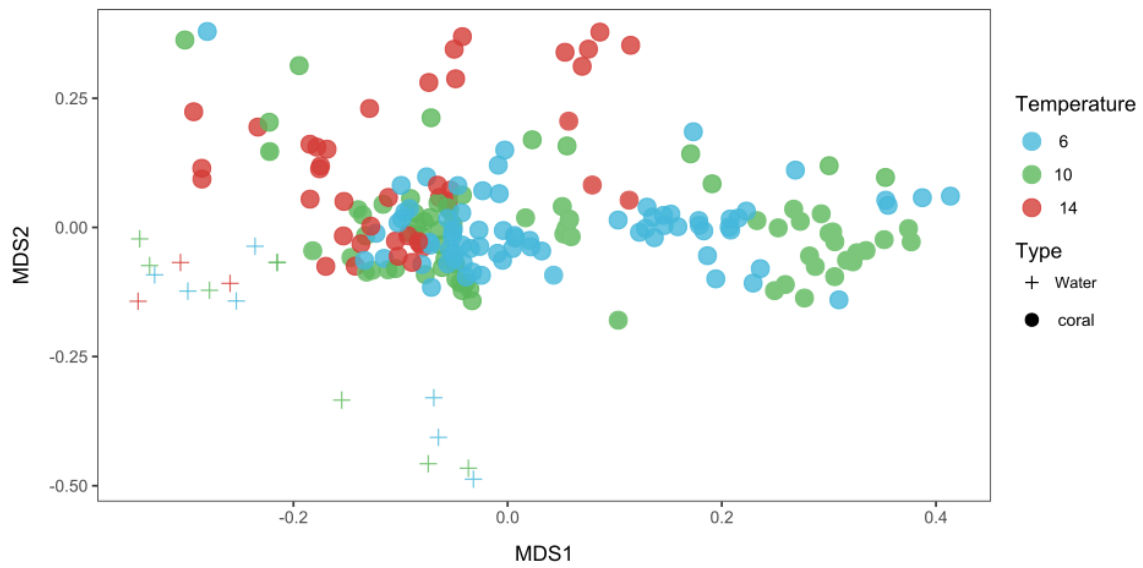
Time	Temperature	R	F	p-value
T0	10 vs 6	0.163	4.688	0.001
	10 vs 14	0.172	4.992	0.001
	6 vs 14	0.129	3.557	0.001
T8	10 vs 6	0.195	5.802	0.001
	10 vs 14	0.179	5.002	0.002
	6 vs 14	0.157	4.289	0.001
T15	10 vs 6	0.206	6.216	0.001
	10 vs 14	0.185	5.443	0.001
	6 vs 14	0.320	11.319	0.001
T30	10 vs 6	0.210	5.054	0.001
	10 vs 14	0.358	7.823	0.001
	6 vs 14	0.349	9.102	0.001
T60	10 vs 6	0.319	9.845	0.001
T90	10 vs 6	0.326	8.716	0.001
T120	10 vs 6	0.266	6.537	0.001



Supplementary Fig. 3.1. Photos of two nubbins used for growth measurements at a) and c) the beginning of the experiment and b) and d) the same nubbins at the end of the experiment; Observed nubbins showed more faded color at the end of the experiment.



Supplementary Fig. 3.2. Average prey capture rate of *L. pertusa* during 4-hours measurement every two weeks along 120 days of experiment.



Supplementary Fig. 3.3. Multi-dimensional scaling plot (nMDS) based on the Bray-Curtis similarity index showing the similarity between bacterial community compositions of *L. pertusa* and seawater along the eight weeks experiment at 6, 10 and 14°C.

***PART II: DETERMINATION OF BIOLOGICAL TRAITS OF L. PERTUSA AND M.
OCULATA ACROSS THE NE ATLANTIC OCEAN AND MEDITERRANEAN SEA***



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The second section of the manuscript presents *in situ* studies aiming at increasing our present understanding of the biology of CWC from the NE Atlantic Ocean and the Mediterranean Sea, to ultimately find out if climate change would impact differently the two populations. This section is divided into three chapters, describing respectively the reproductive biology of the two main reef-building CWC species in the Mediterranean Sea, *L. pertusa* and *M. oculata* (Chapter 4), investigating the patterns of microbe-host association between the NE Atlantic Ocean and the Mediterranean Sea for *L. pertusa* and *M. oculata* (Chapter 5), and measuring the first *in situ* growth rates of *L. pertusa* in the Bay of Biscay (NE Atlantic Ocean) (Chapter 6).

Chapter four: First report of the reproductive biology of the two main reef-building cold-water coral species in the Mediterranean Sea

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Abstract

Although cold-water coral (CWC) biology, physiology, and connectivity, have been extensively studied, no study has so far focused on the reproductive biology of these two species in the Mediterranean Sea, an area particularly exposed to the effects of anthropogenic pressures. The reproductive biology of colonies of the reef-building *Lophelia pertusa* (Linnaeus 1758), now synonymised as *Desmophyllum pertusum*, and *Madrepora oculata* (Linnaeus 1758) from the Mediterranean Sea was investigated for the first time using histological techniques. Samples were collected from the Lacaze-Duthiers canyon in the Gulf of Lion, northwestern Mediterranean Sea. The gametogenic cycle suggests a seasonal spawning of *L. pertusa* in autumn to early winter, similarly to population from the Gulf of Mexico. This period corresponds to the formation of storm-induced deep-sea water plumes, likely acting as a time giver for coral spawning and facilitates larval dispersal. *Madrepora oculata* shows continuous reproduction as already identified in eastern and western North Atlantic, with reproductive features of a species less opportunistic than *L. pertusa*, consistent with its other biological characteristics. This difference between the two species is of paramount importance to define strategies of conservation.

Keywords: *Desmophyllum pertusum*, *Madrepora oculata*, scleractinian coral, reproductive strategy, seasonality



Reproductive biology of the two main reef-building cold-water coral species (*Desmophyllum pertusum* and *Madrepora oculata*) in the Mediterranean Sea

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ABSTRACT

Although cold-water coral (CWC) biology, physiology, and connectivity, have been extensively studied, few studies have so far focused on their reproductive biology. The reproductive biology of colonies of the reef-building *Lophelia pertusa* (Linnaeus 1758), now synonymised as *Desmophyllum pertusum*, and *Madrepora oculata* (Linnaeus 1758) from the Mediterranean Sea, an area particularly exposed to the effects of anthropogenic pressures, was investigated for the first time using histological techniques. Samples were collected from the Lacaze-Duthiers canyon in the Gulf of Lion, northwestern Mediterranean Sea, where the corals are living at 13 °C, close to their upper limit of thermal tolerance. The gametogenic cycle suggests a seasonal spawning of *D. pertusum* in autumn to early winter, similarly to population from the Gulf of Mexico. This period corresponds to the formation of storm-induced deep-sea water plumes, likely acting as a time giver for coral spawning and facilitates larval dispersal. *Madrepora oculata* shows continuous reproduction as already identified in eastern and western North Atlantic, with reproductive features of a species less opportunistic than *D. pertusum*, consistent with its other biological characteristics. This difference between the two species is of paramount importance to define strategies of conservation.

1. Introduction

Cold-water corals (CWC) are deep sea engineers which form extensive reefs in cold and deep environments around the world (Freiwald et al., 2011), providing rich habitats, nursery grounds and abundant source of food for an amount of associated species (Buhl-mortensen et al., 2016). But those corals now face severe anthropogenic threats including non-sustainable fishing activities (e.g. trawling), pollution (by hydrocarbon, seabed mining, plastics) and global changes (increase in temperature, ocean acidification, deoxygenation of deep waters) (Roberts et al., 2009; Chapron et al., 2018; Morato et al., 2020).

Reproduction is a key biological factor to predict population resilience to environmental stressors and anthropogenic pressures (reviewed in Richmond et al., 2018). The fecundity, sex ratio, gamete quality and periodicity, and the environmental parameters that control spawning are essential to better evaluate reproductive output, recruitment, biogeographic dispersion and connectivity of marine species. Although reproduction of scleractinian corals has been extensively studied (Fadlallah, 1983; Baird et al., 2009; Harrison, 2011), few have focused

on the reproductive biology of azooxanthellate species, particularly in the deep habitats linked to the scarcity and the difficulty of sampling. To date, reproductive features and strategies have been partially described in only eight genera: *Fungiacyathus*, *Oculina*, *Desmophyllum*, *Madrepora*, *Caryophyllia*, *Solenosmilia*, *Ellanopsammia* and *Flabellum* (Waller et al., 2002, 2005; Brooke and Young, 2003; Waller and Tyler, 2005, 2011; Burgess and Babcock, 2005; Flint et al., 2007; Mercier et al., 2011a; Brooke and Järnegren, 2013; Waller and Feehan, 2013; Pires et al., 2014; Strömberg and Larsson, 2017). The most widespread and studied CWC species *Lophelia pertusa* (now synonymised as *Desmophyllum pertusum*, Addamo et al., 2016) and *Madrepora oculata* have been described as gonochoristic broadcast spawners, releasing gametes in the surrounding water (Waller and Tyler, 2005). Although often living in close association, including forming chimera-like colonies when the skeleton of living branches of the two species merge (Corbera et al., 2019; Lartaud et al., 2019), these corals exhibit distinct reproductive strategies. *Madrepora oculata* shows clear continuous reproduction (Pires et al., 2014), while *D. pertusum* has a seasonal pattern, releasing a single cohort of small gametes (Waller and Tyler, 2005; Brooke and Järnegren, 2013).

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In Norway, female gametogenesis of *D. pertusum* is initiated in January and spawning occurs from late January to early March of the following year (Brooke and Järegren, 2013). In the Northeast Atlantic, vitellogenesis occurs in late summer to autumn (i.e., August–October) and spawning in January–February (Waller and Tyler, 2005). In the Southwestern Atlantic, off Brazil, gametogenesis is initiated in early summer and vitellogenesis seems to occur between February and April, while spawning occurs from May to July (Pires et al., 2014). Finally, in the Southeastern US and the Gulf of Mexico, Brooke and Schroeder (2007) observed mature oocytes between September and November, suggesting a spawning season in autumn. The environmental factors affecting reproduction therefore do not occur at the same time in these geographical areas. But these data are still too fragmented to determine which parameters control CWC reproduction.

In the Mediterranean Sea, an almost land-locked basin, the effects of climate change are particularly exacerbated (Calvo et al., 2011), while *M. oculata* and *D. pertusum* appears highly sensitive to increasing temperatures (Chapron et al., 2021). The abundance of waste debris in Mediterranean canyons adds further pressure on coral ecosystems (Angiolillo and Fortibuoni, 2020), affecting differently both species (Mouchi et al., 2019). While the biology and ecology of CWC communities from the Mediterranean Sea were widely studied (see review in Orejas and Jiménez, 2019), to date we surprisingly lack a description of the reproductive biology of these deep-sea Mediterranean engineers. This understanding is fundamental to forecast the response of Mediterranean CWC habitats to global changes and adapt the implemented conservation strategies. Assuming the role of the Mediterranean Sea as a refuge for CWCs during the glacial times and a source for coral dispersion in the Atlantic (Boavida et al., 2019), characterization of the reproductive biology of Mediterranean populations is also essential to predict the resilience of CWCs from Northeast Atlantic and northernmost areas.

The aim of this study was to investigate the reproductive features and periodicity of the two main reef forming CWC species in the Mediterranean Sea, *D. pertusum* and *M. oculata*.

2. Material and methods

2.1. Study site and specimens collection

Corals were sampled at 520 m depth in the Lacaze-Duthiers canyon in the Gulf of Lion, Northwestern Mediterranean Sea (42°32.728'N/3°25.267'E) on a long-term experimental site, monitored since 2010 under the programme 'Biodiversity, extreme marine environment and global change' (LECOB, Banyuls-sur-mer Oceanological Observatory), as described in Chapron et al. (2020a) (Table 1). Coral fragments were sampled from nine distinct live colonies of *D. pertusum* and nine distinct live colonies of *M. oculata* using a Remotely Operated Vehicle (ROV) deployed from the R/V Janus II (COMEX company) successively in July 2012, April 2013, October 2014 and June 2015 (Table 1). Two colonies of *D. pertusum* and one colony of *M. oculata* have been resampled at different years (see Sample and Colony ID L62, L67 and O43 in Table 1). On board, corals were fixed in 10% formaldehyde for 48 h and then stored in 70° ethanol until histological processing at laboratory.

2.2. Histology

Colony fragments stored in ethanol were first decalcified in 10% hydrochloric acid/EDTA (Ethylenediaminetetraacetic) solution for four to six days until complete dissolution of the calcareous skeleton. The solution was renewed twice a day during decalcification. Soft tissues were rinsed and then dehydrated by successive ethanol baths at increasing concentrations (70°, 80°, 95° and 100°), followed by three 2-min clearing limonene (xylene substitute) baths. Tissues were then embedded by being left for 24 h in paraffin wax (ParaPlast Plus) and poured into standard blocks. From the blocks obtained, 7 µm thick sections were made every 114 µm using a Leica® RM2245 microtome (Leica, Wetzlar, Germany) as described in Stoddart et al. (2012) in the total section of the polyp. Sections were stained using Movat stain, revealing mucopolysaccharides, fibers and collagens in blue and nuclei and nucleic acid in red. Section of the total number of mesenteries of three to five polyps per colony were examined using the same procedure than described in Waller and Tyler (2005), and polyps were photographed using an Olympus BX61 optical microscope.

The number of oocytes per polyp were counted to determine fecundity index and images were analysed using Image J software (NIH) to

Table 4.1

Data collection for *Desmophyllum pertusum* and *Madrepora oculata* from the Lacaze-Duthiers canyon. Gender was determined according to histological analysis: F=Female, M = Male and I=Immature, U=Undetermined.

Species	Date	Sample	Colony ID	Depth	Coordinates		Gender	
					Latitude	Longitude		
<i>Desmophyllum pertusum</i>	03/07/2012	A4-L1	L67	515	42°32.721	3°25.269	F	
	06/07/2012	A4-L2	L188	526	42°32.715	3°25.299	M	
		A4-L3	ZE2	520	42°32.708	3°25.296	F	
	24/04/2013	A5-L2	L92	521	42°32.708	3°25.279	I	
		A5-L3	L58	525	42°32.728	3°25.279	I	
	27/10/2014	A7-L1	L32	512	42°32.723	3°25.271	I	
		A7-L2	L62	515	42°32.716	3°25.272	I	
		A7-L3	L88	517	42°32.716	3°25.284	I	
	29/10/2014	A7-L4	L67	513	42°32.725	3°25.274	F	
		A7-L5	L62	514	42°32.725	3°25.277	F	
	03/06/2015	A8-L1	L62	515	42°32.723	3°25.267	U	
		A8-L2	L122	515	42°32.725	3°25.275	F	
	<i>Madrepora oculata</i>	06/07/2012	A4-M1	O52	512	42°32.719	3°25.276	F
		24/04/2013	A5-M1	O43	520	42°32.716	3°25.281	M
			A5-M2	O35	521	42°32.702	3°25.294	F
		A5-M3	O40	516	42°32.713	3°25.282	M	
29/10/2014		A7-M1	O54	511	42°32.712	3°25.276	F	
		A7-M2	O43	517	42°32.716	3°25.284	M	
		A7-M3	O12	513	42°32.725	3°25.274	M	
		A7-M4	O56	517	42°32.730	3°25.268	F	
03/06/2015		A8-M1	O64	515	42°32.725	3°25.270	F	
		A8-M2	O48	515	42°32.726	3°25.275	M	

measure oocyte diameters (*i.e.*, diameter of an oocyte as it was perfectly circular (Waller and Tyler, 2005)). Histological study, based on this identification criteria, allowed to identify different stages of maturation in male and female, to describe gametogenesis cycle and determine potential spawning date of *D. pertusum* and *M. oculata* in the Mediterranean Sea. Small polyps for which no gamete was observed were described as immature ones. When the size of the polyp corresponded to the potentially sexually mature adult size but no gamete could be observed, this could indicate either an at rest or post-spawning stage and they were classified as undetermined (Table 1).

2.3. Recruitment

As mentioned above, the site was monitored since 2010 by the LECOB laboratory from Banyuls-sur-mer Oceanological Observatory, particularly for coral growth survey based on the use of deep-sea experimentation platforms equipped with coral nubbins (see Chapron et al., 2020a). The platforms were deployed and recovered each year at the same period (in May–June), corresponding to a time of deployment of one year. After recovery, the platforms were cleaned with freshwater to remove all epibionts and stay onboard on the ship one to three days before redeployment (Fig. 1A).

In early June 2017 and 2018, during the recovery of the platforms,

tiny coral recruits fixed on the lower parts of the platform were observed (Fig. 1B). It corresponded mostly to single polyps of *Desmophyllum dianthus* (light pink to orange colour, Fig. 1C), and some single or dual polyps of *D. pertusum* and few *M. oculata* (whites) (Fig. 1D–F). The polyp length of *D. pertusum* were measured on board with a caliper.

3. Results

3.1. Gametogenesis

The same stages of oogenesis as described in Waller and Tyler (2005) were observed for *M. oculata*: stage I oogonia ($<37\ \mu\text{m}$), stage II pre-vitellogenic oocytes ($37\text{--}200\ \mu\text{m}$), stage III vitellogenic granulated oocytes ($200\text{--}350\ \mu\text{m}$) and stage IV late vitellogenic oocytes ($>350\ \mu\text{m}$) ready for spawning. In *D. pertusum*, no stage I oogonia ($<5\ \mu\text{m}$) were observed and only stage II ($5\text{--}30\ \mu\text{m}$) and III ($30\text{--}140\ \mu\text{m}$) oocytes were found in mesenteries. Moreover, no stage IV late vitellogenic oocyte ($>140\ \mu\text{m}$, inferred stage in Waller and Tyler (2005)), were observed. The different stages of oocytes and cysts are shown in Figs. 2 and 3 for *D. pertusum* and *M. oculata*, respectively.

Colonies of both genders were observed, supporting that these species are gonochoristic. Four of the *D. pertusum* colonies were female (Table 1), while only one male was observed, in July 2012. According to

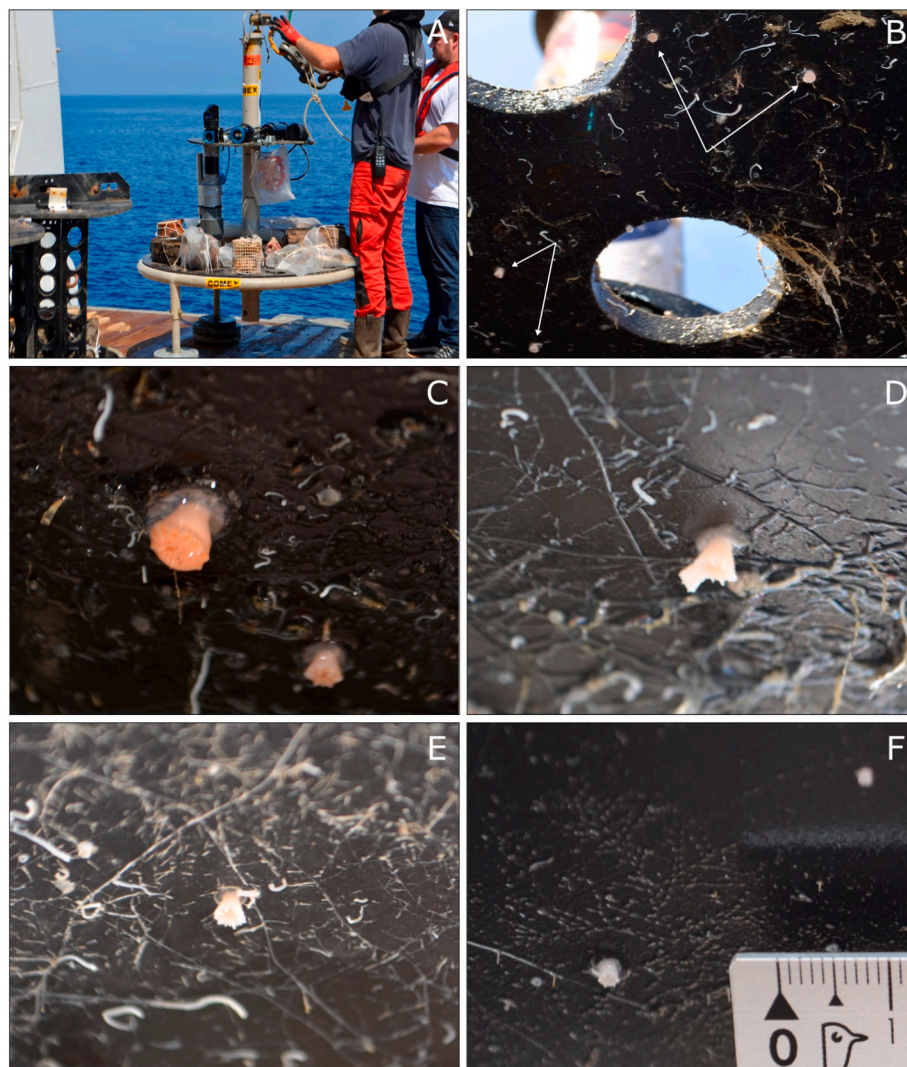


Fig. 4.1. Photos of *in situ* platforms deployed in the Lacaze-Duthiers canyon between 2016 and 2018 (A) with recruits of solitary polyp of *Desmophyllum dianthus* (B and C) and colonial polyps of *Desmophyllum pertusum* (D to F) fixed on the lower part of the platform.

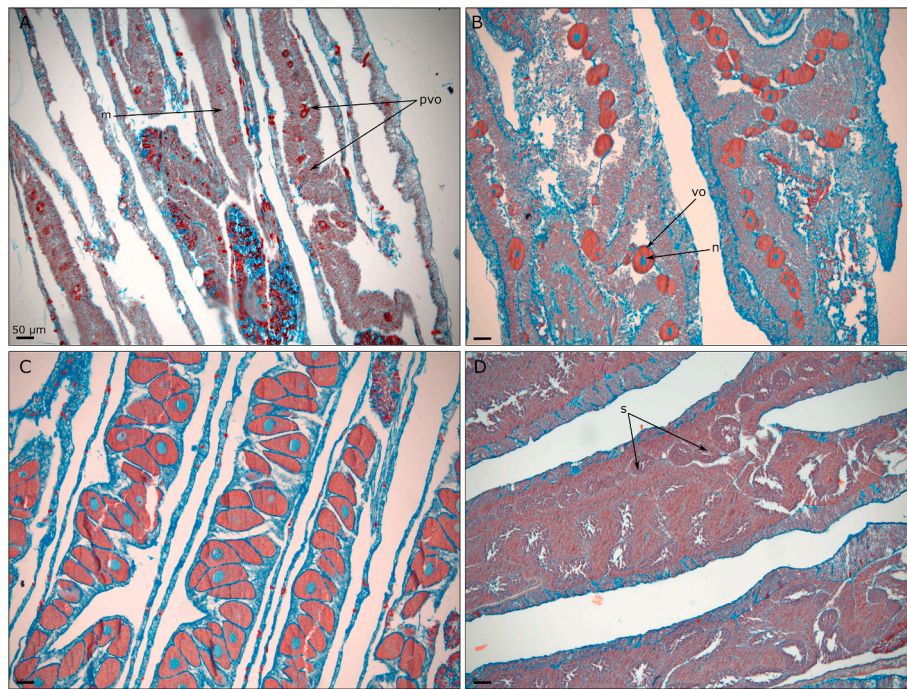


Fig. 4.2. Photos of *Desmophyllum pertusum* colonies: A) small stage II previtellogenic oocytes, B) stage II oocytes and C) late stage III vitellogenic oocytes filling the mesenteries, D) male stage II. *m* female mesenteries, *pvo* previtellogenic oocyte, *vo* vitellogenic oocyte, *n* nucleolus, *s* spermatocyte. Scale bars A, B, C, D = 50 µm.

identification criteria of Waller and Tyler (2005), all spermatocytes from this colony appeared to be at stage II (Fig. 2D). Finally, five of the colonies collected in April 2013, October 2014 and June 2015 could not be assigned a gender because no gamete was discernible and they were undetermined corresponding to immature stage or to the rest or post-spawning period (Table 1).

