Cold-water coral 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 deep-sea communities. The deep ocean is, however, warming and it's therefore essential to assess the resilience of cold-water corals to future conditions. Here we investigate the effects of elevated temperatures on the cold-water coral *Lophelia pertusa* (now named *Desmophyllum pertusum*) from the north east Atlantic Ocean at the holobiont level, the coral host and its microbiome. We show that at temperature increases of + 3 and + 5°C, *L. pertusa* exhibits significant mortality concomitant with changes in its microbiome composition. In addition, a metagenomic approach revealed the presence of genes markers for bacterial virulence factors suggesting that coral death was due to infection by pathogenic bacteria. Interestingly, different coral colonies had different survival rates, as well as colony-specific microbiome signatures, indicating strong colony variability in response to warming waters. Our results suggest that *L. pertusa* can only survive a temperature increase of < 3°C over the long term. Regional variations in deep-sea temperature increase should therefore be considered in future estimates of the global distribution of cold-water corals.

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 enhancers 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 (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 content, respiration, feeding behavior 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, had 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 temperatures, 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 has been demonstrated to be 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 for tropical corals, the associated microbial communities might be good indicators of 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 role 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 *L. pertusa* in the Atlantic are already living at their thermal optimum, which would expose 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 Atlantic reef-forming cold-water coral *Lophelia pertusa*. We conducted a two-month aquaria experiment in which corals from a submarine canyon in the Bay of Biscay (north-east Atlantic Ocean) 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 the microbiome by metabarcoding, and its functions by metagenomics.

2. Material and methods

2.1 Specimen collection and maintenance

Corals were sampled in the Lampaul canyon in the Bay of Biscay, North-east Atlantic Ocean (47°36.703 N, 07°32.192 W). 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 Experimental design

Lophelia pertusa 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) corresponded to the *in situ* temperature in the Mediterranean Sea, and 15°C (+ 5°C) represented a severe warming near the presumed upper limit of thermal tolerance for this species (Brooke et al., 2013). Experiments were conducted following the protocol detailed in Chapron et al. (2021). Between 22 and 24 nubbins were randomly distributed in each of the three 36L experimental tanks (376 polyps in total), with sufficient distance between the nubbins to avoid any contact between the different colonies and the 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 experimental tank was placed in a larger water bath tank to ensure stable temperature regulation. Each experimental 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 to the cooling unit (ICE400, Aquavie, Connaux, France) of the water bath tank. 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.

2.3 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 for each 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 (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, and glued with Patafix© in order to identify polyp's septs 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).

2.4 Bacterial community 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. 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, Ilkirch-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.

2.5 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-AGRGTTTGATCMTGGCTCAG 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). All 16S rRNA sequences were deposited in GenBank under SRA accession number PRJNA1085650.

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., 2022).

2.6 Metagenomic sequencing and data analysis

A total of 12 samples (two per temperature conditions at T0 and after 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 removing adapters and quality filtering using Cutadapt, sequences from each metagenome were assembled individually with MegaHIT (Li et al., 2015) using the *meta-large* options. Subsequently, EukRep (West et al., 2018) was used to identify and segregate eukaryotic from prokaryotic contigs, using a minimum contig size of 2 Kbp. Eukaryotic contigs (coral host and protistan symbionts) were then removed from the dataset. 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 from the different samples were pooled and dereplicated at 95% similarity and 80% alignment coverage with 'linclust' (Steinegger et al., 2017). Metagenome reads were back-mapped to the catalog using BWA (Li and Durbin, 2009), and the number of counts *per* gene was obtained using HTSeq (Anders et al., 2015). Counts *per* gene were normalized by gene length and the geometric mean abundances of 10 selected single-copy genes in each sample (Salazar et al., 2019). Normalized gene abundance tables were generated, including the abundance of each gene (ORF) in each sample. The corresponding functional abundance tables were generated by adding all the normalized abundances of all genes annotated to a specific function within a given database (e.g., KEGG). 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.

All metagenomic sequences were deposited at the ENA under accession number PRJEB68224.

2.7 Statistical analysis

Tests for normality of variance were performed using the Shapiro–Wilk test with the R software (v. 4.2.2). The 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 simper 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.

