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## Diet shapes cold-water corals bacterial communities

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### Originality-Significance Statement

For the cold-water corals (CWC), living in the deep and dark ocean, heterotrophs are the key microbiome partners of the host. The role of the CWC microbiome and the type of association to the host remain however poorly known. In this paper we tested whether diet shapes the composition of the bacterial community associated to the two most common cold water coral species: *L. pertusa* and *M. oculata*. We demonstrate that a large portion of the corals' bacterial community represents a food influenced microbiome. The differences between diets were seen in terms of beta diversity, richness, individual OTU dynamics and coral metabolisms (energy reserves). The microbiomes remained, however, species-specific independently of the diet, which suggest that the niche offered to the microbes by the host also shapes community composition. This study, which is the first to test the effect of diet on coral microbiomes, casts a new light on coral microbial ecology by showing that the coral specific bacterial communities should also be considered as a food influenced microbiome. This result is important and will certainly inspire further research on CWC but also on tropical coral microbiomes.

## Summary

Different cold-water coral (CWC) species harbor distinct microbial communities and the community composition is thought to be linked to the ecological strategies of the host. Here we test whether diet shapes the composition of bacterial communities associated with CWC. We compared the microbiomes of two common CWC species in aquaria, *Lophelia pertusa* and *Madrepora oculata*, when they were either starved, or fed respectively with a carnivorous diet, two different herbivorous diets, or a mix of the 3. We targeted both the standing stock (16SrDNA) and the active fraction (16SrRNA) of the bacterial communities and showed that in both species, the corals' microbiome was specific to the given diet. A part of the microbiome remained, however, species-specific, which indicates that the microbiome's plasticity is framed by the identity of the host. In addition, the storage lipid content of the coral tissue showed that different diets had different effects on the corals' metabolisms. The combined results suggest that *L. pertusa* may be preying preferentially on zooplankton while *M. oculata* may in addition use phytoplankton and detritus. The results cast a new light on coral microbiomes as they indicate that a portion of the CWC's bacterial community could represent a food influenced microbiome.

## Introduction

Coral growth and health are tightly linked to their associated microbial communities (Bourne et al., 2016). The coral microbiome is extremely diverse (Blackall et al., 2015; Huggett and Apprill, 2019) and has been shown to contribute strongly to coral nutrition and metabolism. Energy acquisition is one of the key microbial process for corals, among which, microbial photosynthesis is the most studied in tropical coral living in shallow waters (Davy et al., 2012). In the deep sea where light is absent, corals are not associated with photosynthetic micro-algae, and heterotrophy becomes the dominant metabolism available for growth. Carbon can then be acquired by predation of plankton, and prey digestion in the gastric cavity is associated to the processes mediated by microorganisms (Agostini et al., 2012). These prokaryotic microorganisms, bacteria and archaea, are involved in cycling key elements such as carbon, nitrogen or sulfur (Bourne et al., 2016) and contribute to the transfer of nutrient and co-factors to the host. The coral bacterial microbiome and its functional role is, however, still not clearly understood, but the host associated bacterial communities should play a major role for nutrient acquisition in deep sea corals.

Deep sea corals, often named cold-water corals (CWC), can form large reefs in the dark ocean. Cold-water corals form structures that are homes for both juvenile and adult marine organisms and they thus play an important role for maintaining deep sea biodiversity (Roberts et al., 2006). Among scleractinian cold-water corals, *Lophelia pertusa* and *Madrepora oculata* are the two key species found globally and these emblematic deep sea species have attracted attention due to their sensitivity to man-made disturbances such as trawling (Hall–Spencer et al., 2002), sedimentation (Larsson and Purser, 2011), microplastic pollution (Chapron et al., 2018) or global warming (Hennige et al., 2015). *Lophelia pertusa* and *M. oculata* are azooxanthellate and are hypothesized to rely on their associated bacterial communities for nutrient acquisition (Neulinger et al., 2008; Middelburg et al., 2015). The

microbiomes of both corals have been investigated separately or together with a range of molecular tools in a number of oceanic regions covering the Mediterranean Sea (Yakimov et al., 2006; Meistertzheim et al., 2016; Galand et al., 2018), the Gulf of Mexico (Kellogg et al., 2009) (Galkiewicz et al., 2011), the Norwegian Sea (Neulinger et al., 2008; Neulinger et al., 2009; Schöttner et al., 2009) and the Atlantic Ocean (Hansson et al., 2009; Van Bleijswijk et al., 2015; Kellogg et al., 2017). Comparisons between the two species and between sites indicate that each coral has a species-specific microbiome (Hansson et al., 2009; Schöttner et al., 2012; Meistertzheim et al., 2016; Galand et al., 2018). In addition, *M. oculata*'s microbiome appears less variable than the *L. pertusa*'s microbiome, which changes within and between sites (Meistertzheim et al., 2016; Kellogg et al., 2017). It suggests that the different microbiomes reflect different ecological strategies between the two species (Meistertzheim et al., 2016). The two coral species have different growth patterns even if they share the same *in situ* habitat (Lartaud et al., 2014; Lartaud et al., 2017), they have different prey capture rates (Tsounis et al., 2010), and they probably have different diets (Mueller et al., 2014; Naumann et al., 2015).

Diet is known to affect the host microbiome, and in the marine environment, a link between food type and the composition of the microbial communities has been established in different fish species (Ringø et al., 2016), but also for octopus paralarvae (Roura et al., 2017), the Norway lobster (Meziti et al., 2012), the Pacific White Shrimp (Anuta et al., 2011) or for the abalone (Tanaka et al., 2004; Gobet et al., 2018). For corals, the effect of diet has been linked to growth and tissue composition in CWC fed with different prey densities (Larsson et al., 2013), but, to our knowledge, no published study has ever experimentally tested the effect of diet on the coral microbiome.

The effect of diet is usually seen on the microorganisms inhabiting the gastric cavity of their host. The gut microbiome, which has a key role for nutrient uptake and for increasing

resistance to pathogens, has been extensively studied in some species, such as human and mouse (Hacquard et al., 2015). In corals, diet should also directly impact the gut microbiome, but it could also impact the microorganisms associated with the mucus. Mucus plays a key role for coral feeding through mucociliary transport of food trapped by mucous secretions, and by the formation of mucous nets to catch preys (Brown and Bythell, 2005). Mucus hosts a diverse microbial community (Glasl et al., 2016), which feed on that source of organic matter, as shown in the cold water corals *L. pertusa* and *M. oculata* (Wild et al., 2008; Wild et al., 2009). The composition of mucus may change depending on the food source (Goldberg, 2018), which in turn can change the composition of the associated bacterial community (Lee et al., 2016).

The aim of our study was to test whether diet shapes the composition of the bacterial community composition associated to two CWC species: *L. pertusa* and *M. oculata*. Both coral species are thought to prey principally on zooplankton but phytoplankton or particles may also be ingested (Carlier et al., 2009; Dodds et al., 2009; Orejas et al., 2016; van Oevelen et al., 2016). Our hypothesis was that the microbiome reflects the diet of the corals, and that the impact of the diet would not be the same for corals species that have different ecological strategies. We also hypothesis that the type of diet will impact the fatty acid composition of the corals, as seen earlier with both structural and storage fatty acids of *L. pertusa* (Larsson et al., 2013). To test our hypothesis, we conducted a five-weeks aquaria experiment in which corals were either starved, or fed respectively with a carnivorous diet (*Artemia*), an herbivorous diet composed of Eustigmatophyceae (*Nannochloropsis*) or a diatom (*Phaeodactylum*), or a mix of the 3 food sources. The bacterial communities were analyzed by sequencing the 16S rRNA genes (DNA) and 16S rRNA transcripts (RNA) of corals from aquaria experiments and sampled in the field. The nutritional condition of corals was assessed to provide insights on the energy reserves by measuring the sterols, and the concentration and

composition of the storage lipids (wax esters and triacylglycerols), with the aim of determining the corals favorite diet.

## Results

### Sterol content and storage lipid composition

We measured the concentration of sterols in the polyp tissues, which reflects the balance between sterol dietary inputs and metabolic needs, under the different feeding conditions. For *L. pertusa*, there were similar sterol concentrations between conditions although *in situ* samples showed higher values (Table 1). For *M. oculata*, the polyps contained more sterols when fed with an herbivorous diet (*Nannochloropsis* and *Phaeodactylum*). *In situ* polyps also had higher concentrations than polyps that were starved or that were fed with *Artemia* or a mix diet.

We also analyzed storage lipids that provide insights on the energy reserves. In *L. pertusa*, polyps fed with *Artemia salina* contained ca. 4 times more wax esters than the *in situ* polyps, whereas polyps fed with *Nannochloropsis* and mix diet had lower wax ester contents (Fig. 2a). *L. pertusa* fed with *Phaeodactylum* sp. and the starved corals had wax ester concentration close to 0 (no long-term reserves). Triacylglycerol concentrations were highest in polyps fed with *Artemia salina*, *Nannochloropsis* sp. or the mix diet, and lowest in the starved *L. pertusa* polyps, as well as in those fed with *Phaeodactylum* sp. and the *in situ* condition (Fig. 2a). Feeding with *Artemia salina* and *Nannochloropsis* sp. yielded high PUFA contribution, but the triacylglycerols from the mix diet were depleted in PUFA (Fig. 2b). Low PUFA percentages were measured in polyps fed with *Phaeodactylum* sp., the mix diet and the *in situ* (Fig. 2b). The waxes of the corals fed with *Phaeodactylum* sp. were furthermore depleted in polyunsaturated fatty acids (PUFA) in comparison to the other diets and the *in situ* condition (Fig. 2b).



In *M. oculata*, the highest wax ester concentration was observed with the mix diet (Fig. 2a) and it corresponded to a higher PUFA content of the wax ester (Fig. 2b). Triacylglycerol concentration was higher for corals fed with *Nannochloropsis* and *Phaeodactylum*, whereas the *Artemia* and mix diets had similar lowest values (Fig. 2a). Overall, the triacylglycerols contained low percentages of PUFA in *M. oculata* (Fig. 2b).

### Community composition

We compared the corals' bacterial community composition between feeding protocols based on both the active fraction of the community (16S rRNA) and the standing stock (16S rDNA). For both coral species, *in situ* communities were different from all experimental communities (Table 1). Within experimental communities, the RNA fraction was separated from the DNA fraction on the MDS plot (PERMANOVA,  $p=0.01$ ). Further, communities from the DNA fraction for both *M. oculata* and *L. pertusa* were more dispersed than communities from the RNA fraction (mean of Bray Curtis dissimilarity, t-test  $p>0.001$ , Supplementary Fig. 1).