All *M. oculata* colonies were mature, corresponding to five females and four males respectively (Table 1).

For *D. pertusum*, gametes were observed in colonies sampled in June, July and October and no oocyte was found in colonies sampled in April (Fig. 4). In details, stage II oocytes were observed in June 2015. The size of the smallest detectable and measurable oocyte was 6.13 µm (Fig. 4A), which corresponds to very small stage II oocyte according to Waller and Tyler (2005). Thus, no stage I oocyte (<5 µm) was observed in any sampled colony. One of the three polyps in the colony L122 (polyp a), sampled in June, had no oocyte. Considering its position at the apex of a branch, it might correspond to an immature polyp.

Small stage III oocytes were observed in July 2012 in two colonies (Fig. 4A). Three of the four polyps analysed from colony ZE2 have no oocyte (polyps b, c and d) and considering their smaller size (height <5 mm compared to 8 mm for the polyp a), we assumed they correspond to immature polyps.

Large mature oocyte (stage III according to Waller and Tyler, 2005) were observed in October 2014. The largest average oocyte diameter, registered for colony L67, was 89.70 ± 13.79 µm, with a maximum oocyte diameter of 121.69 µm (Fig. 4A). No stage IV oocytes (>140 µm) were found. Furthermore, oocyte was missing in two of the three polyps analysed from the colony L62 (polyps b and c), while the third polyp contained large mature stage III oocytes (83.6 ± 14.0 µm, Fig. 1C). All polyp's from this colony were similar in length and diameter, suggesting that polyps b and c as mature adults that have already spawn, while polyp a had not yet spawn. Consecutively, the two polyps without oocyte were in stage post-spawning (Fig. 4A).

Madrepora oculata oocytes and spermatocytes were observed in colonies sampled at each period (April, June, July and October). Oocytes from stage II, III and IV were observed simultaneously in the same polyp in most of the sampled colonies (e.g., O52, O56, O64, Fig. 3B). Stage II

oocytes were predominant in April and October, while stage III and IV were predominant in June. Stage II and III oocytes were in equal proportion in July. Very few stage I oocytes were quantified in our sample (polyp a from colony O35 in April 2013 and polyp c from colony O54 in October 2014, Fig. 4B). The minimum oocyte diameter measured was 20.73 µm (in April 2013) and the maximum diameter was 594.46 µm (in June 2015).

3.2. Fecundity

Among the nine *D. pertusum* colonies sampled, three polyps from a single colony (L67) were sectioned entirely, allowing us to estimate the fecundity index. *Desmophyllum pertusum* fecundity index varied between 4052 and 8976 oocytes per polyp in July 2012, which corresponds to 1738 ± 821 oocytes per cm². For *M. oculata*, oocytes from two colonies were counted in October 2014 (three polyps from O54) and in June 2015 (four polyps from O64) and fecundity index varied between 7 and 25 oocytes per polyp, which corresponds to 25 ± 8 oocytes per cm².

3.3. Timing of reproduction

Histological analysis and study of *D. pertusum* oocyte diameter showed that mature stage III oocytes were observed in July to October, with higher mean size of oocytes in October (mean diameter of 83.64 ± 14.01 µm, *t*-test $t = -18.38$, *p*-value <0.001). No stage IV oocyte corresponding to a mean diameter >140 µm was observed, as reported in Pires et al. (2014) (Fig. 4A). Evidence of coral spawning has been described in October 2014 with two mature polyps free from oocytes and one stage III polyp in the same coral colony (L62, Fig. 4A). This suggests that, in 2014, *D. pertusum* started to lay during the autumn season.

Madrepora oculata showed a very different pattern since all stages of maturation were present simultaneously in the same colony for each sampling dates (Fig. 4B). Moreover, oocyte diameter showed that oocytes measured in October 2014 were not predominantly mature. Thus, in contrast to *D. pertusum*, with a predominant period of reproduction, the species *M. oculata* seems to be able to breed all year round.

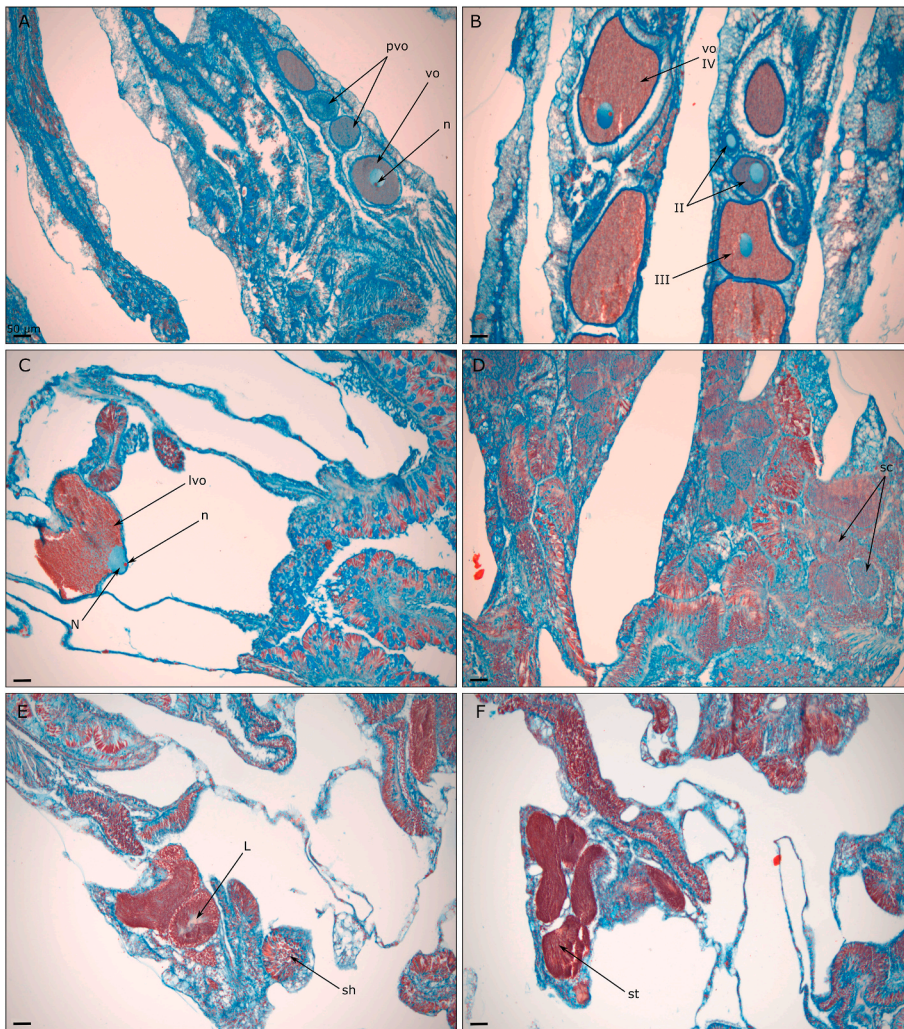


Fig. 4.3. Photos of *Madrepora oculata* colonies: A) Small previtellogenic and vitellogenic oocytes within mesentery; B) Female polyp showing stage II, III and IV oocytes; C) Large late vitellogenic oocyte with nucleolus in the periphery of nucleus; D) Stage II sperm cysts; E) Stage I and II sperm cyst; F) Stage IV sperm cyst showing sperm tails. Pvo previtellogenic oocyte, vo vitellogenic oocyte, lvo late vitellogenic oocyte, sc sperm cyst, sh sperm heads, L lumen, st sperm tails. Scale bars A, B, C, D, E, F = 50 μ m.

3.4. Heights of polyp recruits

Only polyps that could clearly be assigned to *D. pertusum* were selected for size measurements. The *D. pertusum* recruits observed in 2017 and 2018 during platforms recovery exhibited a mean height of 2.5 \pm 1.2 mm (with values ranging from 1 to 5 mm, n = 42).

4. Discussion

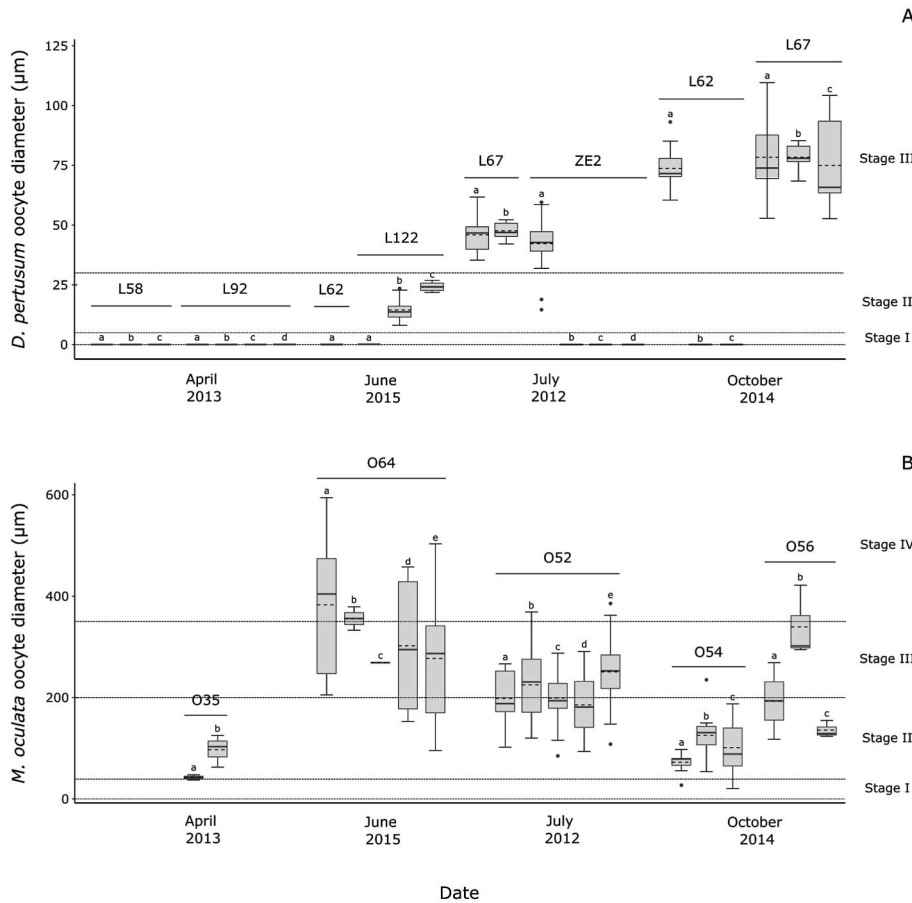
This study describes for the first time some aspects of the reproductive biology of *D. pertusum* and *M. oculata* in the Mediterranean Sea. Colonies of both sexes were observed in each species, confirming that *D. pertusum* and *M. oculata* are gonochoric species, as already determined by Waller and Tyler (2005), Burgess and Babcock (2005), Brooke and Järnegren (2013) and Pires et al. (2014).

4.1. Reproductive cycle of *D. pertusum*

Our results show the presence of *D. pertusum* previtellogenic oocytes in June 2015, vitellogenic oocytes in July 2012 and mature oocytes in October 2014. These observations confirm a seasonal reproduction described for this species in other areas from the Atlantic and Gulf of Mexico (Waller and Tyler, 2005; Brooke and Schroeder, 2007; Brooke and Järnegren, 2013; Pires et al., 2014). Although this present study was not conducting during the same year, due to difficulties in assessing deep-sea habitats, the results suggest a seasonal trend in the oogenesis of

D. pertusum from the Mediterranean Sea. As stage II oocytes were already present in corals in July, oogenesis of *D. pertusum* is likely to be initiated during spring (April) in this area of the Mediterranean Sea. The simultaneous observations within the same colony in October 2014 of mature polyps and polyps at rest, likely corresponding to a post-spawning stage, suggest that spawning should partly occur at this period in the Lacaze-Duthiers Canyon. As stage III oocytes fell within the lower range observed for this species (Waller and Tyler, 2005; Pires et al., 2014) in October, and spawning may occur over 6–8 weeks for this species (Brooke and Järnegren, 2013), spawning season in this area of the Mediterranean sea should occur from October to early winter. Assuming a mean new polyp growth rate for this species in this canyon of 7.5 mm yr⁻¹ (Lartaud et al., 2013, 2014), the average size measured for new polyp recruits during the recovery of the experimental platforms in early June after one year of deployment (2.5 \pm 1.2 mm), would expect a settlement ~4 months earlier (*i.e.*, early February). Aquaria experiments suggest a larval development and the ability to settle after 1–2 months (Larsson et al., 2014), corresponding here to an expected spawning in early January to early December, which is consistent with the observed oocyte maturation and suggested spawning time.

This reproductive cycle is close similar to the one described for *D. pertusum* from the southeastern US and the Gulf of Mexico, with presence of vitellogenic oocytes in July and mature oocytes in autumn with potential partial spawning season in October (Brooke and Schroeder, 2007; CSA International Inc, 2007). Brooke and Schroeder, 2007 We lack of data to accurately estimate the duration of the



A Fig. 4.4. Oocyte diameter (in μm) of six different colonies of *Desmophyllum pertusum* (A) and five different colonies of *Madrepora oculata* (B) between July 2012 and June 2015 in the Lacaze-Duthiers Canyon, plain lines indicating the median, dashed lines representing the mean, and full circles represent extreme values exceeding the 95% quantile for each polyp. We hypothesised that gametogenic cycle was cyclic and represented the month sampled of different years on a continuous scale. Lower case letters identify each polyp from a single colony (colony ID corresponds to the ID in Table 1). Dotted lines determine limits from each stage of oocyte maturation: stage I (0–5 μm), stage II (5–30 μm) and stage III (30–140 μm) for *D. pertusum* and stage I (0–37 μm), stage II (37–200 μm), stage III (200–350 μm) and stage IV (>350 μm) for *M. oculata* (see Waller and Tyler, 2005).

B

spawning period in this area of the Mediterranean Sea for *D. pertusum* but this study revealed that in spring (*i.e.*, April) all observed corals were undifferentiated indicating that spawning is over at this time.

We could not conclude for any pattern concerning spermatogenesis as only one male colony of *D. pertusum* was sampled in July 2012. However, at this period spermatocytes were at stage II, which corresponds to the maturation phase described by Brooke and Järnegren (2013) in the northeast Atlantic, which occurs between May and November. As the gametogenesis are almost synchronized in both genders, we can hypothesise a similar pattern for males spawning.

Reproductive periodicity, both in term of gamete maturation or spawning synchronization, is most likely controlled by hormones and exogeneous factors, including seasonal changes in temperature, salinity, food availability, lunar and tidal cycles, wind and current (*i.e.* Babcock et al., 1986; Hayashibara et al., 1993; Hayashibara et al., 1997; Mercier et al., 2011b). In shallow-water scleractinian corals, regulation and synchronization of reproductive cycles are known to be primarily triggered by changes in temperature, current patterns and lunar cycles among other environmental factors (Harrison, 2011).

In deep-sea environments, the main factors controlling the reproductive periodicity are still unknown, particularly because of the lack of time series of environmental data in coral ecosystems and long-term monitoring of the biology of species. In this area of the deep Mediterranean Sea, the temperature is very stable (average of 13.5 °C without seasonal cycle), only disrupted by occasional and briefly short-term (few days) drops or peaks of 1–3 °C depending on the year (Durrieu de Madron et al., 2013; Chapron et al., 2020a; see below). It is thus unlikely this parameter is a primary driver of the seasonal reproduction of *D. pertusum*.

Lunar cycle, another important factor controlling reproduction for shallow-water corals, was previously described as a potential

synchronizer of deep-sea benthic invertebrates (Mercier et al., 2011b). Lunar changes were described controlling deep cyclic currents and the associated supply of particulate organic matter, as in the Rockall Bank and Whittard Canyon in the Atlantic (Mienis et al., 2007; Ingels et al., 2011). These periodical changes were reported as responsible of the formation of growth increments in the skeleton of *D. pertusum* from the Northeastern Atlantic (Mouchi et al., 2014). The authors also observed growth rates fluctuations with a lower skeletal growth period supposedly occurring between July and October in the Whittard Canyon, for which gametogenesis was suggested as probable cause linked to energy budget. Nevertheless, lunar inducing deep currents might be limited in the Mediterranean Sea, an area submitted to a microtidal regime, and were not described in the hydrology monitoring of this canyon (Chapron et al., 2020a).

This area is however subject to important downwelling current changes, occurring seasonally at the autumn-winter period (Canals et al., 2006; Palanques et al., 2006; Bonnín et al., 2008). These episodic currents result from severe coastal storms induced by easterly winds or from the formation and sinking of dense water masses (called cascading event), associated to the evaporation of cold and dense water at the coast during strong northward winds. Winter storm and dense water shelf cascade down currents are associated to current speeds peak at up 60–80 cm s^{-1} , drop of temperature to 3 °C below the normal conditions in the habitat, and the transport of large amounts of sediment and organic matter (Heussner et al., 2006; Sanchez-Vidal et al., 2008). Due to stratified water masses, the autumn storm down currents are associated with lower current speeds peak (20–40 cm s^{-1}) but up to 2 °C increase in temperatures, corresponding to temperatures of the inner and mid-shelf water in that season (Palanques et al., 2006). Thus, the release of gametes in autumn to winter could be linked to these high current episodes, that may participate in the larval dispersal strategy of this

species, since Strömberg and Larsson (2017) showed that *D. pertusum* planktotrophic larvae exhibit traits indicating high potential for long distance dispersal. Such episodic down currents have been recognized to impact fauna biodiversity and function in canyon ecosystems, particularly to displace small mobile species such as shrimps, modify assemblages of deep-sea microbes, and decrease the organic matter content in sediment (Company et al., 2008; Pusceddu et al., 2013; Luna et al., 2016). Additionally, effects of these currents on the growth rate of *D. pertusum* were also reported (Lartaud et al., 2017; Chapron et al., 2020a).

Seasonal phytoplankton blooms produce high flux of organic matter reaching the deep-sea floor, ultimately bringing food to many benthic communities (Billett et al., 1983). This detrital material rapidly sinking may represent a physical cue inducing synchronized reproductive cycles for many deep-sea species, since gametogenesis and vitellogenesis are particularly energetically expensive (Ramirez Llodra, 2002). Eckelbarger and Watling (1995) identified three strategies of seasonal reproductive periodicity in relation to food availability: organic impulses can 1) initiate gametogenesis with spawning following soon after, 2) trigger spawning for planktotrophic larvae, 3) initiate and synchronize gametogenesis with spawning occurring later when vitellogenesis is completed. Indeed, vitellogenesis or spawning are likely induced by high deposition rates of organic matter in *Flabellum angulare* (Mercier et al., 2011a; Waller and Tyler, 2011), *Enallopsammia rostrata* (Burgess and Babcock, 2005) and five deep-sea echinoderms Tyler et al. (1982). Waller and Tyler (2005) suggested that *D. pertusum* and *M. oculata* initiate gametogenesis in response to seasonal phytoplankton blooms in the North East Atlantic. This assumption is based on the fact that *D. pertusum* initiate oogenesis following summer phytoplankton blooms in the Porcupine region (Lampitt et al., 2001). A potential spawning of *D. pertusum* in autumn and/or start of winter in the Mediterranean Sea suggests an initiation of gametogenesis in spring and potential vitellogenesis in summer, attested by the presence of no oocyte in April 2013, stage I and II oocytes in June 2015 and stage III oocytes in July 2012. Long-term time-series data of the environmental conditions in the Lacaze-Duthiers canyon show a certain pattern of higher total mass fluxes during autumn and winter (Heussner et al., 2006). However, the organic content of this transported material is very low (2–3%) (Heussner et al., 2006). On the contrary, these time series show that lower currents during spring and summer are associated with lower inorganic fluxes and the material transported to the bottom is comparatively richer in organic carbon (10%) (Heussner et al., 2006). Thus, the particulate organic matter sinking in spring and summer is less diluted by mineral material of sedimentary origin, and therefore provides much better energetically quality for the organisms. As oogenesis and then vitellogenesis are extremely expensive, if *D. pertusum* colonies were increasing reproductive effort and reserves in response to environmental and seasonal food availability, spring and summer would probably be the most favourable time of the year in the Mediterranean Sea to initiate vitellogenesis.

4.2. The reproductive strategy of *M. oculata* differs from *D. pertusum*

In *M. oculata*, we observed previtellogenic, vitellogenic and large mature vitellogenic oocytes simultaneously in the same polyp in most of the colonies studied and nearly every sampled month. This pattern clearly indicates a continuous reproduction for this species in the Mediterranean Sea, as already described in the north and south Atlantic by Waller and Tyler (2005) and Pires et al. (2014). The production of less but large oocytes for *M. oculata* may suggest lecithotrophic larvae, not relying on any suitable condition in the environment regarding food availability, at least for the first stages of development, as already suggested by Waller and Tyler (2005).

The reproductive strategy of *M. oculata*, based on the release of few but large oocytes, strongly differs from the one of *D. pertusum*, producing large amounts of small oocytes (Waller and Tyler, 2005). These two

species differ on various other biological features such as the skeletal growth, feeding, the internal anatomy and their microbiome (Tsounis et al., 2010; Lartaud et al., 2017; Meistertzheim et al., 2016). They thus might rely on different ecological niche.

Although the two-strategy *r-K* framework is not completely satisfying for corals, we lack to date similar three axes life-history models as described for shallow-water tropical scleractinian (Darling et al., 2012). *Desmophyllum pertusum* exhibit thus traits of a *r*-selection species, based on the MacArthur and Wilson (1967) definition, with the ability for a large larval dispersal through the production of a large number of small oocytes. Interestingly, this feature is combined with a volatile microbiome, leading to the rapid acquisition of the microbial signature of their habitat (Chapron et al., 2020b), an opportunistic feeding strategy (Mueller et al., 2014; Baussant et al., 2017) and a rapid growth rate (Chapron et al., 2020a), likely enabling to dominate the habitat after settlement. On this basis, *D. pertusum* would fit better with the typical characteristics of 'r-selected' species in clonal organisms (Pianka, 1983; Hughes, 1990). By contrast, the reproductive strategy of *M. oculata* is closer to a *K*-selection species. This species also displays microbial composition which fits the initial definition of holobiont for corals, following Rohwer et al. (2002), based on a highly resilient consortium and a strong association with specific bacteria such as *Endozoicomonas* (Meistertzheim et al., 2016; Chapron et al., 2020b). While the *r*-strategy seems to give more plasticity to *D. pertusum*, which may explain its wide dispersion in the oceans where they frequently dominate cold-water coral communities, they appear less resilient than *M. oculata* to further anthropogenic changes, such as the effects of deep-sea temperature increase or plastic pollution (Mouchi et al., 2019; Chapron et al., 2021). Although *D. pertusum* shows more opportunistic features than *M. oculata*, they might be more dramatically affected by the on-going threats in deep-sea habitats.

5. Conclusion

This study provides, for the first time, data on the reproductive biology of the two main reef-building species *Desmophyllum pertusum* and *Madrepora oculata* in the Mediterranean Sea. The results presented here add new data on the gametogenic cycle of these two species and the potential spawning date, which occurs seasonally in autumn and/or early winter for *D. pertusum*, as in the Gulf of Mexico. Vitellogenesis might be related to the organic matter supplies during spring and summer, when inorganic sedimentary fluxes are low. Spawning is thought to be triggered by strong current events induced by severe storms or dense water formation on the shelf, likely promoting larval dispersal. Differently, *M. oculata* shows continuous reproduction as already observed in Atlantic populations. The information provided on Mediterranean CWC reproduction traits will contribute to comprehensively understand differences in population's timing and so the endogenous or exogenous factors responsible for determining this periodicity. Further studies on larval ecology are needed for modelling larval dispersal and understanding connectivity and potential differences in responses in geographical different populations, allowing us to establish protection and management plans regionally adapted.

Author contributions

FL and ALM designed the study. MC, VM and ALM analysed the data and MC, ALM, VM and FL wrote the paper.