3. Results

3.1 Polyp survival and skeletal growth

Survival rates differed significantly between temperature conditions ($F_{2,3}$ = 110.266, $p < 0.001$) (Fig. 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 a continuous decrease in survival until the end of the experiment (survival of 31 ± 13% at 8 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 the 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-value < 0.001). Some colonies displayed lower survival rates than others (e.g., colonies 5 and 7, HSD post hoc test p-value < 0.01, Supplementary Fig. 3).

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, $p > 0.05$) (Fig. 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). 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 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.

3.2 Coral microbiome

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 (Fig. 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 4).

The bacterial community composition of the water was different from the coral bacterial communities (Supplementary Fig. 4, Adonis, $R^2 = 0.31$, $p < 0.001$), and was similar between temperature conditions during the experiment.

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 (Fig. 3). Gammaproteobacteria were also one of the main classes characterizing the bacterial communities, but tended to decrease slightly at the end of the experiment in all temperature conditions. Desulfobacteria and Clostridia both had the highest abundance after 4 weeks at 13 and 15°C (Fig. 3). Acidimicrobiia had highest relative abundance after 8 weeks.

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 always highest at the start of the experiment and then decreased with time (Supplementary Fig. 5).

We sequenced metagenomes from samples taken at the beginning and at the end of the experiment for all temperature conditions to identify the bacterial genes that could vary in relative abundance between experimental conditions. The nMDS based on the genes that varied the most, identified with DESeq2, 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, $p = 0.04$) (Fig. 4a). There were no patterns of sample separation when looking at all genes together (Supplementary Fig. 6).

We then specifically compared gene composition 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. This gene was annotated as coding for hemolysin A secretion system involved in pathogenicity (KEGG K11004). It prompted us to search for other gene 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 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 (Fig. 4b, t-test, $p < 0.01$).

The taxonomic annotation of the functional genes allowed us to find some 16S rRNA ASVs with the corresponding taxonomy. Among the genes coding for flagella, we identified 3 corresponding ASVs annotated at the genus level as Vibrio (*Vibrionaceae*) and uncultured P3OB-42 (*Myxococcaceae*) (Fig. 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) (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 (Fig. 5).

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. 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. 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. 8). Within their colonies, these ASVs were present under different temperature conditions and along the different times of the experiment (Supplementary Fig. 8).

4. Discussion

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 specific disease-associated microbiome, with low abundance of Vibrio sp., and distinct microbiome metabolisms and functioning. Our results show for the first time that stressed cold-water corals are subject to invasion by pathogenic bacteria in the same way as tropical corals. Changes in bacterial community composition (dysbiosis) 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*, normally living at 13°C, were strongly affected by water temperatures of 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 at 14°C and 16°C respectively, 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 a changing environment than others. Interestingly, we also observed colony-specific differences at the microbiome level. Some colonies 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* found in colony L9 had only 97% similarity to the closest hit in the databases (a sequence found in the sponge *Halicona tubifera* (Erwin et al., 2011), and the *Spiroplasmataceae* from colony L8 had only 91% similarity to reference sequences, so we could not directly relate our data to the 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 us to infer a direct relationship between colony-specific microbiome and survival, we can hypothesize 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 global change.

We reported here the first 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 depths 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 months), 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 the different ecological properties of the Lampaul canyon corals. A better characterization of *in situ* coral biology is thus required.

In the present study, 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 at both 10 and 15°C. A decrease of calcification rates with lower temperatures was described by Naumann et al. (2014) on Mediterranean *L. pertusa* when exposed to 12°C and 6°C, but these corals where 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 with a good health status, but the fact that temperature had no effect on polyp's growth rate during our two-month 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).

Conclusion

We showed that an increase in water temperature of + 3°C and + 5°C was responsible for dramatic mortality in *L. pertusa*. Mortality appears to be associated with 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 the associated bacterial community composition (dysbiosis). Interestingly, some specific colonies had specific microbiomes. Such colony-specific features may explain why 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.