DNA had higher community richness than RNA communities for both *M. oculata* and *L. pertusa* (Fig. 2). For *M. oculata*, *in situ* richness was always lower. For *L. pertusa*, *in situ* richness was lowest only for the DNA fraction. The different feeding experiments did not have a significant effect on the richness of the communities (pairwise t test,  $p>0.05$ ).

We further focused on the RNA fraction that showed less variability within experimental conditions, and that better represents the active fraction of the bacterial community. At the RNA level, independently of the treatment, *L. pertusa* bacterial communities always clustered away from *M. oculata* communities (Fig. 3). For both species, the *in situ* communities were different from the aquaria communities. For *L. pertusa*, bacterial communities grouped according to feeding conditions *Artemia*, *Nannochloropsis* and *Phaeodactylum* (PERMANOVA,  $R^2=0.46$ ,  $p=0.005$ ), while the communities of the conditions

starved and mix where dispersed in the dendrogram (Fig. 3). For *M. oculata*, bacterial communities grouped according to all feeding conditions: *Artemia*, *Nannochloropsis*, *Phaeodactylum*, starved and mix ( $R^2=0.51$ ,  $p=0.001$ ) (Fig. 3). Notably, the *Artemia* fed corals bacterial communities grouped together close to the starved communities, and the mix and *Phaeodactylum* grouped together.

At the phylum/class level, the *Alphaproteobacteria* dominated in all *L. pertusa* samples but there were fewer *Alphaproteobacteria* sequences under herbivorous diets and *in situ*, when some additional phyla increased such as *Firmicutes* under *Phaeodactylum* diet, *Betaproteobacteria* with *Nannochloropsis*, and *Deltaproteobacteria* and *Lentishpaerae* for *in situ* corals (Fig. 4). *M. oculata* had overall more *Gammaproteobacteria* but *Alphaproteobacteria* were dominant for starved corals and under *Artemia* and *Nannochloropsis* diet.

*In situ L. pertusa* had OTUs that disappeared during captivity (OTU 260, *Alphaproteobacteria*) while others had reduced abundance (OTU 67, *Deltaproteobacteria*). The OTU 4 (*Alphaproteobacteria*) was maintained under mix food feeding conditions (Fig. 5). All these OTUs were distantly related to sequences from the databases found earlier associated to corals (Table 2). *In situ M. oculata* also had OTUs that decreased in sequence abundance during captivity. OTU 1, identified as *Endozoicomonas sp.* (Table 2), remained more abundant under *Phaeodactylum* and mix feeding, while others (OTU 16 and OTU 127, *Spirochaetes* and *Epsilonproteobacteria* respectively) remain high in the mix diet only (Fig. 5). *M. oculata* OTUs were also similar to sequences previously detected in different coral species (Table 2).

We also identified the OTUs that were typical for specific feeding conditions with SIMPER statistics. SIMPER ranks the OTUs that most contribute to the differences between groups. Corals fed with *Artemia* were characterized by OTUs associated to *Planctomycetes*

(OTU 36 for *L. pertusa* and *M. oculata*), *Bacteroidetes* (OTU 81 for *L. pertusa*) and *Chloroflexi* (OTU 57 for *M. oculata*). Corals that were starved had more *Alphaproteobacteria* represented by the OTU 9 and 11 in *L. pertusa* and OTU 110 in *M. oculata*. The mixed diet highlighted OTUs from *Proteobacteria* (OTU 4 for *L. pertusa* and OTU 25 for *M. oculata*) and *Bacteroidetes* (OTU 34). Corals that were fed with *Nannochloropsis* were characterized by two *Alphaproteobacteria* for both *L. pertusa* and *M. oculata* (OTU 5 and OTU 6, and OTU 5 and OTU 2 respectively). *Phaeodactylum* feeding was characterized by two OTUs belonging to the *Bacteroidetes* (OTU 37 and OTU 41, Table 2) for *L. pertusa* and *Gammaproteobacteria* (OTU101) and *Acidobacteria* (OTU169) in *M. oculata* (Fig. 6, Table 2).

Some OTUs were present in both coral species. It was the case for OTU36 (*Planctomycetes*, Table 2) for corals fed with *Artemia*, OTU9 (*Alphaproteobacteria*) for corals that were not fed, OTU34 (*Bacteroidetes*) for corals fed with a mix of all food, and OTU5 (*Alphaproteobacteria*) in *Nannochloropsis* feeding (Fig. 6).

We detected chloroplastic *Nannochloropsis* and *Phaeodactylum* sequences in polyps fed with these respective diets (Supplementary Fig. 2).

## Discussion

Here we show that the bacterial communities associated to the cold-water corals *L. pertusa* and *M. oculata* changed according to the type of food given to the colonies in aquaria. The changing bacterial communities may represent the gastric cavity microbiomes, which have been shown in other animals to change with diet (Muegge et al., 2011), but it could also be part of the mucus microbiome. The mucus microbial communities, which is different from the one associated with the polyps in *M. oculata* (Hansson et al., 2009), may change according to the chemical composition of the mucus (Lee et al., 2016). A food induced change in mucus

composition (Goldberg, 2018) could then induce a change of the mucus microbiome. The bacteria that adapt to the food taken by the corals may thus represent a portion of the environmentally responsive bacteria, earlier described in tropical corals (Hernandez-Agreda et al., 2018), rather than symbiotic microorganisms *sensu stricto*. We can hypothesize that some of the species-specific microbiomes observed in many corals reflect the specific feeding habit of the coral species, rather than the selection of a host adapted community with strict partner fidelity. Our results confirm that the microbiome's relationship to the host, at the species level, may not always be very strong (Hernandez-Agreda et al., 2016; Hernandez-Agreda et al., 2018).

Interestingly, the two CWC species that we studied retained different microbiomes even when they were fed on a common diet and reared in the same aquarium. The fact that the microbial community composition did not converge between species eating the same food shows that the microbiomes, although being plastic, remained species-specific. Species-specific microbiomes have been observed in both tropical and cold water corals (Bourne et al., 2016; Meistertzheim et al., 2016). The overall coral microbiome can thus probably only vary within the unique ecological niche given by the host. The composition of the corals' microbial communities can thus be defined by both the type of food ingested and the coral's phenotype.

Even though similar diet did not promote the appearance of similar communities between *L. pertusa* and *M. oculata*, some diet specific OTUs were indeed common between the two species. Some of the OTUs found in the corals were similar to bacteria found earlier associated with CWC and many were similar to bacteria detected in marine host such as sponges or gorgonian. All of these sequences originate from uncultured microorganisms so that their metabolisms remain unknown. In all case, the OTUs detected under the different diets were different from the ones detected *in situ*. Reared coral microbiomes are known to change more or less rapidly after captivity, depending on the host species (Röthig et al., 2017;

Galand et al., 2018). Our data suggest that the differences observed between *in situ* and reared corals' microbiomes may be due to the aquaria diet that is different from the *in situ* feeding habits of corals in the natural environment. In the ocean, *M. oculata* microbiomes are dominated by bacteria from the genus *Endozoicomonas* (Meistertzheim et al., 2016), a genus associated to a large panel of marine hosts (Neave et al., 2016). In aquaria, *Endozoicomonas* remained most abundant under the diatom *Phaeodactylum* diet (Fig. 5). We can thus hypothesize that the diatom diet may be the one that is the most similar to the diet that *M. oculata* has *in situ*. In the deep sea, microalgae can be rapidly transported from the surface with sinking surface water during downwelling events, particularly in the Gulf of Lion where dense water shelf cascades enriched in siliceous material are synchronous with high biological production levels (Buscail et al., 1990; Canals et al., 2006). It's a mechanism known in submarine canyons like the Lacaze-Duthiers canyon where the corals for this study were collected. Microalgae can also sink in the form of detrital particles. These algae containing particles could also be an important source of food for *M. oculata*.

We are not aware of any coral studies linking microbiome and diet. The effect of diet on the physiology or behavior of CWC has, however, been studied. Experiments showed for instance that in *L. pertusa*, zooplankton was predominantly captured at low flow velocities whereas phytoplankton was captured at higher flow speed (Orejas et al., 2016). It has also been shown that prey density, in that case nauplii of *Artemia*, had no significant effect on structural and storage fatty acids concentrations (Larsson et al., 2013). Here we assessed the nutritional condition of corals by measuring the sterol content, which reflects the balance between sterol dietary inputs and metabolic needs, and the concentration and composition of 2 storage lipid classes, triacylglycerols and wax esters, which provide insights on the energy reserves (Martin-Creuzburg and Von Elert, 2009; Lesser, 2013). The triacylglycerols are used for short-term energy needs and the wax esters serve as long-term storage deposits. For *L.*

*pertusa*, wax esters were overall the main lipid storage component. The *Artemia* diet resulted in higher concentrations of both storage lipids, but yielded a slightly lower sterol content than for the *in situ* condition. It could mean that the zooplankton diet was the most suitable for *L. pertusa* as illustrated by a better ability to store short-term and long-term forms of energy reserve and PUFAs, which originate directly from the food and are essential for the host metabolism. In contrast, *L. pertusa* relied on its lipid reserves when fed on *Phaeodactylum*, showing a negative energetic budget and a strong depletion in the amount of PUFAs. PUFA are generally not well biosynthesized by marine animals that rather rely on their dietary inputs for somatic growth and metabolism regulation (Brett and Muller-Navarra, 1997). The negative effect of the *Phaeodactylum* diet could be explained by an inefficient digestion (Robert and Trintignac, 1997) or by the siliceous frustules of the diatom that could impair the feeding process (Petersen et al., 2008). This could result in the consumption of the PUFAs stored in the lipid reserves. For *M. oculata*, whose tissues contained overall 3 times less lipid components, the effect of *Artemia* feeding was not as strong as for *L. pertusa*. *Madrepora oculata* seemed to store more wax ester reserves when fed with a mixed diet, but also stored more triacylglycerols and sterols when fed with an herbivorous diet. The latter observation comforts the idea of *M. oculata* thriving with an herbivorous diet, but contradict earlier results suggesting a preference for live zooplankton (Naumann et al., 2015). Overall, the experiment suggests that a carnivorous diet provides more reserves to *L. pertusa*, while the contribution of an herbivorous or mixed diet is more suitable for *M. oculata*.

*M. oculata* has been shown to have a faithful association to its microbiome that does not vary much in nature (Meistertzheim et al., 2016; Galand et al., 2018). Our feeding experiment thus suggests that *M. oculata* may have a preferred *in situ* diet that does not vary much with time or space. Inversely, *L. pertusa* exhibits much larger variations in its *in situ* microbiome (Meistertzheim et al., 2016; Galand et al., 2018). It could suggest that *L. pertusa*

is much more opportunist in its feeding habits (Mueller et al., 2014) and that the different microbiomes observed in the natural environment represent the type of food available at the time of sampling.