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Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Chapter five: Microbiomes of *Lophelia pertusa* and *Madrepora oculata* in deep-sea canyons of the NE Atlantic and the NW Mediterranean

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Abstract

Cold-water corals harbour specific microbial communities that are thought to contribute to the metabolisms and health of the coral host. However, the patterns of host-microbe associations between different coral species and geographical locations remain poorly characterised. Here we study the microbiome diversity of the two main framework-forming cold-water corals, *Lophelia pertusa* and *Madrepora oculata*, across different geographical scales by using standardize protocols. In an unprecedented sampling effort, 116 different coral colonies were collected in replicates (336 samples) from a canyon of the NE Atlantic Ocean (Lampaul canyon) and from two canyons of the Mediterranean Sea (Lacaze-Duthiers and Blanes canyons), and communities were analysed by 16S rRNA metabarcoding. *Lophelia pertusa* showed variable microbial community composition between the two seas, with high spatial variability within canyons. Inversely, *M. oculata* exhibited stable microbiome across the different regions with strong association with some bacteria, including the ubiquitous symbiont *Endozoicomonas*. These differences in the microbe-host association patterns between species suggest different host-bacteria association strategies, which could be related to the occupation of different ecological niches within a same habitat.

Key words: Microbiome, Holobiont, Cold-water corals, DNA, Bacteria, *In situ*

5.1 Introduction

Corals live in very close association with bacterial communities, together forming the holobiont. The role of the microbiome in maintaining and contributing to the coral function and health is still poorly known, but it surely contributes substantially to the metabolism of the host (Bourne et al., 2016). Recent works on tropical corals have highlighted the importance of characterising host-associated microbial communities to better understand the impact of the environment on coral ecology and physiology, as the microbiome may offer the flexibility needed to adapt to local environmental variations (Reshef et al., 2006; McDevitt-Irwin et al., 2017). As a result, the microbiome may contribute to stress tolerance (Rosenberg et al., 2007; Bénard et al., 2020), including against warming in tropical corals (Ziegler et al., 2017).

The microbiomes of cold-water corals have been much less studied, and their role and diversity are still poorly known. Cold-water corals are main engineers of the deep sea (Roberts et al., 2006), which provide important ecosystem services (Armstrong et al., 2014) by forming large reefs offering shelter for a large diversity of associated species in many regions of the world (Roberts et al., 2006). Among the scleractinian cold-water corals, *Lophelia pertusa* (now synonymized *Desmophyllum pertusum*, Addamo et al., 2016) and *Madrepora oculata* are the two key reef-forming species distributed worldwide. Microbiome studies using culture-independent molecular techniques have until now mainly focused on different populations of *L. pertusa* and *M. oculata* from the Mediterranean Sea, the North Atlantic Ocean and the Gulf of Mexico. They showed that *L. pertusa* from Rockall Bank (North Atlantic Ocean) were dominated by *Endozoicomonas* and *Mycoplasma* (Van Bleijswijk et al. 2015), while *Anaplasmataceae* (or more broadly *Rickettsiales*) and *Sphingomonadales*, respectively, dominated the bacterial community from *L. pertusa* in the Mediterranean Sea (Meistertzheim et al. 2016; Galand et al., 2018), and in the Gulf of Mexico (Kellogg et al., 2017). Inversely, bacterial community of *M. oculata* were dominated by *Endozoicomonas* (Gammaproteobacteria) in the North Atlantic (Hansson et al., 2009) and the Mediterranean Sea samples both *in situ* and in aquaria (Meistertzheim et al. 2016; Galand et al., 2018), while *Flavobacteria* were dominant in Norwegian samples (Jensen et al., 2019). These studies showed differences in the microbiome composition between *L. pertusa* and *M. oculata*, *L. pertusa* microbiome being more variable than *M. oculata* which displayed a more stable microbiome (Meistertzheim et al., 2016; Galand et al., 2018; Chapron et al., 2020).

Most of the previous microbiome studies have described microbial diversity of *L. pertusa* and *M. oculata* from single geographic locations or across small latitudinal gradients

(Schröttner et al., 2012), including a relatively low number of samples. To date, only one study focused on describing differences in the bacterial community of these species across a larger geographic scale (Jensen et al., 2019). However, this meta-analysis included 165 samples from studies using different approaches and protocols (including different DNA extraction protocols, 16S rRNA primers, and targeted coral compartments), limiting the possibility for direct comparisons. Understanding the main patterns of bacterial community composition on large spatial scales is essential for a better understanding of the factors contributing to the stability and functioning of cold-water coral reefs. However, for direct comparisons between geographical distant regions the use of homogenized sampling and analysis protocols are needed (Galand et al. 2023).

The aim of this study was to describe the microbiomes of *L. pertusa* and *M. oculata* at a broad geographical scale by targeting corals from the NE Atlantic Ocean and the Mediterranean Sea, and at a local scale by studying corals from two neighboring canyons of the NW Mediterranean Sea. In an unprecedented effort, a total of 336 samples covering 116 different coral colonies of both species were sampled in the Lampaul canyon (NE Atlantic Ocean), and in the Lacaze-Duthiers and the Blanes canyons (Mediterranean Sea), and bacterial communities were analysed by sequencing the 16S rRNA genes (DNA).

5.2 Material and methods

5.2.1 Study sites and specimen collections

In the Atlantic Ocean, the Lampaul canyon is located off North-West coast of France, in the Bay of Biscay (Northeast Atlantic Ocean), and is included in a Natura 2000 network, aiming at preserving deep-sea reef habitats in the Bay of Biscay. Both *L. pertusa* and *M. oculata* are present in the Lampaul canyon in which they live in different habitats (Van den Beld *et al.* (2017). In the Mediterranean Sea, the Lacaze-Duthiers canyon is located in the Gulf of Lion Marine Nature Park, a Marine Protected Area and is historically known to host cold-water coral reefs (Reyss, 1964). The Blanes canyon is one of the most prominent submarine canyons incising the Catalan margin, North West Mediterranean Sea, where dense cold-water coral colonies were recently described (Santi et al., 2021) (Figure 5.1).

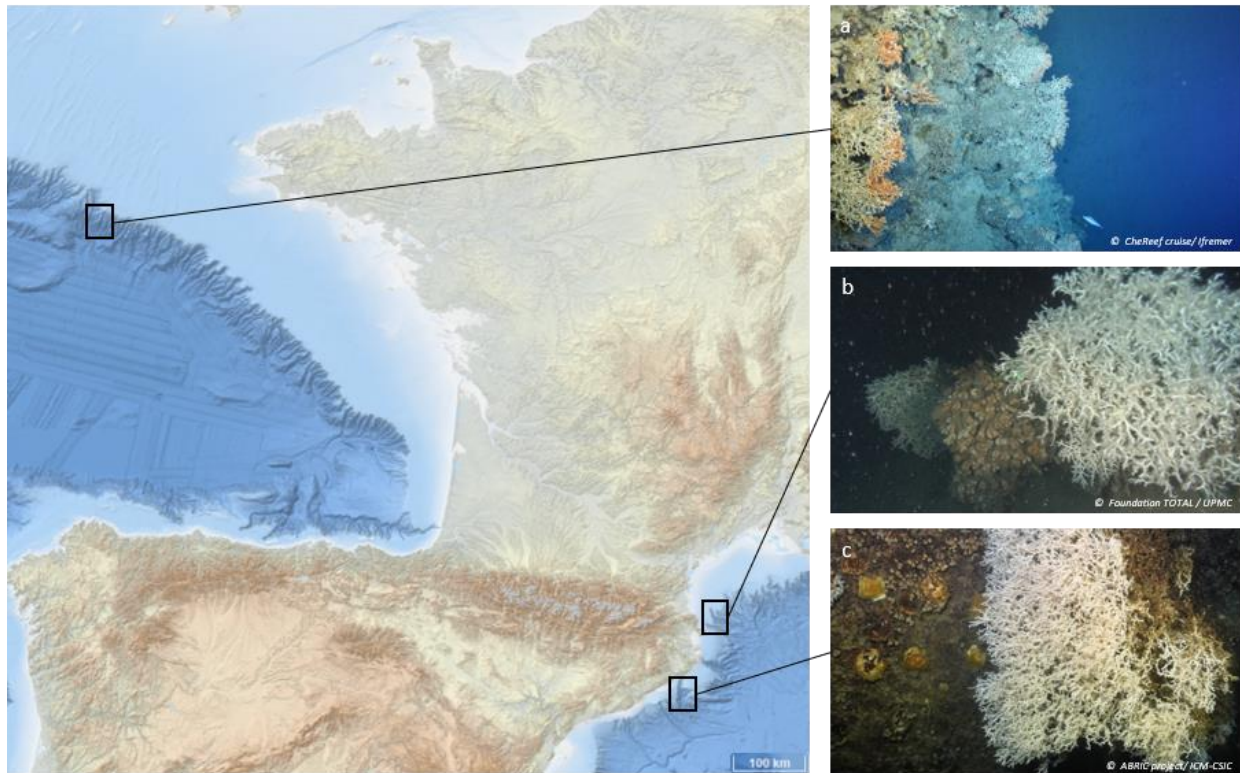


Figure 5.1. Map of the study sites: the Lampaul canyon in the NE Atlantic Ocean, with fringes of *L. pertusa* on cliffs concentrated between 800 and 1000 m depth (a), the Lacaze-Duthiers canyon in the NW Mediterranean Sea, with large reefs of *L. pertusa* and *M. oculata* between 300 and 600 m depth (b), and the Blanes canyon, also in the Mediterranean Sea, with cliffs covered with *L. pertusa* around 1000 m depth (c).

For the Lampaul canyon, corals were collected during two different cruises in 2021 and 2022 using Ifremer's deep-sea Remotely Operated Vehicles (ROV) Ariane and Victor 6000 from the R/V Thalassa. Colony fragments of *L. pertusa* and *M. oculata* were collected between 800 and 1600 m depth and then transported to the surface using thermally insulated boxes maintaining ambient temperature of deep seawater from the site of collection (i.e., 10°C). For the Lacaze-Duthiers canyon, corals were sampled between 300 and 600 m depth, during 6 distinct cruises (2012-2017) using the ROV Super Achille on the R/V Minibex, or the R/V Janus II, from COMEX S.A. Finally, for the Blanes canyon, *L. pertusa* and *M. oculata* colonies were sampled in 2020 around 1050 m depth, using the ROV Liropus 2000 on the Spanish National Research Council (CSIC) oceanographic vessel Sarmiento de Gamboa.

5.2.2 DNA extraction

Three polyps from each colony were individualized in cyotubes on board the research vessels and immediately stored at -80°C until nucleic acid extractions. For *L. pertusa*, 32 different

colonies were collected from the Lampaul canyon, 22 different colonies from the Lacaze-Duthiers canyon and 6 different colonies from the Blanes canyon, and for *M. oculata*, 37 different colonies of the Lampaul canyon, 17 different colonies from the Lacaze Duthiers canyon and 2 colonies of the Blanes canyon. In total, the study is based on 179 polyp samples from 60 different colonies of *L. pertusa*, and 157 samples from 56 different colonies for *M. oculata*.

Once in the laboratory, coral bacterial communities were analysed following the protocol described in Chapron et al. (2020). Individual polyps (including skeleton, tissues and mucus) were first crushed using a sterile hammer, then grounded in tubes containing a garnet matrix, and lysed mechanically using a FastPrep Instrument (MP, Biomedical, Illkirch-Graffenstaden, France). DNA extraction was performed using the Maxwell RSC Blood DNA Kit LEV and the Maxwell 48 MDx Instrument (Promega, Madison, WI, United States) following the manufacturer's instructions. All samples were subjected to RNase A (4 mg mL⁻¹) treatment for 30 min at 37°C.

5.2.3 16S rRNA amplicon sequencing and data analysis

The V1–V3 region of the bacterial 16S rRNA gene was amplified with the primers 27F AGRGTTTGATCMTGGCTCAG (Lane, 1991) and 519R GTNTTACNGCGGCKGCTG (Turner *et al.*, 1999) using the high-fidelity Advantage2 polymerase (Takara Holdings, Kusatsu, Japan) under the following PCR conditions: 30 s at 94°C, 40 s at 56°C, 1 min at 68°C for 35 cycles. PCR products were sequenced in a commercial laboratory (Integrated Microbiome Resource, Halifax, Canada) by mixing the amplicon products from the different samples in equal concentrations and purifying using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, United States). The DNA library was prepared using the purified PCR products following the Illumina TruSeq DNA library preparation protocol. All the samples were sequenced on the same Miseq Illumina sequencer run using Miseq reagent kit V3 (Illumina, CA, United States) producing 2 × 300-bp long reads. PCR and sequencing were conducted. All sequences were deposited in GenBank under SRA accession number xx.

Sequence analysis was performed with DADA2 in R (Callahan *et al.*, 2016, v 1.26.0). The reverse reads were of low quality so we applied the pipeline on forward reads only with the following parameters: trimLeft = 20, truncLen = 260, maxN = 0, maxEE = 5, truncQ = 2. The sequences were filtered, dereplicated, and chimeras removed, to obtain amplicon sequence variants (ASVs). ASVs were classified against the SILVA v. 128 database (Quast *et al.*, 2012)

for taxonomic assignment. A total of 13 samples having less than 1000 sequences were removed, and 323 samples were included in the analysis.

5.2.4 Statistical analysis

To enable comparison of the bacterial community composition and diversity between different sites, the sequences abundance table was normalized with Hellinger transformation (Legendre and Gallagher, 2001). The Shannon diversity index (Shannon, 1948) was computed between and within samples, and a nMDS based on Bray–Curtis similarity was conducted to visualize similarities in community composition between samples with the *vegan* package in R (Oksanen *et al.*, 2013). Significant differences between community composition were tested with PERMANOVA with the *adonis* function of the *vegan* package. Finally, to identify the ASVs that contributed the most to the differences between the study sites, an indicator species analysis (De Cáceres & Legendre, 2009) was performed, using the *multipatt* function of the *indicspecies* package. From the ASVs identified as indicator species, we statistically selected the top 15 % of the ASVs contributing the most to differentiate each site and graphically represented them, using *scale* function and *heatmap* package (Kolde, 2015).

5.3 Results

The non-metric multidimensional scaling ordination (nMDS) plot showed that *L. pertusa* samples grouped together, and were separated from the *M. oculata* samples, which grouped together (Figure 5.2a). *Lophelia pertusa* samples were more dispersed than the *M. oculata* samples that were more densely grouped. A non-parametric multivariate analysis of variance test showed significant difference between the bacterial composition of the two species for all three sites combined (ANOSIM, $R = 0.504$, p -value < 0.001).

Considering the two species separately, the nMDS plot showed that *L. pertusa* samples from the Lampaul canyon grouped together and were separated from the Lacaze-Duthiers and the Blanes canyons samples (Figure 5.2b). The Blanes samples fully overlapped with the Lacaze-Duthiers samples. A non-parametric multivariate analysis of variance test showed significant difference between the bacterial composition of the three sites (ANOSIM, $R = 0.411$, p -value < 0.001).

For *M. oculata*, samples from the three study sites were mixed and did not show any highly differentiated clusters on the nMDS plot (Figure 5.2c), despite the significant Permanova showing differences between the sites (ANOSIM, $R = 0.429$, p -value < 0.001).

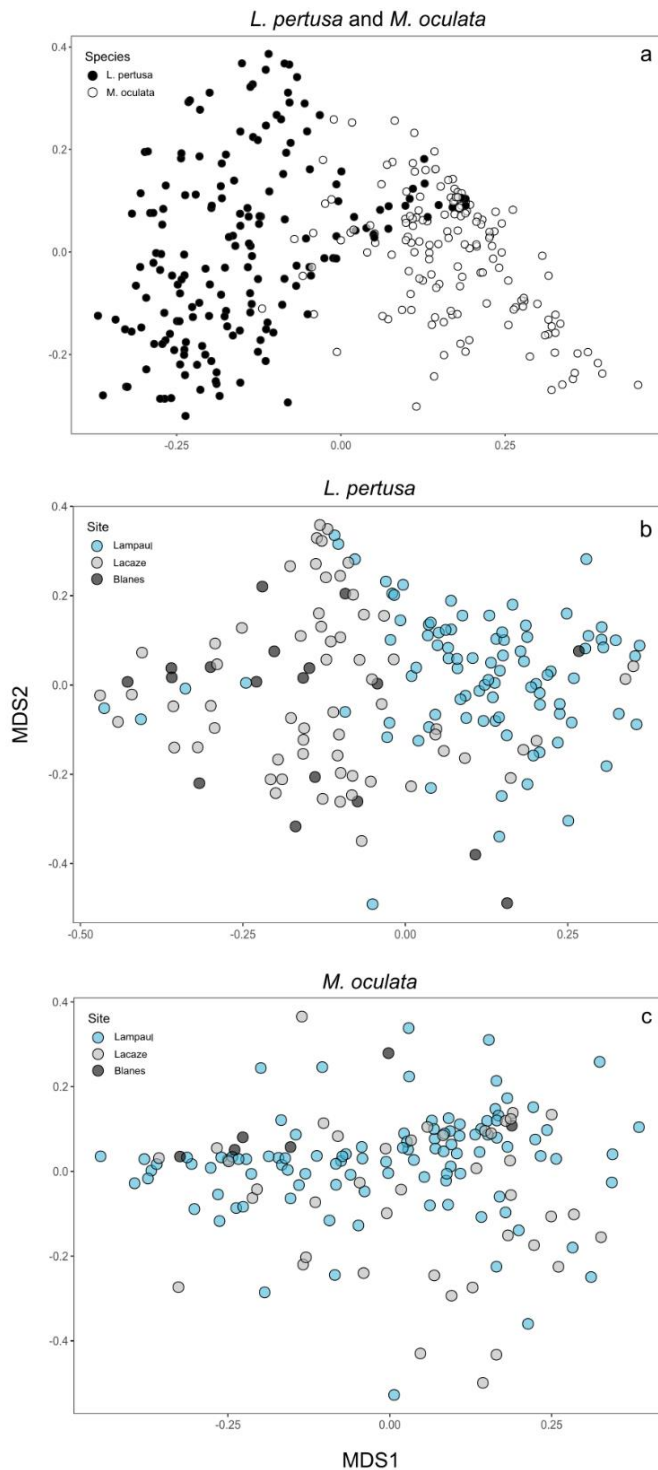


Figure 5.2. Non-metric multi-dimensional scaling plot (nMDS) based on the Bray-Curtis similarity index showing the similarity between bacterial community compositions of *L. pertusa* and *M. oculata* for the three sites: the Lampaut canyon (NE Atlantic Ocean), the Lacaze-Duthiers and the Blanes canyons (both in the Mediterranean Sea) (a), similarity between *L. pertusa* bacterial community compositions from the three study sites (b) and similarity between *M. oculata* bacterial community from the three study sites (c).

L. pertusa bacterial communities were mostly dominated by ASVs belonging to *Haliaceae*, *Anaplasmataceae* and *Propionibacteriaceae*, which represented in average 36, 6 and 7 % of the sequences respectively in the Lampaul canyon (Figure 5.3a). We observed no clear geographical pattern of bacterial community composition between coral colonies. In some cases, colonies sampled from very close sampling sites (1L6, 1L7 and 1L8 or 2L7 and 2L8 or 2L26, 2L27 and 2L28) had very different bacterial communities (Figure 5.3a and Supplementary Fig. 5.1). Moreover, there was no clear temporal patterns between *L. pertusa* colonies sampled in 2021 and 2022.

In *L. pertusa*, the bacterial family that dominated Lampaul samples, *Haliaceae*, represented only 16 and 7 % of the sequences in the Lacaze-Duthiers and Blanes canyons, except for a few samples. Interestingly, colonies collected in 2012 in the Lacaze-Duthiers canyon (A4-L2 and A4-L3) grouped with Lampaul canyon samples on the nMDS (Supplementary Fig. 5.1), with a higher abundance of *Haliaceae*, whereas the other Lacaze-Duthiers samples were different from the Lampaul samples.

More generally, the *L. pertusa* bacterial communities from the Lacaze-Duthiers canyon were mostly dominated by ASVs belonging to non-assigned families (29 %), *Pirellulales* (10 %) and *Propionibacteriaceae* (9 %). The family *Micrococcaceae* showed higher abundance in 6 colonies in the Lacaze-Duthiers canyon, while it was very scarce in the others colonies in the Mediterranean canyons, as well as in the Lampaul canyon (Figure 5.3a).

Finally, bacterial communities from the Blanes canyon showed a more balanced composition, with *Anaplasmataceae*, *Pirellulaceae*, *Propionibacteriaceae* and *Phycisphaeraceae* present in similar proportions, and ASVs belonging to the *Thioglobaceae* in high abundance in 2 colonies (30 %, Figure 5.3a).

M. oculata bacterial communities were mostly dominated by ASVs belonging to the family *Endozoicomonadaceae*, which represented almost 40 % of the bacterial community in the Lampaul canyon and respectively 16 and 15 % in the Lacaze-Duthiers and Blanes canyons (Figure 5.3b). Interestingly, different *Endozoicomonadaceae* dominated in the different canyons. Some ASVs present had different abundances in the three canyons, with some prevalent only in the Lampaul canyon, while others were in higher abundance in the Mediterranean canyons (Supplementary Fig. 5.2). The rest of the bacterial community belonged to non-assigned families in the three canyons, but also *Rickettsiaceae*, *Propionibacteriaceae*, *Rhodobacteraceae* in some colonies of the Lampaul canyon, and *Anaplasmataceae*, *Micrococcaceae*, *Flavobacteriaceae* and *Mycobacteriaceae* in some colonies of the

Mediterranean canyons (Figure 5.3b). Interestingly, we noted higher abundance of *Micrococcaceae* in both *L. pertusa* and *M. oculata* concomitantly in 2014.

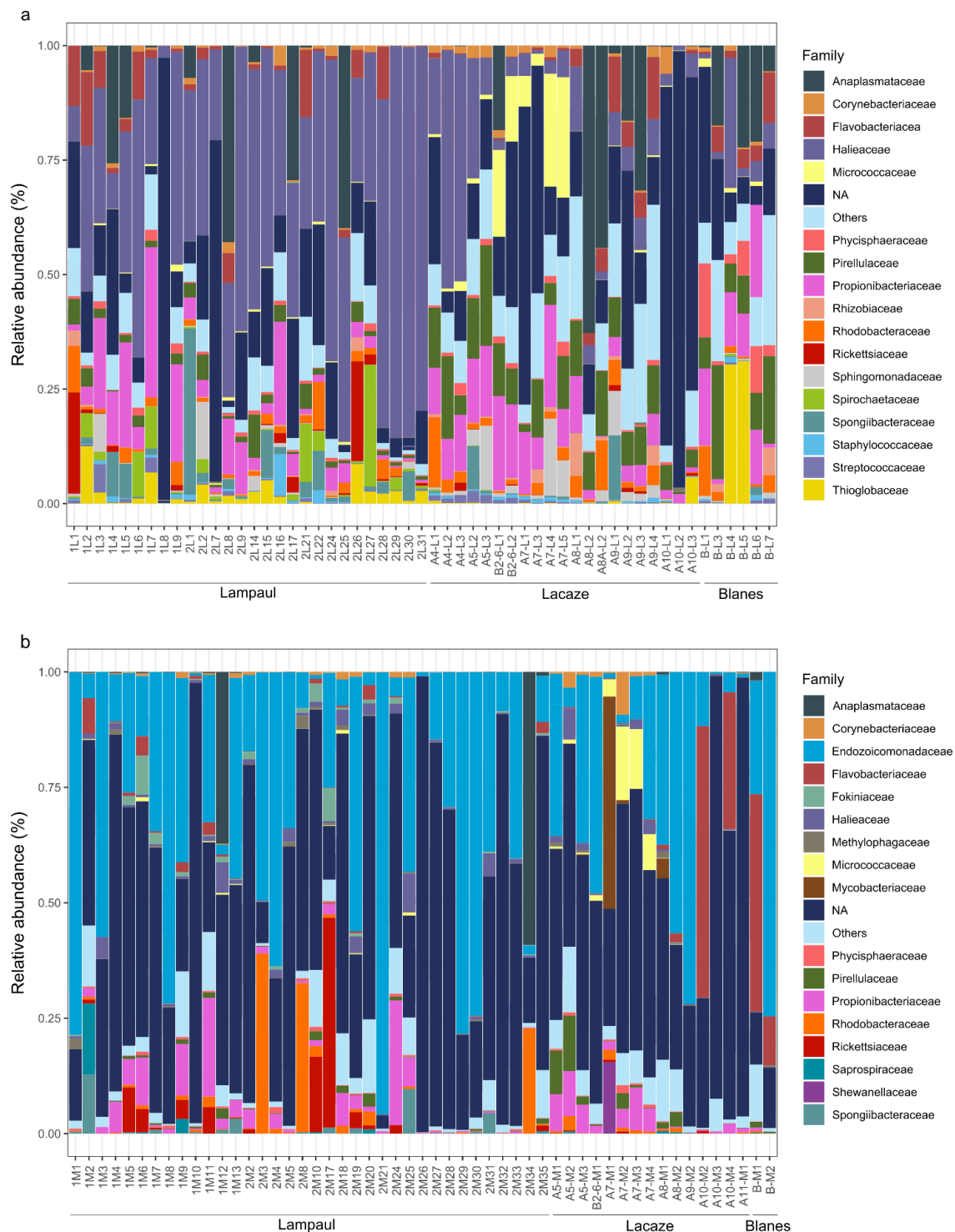


Figure 5.3. Relative abundance of bacterial sequences at the family level (top 18 families, all other families showing less abundance were summed into “Others”) in a) *L. pertusa* and b) *M. oculata*. The composition is based on average values over triplicate samples of each colony for the three different study sites: the Lampaul, the Lacaze-Duthiers and the Blanes canyons.