Declarations

Data Accessibility

Samples and their metadata were registered in the ENA and Genbank biosample database. All sequencing files were submitted to the European Nucleotide Archive (ENA) at the EMBL European Bioinformatics Institute (EMBL-EBI) and GenBank under accession number PRJEB68224 (metagenomic sequences) and PRJNA1085650 (metabarcoding sequences) respectively.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contribution

PG and FL designed the study. MC, EP, MMB, RL, FL and PG conducted the study, analyzed data, and wrote the paper.

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Figures

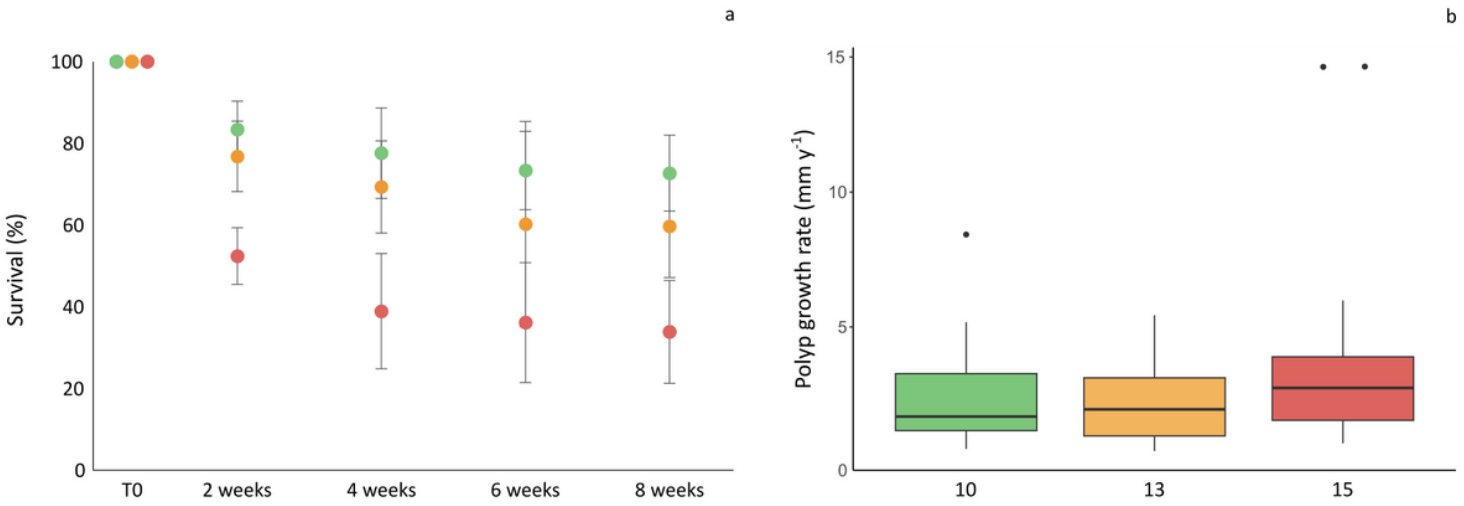

Figure 1

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 the experiment. Box plots plain lines indicate the median and quartiles, and points represent extreme values exceeding the 95% quantile for each treatment.