To end on a methodological note, it should be mentioned that although the use of RNA has been shown to be a good indicator of the metabolic state or activity of certain marine microbes (Salter et al., 2015), its use has been criticized (Blazewicz et al., 2013). In our study, the fact that the DNA fraction showed highest diversity and highest variability may indicate that it included non-active microbes that were present randomly and not selected by the host. Inversely, the less dispersed RNA data may better represent the bacteria that were metabolically active at the time of sampling and that were more specific to the host. The fact that prey sequences were found in the DNA fraction only and not in the RNA is an indication that the use of RNA can be very useful in experimental studies.

### **Conclusions**

In conclusion, our experiment casts a new light on coral microbiomes as it shows that the CWC bacterial community varies with the type of diet. The communities remained, however, species-specific independently of the diet, which suggest that the niche offered to the microbes by the host also shapes community composition. It could also mean that within the host associated bacterial communities, one portion represents a classical gut microbiome while another part represents a steady community faithfully associated to the host. Further gut microbiome researches, with dedicated sampling (Agostini et al., 2012), should be conducted to better understand the role of the bacteria in the digestive process of the coral and to disentangle possible host specific versus food specific compartments of the microbiome.

## Experimental Procedures

Coral fragments of *L. pertusa* and *M. oculata* were sampled in the Lacaze-Duthiers submarine canyon off the Gulf of Lion coast in the northwestern Mediterranean Sea (42°32'0.72" N; 03°25'0.26" W) at ca. 530 m depth in July 2012 using the R/V Minibex and ROV SuperAchille (COMEX) as described earlier (Chapron et al., 2018). The coral fragments meant as 'in situ samples' were immediately flash frozen in liquid nitrogen on board and the rest of the coral fragments were transferred to an aerated 30 L seawater tank maintained in the dark at 13°C using a chiller. Once in the laboratory, live corals were fixed to cement blocks using an aquatic epoxy resin and kept in a 80 L aquarium in the dark and at constant temperature (13°C) with a continuous flow (>1 renewal day<sup>-1</sup>) of filtered (5 µm) Mediterranean seawater pumped from 5 m depth (Chapron et al., 2018; Orejas et al., 2019), and fed every 3 days with freshly hatched *Artemia salina* nauplii (1000 L<sup>-1</sup>). Corals were starved for 2 weeks before the start of the experiment.

## Experimental design

A total of five aquaria (10 L) were used for the experiment, each dedicated to a specific experimental diet. Each aquarium contained fragments of both *L. pertusa* and *M. oculata*. For each experimental condition, corals were fed every 3 days with respectively freshly hatched *Artemia salina* (1000 nauplii L<sup>-1</sup>), the diatom *Phaeodactylum tricornutum* (20 mL of in house culture taken during the exponential growing phase of the algae), the algae *Nannochloropsis gaditana* (3 mL of commercial culture, 3x10<sup>9</sup> cells mL<sup>-1</sup>, Greensea, Mèze, France), a mix of the 3 diets (500 nauplii, 10 mL *Phaeodactylum* and 1.5 mL *Nannochloropsis*) or not fed at all. For simplicity, the different diet will be further mentioned as respectively *Artemia*, *Phaeodactylum*, *Nannochloropsis*, mix and starved. The experiment lasted for 5 weeks. Water temperature was maintained at the canyon's *in situ* temperature (13°C), with a continuous



flow (>1 renewal day<sup>-1</sup>) of oxygenated and filtered seawater (5 µm) and corals were maintained in the dark.

Coral fragments were sampled at the end of the experiment. A total of 3 polyps from each species and each experimental condition were taken for microbial analyses whereas 3 polyps of *L. pertusa* and 10 polyps of *M. oculata*, which are smaller, were preserved for the lipid analysis. Coral samples were flash frozen in liquid nitrogen and then stored at -80°C.

### **Lipids**

Total lipids were extracted from the freeze-dried polyps with chloroform: methanol (2:1) (Bligh and Dyer, 1959). The 3 lipid classes that dominate in these CWC species (sterols, wax esters and triacylglycerols) (Pruski et al. personal observation) were then separated by solid phase extraction (SPE) on aminopropyl strata-NH<sub>2</sub> cartridges (Phenomenex, Le Pecq, France) using solvent of increasing polarity (Kaluzny et al., 1985). The sterol fraction was assayed calorimetrically by the sulfovanillic method (Barnes and Blackstock, 1973) using a cholesterol standard. Sterol contents are expressed in mg of cholesterol equivalent and normalized per gram of polyp. The wax ester and triacylglycerol fractions were transesterified to give fatty acid methyl esters (FAME) and the derivatives of each fractions were separated and analysed by gas chromatography coupled to mass spectrometry (Pruski et al., 2017). Wax ester and triacylglycerol concentrations are calculated as the sum of all of fatty acids and normalized per gram of organic matter. Among all fatty acids, the contribution of polyunsaturated fatty acids (PUFA) is presented separately.

### **DNA and RNA extractions and sequencing**

DNA and RNA was extracted from three different polyps, originating from a same colony, for each species (supplementary Table 1). Polyps were crushed separately using a hammer and

the tissues were homogenized with homogenization buffer (Maxwell® simply RNA Tissues Kit LEV) in tubes containing a garnet matrix using a FastPrep Instrument (MP Biomedical, Santa Ana, CA, United States). The samples were then divided into two tubes, one for RNA extraction and one for DNA extraction. RNA and DNA were extracted using, respectively, the Maxwell® simply RNA Tissues Kit LEV and the Maxwell® Blood DNA Purification Kit LEV (Promega, Madison, WI, United States) on a Maxwell 16 MDx Instrument (Promega) following the manufacturer instructions. DNA and RNA concentrations were measured by spectrophotometry (Nanodrop ND-1000, Thermo Fisher Scientific, Waltham, MA, United States). The RNA samples were reverse-transcribed to cDNA with random primers using the RevertAid™ H Minus First Strand cDNA Synthesis kit (Life Technologies).

For both the DNA and cDNA, the V1–V3 region of the bacterial 16S rRNA genes were amplified using bacteria specific primers 27F - AGRGTTTGATCMTGGCTCAG and 519R - GTNTTACNGCGGCKGCTG with a single step and 28 cycles of PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, United States) under the following conditions: 94 °C for 3 minutes, followed by 28 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, after which a final elongation step at 72 °C for 5 minutes was performed. Barcodes were added to the sequences during the PCR step. Following the PCR, all the amplicon products were quantified by spectrophotometry (Nanodrop ND-1000, Thermo Fisher Scientific, Inc., Waltham, MA, United States) and the different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, United States). Purified PCR products were used to prepare a DNA library by following the Illumina TruSeq DNA library preparation protocol. All samples were sequenced on the same Miseq Illumina sequencer run (Illumina, San Diego, CA, United States) using Miseq reagent kit V3 (Illumina) producing 2 × 300-bp long reads. Negative controls were added to the PCR and sequencing, and the reactions were conducted in a

commercial laboratory (MR DNA, Shallowater, TX, United States). The raw data of 16S rRNA gene sequences have been submitted to the NCBI Sequence Read Archive (SRA) database under the BioProject number PRJNA514441.

### Sequence Analysis

All the reads that had a mismatch with the 16S rRNA primers, contained ambiguous nucleotides (N) or were <300 bp long beyond the forward primer were removed. In addition, a stringent quality trimming criteria was applied to remove reads that had  $\geq 10\%$  of bases with Phred values <27. This procedure is recommended to ensure that when clustering at 97% or more, the influence of erroneous reads is minimized (Huse et al., 2010; Kunin et al., 2010). The sequences were then de-replicated and clustered at a 97% threshold using UCLUST (Edgar, 2010) for de novo operational taxonomic unit (OTU) picking. Representative sequences were classified against the SILVA v.128 database (Quast et al., 2013). Sequence data analyses were conducted with Pyrotagger (Kunin and Hugenholtz, 2010). Sequences selected for further analysis were compared manually to the Genbank database by BLAST. Putative chimeric sequences were removed. They were identified as sequences having a best Blast alignment <90% of the trimmed read length to the reference database, >90% sequence identity to the best Blast match and OTU size  $\leq 2$ .

### Statistics

All chloroplast sequence were removed and the samples were randomly re-sampled to match the size of the sample containing the fewest sequences (n = 6981). The sequence abundance table was transform with Hellinger transformation, which is recommended before ordination (Legendre and Gallagher, 2001). Four samples were removed from further analysis because they were dominated by contaminating sequences matching human skin (Supplementary

Table 1). A multidimensional scaling ordination (MDS) 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. The PERMANOVA assumption of 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 RNA versus DNA community composition for both *L. pertusa* and *M. oculata*. When comparing feeding conditions, for *M. oculata*, the assumption of homogeneity of variances was respected when comparing the conditions *Artemia*, *Nannochloropsis*, *Phaeodactylum*, mixed and starved. For *L. pertusa*, the assumption was respected when comparing the conditions *Artemia*, *Nannochloropsis* and *Phaeodactylum*. The statistical results given in the result section met the assumption. A SIMPER test was performed to identify OTUs that contributed the most to the differences between feeding groups.

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## Figure legends

Figure 1. Multi-dimensional scaling plot (MDS) based on the Bray–Curtis similarity index showing the similarity between bacterial community compositions for the DNA (open circles) and RNA (full circles) fraction in *L. pertusa* (a) and *M. oculata* (b) under *in situ* conditions or fed with *Artemia*, *Nannochloropsis*, *Phaeodactylum* or a mix of all, or starved. The *in situ* *L. pertusa* and *M. oculata* bacterial communities were different.

Figure 2. Boxplots showing bacterial community richness for the DNA and RNA fraction in *L. pertusa* (a) and *M. oculata* (b) under *in situ* conditions or fed with *Artemia*, *Nannochloropsis* (Nanno), *Phaeodactylum* (Phaeo) or a mix of all, or starved. All values are based on triplicates with 4 exceptions (supplementary Table 1).

Figure 3. Dendrogram based on the Bray–Curtis index showing the similarity between bacterial community compositions for the RNA fraction in *L. pertusa* and *M. oculata* under *in situ* conditions (in bold) or fed with *Artemia*, *Nannochloropsis* (Nanno), *Phaeodactylum* (Phaeo), or mix of all (Mix), or starved.

Figure 4. Relative proportion of bacterial sequences at the Phylum/Class level in the RNA fraction of *L. pertusa* (Lp) (a) and *M. oculata* (Mo) (b) under *in situ* conditions or fed with *Artemia*, *Nannochloropsis* (Nanno), *Phaeodactylum* (Phaeo) or a mix of all, or starved. Each sample is represented by the average of 3 replicates.

Figure 5. Sequence abundance selected RNA OTUs characterizing *in situ* corals (SIMPER analysis). See Table 2 for OTU taxonomic affiliation.