The Shannon diversity index was significantly higher in *L. pertusa* than *M. oculata* (KW Chi-squared = 59.889, df = 1, p-value < 0.001). The bacterial community diversity in *L. pertusa* was significantly lower in the Lampaul compared to the Lacaze-Duthiers canyons (Dunn test, p-value = 0.042), while no differences were detected between the Lampaul and the Blanes canyons, neither between the two Mediterranean canyons. No differences in the bacterial community diversity was observed in *M. oculata* between the different sites. However, high variability was observed in the Lampaul and the Lacaze-Duthiers canyons (Figure 5.4).

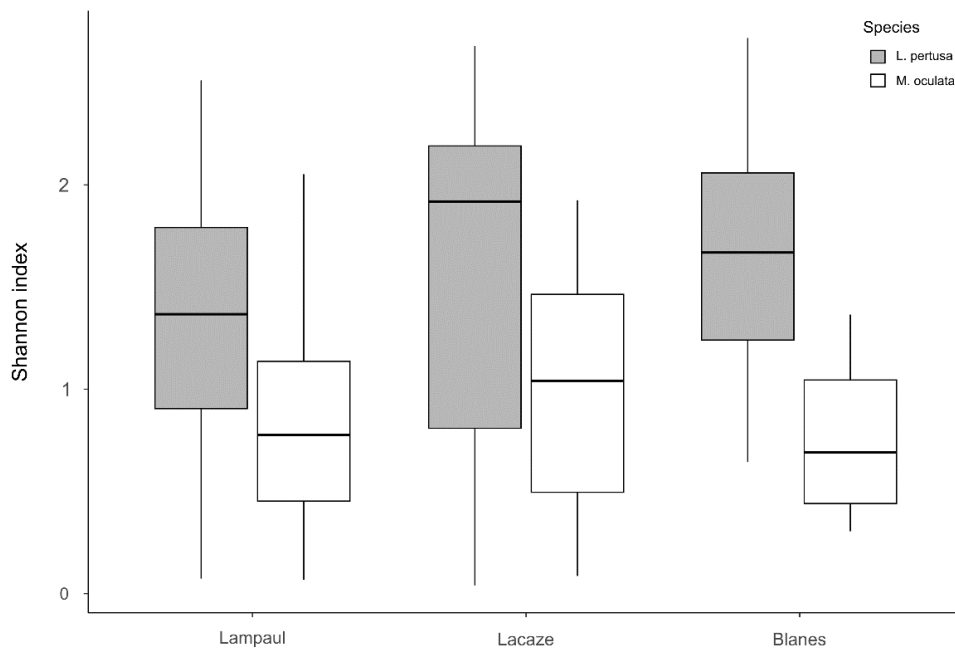


Figure 5.4. Shannon diversity index of *L. pertusa* and *M. oculata* bacterial communities at the three different study sites: the Lampaul, the Lacaze-Duthiers and the Blanes canyons. Values presented correspond to the median and quartile.

To further identify the bacteria contributing the most to the differences between the three study sites in *L. pertusa*, we statistically selected the ASVs that were indicator species for each site (Figure 5.5). Several ASVs from the *Phycisphaeraceae* and the *Pirellulacea* families contributed the most to the Blanes canyon (Figure 5.5). In contrast, *Micrococcaceae* ASVs characterised the Lacaze-Duthiers canyon, (Figure 5.5), although they were not very abundant (Figure 5.3a). ASVs from the *Haliaceae*, which dominated Lampaul’s bacterial community (Figure 5.3a), contributed the most to the difference between the Atlantic canyon and the Mediterranean ones (Figure 5.5).

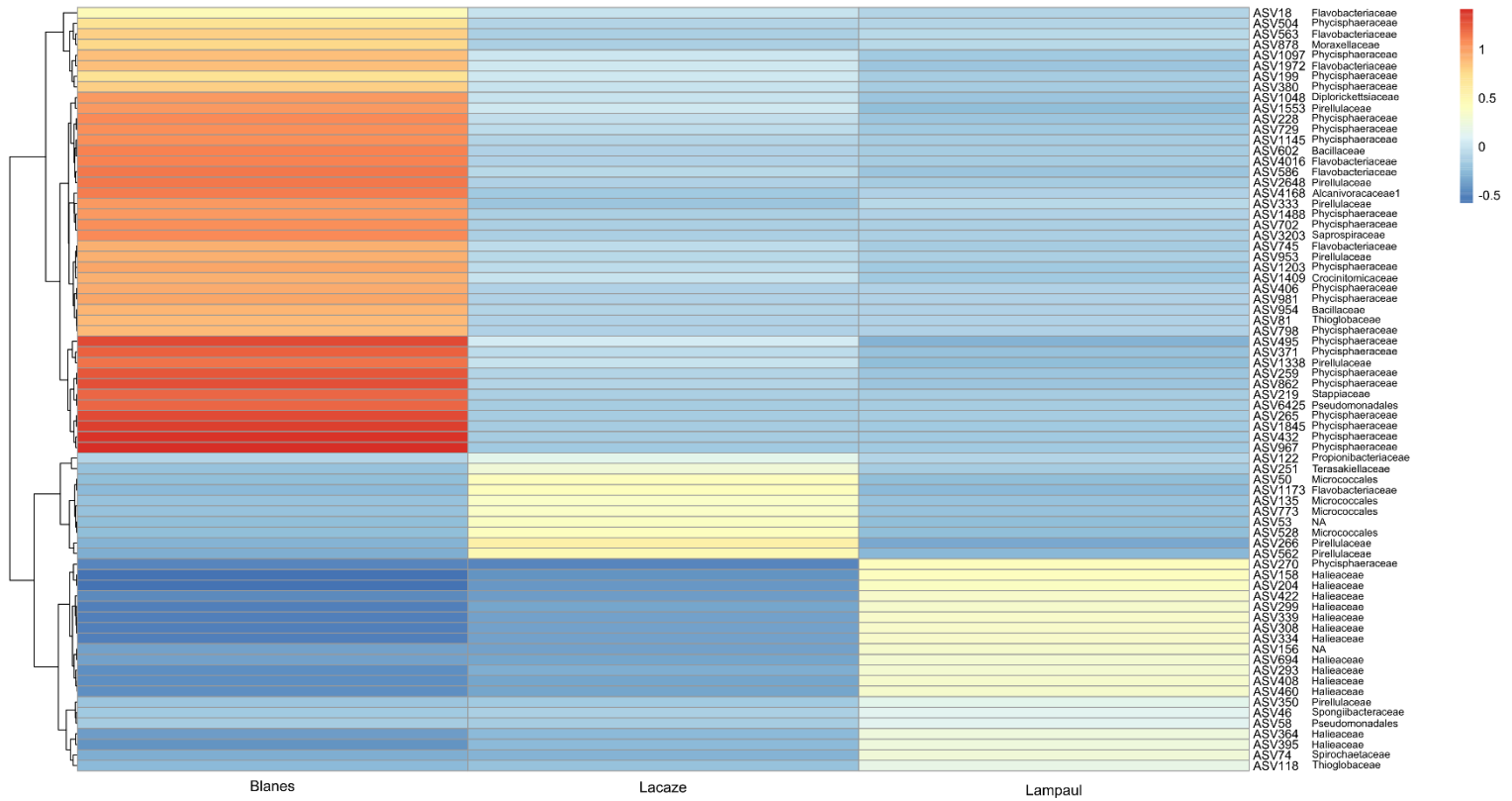


Figure 5.5. Heatmap illustrating Z-score transformed abundance of the *L. pertusa* ASVs that best explained the difference between the Lampaul, the Lacaze-Duthiers and the Blanes canyons. Taxonomic affiliation of each ASV is given at the family level, except for ASV6425 and ASV58 for which taxonomic affiliation is given at the order level.

5.4 Discussion

This first standardized large-scale study of bacterial communities associated with *L. pertusa* and *M. oculata* showed highly differentiated microbiomes between *L. pertusa* and *M. oculata*, across regional and local scales.

Dissimilarity between *L. pertusa* and *M. oculata* microbiomes

Our study showed that *L. pertusa* and *M. oculata* collected at the same time and location, both in the NE Atlantic Ocean and the Mediterranean Sea, harboured different microbial communities. Several studies conducted in the Lacaze-Duthiers canyon, in the Mediterranean Sea, have earlier shown that the microbiomes of *L. pertusa* are more variable than those of *M. oculata* (Meistertzheim et al., 2016; Galand et al., 2018; Chapron et al., 2020). They are also more diverse and variable in space and time. However, it is the first time that this same trend is confirmed for another canyon in the Mediterranean Sea (i.e., the Blanes canyon) and beyond,

in the Atlantic Ocean. Our comparative study is the largest large-scale study of bacterial communities associated with *L. pertusa* and *M. oculata* comparing two distant populations using a standardized protocol. The sampling effort including 336 samples from 116 different colonies of both *L. pertusa* and *M. oculata* allow to confirm the trends already observed earlier in the literature, with more robustness and confidence.

Here, the fact that *M. oculata* microbiomes were stable across geographical scales corroborates findings from a local study in the Mediterranean Sea (Meistertzheim et al., 2016), and from *in situ* transplant experiment at the same site (Chapron et al., 2020), as well as from experiments of several months in aquaria (Galand et al., 2018). In contrast, bacteria from *L. pertusa* were more variable, showing spatial and temporal changes within Atlantic and Mediterranean canyons, as earlier reported in the Mediterranean Sea (Meistertzheim et al., 2016; Chapron et al., 2020a). Although, we observed some common groups shared between the two species, such as *Propionibacteriaceae* in the two seas, most of the diversity between the two species differed.

Lophelia pertusa and *M. oculata* have distinct living strategies, including differences in the microbe-host association patterns (Meistertzheim et al., 2016; Galand et al., 2018; Chapron et al., 2020), growth patterns (Lartaud et al., 2014), reproductive biology (Waller & Tyler, 2005; Chemel et al., 2023), and different feeding strategies (Tsounis et al., 2010; Galand et al., 2020) suggesting that they do not share the same ecological niches within the same habitat. Feeding may in particular influence the composition of the microbiome since diet is a major factor influencing bacterial communities, including in cold-water corals (Galand et al., 2020). Although *L. pertusa* and *M. oculata* rely mainly on a zooplankton-based diet within their habitat (Kiriakoulakis et al., 2005; Naumann et al., 2015), they exhibit different feeding strategies and possible different assimilation and storage performances (Kiriakoulakis et al., 2005), which may influence their microbial community. *Lophelia pertusa* is more tolerant to changing environmental conditions (Chapron et al., 2020), and has been hypothesized as a more opportunistic species, adapting its nutritional strategy. On the contrary, *M. oculata* is less tolerant to environmental changes and so may be less resilient to disappearance of its specific prey, which may partially explain its more conserved microbiome. Finally, as the source of food and the delivery dynamics may differ between the Mediterranean and Atlantic canyons, with supposedly richer food and more regular delivery in the Atlantic, this could partly explain differences in microbiomes between *L. pertusa* and *M. oculata* from Atlantic and Mediterranean canyons.

Spatial differences in *L. pertusa* microbiomes

Bacteria associated to *L. pertusa* in the Lampaul canyon were dominated by *Haliaceae* and *Anaplasmataceae*, both belonging to *Proteobacteria*, which contributed the most to the differentiation of the microbial community from this canyon compared to the Mediterranean ones. This phylum was reported in *L. pertusa* from the Atlantic Ocean, but was represented by other families. *Spongiibacteraceae* dominated *L. pertusa* samples from Rockall Bank (off UK) (Van Bleijswijk et al. 2015), while samples from the Gulf of Mexico were dominated by *Sphingomonadaceae*, *Rhodobacteraceae* and bacteria from others phyla (Kellog et al., 2009; 2017). The study from Jensen et al. (2019) targeting the same variable regions of the 16S rRNA gene (V1–V3) showed Norwegian *L. pertusa* bacterial community also dominated by *Proteobacteria*, but from *Rhizobiales* order. *Lophelia pertusa* microbial community from Norwegian fjords were very similar to Rockall Bank microbiomes (Jensen et al., 2019), suggesting more similar microbiomes within Atlantic specimens than with Mediterranean ones. Here, the Mediterranean samples were dominated by non-assigned families, *Pirelullales* (*Planctomycetota*), and *Propionibacteriaceae*. Earlier studies in the Mediterranean Sea have identified *Spirochaetales*, *Rickettsiales* as dominant orders, and more generally *Alphaproteobacteria*, in *L. pertusa* samples (Meistertzheim et al., 2016; Galand et al., 2018). However, Meistertzheim et al. (2016) did not consider the microbial community of the skeleton since only the tissues were extracted. As different compartments of the corals harboured different microbial communities, including in *L. pertusa* (Van Bleijswijk et al. 2015), this further limit direct comparisons.

Interestingly, *Propionibacteriaceae* were abundant in both the Atlantic and Mediterranean *L. pertusa* samples, and have also been previously detected in different regions (Neulinger et al., 2008; Galkiewicz et al., 2011; Meistertzheim et al., 2016). *Propionibacteriaceae* have been identified in both sides of the Atlantic Ocean (Neulinger et al., 2008; Kellog et al., 2017) and consistent identification of certain phylotypes across the Ocean suggests that they are typically associated to the coral independently of the region the host comes from (i.e., conserved symbiont) (Kellog et al., 2017). *Propionibacterium* appears to be conserved across Anthozoa, including *Scleratinia* and *Alcyonacea* (Kellog, 2019), and in some *Actinaria* and *Zoantharia*, however their role in the host metabolism is still unknown. Contrarily to *M. oculata*, we observed very few *Endozoicomonas* in *L. pertusa*, as earlier reported in the NE Atlantic Ocean (Neulinger et al. 2008; van Bleijswijk et al. 2015) and the Gulf of Mexico (Kellogg et al. 2009; 2017).

Finally, high spatial variability was observed in the Atlantic Ocean, with very close colonies exhibiting distinct bacterial communities. Kellog et al. (2017) also reported such colony-specific variability within a sampling site in the Gulf of Mexico, suggesting a site-specific influence of *L. pertusa* microbiomes. In addition, Jensen et al. (2019) showed that environmental conditions, including depth, influenced coral microbiomes. More generally, microbial communities of *L. pertusa* appeared more structured by spatial variability of the environmental conditions. Since oceanographic and hydrodynamics conditions strongly differ within coral habitats between the Atlantic Ocean and the Mediterranean Sea, this may support the apparent greater differences between the NE Atlantic Ocean samples and the Mediterranean ones than between *L. pertusa* coming from canyons close to each other.

Similarity between *M. oculata* microbiomes across NE Atlantic Ocean and the Mediterranean Sea

Madrepora oculata associated bacteria have been described in the Mediterranean Sea (Yakimov et al., 2006, Meistertzheim et al., 2016; Galand et al., 2018; Chapron et al., 2020), but rarely in the NE Atlantic Ocean (Hansson et al., 2009; Appah, 2022). Here, we report lower community diversity in *M. oculata* than in *L. pertusa*, as already shown in the Mediterranean Sea (Meistertzheim et al., 2016), concurrently with a stable and homogenous bacterial community across NE Atlantic and Mediterranean canyons. Interestingly, different *Endozoicomonas* ASVs dominated corals across the large geographical scale, with specific ASVs found for each canyon. The dominance of *Endozoicomonas*, reported here as well as in other studies (Hansson et al. 2009, Meistertzheim et al. 2016, Galand et al. 2018; Kellog & Pratte, 2021) might suggest the presence of a true host-associated bacteria in *M. oculata*. In fact, even after several months in aquaria, *Endozoicomonas* still dominated *M. oculata* bacterial community and remained active as seen in the RNA fraction (Galand et al., 2018). *Endozoicomonas* are typical bacterial associates in various marine hosts (Neave et al., 2017), including in tropical corals (Neave et al., 2016, Hochart et al., 2023). They are usually abundant in healthy corals, but less abundant in stressed, diseased, and bleached corals (Meyer et al., 2014; Pogoreutz et al., 2018) and so have been proposed as symbionts, involved in DMSP transformation or amino acid and carbohydrate metabolism (Hochart et al., 2023). The dominance of the genus *Endozoicomonas* in bacterial community of *M. oculata* may reflect a true symbiosis between this genus and cold-water corals, as this genus has also been described in others deep scleractinians (Kellog & Pratte, 2021). However, their function in cold-water coral metabolisms have not been yet

studied. Reconstitution and comparison of whole genomes from *Endozoicomonas* in deep-sea corals against those reported in zooxanthellate corals would allow to determine the roles that these apparently pervasive symbionts are playing in cold-water coral health. As hypothesized by Meistertzheim et al. (2016), the similarity in *M. oculata* bacterial communities across the NE Atlantic Ocean and the Mediterranean Sea, in both their composition and diversity, may indicate that the microbiome is more structured by the host species than by the spatial variability of the environmental conditions, or the population origin as possibly seen in *L. pertusa*.

5.5 Conclusion

Our large-scale comparative study of the bacterial communities associated with the two main reef-building species *L. pertusa* and *M. oculata* between a NE Atlantic and two NW Mediterranean canyons show a strong spatial variability in the microbial assemblages of *L. pertusa*. Inversely, *M. oculata* microbiomes exhibited higher stability and specificity across regional scales, harbouring some conserved symbiont such as *Endozoicomonas*. This indicates differences in the microbe-host association strategy between the two species, reflecting the different ecological niches occupied by two species sharing a same habitat.

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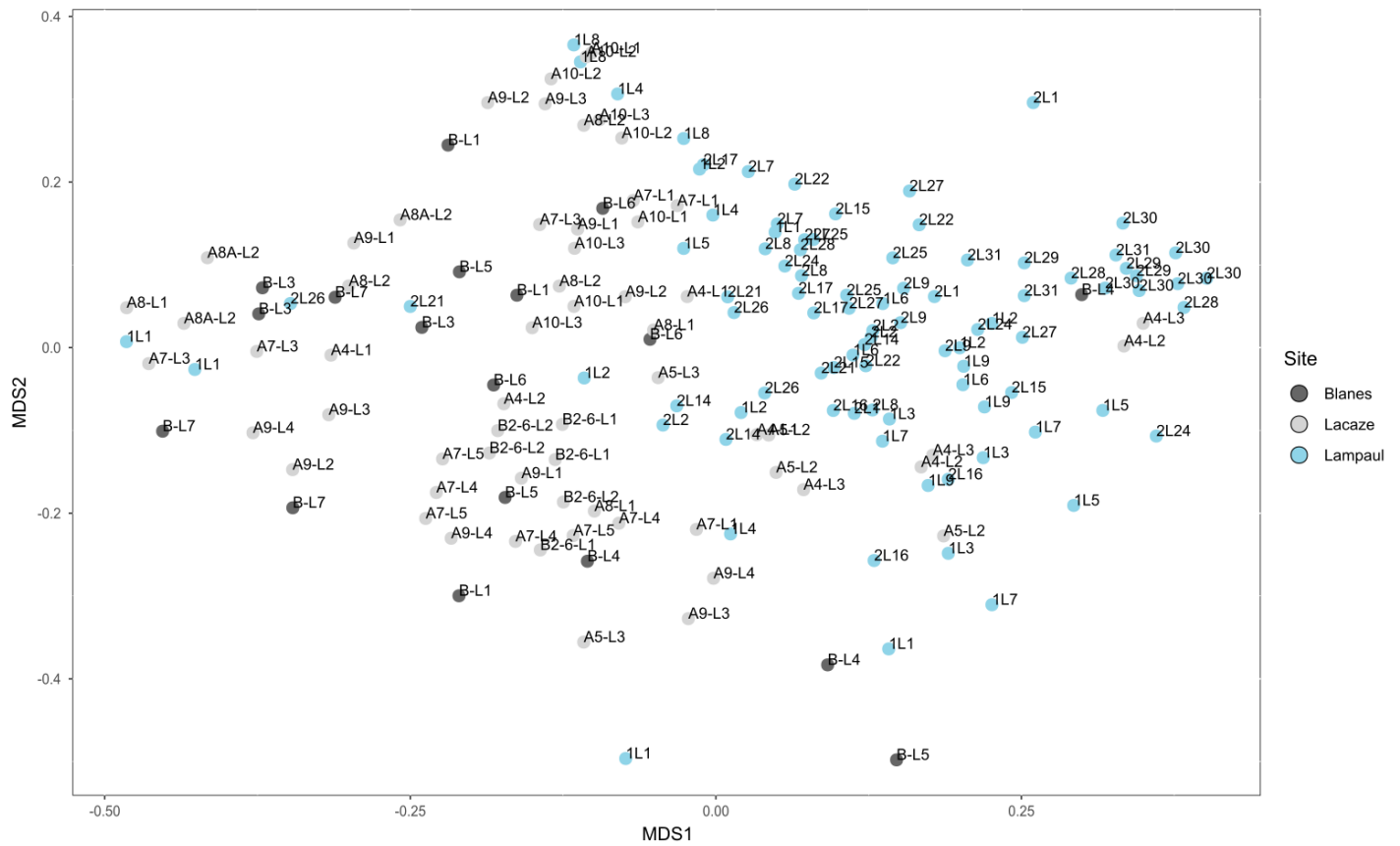
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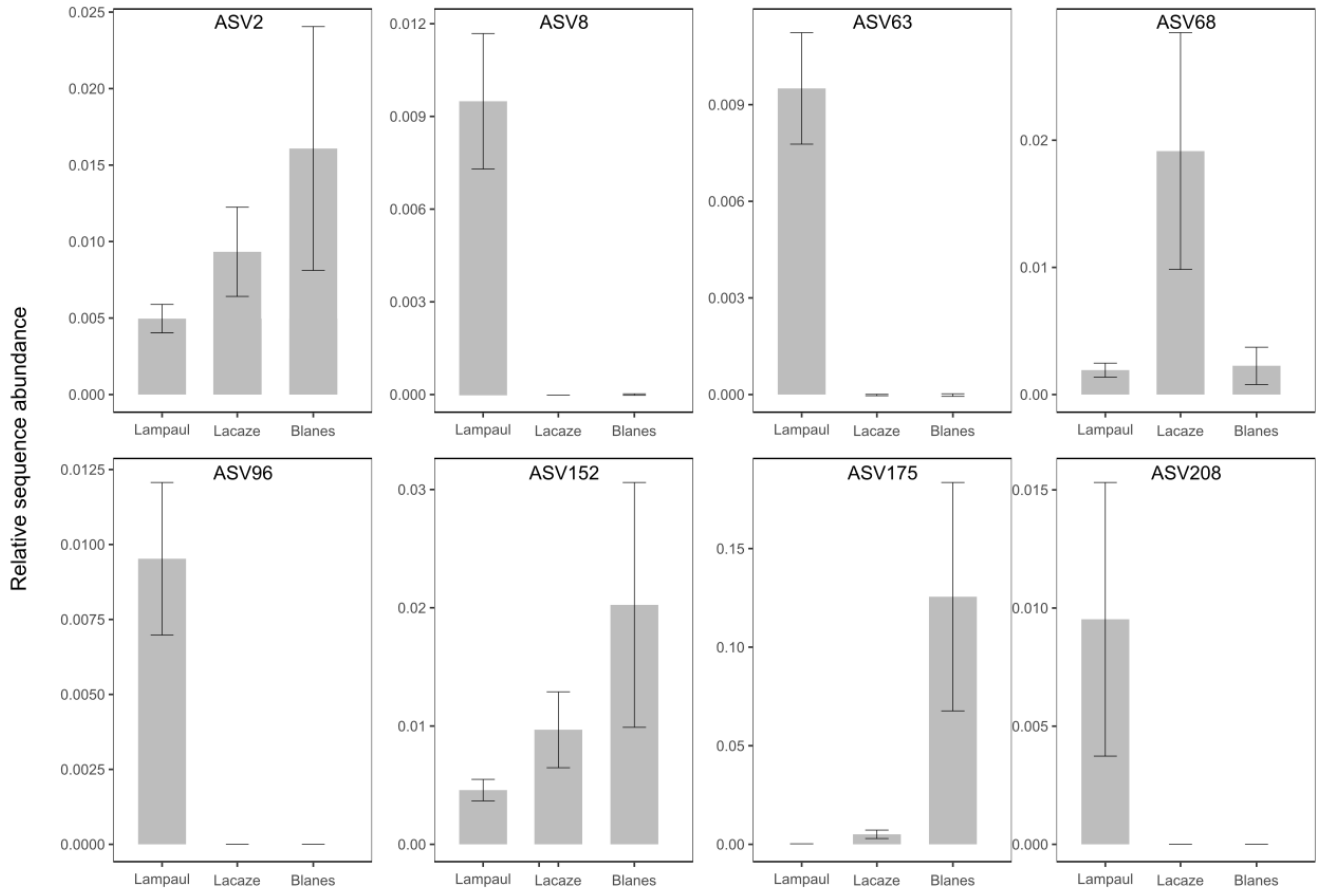
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Supplementary material



Supplementary Fig. 5.1. Non-metric multi-dimensional scaling plot (nMDS) based on the Bray-Curtis similarity index showing the similarity between bacterial community compositions of *L. pertusa* colonies in the three study sites.