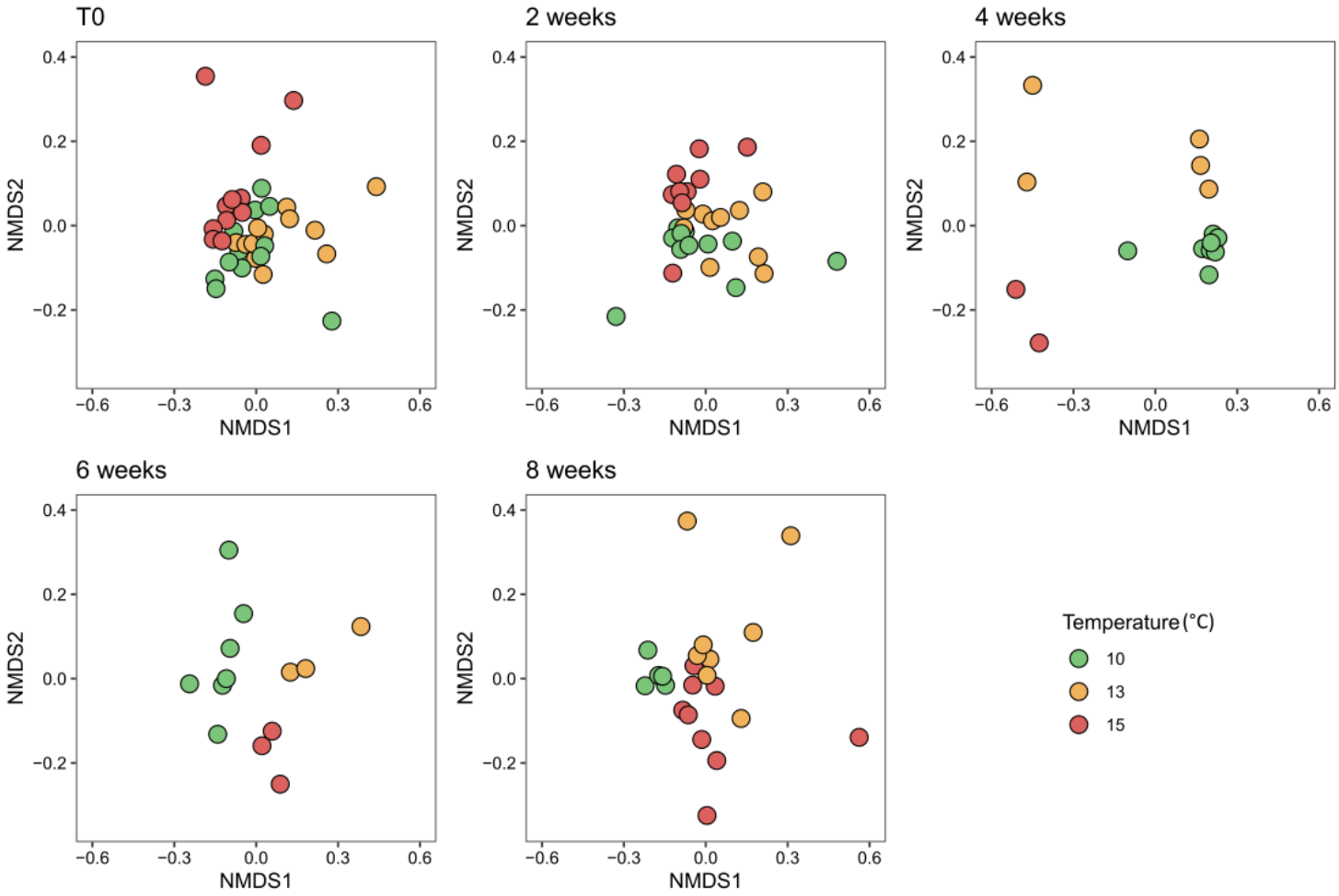

Figure 2

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.