Figure 6. Sequence abundance of selected RNA OTUs characterizing *L. pertusa* and *M. oculata* under *in situ* conditions or fed with *Artemia*, *Nannochloropsis* (Nanno), *Phaeodactylum* (Phaeo) or a mix of all, or starved (SIMPER analysis). See Table 2 for OTU taxonomic affiliation.

Table 1 . Concentrations of the main lipid classes within tissues of *L. pertusa* and *M. oculata* under *in situ* conditions, or fed with *Artemia*, *Nannochloropsis*, *Phaeodactylum* or a mix of all, or starved, and the proportion of polyunsaturated fatty acids (PUFA) in the triglycerids and waxes.

|                   |                 | Sterols<br>(mg g <sub>OM</sub> <sup>-1</sup> ) | Triglycerids<br>(mg g <sub>OM</sub> <sup>-1</sup> ) | Waxes<br>(mg g <sub>OM</sub> <sup>-1</sup> ) | PUFA in<br>triglycerids (%) | PUFA in<br>waxes (%) |
|-------------------|-----------------|--|---|--|-----------------------------|----------------------|
| <i>L. pertusa</i> |                 |  |   |  |                             |                      |
|                   | In situ         | 7.0  | 0.4   | 5.0  | 4.5                         | 46.4                 |
|                   | Artemia         | 2.5  | 0.7   | 22.1   | 66.4                        | 52.9                 |
|                   | Nannochloropsis | 3.9  | 1.3   | 2.2  | 48.8                        | 66.1                 |
|                   | Phaeodactylum   | 4.0  | 0.3   | 0.4  | 2.8                         | 15.1                 |
|                   | Mix             | 5.2  | 0.9   | 1.9  | 1.3                         | 33.3                 |
|                   | Starved         | 4.6  | 0.4   | 0.3  | 9.2                         | 34.3                 |
| <i>M. oculata</i> |                 |  |   |  |                             |                      |
|                   | In situ         | 10.8   | 0.4   | 2.2  | 3.8                         | 23.8                 |
|                   | Artemia         | 1.9  | 0.1   | 5.5  | 11.7                        | 43.9                 |
|                   | Nanno           | 17.8   | 1.0   | 2.8  | 1.6                         | 40.0                 |
|                   | Phaeo           | 11.7   | 0.9   | 3.5  | 1.7                         | 43.1                 |
|                   | Mix             | 3.1  | 0.2   | 7.7  | 7.9                         | 52.5                 |
|                   | Starved         | 3.7  | 0.4   | 3.9  | 4.0                         | 34.0                 |

**Table 2. List of the RNA OTUs associated to each feeding conditions for *L. pertusa* and *M. oculata* as determined by SIMPER analysis**

| OTU    | Host              | Feeding       | Blaster result    | Identity | Isolation source     | Reference   | Silva annotation                                  | Reference sequence  |
|--------|-------------------|---------------|-------------------|----------|----------------------|-------------|---|---|
| OTU101 | <i>M. oculata</i> | Phaeodactylum | <u>JQ347359</u>   | 97%      | Acropora pruinosa    | unpublished | Proteobacteria_Gammaproteobacteria_Incertae_Sedis | GATTGAACGCTGGAGGTATGCTTAACACATGCAAGTCGAACGCGAAATTCCTTCGGGAAAGAGTAGAGTGGCGGACGGGTGAGTAACCGTAGGAATCTACCTAAGTGTGGGGGATAACATGGAGAAATTCATGCTAATACCGCATACGCACTACGGGTAAAGAGGGCCTCTTCTTGAAAGCTTTGCATTTAGATGAGCCTGCGTCGGATTAAGCTTGTGGTGGGGTAATGGCCTACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCAATACCTCGTGTGTGAAGAAGGCCTTAGGGTTGTAAAGCACTTTCAATTGGGACGAAGGTTGGTAATTAATACATTGC      |
| OTU169 | <i>M. oculata</i> | Phaeodactylum | <u>FJ403093.1</u> | 95%      | Montastrea faveolata | unpublished | Acidobacteria_Holophagae_Subgroup_10_CA02         | GAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGCGAACGTCCTTCGGTGGCTAGTAGAGTGGCGAACGGGTGAGTAACACGTGGACAACCTGCCTGATGAGGGGGATAACTTTTGAAACGGAAGCTAATACCGCATTTCGCTCGGAGATCGCATGGTCTCTGAGGAAAGACCGCCTATCCTTGGAAAGCGGTTGCATTAGAGGGGTCCGCGGCTGATTAGCTAGTTGGTGGGGTAACGGCCTACCAAGGCGACGATCAGTAGCCGGCCTGAGAGGGCGATCGGCCACACTGGAAGTACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCAACGCCGCGTGGAGGATGAAGGCCTTCGGGTTGTAACTCCTGTGAGGTGGAACGAAAAGCTTT |

|                  |                                      |                                 |  |             |   |                       |  |   |
|------------------|--------------------------------------|---------------------------------|--|-------------|---|-----------------------|--|---|
| O<br>T<br>U<br>2 | M<br>O<br>c<br>u<br>l<br>a<br>t<br>a | Na<br>nno<br>chl<br>oro<br>psis | <u>KX</u><br><u>17</u><br><u>75</u><br><u>02</u><br><u>.1</u>            | 9<br>9<br>% | mild<br>steel -<br>sedimen<br>t                   | Barc<br>o<br>201<br>7 | Proteobacteria_Alphap<br>roteobacteria_OCS116<br>_clade  | GAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGCCCTCTTCGGA<br>GGGAGTGGCAGACGGGTGAGTAACGCGTGGAATCTACCTAGTGGTGGAGGA<br>TAACTTCGGGAAACCGGAGCTAATACTCCATAAGCCCTTCGGGGAAAGTTTT<br>TCGCCATTAGATGAGCCCGCTTAGATTAGCTTGTGGTAGGGTAATGGCCTAC<br>CAAGGCGACGATCTATAGCTGGTCTGAGAGGATGATCAGCCACACTGGA<br>ACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG<br>GCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGATGAAGGCCTTAGGGTTGTA<br>AAACTTTTCATCGGTGAAGATAATGACGGTAGCCGAAGAAGAAGCCCCGGCT<br>AACTCCGTGCCAGCCGCC |
| O<br>T<br>U<br>5 | M<br>O<br>c<br>u<br>l<br>a<br>t<br>a | Na<br>nno<br>chl<br>oro<br>psis | <u>H</u><br><u>Q7</u><br><u>26</u><br><u>81</u><br><u>1.</u><br><u>1</u> | 9<br>9<br>% | Callyspo<br>ngia<br>diffusa<br>(marine<br>sponge) | unp<br>ublis<br>hed   | Proteobacteria_Alphap<br>roteobacteria_Rhodob<br>acterales_Rhodobacter<br>aceae_uncultured_Ros<br>eobacter | GAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGCACCTTCGGG<br>GCGAGCGGCGGACGGGTAGTAACGCGTGGAACGTACCCTTTCTACGGAAT<br>AGCCTCGGGAAACTGAGAGTAATACCGTATACGCCCTTTGGGGGAAAGATTTAT<br>CGGAGAAGGATCGGCCCGCTTAGATTAGATAGTTGGTGGGGTAACGGCCTAC<br>CAAGTCTACGATCTATAGCTGGTTTTAGAGGATGATCAGCAACTGGGACTGA<br>GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAATGGG<br>CGCAAGCCTGATCTAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTCGTA<br>AAAGCTCTTTCCGAGAGATGATAATGACAGTATCTGGTAAAGAAACCCCGGCTAAC<br>TCCGTGCCAGCCGCC  |
| O<br>T<br>U<br>9 | M<br>O<br>c<br>u<br>l<br>a<br>t<br>a | No<br>foo<br>d                  | <u>JQ</u><br><u>25</u><br><u>68</u><br><u>33</u><br><u>.1</u>            | 9<br>8<br>% | surface<br>sedimen<br>t                           | Lenk<br>201<br>2      | Proteobacteria_Alphap<br>roteobacteria_Rhodob<br>acterales_Rhodobacter<br>aceae_uncultured_Ros<br>eobacter | GAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGTGCGAGACCTTCGGG<br>TCTAGCGGCGGACGGGTAGTAACGCGTGGAACATACCCTTCTCTAAGGAATA<br>GCCACTGGAAACGGTGAGTAATACCTTATACGCCCTTCGGGGAAAGATTTATC<br>GGAGATGGATTGGCCCGCTTAGATTAGATAGTTGGTGGGGTAACGGCCTACC<br>AAGTCTACGATCTATAGCTGGTTTTAGAGGATGATCAGCAACTGGGACTGAG<br>ACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAATGGGC<br>GCAAGCCTGATCTAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTCGTA<br>AAAGCTCTTTCCGAGAGATGATAATGACAGTATCTGGTAAAGAAACCCCGGCTAAC<br>TCCGTGCCAGCCGCC |
| O<br>T<br>U      | M<br>O<br>d                          | No<br>foo<br>d                  | <u>FJ</u><br><u>20</u><br><u>32</u>                                      | 9<br>8<br>% | Montast<br>raea<br>faveolat                       | Sun<br>aga<br>wa      | Proteobacteria_Alphap<br>roteobacteria_Rhodob<br>acterales_Rhodobacter                                     | GAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGCACCTTCGGGT<br>GAGCGGCGGACGGGTAGTAACGCGTGGAACGTACCCTTTCTGCGGAATAG<br>CCACTGGAAACGGTGAGTAATACCGCATAACGCCCTTCGGGGGAAAGAATTCG  |