Supplementary Fig. 5.2. Relative sequence abundance of the most abundant *Endozoicomonas* ASVs in *M. oculata* in the different canyons of the NE Atlantic Ocean and the Mediterranean Sea. Mean values and standard deviations are presented.

Chapter six: First report of *in situ* growth dynamics of the reef-building *Lophelia pertusa* in the Bay of Biscay

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Abstract

Growth patterns of reef-building scleractinians was documented since the 19th and growth of *L. pertusa* were reported in Norway, in the North Sea, in the Mediterranean Sea, and in the Gulf of Mexico. We conducted catch and recovery experiments to investigate the skeletal growth patterns of two reef-building cold-water coral species, *Lophelia pertusa* (now synonymized *Desmophyllum pertusum*) and *Madrepora oculata*, from the deepest area reported yet for these species (800-1145m), in the Laumpaul canyon in the North East (NE) Atlantic Ocean. Coral fragments were collected, stained using calcein and deployed for both short-term (one month) and long-term (one year) growth experiments at three different sites of the canyon. This study presents the first *in situ* estimation of the growth patterns, including budding and linear extension of *L. pertusa* and *M. oculata* in the Bay of Biscay. The average polyp linear extension measured for *L. pertusa* was 2.4 ± 1.6 mm yr⁻¹, which fall within the lower range compared to previous estimations for this species in other geographical regions. For *M. oculata*, all specimens died for the two consecutive years and thus no characterization of growth was feasible. *In situ* long-term experiments are difficult to conduct in deep-sea environments, however, they are essential for understanding the biology and ecology of these species, which are particularly threatened by anthropogenic activities in submarine canyons, including climate change.

Keywords: Cold-water corals, Linear extension, Skeletal growth, Lampaul canyon, Mortality

6.1 Introduction

Cold-water corals are known to form complex three-dimensional structures in the deep-sea, attracting large numbers of organisms, accumulating suspended particulate matter, providing food to this associated fauna (Freiwald et al., 2004), and influencing the local reef hydrodynamics conditions (e.g., Cyr et al., 2016). *Lophelia pertusa* (now named *Desmophyllum pertusum*, Addamo et al., 2016) and *Madrepora oculata* are the major, globally distributed, engineer species of submarine canyons (Freiwald et al., 2021). A single colony of *L. pertusa* can be up to 2 m high and 1.5 m large in the Northern Gulf of Mexico (Schroeder, 2002) and the North East Atlantic Ocean (Freiwald, 2002), and the assembly of several colonies can form extensive reefs, which can reach several kilometres long (e.g., Rost and Sula reefs in Norway and giant mound barrier reef off Mauritania, Ramos et al., 2017). Since the 19th century, growth of reef-building scleractinians, mostly *L. pertusa*, has been documented in the natural environment, starting with the discovery of reefs growing on man-made structures such as telegraph sea cables (Duncan, 1878; Wilson, 1979), ship wrecks (Roberts et al., 2003) or oil and gas platforms (Gass and Roberts, 2006). Since then, the development of new technologies, in particular the access to Remotely Operated Vehicles (ROV), has enabled studies implementing *in situ* experiments to characterise the growth of these reef-building species in the deep environment from many geographical regions.

Growth rates reported for *L. pertusa* vary widely from 2 to 32 mm y⁻¹ in the Gulf of Mexico (Brooke & Young, 2009; Larcom et al., 2014), 1 to 40 mm y⁻¹ in the Mediterranean Sea (Lartaud et al., 2017; Chapron et al., 2020a), from 1 to 26 mm y⁻¹ in Norway (Mikkelsen et al., 1982; Büscher et al., 2019) and up to 26 mm y⁻¹ in the North Sea (Gass and Roberts, 2006). *Madrepora oculata* has been less studied, but is known to exhibit lower growth rates, ranging from 1 to 18 mm y⁻¹ in the Mediterranean Sea as measured from both *in situ* and aquaria experiments (Orejas et al., 2008; Lartaud et al., 2017), and up to 14 mm y⁻¹ in Norway (Sabatier et al., 2012).

The Bay of Biscay is part of the North-East Atlantic Ocean, west of France and north of Spain, and is incised by more than a hundred submarine canyons (Bourillet et al., 2006a), of which some harbour dense and rich cold-water coral habitats (van den Beld et al., 2017). Joubin (1922) and Le Danois (1948) produced the first report and map of the occurrence of coral reefs along the continental slope of this region. While continental slopes of the Bay of Biscay are subjected to intense fishing activity (Bourillet et al., 2006b), including bottom trawling, submarine canyons might offer the last natural refuges less accessible to trawling (Fabri et al., 2014; Fernandez-Arcaya et al., 2017) for reef-forming species to flourish. Despite this, few

studies have examined cold-water corals distribution and biology in the French part of the Bay of Biscay (Zibrowius, 1980; Reveillaud et al., 2008; De Mol et al., 2011; van den Beld et al., 2017). And to our knowledge, no study has so far described their growth dynamics in this region, despite their ecological importance and the potential role of the Bay of Biscay in the maintenance of these ecosystems and the connectivity between regions (van den Beld et al., 2017).

The aim of this study was to investigate the growth patterns of *L. pertusa* and *M. oculata* from the Bay of Biscay. An *in situ* experimental approach implemented to study coral growth was based on yearly deployment and recovery of coral fragments in the Lampaul canyon, NE Atlantic Ocean, using the methods described in Lartaud et al. (2017) for the Mediterranean Sea. Corals were deployed in contrasted study sites located between 800 m depth, where corals grow both in coral gardens or on steep cliffs, and 1150 m depth, where they are much less abundant.

6.2 Material and methods

6.2.1 Study sites and specimen collections

The Lampaul canyon (Figure 6.1) is located off the North-West coast of France, in the Bay of Biscay (Northeast Atlantic Ocean) and is included in a Natura 2000 network, aiming at preserving deep-sea reef habitats in the Bay of Biscay. Van den Beld *et al.* (2017) described the different cold-water coral habitats in 24 submarine canyons of the Bay of Biscay, including Lampaul canyon, where populations of *Lophelia pertusa* and *Madrepora oculata* are found. Coral collection for this study were obtained from three different cruises during the ChEReef program, aiming at characterizing the habitat and ecology of cold-water coral reefs in the bay of Biscay, in August 2021, 2022, and 2023 using Ifremer's deep-sea Remotely Operated Vehicles (ROV) Ariane and Victor 6000 from the R/V Thalassa and Pourquoi pas?. Colony fragments of *L. pertusa* and *M. oculata* were collected on the western interfluvium of the canyon between 780 and 805 m depth, and then transported to the surface using thermally insulated boxes maintaining ambient temperature of deep seawater from the site of collection (i.e., 10°C). On board, the collected corals were used for *in situ* growth examination with redeployment of colony fragments for both one-month and one-year monitoring.

Once colonies were on board, they were transferred into aerated seawater tanks maintained at 10°C using a chiller. Coral colonies were cut into nubbins ~10-15 polyps for *L. pertusa* and ~20-30 polyps for *M. oculata*. Fragments were then glued with an epoxy resin on transplant structures called Ranch (Redeployment structure to ANalyse Coral Health) following

the protocols described in Lartaud et al. (2014) (Figure 6.2a). Three sites for growth monitoring were then selected (Figure 6.1). The first site, called Marley station (N.47°36.703, W07°32.192), is a coral garden located on a flat area with a medium density of small coral colonies (mainly *M. oculata*), from which the main part of coral collection originates. Redeployment structures have been successively deployed on this site in 2021 and 2022 to monitor long-term growth of corals between 2021-2022 and 2022-2023, and on shorter term for a month in august 2022 (Figure 6.2b, Table 6.1). The second site, called cliff top (N47°36.692, W07°32.117), is located in the vicinity of the Marley station, in an area of steep cliffs. Very large colonies of *L. pertusa* and *M. oculata* grow on the cliffs covered by deep sea oysters *Neopycnodonte zibrowii* (Figure 6.2c) but as the topography was too steep, the structure was placed on a short slope breakage, just below the cliff wall, at 805 m (Figure 6.2d), on an area covered by dead coral fragments with a dense associated fauna. The temperature measured in these two sites was ~10°C. The third site, called cliff bottom (N 47 36.7863, W 007 31.9206), is situated at the bottom of deeper cliffs at 1145 m depth, where the coral density is lower (Figure 6.1e) and the temperature colder (~ 8.5°C, Supplementary Fig. 6.1) (Figure 6.2f). This area is also more sedimented, and likely subject to occasional turbidity events as suggested by the cloud of sediment fall observed the 13/08/2022 during the PL817-08 dive (Figure 6.2).

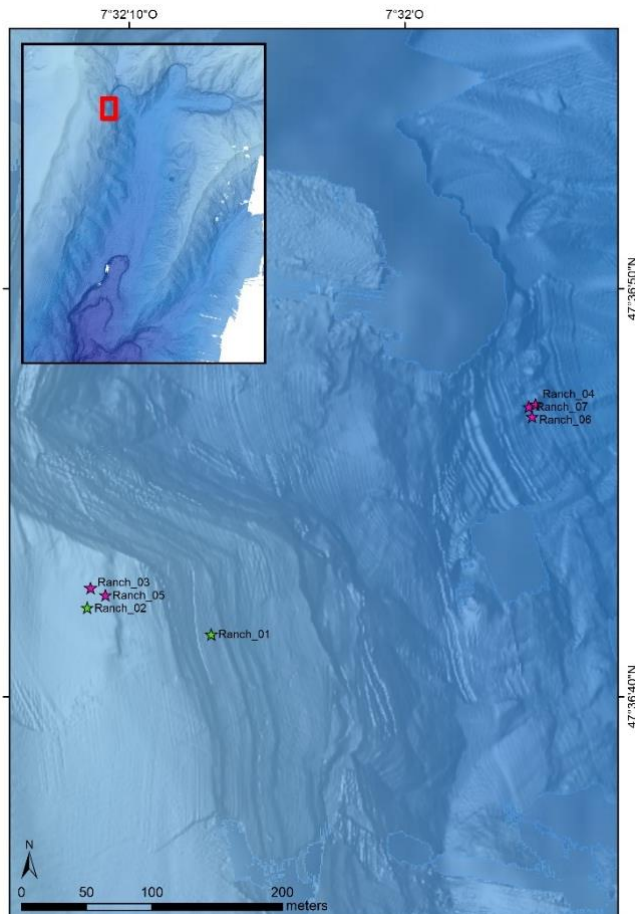


Figure 6.1. Map of the study site, the Lampaul canyon, with all the redeployment structures at the three different sites for growth monitoring: Ranch 2, R3 and R5 at Marley station above the cliff, R1 at cliff top site and R4, R6 and R7 at cliff bottom site.

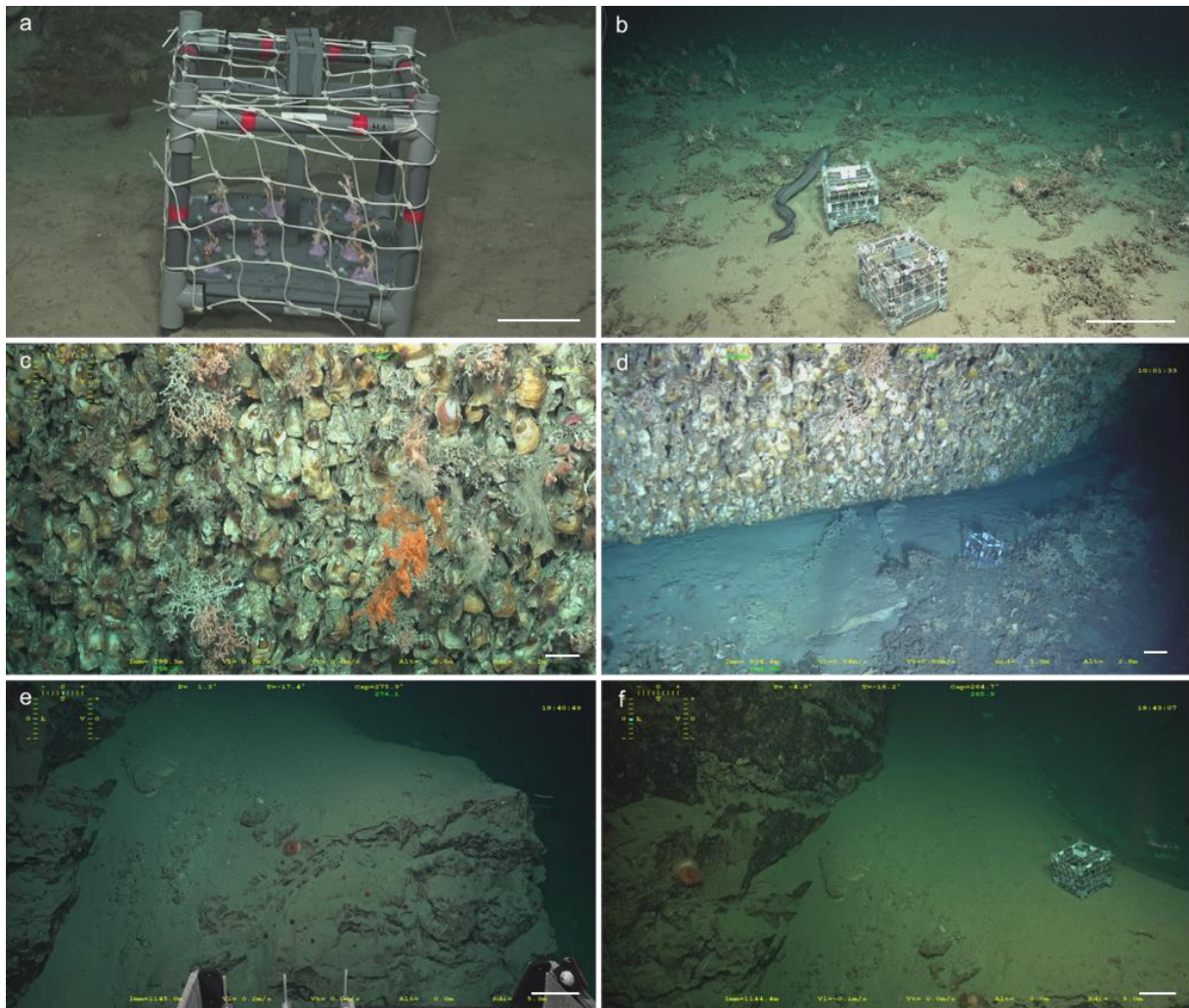


Figure 6.2. Pictures of Ranch structures and canyon habitats. A Ranch equipped with coral nubbins and a net to prevent breakage by megafauna (a). Coral garden at the Marley station with two Ranch structures deployed (b). Landscape of the cliff top located just below the Marley station (c), where a Ranch was deployed on dead coral fragments (d). The deeper, colder and more sedimented site at the bottom of the large cliff (e) where Ranchs were deployed on small steps (f). Scale bars are 10 cm (a, c), 50 cm (b, d, e, f). © Ifremer / Chereef

6.2.2 Determination of linear extension and skeletal growth

Determination of growth was assessed following the protocol described in Chapron et al. (2020a). Each living and dead polyp of the coral nubbins and potential breakage of branches were identified (Table 6.1), counted and photographed before and after deployment with the same orientation, or the nubbin for comparison. The use of a scale bar on the pictures allows to

measure the linear extension of the coral fragments, which corresponds to the calcification between the last polyp observed before deployment to the summit of the calix of the last polyp observed after recapture. The addition of new polyps, growing on top of previous generation of polyps at the top of a branch, was counted and measured using the protocol established by Lartaud et al. (2014) to determine the budding rates. The budding rate corresponds to the percentage of new polyp *per* polyp initially present *per* year, reflecting the density development of polyps within a colony.

The colonies redeployed for a month followed the same protocol described above with the addition of skeletal growth measurement *via* a sclerochronological approach. Polyp growth was measured using fluorescent calcein staining (Lartaud et al., 2013). Before redeployment, nubbins were stained with calcein fluorescein at 150 mg L⁻¹ following the protocol of Lartaud et al. (2013).

At the end of the one-month *in situ* experiment, redeployment structures were recovered, and living and dead polyps, as well as breakage were counted before nubbins were cleaned in a hydrogen peroxide solution (H₂O₂, 4 %) for 12 hours, to remove all organic tissues, and then rinsed with demineralised water. Each polyp calyx was individualised and placed on a slide, glued with Patafix©, in order to identify polyp's septa under microscope. The calcein labelling was observed under fluorescence microscope (Olympus IX51, Olympus, Tokyo, Japan), with excitation at 495 nm. Images were taken using a camera and image analysis was carried out using Image J software. Growth was assessed by measuring the distance between the calcein label and the outer edge of the septum of the calyx (repeated 10 times), following Chapron et al. (2018). Apical and subapical polyps were identified and their growth was compared.

Table 6.1. Characteristics of the structure redeployed between 2021 and 2023 to monitor *L. pertusa* and *M. oculata* growth at three sites of the Lampaul canyon, NE Atlantic Ocean.

Site	Redeployment structure	Time period	Species	Colony	Replicates	Initial number of polyps	Number of new polyps	Mortality (%)	Breakage (%)
Cliff top	Ranch 1	Aug 2021-Aug 2022	<i>L. pertusa</i>	2	8	77	1	90 ± 19	10 ± 19
			<i>M. oculata</i>	3	6	130	0	89 ± 14	11 ± 14
Marley station	Ranch 2	Aug 2021-Aug 2022	<i>L. pertusa</i>	3	6	84	28	0 ± 1	0 ± 0
			<i>M. oculata</i>	4	7	98	0	46 ± 42	51 ± 45
Marley station	Ranch 3	Aug 2022	<i>L. pertusa</i>	4	7	31	0	0 ± 0	0 ± 0
Cliff bottom	Ranch 4	Aug 2022	<i>L. pertusa</i>	4	7	22	0	0 ± 0	0 ± 0
Marley station	Ranch 5	Aug 2022-Aug 2023	<i>L. pertusa</i>	7	10	150	0	85 ± 20	10 ± 16
			<i>M. oculata</i>	3	6	114	0	100 ± 0	0 ± 0
Cliff bottom	Ranch 6	Aug 2022-Aug 2023	<i>L. pertusa</i>	4	7	73	3	51 ± 34	0 ± 0
			<i>M. oculata</i>	4	7	120	0	100 ± 0	0 ± 0

6.2.3 Statistical analysis

Tests for normality of variance were performed using the Shapiro–Wilk test with the R software (v. 4.2.2). Distribution of survival and growth were not normal and variances were not homogenous, so non-parametric Kruskal Wallis tests were performed to test possible statistical differences between different sites of redeployment for growth monitoring. Non-parametric post-hoc tests were then performed to determine which sites differed using dunn test.

6.3 Results

6.3.1 Growth

Mortality was significantly different between the two species (Chi squared = 10.783, df = 1, p-value = 0.001) and the three sites (Chi squared = 16.982, df = 4, p-value = 0.002), with higher mortality in *M. oculata* (dunn test, p = 0.001) (Figure 6.3). Within a single site, mortality exhibited a wide variability in time, ranging from 0 to 85 ± 20 % for *L. pertusa* and 46 ± 42 to 100 % for *M. oculata* during the deployments in 2021-2022 and 2022-2023, respectively.

Breakage was not different between the two species (Chi squared = 1.313, df = 1, p-value = 0.252), or between the sites (Chi squared = 7.115, df = 4, p-value = 0.130). *Madrepora oculata* exhibited a high average breakage of 54 ± 45 % at the Marley station between 2021 and 2022, while similar breakage as *L. pertusa* at the cliff top site (11 ± 14 % and 10 ± 19 % respectively for *M. oculata* and *L. pertusa*), and no breakage at the bottom of the cliff (Figure 6.2). It should be noted that a net was added to the Ranch structures following these breakage observed in 2022, to prevent megafauna crossing through coral nubbins, as large fishes and sharks have been observed around the structures (Figure 6.1b).

Budding rates were different between species (Chi squared = 6.542, df = 1, p-value = 0.011), with highest budding rate of 28 ± 27 % for *L. pertusa* at the Marley station between 2021 and 2022 while no or very few budding rate were observed for the both species at the other sites (Figure 6.3).

Lophelia pertusa showed average linear extension of 2.2 ± 1.8 mm y⁻¹ and 1.6 ± 0.5 mm y⁻¹ respectively at Marley station between 2021 and 2022 and at cliff bottom site between 2022 and 2023, while no growth was monitored for the other sites during the subsequent deployments. Finally, *M. oculata* showed average linear extension of 0.5 ± 0.5 mm y⁻¹ at cliff bottom site between 2022 and 2023, but no further characterization of growth was available for this species, as no other linear extension could be determined in the other study sites.

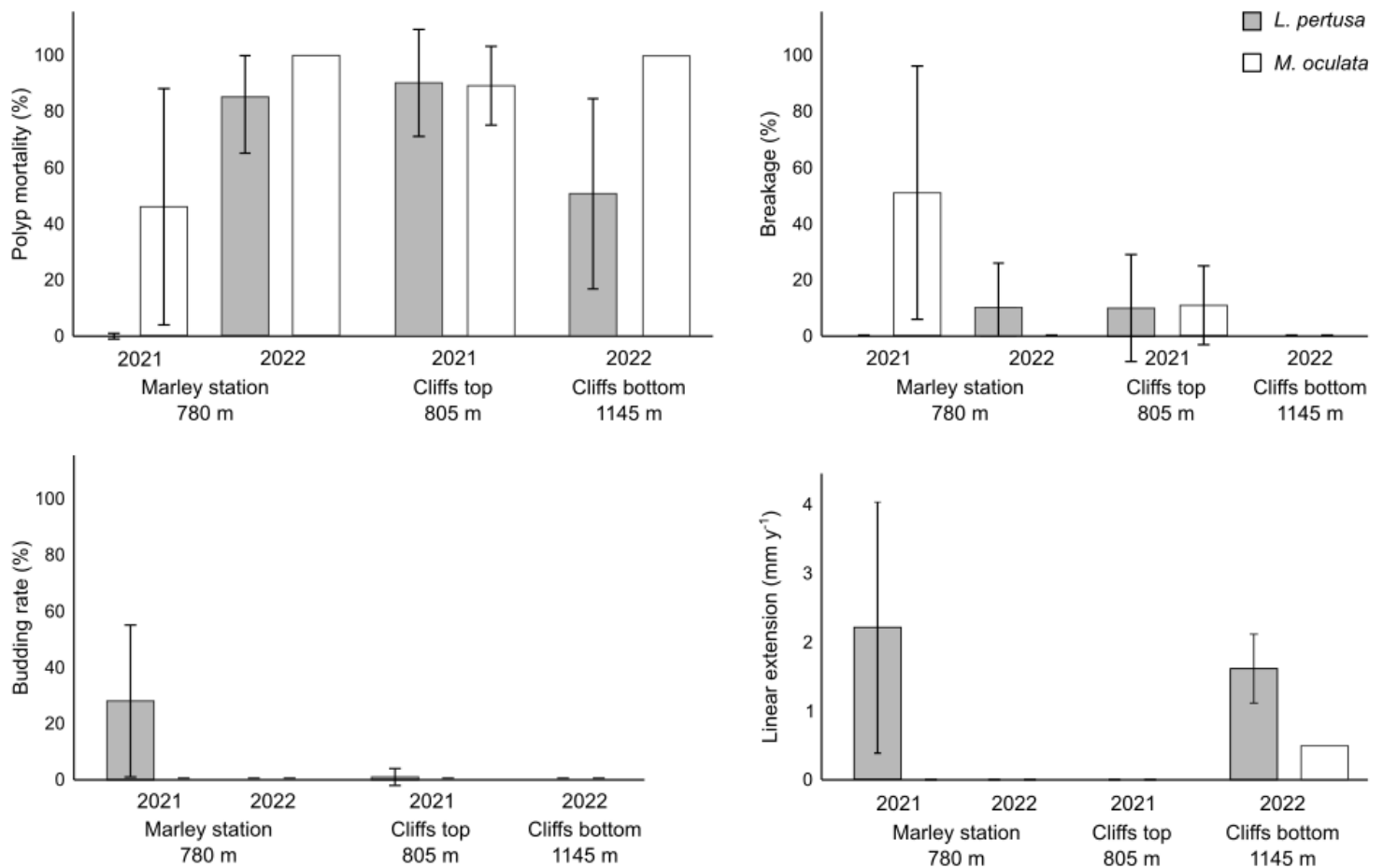


Figure 6.3. *In situ* polyp mortality, breakage, budding rate and linear extension of *L. pertusa* and *M. oculata* at the three sites of redeployment in the Lampaul canyon: Marley station between 2021-2022 and 2022-2023, cliffs top between 2021-2022 and cliffs bottom between 2022-2023.

Polyp linear growth rate, measured on septae through the calcein staining, was not significantly different between the two sites of redeployment in August 2022 (Chi squared = 0.0390, df = 1, p-value = 0.844), with an average of 3.0 ± 1.3 and 2.7 ± 1.1 mm y⁻¹ at Marley station and Cliffs bottom, respectively (Figure 6.3). Apical (i.e., younger polyps at the summit of the branch, driving the linear extension) and subapical polyps showed no significant differences in linear growth rate (Chi squared = 0.575, df = 1, p-value = 0.448).

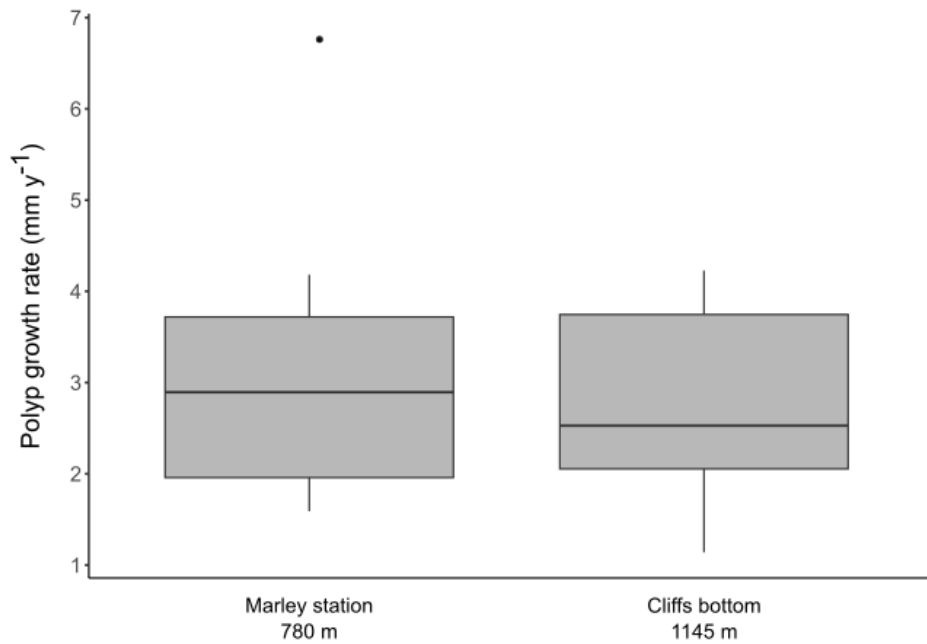


Figure 6.4. *In situ* polyp growth rate (mm y^{-1}) of *L. pertusa* in the Lampaul canyon for a month in august 2022 at Marley station and at the bottom of the cliffs.

6.3.2 *In situ* observations

During the deployment of a Ranch at the Cliffs bottom site in 2022, we observed a small turbidity-like events with sediment deposited on the colonies redeployed at the site (Figure 6.5).

We also observed a high abundance of associated fauna, including large fishes that approach the structures when they are deployed on the seabed (Figure 6.6a and 6.6b), and associated fauna within the structures. Very soon after deployment, coral nubbins were colonised by snails and shrimps (Fig. 6.6c and 6.6d). A high abundance of large and smaller shrimps was observed directly on the corals.

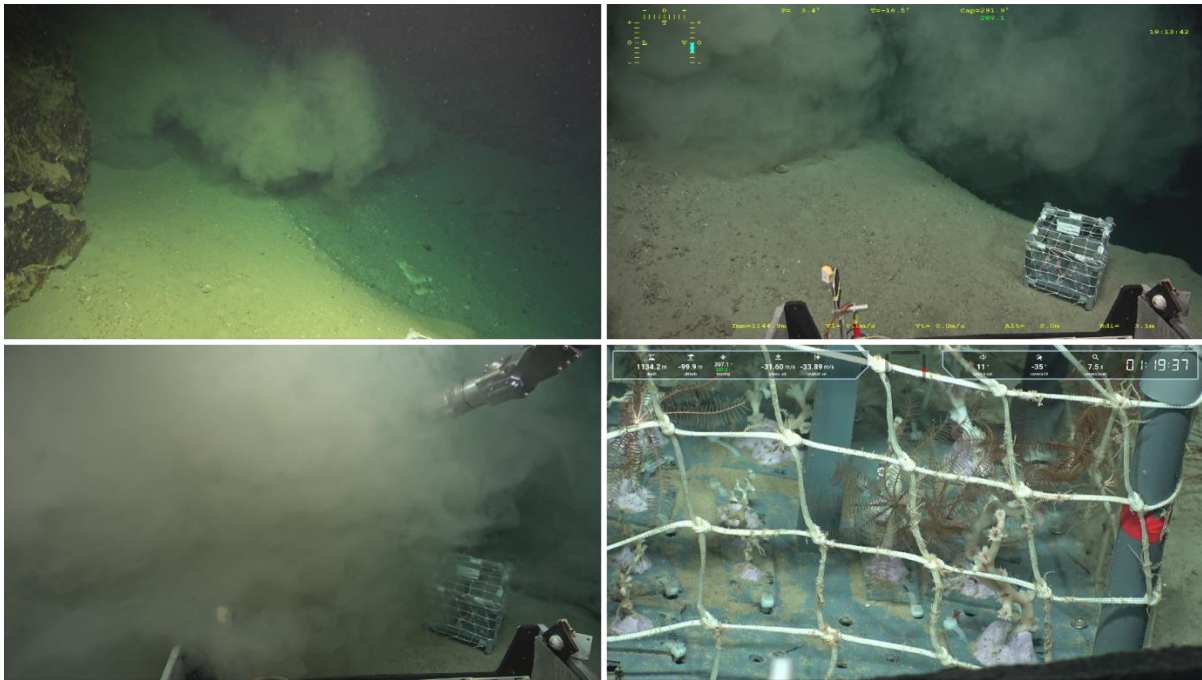


Figure 6.5. Micro-turbidity event observed on August 13th 2022 (dive PL817-08) at the “Cliff bottom” site (1145 m depth). Sediment covered the structure surface of a Ranch and polyps produced mucus to clean themselves. © Ifremer / Chereef

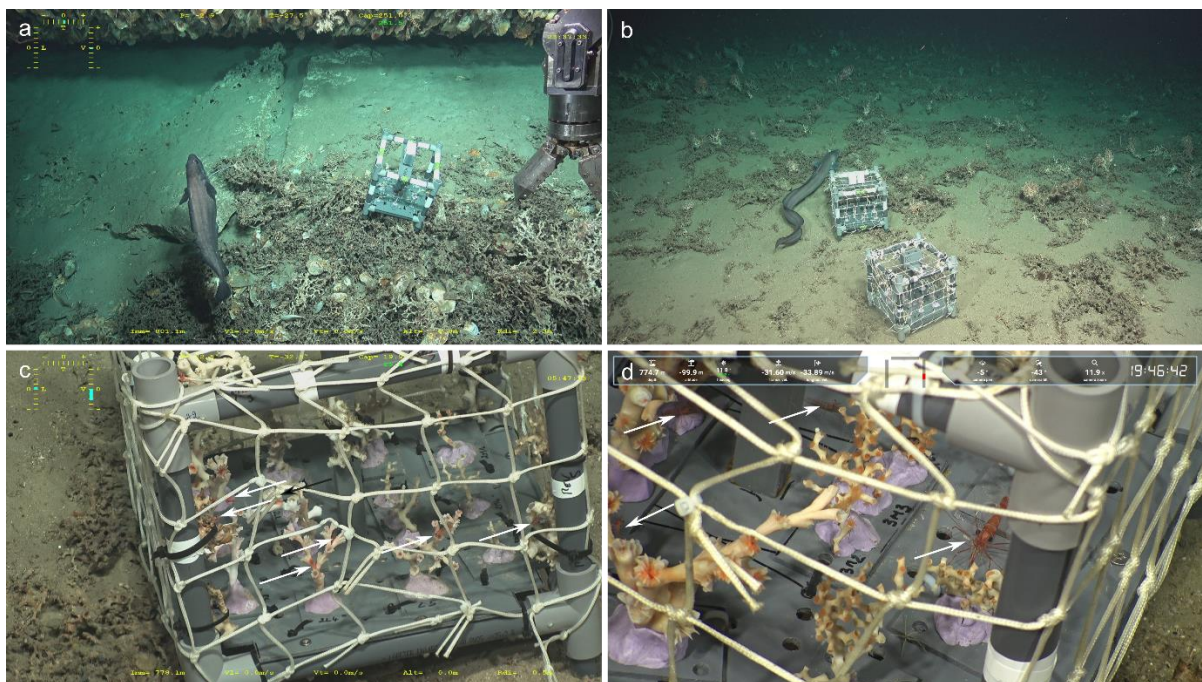


Figure 6.6. Associated fauna observed during ROV dives, including fishes, such as forkbeard here (a) and conger eels (b) and high density of snails (black arrow, c) and shrimps on redeployed structures (white arrows on c and d). © Ifremer / Chereef

6.4 Discussion

In the present study, we report the first *in situ* growth rate estimations for *L. pertusa* from the Bay of Biscay (NE Atlantic Ocean), obtained during a short-term experiment and two consecutive long-term experiments (one-year each). Considering the very high mortality and breakage reported for *M. oculata*, we were unable to provide accurate data on the growth dynamics of this species in the study area. For *L. pertusa*, linear extension values measured over the long-term monitoring and the growth rates measured by sclerochronology after the short-term experiment are both in the same range of values ($2.4 \pm 1.6 \text{ mm yr}^{-1}$). However, we observe that *L. pertusa* growth estimations are in the lower range compared to values reported for this species in the literature for other geographical areas, both from aquaria or *in situ* experiments (Table 2). In fact, *in situ* measurements showed growth rates of *L. pertusa* from 2.44 to 32 mm yr^{-1} in the Gulf of Mexico (Brooke & Young, 2009; Larcom et al., 2014), 1 to 40 mm yr^{-1} in the Mediterranean Sea (Lartaud et al., 2017; Chapron et al., 2020a), from 1 to 26 mm yr^{-1} in Norway (Mikkelsen et al., 1982; Büscher et al., 2019) and up to 26 mm yr^{-1} in the North Sea (Gass and Roberts, 2006). However, it is somewhat difficult to strictly compare growth responses from corals originating from different geographic locations and depths, as variability is strongly related to local environmental conditions (e.g., Chapron et al., 2020a) and temporal dynamics (Chapron et al., 2020b), including at seasonal scale (Lartaud et al., 2014). Furthermore, linear extension values determined for different purposes (maximal or average growth rates) and obtained from various measurement methods, such as observations and measurements *via* video recordings, sclerochemistry, isotopic dating, catch and recapture using different staining, or buoyant weight, are difficult to compare. Nevertheless, considering the literature for which the methods used to monitor linear extension were similar to the current study, we observed similar growth rates to those found by Büscher et al. (2019) in a Norwegian fjord and Brooke & Young (2009) in the Gulf of Mexico. Using a setup of catch-recapture and alizarin staining of coral colonies, they found that *L. pertusa* grow in average from 1 to 4.05 mm yr^{-1} (Büscher et al., 2019) and between 2.44 and 3.77 mm yr^{-1} (Brooke & Young, 2009). Differently, in the Mediterranean Sea, a similar experimental setup as ours using calcein staining, showed significantly higher growth rates, ranging from $11.3 \pm 7.3 \text{ mm yr}^{-1}$ (Lartaud et al., 2017) up to 40 mm yr^{-1} (Chapron et al., 2020b). They also monitor much higher budding rate, ranging from 41 % (Chapron et al., 2020a) to $104 \pm 124 \%$ (Lartaud et al., 2017). In our experiment, budding rate were considerably lower and ranged from $2 \pm 6 \%$ to $28 \pm 27 \%$, showing a very slow turnover of new polyps. Further long-term experiments on this population

are still needed to increment our data, in order to better characterize *in situ* coral biology and dynamics in the Bay of Biscay.

It is challenging to obtain *in situ* growth data for cold-water coral, as their habitats are difficult to access and *in situ* long-term studies are particularly challenging to carry out. Mortality in *in situ* experiments may be high, depending on different factors. Handling of colonies and staining may be a source of stress before redeployment of colonies *in situ*, as suggested by Büscher et al. (2019). They observed relatively high mortality rate, with 10-30 % depending on location of redeployment and maximal mortality of 48 ± 26 %, supposedly due to stronger variability in environmental fluctuations in terms of temperature, salinity, and currents in certain sites. The difference of temperature between the shallower and deepest sites at Lampaul canyon is low (expected range of 1.5°C) and unlikely to affect growth rates and mortality. Sedimentation is also an important factor that influence the growth patterns of cold-water corals. During a 8-year monitoring program evaluating *in situ* growth of cold-water corals in the Lacaze-Duthiers canyon, Chapron et al. (2020a) showed that the lowest budding rates and linear extension along with the highest mortality occurred during high sedimentation periods in the absence of water plume event, which normally supply food. During these years, mortality reached almost 100 % both in *L. pertusa* and *M. oculata*, while budding rate did not exceed 1 ± 3 % in *L. pertusa*. In our study, we observed a small turbidity-like events during a ROV dive with sediment deposited on the colonies redeployed at the site Cliffs bottom. Surprisingly, we noticed lower mortality on the more sedimented site (Cliff bottom) compared to Marley station, and no significant difference on growth rates. *Lophelia pertusa* has proved to be relatively resilient to low sediment loading (Brooke et al., 2009; Büscher et al., 2019), while mortality occurred only when corals are partly or completely buried for several days (Brooke et al., 2009; Larsson and Purser, 2011).

Table 6.2. Linear extension (mm year⁻¹) measured for *L. pertusa* from different geographical area (adapted from Lartaud et al. (2019)).

Study site	Depth (m)	Growth rate (mm year ⁻¹)	Experimental design	Method	References
Gulf of Mexico	460-510	2.44 - 3.77	<i>in situ</i>	Catch, alizarin staining and recapture	Brooke and Young, 2009
Gulf of Mexico	200-800	3.2 - 32.3	<i>in situ</i>	Measurements on video	Larcom et al., 2014
Central Mediterranean Sea	300	15 - 17	aquarium	Measurement of lengthening of branches	Orejas <i>et al.</i> , 2008
Central Mediterranean Sea	300	8.8 ± 6.6	aquarium	Buoyant weight technique	Orejas <i>et al.</i> , 2011
Western Mediterranean Sea	520	7.5 ± 1.2	<i>in situ</i>	Calcein staining and sclerochronology	Lartaud <i>et al.</i> , 2013
Western Mediterranean Sea	520	0.01 - 12.1	<i>in situ</i>	Catch and recapture	Lartaud <i>et al.</i> , 2014
Western Mediterranean Sea	480-520	2.2 - 38.1	<i>in situ</i>	Catch and recapture	Lartaud <i>et al.</i> , 2017
Western Mediterranean Sea	380-530	1 - 25	<i>in situ</i>	Catch and recapture	Chapron <i>et al.</i> , 2020a
Western Mediterranean Sea	300-600	1 - 40.2	<i>in situ</i>	Catch and recapture	Chapron <i>et al.</i> , 2020b
Western Mediterranean Sea	540	2.5 ± 2.8	aquarium	Calcein staining and sclerochronology	Chapron <i>et al.</i> , 2021
Northeast Atlantic	800	4.1- 7.5	<i>in situ</i>		Wilson, 1979
Northeast Atlantic	220	2.5 - 5	<i>in situ</i>	U-Th dating	Pons-Branchu <i>et al.</i> , 2005
Northeast Atlantic (Bay of Biscay)	800-1145	2.4 ± 1.6	<i>in situ</i>	Catch-recapture, calcein staining and sclerochronology	This study
Northeast Atlantic (South Bay of Biscay)	955-1005	maximum 7 mm	<i>in situ</i>	Observations of colonies from a submarine cable	Duncan, 1878
North Sea (UK)	90-115	19 - 34	<i>in situ</i>	Measurements on videos	Gass and Roberts, 2006
North Sea	60-110	up to 26	<i>in situ</i>	Observations of colonies from oil platform	Bell and Smith, 1999
North Sea	100	5	<i>in situ</i>	Observations of colonies from oil platform	Roberts, 2002
Norway	300	25	<i>in situ</i>	Sclerochemistry	Mikkelsen <i>et al.</i> , 1982
Norway	250	19	<i>in situ</i>	Sclerochemistry	Freiwald <i>et al.</i> , 1997
Norway	200-350	5.5 - 6	<i>in situ</i>	Sclerochemistry	Mortensen et al., 1998
Norway	80-315	9.4	aquarium		Mortensen, 2001
Norway	340	8	<i>in situ</i>	Pb-Ra dating	Sabatier <i>et al.</i> , 2012
Norway	150-300	1.00 - 4.05	<i>in situ</i>	Catch, alizarin staining and recapture	Büscher <i>et al.</i> , 2019

The mortality observed at the Marley station but also the cliffs top is thus surely due to other factors than temperature and sedimentation. We observed a dense fauna around the corals, as well as large fishes (notably conger eels ~1.5 m long) that approach the redeployment structures as soon as they are deposited on the seabed. We hypothesise that these fishes could be partly responsible for the high percentage of breakage that we observe the first year. Since the 2022 redeployments, we covered the ranch structures with nets to protect them from large organisms that could break the nubbins, which have been highly effective, since breakages were considerably reduced in 2023. Finally, we observed a high abundance of smaller fauna like snails and shrimps in the structures when they were redeployed (Supplementary Fig. 3c and 3d). Shrimps were observed directly on the corals and seemed to clean or feed on them. Buhl-Mortensen & Mortensen (2004; 2005) already noticed the presence of shrimps in the associated fauna of deep corals and gorgonians, as they provide microhabitats for resting and avoiding predators. Other studies observed elevated shrimp densities in living colonies of *L. pertusa* (Purser et al., 2013) and hypothesized that they may clean corals and feed on coral mucus, as already observed in tropical corals (Patton, 1994). This also may stress corals, which are potentially already weakened by a decompression to the surface, handling and then recompression before resettled to the deep-sea floor. These factors could contribute to the high mortality observed in the field.

6.5 Conclusion

The present study provides, through long and short-term *in situ* experiments, the first estimations of skeletal growth rate of *L. pertusa* in the Bay of Biscay, NE Atlantic Ocean. We report average linear extension of $2.4 \pm 1.6 \text{ mm yr}^{-1}$ for *L. pertusa*. Those values of growth rate are similar to the one monitored in a study in a norwegian fjord, but are predominantly lower compared to values reported for these species from other geographical regions, especially the Mediterranean Sea. Concurrently, high mortality and breakage rates in *M. oculata* prevented the characterization the growth dynamics for this species in the study area. Further long-term experiments and repeated temporal surveys are thus needed to better characterize the growth of the two main reef-building cold-water coral species and its link to environmental changes in this canyon, as canyon are particularly highly variable habitats.

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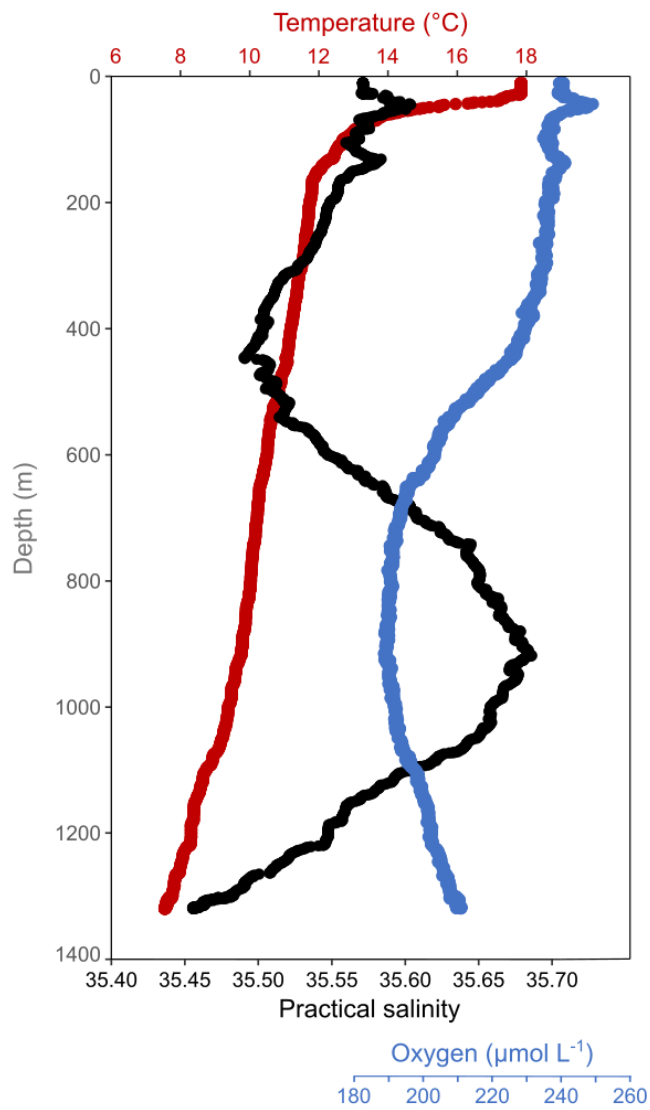
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Supplementary material



Supplementary Fig. 1. CTD profiles of temperature, salinity, and dissolved oxygen concentration in the water column along the cliff of the canyon's head.

Chapter seven: Discussion and perspectives



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7.1 Discussion

During this PhD, we investigated the response of Atlantic *L. pertusa*, the main reef-building and most widespread CWC species, to temperature change, and compare their biology to their Mediterranean counterparts. We conducted short and long-term experiments to test the effects of lower and elevated temperatures on *L. pertusa* holobiont by considering different levels of biological organization (from molecular and cellular levels to the whole organism). We then conducted *in situ* studies to compare *L. pertusa* from the NE Atlantic and the Mediterranean Sea using homogenized sampling and analysis protocols. We observed differences between these populations for patterns of reproductive biology, growth and microbiome diversity. Finally, we compared *L. pertusa* and *M. oculata* microbiomes across different Atlantic and Mediterranean sites. The following section summarises the main findings of our work and discusses them in light of previous studies from the literature.

7.1.1 Effect of temperature on the NE Atlantic *L. pertusa* holobiont

7.1.1.1 Effects of elevated temperatures

The present study shows that *L. pertusa* holobionts from the NE Atlantic Ocean were strongly negatively affected by elevated sea water temperatures. During both aquaria experiments, mass mortality of polyps maintained at elevated temperatures was registered, as earlier observed for other populations (Brooke et al., 2013; Chapron et al., 2021). Considering both experiments, as soon as one month after the start of the experiment, between 61 % and 100 % of all polyps were dead for temperatures $\geq 14^{\circ}\text{C}$.

Mortality under elevated temperature is associated with rapid dysbiosis and pathogenic bacteria infecting the coral

In both experiments, a rapid stress-induced dysbiosis under warming conditions was registered. The emergence of opportunistic and potentially pathogenetic bacteria, concomitant with the disappearance of some abundant bacteria that persisted in the control temperature, was observed after less than two weeks in both the short and long-term experiments, and lasted until the end of the experiments. This shift in microbial community has been observed in Mediterranean *L. pertusa* and *M. oculata* exposed to temperatures increase of +2 and +4°C after 2 months of experiment (Chapron et al., 2021). Our results bring new knowledge by showing that dysbiosis

occurs rapidly, within weeks, in response to similar temperature increases (+3/4/5°C). We noted in addition that changes took place more rapidly when temperature increase with +4 and +5°C. Several bacteria from the families *Saprospiraceae*, *Vibrionaceae*, *Rhodobacteraceae*, *Fusibacteraceae*, and *Flavobacteriaceae* were found more abundant along the course of the experiment in corals maintained at 13, 14 and 15°C. These bacteria were earlier associated with diseases in tropical corals (reviewed in McDevitt-Irwin et al., 2017), and involved in detrimental health status in Mediterranean CWCs exposed to elevated temperatures (Chapron et al., 2021). In contrast, they were in low abundance or absent in corals maintained at low temperature and control conditions. Our results reflect a limited capacity of *L. pertusa* to maintain or regulate its microbiome under stressing conditions when temperature severely increases (> +3°C). Dysbiosis, and disruption of interaction with some bacteria or symbionts may cause the death of tropical corals (Vega Thurber et al., 2020), and we suggest this could also occur in CWCs.

Furthermore, our use of metagenomic approach revealed that bacterial gene composition differed between coral microbiomes incubated at different temperatures after 2 months, and the presence of gene markers for virulence factors at elevated temperatures. Among the more abundant genes at higher temperatures, several were coding for secretion systems (T3SS and T1SS), flagella and pili. Similar increased abundance of microbial genes involved in virulence, motility, and chemotaxis have earlier been observed in response to stress, including thermal stress, in tropical corals (Thurber et al., 2009) and have been linked to tropical coral lethal diseases (Munn, 2015). Our study is the first to show a correlation between dysbiosis, the possible infection by pathogenic bacteria, and massive CWC death in response to thermal stress.

Higher temperatures induce stress in *L. pertusa*

Metatranscriptomics analysis revealed the significant upregulation of transcripts related to general stress response in the corals at elevated temperatures. They included transcripts related to immune, inflammatory, antioxidant and stress responses, cell death and apoptosis, and DNA repair and maintenance. Several of the genes identified in our study as molecular biomarkers for stress were also upregulated in diseased, bleached, or stressed tropical corals and gorgonians (e.g., Barshis et al., 2013; Maor-Landaw & Levy, 2016; Ricci et al., 2019), and stressed CWCs exposed to suspended particles mimicking deep-sea mining plumes exposition (Carreiro-Silva et al., 2022). In summary, our findings described for the first time the molecular response of *L. pertusa* to higher temperature, and demonstrated that higher temperatures induce stress in

Atlantic *L. pertusa*, within a month, a response similar to the one reported in heat stressed tropical corals.

Energy investment in growth may impair *L. pertusa*'s fitness

In the long-term experiment, higher calcification rates were measured before coral death after one month at 14°C compared to control. The higher growth was observed by direct growth measurements and by the upregulation of carbonic anhydrase genes expression. Similar upregulation of calcification-related genes was earlier reported in *D. dianthus*, and hypothesised to counter the negative effect of low pH on the biomineralization process (Carreiro-Silva et al., 2014). Higher growth was also observed in *D. cornigera* and *L. pertusa* when temperature increase by 4 and 5°C (Naumann et al., 2013; Gori et al., 2014; Büsher et al., 2017). However, in our experiment, despite this enhanced calcification rate corals did not feed much. We can thus hypothesise that corals were able to invest in growth as long as they had sufficient reserves. Once these reserves were depleted, polyps started dying as reserves were not renewed by feeding. It is difficult to know whether the corals didn't feed because they didn't need to (i.e., sufficient reserves during the experiment), or couldn't, or if they were too stressed to feed.

During the 2-months experiment, corals exhibited similar growth rates at 13, 15°C and in the control condition. Several hypotheses may explain the different growth response between the two experiments. During the short-term experiment, the lower number of available samples for growth measurement may have lowered the statistical power. Similarly, we were not able to detect any differences in volume or surface growth using a 3D scanner protocol since growth was too slow and too low to detect differences within 2 months. This 3D protocol might be tested and effective over longer periods of growth determination, particularly for *in situ* experiments. It is also possible that the corals had simply not invested in their growth at elevated temperatures. This trend was earlier observed in Mediterranean *L. pertusa*, where growth under control conditions was similar to the one at +2°C conditions, probably due to an alteration of the coral's metabolism (Chapron et al., 2021).

Considering overall growth rates, they were similar between corals exposed to low temperature and control conditions for both experiments, elevated temperatures from 13 to 15°C and *in situ* experiments. However, they were in the lower range compared to values published for this species in other geographical areas. Moreover, we reported no differential growth between subapical and apical polyps in Atlantic *L. pertusa* in aquaria and *in situ* experiments, while younger polyps grow faster in the Mediterranean Sea (Lartaud et al., 2013;

Chapron et al., 2021). This indicates different growth dynamics between Atlantic and Mediterranean *L. pertusa*.

7.1.1.2 No negative effects of lower temperature on Atlantic *L. pertusa* health

We observed similar health conditions for *L. pertusa* maintained at 6 and 10°C, although their microbiome differed during the experiment. Metatranscriptomics analysis revealed that most of the stress-related genes were downregulated at lower temperature, which may partially explain the higher survival rate at 6°C compared to the control condition along the experiment. Slower metabolism of corals maintained at 6°C may indicate that corals reduced their energy demand at a minimum while maintaining their overall fitness, as attested by the similar growth rates between low temperature and control conditions. Such decreased metabolism in response to lower temperature has been reported in several CWCs. *Lophelia pertusa* respiration decreased when temperature rapidly dropped from 9 to 7.5°C in the NE Atlantic Ocean (Dodds et al., 2007). Furthermore, Brooke et al. (2013) observed no mortality of *L. pertusa* when temperature decreased by 3°C in the Gulf of Mexico, suggesting high tolerance to short-term exposure to low temperature. In the Mediterranean Sea, *D. cornigera* had lower growth, respiration, and calcification rates when the water temperature was lowered by 4°C. Similarly, reduced calcification, growth, prey capture rates and lipid content of tissues were reported in Mediterranean *L. pertusa* when the water temperature was lowered by 6°C (Naumann et al., 2014) and 3°C (Chapron et al., 2021). However, it appeared that a gradual decrease in temperature of 6°C over three months did not result in lower metabolism (Naumann et al., 2014), suggesting long-term acclimation to low temperature.

Interestingly, although *L. pertusa* from the NE Atlantic appeared relatively well adapted to lower temperatures (Chapter 3), they nevertheless do not colonise very cold environments, deeper in the Atlantic Ocean, or in boreal and Arctic regions. If we assume that low temperatures are not deleterious to Atlantic *L. pertusa* performances (Brooke et al., 2013; Manuscript's Chapter 3), then its absence in colder waters (<4°C) may be linked to other limiting parameters, such as aragonite saturation or potential larval dispersal fluxes.

7.1.2 On the future of Atlantic and Mediterranean populations of *L. pertusa* facing climate changes

7.1.2.1 Proposed thermal niches of Atlantic and Mediterranean *L. pertusa*

A comparison between NE Atlantic and Mediterranean populations revealed differences in the holobiont biology, including physiological differences *in situ* (Manuscript's Chapters 4 and 6), and in response to temperature (Manuscript's Chapters 2 and 3), as well as microbiome composition (Manuscript's Chapter 5). Altogether, these differences are supposedly linked to adaptation or specific acclimation to environmental conditions for each population within its habitat, further supporting intraspecies variations.

Under future climate change scenarios, the distribution limits and survival of CWCs, including *L. pertusa*, will depend, among other factors, on how their physiological performance is impacted when the environment changes from optimal to extreme conditions (Reynaud et al., 2021). Based on the aquaria studies we conducted, and previous studies conducted on the Mediterranean population, we suggest the following model for the thermal niche of *L. pertusa* (Figure 7.1). Here, the shape of the thermal performance curves is arbitrary since the data we collected did not allow to accurately inform on the exact thermal performance curve of the organism. We rather provide information on the temperature characterizing the tolerance range for each population. Our results should be considered cautiously as they originate from laboratory experiments with single replication of conditions and maintenance of deep specimens at atmospheric pressure.

The thermal niche of *Lophelia pertusa* from the NE Atlantic has an optimum at lower temperature compared to one of populations from the Mediterranean Sea. In addition, it shows relatively good tolerance to low temperature but higher sensitivity to higher temperatures. Although Atlantic populations of *L. pertusa* seem adapted to lower temperatures, corals have a slower metabolism, with, for example, much lower growth rates recorded in the Bay of Biscay (Manuscript's Chapter 6), in Norwegian fjords (Büscher et al., 2019), and in the Gulf of Mexico (Brooke & Young, 2009) compared to those recorded in the Mediterranean Sea (Lartaud et al., 2017; Chapron et al., 2020b). On the contrary, Mediterranean *L. pertusa* seemed less resilient to lower temperature than Atlantic specimens, with reduced metabolic performances at low temperature, but relatively good tolerance to slightly higher temperatures as showed by Chapron et al. (2021). These authors demonstrated that a +2°C temperature increase caused dysbiosis and lower metabolism, while survival was unaffected. However, when temperature increases by +4°C, Mediterranean *L. pertusa* exhibited great sensitivity, resulting in very high mortality after 2 months of the experiment, dysbiosis with the emergence of opportunistic and potentially pathogenic bacteria, and altered metabolism through changing reserves, and lowered polyp activity and prey capture rates. Our study showed a similar high sensitivity of Atlantic *L. pertusa* to short and long-term +3/+4/+5°C increase in temperature with massive mortality due

to dysbiosis and infection of actively pathogenic bacteria, concomitant with altered metabolism (Manuscript's Chapters 2 and 3). We thus suggest that the upper thermal limit of the Atlantic population of *L. pertusa* is below 13°C. Here we show that although the NE Atlantic *L. pertusa* has a lower upper thermal limit than the Mediterranean and the Southeastern US *L. pertusa* populations (<13°C vs <15°C) (Brooke et al., 2013; Chapron et al., 2021), it appears that all *L. pertusa*, regardless of the region they originate from, and the water temperature in which they live, will be strongly impacted by a long-term +3°C increase in water temperature.

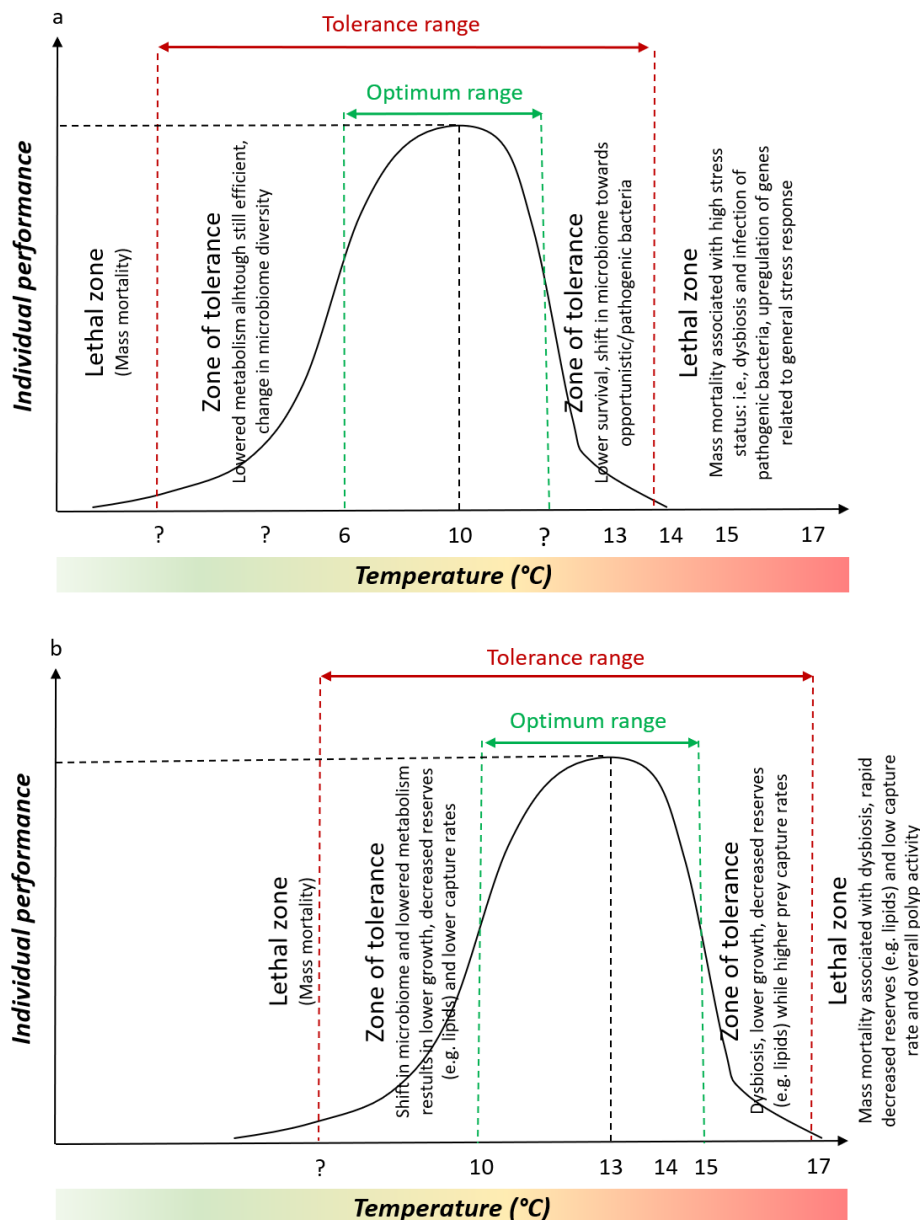


Figure 7.1. Hypothetical thermal niches of *L. pertusa* in the Lampaul canyon (NE Atlantic Ocean) (a) and in the Lacaze-Duthiers canyon (Mediterranean Sea) (b) based on the study from Chapron et al. (2021).

Although Atlantic and Mediterranean populations both are sensitive to temperature increase, the major difference will probably be the speed of changes they will face in their habitat in the context of the ongoing climate changes. Mediterranean *L. pertusa* was shown to be more plastic and resilient to variations of environmental conditions both spatially and temporally at the microbiome level and for skeletal growth compared to *M. oculata* (Meistertzheim et al., 2016; Chapron et al., 2020a). In the deep Mediterranean Sea, environmental conditions are very stable along the year, with some very episodic seasonal cascading in submarine canyons, lowering by 3°C the normal temperature within a day. Differently in the Bay of Biscay canyons, temperature may range from 8 to 12°C along the year (van den Beld et al., 2017) as a result of tidal and lunar cycles. Corals in the Lampaul canyon may thus be subjected to high variability within their environment. In Norway, *L. pertusa* from close locations showed different local response and sensitivity to acute exposition to a temperature rise of +10°C. The most sensitive corals were the ones experiencing the least variable, and the coldest temperatures along the year, compared to more plastic *L. pertusa* from other sampling sites experiencing more variable temperatures along the year (Dorey et al., 2020). The authors suggested that Norwegian *L. pertusa* tolerates a broad thermal range of temperatures, as a result of exposition to high thermal variability within their natural environment. Differently, NE Atlantic populations, supposedly exposed to variable conditions, appeared relatively tolerant to lower temperature but very sensitive to warming.

Finally, Mediterranean *L. pertusa* showed high sensitivity to lower temperatures, while being relatively tolerance to slightly higher temperatures, although they were inhabiting very stable environment. In light of our results, and the one reported for the Mediterranean population, global scale generalization regarding *L. pertusa* tolerance to temperature is not possible. A wide variability in thermal tolerance is reported for this species with apparently high local acclimation. It appears then essential to characterise the response of each populations, as they show distinct specific responses, perhaps due to (potentially genetic) isolation between populations.

7.1.2.2 Ecological implications of climate changes on *L. pertusa* across large geographic scale

Climate change, including warming, acidification, and deoxygenation leading altogether to a decrease in food availability at the seafloor, is expected to have rapid and profound impacts on the deep Ocean (Gehlen et al., 2014; Sweetman et al., 2017). Climate change is expected to

decrease cascading events (Canals et al., 2006), which deliver food to deep Mediterranean communities in submarine canyons. Considering that the reproduction timing of *L. pertusa*, including the start of oogenesis, vitellogenesis and the spawning event, is associated to food delivery and seasonally triggered by downwelling currents, we can hypothesize that the reproduction of Mediterranean *L. pertusa* might be disrupted in the future. This may not affect the Atlantic population in the same way, as food-delivering currents and more generally water masses dynamics are different in the NE Atlantic Ocean.

Furthermore, in the context of ongoing climate changes, prolonged exposure to high temperatures is likely to affect the survival of *L. pertusa* and *M. oculata* in the Mediterranean Sea (Chapron et al., 2021) and of NE Atlantic *L. pertusa*. Large-scale warming of the deep Mediterranean Sea and Atlantic Ocean may contribute to a shift in coral community composition. The dominance of species, such as *D. dianthus* or *D. cornigera* (in shallower waters of the Mediterranean Sea) is anticipated, especially since these species may better cope with climate changes and show remarkable phenotypic plasticity, as reported in the Mediterranean Sea (Naumann et al., 2013; Gori et al., 2014) and in Chilean fjords (Beck et al., 2022). On the contrary, *L. pertusa* communities are expected to decrease. By 2100, models projected a dramatic decrease of 79 % in suitable habitat for *L. pertusa* in the North Atlantic Ocean, including the Bay of Biscay, under RCP8.5, while *M. oculata* and *D. dianthus* might experience a less severe reduction in suitable habitat of about 30 % and 55 %, respectively (Morato et al., 2020). The dominance of these species may offer less complex habitats or smaller framework for the associated benthic fauna, affecting community structure and functioning. In addition, the potential *L. pertusa* decrease in the Atlantic Ocean, forming sometimes prime habitats for fish, offering spawning and nursery grounds (Corbera et al., 2019), is expected to negatively impact some fish stocks, while they are in parallel facing climate change (Morato et al., 2020).

Furthermore, other factors may be even more detrimental for CWC reefs. Although our work has showed the effects of temperature on the biology of *L. pertusa* and revealed very high sensitivity of this species to ocean warming, the impacts of temperature remain minor when compared to those of deep-sea fishing and trawling. The NE Atlantic fishing grounds, exploited by the UK and other UE countries, represent one of the most productive and intensively exploited in the world (Thurstan et al., 2010). Bottom trawling has severely impacted CWC reefs in the Atlantic Ocean, destroying at least one-third of deep-sea reefs in Norway (Fosså et al., 2002). Bottom fishing is now banned below 800 m in all European waters, and even shallower in some more restricted areas in the NE Atlantic Ocean. Despite this, CWCs species

are particularly threatened, particularly in the NE Atlantic Ocean, given their extremely slow growth, their long-life spans, their sensitivity to other factors, including warming. The impacts of some human activities, such as past bottom trawling, are so detrimental that they are almost irreversible nowadays. It is thus crucial to get more knowledge about a population's potential for extinction and its connectivity to other populations.

Despite contrasting experimental results, uncertainties regarding species' potential for acclimation and (local) adaptation to climate change (Georgian et al., 2016), and interspecific genetic variability (Kurman et al., 2017), there is growing evidence that the long-term capacity for adaptation to multiple stressors associated with climate change is limited in CWCs, and particularly in *L. pertusa* (Kurman et al., 2017).

7.2 Perspectives

7.2.1 Deeper understanding at the holobiont level

a. Metabolomics as indicator of coral stress

Metatranscriptomics provides a good overview of the potential changes in metabolisms in corals. To further describe cellular pathways implicated in coral stress response, the analysis of changes in metabolite composition (i.e., metabolomic profiling) would provide crucial information regarding the real metabolites production, metabolic pathway utilisation, and thus information on changes in coral performance and individual response to stress. In tropical corals, studies have generally shown shifts in metabolic pathways involved in carbohydrate and lipid metabolisms, cell structural maintenance and signalling, and osmotic balance to maintain cellular activity necessary for survival in response to ocean warming and/or acidification, often at the expense of growth and reproduction (e.g., Sogin et al., 2016; Haydon et al., 2023). Metabolomic fingerprints and diversity have been described in some deep-sea corals from the Gulf of Mexico (Vohsen et al., 2019), but never for *L. pertusa* in the context of thermal stress.

As part of a collaboration with D. Stien and E. Houelle (LBBM laboratory), we started investigating the metabolomic profiles of *L. pertusa* holobionts in response to long-term exposure to different temperatures (Chapter 3). Preliminary results suggest that there was no major influence of the lower temperature (6°C) on the metabolome during the whole experiment when compared to control conditions, further suggesting an effective acclimation to lower temperatures. Preliminary results also showed that the metabolomes of corals maintained at

elevated temperature was different from the ones of corals maintained under control conditions and at lower temperature. We observed different production of metabolites between corals at 10 and 14°C after 15 days, before the massive mortality registered at 14°C, as seen by both under-expressed (green zone) and over-expressed compounds (red zone) (Figure 7.2). It appeared that metabolites were more down regulated than up regulated at 14°C. The annotation of these metabolites has yet to be determined in order to understand the mechanisms underlying the response of *L. pertusa* to thermal stress.

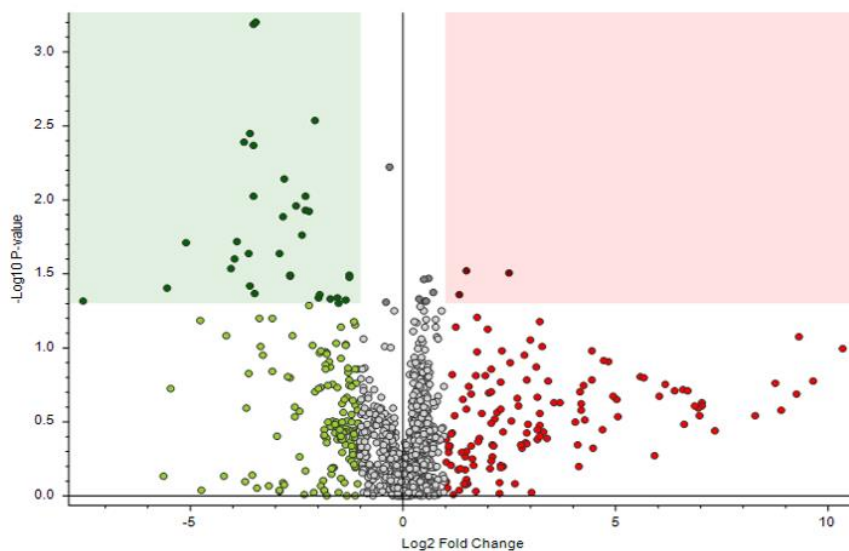


Figure 7.2. Volcano plots comparing the metabolites at 10 and 14°C after 15 days of experiment. The coloured zones indicate a p value of 0.05. All metabolites within these zones are significantly up-regulated (red zone) or down-regulated (green zone) compared to the metabolites outside these zones. Vertical dashed lines indicate Log₂ fold changes of -1 and +1.