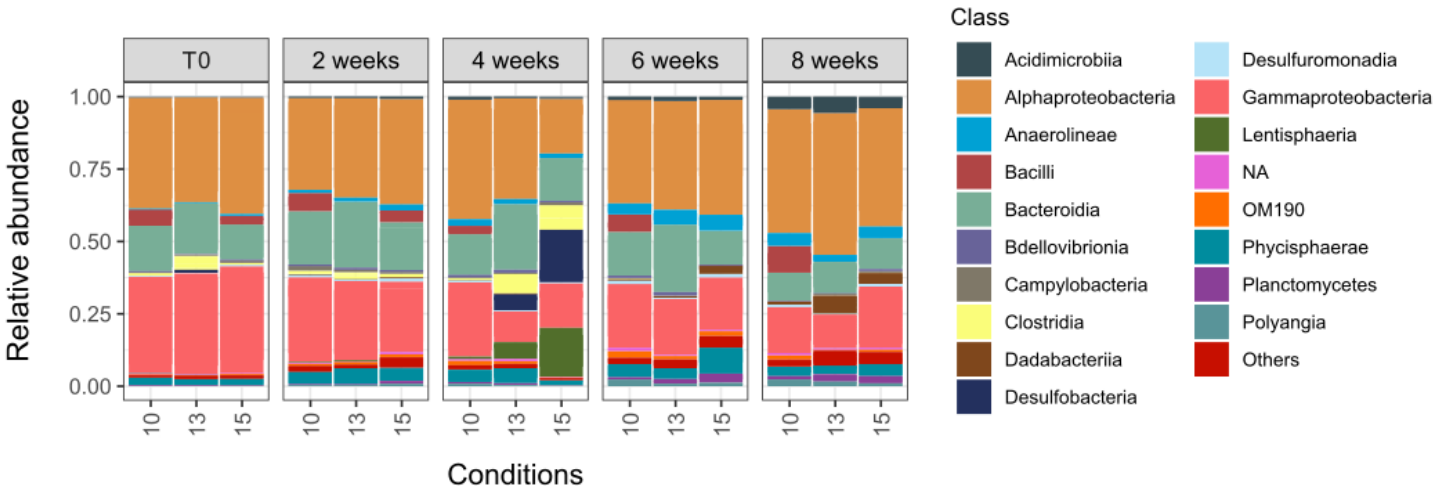

Figure 3

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.

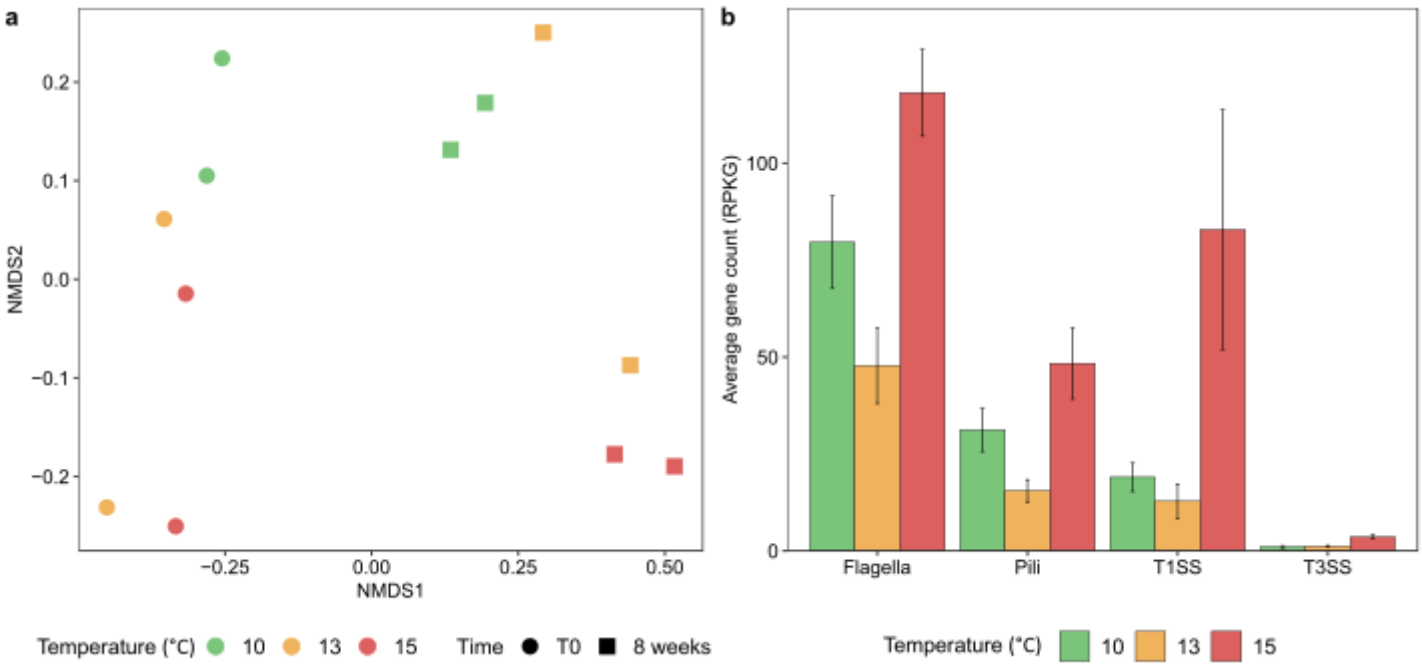

Figure 4

Non-metric multi-dimensional scaling plot (nMDS) based on the abundance of the genes that varied the most between temperature 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).