|                       |                          |                 |   |             |  |                             |   |  |
|-----------------------|--------------------------|-----------------|---|-------------|--|-----------------------------|---|--|
| 1<br>1<br>0           | cu<br>la<br>ta           |                 | <u>03</u><br><u>.1</u>  |             | a -<br>diseased<br>tissue                        | 200<br>9                    | aceae_uncultured_Ros<br>eobacter  | GGGAAGGATCGGCCCGGTTAGATTAGGTAGTTGGTGGGGTAACGGCCTACCA<br>AGCCTACGATCTATAGCTGGTTTTAGAGGATGATCAGCAACACTGGGACTGAGA<br>CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGGACAATGGGCG<br>CAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTCGTAAAG<br>CTCTTCGCCAGGGATGATAATGACAGTACCTGGTAAAGAAACCCCGGCTAACT<br>CCGTGCCAGCCGCCGCC   |
| O<br>T<br>U<br>5<br>7 | M<br>O<br>cu<br>la<br>ta | Art<br>emi<br>a | <u>KF</u><br><u>18</u><br><u>07</u><br><u>13</u><br><u>.1</u> | 9<br>9<br>% | Eunicella<br>verrucos<br>a                       | Ran<br>som<br>e<br>201<br>4 | Chloroflexi_Anaeroline<br>ae_Anaerolineales_An<br>aerolineaceae         | GGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGGGAAGCGACCT<br>TCGGGTCGTGGAGAGTGGCGAACGGGTGAGTAACACGTAGCTGACCTGCCCTC<br>AAGACGTGGATAACTATTGGAAACAGTAGCTAATACACGATAAGCTCACGTATA<br>ATAGAGATATGTGAGAAAAGTTAAGGCGCTTGAGGATGGGGCTGCGAGCCATC<br>AGCTAGTTGGTGAAGTAAAAGCTCACCAAGGCGACGACGGCTAGGGGACCTGA<br>GAGGGTGACCCCCACACTGGAAGTGAAGACACGGTCCAGACTTCTACGGAAGG<br>CAGCAGTGAGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCG<br>TGGGTGAAGACGGTTTTTCGGACTGTAAAGCCCTTTTCTAGGTGACGAGAGTGGA<br>CGGTAGCCTAGGAATAA |
| O<br>T<br>U<br>3<br>6 | M<br>O<br>cu<br>la<br>ta | Art<br>emi<br>a | <u>JQ</u><br><u>19</u><br><u>56</u><br><u>54</u><br><u>.1</u> | 8<br>8<br>% | seawater   | unp<br>ublis<br>hed         | Planctomycetes_BD7-<br>11   | GAACGAACGCTAGCGGCGTGGATTAGGCATGCAAGTCGAGCGAGAAAGCCTTT<br>CGGGGTTAGTAAAGCGGCAAAAGGGGTAGTAATGAATAGGTAACGTGCCCAAC<br>GGTACGGGATAACTGTTAGAAATGACAGCTAATACCGTATACGCTATACGTAGG<br>AAAGCAGGGGATCTTCGGACCTTGCGCCGATGGAGCGGCCTATTTGACATTAGA<br>TAGTTGGAGAGGTAACGGCTCACCAAGTCATAGATGTCTAGGGGACCTGAGAG<br>GGTGACCCCCACCACCGGAAGTGAAGTCTGACGGAGCGACGCCGCGTGAG<br>GGATGAAGGCCCTAGGGTTGTAAACCTCTGACAGGTATTAAGAATAACTAACT<br>CTAATATAGTTTAAAG   |
| O<br>T<br>U<br>3<br>4 | M<br>O<br>cu<br>la<br>ta | Mix             | <u>KU</u><br><u>68</u><br><u>88</u><br><u>80</u><br><u>.1</u> | 9<br>4<br>% | Cystoseir<br>a<br>compres<br>sa<br>(seawee<br>d) | Man<br>cuso<br>201<br>6     | Bacteroidetes_Sphingo<br>bacteria_Sphingobact<br>eriales_Saprospiraceae | GGATGAACGCTAGCGGGAGGCTTAATACATGCAAGTCGAAGGACCATTTCCGGT<br>GGGACTGGCGCACGGGTGAGTAACGCGTACACTACCTACCTTTTACTGGGGGAC<br>AGCCTTTGGAACGAAGATTAATACCCCATAGTATCGAGAGATTAAAGCTTCGG<br>CGGTAGAAGATGGGTGTGCGTATCATTAGATAGTTGGTGAAGTAAAGGCTCAC<br>CAAGTCAGCGATGATTAGGGGGCGTGAGAGCGTGACCCCCACACGGGTAAGT<br>AGACACGGACCCGACTCCTACGGGAGGCAGCAGTAAGGAATATTGGACAATGG  |

|                       |   |                               |  |                  |                                 |                                      |   |   |
|-----------------------|---|-------------------------------|--|------------------|---------------------------------|--------------------------------------|---|---|
|                       |   |                               |  |                  |                                 |                                      | GCGGAAGCCTGATCCAGCCATCCCGCGTGTAGGATGACTGCCCTATGGGTTGTA<br>AACTACTTTTGTATGAGAAGAAACGCCTGTATTTATACGGGTTTGACGGTATCAT<br>AAGAATAAGCACCG |   |
| O<br>T<br>U<br>2<br>5 | M<br>.<br>O<br>c<br>u<br>l<br>a<br>t<br>a | Mix                           | <u>KX</u><br><u>26</u><br><u>14</u><br><u>82</u><br><u>.1</u>            | 9<br>9<br>9<br>% | Marine<br>biofilm               | unp<br>ublis<br>hed                  | Proteobacteria_Alphap<br>roteobacteria_Rhodob<br>acterales_Rhodobacter<br>iaceae  | GAACGAACGCTGGCGGTAGGCCTAACACATGCAAGTCGAGCGATCTCTTCGGA<br>GATAGCGGCGGACGGGTGAGTAACGCGTGGGAACATACCCAGAGGTACGGAA<br>CAACAGTTAGAAATGACTGCTAATACCGTATACGCCCTACGGGGAAAGAATTT<br>CGCCTTTGGATTGGCCCGCGTTGGATTAGATAGTTGGTGGGGTAACGGCCTACC<br>AAGTCTACGATCCATAGCTGGTTTGAGAGGATGATCAGCCACACTGGGACTGAG<br>ACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGAATCTTAGACAATGGGC<br>GCAAGCCTGATCTAGCCATACCGCGTGAGTGATGAAGGCCCTTAGGGTCGTAAA<br>GCTCTTCGCCAGGGAAGATAATGACTGTACCTGGTAAAGAAGTCCCGGCTAAC<br>TCCGTGCCAGCCGCCG  |
| O<br>T<br>U<br>3<br>7 | L.<br>p<br>e<br>r<br>t<br>u<br>s<br>a     | Pha<br>eod<br>act<br>ylu<br>m | <u>KU</u><br><u>68</u><br><u>91</u><br><u>22</u><br><u>.1</u>            | 9<br>9<br>9<br>% | Cystoseir<br>a<br>compres<br>sa | Man<br>cuso<br>et<br>al.<br>201<br>6 | Bacteroidetes_Flavoba<br>cteriia_Flavobacteriale<br>s_Flavobacteriaceae   | GGATGAACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGTAACATTGGT<br>GCTTGCACCAGATGACGACCGGCGCACGGGTGCGTAACGCGTATGAAACCTAC<br>CTAATACAGAGGGATAGCCCAGAGAAATTTGGATTAATACCTCATGGTACTGTG<br>ATCTCGCATGGGATTATAGTTAAAGATTTATCGGTATTAGATGGTCATGCGTTCT<br>ATTAGTTAGTTGGTAAGGTAACGGCTTACCAAGACGGCGATAGATAGGGGCCCT<br>GAGAGGGGGATCCCCCACTGGTACTGAGACACGGACCAGACTCCTACGGGA<br>GGCAGCAGTGAGGAATATTGGACAATGGAGGCAACTCTGATCCAGCCATGCCG<br>CGTGAAGGAAGACTGCCCTATGGGTTGTAAACTTCTTTTATAGAGGAAGAAACG<br>TGATTACGTGTAAT |
| O<br>T<br>U<br>4<br>1 | L.<br>p<br>e<br>r<br>t<br>u<br>s<br>a     | Pha<br>eod<br>act<br>ylu<br>m | <u>G</u><br><u>U2</u><br><u>35</u><br><u>10</u><br><u>1.</u><br><u>1</u> | 9<br>5<br>%      | seawater                        | Grzi<br>mski<br>201<br>2             | Bacteroidetes_Cytopha<br>gia_Cytophagales_Fla<br>mmeovirgaceae_Reich<br>enbachiella   | GGATGAACGCTAGCGGCAGGCCTAATACATGCAAGTCGAGGGGCAGCAGATT<br>TTCGGATTGGTGGCGACCGGCGCACGGGTGCGTAACGCGTATGCAACCTACCTT<br>ATACACTGGGATAGCCCCGGGAAACTCGGATTAATACCGGATAGCATTATAAAG<br>TGACATCACTTAATAATTAAGATTTATTGGTATAAGATGGGCATGCGTACCATT<br>AGCTAGTTGGTGGGTAACGGCTCACCAAGGCGACGATGGTTAGGGGGCCTGA<br>GAGGGTGGTCCCCCACTGGTACTGAGATACGGACCAGACTCCTACGGGAGG<br>CAGCAGTAGGGAATATTGGTCAATGGACGAGAGTCTGAACCAGCCATGCCGCG<br>TGCAGGAAGACGGCCTTCTGGGTTGTAAACTGCTTTTGACAGGGAAGAAAACG<br>GCTATGAGTAGCTAA    |

|       |            |                 |                   |     |                                      |              |   |   |
|-------|------------|-----------------|-------------------|-----|--------------------------------------|--------------|---|---|
| OTU6  | L. operusa | Nannochloropsis | <u>JN092219.1</u> | 99% | Nephrops norvegicus                  | Meziti 2012  | Proteobacteria_Alphaproteobacteria_Rhodobacterales_Rhodobacteraceae | GAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGGACGCATCCTTCGGGATGAGTGGCGGACGGGTTAGTAACGCGTGGGAATATGCCCTGGGTAAGGAAC AACAGCTGAAAACGGCTGCTAATACCTTATGATGTCTACGGACCAAAGATTTATCGCCAGGGATTAGCCCGCGTTGGATTAGCTAGTTGGAGAGGTAACGGCTCACC AAGGCAACGATCCATAGCTGGTTTGGAGAGGATGATCAGCAACACTGGGACTGA GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAATGGG GGCAACCCTGATCTAGCCATGCCGCGTGATCGATGAAGGCCTTAGGGTTGTAAGATCTTTCCCGGGGACGATAATGACGGTACCCGAGAAAGTCCCGGCTAACTTCGTGCCAGCCGCC       |
| OTU5  | L. operusa | Nannochloropsis | <u>HQ72681.1</u>  | 99% | Callyspongia diffusa (marine sponge) | unpublished  | Proteobacteria_Alphaproteobacteria_Rhodobacterales_Rhodobacteraceae | GAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGCACCTTCGGG GCGAGCGGCGGACGGGTTAGTAACGCGTGGGAACGTACCCTTTCTACGGAAT AGCCTCGGAAACTGAGAGTAATACCGTATACGCCCTTTGGGGGAAAGATTTAT CGGAGAAGGATCGGCCCCGCGTTAGATTAGATAGTTGGTGGGGTAACGGCCTAC CAAGTCTACGATCTATAGCTGGTTTTAGAGGATGATCAGCAACACTGGGACTGA GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAATGGG CGCAAGCCTGATCTAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTCGTAAGCTCTTTCCCGAGAGATGATAATGACAGTATCTGGTAAAGAAACCCCGGCTAAC TCCGTGCCAGCCGCC |
| OTU11 | L. operusa | Noctuid         | <u>AM911356.1</u> | 99% | Lophelia pertusa                     | Neuling 2008 | Proteobacteria_Alphaproteobacteria_Rhizobiales_Hyphomicrobiae       | GAACGAACGCTGGCGGCAGGCTAACACATGCAAGTCGAACGCTCTCTTCGGA GAGAGTGGCAGACGGGTGAGTAACACGTGGGAACCAACCCTTCGGTACGGAAT AGCTCAGGGAAACTTGGGGTAATACCGTATACGCCCTTAGGGGGAAAGATTTAT CGCCGAAGGACGGGCCCCGCGTCTGATTAGCTTGTGGTGGAGTAATGGCTCACC AAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGA GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGG GGAACCTGATCCAGCCATGCCGCGTGAGTGACGAAGGCCTTAGGGTTGTAAGCTCTTTGGTGGGGACGATAATGACGGTACCCACAGAATAAGCTCCGGCTAACTTCGTGCCAGCCGCC    |
| OTU   | L. operusa | Noctuid         | <u>JQ2568</u>     | 98% | surface sediment                     | Lenk 2012    | Proteobacteria_Alphaproteobacteria_Rhodobacterales_Rhodobacter      | GAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGTGCGAGACCTTCGGG TCTAGCGGCGGACGGGTTAGTAACGCGTGGGAACATACCCTTCTTAAGGAATA GCCACTGGAAACGGTGAGTAATACCTTATACGCCCTTCGGGGAAAGATTTATC   |

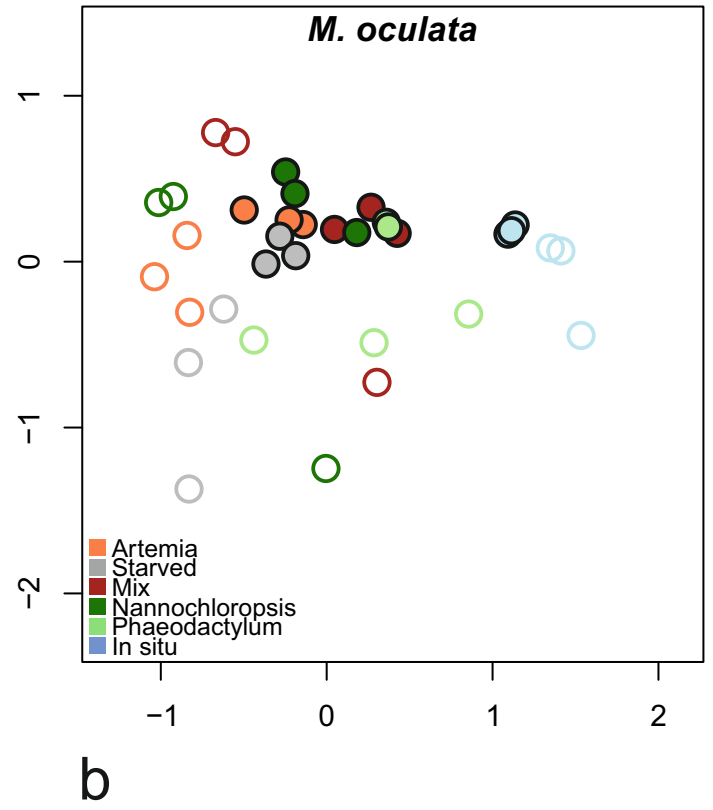
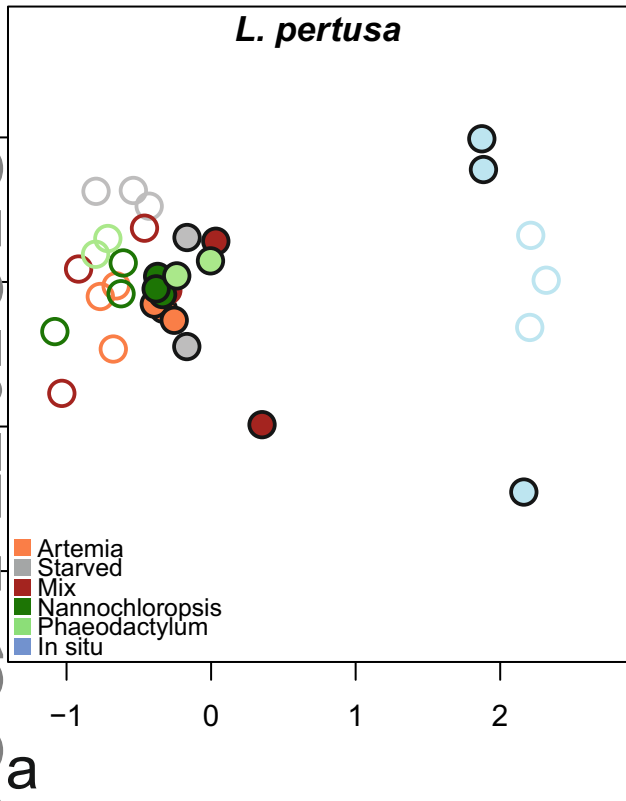


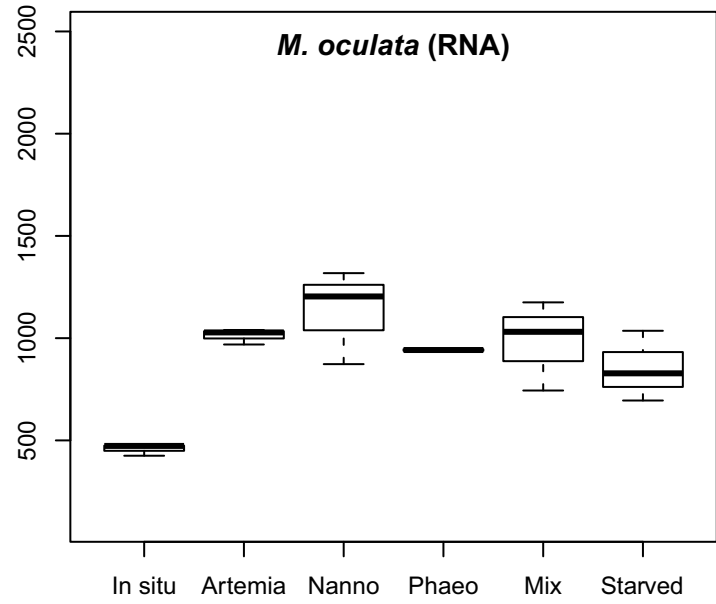
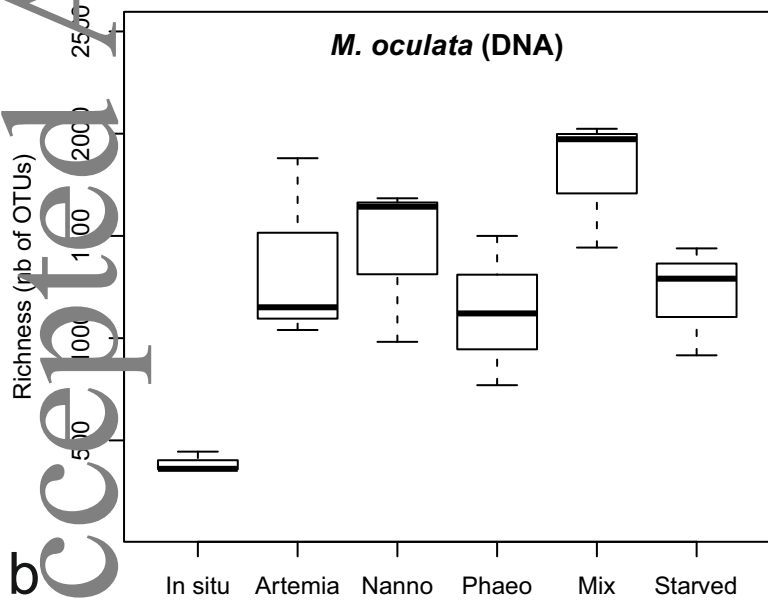
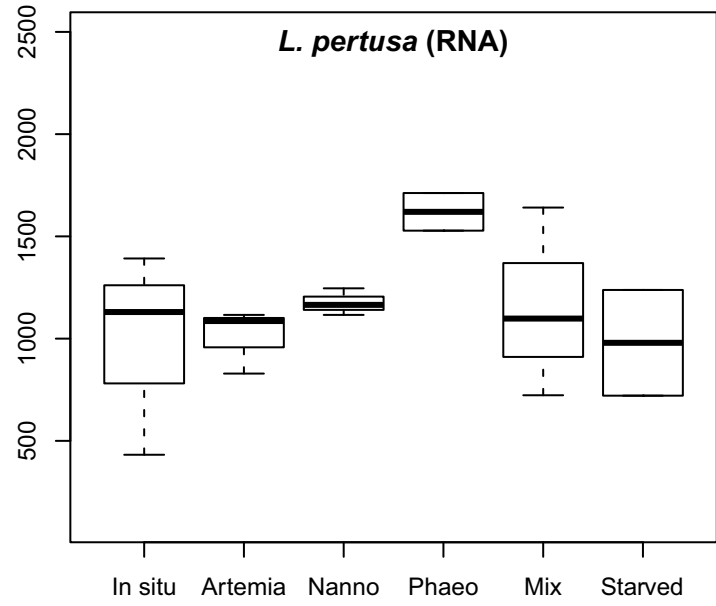
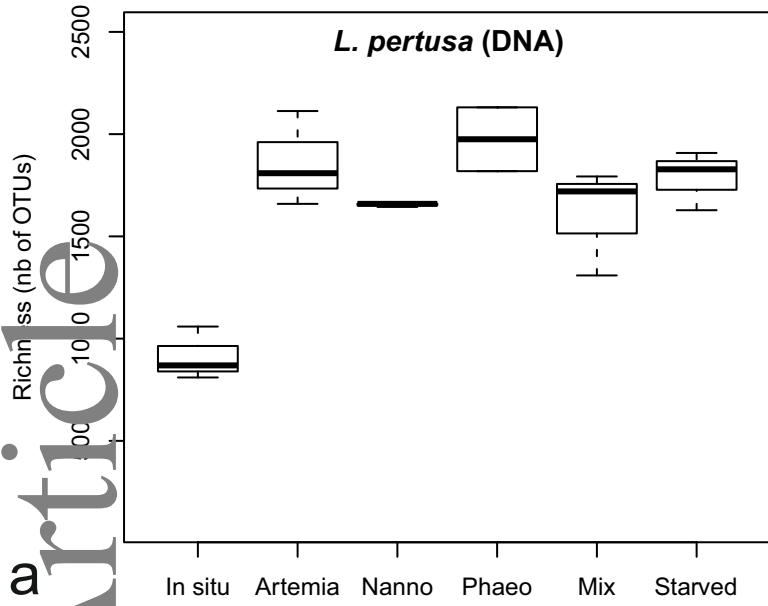
|       |            |          |  |             |                    |                  |  |   |
|-------|------------|----------|--|-------------|--------------------|------------------|--|---|
| 9     | usa        |          | <u>33</u><br><u>.1</u>   |             |                    |                  | aceae  | GGAGATGGATTGGCCCGCTTAGATTAGATAGTTGGTGGGGTAACGGCCTACC<br>AAGTCTACGATCTATAGCTGGTTTTAGAGGATGATCAGCAACACTGGGACTGAG<br>ACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAATGGGC<br>GCAAGCCTGATCTAGCCATGCCCGGTGAGTGATGAAGGCCTTAGGGTCGTAAA<br>GCTCTTCGCCAGAGATGATAATGACAGTATCTGGTAAAGAAACCCCGGCTAAC<br>TCCGTGCCAGCCGCC   |
| OTU36 | L. pertusa | Art emia | <u>JQ</u><br><u>19</u><br><u>56</u><br><u>54</u><br><u>.1</u>            | 8<br>8<br>% | seawater           | unpublished      | Planctomycetes_BD7-11  | GAACGAACGCTAGCGGCGTGGATTAGGCATGCAAGTCGAGCGAGAAAGCCTTT<br>CGGGGTTAGTAAAGCGGCAAAAGGGTAGTAATGAATAGGTAACGTGCCCAAC<br>GGTACGGGATAACTGTTAGAAATGACAGCTAATACCGTATACGCTATACGTAGG<br>AAAGCAGGGGATCTTCGGACCTTGCGCCGATGGAGCGGCCTATTTGACATTAGA<br>TAGTTGGAGAGGTAACGGCTCACCAAGTCATAGATGTCTAGGGGACCTGAGAG<br>GGTGACCCACCACCGGAAGTACTGAGACTGTCCGGACACCTACGGGTGGCTGC<br>AGTCGAGAATCTCCGCAATGGACGAAAGTCTGACGGAGCGACGCCGCGTGAG<br>GGATGAAGGCCCTAGGGTTGTAACCTCTGACAGGTATTAAGAATAACTAACT<br>CTAATATAGTTTAAAG |
| OTU81 | L. pertusa | Art emia | <u>KF</u><br><u>78</u><br><u>65</u><br><u>46</u><br><u>.1</u>            | 9<br>5<br>% | oil sheen          | unpublished      | Bacteroidetes_Cytophagia_Cytophagales_Cytophagaceae_Microscilla  | GGATGAACGCTAGCGGCAGGCCTAATACATGCAAGTCGAACGATCTCTTCGGA<br>GAGAGTGGCGCACGGGTGCGTAACGCGTATGCAACTTACCTTACTGGGGAAT<br>AACCCCGCGAAAGCGGGACTAATACCGCATAATAGATATTGAGGCATCTCATAA<br>TCTTAAAAGGTTTACGGTAGGAGATGGGCATGCGTCCCATTAGCTAGTTGGTAA<br>GGTAATGGCTTACCAAGGCAACGATGGGTAGGGGAACTGAGAGGTTGATCCCC<br>CACACTGGTACTGAGATACGGACCAGACTCCTACGGGAGGCAGCAGTAAGGAA<br>TATTGGTCAATGGACGAGAGTCTGAACCAGCCATGCCGCGTGTAGGAAGAAGG<br>CGTTCTGCGTCGTAACACTTTTTATATAGGAAGAAAAGTTTCTGCGGAAATAA<br>TTGACGGTACTATA |
| OTU4  | L. pertusa | Mix      | <u>H</u><br><u>M</u><br><u>17</u><br><u>32</u><br><u>65</u><br><u>.1</u> | 9<br>6<br>% | Cryogorgia koolsae | Gray et al. 2011 | Proteobacteria_Alphaproteobacteria_Rickettsiales_Anaplasmataceae | GAACGAACGCTAGCGGCAGGCCTAACACATGCAAGTCGAACGAACTAAAATTT<br>AGCTTGCTAAATTTTAGTTAGTGGCAGACGGGTGAGTAACACATGAGAATCTAC<br>CTGATAGTAAGGAATAACCACCAGAAATGGTGGCTAATACCTTATATACCCTGA<br>GGGGAAAGATTTATCGCTATCAGATGAGCTTGTGCTAGATTAGCTTGTGGTA<br>GGGTAATTGCCTACCAAGGCGATGATCTATAGCTGGTCTGAGAGGACGGACAG<br>CCACATTGGAAGTACTGAGATACGGTCTAGACTCCTACGGGAGGCAGCAGTGGGGA  |

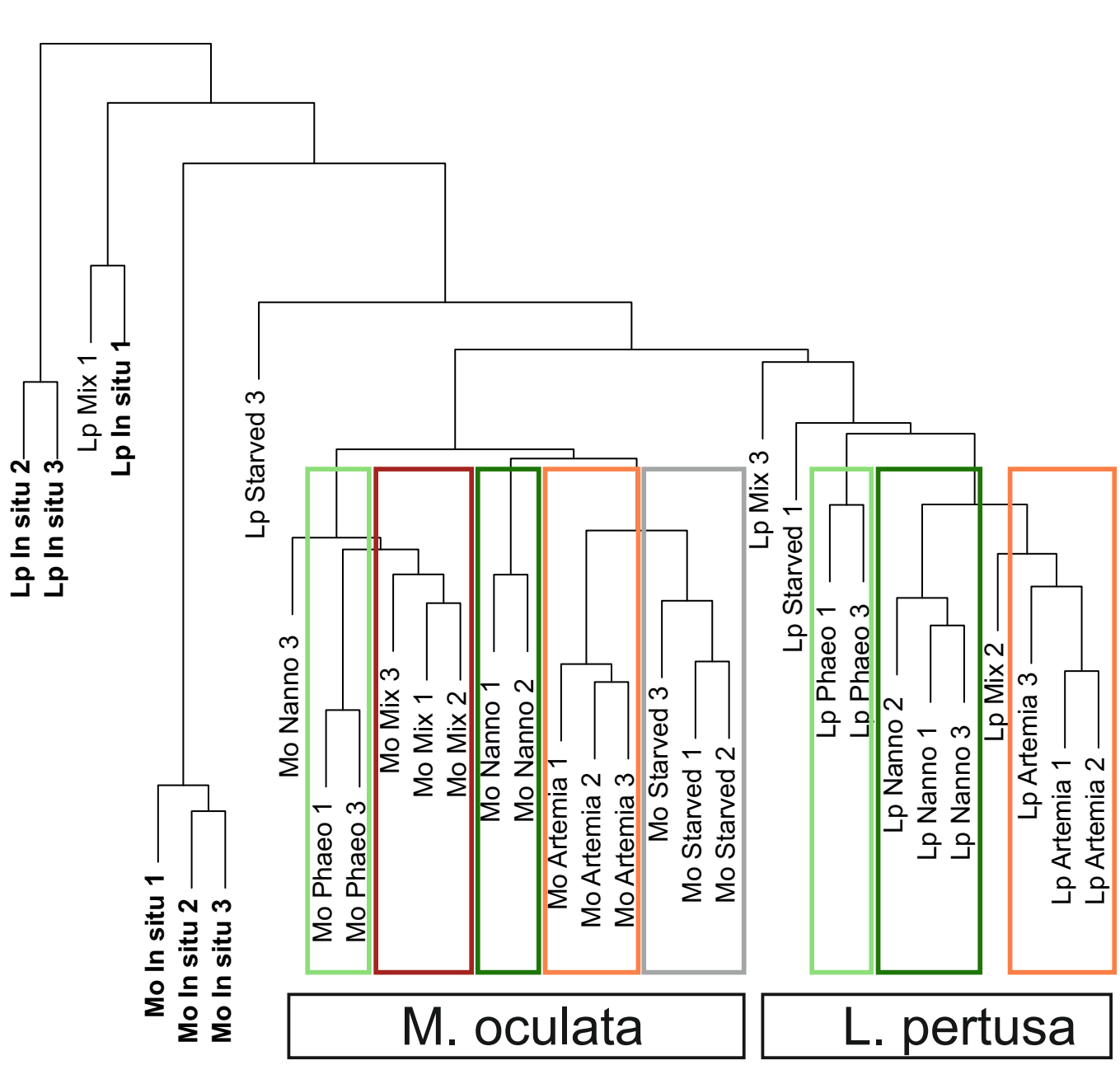
|        |            |         |  |     |                                |             |   |   |
|--------|------------|---------|--|-----|--------------------------------|-------------|---|---|
|        |            |         |  |     |                                |             |   | ATATTGGACAATGAGCGAAAGCTTGATCCAGCCATGCCGCATGAGTGAAGAAG<br>GCTCTAGGGTTGTAAACTCTTTTCAGTGGGAAAGATAATGACGGTACCCACAGA<br>AGAAGTCCTGGCTAA  |
| OTU34  | L. pertusa | Mix     | <u>KU</u><br><u>68</u><br><u>88</u><br><u>80</u><br><u>.1</u>            | 94% | Cystoseira compressa (seaweed) | Manuso 2016 | Bacteroidetes_Sphingobacteriales_Saprospiraceae   | GGATGAACGCTAGCGGGAGGCTTAATACATGCAAGTCGAAGGACCATTTCCGGT<br>GGGACTGGCGCACGGGTGAGTAACGCGTACACTACCTACCTTTTACTGGGGGAC<br>AGCCTTTGGAAACGAAGATTAATACCCCATAGTATCGAGAGATTAAGCTTCGG<br>CGGTAGAAGATGGGTGTGCGTATCATTAGATAGTTGGTGAGGTAACGGCTCAC<br>CAAGTCAGCGATGATTAGGGGGCGTGAGAGCGTGACCCCCACACGGGTA<br>AGACACGGACCCGACTCCTACGGGAGGCAGCAGTAAGGAATATTGGACAATGG<br>GCGGAAGCCTGATCCAGCCATCCCGGTGTAGGATGACTGCCCTATGGGTTGTA<br>AACTACTTTTGTATGAGAAGAAACGCCTGTATTTATACGGGTTTGACGGTATCAT<br>AAGAATAAGCACCG        |
| OTU260 | L. pertusa | in situ | <u>KC</u><br><u>66</u><br><u>84</u><br><u>22</u><br><u>.1</u>            | 95% | Acropora humilis               | Bay er 2013 | Proteobacteria_Alphaproteobacteria_Rickettsiales_SM2D12                                 | GAACGAACGCTTGCGGCAGGCTTAACACATGCAAGTCGGACGGTAAAGAGACT<br>TCGGTTTCTTTATAGTGGCGAACGGGTGCGTAACACGTGGGAACATGCCCATAG<br>GTAGGGGATAACTGCGGGAACTGCAGCTAATACCGTATATGCTCTACGGAGTA<br>AAGATTTATCGCCTATGGATTGGCCCGCGTTCGATTAGATAGTTGGTGGGGTAA<br>TTGCCTACCAAGTCCGTGATCGATAGCTGGTTTGAGAGAATGATCAGCCACATT<br>GGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG<br>ACAATGGAGGAACTCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTCG<br>GGTTGTAAAGCTCTTTTAGCGAGGAAGATAATGACTGTA<br>CTCGCAGAAAAAGCC<br>CCGGCTAACTCCGT |
| OTU4   | L. pertusa | in situ | <u>H</u><br><u>M</u><br><u>17</u><br><u>32</u><br><u>65</u><br><u>.1</u> | 96% | Cryogorgia koolsae             | Gray 2011   | Proteobacteria_Alphaproteobacteria_Rickettsiales_Anoplasmataceae_Candidatus_Xenohalotis | GAACGAACGCTAGCGGCAGGCCTAACACATGCAAGTCGAACGAACTAAAATTT<br>AGCTTGCTAAATTTAGTTAGTGGCAGACGGGTGAGTAACACATGAGAATCTAC<br>CTGATAGTAAGGAATAACCACCAGAAATGGTGGCTAATACCTTATATACCCTGA<br>GGGGGAAAGATTTATCGCTATCAGATGAGCTTGTGCTAGATTAGCTTGTGGTA<br>GGGTAATTGCCTACCAAGGCGATGATCTATAGCTGGTCTGAGAGGACGGACAG<br>CCACATTGGAAGTGAAGTACGGTCTAGACTCCTACGGGAGGCAGCAGTGGGGA<br>ATATTGGACAATGAGCGAAAGCTTGATCCAGCCATGCCGCATGAGTGAAGAAG<br>GCTCTAGGGTTGTAAACTCTTTTCAGTGGGAAAGATAATGACGGTACCCACAGA<br>AGAAGTCCTGGCTAA     |

|                       |                           |            |   |             |                              |                       |   |  |
|-----------------------|---------------------------|------------|---|-------------|------------------------------|-----------------------|---|--|
| O<br>T<br>U<br>6<br>7 | L.<br>pe<br>rt<br>us<br>a | in<br>situ | <u>FJ</u><br><u>19</u><br><u>74</u><br><u>44</u><br><u>.1</u> | 9<br>3<br>% | Sedimen<br>t                 | unp<br>ublis<br>hed   | Proteobacteria_Deltap<br>roteobacteria_Myxoco<br>cales_Haliangiaceae                        | GAGCGAACGTTAGCGGCAGGCTTAACACATGCAAGTCGAGCGAGAACGGGTTCTTCGGAACCTTATTAAAGCGGCGGACGGGTGAGTAACACGTGGGTAATCTGCCCTCCGATGGGGGACAACCATTTCGAAAGAGTGGCTAATACCGCATAACGGCCACCGAATCTGCGGATTTGGTGGGGAAAGGCTTCGGCCGTCCGAGGAGGAGCCCGCGGCGCATCAGCTTGATGGTGGGGTAATGGCCTACCATGGCATAGACGCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTCGGGTCGTAAAGCTCTGTGGAGAGGGACGAACACAATGACGGTACCTC |
| O<br>T<br>U<br>1      | M<br>.O<br>cu<br>la<br>ta | in<br>situ | <u>KC</u><br><u>66</u><br><u>84</u><br><u>20</u><br><u>.1</u> | 9<br>7<br>% | Acropora<br>humilis          | Bay<br>er<br>201<br>3 | Proteobacteria_Gamm<br>aproteobacteria_Ocea<br>nospirillales_Hahellace<br>ae_Endozoicomonas | GATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAACAGGACTAGCTTGCTAGTTGCTGACGAGCGGCGGACGGGTGCGTAACACGTAGGAATCTGCCGGTAGTGGGGGATAGCCCGGAGAAATCCGGATTAATACCGCATAACCCCTAAGGGGAAAAGCAGGGGATCAGTTTACTGACCTTGCCTATCGGATGAGCCTGCGTCCGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCTCTAGGGTTGTAAAGCACTTTCAGCGAGGAGGAAAGGTTTAAGATTAAT     |
| O<br>T<br>U<br>1<br>6 | M<br>.O<br>cu<br>la<br>ta | in<br>situ | <u>KC</u><br><u>66</u><br><u>89</u><br><u>83</u><br><u>.1</u> | 9<br>2<br>% | Stylopho<br>ra<br>pistillata | Bay<br>er<br>201<br>3 | Spirochaetae_Spirocha<br>etes_Spirochaetales_S<br>pirochaetaceae                            | GAACGAACGTTAGCGAGATGTTTTAAGCATGCAAGTCGAGCGGTAACACTGCCTTCGGGTGAAGACGAGCGGCAAACGGGTGAGTAATGAGAAGTTATCTGCCTATTAGACTGGAATAGCCCAGGGAAACCTGGATTAATGCCGGATATGAGGAACTTGAAAGATGCGTTTTGCATCACTAGTAGATGAGACTTCTTCTATTAGCTAGTTGGTGGGGTAATGGCCTACCAAAGTGATTATAGGTAGCCGGCCTGAGAGGGTGATCGGCCACATTGGGACTGAGATACGGCCAGATTCTACGGGAAGCAGCAGCTAAGAATATTCCGCAATGGGGGAAACCCTGACGGAGCAATCTCGCATGGATGATGAAGGCTTCGGATTGTAATACTTTTCGACAGGGGAAGAATGGCTACAGTAGGGAATGACTGTAGAATGACGGTA    |
| O<br>T<br>U           | M<br>.O                   | in<br>situ | <u>G</u><br><u>U1</u><br><u>17</u>                            | 9<br>5<br>% | Acropora<br>cervicor<br>nis  | Sun<br>aga<br>wa      | Proteobacteria_Epsilon<br>proteobacteria_Campy<br>lobacterales_Helicobac                    | GAGTGAACGCTGGCGGCGTGCTTAATACATGCAAGTCGAACGAGAACGGACATAGCTTGCTATGTTGTGCTAGCTAAGTGGCGCACGGGTGAGTAATGTATAGCTAACA<br>TGCCCTTAGCGGGGGATAACAGTTGGAAACAGCTGCTAATACCCCATATTCCTT  |

|   |    |  |           |  |     |          |   |
|---|----|--|-----------|--|-----|----------|---|
| 1 | cu |  | <u>96</u> |  | 201 | teraceae | AATATCGTAAAGTTGTTAAGGGAAAGATTTATCGCTAAAGGATTGGGCTATATG  |
| 2 | la |  | <u>1.</u> |  | 2   |          | GTATCAGCTTGTTGGTGGGGTAAGAGCCCACCAAGGCTATGACGCCTAACTGGT  |
| 7 | ta |  | <u>1</u>  |  |     |          | CTGAGAGGACGAACAGTCACACTGGAAGTGGAGACACGGTCCAGACTTCTACGG<br>AAGGCAGCAGTAGGGAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGC<br>CGCGTGGAGGATGACGGCCTTAGGGTTGTAACTCCTTTTATATGAGAAGATTA<br>TGACGGTATCATA |

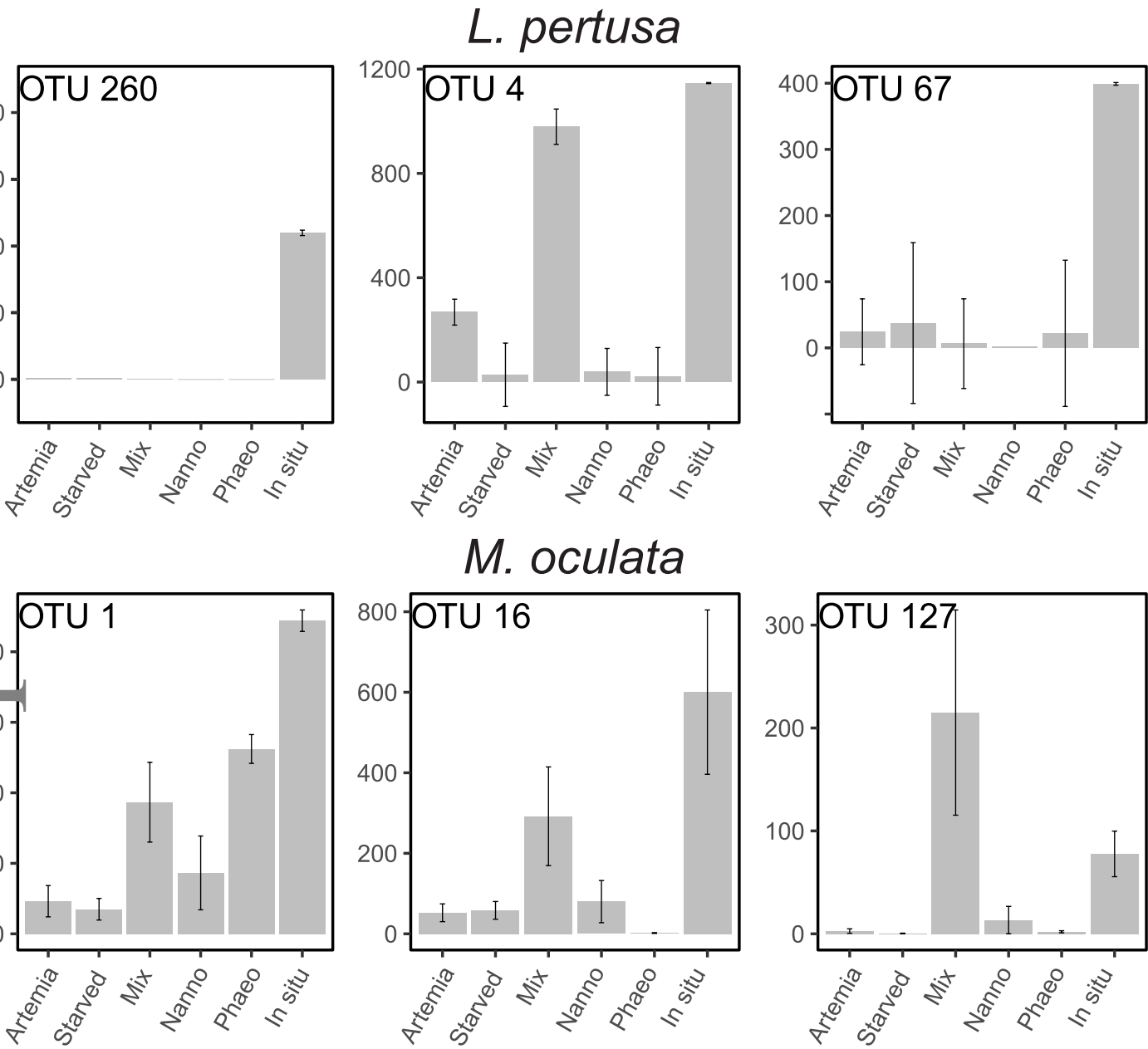












# *L. pertusa*

# *M. oculata*