The combined use of metabolomics and transcriptomics will allow us to see what is ultimately produced in terms of metabolites and for which use. Further work should investigate how well CWC holobiont metabolomics and transcriptomics correlate under high temperature condition, including the response of the bacteria consortium.

b. Role of the microbiome in coral health: who and where?

In Chapter 5, we explored the diversity of microbial communities of *L. pertusa* and *M. oculata* across the NE Atlantic Ocean and the Mediterranean Sea. We observed some conserved symbionts in *L. pertusa*, such as *Propionibacteriaceae*, earlier identified in both sides of the Atlantic Ocean with consistence of particular genus (Neulinger et al., 2008; Kellog et al., 2017).

However, their role in the host metabolism is still unknown. Moreover, we showed that in *M. oculata* from both the Lampaul canyon and the Mediterranean Sea, bacteria from the genus *Endozoicomonas* dominated the microbial community, as already observed earlier across a large geographical scale (Galand et al. 2018; Kellog & Pratte, 2021), further suggesting a true host-association. *Endozoicomonas* are typical host associated bacteria that are common in healthy tropical corals, and that conduct important functions such as vitamin production, DMSP transformation or amino acid and carbohydrate metabolisms (Hochart et al., 2023). Further studies may identify if there are conserved strains of *Endozoicomonas* in CWC by comparing the 16S rRNA across distant populations, as well as with tropical corals. In addition, the reconstitution of bacterial genomes and their annotation, together with comparative analysis with genomes found in zooxanthellate corals, would allow to determine the potential role that these bacteria are playing in CWC health, and their interaction within the holobiont.

Finally, it is known that tropical corals harbour distinctive populations of bacteria in different compartments of the coral, including the mucus, the tissues, and the skeleton (Bourne & Munn, 2005; Koren & Rosenberg, 2006), and that these bacteria are associated to different function in coral physiology (Rosenberg et al., 2007). However, tissue specific studies have rarely been conducted in azooxanthellate corals (e.g., Biagi et al., 2020) and even less in CWC (Neulinger et al., 2009). Neulinger et al. (2009) found spatially constant fraction of tissue-bound bacteria, mostly concentrated in Norwegian *L. pertusa*' tentacles, while a more variable "liquid-associated" bacterial fraction inhabited the mucus and gastric fluid. Further work could aim at determining the localisation of bacteria and their specific metabolisms in CWC. Within the ARDECO project (Assessing Resilience of Deep-Corals, 2021-2026, PI L. Menot), one of the objectives is to describe the diversity of microbial communities considering their precise location and abundance in CWC compartments (partners: P. Galand & M. Zbinden). Fluorescence *In Situ* Hybridization (FISH) is a powerful tool for direct imaging of bacteria associated with corals. It could allow the localisation and quantification of specific bacterial phylotypes in coral mucus, tissues and skeleton separately. Determination of bacterial communities within *L. pertusa* from the Lampaul canyon will allow to compare compartment's bacterial diversity and possibly confirm patterns found by Neulinger et al. (2009), with maybe identical strains of abundant *Mycoplasma*, hypothesized as commensal bacteria in Norwegian *L. pertusa*.

c. Calcification: promoting linear extension over quality?

During our long-term experiment, we observed almost 3 times higher growth rates under elevated temperature than the ones measured at 6 and 10°C. Here, we determined growth rate as the polyp linear extension. Skeleton calcification corresponds to both linear extension, creating new skeletal elements, and thickening by the growth of bundles of mature, aligned aragonite fibres. This thickening affects the bulk density of the skeleton, as the thicker or longer the fibre bundles are, the lower the porosity of the skeleton (Cohen & McConnaughey, 2003). Considering that the calcification process is very energetically costly, it is currently unknown whether energetic costs are distributed into different types of growth (linear extension vs calcification thickening) in response to environmental changes. Although no differences in calcification have been reported in *L. pertusa* exposed to ocean acidification for 12 months, there were changes in crystal organization, with less organized fibrous aragonite crystals within the skeleton (Hennige et al., 2015). Although this trend was not reported in *L. pertusa* exposed to elevated temperatures, the reduction in respiration rates in corals exposed to warming for a year suggests energy trade-off occurring within the coral (Hennige et al., 2015).

In our experiment, it is possible that despite the higher growth reported after one month at elevated temperature, the quality of *L. pertusa* skeleton biomineralization or the crystallography changed. A more fragile skeleton would confer weakened structure and may have negative implication for the reef survival (Büscher et al., 2017). Further studies may investigate the impact of long-term exposure to elevated temperatures on skeleton biomineralization to determine potential negative impacts of warming on coral skeleton quality. Tomography scanner may be used to determine coral skeletal density, while skeletal microstructures may be characterized using microscopic analysis techniques, combining transmitted light microscope and scanning electron microscope (SEM) to determine molecular-scale bonding of the skeleton. Crystallographic arrangement of thickening deposits of aragonite crystals may be determined using Electron Back-Scatter Diffraction (EBSD), an electron-beam technique allowing to identify mineral phases, measure the crystallographic orientation and evaluate the spatial and crystallographic relationships between crystals. This would allow the production of orientation maps to determine eventual misorientation of the fibres.

7.2.2 Integrative aquaria experiments simulating *in situ* conditions

Experiments carried out at atmospheric pressure instead of *in-situ* pressure, may misjudge the response of deep-sea species such as CWCs to changing environmental drivers, especially if

the studied population inhabits very deep environment. For example, as carbonate dissolution kinetics and patterns are linked to depth (Sulpis et al., 2021), experiments investigating the impacts of ocean acidification on CWC might underestimate the impact of low pH. In our experiments (Chapters 2 and 3) we might have observed an impact of atmospheric pressure on *L. pertusa* since we observed mortality in the control conditions (i.e., 10°C). Since the corals we collected are, to our knowledge, the deepest specimens maintained in aquaria at atmospheric pressure, both their collection and maintenance could have been detrimental to their health.

The ARDECO project plans to carry out experiments on the impact of the combined effect of ocean warming and acidification on different CWC species in pressurised aquaria compared to atmospheric pressure (partner: AMEX laboratory). Flow-through aquaria simulating *in situ* depth (up to 3000 m) and isobaric feeding line will allow to keep CWCs, such as the deep-living *Solenosmilia variabilis*, several months at *in situ* pressure without repeated decompressions. The comparison between CWCs maintained at *in situ* and atmospheric pressures will allow to determine the impact of deep CWC maintenance at atmospheric pressure, in addition to determine the combined effects of temperature and pH on biological response at the holobiont scale.

7.2.3 Resilient coral genotypes and potential for population resilience

In Chapter 5, we observed *in situ* colony-specific microbiome variability within submarine canyons both in the Atlantic and the Mediterranean Sea, as already observed in Kellog et al. (2017) between close sampling sites in the Gulf of Mexico. Furthermore, in Chapter 2, we observed intra-species variability, through differences in colony survival, and colony specific bacterial communities. We identified *Spiroplasmataceae* (class *Mollicutes*) in high abundance in only one colony (L8). The taxonomic affiliation of this class has changed several times and still appears sometimes (especially using the Silva database) as *Firmicutes*. They were suggested as potential pathogens in corals, since they are more abundant in tropical corals infected by black and white band diseases (Frias-Lopez et al., 2004; Mhuantong et al., 2019). Neulinger et al. (2009) described a *Mycoplasma* (class *Mollicutes*) in *L. pertusa* tentacle ectoderm, and they suggested a commensal relation between the bacteria and the coral, rather than a parasitic relation as usually proposed for other *Mollicutes*. In our experiment, the *Spiroplasmataceae* had only 91 % similarity to reference sequences, so we could not directly relate our data to existing literature. However, considering that this bacterium is found in the coral colony that exhibited the highest survival during the experiment, investigating its potential

metabolism may possibly inform us on a potential association between this bacterium and a resistant character of the host (here regarding survival). Variability between *L. pertusa* colonies have been earlier reported for different physiological parameters (e.g., Hennige et al., 2015; Kurman et al., 2017), and was hypothesized as a potential support for adaptation to changing environment. Here we showed for the first time colony specific microbiomes that may correlated to a response to temperature change. These differences between colonies may illustrate a potential for adaptation through more resilient coral genotypes at the holobiont level.

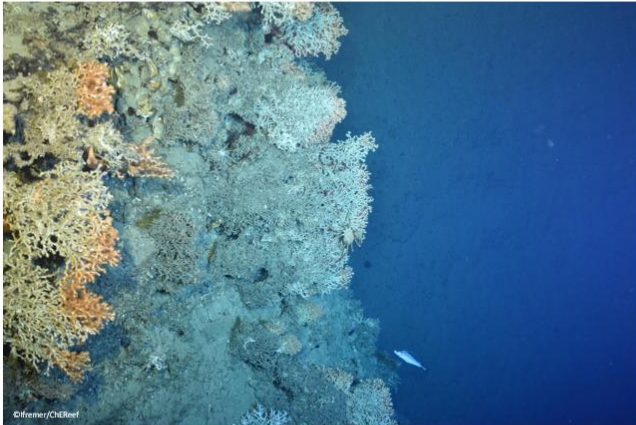
In future experiments, it would be relevant to keep polyps from the most and least resistant colonies, when intra-species variability is observed, to further conduct dedicated experiments to compare coral phenotypes. The following questions could be tested. Is there any genome modification in the most resilient colonies? Are there differences in the genome across different populations and are they in accordance with a more resilient character within a population? What are the microbial communities associated to these resilient genotypes? As we used identical protocols between *in situ* and *ex situ* experiments, we may use the same approach as the one described above (Perspective 7.2.1) to identify important resilient bacterial consortia in healthy corals from aquaria experiments (Chapters 2 and 3) and compare them with *in situ* microbiomes to determine possible links between *in situ* and *ex situ* healthy colonies. Further metagenomics analysis may allow to describe the metabolism of potential important resilient bacterial consortia in healthy corals. This may help understand mechanisms responsible for individual success and extrapolate on the potential for population adaptation within the context of climate changes.

7.3 Conclusion

This thesis contributed to increase our understanding on *Lophelia pertusa* sensitivity to sea water temperature changes across different populations from the NE Atlantic Ocean and the Mediterranean Sea. Notably, we show a very high sensitivity of Atlantic *L. pertusa* to a +3/+4°C increase in temperature, with strong changes in microbiome composition and coral metabolism. This suggests a low resilience and limited long-term capacity for adaptation to increasing temperature in the context of climate change. NE Atlantic *L. pertusa* appeared to have a lower upper thermal limit than Mediterranean *L. pertusa* (<13°C vs <15°C). However, regardless of their region of origin, the differences in biological parameters, and their differential apparent resilience, both populations will be strongly impacted by a long-term warming of +3°C. With temperatures set to rise by up to 4°C in the deep Atlantic and up to 2°C in the Mediterranean Sea by 2100, the NE Atlantic population is particularly exposed to warming. This sensitivity strongly threatens the survival of CWC reefs across oceanic basins, and jeopardizes the associated species relying on them. Nevertheless, the high variability between individuals illustrates potentially more resilient coral genotypes, which may support an adaptive capacity in response to climate change.

Appendices: Oral and poster communications

Talk for the International Symposium on Deep-Sea Corals, Edinburgh, 2023



EFFECTS OF TEMPERATURE ON COLD-WATER CORAL HOLOBIONT IN THE ATLANTIC OCEAN

Mathilde Chemel

Under supervision of Pierre E. Galand & Franck Lartaud

Sorbonne University, CNRS, Benthic Ecogeochemistry Laboratory, LECOB,
Banyuls Oceanological Observatory, France



RÉCHAUFFEMENT DES EAUX PROFONDES :

Les coraux atlantiques sont-ils plus résilients que les méditerranéens ?

Mathilde Chemel, Erwan Peru, Pierre E. Galand, Franck Lartaud
Observatoire Océanologique de Banyuls

Les récifs de coraux d'eau froide créent de riches habitats dans les eaux profondes mais ils sont menacés par les activités humaines.

En Méditerranée, les coraux profonds vivent à 13 °C, ce qui les rend particulièrement exposés au réchauffement climatique. Dans l'Océan Atlantique, ils vivent à 10 °C et les effets du réchauffement pourraient déjà être dévastateurs pour ces populations.



L'effet du réchauffement sur les coraux profonds a été testé expérimentalement sur des populations méditerranéenne et atlantique

MER MÉDITERRANÉE

OCÉAN ATLANTIQUE



Polype de coraux d'eau froide



Sous-marin pour récolter les coraux



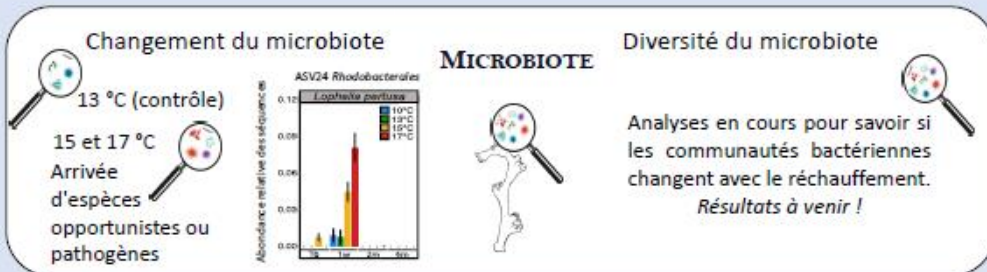
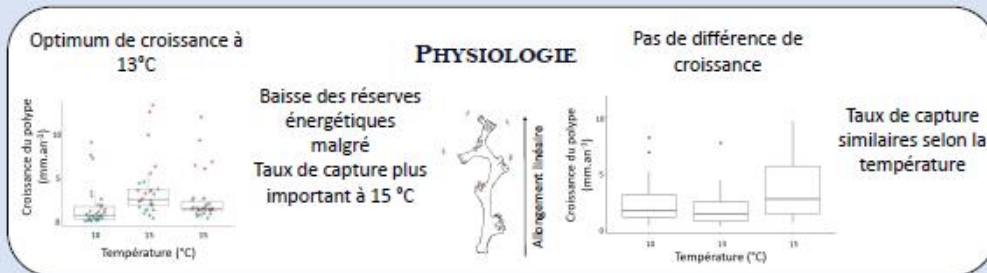
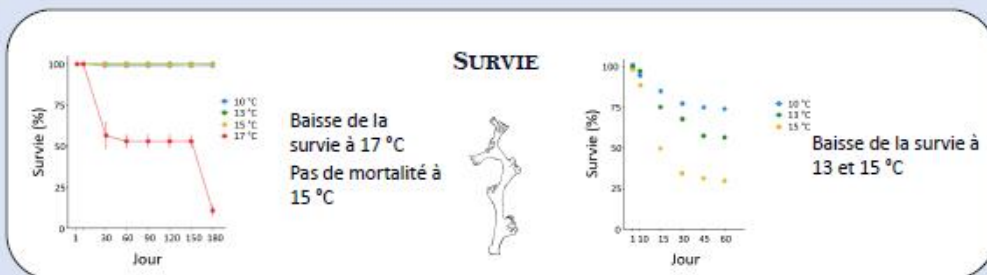
Pince du sous-marin au travail



Echantillon de corail



Expérience en laboratoire



A RETENIR !

Le réchauffement semble avoir un impact différent sur les populations de coraux de Méditerranée et d'Atlantique.

L'impact différentiel du changement climatique doit être pris en compte dans les plans de conservation de ces espèces, d'autant plus que d'autres menaces locales peuvent s'ajouter (pollution plastique, pression de pêche en milieu profond...)





Effects of temperature on deep corals from the Atlantic Ocean



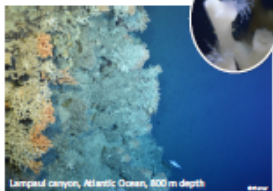
Mathilde Chemel (chemel@obs-banyuls.fr), Erwan Peru, Pierre E. Galand, Franck Lartaud
Observatoire Océanologique de Banyuls, Banyuls-sur-mer, France

CONTEXT

- Cold-water corals (CWC) are deep sea engineers, as they form extensive reefs, providing complex habitats and abundant source of food for a large biodiversity¹. They constitute ecosystems of high ecological values.
- CWC reefs are now facing serious anthropogenic threats, because of unsustainable fishing, pollution, and global changes².
- Mediterranean CWC, living at 13 °C, are particularly sensitive to ocean warming³. For Atlantic CWC, living between 8-12 °C, the effects of ocean warming is poorly described even though the impacts may already be devastating for these populations.

Here, we studied the effects of elevated temperature on cold-water coral holobionts from the Atlantic Ocean

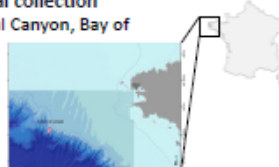
METHODS



- *Desmophyllum pertusum* is the main reef-forming CWC in deep environments
- This species is highly sensitive to temperature changes³

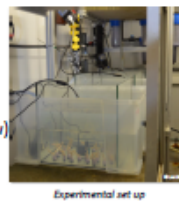
Cold-water coral collection

- In the Lampaul Canyon, Bay of Biscay, France
- 800 m depth
- August 2021
- Ariane HROV



Experimental design

- 2 months experiment in aquaria
- 4 colonies under 3 temperatures: 10 °C (*in situ*) 13 °C (+3 °C) and 15 °C (+5°C)



Prey capture rate

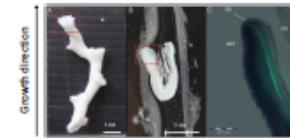
- Every two weeks on a 4-hours period

Growth assessment

- Coral fragments at macroscopic scale, with a 3D scanner
- Polyp growth at microscopic scale, using a calcein staining



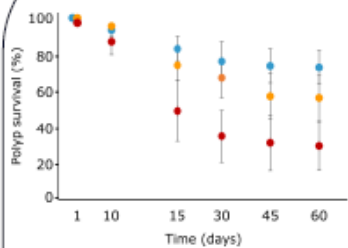
3D scan of coral nubbin at the beginning of the exposure



A. Coral nubbin; B. Isolated polyp; C. Polyp under fluorescent microscope with calcein staining (SS, summit of the septum)⁴

PRELIMINARY RESULTS

Survival



Polyp survival rate (% ± SE) of *D. pertusum* at 10, 13 and 15 °C

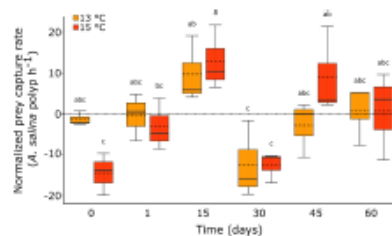
- Polyp survival decreased significantly at 15 °C ($p < 0,01$)
- Negative effect of elevated temperature also observed for the same species in other regions^{5,9}

- Mortality increases with time ($p < 0,01$)
- Deepest corals maintained in aquaria experiment (800 m) - potential impact of the pressure?

Prey capture rate

- No effect of temperature on prey capture rate

→ Lower prey capture rate and higher variability compared to corals from the Mediterranean Sea



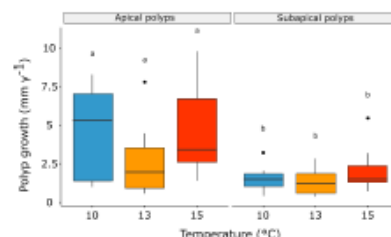
Prey capture rate per polyp during four hours of feeding. Values are normalized against controlled conditions (dotted line)

Growth

Macroscopic scale: below the detection limit of the 3D scanner

Microscopic scale:

- Apical polyps grew significantly faster than subapical ones ($p < 0,05$)
- No effect of temperature on polyp's growth



Polyp linear growth rate (mm y⁻¹) of apical and subapical polyps at 10, 13 and 15 °C after two months of exposure

- Growth rates are in the lower range of previous measurements of corals from the Mediterranean Sea^{4,5,6,7} and the Atlantic Ocean⁸
- We observed no impact of warming on growth, contrary to observations on Mediterranean *D. pertusum*⁵

CONCLUSION

Atlantic CWC had different physiological response to warming compared to the Mediterranean population. However, survival was significantly reduced with temperature rise.

→ What's next? Characterization of the microbiome of Atlantic specimens

Reproductive biology of the two main reef-building cold-water coral species (*Desmophyllum pertusum* and *Madrepora oculata*) in the Mediterranean Sea



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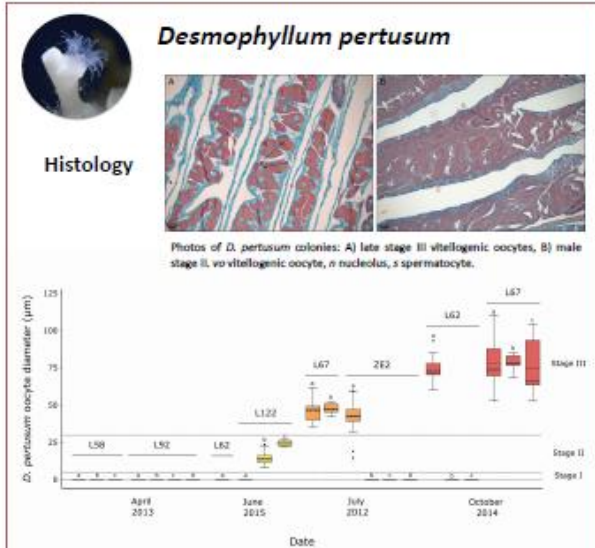


CONTEXT

- Reproduction is a key biological factor to predict population resilience to environmental stressors and anthropogenic pressures¹, but information of CWC reproduction is sparse.
- We lack a description of the reproductive biology of the main deep-sea Mediterranean engineers, even though they are particularly threatened by global changes.

METHODS

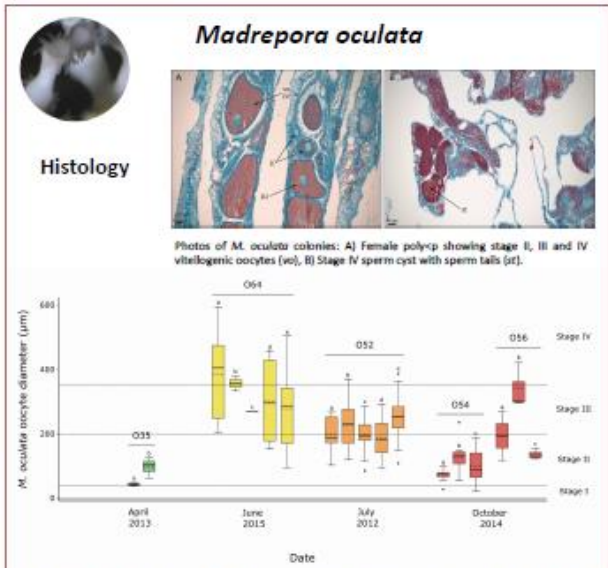
- Collection of colonies fragments from the Lacaze-Duthiers canyon, NW Mediterranean Sea
- Discovery of coral recruits on deepsea platforms (A)
- Histology on 9 colonies *per* species (both gender), using Movat stain
- Measurement of oocyte diameter to determine stage of maturation and size of recruits



Stage III observed from July to October, evidence of coral spawning described in October (L62) and corals were undifferentiated in April

→ Seasonal reproduction

→ Reproductive cycle is similar to the one described from the southeastern US and the Gulf of Mexico²



All stages of maturation were present simultaneously

→ continuous reproduction

→ reproductive strategy of *M. oculata* differs from *D. pertusum*: closer to a *K*-selection species

TAKE HOME MESSAGE

Spawning occurs seasonally in autumn/early winter for *D. pertusum*

- Vitellogenesis: related to the organic matter supplies during spring and summer
- Spawning: related to winter dense water shelf currents

Differently, *M. oculata* shows continuous reproduction

1. Reviewed in Richmond et al., 2018; 2. Brooke and Schroeder, 2007; 3. Lartaud et al., 2011; 4. Larson et al., 2014

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