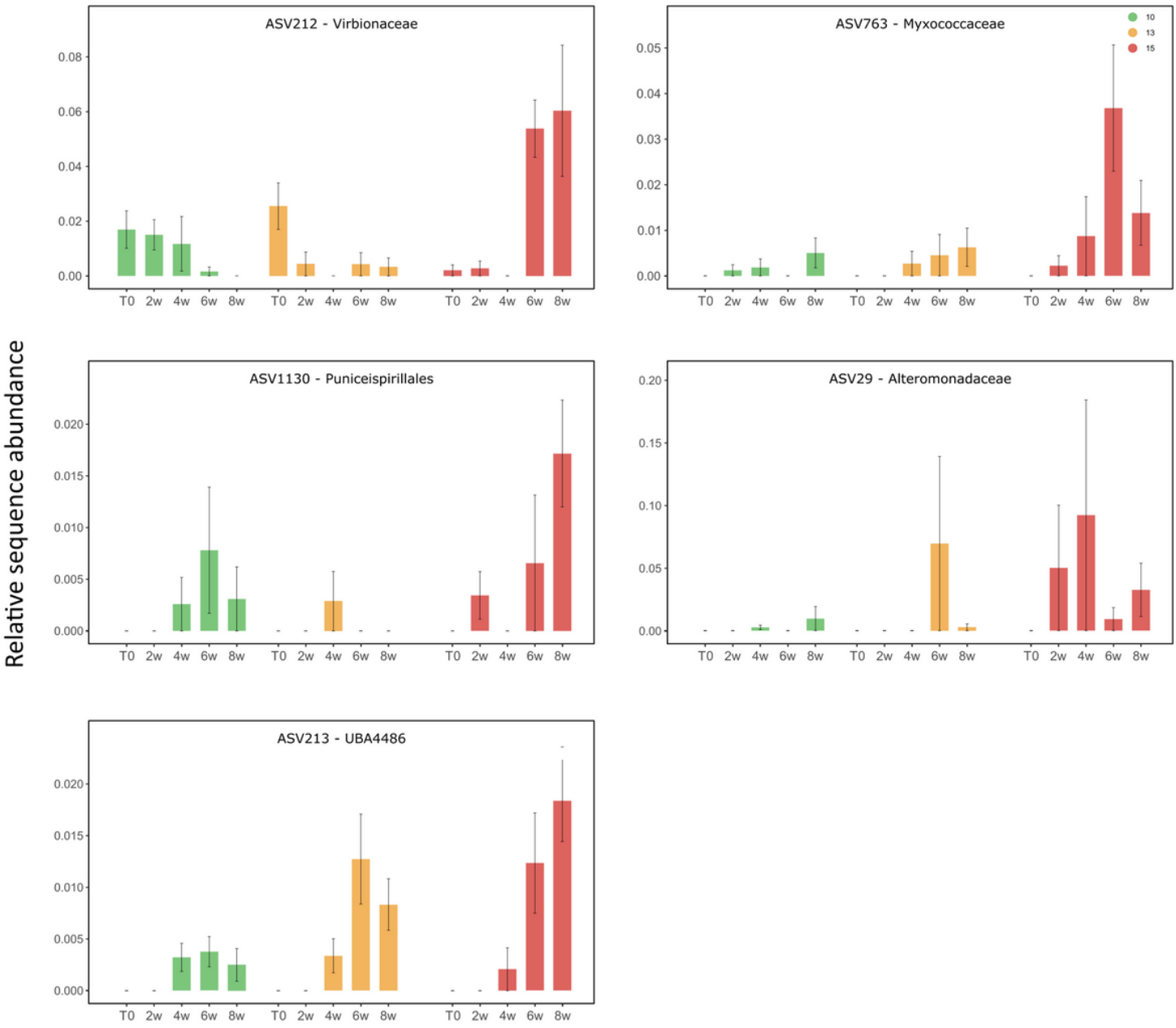

Figure 5

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx