‘OCEANS AND LAKES’

INTERUNIVERSITY MASTER OF SCIENCE IN MARINE AND LACUSTRINE SCIENCE AND MANAGEMENT

The effects of probiotic treatments on *Laminaria ochroleuca*

Assessing their potential to enhance kelp resilience under heat stress

Axelle Defossez
June 2025

Thesis submitted in partial fulfilment for the master’s degree in
Marine and Lacustrine Science and Management

Prof. Dr. Olivier De Clerck
Prof. Dr. Aschwin Engelen
Sofie Peeters

Table of Contents

Acknowledgments

This master thesis would not have been possible without the support and involvement of many, to all of whom I would like to express my gratitude.

Firstly, to Ghent University and Professor De Clerck for granting me the opportunity to undertake an exchange at the University of the Algarve in Faro. I am especially grateful to Professor Martins, not only for her excellent supervision, but also for the warm welcome into her lab and team. I would also like to thank Professor Engelen for guiding us. Together with Professor Pearson, you all ensured our stay went smoothly. I am also deeply thankful to Joana, Tiago, Cicero, Rita, and all others in the lab for helping me find my way around, sharing valuable tips and never getting tired of all my questions. To all of you, I owe a special thanks for making my time in and outside the lab truly unforgettable (and for ordering coffee and *pastéis de nata* for me in the cafeteria).

Secondly, to my supervisor Sofie Peeters, for her guidance during the intensive set-up days in Faro and her continued support throughout the follow-up experiments in Ghent. To Professor De Clerck, for brainstorming with us as we explored our next steps following the exciting discoveries in Faro. I would also like to thank everyone in the lab in Ghent for their patience when I fully booked the microscope time slots, and for the pleasant breaks in between endless counting sessions.

Counting all these kelp individuals would not have been possible without being able to count on some keystone individuals in my own life: my family and friends. Especially my parents, my sister, Sarah, Luna, Anne-Sophie and my roommates — thank you for your ongoing support during these challenging times (and for feeding me more times than I can count).

To my colleague and friend Kato, who has been with me throughout this entire journey — from day one in Faro to the final struggles of writing this thesis, and everything in between. Thank you for sharing in the laughter and tears, and for creating memories I will cherish forever.

And lastly, I want to acknowledge that, in a time when many around the world are denied access to safety, education, and freedom, being able to pursue my studies is a privilege I do not take for granted.

Abstract

Kelp forests are one of the most productive marine ecosystems, yet due to climate change and other anthropogenic stressors, they have known serious declines. These forests are characterized to provide ecosystems services as well as primary and secondary habitats. One of the main drivers of their declines is the rising seawater temperature, with marine heat waves increasing in frequency and intensity. This is a trend unlikely to reverse soon.

In recent years, the concept of the kelp holobiont - encompassing the host and its associated microbiota - has gained increasing attention. The stability of this entity is crucial for the survival of kelp, yet the previously mentioned stressors may disrupt this functional host-microbiome symbiosis. Therefore, with increasing external pressures, maintaining or restoring this equilibrium is important. As traditional restoration efforts struggle to keep pace with the rapid rate of climate change, other strategies must be explored.

In this thesis, we investigated the potential of enhancing kelp resilience and reproductive success by adding bacteria we thought to be beneficial to its microbiome. The results indicate that the bacterial strain *Cobetia amphilecti* could be a promising probiotic. This bacterium enhanced the onset and output of reproduction in *Laminaria ochroleuca* and boosted its resilience to heat stress significantly, while maintaining overall physiological health. Although previous studies have shown similar positive effects of microbiota enhancing macroalgae, detrimental outcomes for these microbiome alterations have also been reported. In our study, the effects varied depending on the population to which certain bacterial strains were applied. *Kocuria polaris,* for example*,* inhibited growth in one population while promoting it in others. This highlights the importance of host-specific interactions and the need for a precautionary approach.

Nonetheless, this research supports the potential of probiotic treatments for kelp forest restoration, especially when combined with restoration methods such as green gravel, where kelp spores or gametophytes are applied to substrates and cultivated under controlled conditions before outplanting. This research opens new avenues for enhancing the resilience of these vital ecosystems in a rapidly changing ocean.

Keywords*: Laminaria ochroleuca*, kelp holobiont, probiotic treatment, marine heatwaves, microbiome engineering, climate change resilience, recruitment success of kelp, kelp restoration, *Cobetia amphilecti*, *Kocuria polaris*

1. Introduction

1.1. Climate change and marine ecosystems

Marine ecosystems are undergoing rapid changes due anthropogenic stressors like climate change (Wernberg et al., 2018). Rising seawater temperatures, ocean acidification, and deoxygenation are already exerting significant negative effects on biodiversity, coastal resilience, and global carbon cycling (IPCC, 2019). These impacts are expected to intensify with continued greenhouse gas emissions, leading to the disruption of ecological communities and the loss of ecosystem services (Smale, 2019).

Among these ecosystems, one stands out as highly productive and ecologically critical habitats: kelp forests (Mann, 1973). These underwater forests, formed by large brown macroalgae, are found in temperate waters of both hemispheres. They typically dominate shallow rocky coasts (Teagle et al., 2017) but can also occur at depths of up to 30–60 meters and are even predicted to extend beyond these depths under certain conditions (Graham et al., 2007).

Vegetated coastal habitats represent up to 10% of the global marine net primary production (Duarte, 2017). Kelp contributes substantially to the carbon fluxes, with kelp detritus representing an average of 301 C m^{-2} yr^{-1} (Smale et al., 2021), this detritus can be transferred to "sink" habitats where it can be stored, highlighting its role in carbon sequestration (Krause-Jensen et al., 2018). With the 2015 Paris Agreement aiming to limit the global temperature increase to 1.5°C above pre-industrial levels, blue carbon is gaining increasing attention as a natural solution to enhance carbon sequestration potential (Macreadie et al., 2019).

1.1.1. Ecological role of kelp

Kelp forest provide food and shelter to a diverse range of marine life (Teagle et al., 2017), protect coastlines by attenuating wave energy (Morris et al., 2019) and serve as both primary and secondary habitats for organisms such as epiphytes (Teagle et al., 2017) and endophytes (Egan et al., 2013).

Besides their ecological function, their economic role is gaining importance with an estimated average annual revenue of $500 billion (Eger et al., 2023). It can be concluded that kelp ecosystems are of significant importance, and their disruption and decline can lead to cascading negative effects on biodiversity and the services they provide (Wernberg et al., 2019). Over recent decades, these forests have experienced severe declines due to increasing anthropogenic stressors, with warming sea temperatures acting as a major amplifier (Filbee-Dexter et al., 2016).

1.1.2. Warming seawater temperatures

Franco et al. (2017) showed that temperature will be the most critical physiological factor for the survival of *L. ochroleuca*, a conclusion also supported by Biskup et al. (2014). In addition to long-term warming trends, short-term extreme events such as marine heatwaves (MHWs) pose an increasingly urgent threat. MHWs refer to anomalous warm seawater events that possibly affect marine ecosystems (Oliver et al., 2021). Because of prolonged ocean warming, due to ongoing climate change, a rise in MHWs is anticipated to persist (Frölicher et al., 2018).

Warming sea temperatures impact kelp both directly and indirectly (Wernberg et al., 2019). MHWs can directly cause mortality through thermal stress and disrupting reproduction (Smale et al., 2019). Given their non-mobile nature and restricted distribution within a specific temperature range (Hobday et al., 2016), they may be particularly vulnerable to temperatures increasing beyond their upper physiological limits (Wiens, 2016). The effects vary with species, local thermal history, and the intensity of the MHW (Wernberg et al., 2019). While moderate warming may boost growth below thermal optima (Smale & Wernberg, 2013), temperatures above these limits lead to mortality (Wernberg et al., 2012).

Although kelp possesses the ability to mitigate negative effects of climate-related disturbances (Bertocci et al., 2014), they still face severe declines or changes in their distribution patterns (Krumhansl et al., 2016; Figure 3). Especially in low latitudes, declining trends in macroalgae linked to temperature increases have been observed (Fernández, 2011).

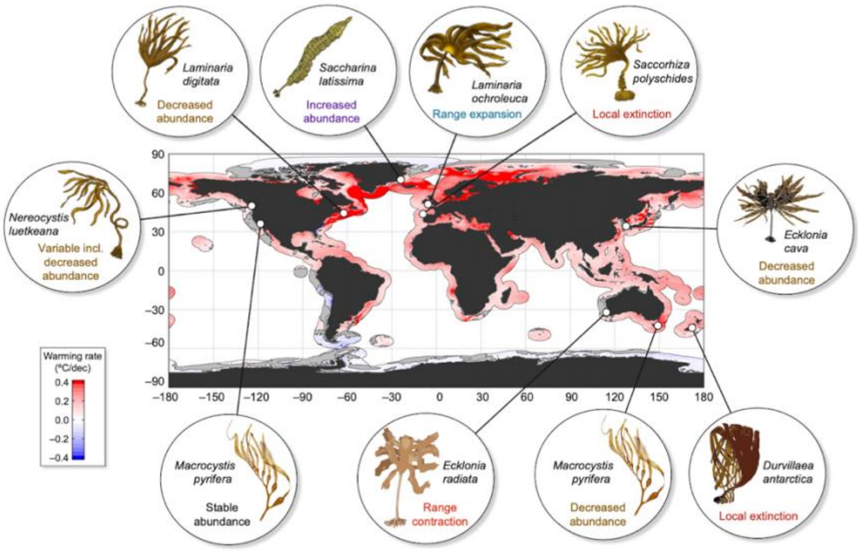

Figure 1. Distribution and responsens of kelp populations and ecosystems to ocean warming. Source: Smale (2019).

1.2. The holobiont perspective

To understand this functional entity, it is not only important to understand the host (section 1.2.1.) and its associated microbiota (section 1.2.2.), but also how they assemble and interact (section 1.2.3.).

1.2.1. The golden kelp *Laminaria ochroleuca*

Laminaria ochroleuca – or the 'Golden Kelp' – is a species from the order Laminariales (Dayton, 1985) and falls under the scope of this thesis. The species is distributed from Morocco to southern UK (Franco et al., 2017). Unlike many other kelp species, *L. ochroleuca* is adapted to warmer temperate waters (Smale et al., 2014).

Like other marine macroalgae, they are multicellular, photosynthetic eukaryotes. Even though they have a plantlike morphology (Figure 2), due to their lack of specialized tissues (e.g. root system and vascular structures), they are differentiated from plants (Schiel & Foster, 2006). The holdfast, resembling the root-system, is purely for attachment to the substrate and does not serve nutritional purposes (Mann, 1973).

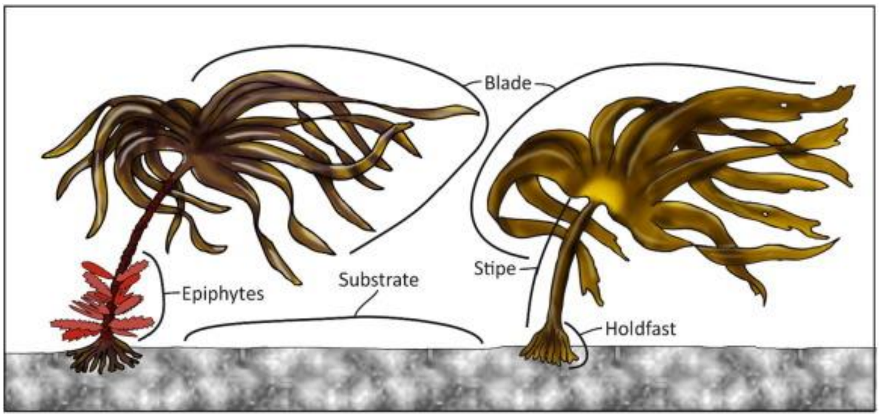

Figure 2. An illustration of Laminaria hyperborea (left) and Laminaria ochroleuca (right) showing the blade, stipe and holdfast alongside the epiphytes as secondary habitat components. Source: Teagle et al. (2017)

1.2.1.1. Lifecycle of L. ochroleuca

L. ochroleuca has a pluriannual haplo-diplontic lifecycle (Biskup et al., 2014), meaning it contains of two free-living stages: a macroscopic diploid sporophyte alternating with microscopic haploid gametophytes (Steneck et al., 2002).

The stages have distinct optimal temperatures. Optimal reproductivity occurs at 15°C to 18°C (Izquierdo et al., 2002). While sporophytes can grow vegetatively in warmer waters, the production of microscopic gametophytes fails when temperatures exceed the range of 10 – 15°C (Pereira et al., 2011). This helps explain their northern distributional limit (Van Den Hoek, 1989). When conditions deteriorate, these microscopic forms enter a slow-developing phase known as the "seed banks". They remain in this vegetative state until conditions become favorable again and the development resumes (Dieck, 1993).

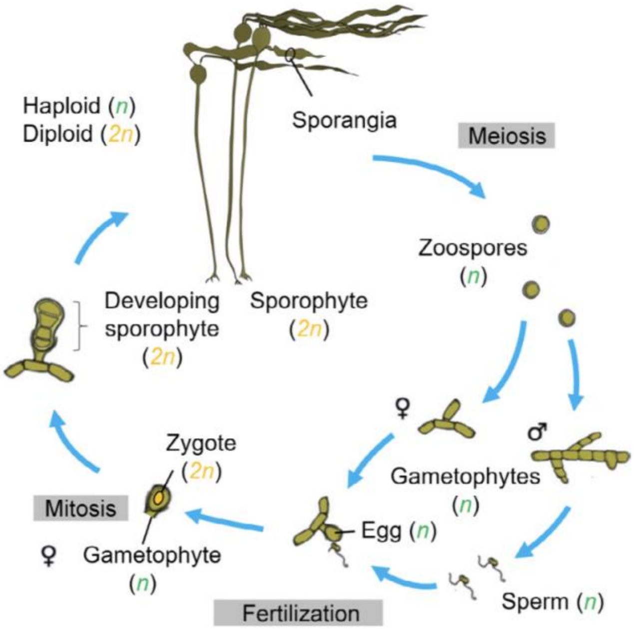

Figure 3. The life cycle of kelp with an alternation of two life stages: a haploid gametophyte and a diploid sporophyte. Source: Wernberg et al. (2019)

Figure 3 illustrates this lifecycle. Once this gametogenesis is initiated by blue-light (Lüning, 1981), male gametophytes produce motile, biflagellate sperm, while female gametophytes develop elongated oogonia containing eggs. Each oogonium releases a single egg prior to fertilization (Kim & Bhatnagar, 2011). The eggs secrete a pheromone called lamoxirene (Maier et al., 2001). This serves as a chemotaxis for male gametophytes to release their sperm cells and attract them to eggs (Mamer et al., 1984). After fertilization, the zygote forms a flat proembryo that matures into the large, familiar kelp sporophyte structure (Dayton, 1985).

1.2.2. Marine microbiota

Marine macroalgae host dense and diverse microbial communities. Microbiota - consisting of communities of bacteria, archaea, protists, and unicellular fungi - are omnipresent in the oceans waters with abundances exceeding 10^5 mL^{-1} (Sogin et al., 2006). These microorganisms can be found from surface water to deep-sea sediments (Huber et al., 2007). Therefore, they are continuously exposed to changing environmental factors, making them ideal for potential bioremediation or bioindicators of ecosystem change (Dash et al., 2012).

Abundances vary by species, sporophyte tissue region, and season with densities varying from 10^2 to 10^7 cells cm^{-2} (Bengtsson et al., 2010). They form a unified functional entity or holobiont (Figure 3; Egan et al., 2013) and are characterized by their complex interactions (van der Loos et al., 2019). Marine bacteria are involved in key functions that support algal development and health. This includes nutrient exchange, growth hormone production, morphogenesis and biofilm formation. These functions occur through complex biochemical and signaling interactions (Amin et al., 2015; Bengtsson et al., 2012; Singh & Reddy, 2014; Cirri & Pohnert, 2019). A detailed overview of a selection of genera and their functional roles is provided in Appendix Table A - 1.

1.2.3. Microbiome assembly and functional interactions

Some algae rely on their epiphytic bacterial communities for normal morphological development (Spoerner et al., 2012). These bacteria can also provide protection against harmful or secondary colonization by other micro- and macroscopic epibiota, due to their antifouling properties (Rao et al., 2005). These interactions can result in mutualistic, commensal, competitive, or antagonistic relationships, depending on the processes involved (Egan et al., 2013). Thus, the health and functioning of the algal holobiont depend on stable and context-sensitive interactions between the host and its microbial partners (Dittami et al., 2021). The following sections highlight key principles that shape these interactions.

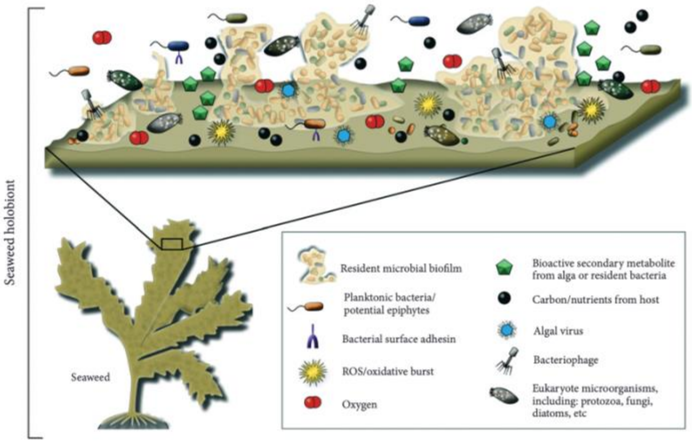

Figure 4. The seaweed holobiont and the various factors or organisms proposed to influence bacterial colonization on macroalgal hosts. Source: Egan et al. (2013)

1.2.3.1. Chemical gardening

How these organisms communicate has been studied by Thøgersen et al. (2018), showing that algae–bacteria interactions are primarily mediated through chemical signalling, rather than direct physical contact between cells. Key mechanisms – such as polysaccharides, secondary metabolite signalling and quorum sensing (QS)— shape the dynamics within the algal holobiont (Saha et al., 2012). QS enables bacteria to detect their own population density and upon reaching a threshold or quorum of cells, initiate changes in gene expression (Vannini et al., 2002). Algae can influence microbial communities by releasing bioactive compounds (Inderjit et al., 2011) and can detect and respond to bacterial QS signals (Zhou et al., 2016)

This selective microbial recruitment is sometimes referred to as “chemical gardening”, a process where macroalgae attract beneficial bacteria while deterring harmful ones (Saha et al., 2020).

1.2.3.2. Host-specifity

This “chemical gardening” further supports the idea that the microbiome composition is largely shaped by the host’s identity, implying host-specificity (Grossart et al., 2005). This means that a specific set of bacterial epiphytes can be found on one species but be absent on another (Egan et al., 2013). Such specificity likely contributes to holobiont stability, as the host selectively maintains beneficial microbial partners across different conditions and locations.

Lachnit et al. (2009) showed that the microbiota of the same algae species from different geographic locations are more similar to each other than to those of other macroalgae species from the same environment or to the surrounding seawater. This supports the idea of species-specific selection process that shapes the microbial community (Egan et al., 2013).

1.2.3.3. Competitive lottery model

While host identity influences microbial community composition, randomness also plays a role. With the competitive lottery model, Burke et al. (2011) suggested that coexisting bacteria randomly colonize ecological niches with similar ecological functions. This highlights the concept of functional redundancy, the notion that multiple microbial taxa can fulfill equivalent roles within the holobiont (Grueneberg et al., 2016).

This suggests that maintaining key microbial functions—rather than specific taxa—may be more important for holobiont health and probiotic applications. This is further supported by the study of van der Loos et al. (2025) on the genus *Ulva* across a 2000-km salinity gradient, where functional changes remain low despite a great taxonomic turnover.

1.2.3.4. Ulva mutabilis: example of interkingdom communication

A well-studied example involves the green macroalga *Ulva mutabilis*. This symbiosis, known as the tripartite interaction *Ulva–Maribacter–Roseovarius* (Burgunter-Delamare et al., 2024), exemplifies quorum sensing (QS)-mediated communication between algae and bacteria. *U. mutabilis* releases dimethylsulfoniopropionate (DMSP) to attract *Roseovarius* species, which in turn use QS signals to coordinate their activity and stimulate algal thallus development (Kessler et al., 2017).

However, development remains incomplete without *Maribacter*, which secretes complementary morphogens. *Roseovarius* and *Maribacter* resemble cytokinin- and auxin-like signaling roles, respectively (Spoerner et al., 2012), and their effects cannot be mimicked by known phytohormones (De Clerck et al., 2018). This indicates that *U. mutabilis* requires at least two bacterial partners with distinct but synergistic functions (Ghaderiardakani et al., 2017), supporting the competitive lottery model of microbial niche colonization (Grueneberg et al., 2016; Burke et al., 2011).

1.2.4. Environmental changes influencing the holobiont

Besides an algae-bacteria interaction, the environment plays a crucial role in shaping the structure and function of the holobiont (Rosenberg & Zilber-Rosenberg, 2016). Van der Loos et al. (2019) proposed three pathways how they can influence the holobiont (Figure 6). Firstly, environmental stressors such as warming or acidification can directly affect the microbial communities on the host (Bengtsson et al., 2010; Erwin et al., 2012). Secondly, factors like MHWs can have a direct effect on the host, e.g. impacting algal physiology (Wernberg et al., 2019; Strasser et al., 2022). Similarly environmental shifts can mediate the effects on interactions and modify the functional roles of microbes or host physiology (Van der Loos et al., 2019). These complex and dynamic interactions highlight the sensitivity of the holobiont to environmental perturbations.

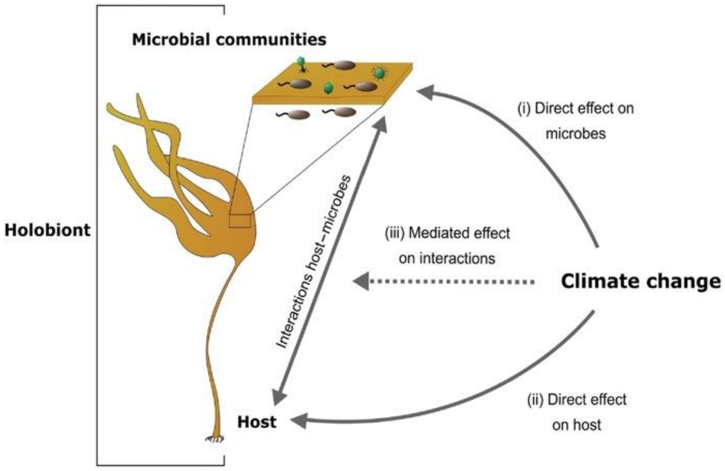

Figure 5. Overview of the holobiont and its interaction with changes in climate either directly effecting the microbiota, the host or the interactions between the host and the microbiota. Source: van der Loos et al. (2019)

1.2.5. Microbial dysbiosis

Holobiont health depends on a dynamic balance between resistance (the ability to maintain stability) and resilience (the ability to recover after disturbance) (Pita et al., 2018). When biotic or abiotic stressors disturb this equilibrium, it may result in dysbiosis, a break-up of the holobiont (Egan & Gardiner, 2016). This disruption increases host susceptibility to disease and stress (Egan & Gardiner, 2016). In marine macroalgae, microbial dysbiosis has been linked to bleaching, tissue necrosis, and reduced reproductive success, threatening the stability of coastal ecosystems (Fernandes et al., 2012; Li et al., 2022; Veenhof et al., 2025; Campbell et al., 2011).

A compelling example of how delicate this balance is can be found in the “Jekyll and Hyde” behavior of certain bacteria. For example, Seyedsayamdost et al. (2011) illustrated that *Phaeobacter gallaeciensis* typically supports algal growth but can switch to a pathogenic role as the host ages.

This duality illustrates that microbial function is not fixed but context-dependent, shaped by both abiotic and biotic conditions. Therefor studying them can reveal mechanisms that maintain holobiont stability. Applying ecological frameworks to host-microbe interactions can help identify key microbial functions, improve predictions of host resilience under stress (Bourne et al., 2009), and guide restoration efforts.

1.3. Previous restoration efforts

In their review, Eger et al. (2022) identified four main strategies for kelp reforestation: transplanting, seeding, grazer control, and artificial reefs. Transplanting adult donor sporophytes in degraded reefs remains a commonly applied approach, happening via various techniques e.g. gluing holdfast to substrate, tying to ropes or attaching to small rocks (Eger et al., 2022). Yet they face many challenges to overcome before reproduction can occur (Campbell et al., 2014).

Multiple efforts to restoration have occurred with variable success. Although short-term improvements, long-term successes have been limited (Layton et al., 2020). Combining seeding - the deployment or growth of juvenile gametophytes in the ocean - with transplanting might increase the chances of successful restoration (Verdura et al., 2018). Chemello et al. (2024) studied the potential of “green gravel”, where kelp spores or gametes are put on substrates and grown under laboratory conditions before outplanting.

An overall conclusion is that all efforts are small scale, labor intensive and cost prohibitive (Eger at al., 2022). Successful restoration should advance beyond current limitations. Synthetic biology offers potential tools to "future-proof" existing kelp forests (Coleman & Goold, 2019).

1.4. Probiotics as a solution

Derived from the Greek meaning *“for life”*, probiotics are widely recognized in human health for their immune-boosting properties (Isolauri et al., 2001). Chauhan and Singh (2018) suggested the analogous use of probiotics for marine holobionts, particularly to enhance the resilience of kelp to rising ocean temperatures and MHWs as seen in corals (Peixoto et al., 2017). Manipulating the kelp microbiome has already shown promise in boosting productivity, recruitment and algal growth (Flórez et al., 2021; Amin et al., 2009; Wichard, 2023), supporting the potential of probiotics as a tool for enhancing algal resilience under environmental stress.

The study of Li et al. (2022) showed promising results for *Phaeobacter* spp. and *Pseudoalteromonas* spp. to be general marine probiotics. These bacteria may protect their host not by directly inhibiting pathogens, but by mitigating dysbiosis and stabilizing the native microbiota of *Delisea pulchra* (Figure 6). This ability for a probiotic strain to establish itself within the host microbiome is an important quality for its effectiveness (Chauhan & Singh, 2018).

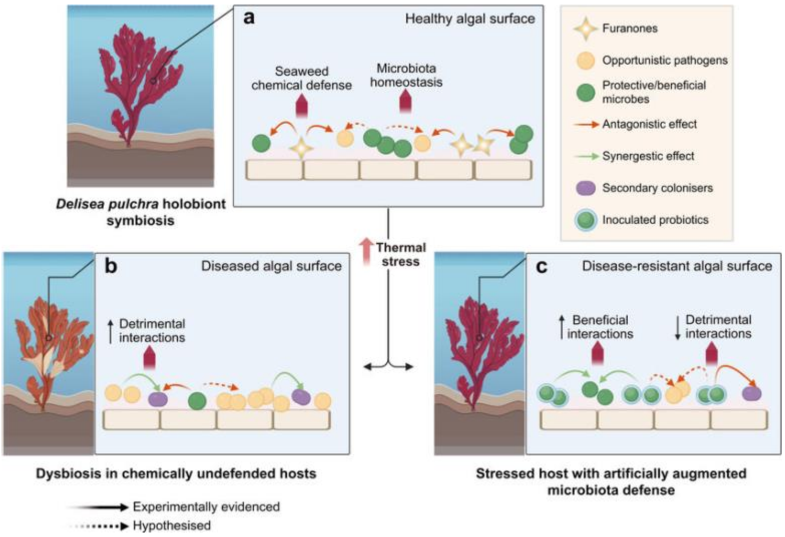

Figure 6. The states of symbiosis and dysbiosis of the Delisea pulchra holobiont. Source: Li et al. (2022)

1.5. Expected challenges

Whilst literature about terrestrial and aquatic holobionts bring valuable insights, Dittami et al. (2021) emphasize the need for caution when applying successful strategies from one to the other. Aquatic systems exhibit higher phylogenetic diversity, with much still to be discovered (Thompson et al., 2017). This implies complex cross-lineage interactions that remain largely unexplored in marine holobionts (Dittami et al., 2021). Due to the open nature of marine ecosystems, microbial communities can shift more rapidly in response to environmental changes (Martin-Platero et al., 2018).

Field conditions are inherently more variable and less predictable than laboratory settings, increasing the risk of unintended negative outcomes with cascading effects on the ecosystem functioning and services provided by kelp (Coleman & Goold, 2019; Coleman and Wernberg, 2020).

The expected challenges include the complexity of the kelp life cycle, which involves an alternation of generations (Biskup et al., 2014). Gametophytes and sporophytes may exhibit different physiological responses to laboratory conditions, potentially affecting the consistency and interpretation of experimental outcomes (Coleman & Goold, 2019). Additionally, the kelp holobiont microbiome varies with factors such as tissue region, season, geography, and host specificity (Girão et al., 2019; Lemay et al., 2021; Lachnit et al., 2009; Egan et al., 2013). It is therefore essential to highlight that working with microbiomes is a "snapshot" reflecting the environmental conditions present at the time.

1.6. Research objective

This thesis aimed to evaluate whether selected bacterial strains can improve the reproductive success and resilience of *Laminaria ochroleuca*, and potentially other kelp species, to support holobiont-based approaches for kelp forest restoration.

The primary focus was to test whether the selected probiotic treatments can enhance the reproduction and recruitment under controlled laboratory conditions. To assess potential microbial shifts, the kelp's microbiome was analyzed using 16S rRNA gene sequencing. A follow-up experiment using only female gametophytes explored potential sex-specific effects. Additionally, the study examined whether a single bacterial isolate could improve kelp resilience under elevated temperature conditions, which is particularly important given the rise in both frequencies and intensities of marine heatwaves.

This work is a collaboration between the Phycology Research Group at the Ghent University (Belgium) and the Centre of Marine Sciences (CCMAR) at the University of the Algarve (Portugal). It is part of the RestoreSeas project, aiming to develop effective restoration measures for kelp forests alongside seagrasses and deep-sea corals.

2. Material and Methods

During this thesis, three different experiments were set up. A "bacteria experiment" was conducted to study the effects of four different bacteria on the development of kelp, followed by a microbiome analysis. A follow-up experiment where the effects of two selected bacteria were studied on the production of ontogenetic structures was called "ontogenetic experiment". The last experiment was the "heatwave experiment" to study the effects of a bacteria strain on kelp gametophytes exposed to heat stress.

The first experiment was conducted in the lab of CCMAR (University of Algarve, Portugal) until DNA analysis. The latter, together with the ontogenetic and heatwave experiment, took place in the lab of Prof. De Clerck at UGent (Ghent University, Belgium).

2.1. Bacteria experiment

2.1.1. Origin and preparation of *L. ochroleuca* strains

For the experiment, *L. ochroleuca* strains originating from mature specimens collected in the Strait of Messina, Italy and Gatteville-le-Phare were used. Three different strain compositions were tested and hereafter referred to as 'populations':

1. IT MONO: A monoculture of Italian strains consisting of male and female individuals from different specimens—specifically, individual 10.4 (female) and 11.2 (male), with the first number referring to the parent strain.
2. IT POOL: A mix of male and female strains from both IT 10 and IT 11 of the Italian population.
3. FR MONO: A monoculture consisting of male and female individuals from different French specimens.

All metadata is provided in Table 1.

Table 1. Metadata of the Laminaria ochroleuca sampled and used in this experiment. Lab cultured refers to the research group that had sampled and maintained theses kelp strains.

Population	Sampling date	Site	Coordinates	Depth (m)	Lab cultured
France (FR)	16/08/2022	Gatteville-le-Phare	49°41'47.2"N 1°15'53.1"W	4	UGent
Italy (IT)	16/11/2019	Messina Strait	38°15'27.97"N 15°37'40.04"E	50	CCMAR

The experimental set-up can be found in Figure 7. Gametophytes were grinded with pestles and mortars that were precooled at 15°C to avoid extra heat stress. Followed by a size-selective sieving (< 100µm) via filtering the grinded *L. ochroleuca* through sterile sieves with tropical marine in falcon tubes of 50mL. Tropical marine was made by autoclaving 2L of distilled water. After cooling down, sea salt (Classic Meersalz, Tropic Marin, Germany) was added to obtain a concentration of 32 ‰ per Liter. When this concentration was confirmed by a salinity refractometer (Atago, Japan), Provasoli-Enriched Seawater (PES) with a mix of vitamins was added with a concentration of 10mL/L. To limit stress to the gametophytes, the tropical marine was stored at 15°C.

This solution was then stirred with magnetic stirrers in crystalizing dishes for minimum one minute before the densities were checked under the microscope with a counting grid (Zeiss Axio Observer D1, Carl Zeiss MicroImaging GmbH, Göttingen, Germany) on 100x magnification.

For an optimal experiment, the previous steps were repeated until a density of a minimum of 150 gametophytes per cm^2 per sex was reached. This is a trade-off between a dense medium that limits movement and having too few gametophytes that reduce encounter chances. The treatments were prepared in Petri dishes (55 mm ⌀) with a total volume of 12 mL (Equation 1). Each kelp population received four distinct mono-bacteria treatments and had a control group (no bacteria added). Four replicates were made per kelp population.

2.1.2. Origin and preparation of bacteria strains

The bacteria used in this experiment are in Table 2 and their taxonomy in Table 3. The bacteria were isolated during experiments that preceded this thesis. From their stock solution (30% glycerol, 70% marine broth) 5 µl of suspension was taken and plated out on agar plates (BD DIFCO™, USA) under the Vertical Flow (Aura Vertical S.D.4, BioAir, Italy). A single colony of each isolate was transferred to 50mL Marine Broth (Marine agar 2216, BD DIFCO™, USA) in 200 mL Erlenmeyer flasks. Marine Broth was made by adding 37.4g in 1L of distilled water that was subsequently autoclaved and stored at 15°C.

Table 2. Overview of the metadata of the bacteria strains used in the experiments. Their code for internal use in the lab of UGent, the harvested region of the kelp individual and the country of origin.

Species	**Code**	**Harvested region *L. ochroleuca***	**Country of origin**
Cobetia amphilecti	RosG3M4	Blade individual G-Roscoff	France
Kocuria polaris	RosH3M4	Blade individual H-Roscoff	France
Tritonibacter scottomollicae	EngE-AKF1	Gametophyte of parent E-Plymouth	United Kingdom
Zobellia alginiliquefaciens	EngO – AKR1	Gametophyte of parent O-St Mawes	United Kingdom

Table 3. Taxonomic classification of the bacteria strains used, up to family level. The genus corresponds to the first word of the species name.

Species	**Phylum**	**Class**	**Order**	**Family**
Cobetia amphilecti	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomondaceae
Kocuria polaris	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae
Tritonibacter scottomollicae	Proteobacteria	Rhodobacterales	Rhodobacterales	Rhodobacteraceae
Zobellia alginiliquefaciens	Bacteroidota	Flavobacteriales	Flavobacteriales	Flavobacteriaceae

They were incubated at 15ºC for 48 hours on a shaker (Orbital mini shaker, VWR International, LLC., The Netherlands). Cultures were then centrifuged (3000RPM, 15°C, 5 minutes; Centrifuge 5180 R, Eppendorf SE, Germany) and the supernatant discarded. Pellets were washed twice in tropical marine water, each time vortexed and centrifuged under the same conditions. After the final wash, the suspension was vortexed again and the bacterial density was assessed.

To assess optical density (OD), 100µl of the bacteria culture was diluted 1:1000 in a 96 well-plate and measured at 600nm using a microplate reader (Agilent Biotek synergy 4 Hybrid Microplate Reader, Agilent BioTek, USA). An OD of 0.015, corresponding to 10^6-10^7 colony forming units per mL (CFU/mL) was used to calculate the required volume for a 12mL filled Petri dish (Equation 1).

2.1.3. Experimental set-up

The experimental set-up (Figure 7) was as follows: after cultivating bacteria and grinding gametophytes followed by size-selective sieving (≤100 µm), they were added to the Petri dishes. The volume was calculated as follows:

$$12\ \mathrm{mL} = \mathrm{w\ mL}\ + \mathrm{x\ mL}\ + \mathrm{y\ mL}\ + \mathrm{z\ mL}$$

Equation 1. The total volume of the Petri dishes. The formula is as follows: w = solution solution female gametophytes (150 gametophytes cm^{-2}), x = solution of male gametophytes (150 gametophytes cm^{-2}), y = bacterium solution (OD$_{600}$ = 0.0015 mL^{-1}), z = tropical marine medium.

The set-up of the experiment happened over two days: IT MONO (all replicas) and IT POOL (replicas 1-2) were given bacteria treatments first. IT POOL (replicas 3-4) and FR MONO (all replicas) the next day. After a 5-day recovery at 15°C under red light (3-7 µmol photons m^{-2} s^{-1}; ULM-500 Walz MQSC 0301, Heinz Walz GmbH, Germany), the Petri dishes were placed into white light (18-22 µmol photons m^{-2} s^{-1}) with a light-dark cycle of 14:10 at 15 °C. This induces gametogenesis and is considered as DAY 0 of the experiment.

They were randomly counted on DAY 0, 7, 14, 21 and 26. Dataloggers tracked the temperature levels throughout the entire experiment.

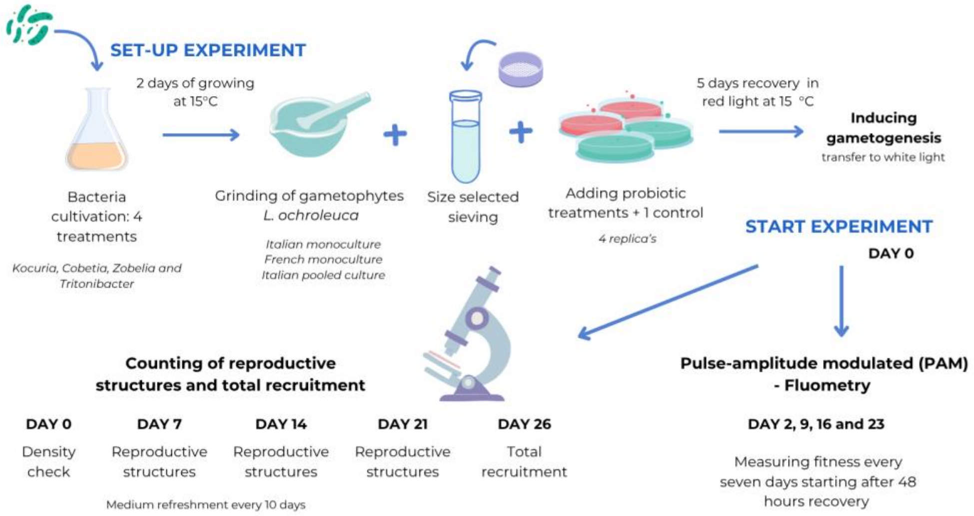

Figure 7. Schematic overview of the experimental set-up, including preparations of both gametophytes and bacteria, recovery under red light, induction of gametogenesis (DAY 0), and counting overview together with the PAM fluorometry. Graph was made in Canva.

The medium refresh was done every 10 days under the vertical flow where 5mL was removed and replaced with newly made Tropical Marine. This was done with caution to avoid disturbing the settled gametophytes.

Pictures of the gametophytes were taken with Nikon D800 (Nikon Corporation, Japan) mounted on the microscope. The counting of different structures was done via the Simplest Manual Counter (version 1.1.1., GNU General Public License, Open source).

2.1.3.1. Counting scheme

On DAY 0, the density was checked counting all gametophytes (male and female). On DAY 7, 14, and 21, the occurrence of the reproductive structures *(vegetative, gametophytes with eggs, or gametophyte with sporophytes*) was classified by the most developed stage observed. The first visible cell division marked the onset of the sporophyte stage. At the end of the experiment (DAY 26), the reproductive success was assessed by counting the total number of sporophytes per gametophyte. Sporophytes with irregular morphology were counted separately. Presumed parthenogenic sporophytes (not attached to a gametophyte) were not counted.

2.1.4. Pulse amplitude modulation (PAM) fluorometry

The maximum quantum yield of photosystem II (PSII) was measured as a proxy for the gametophyte fitness via PSI AquaPens (AquaPen-P AP 110-P fluorometer, Photon Systems Instruments, Czech Republic). A dark room was recreated where three replicas simultaneously could be measured (Figure 8). Samples were dark-adapted for 10 minutes to open their reaction centres (Murchie & Lawson, 2013). The measurements could be retained via FluorPen 1.2.0.0 software. The intensity settings for the actinic, super and flash pulse lights were respectively 20%, 70% and 20%. The measurements were carried out on DAY 9, 16 and 23.

Figure 8. Set-up for PAM (Pulse Amplitude Modulated) fluorescence measurements designed to eliminate ambient light interference. A dark room environment was simulated using two stacked plastic boxes. The PAM probes were inserted through the lower box, while the

2.1.5. Microbiome analysis

2.1.5.1. Transferring samples

To check how the bacteria had established themselves on the kelp compared to their original gametophyte microbiome, a 16S rRNA analysis was done. To transfer the samples from the lab in Faro (CCMAR) to Ghent, they were put in RNA later and frozen upon arrival.

Working under the vertical flow, 8mL of the tropical marine was removed from each Petri dish. The gametophytes attached to the bottom were scraped by the same pipet tip and 1.5 mL of the suspension was transferred into 1.5 mL microcentrifuge tubes. Once the gametophytes settled to the bottom of these tubes, as much excess Tropical Marine as possible was removed. Then, 1 mL of RNA later (Merck, Germany) was added and the tube was sealed with parafilm.

2.1.5.2. DNA-Sequencing

Extracting the DNA of the samples was done by following the QIAamp DNA Mini Kit protocol (QIAGEN, Aarhus, Denmark). The samples were defrosted at room temperature, followed by centrifuging (Centrifuge 5425 R9, Eppendorf SE, Germany) a first time for 15 min with 1000 RPM at 4°C followed by a second time (20 min, 16000 rcf, 4°C). An additional step was added to the protocol wherein paramagnetic (PAE) beads were added to the samples with lysis buffer whereafter they were shaken for 5 minutes with a bead beater (30beats/sec). After finalizing the extraction, a polymerase chain reaction (PCR) was done by putting DNA, the pre-mix (Phire Tissue direct PCR Master Mix, Thermo Fisher Scientific INC., USA) and 16S primer together. The primers used were:

1. 27F_BCtail-FW (TTTCTGTTGGTGCTGATATTGC_AGAGTTTGATCMTGGCTCAG)
2. 1492R_BCtail-RV (ACTTGCCTGTCGCTCTATCTTC_CGGTTACCTTGTTACGACTT).

With PCR conditions being 98 °C 3min, 30x (98°C 8sec , 60°C 8sec, 72°C 30 sec), 72°C 3min, 12°C ∞.

The DNA was transferred onto an agarose gel and run on 100 V, variable mA for 30 minutes. Afterwards, the gel was placed in a bath of ethylbromide to stain the DNA and a UV picture was made (ChemiDoc™ XR+ Imaging System, Bio-Rad Laboratories, Inc. United States) to check the quality of the DNA samples. Each sample was tagged with a barcode following the protocol of Oxford Nanopore PCR Expansion 1-96 barcode primers (Oxford Nanopore Technologies, UK) under PCR conditions 95 °C 3min, 13x (95 °C 15sec, 62 °C 15sec, 65°C 40sec), 65°C 2min, 12°C ∞. The concentration of DNA was measured via the Qubit fluorometer (Thermo Fisher Scientific INC., USA), followed by the library preparation via the Oxford Nanopore Technologies Ligation and PCR barcoding kit protocol.

Sequencing was performed using a MinION Mk1B (Oxford Nanopore Technologies). Base calling and read assignment were conducted using the EMU pipeline (v3.4.5) with the SILVA v138.1 reference database. Initial quality control was assessed using NanoPlot. Low-quality and non-target reads, such as chloroplast sequences, were filtered out using Chopper (v0.7.0), applying a minimum quality threshold of Q15 and retaining fragments between 1300 and 1800 bp. With this curated dataset, statistical analyses were performed.

2.2. Ontogenetic experiment

This experiment aimed to assess whether the bacteria treatments influenced the development of ontogenetic stages, using cultures with only female gametophytes. Two bacteria treatments were tested: the best-performing strain (*C. amphilecti*) and the most inhibitory strain (*K. polaris*) which were determined by the first treatment experiment. Only the populations IT MONO and FR MONO were tested, maintained under the same culture conditions as previously described.

Due to limitations of the shaker available (Gyrotory Shaker Model G2, New Brunswic Scientific Co., inc. Edison, USA), the bacteria were cultivated at 125 RPM in plastic culture flasks (25cm^2). The bacteria treatment was added to both strains on the same day. All other conditions for the set-up were the same as the treatment experiment, with the exception of some equipment and a light-dark cycle of 12:12 (Table 4).

Table 4. An overview of the equipment used in the phycology lab of uGent that differed primarily in term of brands from the ones used in CCMAR.

Material	**Series**	**Company**	**Country**
Camera	Olympus SC50 + Olympus CellSens Entry	Olympus Corporation	Japan
Plate reader	Cytation 3 Imaging reader	Biotek	USA
Laminar flow	Airstream Class II Type A2 Biological Safety Cabinet - K series	Esco Micro Pte. Ltd.	Singapore
Centrifuge	Sigma 4k15	DJB Labcare	UK

After a 5-day recovery at 15ºC in a red-light incubator (Thermostatschrank aqualytic, VWR International LLC., Belgium), the replicates were transferred into white light at 15 °C (DAY 0) in the climate control room (Fermod, France). The ontogenetic stages were counted and afterwards categorized as "eggs" or "no eggs". When eggs were present, their number per gametophyte was counted, including loose eggs in the medium. The counting happened on DAY 10 and DAY 18. The latter marks the end of the experiment.

Their medium was refreshed once (DAY 15).

2.3. Heatwave experiment

To study the effect of our best-performing bacteria on kelp when exposed to heat stress, a heatwave experiment was performed with three different temperatures (15, 19 and 23 °C) after referred to as HW15, HW19 and HW23. HW15 served as the control condition, representing the baseline temperature. Temperatures were gradually increased by 2°C per day until the respective target temperature were reached. The temperature range for optimal reproductive development of *L. ochroleuca* is 15-18 °C (Izquierdo et al., 2002) but tolerates temperatures up to 24°C (Franco et al., 2017).

The same protocol was followed, and material used as in the ontogenetic experiment for the preparation of the replicates.
The heatwave experiment followed the timeline shown in Figure 9. After being transferred to white light with a 12:12 dark-light cycle (DAY 0), samples were acclimated at 15 °C before being placed in their respective heat treatment incubators (HW19: Thermostatschrank Lovibond, Tintometer GmbH, Germany; HW23: Thermostatschrank Aqualytics, VWR International, LLC, Belgium). The heatwave peaked on DAY 4 and lasted for 10 days, after which temperatures were gradually reduced by 2 °C per day to 15 °C, followed by a recovery phase of 25 days (HW19) and 23 days (HW23).

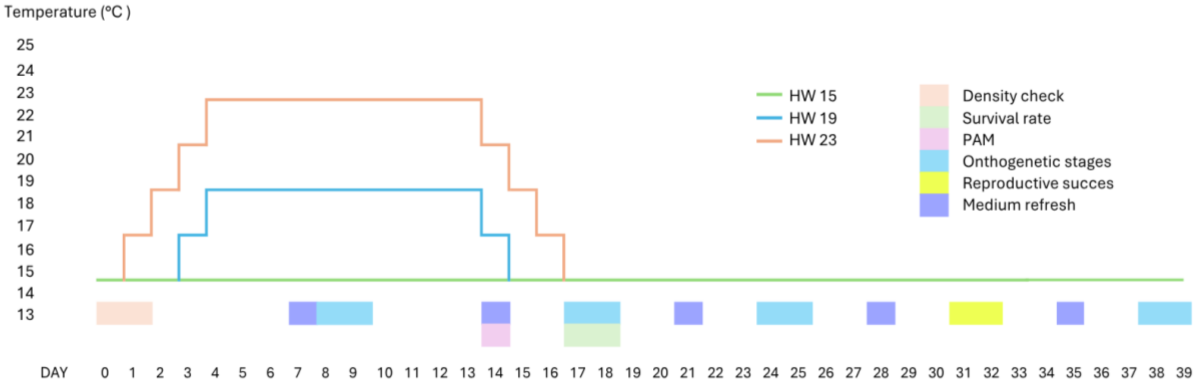

Figure 9. Timeline of the heatwave experiment with the three heatwave (HW) treatments. The corresponding temperatures (ºC) and days are marked in the y- and x-axis. The colored blocks refer to the corresponding counting day or measurements conducted.

At DAY 0, densities of all replicas were counted. Due to unforeseen circumstances, 9 out of 48 replicas were counted on DAY 1. The reproductive structures of replicas 1-2 were randomly counted on DAY 8, those of replicas 3-4 on DAY 9. Medium refresh was done every seven days and tropical marine was stored for each corresponding HW treatment.

PAM-measures (Walz IMAG k4, Heinz Walz GmBH, Germany) were conducted with intensity settings for the actinic, super and flash pulse lights all set to 8. The samples were dark-adapted for a minimum of 10 minutes.

2.4. Statistical Analysis

All statistical analyses were performed in R (version 2024.12.1) using the vegan, emmeans, multcomp, car, ggplot2 packages or otherwise specified. Normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were confirmed ($p > 0.05$) prior to analyses.

2.4.1. Bacteria experiment

2.4.1.1. Statistical analysis

Two replicates (IT TRIT 2 and MIX ZOB 1) were identified as invalid due to spills during bacteria treatments applications. These data points were therefor replaced with the average of the remaining replicates within their respective treatment group and population to ensure consistency in the analysis.

The gametophyte densities were compared across populations using one-way ANOVA.

Counts of reproductive structures were transformed into relative abundances and reshaped with dplyr and tidyr packages to enable comparisons across populations and bacterial treatments. A generalized linear model (GLM) with a quasibinomial family was fitted with Bacteria × Stage interaction, and Day and Population as fixed factors. Estimated marginal means (EMMs) were calculated for each combination of Day and Population, with pairwise comparisons performed using the Dunnett method against the control.

To assess the recruitment success, sporophyte counts were averaged per field and normalized to the population-specific control. Due to heteroscedasticity (Levene's $p < 0.05$), a GLM with Gaussian distribution was fitted with population, bacteria, and their interaction as fixed factors. EMMs of bacteria treatments were calculated within each population, with Dunnett-adjusted comparisons to the control.

For the fitness of the gametophytes, Y(II) values were analyzed using a GLM (Gamma family, log link) with Day × Bacteria × Population as fixed factors. The null deviance was assessed to evaluate model fit. EMMs were extracted for the bacteria treatment within each Day and Population, using Dunnett contrasts. Repeated measures were tested but excluded due to boundary warnings (singular fit).

2.4.1.2. Microbiome analyses

Microbial counts were aggregated at the genus level using the tax_glom**()** function in the phyloseq package (McMurdie & Holmes, 2013). Genera with undefined taxonomy were excluded, and relative abundance transformations were applied for visual and statistical analysis.

Microbial community structure was explored using Principal Coordinates Analysis (PCoA) and differences in community dispersion were tested with the betadisper() and anova() functions. Analysis of Principal Coordinates (CAP) based on Bray–Curtis dissimilarities was performed, with Treatment and Population as constraining factors. The significance was assessed using PERMANOVA via the adonis2() using 999 permutations and marginal tests (by = "margin").

Exploratory barplots were generated to explore the top genera, the establishment of bacteria treatments in the core microbiome and the relative abundance of different orders using the viridis and patchwork package. The core microbiome was defined (80% prevalence, ≥0.01% mean abundance), using the microbiome package. Core genera were identified within each population and treatment group. Venn diagrams were used to visualize shared and unique taxa across populations and within populations using the forcats package.

2.4.2. Ontogenetic experiment

Starting densities were checked via ANOVA after log transformation (all assumptions met). Absolute count of gametophytes with ontogenetic structures (eggs or sporophytes) data was converted to relative values by dividing per total gametophytes.

Reproductive success was calculated by dividing the total number of eggs (loose + attached) by the number of observed fields and normalized by the relative abundance of gametophytes with reproductive structures. This was repeated for sporophyte counts. To assess bacterial effects over time within each population, separate day-by-day GLMs (Gamma family, log link) were performed with bacteria as the sole fixed factor.

The presence of ontogenetic structures on gametophytes was analyzed as binary data. Due to complete separation in the FR MONO population, Firth's penalized logistic regression was applied using the logistf package to obtain reliable estimates.

2.4.3. Heatwave experiment

Both males and female gametophytes were counted until a total of 150 gametophytes was obtained at DAY 0 and at DAY 17. The initial densities between the two populations were checked via a one-way ANOVA, as well as a one-way ANOVA for each HW treatment within each population to check densities within bacteria treatments (assumptions met). Survival trends were analyzed using a four-way GLM (Day × Population × Temperature × Bacteria) with a Gaussian distribution. EMMeans and pairwise comparisons evaluated factor differences.

The counts of the different reproductive structures were transformed to relative abundance. A quasibinomial GLM tested for Bacteria, Temperature, Stage, Population and Day including a three-way interaction (Bacteria × Temperature × Stage). Separate GLMs were performed for each stage and EMMeans with Tukey adjustments assessed HW treatments within each Bacteria, Day, and Population.

A GLM with quasibinomial family was fitted for (Bacteria x Population x Treatment) to check the reproductive success on DAY31. Recruitment of healthy sporophytes per gametophyte was modeled with a Gamma GLM (log link), correcting for zeros by adding a small constant.

To assess the PAM-measures, a three-way ANOVA (Treatment × Population × Bacteria) was performed (assumptions met). TukeyHSD post-hoc tests were used for pairwise comparisons. Plots were finetuned with the ggh4x package.

2.5. Digital tools

Some pictures and figures were edited for clarity using Canva (Canva Pty Ltd, Sydney, Australia). Support with statistical coding and grammar improvements was provided by ChatGPT (OpenAI, 2024).

3. Results

To avoid confusion, when referring to the bacteria treatment itself the genus name was used (e.g. *Cobetia*). The full species name was used (e.g. *C. amphilecti*) when the bacteria itself was addressed. Figures and tables in appendix are referred to as “Figure A – x“ and “Table A – x“.

3.1. Bacteria experiment

Mean initial densities of gametophytes per cm^2 with standard deviation (SD) for each population at DAY 0 were above 150; IT MONO (201,5 ± 45,27 SD), FR MONO (195,7 ± 29,31 SD) and IT POOL (159,3 ± 32,46 SD). The one-way ANOVA showed IT POOL significant lower compared to the other populations ($p = 0.0018$ and $p < 0.001$ respectively, Figure 10).

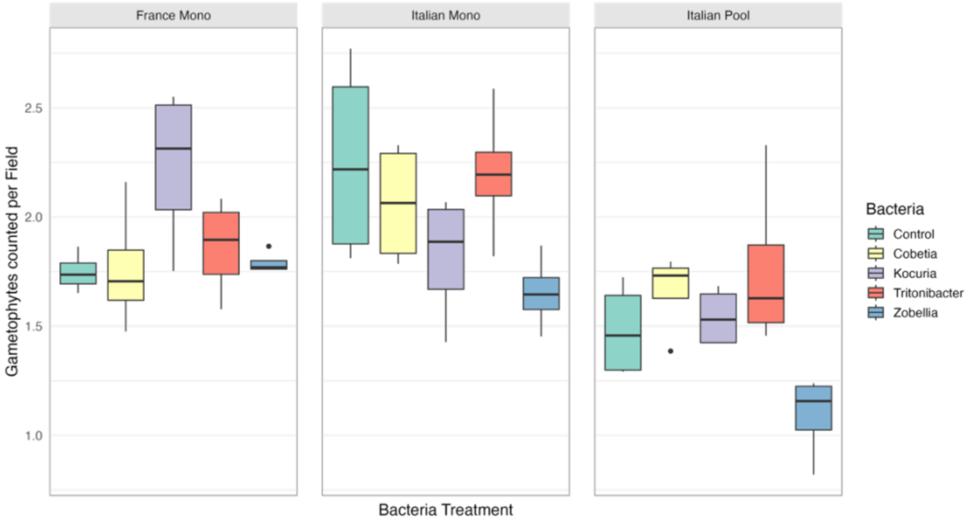

Figure 10. Boxplots of starting densities of the total gametophytes (males and females) for different bacteria treatments after a 5-day recovery in red light at 15 ºC for all three populations. This is considered as DAY 0 of the experiment. The legend shows the bacteria treatments with their corresponding color.

3.1.1. Reproductive structures

The relative abundances of the reproductive structures are visualized in Figure 11. A significant interaction between bacteria treatment and reproductive structure was found ($p < 0.001$), indicating that the effect of bacteria differed across stages. No significant main effects were found for Day or Population.

The most pronounced overall differences on the relative abundance of reproductive structures were observed within IT MONO, where *Cobetia* enhanced reproduction ($p < 0.001$), while *Kocuria* had an inhibitory effect ($p < 0.001$). Across populations, the effects also differed. By DAY 7, reproductive activity was observed across all populations. With FR MONO showing the greatest effects of the bacteria treatments (*Cobetia*, *Tritonibacter*, and *Zobellia:* $p < 0.001$) for both reproductive structures. The effect of the treatments only became apparent in IT MONO and IT POOL for gametophytes with sporophytes with the latter having an overall relative abundance of ~75%. Table 5 summarizes the significance of effects of this growth across treatments during the experiment and their relative abundances are visualized in Figure 12. The vegetative and eggs stages are presented in the Figure A - 1 and 2.

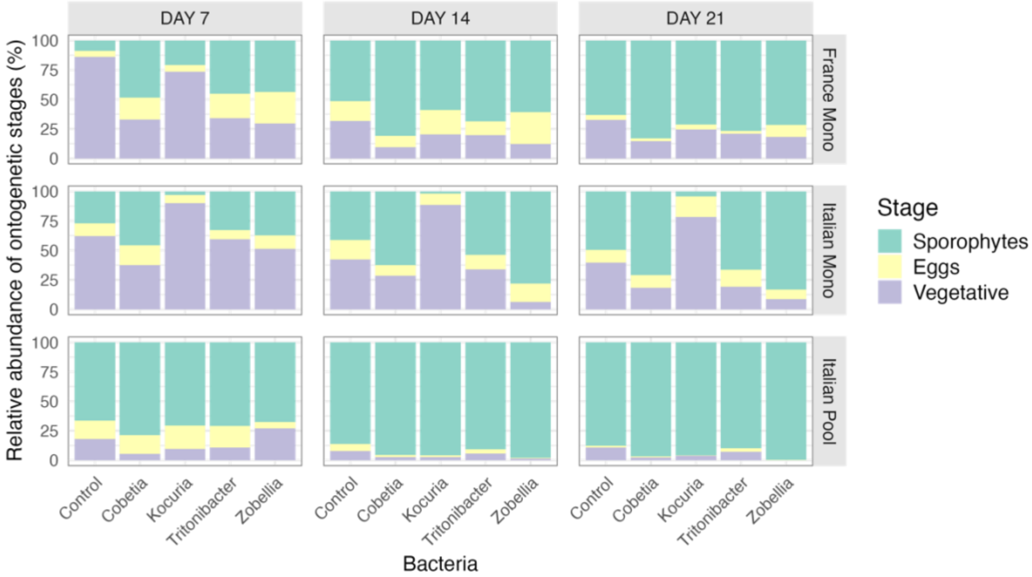

Figure 11. Relative abundance of reproductive structures per population and day for female gametophytes. The most advanced staged was counted when both eggs and sporophytes were observed on the same individual.

Table 5. The significant effects of each bacteria treatment on the Relative abundances of gametophytes with sporophytes per population and day. A positive effect is resembled by '+' , negative by '-'. The significance of the effect depends on the number of symbols: (+++) = p <0.001, (++) = p < 0.01, (+) = p <0.05.

Sporophyte growth	**FR MONO**			**IT MONO**			**IT POOL**		
	DAY 7	DAY 14	DAY 21	DAY 7	DAY 14	DAY 21	DAY 7	DAY 14	DAY 21
Cobetia	+++	+++	+++	+++	++	+++	+++	+	++
Kocuria	++	+		---	---	---	+++	+	+
Tritonibacter	+++	++	++			+++	+		
Zobellia	+++	+++	++	+	+++	+++	+	+	

On the final counting (DAY 21), *Cobetia* stood out as the only bacterial strain that consistently promoted sporophyte formation across all three populations. Its effect was strongest in FR MONO (p < 0.001), but it also significantly enhanced development in IT POOL (p = 0.0022) and IT MONO (p < 0.001). This consistent response contrasts with the more variable effects of *Kocuria*. This had a positive effect in IT POOL (p = 0.0108), no effect in FR MONO, and a significantly negative impact in IT MONO (p < 0.001).

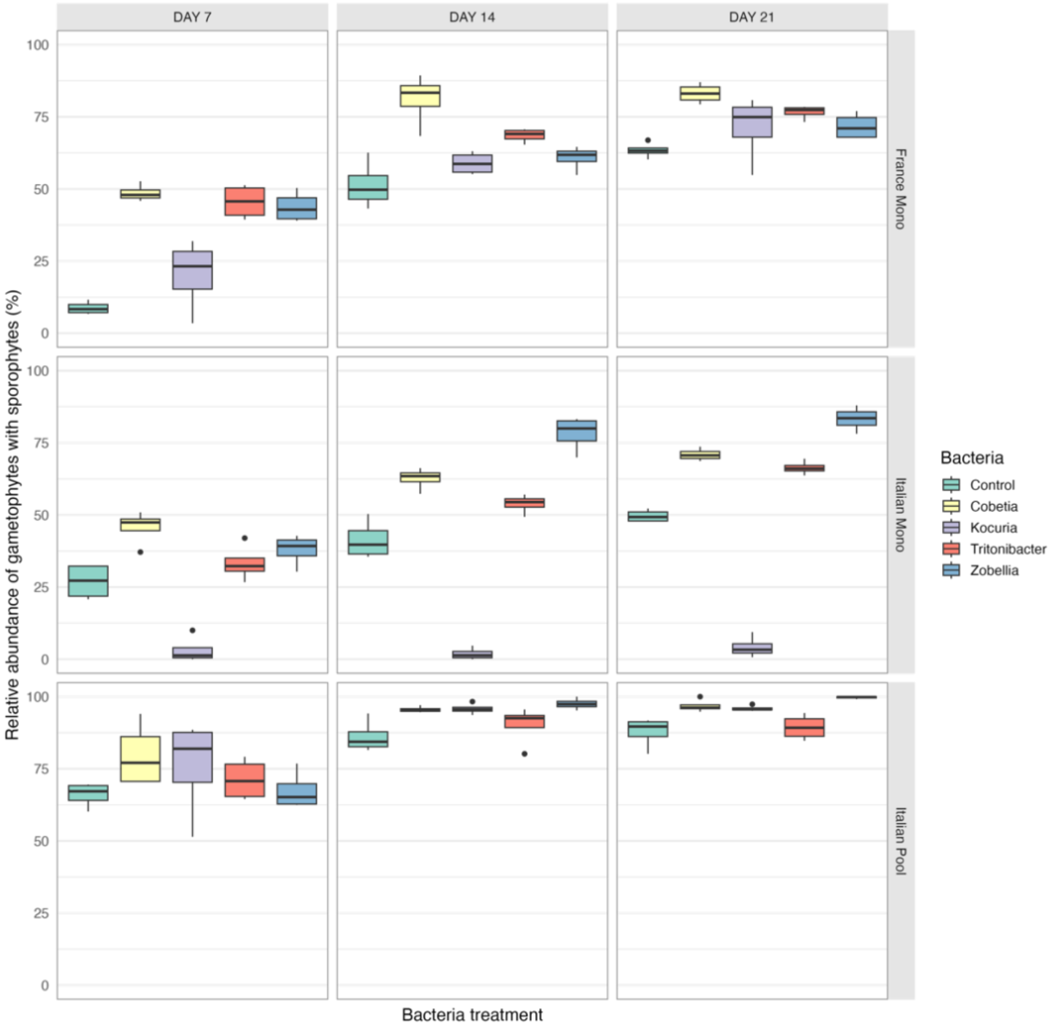

Figure 12. Boxplot showing the relative abundance of gametophytes with sporophytes in percentages across different bacteria treatments. The plot is faceted by the three populations and different counting days. Legend shows the bacteria with their corresponding color.

3.1.2. Morphology observations

Observations were made regarding the growth of the gametophytes. The three different reproductive stages are shown in the appendix (Figure A - 3) *Kocuria* sporophytes of IT MONO appear to be smaller on DAY 21 compared to the others (Figure 13). All other populations are presented in the appendix (Figure A - 4 and 5).

Unidentified organisms were observed for all IT POOL samples (Figure 14). At DAY 7, they appeared to be motile on DAY 7 and were often found directly on the gametophytes. Whether they were feeding on them or engaging in other interactions remained unclear. By DAY 21, these motile microorganisms were considered dead or immobilized (personal observation).

Figure 13. Gametophytes with sporophytes on DAY 21 for Italian Mono. Pictures are taken on a magnitude of x100. Scale is added for reference (ruler = 1mm). Labels on the pictures refer to: CONT = Control; COB = Cobetia; KOC = Kocuria; TRIT = Tritonibacter; ZOB = Zobellia.

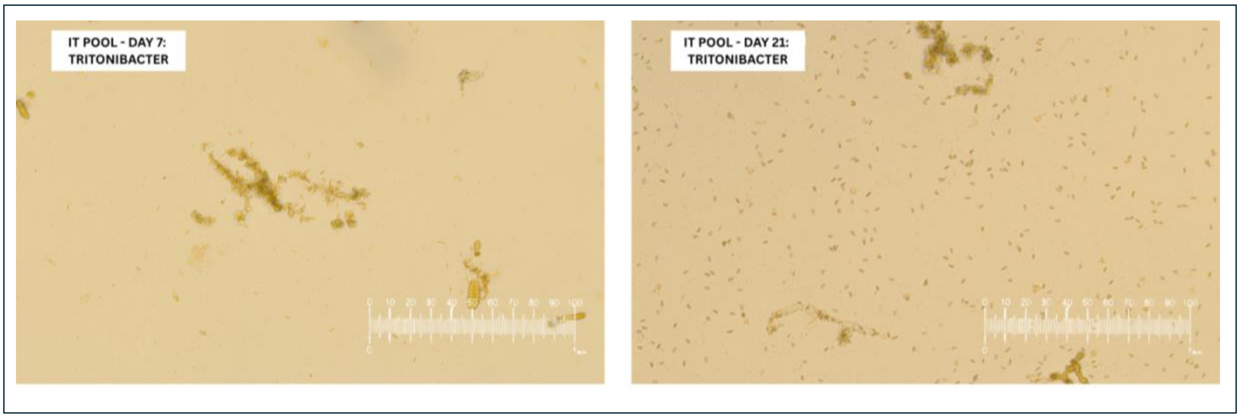

Figure 14. Pictures of IT POOL for Tritonibacter treatment with living organism on the gametophyte on DAY 7, at DAY21 these organisms are still present but stopped moving. Pictures are taken on a magnitude of x 100. Scale is added for reference (ruler = 1mm).

3.1.3. Reproductive success

A significant interaction between bacteria treatment and population ($p < 0.001$) indicated that the effect of bacterial strains on sporophyte recruitment differed depending on the population. While bacteria treatment had a significant overall effect ($p < 0.001$), there was no main effect of population alone.

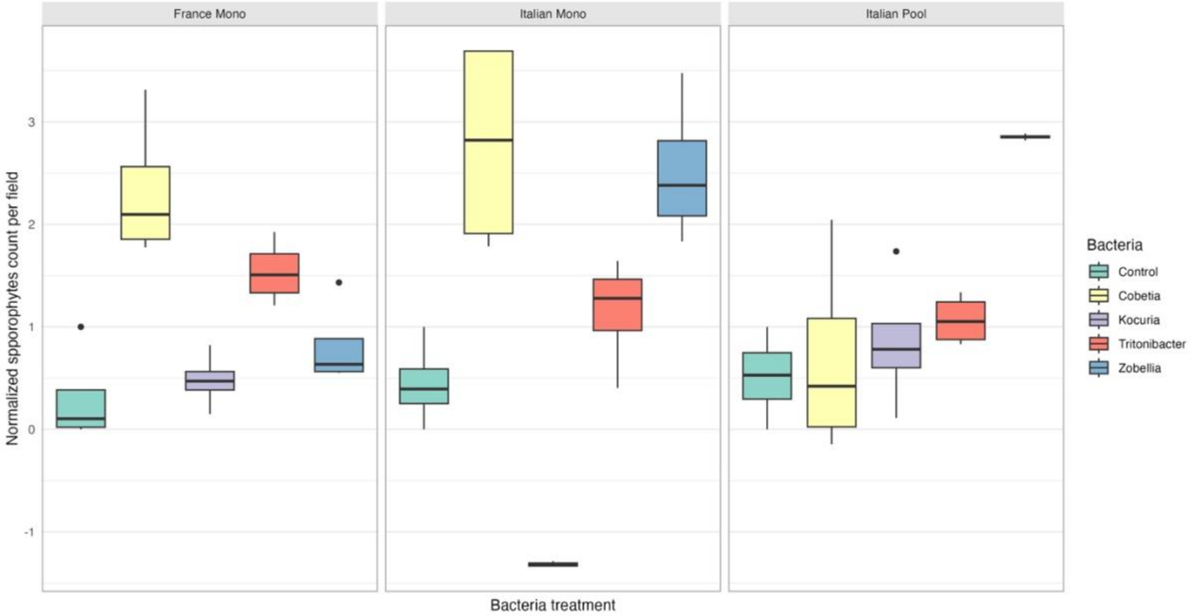

Figure 15. Sporophyte recruitment per field for every bacteria treatment for all populations. The data was normalized within each population compared to their control group. The graph is faceted by the three different populations and the bacteria treatments are in the legend with their corresponding colors.

Cobetia's positive role remained affective in FR MONO and IT MONO ($p < 0.001$). This effect was not observed for IT POOL. Unlike the other populations, where multiple treatments showed beneficial effects, only *Zobellia* improved recruitment in IT POOL ($p < 0.001$). *Kocuria* consistently had a negative impact on IT MONO ($p < 0.001$).

Looking at the abnormal sporophyte growth, *Zobellia* had significant more abnormal sporophytes for FR MONO ($p = 0.0319$) and IT MONO ($p = 0.0111$). Yet the relative abundance of the abnormal structures never exceeded 3%.

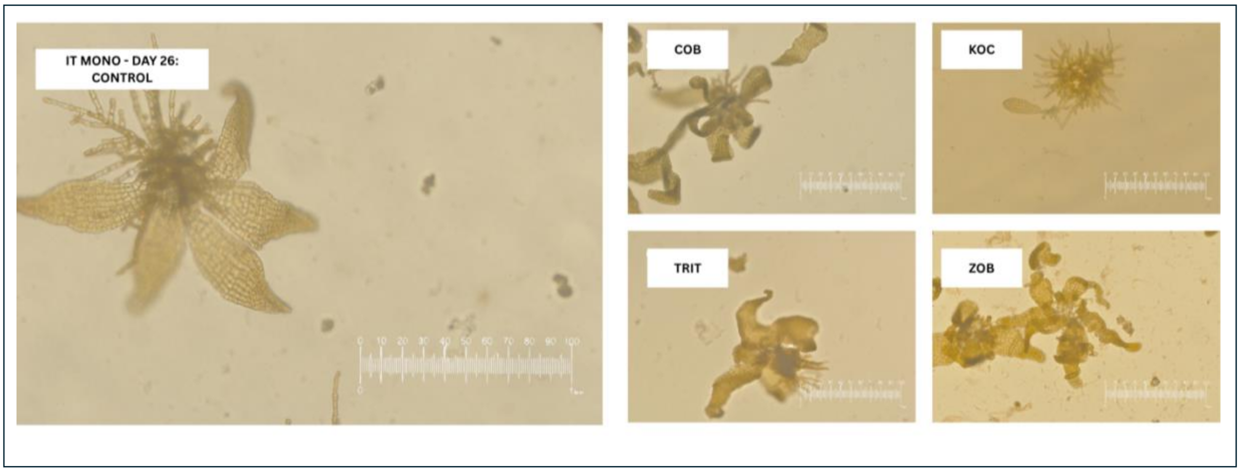

Figure 16. Gametophytes with sporophytes of IT MONO at DAY 26. Pictures are taken on a magnitude of x 100. Scale is added for reference (ruler = 1mm). Each picture represents a bacteria treatment. Left: Control – Right: COB = Cobetia, KOC = Kocuria, TIRT = Tritonibacter and ZOB = Zobellia.

3.1.4. PAM measures

Photosynthetic yields (Fv/Fm) were significant influenced by all fixed factors, including their Bacteria x Day x Population interaction ($p < 0.001$). The only non-significant interaction was between Population x Day.

The temporal trend in yields across treatments, including control, holds an increase at DAY 9 and remains on this level at DAY16, to decrease again at DAY 23.

Despite variable baseline values across populations, treatment effects relative to controls revealed distinct and time-dependent patterns in photosynthetic performance (Figure 17). *Kocuria* showed a striking shift over time. In IT MONO, it significantly reduced yields at DAY 2 and 16 ($p < 0.001$). However, by DAY 23, this effect reversed, with significantly enhancing yields ($p < 0.001$). A similar late-stage improvement was observed in IT POOL, where both *Kocuria* and *Zobellia* positively affected Fv/Fm ($p < 0.01$ and $p < 0.001$ respectively).

Other treatments followed these similar delayed benefits. For instance, *Cobetia* led to improved yields at DAY 23 in both FR MONO and IT MONO ($p < 0.001$).

Although control yields at DAY 2 varied across populations, all treatment effects were evaluated within populations relative to their respective controls, thereby avoiding confounding effects due to baseline differences.

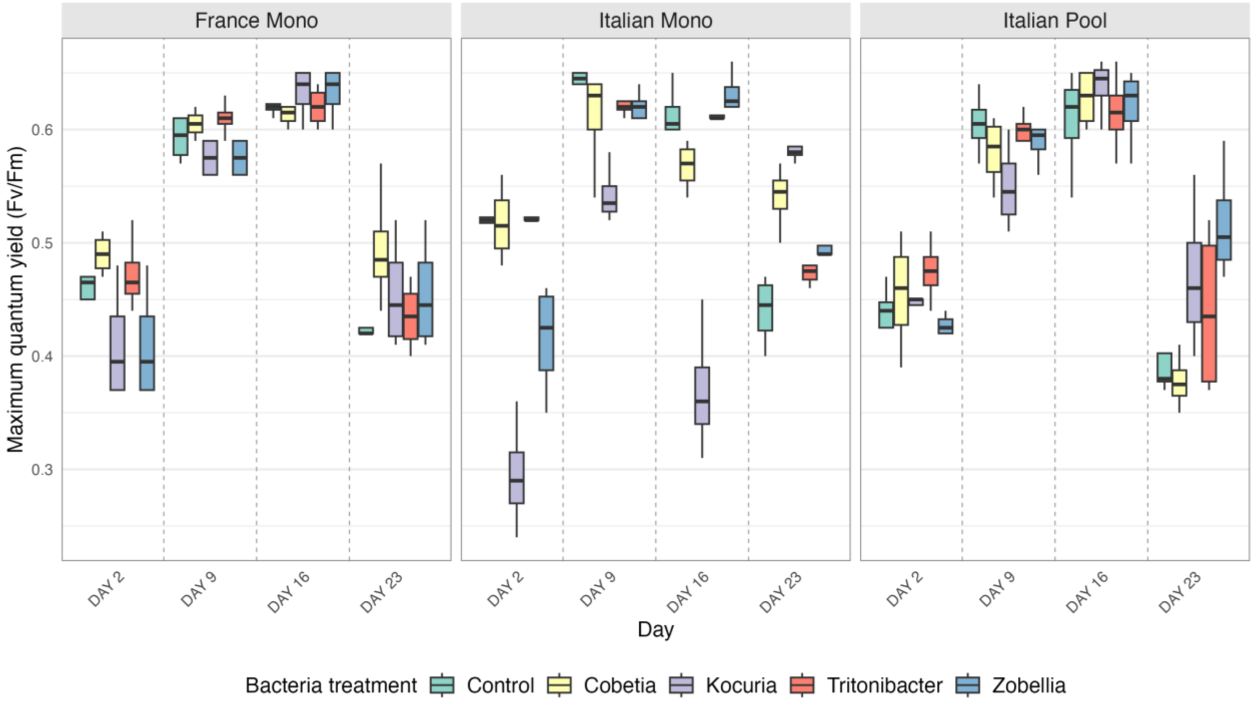

Figure 17. Boxplot of the photosynthetic yields (Fv/Fm) of female gametophytes across different populations and different time points for different bacteria treatments. These bacteria treatments with their corresponding colors can be found in the legend.

3.1.5. Microbiome analysis

3.1.5.1. Composition of the different taxa

After merging the data on genus level, 190 unique taxa were left in the dataset. The CAP (Figure 18) shows a distinct separation of the three populations. PERMANOVA confirmed this significance ($p < 0.001$). The composition within populations is discussed further (section 3.1.5.4.) Patterns can be observed within the populations between treatments with IT POOL and IT MONO sharing the same CAP 1 coordinates.

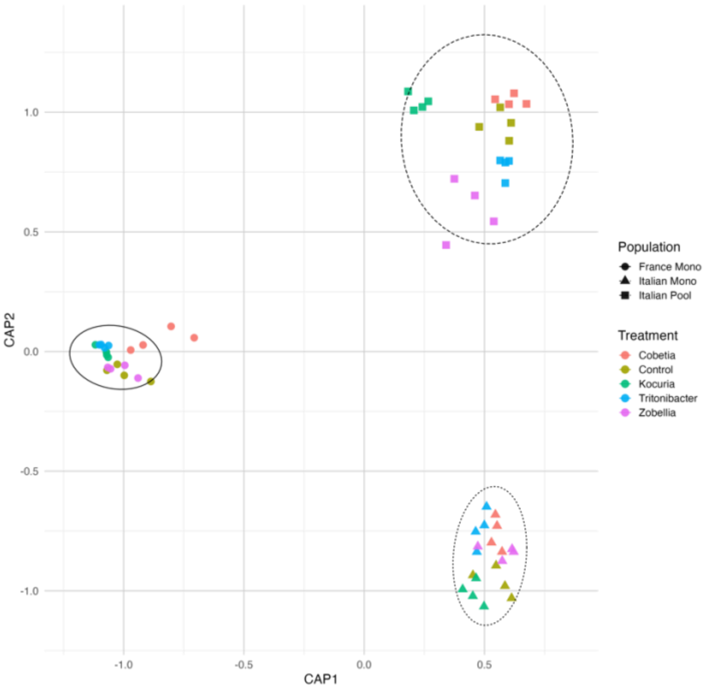

Figure 18. Constrained Analysis of Principal Coordinates (CAP) based on Bray–Curtis dissimilarities, showing bacteria treatment effects across populations. Ellipses represent 95% confidence intervals for each treatment–population combination.

The stacked bar chart (Figure 19) illustrates key compositional differences in bacterial classes among the three populations. While Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia dominate across all groups, population-specific patterns emerged. Figure 20 explores these differences on genus level.

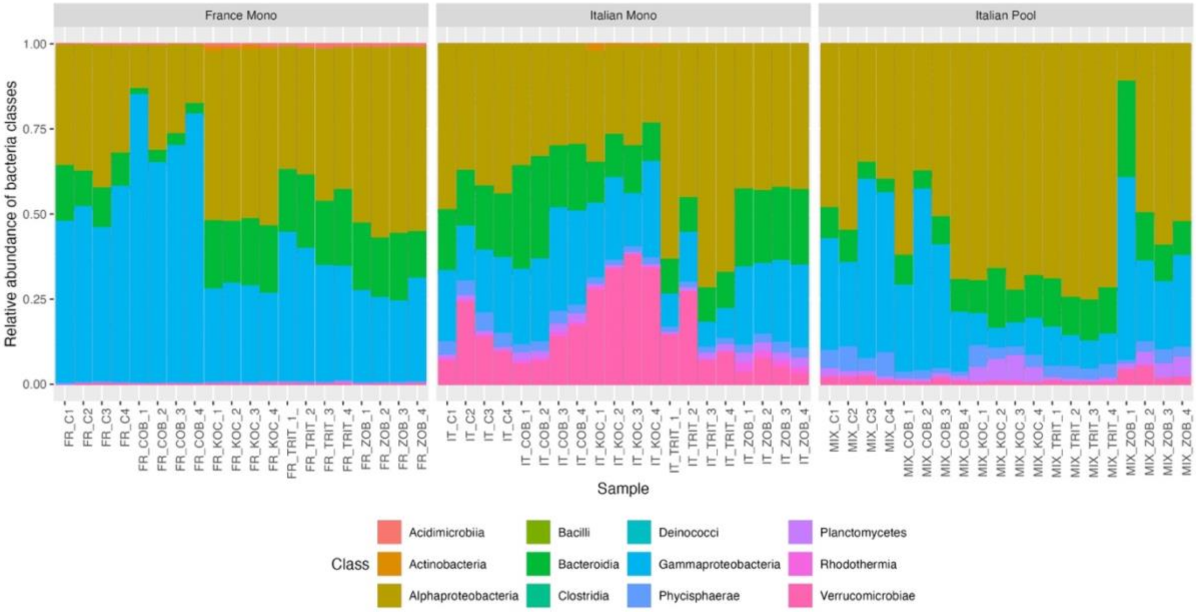

Figure 19. Barplots of the relative abundances for the different classes of bacteria for all three populations.

Proteobacteria dominated, with *Marinobacter*, *Nisaea*, and *Epibacterium* among the key genera. The first two belong to the phylum Gammaproteobacteria, the latter belongs to alphaproteobacteria. *Marinobacter* was the only genus consistently detected across all samples. This also counts for *Nisaea,* reaching particularly high relative abundances in several individuals in FR MONO. In contrast in IT MONO, it did not reach thresholds in KOC and TRIT to be considered in the core microbiome. *Epibacterium* was uniquely associated with TRIT-treated samples across all populations.

Lentimonas, from the phylum Verrucomicrobiota, was especially abundant in IT MONO.

Another notable class is *Actinobacteria* present in *Kocuria* for IT MONO only. For FR MONO it is present in all treatments. Additional bar plots showing phylum-level distributions are included in the appendix (Figure A - 6 - 8).

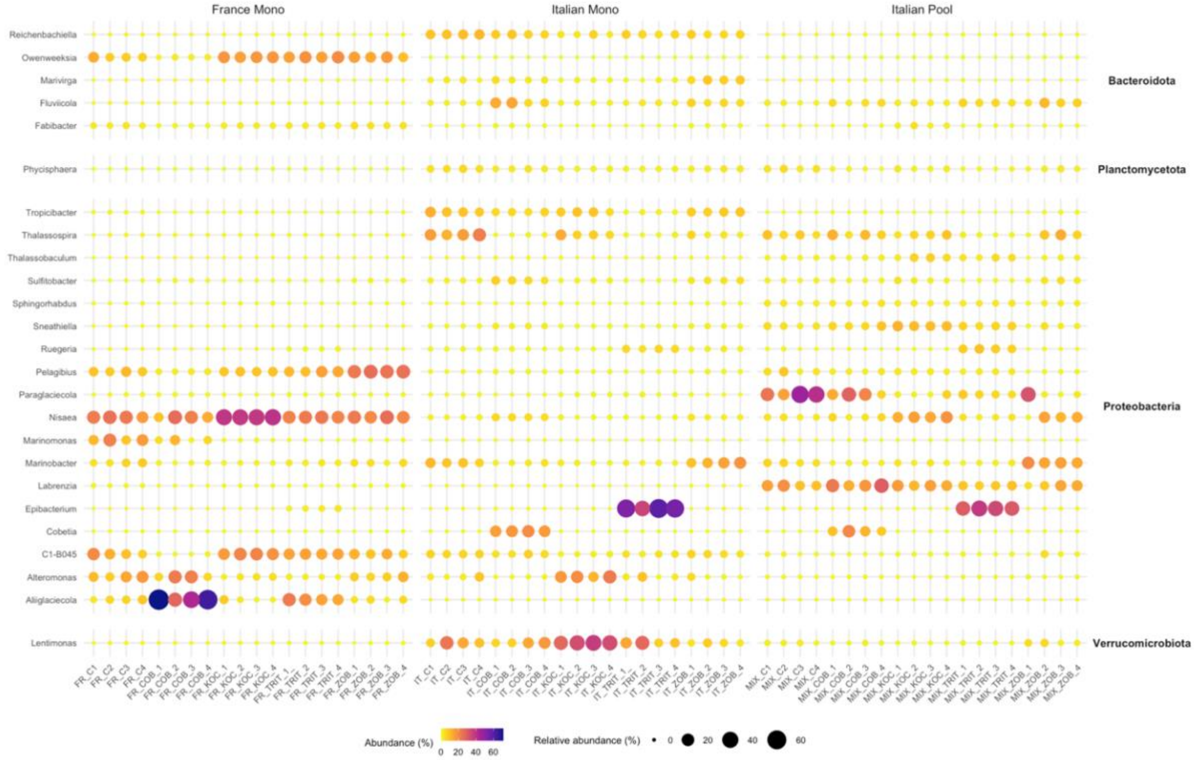

Figure 20. Bubble plot comparing the relative abundance of key bacterial genera across the three populations: FR MONO, IT MONO, and IT POOL. Genera are grouped by corresponding phylum (right side). Relative abundances are represented both by circle size and a color gradient. This allows for a visual comparison between populations and abundances.

3.1.5.2. Core microbiome across populations

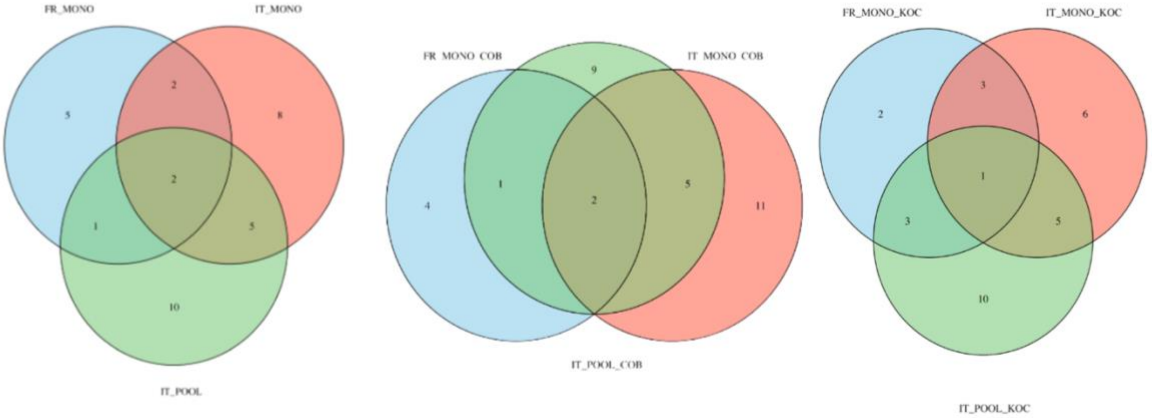

Figure 21. Venn-diagrams showing the number of shared and unique taxa for the three populations IT MONO, FR MONO and IT POOL (left to right) for bacteria treatments Control, Cobetia and Kocuria. The first to share Marinobacter and Nisaea. In contrast, only Marinobacter is shared under the Kocuria treatment.

Given the results following section 3.1., focusing on IT MONO seemed particularly interesting. And specifically zooming in on the bacteria treatments *Kocuria* and *Cobetia*. Figure 21 shows the three different populations and the core microbiome of the bacteria treatments and their overlap.

For the *Kocuria* treatment, there is one unique taxon or all populations. IT MONO and FR MONO share *K. polaris* (phylum Actinobacteriota) as their unique taxa, confirming the successful settlement.

Cobetia has two unique taxa for IT MONO and IT POOL, *C. amphilecti* (Gammaproteobacteria) and *Aestuariicoccus spp.* (Alphaproteobacteria). FR MONO has no unique taxa for this bacteria treatment, yet *Aliiglaciecola spp.* (Gammaproteobacteria) is present with relative abundances exceeding 50% for some samples (Figure 20).

3.1.5.3. Establishment of the bacteria treatments

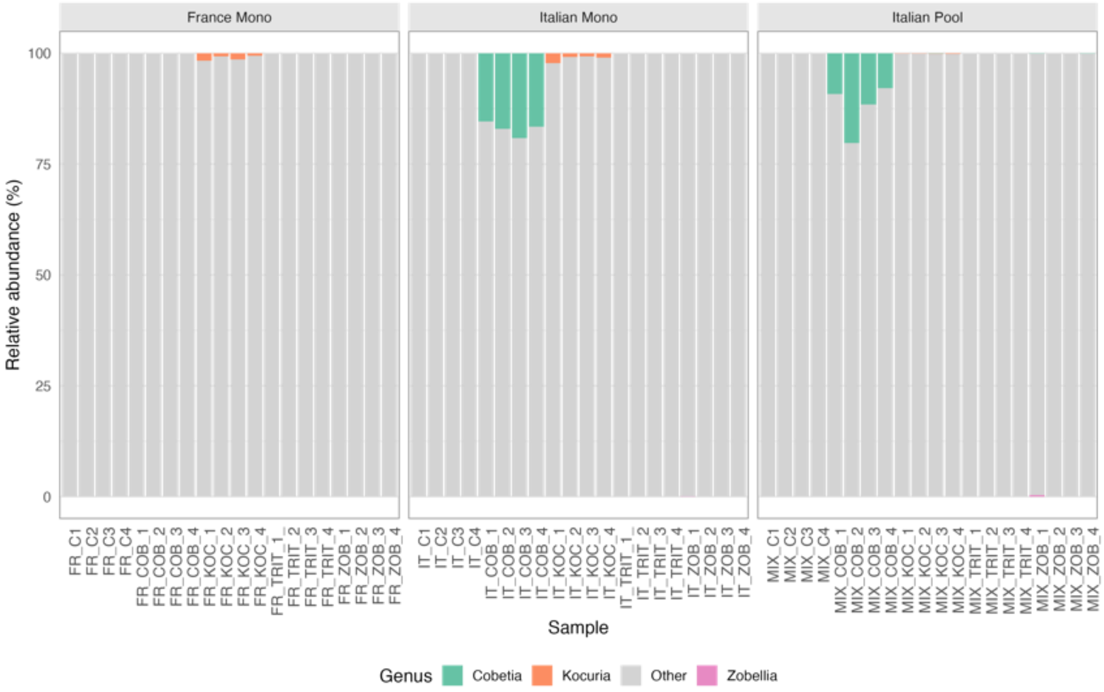

Figure 22. A barplot showing the relative abundances in percentages of the genera corresponding to our treatments and "other". The latter represent all other genera present in the microbiome. This graph helps to visualize the successful settlement of the treatments.

The settlement is successful for *K. polaris* in FR MONO and IT MONO and for *C. amphilecti* in both Italian populations (Figure 22). *Z. scottomollicae* was confirmed but the relative abundances are too little to be visualized (1.09% and 0.89% for IT MONO and IT POOL respectively). *T. alginiliquefaciens* is not included in this graph, meaning it had not known an establishment.

However, when looking at Figure 20, *Epibacterium* is a genus found across all populations unique to the *Tritonibacter* treatment. This proves an effect on the microbiome with relative abundances it both Italian populations around 50%. Therefore, this taxa is included in Figure 23.

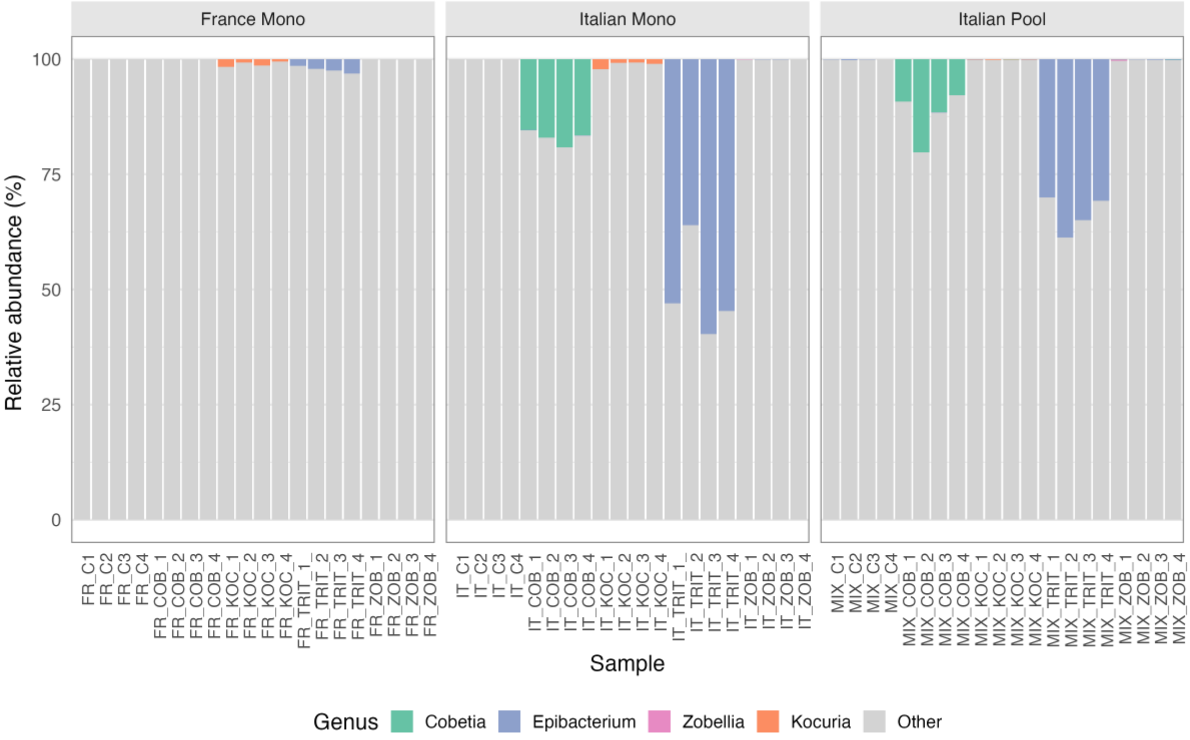

Figure 23. A barplot showing the relative abundances in percentages of the genera corresponding to our treatments with an addition of the genera Epibacterium and "other". The latter represent all other genera present in the microbiome. This graph helps to visualize the successful settlement of the treatment.

3.1.5.4. Shared core microbiome within populations

CAPS per populations (Figure 24) show distinct patterns between bacteria treatments. PERMANOVA analysis confirms this significance ($p < 0.001$). Yet this varied, 91% of the variation in Italian Mono ($R^2 = 0.91$, $F = 36.60$), 85% in FR MONO ($R^2 = 0.85$, $F = 21.75$), and 74% in Italian Pool ($R^2 = 0.74$, $F = 10.85$).

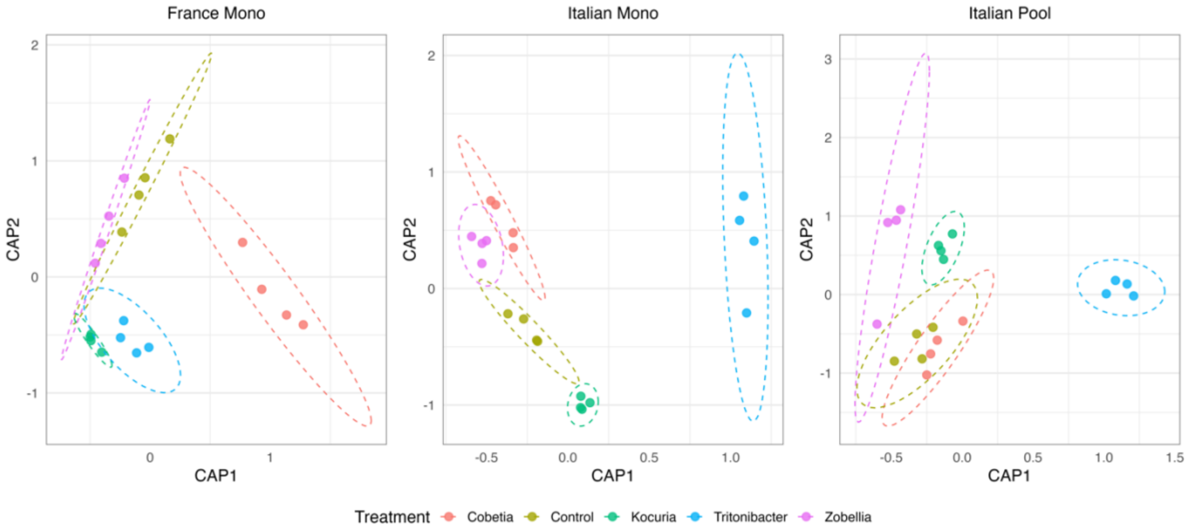

Figure 24. Canonical Analysis of Principal Coordinates (CAP) illustrating microbiome community structure within each Laminaria ochroleuca population, constrained by bacteria treatment. Ellipses represent 95% confidence intervals for each treatment group based on Bray–Curtis dissimilarities.

Looking at the different bacteria within each population the core microbiome, meaning taxa shared across all bacteria treatments, consists for IT MONO of 7 different taxa and are shown in Table 6. Regarding phyla, *Verruocomicrobiota* and *Planctomycetota* are unique to this core microbiome compared to the other populations. Their overview is also shown for FR MONO (Table 7) and IT POOL (Table 8). On class-level, they all share Bacteroidota and α- and γ- proteobacteria (Figure 19). In the appendix an overview of the shared and unique taxa of each population can be found (Table A - 7).

Table 6. Core microbiome taxa of the IT MONO population, shared among all bacteria treatments. Total number = 7.

Phylum	**Class**	**Order**	**Family**	**Genus**
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Porticoccaceae	*C1-B045*
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Marinobacteraceae	*Marinobacter*
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Tropicibacter*
Verrucomicrobiota	Verrucomicrobiae	Opitutales	Puniceicoccaceae	*Lentimonas*
Planctomycetota	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	*Phycisphaera*
Bacteroidota	Bacteroidia	Cytophagales	Cyclobacteriaceae	*Reichenbachiella*
Bacteroidota	Bacteroidia	Flavobacteriales	Crocinitomicaceae	*Fluviicola*

Table 7. Core microbiome taxa of the FR MONO population, shared among all bacteria treatments. Total number = 6.

Phylum	**Class**	**Order**	**Family**	**Genus**
Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	*Aliiglaciecola*
Bacteroidota	Bacteroidia	Flavobacteriales	Cryomorphaceae	*Owenweeksia*
Proteobacteria	Alphaproteobacteria	Kiloniellales	Kiloniellaceae	*Pelagibius*
Proteobacteria	Alphaproteobacteria	Thalassobaculales	Nisaeaceae	*Nisaea*
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Marinobacteraceae	*Marinobacter*
Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	*Alteromonas*

Table 8. Core microbiome taxa of the IT POOL population, shared among all bacteria treatments. Total number = 10.

Phylum	**Class**	**Order**	**Family**	**Genus**
Proteobacteria	Alphaproteobacteria	Thalassobaculales	Nisaeaceae	*Nisaea*
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Marinobacteraceae	*Marinobacter*
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Thalassospiraceae	*Thalassospira*
Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	*Paraglaciecola*
Proteobacteria	Alphaproteobacteria	Rhizobiales	Stappiaceae	*Labrenzia*
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingorhabdus*
Bacteroidota	Bacteroidia	Flavobacteriales	Crocinitomicaceae	*Fluviicola*
Proteobacteria	Alphaproteobacteria	Sneathiellales	Sneathiellaceae	*Sneathiella*
Proteobacteria	Alphaproteobacteria	Thalassobaculales	Thalassobaculaceae	*Thalassobaculum*
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Cohaesibacter*

3.2. Ontogenetic experiment

The starting densities differ significantly with FR MONO having lower starting densities (p = 0.0456) with mean gametophytes per cm^2 (106.74 ± 7.09 SD) and IT MONO (129.20 ± 14.99 SD). The model revealed significant main effects (Table A - 6).

At DAY 7, a first effect of bacteria treatments on the reproductive development was observed (Figure 25). In IT MONO, *Kocuria* significantly increased the proportion of gametophytes with eggs ($p = 0.0164$), and both treatments led to significantly higher egg numbers per gametophyte ($p = 0.0141$ and $p = 0.0057$, respectively). FR MONO responded only to *Kocuria*, with a comparable enhancement in egg production ($p = 0.0042$).

However, by DAY 16, the proportion of egg-bearing gametophytes had declined significantly in both populations (IT MONO: $p < 0.001$; FR MONO: $p = 0.0437$). In terms of sporophyte development, a significant increase was only detected in IT MONO treated with *Kocuria* at Day 16 ($p = 0.0358$).

A notable observation was the absence of ontogenetic structures in the FR MONO control group, except for one replicate. At DAY 7, both bacteria treatments significantly increased the likelihood of egg presence compared to the control ($p = 0.0067$). On DAY 16 this effect had disappeared.

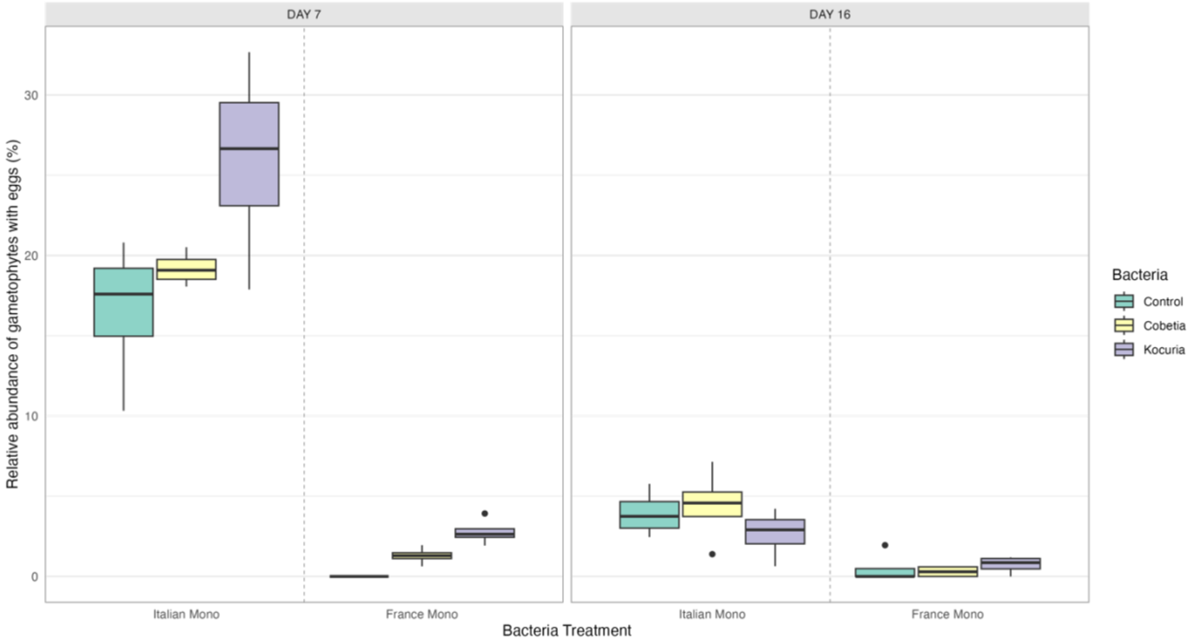

Figure 25. Boxplots show the relative abundance of gametophytes with eggs for DAY 7 and DAY 16 for both populations. The legend shows the corresponding colours to the bacteria treatments.

3.3. Heatwave experiment

3.3.1. Survival rate

Starting densities in IT MONO were 187 ± 27.6 (SD) gametophytes per cm^2 for the control group and 134 ± 27.6 (SD) for the COB treatment. In FR MONO, values were 162 ± 35.6 (SD) and 146 ± 15.6 (SD) for the control and COB treatments, respectively. These initial densities did not differ significantly across populations. However, within them, *Cobetia* consistently showed lower initial densities compared to the control. This pattern was particularly pronounced for HW19 and HW23, with significant differences in both populations (FR MONO: p = 0.0024 and 0.0315; IT MONO: p = 0.0429 and 0.0278, respectively).

The overall model effects are summarized in Table A - 7. The total densities at DAY 17 decreased in the control group of FR MONO for HW19 and HW23 ($p < 0.001$). These were not observed in IT MONO.

Across all heatwave treatments in IT MONO, differences among bacterial groups remained consistent and significant ($p < 0.05$; Figure 26).

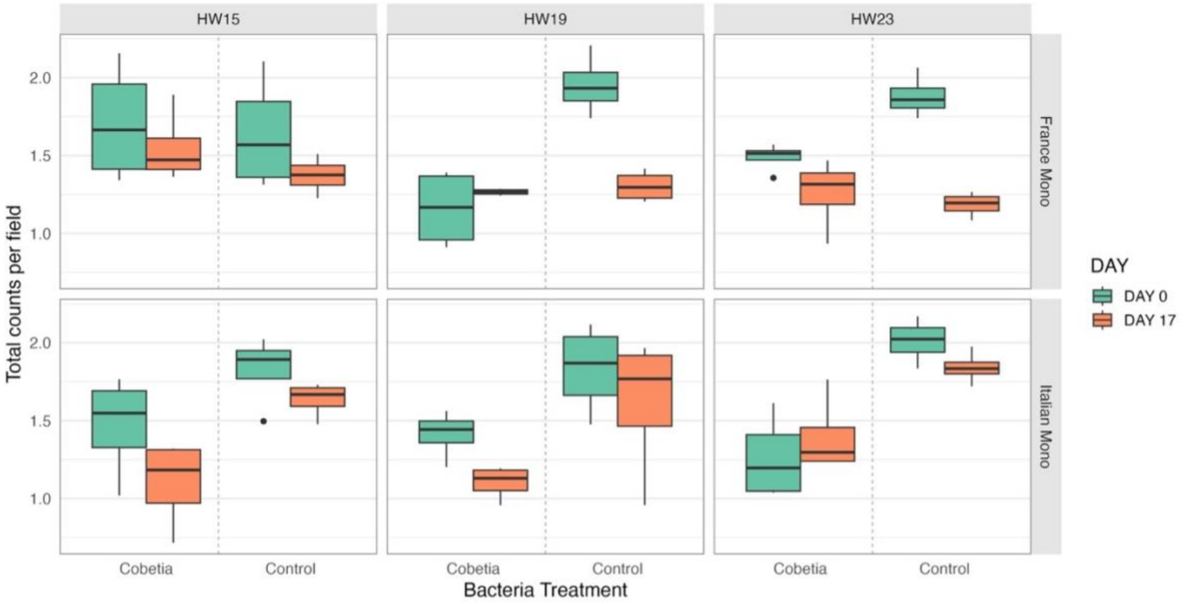

Figure 26. Boxplots of the survival rate of gametophytes at DAY 17 compared to DAY 0. The plot is faceted by heatwave treatment and population. This is the day the heatwave temperatures returned to the reference level of 15ºC.

3.3.2. Reproductive structures

The overall effects of the model are reported in Table A - 8.

Heat stress – based on the control groups - had a strong negative impact on reproductive development, significantly reducing the proportion of gametophytes with eggs (Figure 27; $p < 0.001$). However, even at HW15, remained mostly vegetative with relative abundances up to 80% for IT MONO and 90% for FR MONO.

At DAY 24 under HW23 in FR MONO, an increase in egg-bearing gametophytes was recorded compared to the other temperatures ($p < 0.001$). A similar trend was observed at DAY 38 for gametophytes with sporophyte (Figure 28). While heat stress generally suppressed the relative abundance of gametophytes with sporophytes across all populations ($p < 0.001$), HW23 again counteracted this effect ($p = 0.0194$).

An interesting observation is that no eggs were present at DAY 38 for both populations in any HW treatments.

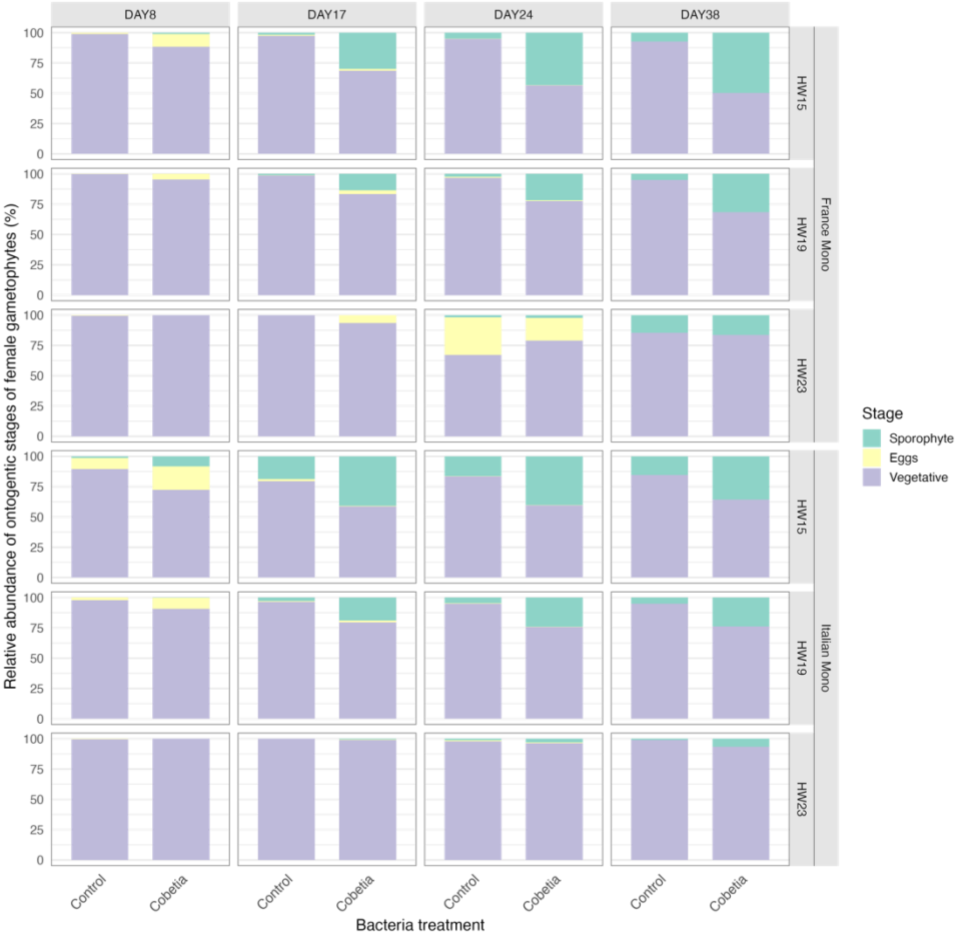

Figure 27. Stacked bars of ontogenetic stages of female gametophytes as a relative abundance in percentages. The boxplots are plotted by population and heatwave (HW) treatment and faceted by different counting days. The legend shows the different reproductive structures.

When looking at the bacteria effect, *Cobetia* had a consistent positive effect ($p < 0.001$) on reproductive development, but the start differed between populations with earlier effects for IT MONO (HW15 & HW19).

Interestingly under HW23, *Cobetia* enhanced egg production from DAY 17 onwards ($p < 0.001$) but was outperformed by the control group by DAY 24 ($p < 0.001$). Boxplots of the vegetative structures and gametophytes with eggs are put in the appendix (Figure A - 10 and - 11).

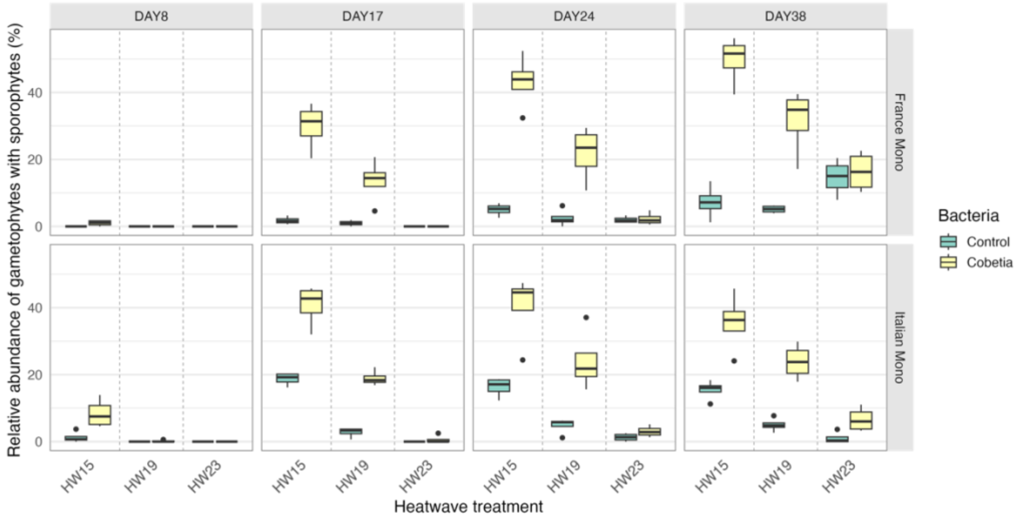

Figure 28. Boxplots showing the relative abundances of gametophytes with sporophytes per heatwave treatment. The plots are faceted by days and population.

3.3.3. Reproductive success

The overall model is significant (Table A - 9) with the 3-way interaction significantly affecting the growth of gametophytes with sporophytes ($p < 0.001$).

At DAY 31 – after two weeks recovery - *Cobetia* enhances the gametophytes with sporophytes for both populations across all HW treatments ($p < 0.001$) except for HW23 in FR MONO. For this population, pictures of sporophytes are shown in Figure 30. For IT MONO they can be found in the appendix (Figure A - 13)

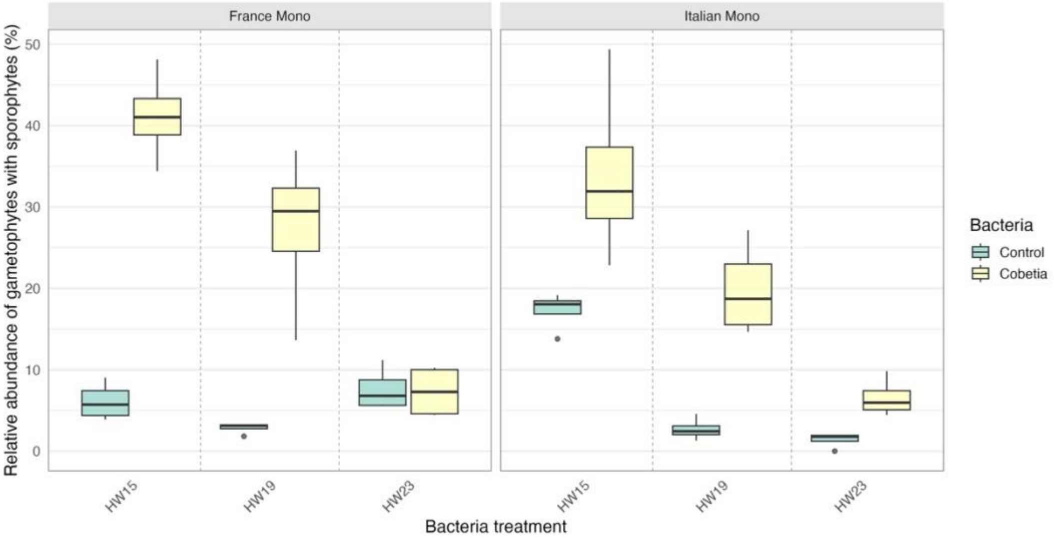

Figure 29. Boxplots showing the relative abundances of gametophytes with sporophytes per heatwave treatment at DAY 31, marking a two-week recovery. The plots are faceted per population.

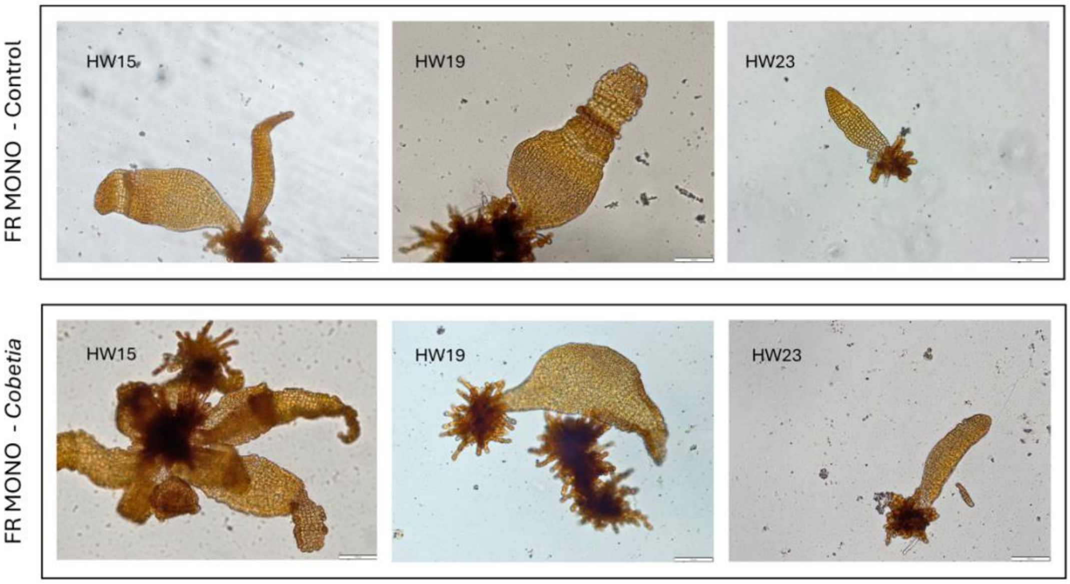

Figure 30. Pictures of gametophytes with sporophytes for FR MONO for both bacteria treatments and heatwave treatments on DAY 31, marking a 14-day recovery, taken at 100× magnification. Scale bar (0.1 mm) is shown in the bottom right corner.

Regarding healthy sporophytes per gametophytes, *Cobetia* only had a positive effect in FR MONO for treatment HW19 ($p = 0.0032$) and in HW15 ($p = 0.0149$). Statistics are in the appendix (Table A - 10). Yet visual observations show more abnormal sporophyte growth for the bacteria treatments (Figure 32).

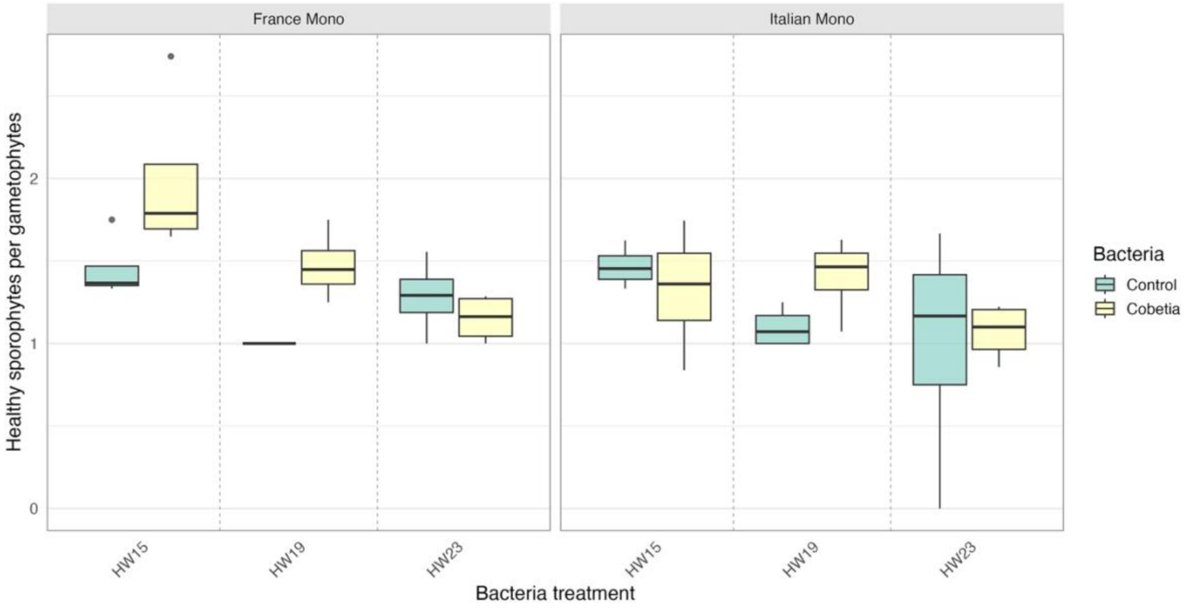

Figure 31. Boxplots showing the healthy sporophytes per gametophyte carrying sporophytes on DAY 31.

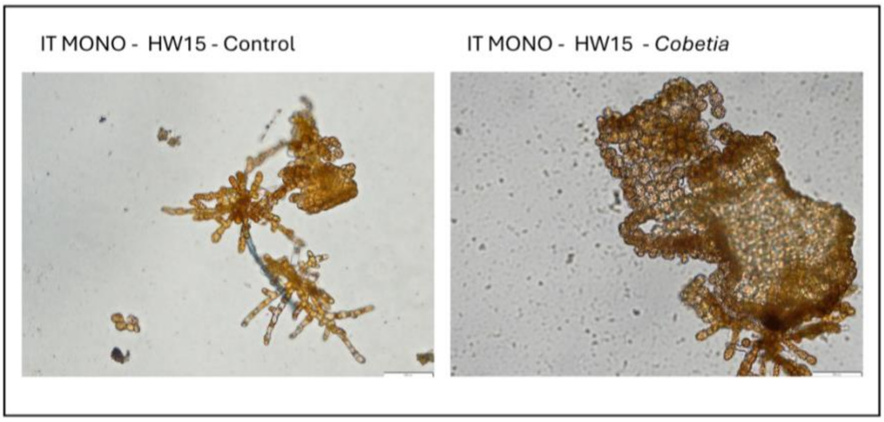

Figure 32. Pictures of abnormal sporophyte growth observed on DAY 31, taken at 100× magnification. Scale bar (0.1 mm) is shown in the bottom right

3.3.4. PAM measures

The three-way ANOVA revealed significant main effects for Treatment, Population, and Bacteria, as well as significant two-way interactions involving Treatment (Table A - 11). The three-way itself was not significant. Despite this non-significant effect, post-hoc comparisons did show biologically meaningful patterns.

No significant heat stress effect was observed within both populations comparing groups without bacteria treatment. *Cobetia* showed a significant positive effect in HW15 for both populations ($p < 0.001$) and in HW23 ($p = 0.0363$) for FR MONO. When considering heat stress for COB itself, HW15 had higher yields with their significance shown in Figure 33.

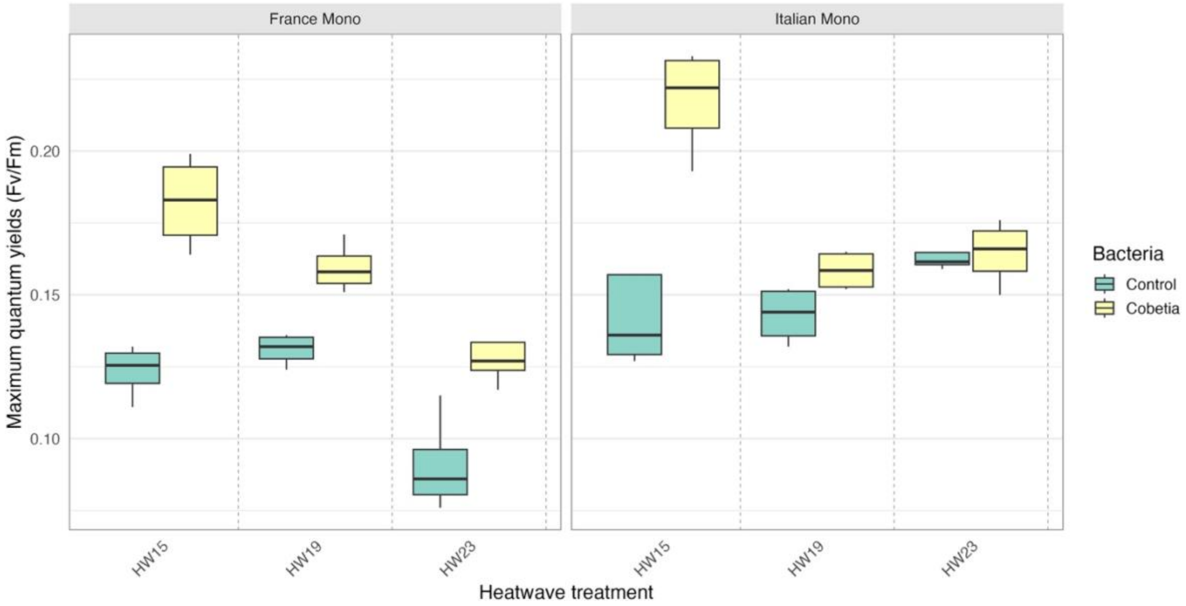

Figure 33. Boxplots with the maximum quantum yields (Fv/Fm) of both populations per heatwave treatment. These were measured on DAY 14, marking the end of the 10-day heatwave.

4. Discussion

4.1. Bacteria experiment

This experiment investigated the effect of bacteria treatments on kelp. First the overall trends and effects are discussed (section 4.1.1.) before focusing on changes in the microbiome specifically in (section 4.1.2.).

4.1.1. Host-specific responses to bacteria treatments

Most bacteria treatments showed a positive effect on the relative abundances of gametophytes with reproductive structure. Enhanced sporophyte production by DAY 7 suggests that these treatments not only improved reproductive output but also accelerated its onset (Figure 11). This is consistent with growing evidence that microbial associations can enhance algae reproduction. Although research on *L. ochroleuca* is limited, similar effects of bacteria on growth and reproduction have been reported in other algae species (Wichard, 2015; Dittami et al., 2016; Tapia et al., 2016).

Although the *Cobetia* treatment consistently enhanced reproduction across all three populations, this did not translate into improved sporophyte recruitment in IT POOL (Figure 12). This underscores the significant role of host-microbiome interactions in determining reproductive outcomes. For a bacterium to be considered a potential probiotic for restoration, it must not only promote reproduction but also lead to successful recruitment—i.e., the formation of healthy sporophytes capable of developing into reproductive adults.

The varying effectiveness of treatments across populations highlights the complex interactions of the microbiome with the macroalgae holobiont (van der Loos et al., 2019; Egan et al., 2013). Although our main interest is the effect of the bacteria itself, exploring these differences across populations might bring valuable insights considering the hypothesis that microbiome functioning is host specific.

4.1.2. Baseline differences across populations

Differences in treatment outcomes may result from underlying variation in baseline performances. The absence of an effect for successful recruitment for IT POOL may reflect a ceiling in reproductive output, where early onset of sporophyte development limited the potential for further enhancement.

IT POOL reached over 75% relative abundance of gametophytes with sporophytes early on, whereas IT MONO and FR MONO achieved similar levels only by DAY 21 (Figure 11). By then, IT POOL had already reached ~90% for treated samples, indicating higher reproductive success. The control group of IT POOL also performed better (80%) compared to the others (~50-60%) suggesting that bacteria were not the sole contributing factor. IT POOL started with lower initial gametophyte densities (Figure 10), which may have reduced nutrient competition supporting stronger performance (Edwards & Connell, 2012).

4.1.2.1. Unidentified organisms

Another difference observed in this population is the presence of unidentified motile microorganisms (Figure 14). Egan et al. (2013) emphasize the role of eukaryotic microorganisms in holobiont systems (Figure 5), indicating that these organisms might contribute indirectly to kelp reproduction through e.g. facilitating nutrient cycling, prey-predator interactions, or modulation of microbial communities. Their interactions—whether mutualistic, commensal, or antagonistic—remain unclear. As their taxonomic identity, origin, and functional role were not determined in this study, their contribution to kelp reproduction remains speculative.

4.1.2.2. Kinship

The strong performance of the control group in IT POOL may also relate to kinship among gametophytes, as this population was derived from pooled samples. Kinship, where female gametophytes produced more eggs in the presence of related males was observed in *Macrocystis pyrifera* —a strategy suggested to ensure reproductive success (Camus et al., 2021) yet how they can recognize kin remains unknown.

4.1.2.3. *Experimental set-up limitations*

On the last counting day (DAY 26), many sporophytes in most treated groups had grown large and appeared curled up (Figure 16). This likely interfered with accurate counting, potentially causing over- or underestimation of recruitment. Such physical distortion probably impaired visibility under the microscope, complicating precise quantification. To address this in future studies, recording sporophyte recruitment earlier could prevent this.

For IT POOL, the treatments may still have had an impact, but this was likely masked by the already high performance of the control group. This is consistent with the stress-gradient hypothesis (SGH), which predicts that facilitative interactions become more frequent under higher stress (Maestre et al., 2009), as well as with context-dependent mutualism theory (Bronstein, 1994).

4.1.3. Mechanisms of bacterial influences on kelp

The quantum yields (Fv/Fm) indicated that bacteria-treated samples generally maintained or had higher photosynthetic efficiency during the experiment, suggesting that presence of bacteria improved the overall health (Figure 17). This improved physiological state could underlie higher reproductive success or be an indication that healthier hosts recruit better. Yet within populations, the extent of each bacteria treatment varied.

This suggest that bacteria may promote kelp reproduction through multiple and varying mechanisms. Some examples are the production of signalling molecules that trigger developmental pathways (Frenkel et al., 2014), excreting hormone-like substances that stimulate morphogenesis (Spoerner et al., 2012) or enhancing nutrient exchange that supports energy-demanding stages such as sporophyte formation (Tapia et al., 2016). These mechanisms potentially contribute to enhanced reproductive success at different lifecycle stages or may lead to inhibition when one of them is disrupted. For example, in IT MONO, *Kocuria* had an inhibitory effect. The possible reasons will be discussed in section 4.5.2.

The consistent positive effect of *Cobetia* is reflected in its ability to stimulate early reproductive structures, accelerating reproductive onset and enhance reproductive success. In contrast, other bacteria like *e.g. Zobellia* exerted their effects mostly later in the life cycle or more indirectly, possibly enhancing sporophyte recruitment or physiological resilience rather than initial gametogenesis. In contrast, *Zobellia* was also the treatment reported to have shown more abnormal sporophyte growth, yet it can be considered negligible given their low abundances.

4.2. Microbiome analysis

4.2.1. Overall composition

The microbiome composition (Figure 18) shows a clear separation between the three populations rather than clustering by bacteria treatment. The Italian populations overlap along Axis 2, indicating both location and strain-specific patterns. This is supported by comparison with the core microbiome of the control groups (Figure 21) where IT MONO and IT POOL shared the most taxa. These patterns reflect strong host-specific associations, as previously observed (Lachnit et al., 2009) and may explain why added bacteria did not override existing population-level microbiome structures.

Grown under common garden conditions, this observed population-level difference suggest vertical transmission of the microbiome (Wilkins et al., 2019). Dash et al. (2012) propose that environmental pressures select for different energy acquisition mechanisms, which can lead to genetic divergence in associated microbial populations. This aligns with the hypothesis by Veenhof et al. (2025), suggesting that juvenile sporophytes actively select microbial symbionts based on their specific functional or metabolic needs. The microbiome is then passed on from adult sporophytes to gametophytes. Such a combination of vertical and horizontal transmission is referred to as mixed-mode transmission (Ebert, 2013).

4.2.2. The core microbiome

The core microbiome phyla observed in this study (Table 6 - 8) aligns with those reported in previous macroalgal microbiome research (Freitas et al., 2012; Wiese et al., 2008). Proteobacteria, along with Bacteroidetes, Actinobacteria, Planctomycetes, and Verrucomicrobia, were dominant across samples, consistent with typical epiphytic communities on brown algae (Wahl et al., 2012). However, notable population-specific differences emerged when looking at class and genus level (Figure 19). These compositional differences reinforce the idea that host population or strain specificity plays a significant role in shaping microbial communities (Martin-Platero et al., 2018).

Egan et al. (2013) also highlighted the difficulty of assigning a core microbiome since specific taxa vary but phyla remain consistent. Besides this, defining a “core microbiome” is highly dependent on the defined thresholds, yet there is limited literature available to standardize this. Taxa that fall below these thresholds may still be functionally relevant, particularly across genetically distinct populations. This may result in varying interpretations and makes comparisons between studies difficult.

4.2.2.1. General taxa of the core microbiome

However, one bacterium *Marinobacter* (Gammaproteobacteria) was consistently shared across all samples, even within bacteria treatments. Its ability to synthesize alginate (Si et al., 2024), a major component of brown algal cell walls (Mabeau & Kloareg, 1987), may explain its persistent presence. *Nisaea* (Alphaproteobacteria) is also commonly present, although below the core microbiome threshold in some treatments.

Additional functions of other prominent genera are listed in the Table A - 1**.** Although these genera belong to different phyla, many perform overlapping functions. This functional redundancy suggests that similar traits can be delivered by taxonomically diverse microbes (Ghaderiardakani et al.,2017; van der Loos et al., 2025).

4.2.2.2. Shifts of the microbiome within populations

While treatments may not override population-level differences - they do influence the microbiome within the same host species (Figure 24).

The strongest effect was observed in IT MONO, where 91% of the variation was explained by treatment, indicating high microbiome plasticity. FR MONO showed comparatively lower microbiome shifts (85%). In literature, a genetic difference is observed between northern and southern populations, with the former having less genetic diversity, mainly due to past climate changes (Assis et al., 2018). Our observed patterns align with the competitive lottery model (Burke et al., 2011). This may further imply that hosts with a greater genetic diversity have less selective pressures on specific microbiomes, allowing for a more variable community composition.

The relative stable core microbiome of IT POOL (74% alteration) – sharing 10 taxa across treatments (Table 8) – might be explained by possible inbreeding, reducing genetic diversity and resulting in reduced microbial plasticity.

4.2.2.3. Settlement within the microbiome

The most important proxy of assigning effects of the bacteria treatments on kelp, is their settlement in its core microbiome (Chauhan & Singh, 2018). This is considered successful for *C. amphiliecti, K. polaris* and *Z. alginiliquefaciens* yet the extent within and across populations differ (Figure 22). These differences might reflect the “chemical gardening” of the host (Saha et al., 2019). *T. alginiliquefaciens* was not included. Yet one genus *Epibacterium,* was unique for the latter treatment in all populations (Figure 23).

A possible hypothesis could be that the EMU tool assigned the sequence to *Epibacterium* whilst average nucleotide identity (ANI) prior to this thesis assigned this to *Tritonibacter* (97.51% similarity). These genera are closely related, and papers discussed assigning one genera to the other (Hördt et al., 2020; Liang et al., 2021). In the study of Li et al.

(2021), *Epibacterium* was officially assigned to *Tritonibacter*. Figure 23 would then not only confirm the settlement but would make it the most successful too. Although this theory is most likely true, without a possibility to verify our data, it remains speculative.

The Italian populations showed the most similarities, with IT MONO having all three bacteria treatments settled. This supports the above-mentioned findings of the greater microbiome alteration in IT MONO and supports the plasticity argument and aligns with the ceiling effect in IT POOL (section 4.1.2)

Similar to findings in other marine organisms like corals (Apprill, 2017), the success of microbial colonization in kelp is shaped by both host genetics and environmental factors. This host-microbiome specificity might explain the varying outcomes observed in different populations.

The distinct effects of *C. amphiliecti* and *K. polaris* are discussed in section 4.5

4.2.3. Summary and implications

The distinct population patterns in the microbiome and the different outcomes of bacteria treatments illustrate that they likely act through a combination of biochemical signalling, nutrient support, physiological modulation and the chemical gardening capacities of the host to influence kelp reproductive success. Fully understanding these complex interactions will require targeted functional analyses beyond the scope of this study, representing a promising direction for future research. Yet some first attempts are made.

4.3. Ontogenetic experiment

To further investigate the variable responses to bacteria treatments, a sex-specific experiment was conducted using exclusively female gametophytes. The experiment tested two bacterial strains: *C. amphilecti* as best performing and *K. polaris* as inhibiting bacteria strain. The populations tested were FR MONO and IT MONO.

4.3.1. Development of reproductive structures and total egg output

Bacteria treatments boosted both populations, yet their patterns regarding the relative abundances of gametophytes with eggs and egg-production were inconsistent (Figure 25). These results support our previous findings that bacteria possibly enhance reproduction via different mechanisms, including chemical gardening tailored to the host’s needs, confirming host-specificity.

Notably, FR MONO started with much lower initial densities, a pattern also observed for IT POOL in the first experiment, where *Cobetia* similarly did not exert a positive effect on the final recruitment.

4.3.2. Temporal trends

A temporal decline in both total counts and relative abundance was observed from DAY 7 to DAY 16, without bacteria counterbalancing this trend. As stated above, the absence of males is unlikely to explain this (Camus et al., 2021). Therefore, female gametophytes may have a limited timeframe following the onset of gametogenesis during which they can sustain egg production or maintain egg viability. However, this remains a hypothesis in the absence of supporting literature.

4.3.3. Formation of parthenosporophytes

In this same study, Camus et al. (2021) showed that female gametophytes of kelp species can produce parthenosporophytes without the presence of males, a suggested strategy for reproductive assurance. Other studies confirm that Laminariales can develop sporophytes via parthenogenesis from unfertilized eggs (Dieck, 1992; Martins et al., 2019).

For both populations *Kocuria* enhances the production of sporophytes. IT MONO showed higher absolute counts, whereas in FR MONO, sporophytes only occurred in the *Kocuria*-treated samples. This supports the idea that *Kocuria* boosts egg-production and without fertilization results in parthenogenesis.

However, this contrasts with the expected inhibitory effect based on the results from experiment 3.1. The effects of *Kocuria* are discussed in greater detail in section 4.5.2.

4.3.4. External stressor

An important observation in FR MONO is that ontogenetic structures were absent for the control group but increased significantly with both treatments. This absence suggest – for an unknown reason – that FR MONO is experiencing stress when grown in the Ghent Lab. Camus et al. (2021) observed higher values of female fecundity and fertility for M. *pyrifera* when unrelated males were absent, making the lack of males an unlikely explanation for the reduced egg production observed. This supports the likelihood that an unidentified stressor is affecting this population in this experiment.

Despite this stressor, this experiment reveals the consistent enhancing effects of bacteria treatments on the reproduction of *L. ochroleuca*. These findings further support – especially for *C. amphiliecti* - the potential of these bacteria as probiotic treatments.

4.4. Heatwave experiment

This experiment provides valuable insights into the response of *L. ochroleuca* to heat stress and explores how the *Cobetia* treatment may enhance the macroalga's resilience to this stressor. First, the response to heat stress independent of bacteria treatments is discussed (Section 4.4.1), followed by an evaluation of *Cobetia*'s potential to improve resilience (Section 4.4.2).

4.4.1. Response to heat stress

Heat stress appeared to negatively affect the survival of gametophytes. By the final day of the heatwave, the relative abundance of gametophytes significantly decreased. This density drop is consistent with previous studies (Strasser et al., 2022). The lower relative abundances of reproductive structures for both HW treatments are another indication that kelp was experiencing heat stress. This low rate of gametogenesis can be explained by temperatures exceeding the optimal range of 15-18°C for optimal gametophyte development in *L. ochroleuca* (Izquierdo et al., 2001). The later onset of gametogenesis as a response to a heatwave aligns with the findings of Alsuwaiyan et al. (2021).

Although not quantified, male gametophytes were observed more frequently under HW19 and HW23 (personal observation). This could support the formation of a "seed bank", where gametophytes remain vegetative under suboptimal conditions (Dayton, 1985; Silva et al., 2022). Alternatively, it may indirectly support the hypothesis that sperm release did not occur. If sperm had been released, the males would likely have died off shortly after (Izquierdo et al., 2002). One possible explanation could be that the few eggs present did not release enough lamoxirene to trigger sperm release in most males.

The lower quantum yields observed under HW23, measured on the last day of the heatwave, confirm a negative impact of heat stress on the overall physiological health (Figure 33). "Seed banks" may explain why there is no significant difference between HW19 and HW15, as dormancy in response to heat stress could counterbalance negative effects and help maintain quantum yields. This would reflect a trade-off between physiological performance and ontogeny (Strasser et al., 2022). Under HW23, the stress might have been too severe, making dormancy insufficient to offset the damage. This greater stress, although not always significant, can be confirmed by the lower relative abundances of sporophyte growth (Figure 27 and Figure 29).

After 14 days of recovery, there was no difference observed between healthy numbers of sporophytes per gametophyte between temperature treatments (Figure 31). The relative abundance of gametophytes with sporophytes was still lower for HW23, suggesting that the extent of recovery depends on the experienced heat stress. This later start of reproduction under stress conditions is in line with literature findings (Strasser et al., 2022; Martins et al., 2020). However, HW23 does not follow these patterns for FR MONO, indicating differences among populations.

4.4.1.1. Differences among populations

These differences are not uniquely to HW23, other trends have been observed across populations. This aligns with population-level variation in heat stress responses reported by Strasser et al. (2022).

A similar finding of our experiment to this study is the greater decline in densities for FR MONO compared to IT MONO. This might indirectly suggest that less genetically fit individuals are less likely to survive the heatwave. Besides the survival rate, differences were observed in the onset of gametogenesis and the relative abundances of reproductive structures (Figure 27). The germination of colder adapted species is expected to be more negatively affected (Gonzalez et al., 2018). The differing capacity to recover and reproduce between populations aligns with findings by Silva et al. (2022), suggesting species-specific thermal tolerance. Assis et al. (2018) highlight possible genetically based thermal responses for their reproductive investments linked to their origin. This is consistent with a study on *M. pyrifera* showing local adaption to thermal gradients (Hollarsmith et al., 2020).

Interestingly, the relatively high performance of the HW23 treatment in FR MONO might not indicate genuine thermal tolerance, but rather reflect an ultimate stress-induced survival response, as previously observed in seagrasses (Marín-Guirao et al., 2019).

4.4.1.2. Marine heatwaves in the Mediterranean Sea

The Mediterranean Sea has faced an increase of MHWs in intensity and frequency in the last two decades (Pastor & Khodayar, 2022). Veenhof et al. (2022) mentioned that different thermal tolerances of gametophytes can possibly have been influenced by the temperatures their parents experienced. Considering that three out of the six MHWs in 2019 reached deep into the water column (Hamdeno & Alvera-Azcaráte, 2023), which corresponds to the location and time of the sampling of our Italian populations, the better performance of IT MONO strains might be explained by this thermal history.

4.4.1.3. Possible external stressor impact

When comparing experiment 3.1. and 3.3. in terms of the relative abundance of reproductive structures, a key difference emerges. In Faro (experiment 3.1, without heat stress), control groups of both populations consistently showed a minimum of 50% relative abundance of eggs and sporophytes. In contrast, the highest relative abundance observed in the current heat stress experiment is only around 20% in the control groups of both populations. This further indicates the possibility of an external stressor being present, as suggested in the ontogenetic experiment.

Possible reasons will be explored in supplementary 7.1.1.

4.4.2. Effect of Cobetia treatment

4.4.2.1. Overall findings

At the end of the heatwave, unlike the control groups, the *Cobetia*-treated groups showed no significant decline in kelp survival across all populations. It resulted in the earlier onset of gametogenesis whereas the control groups remained vegetative.

The overall fitness maintained its positive effect for *Cobetia* treatment under HW15 in both populations, confirming its benefit under normal conditions (Figure 33). Although no significant effect was detected at the higher stress level (HW19), this does not necessarily mean that *Cobetia* had no influence. Based on the seedbank theory, the earlier onset of egg production observed with *Cobetia* treatment (Figure 27) might have been expected to reduce fitness due to higher energy demands. However, this was not the case, suggesting that *Cobetia* enhances reproduction while maintaining healthy fitness levels even under stress conditions, thereby explaining the status quo.

Thermal threshold

The bacterium also consistently boosted the relative abundance of reproductive structures in all populations, yet values appeared to decrease with increasing HW temperatures. This might imply a threshold beyond which *Cobetia* cannot fully counteract the negative effects of heat stress.

This pattern is particularly evident in FR MONO, where baseline levels in control groups for gametophytes with sporophytes remained similarly low across heatwave treatments (Figure 29). This consistent baseline might suggest that the variation in performance is driven by the temperature effect, not in host performance. It highlights that *Cobetia* provided a strong and stable benefit that appears to be lost entirely under HW23.

In contrast, the control groups in IT MONO differed for each HW treatment, yet the increase of *Cobetia* relative to the control appeared stable across treatments. Overall, effect sizes for *Cobetia* are largest under moderate heat stress (HW15), reduced at intermediate stress (HW19), and minimal or absent under severe heat stress (HW23), regardless of how control groups perform.

Successful recruitment

Although healthy sporophyte numbers didn’t differ significantly (Figure 31), abnormal sporophytes were relatively more abundant when treated with *Cobetia* (Figure 32). This suggests that while Cobetia may promote overall sporophyte growth, excessive growth could lead to space limitations or stress, causing a higher proportion of abnormal development. These observations are visual only, so future work should include quantitative measures to confirm these patterns.

4.4.2.2. Population-level differences

Differences across populations are also observed regarding the bacteria treatment. In HW23, *Cobetia* only enhanced gametogenesis in FR MONO (Figure 27). Interestingly, on DAY 24, the control group outperforms *Cobetia* regarding egg-carrying gametophytes. This potentially confirms the earlier suggestion that the strong performance of HW23 in FR MONO is driven by an ultimate stress-induced survival mechanism (section 4.4.1.1.)

Yet, this enhanced egg-production results in a status quo between the control group and *Cobetia*-treated replicas at the end of the experiment regarding sporophytes. This suggests that not all eggs in the control group were fertilized. A reason could be a mismatch with the timing of sperm release - as male gametophytes are known to develop faster - and sperm typically remains viable for only around 12 hours (Li et al., 2013). The heatwave may have impaired sperm motility or fertility. However, studies investigating such post-MHW effects remain lacking. At this time, there is a total absence of eggs in all treatments across all populations (Figure A - 11). Therefore, this non-fertilization hypothesis cannot be confirmed due to the disappearance of all eggs.

Another population difference is that only FR MONO was enhanced by *Cobetia* in healthy sporophytes per gametophytes (HW15 and HW19). The contrast with the relative abundances being significant higher for *Cobetia* in all treatments and populations, supports our previous hypothesis that the bacteria treatment can boost different mechanisms or the chemical gardening depending on the host's need.

4.4.2.3. Summary and implications

These observations suggest a context-dependent interaction, where the beneficial effect of *Cobetia* is modulated by the host' stress level. This ability of the microbial community to buffer negative effects up to a certain threshold has also been observed in the study of Brodie et al. (2016) in corals. The consistent positive effect of *Cobetia* during heat stress aligns with the ability of microbiota to modify the response of the host to changes in the environment (van der Loos., 2019). Rather than altering the microbiome, it may have mitigated dysbiosis or stabilized the microbiota to avoid additional heat stress as previously proposed by Li et al. (2022).

An important limitation in this study is the very low occurrence of reproduction in the FR MONO control group. Possible reasons for this are discussed in the supplementary (7.1.1.).

4.5. Concluding effects for probiotics

Although not consistent, all bacteria treatments did exert a positive effect in some way, which means they should not be dismissed as insignificant. Exploring each one in depth would require more time and resources than were available. As a result, this discussion focuses on *C. amphilecti* and *K. a polaris*. The consistently positive effect of *Cobetia* treatment highlights its promise as a probiotic candidate (4.5.1.). However, fully understanding the inhibitory effect of *Kocuria* treatment is essential to anticipate potential limitations in future applications (4.5.2.).

4.5.1. The positive effect of *Cobetia amphilecti*

Across all three experiments, the *Cobetia* treatment significantly enhances kelp's reproductive success, overall fitness and resilience to heat stress. To better understand these benefits, it is important to consider the biological and ecological characteristics of this bacterium.

4.5.1.1. Key characteristics of Cobetia amphilecti

Cobetia spp. are gram-negative, halophilic and facultative psychrophilic marine bacteria with a temperature range of 0 – 27 ºC (Yumoto et al., 2004). They are motile because of their peritrichous flagella (Arahal et al., 2002). They are oxidase negative (Arahal et al., 2002) and can store energy efficiently via accumulating polyhydroxybutyrate that acts as carbon reserves (Roohi et al., 2017).

C. amphilecti may be considered a functional generalist, based on the generalist hypothesis introduced in the context of *Sargassum muticum* (Aires et al., 2022). It might suggest that as a generalist, *Cobetia* can occupy broad metabolic or ecological niches, making them more likely to persist across varying hosts and environmental conditions.

Literature has shown that *C. amphilecti* possesses a range of functional traits that may contribute to its effectiveness as a probiotic. It exhibits antibiofilm activity (Balabanova et al., 2017) and anti-microbial activity (Balabanova et al., 2016). This can keep away pathogens and prevent diseases (Goecke et al. 2010). It can cleave certain proteins (Noskova et al., 2025) and can tolerate toxic concentrations of heavy metals and suggested that it may adapt its metabolism according to its surrounding environment (Ivanova et al., 2005). A different species, called *Cobetia marina*, is found to have a metabolic co-operation, meaning it helps other microbes break down and use nutrients (Ivanova et al., 2005). This inter-species communication was also confirmed by Balabanova et al. (2016). Noskova et al. (2025) highlighted the potential of *C. amphilecti* for various fields like biotechnology and ecology.

4.5.1.2. Sampling region on the kelp individual

A reason *C. amphilecti* might outperform the other bacteria strains as a probiotic, could be attributed to its context-dependent colonization. Given it was sourced from kelp blades of a French population, it is plausible that it naturally exists of traits beneficial for exposed tissue such as stress-tolerant capacities, capable of biofilm formation or antagonism against pathogens.

Regional and tissue-specific differences in microbiomes have been documented in various *Laminaria* species, with studies showing distinct microbial communities between *e.g.* blade and thallus tissues (Lemay et al., 2021; Ihua et al., 2020), across geographical origins (Morrissey et al., 2019) and seasons (Tujula et al., 2009).

4.5.1.3. Establishments in the core microbiome

For both Italian populations, the establishment was successful. These dynamics suggest distinct colonization strategies: IT POOL retained most control-associated taxa, whereas IT MONO showed a stronger shift, with Cobetia-associated taxa replacing those of the control. This pattern aligns with the competitive lottery model, where introduced microbes compete for niche establishment. The fact that Alphaproteobacteria in the core microbiome replaced other Alphaproteobacteria, confirms this (Table A - 3).

For FR MONO, *C. amphilecti* did not establish in the microbiome yet *Aliiglaciecola* spp. was significantly boosted. In line with the holobiont theory (Rosenberg & Zilber-Rosenberg, 2016) the positive effects of *Cobetia* on FR MONO could be attributed to synergistic interactions with the host, where the microbiome provides functional benefits that enhance host fitness, despite not being fully integrated into the core microbiome. Therefore, the enhancement of *Aliiglaciecola* spp. could be a proof of the above-mentioned metabolic co-operation (Ivanova et al., 2005).

4.5.2. The negative effect of *Kocuria polaris*

4.5.2.1. Key characteristics of Kocuria polaris

K. polaris is an orange-pigmented, gram-positive bacterium (Gundlapally et al., 2015) belonging to the phylum Actinobacteria. It can grow in temperatures ranging from 5 – 28 ºC (Zhou et al., 2008), contains high proportions of fatty acids and can reduce nitrate (Reddy et al., 2003).

Kocuria spp. are known for their strong surface adhesion properties (Ananieva et al., 2018), which may facilitate their integration into the host microbiome and influence community composition more directly. Actinobacteria can produce antimicrobial metabolites (Girão et al., 2019) and might thereby improve the ability to compete on the host surface (Table A - 1).

4.5.2.2. The alteration of the core microbiome of Italian Mono

For both Italian Mono and FR MONO, *K. polaris* was established in the core microbiome (figure 23). The establishment was not successful in IT POOL, supporting our hypothesis that it is more stable and therefore the host-microbiome resisted colonization.

Gametophytes treated with *K. polaris* had the highest relative abundance of *Verrucomicrobia*, indicating that the holobiont equilibrium was altered. The absence of the *Nisaea* genus in the core microbiome is supporting this change. As a result, the core microbiome kelps treated with *K. polaris* only shared *Marinobacter* across populations and treatments. Little literature can be found about a potential interplay of these bacteria, but if so, the absence may reflect the loss of an important microbial partnership.

This alteration of the core microbiome in IT MONO may promote an antagonistic shift in *K. polaris* or other involved bacteria as proposed by the Jeykyll-Hyde theory of Seyedsayamdost et al. (2011) or outcompeted taxa with functional traits. In contrast, since FR MONO already hosted members of the Actinobacteria phylum, it potentially buffered against such disruption through functional redundancy (Figure A - 7).

4.5.2.3. Mechanisms affecting and inhibiting growth

Since *Kocuria* inhibited especially the sporophyte growth in IT MONO, this discussion will explore several mechanisms that might have negatively affected reproduction.

The bacterium *Sulfitobacter*

An important genus in both Italian populations is *Sulfitobacter*, which interacts with the host by promoting growth, morphogenesis, and protects against pathogens (Table A - 1). It is also known to have a quorum-sensing (QS) system (Zhou et al., 2016).

It has been observed that *Sulfitobacter* can switch from mutualistic to algicidal activity. One hypothesis suggests that the bacterium is lysing microalgae to obtain more DMSP, but it is unknown if this is regulated by the QS system (Hu et al., 2021). The establishment of *K. polaris* might have promoted this switch by *e.g.* inducing a stress-response due to the alteration of the microbiome. Besides this antagonistic shift, *K. polaris* may have disrupted quorum sensing or chemical signalling more broadly, thereby destabilizing the symbiosis and impairing functional interactions. However, further research is needed to fully understand this.

The different outcomes in populations - where FR MONO had no *Sulfitobacter* and IT POOL had no establishment of *K. Polaris* – are consistent with this Jekyll-and-Hyde hypothesis where context-dependent shifts in microbial function can dramatically alter holobiont outcomes (Seyedsayamdost et al., 2011).

Possible egg-production impairment

Although only starting from DAY 14, *Kocuria* did start egg production in the first experiment (Figure A - 2Figure A - 11). The ontogenetic experiment confirmed this boost in egg production in both populations (Figure 25). Therefore, it is more likely that other reasons are related to the inhibition of fertilization or sporophyte growth.

The ontogenetic experiment also showed a significant positive effect of KOC on parthenosporophytes forming. This could explain that the little observed sporophytes in the first experiment (experiment 3.1.) were parthenosporophytes.

Possible lamoxirene inhibition

Since egg production itself appeared unaffected, the failure of fertilization may be linked to the secretion of the pheromone Lamoxirene being impaired. This hormone is a chemotaxon that activates sperm production and the motility of these cells towards the eggs (Mamer, 1984). Disruption of this signal — whether by microbial interference, community imbalance, or altered host physiology — could represent a form of interkingdom signalling failure, potentially explaining the absence of sporophyte recruitment despite gametogenesis. Given the strict stereochemical specificity of lamoxirene (Müller et al., 1979), even subtle disruptions could affect gamete release and fertilization success.

Further research could provide more insights in this. A follow-up experiment was considered but due to lack of equipment and expertise to conduct experiments with such volatile and rather expensive molecules, the ontogenetic experiment was conducted first.

Possible affected motility of fertility of males

Another hypothesis might be that affected males prevented fertilization. Disruption could occur at multiple levels, including the general fitness of male gametophytes, the development of antheridia, the release of sperm cells, or the motility and viability of those sperm cells. An example in literature is *Pseudomonas protegens* immobilizing the motile *Chlamydomonas reinhardtii* via deflagellation by accumulating around the algae (Cirri & Pohnert, 2019). However, very limited literature is available on the development and physiology of male reproductive structures in *L. ochroleuca* or other kelps (Veenhof et al., 2021).

Efforts were made to examine male-specific reproductive success but were unsuccessful to bring insights due to the lack of detailed knowledge regarding recognizing antheridia formation and sperm release mechanisms, highlighting a key gap in the field (Supplementary ⍰o-)

4.6. Future outlook

The consistent positive response to *C. amphilecti* highlights its promising potential for microbial enhancement in kelp restoration. This aligns with recent work of Noskova et al. (2025), which identified the potential of *C. amphilecti* for several applications, including biotechnology and ecology. This underscores the value of the bacterium as an especially exciting target for future studies. However, the variability observed across populations and differing effects of other bacterial strains highlight the complexity of host-microbiome interactions and the need for further exploration.

Metagenomic and metabolomic approaches could clarify functional shifts and better identify microbial traits critical for kelp resilience. It will also be essential to screen for context-dependent negative effects, as certain strains—such as *K. polaris* —may inhibit successful recruitment in specific populations while showing neutral or beneficial effects in others. Understanding these population-specific responses is crucial to avoid unintended outcomes in restoration efforts.

Under this precautionary approach, field trials to study ecological interaction should only be considered once such risks have been ruled out. Therefore, research should first prioritize laboratory and mesocosm experiments.

The Baldwin effect suggests that environmentally induced changes can drive evolutionary responses (Crispo, 2007). If microbial manipulation promotes phenotypic flexibility in kelp, repeated exposure to beneficial microbes might influence long-term adaptation, especially under climate stressors like marine heatwaves. Future research should include long-term trials to evaluate the persistence of introduced probiotic treatments.

This cautious approach could be supported by combining strategies like the green gravel approach (Chemello et al., 2024), with controlled probiotic testing prior to outplanting. These frameworks account for the discrepancy between laboratory and real-world conditions, while also minimizing the risk of unintended consequences in open-ocean environments (Coleman & Goold, 2019). Together, they offer a pathway toward the successful, sustainable, and scalable use of probiotics in kelp restoration.

5. Conclusion

With anthropogenic stressors increasing and marine heatwaves expected to more frequently occur, probiotics are gaining attention as a tool to boost the health of marine holobionts and its resilience to environmental stress.

In this study, the application of bacterial strains to kelp revealed that *Cobetia amphilecti* significantly enhanced both the onset and success of reproductive development. Its positive effects were maintained even under heat stress, suggesting that *C. amphilecti* not only supports reproductive processes but may also improve the resilience of *Laminaria ochroleuca* to warming seawater temperatures.

This thesis supported previous findings that elevated temperatures negatively impact kelp reproductive success and survival, with differing outcomes per population. These population-specific differences were also observed regarding the effectiveness of the bacteria treatments. This highlights the importance of host-specificity, where different bacteria have different outcomes depending on the species. This is further supported by our findings that the bacteria treatments could not override the microbiome composition on population-level yet could alter the microbiome within populations.

All four bacteria treatments (*Cobetia, Kocuria, Tritonibacter* and *Zobellia*) exerted positive effects at some point of this research. These variations suggest not only host-specificity, but also that bacteria are probably acting on different mechanisms. Particularly notable is the inhibitory effect of *Kocuria polaris* on the Italian Mono population, despite showing neutral or beneficial effects in others. It is of utmost importance to first fully understand the reason behind these contrasting outcomes, before applying probiotics to kelp ecosystems. Considering the ocean is an open system, unforeseen negative effects can be detrimental.

These findings open exciting avenues for using probiotics in kelp restoration, though a precautionary approach remains essential. Future research should aim to better understand the mechanisms, duration, and stability of probiotic effects. When thoughtfully combined with techniques like the green gravel method, probiotics have the potential to become a powerful tool in strengthening kelp forests against the challenges of a changing ocean.

6. References

Aires, T., Kläui, A., & Engelen, A. (2022). Regional microbiome differentiation of the invasiveSargassum muticum(Fucales, Phaeophyceae) follows the generalist host hypothesis across the North East Atlantic. *European Journal of Phycology, 58(3),* 268–283. https://doi.org/10.1080/09670262.2022.2103738

Alsuwaiyan, N., Vranken, S., Filbee-Dexter, K., Cambridge, M., Coleman, M., & Wernberg, T. (2021). Genotypic variation in response to extreme events may facilitate kelp adaptation under future climates. *Marine Ecology Progress Series*, *672*, 111–121. https://doi.org/10.3354/meps13802

Amin, S. A., Green, D. H., Hart, M. C., Küpper, F. C., Sunda, W. G., & Carrano, C. J. (2009). Photolysis of iron–siderophore chelates promotes bacterial–algal mutualism. *Proceedings of the National Academy of Sciences*, *106*(40), 17071–17076. https://doi.org/10.1073/pnas.0905512106

Amin, S. A., Hmelo, L. R., Van Tol, H. M., Durham, B. P., Carlson, L. T., Heal, K. R., Morales, R. L., Berthiaume, C. T., Parker, M. S., Djunaedi, B., Ingalls, A. E., Parsek, M. R., Moran, M. A., & Armbrust, E. V. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature*, *522*(7554), 98–101. https://doi.org/10.1038/nature14488

Ananieva, M. M., Nazarchuk, O. A., Faustova, M. O., Basarab, Y. O., Loban, G. A., Ананьєва, М. М., Назарчук, О. А., Фаустова, М. О., Басараб, Я. О., & Лобань, Г. А. (2018). Pathogenicity Factors of Kocuria kristinae Contributing to the Development of Peri-Implant Mucositis. *Malaysian Journal of Medicine and Health Sciences*. http://elib.umsa.edu.ua/jspui/bitstream/umsa/8728/1/2018092410472706_MJMHS_Oct_2018.pdf

Apprill, A. (2017). Marine Animal Microbiomes: Toward Understanding Host–Microbiome Interactions in a Changing Ocean. *Frontiers in Marine Science*, *4*. https://doi.org/10.3389/fmars.2017.00222

Arahal, D. R., Castillo, A. M., Ludwig, W., Schleifer, K. H., & Ventosa, A. (2002). Proposal of Cobetia marina gen. nov., comb. nov., within the Family Halomonadaceae, to Include the Species Halomonas marina. *Systematic and Applied Microbiology*, *25*(2), 207–211. https://doi.org/10.1078/0723-2020-00113

Assis, J., Serrão, E. Á., Coelho, N. C., Tempera, F., Valéro, M., & Alberto, F. (2018). Past climate changes and strong oceanographic barriers structured low-latitude genetic relics for the golden kelp Laminaria ochroleuca. *Journal of Biogeography*, *45*(10), 2326–2336. https://doi.org/10.1111/jbi.13425

Balabanova, L. A., Golotin, V. A., Kovalchuk, S. N., Babii, A. V., Shevchenko, L. S., Son, O. M., Kosovsky, G. Y., & Rasskazov, V. A. (2016). The Genome of the marine bacterium Cobetia marina KMM 296 isolated from the mussel Crenomytilus grayanus (Dunker, 1853). *Russian Journal of Marine Biology*, *42*(1), 106–109. https://doi.org/10.1134/s106307401601003x

Balabanova, L., Podvolotskaya, A., Slepchenko, L., Eliseikina, M., Noskova, Y., Nedashkovskaya, O., Son, O., Tekutyeva, L., & Rasskazov, V. (2017). Nucleolytic enzymes from the marine bacterium Cobetia amphilecti KMM 296 with antibiofilm activity and biopreservative effect on meat products. *Food Control*, *78*, 270–278. https://doi.org/10.1016/j.foodcont.2017.02.029

Bengtsson, M. M., Sjøtun, K., Lanzén, A., & Øvreås, L. (2012). Bacterial diversity in relation to secondary production and succession on surfaces of the kelp Laminaria hyperborea. *The ISME Journal*, *6*(12), 2188–2198. https://doi.org/10.1038/ismej.2012.67

Bengtsson, M., Sjøtun, K., & Øvreås, L. (2010). Seasonal dynamics of bacterial biofilms on the kelp Laminaria hyperborea. *Aquatic Microbial Ecology*, *60*(1), 71–83. https://doi.org/10.3354/ame01409

Bertocci, I., Godino, J. D., Freitas, C., Incera, M., Araújo, R., Bio, A., Arenas, F., Sousa-Pinto, I., Reis, P. A., & Domínguez, R. (2014). The regime of climate-related disturbance and nutrient enrichment modulate macroalgal invasions in rockpools. *Biological Invasions*, *17*(1), 133–147. https://doi.org/10.1007/s10530-014-0711-4

Biskup, S., Bertocci, I., Arenas, F., & Tuya, F. (2014). Functional responses of juvenile kelps, Laminaria ochroleuca and Saccorhiza polyschides, to increasing temperatures. *Aquatic Botany*, *113*, 117–122. https://doi.org/10.1016/j.aquabot.2013.10.003

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C. D. (2009). Microbial disease and the coral holobiont. *Trends in Microbiology*, *17*(12), 554–562. https://doi.org/10.1016/j.tim.2009.09.004

Brodie, J., Williamson, C., Barker, G. L., Walker, R. H., Briscoe, A., & Yallop, M. (2016). Characterising the microbiome ofCorallina officinalis, a dominant calcified intertidal red alga. *FEMS Microbiology Ecology*, *92*(8), fiw110. https://doi.org/10.1093/femsec/fiw110

Bronstein, J. L. (1994). Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution*, *9*(6), 214–217. https://doi.org/10.1016/0169-5347(94)90246-1

Burgunter-Delamare, B., Shetty, P., Vuong, T., & Mittag, M. (2024). Exchange or Eliminate: The Secrets of Algal-Bacterial Relationships. *Plants*, *13*(6), 829. https://doi.org/10.3390/plants13060829

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2011). Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(34), 14288–14293. https://doi.org/10.1073/pnas.1101591108

Campbell, A. H., Harder, T., Nielsen, S., Kjelleberg, S., & Steinberg, P. D. (2011). Climate change and disease: bleaching of a chemically defended seaweed. *Global Change Biology*, *17*(9), 2958–2970. https://doi.org/10.1111/j.1365-2486.2011.02456.x

Campbell, A. H., Marzinelli, E. M., Vergés, A., Coleman, M. A., & Steinberg, P. D. (2014). Towards Restoration of Missing Underwater Forests. *PLoS ONE*, *9*(1), e84106. https://doi.org/10.1371/journal.pone.0084106

Camus, C., Solas, M., Martínez, C., Vargas, J., Garcés, C., Gil-Kodaka, P., Ladah, L. B., Serrão, E. A., & Faugeron, S. (2021). Mates Matter: Gametophyte Kinship Recognition and Inbreeding in the Giant Kelp,Macrocystispyrifera(Laminariales, Phaeophyceae). *Journal of Phycology*, *57*(3), 711–725. https://doi.org/10.1111/jpy.13146

Chauhan, A., & Singh, R. (2018). Probiotics in aquaculture: a promising emerging alternative approach. *Symbiosis*, *77*(2), 99–113. https://doi.org/10.1007/s13199-018-0580-1

Chemello, S., Santos, I. a. D., Sousa-Pinto, I., & Pereira, T. R. (2024). Unlocking the Potential of Green Gravel Production for Efficient Kelp Restoration: How Seeding Density Affects the Development of the Golden Kelp Laminaria ochroleuca. *Phycology*, *4*(3), 443–449. https://doi.org/10.3390/phycology4030025

Cirri, E., & Pohnert, G. (2019). Algae−bacteria interactions that balance the planktonic microbiome. *New Phytologist*, *223*(1), 100–106. https://doi.org/10.1111/nph.15765

Coleman, M. A., & Goold, H. D. (2019). Harnessing synthetic biology for kelp forest conservation 1. *Journal of Phycology*, *55*(4), 745–751. https://doi.org/10.1111/jpy.12888

Coleman, M., & Wernberg, T. (2020). The Silver Lining of Extreme Events. *Trends in Ecology & Evolution*, *35*(12), 1065–1067. https://doi.org/10.1016/j.tree.2020.08.013

Crispo, E. (2007). The baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution*, *61*(11), 2469–2479. https://doi.org/10.1111/j.1558-5646.2007.00203.x

Dash, H. R., Mangwani, N., Chakraborty, J., Kumari, S., & Das, S. (2012). Marine bacteria: potential candidates for enhanced bioremediation. *Applied Microbiology and Biotechnology*, *97*(2), 561–571. https://doi.org/10.1007/s00253-012-4584-0

Dayton, P. K. (1985). Ecology of Kelp Communities. *Annual Review of Ecology and Systematics*, *16*, 215–245. https://www.jstor.org/stable/2097048

De Clerck, O., Kao, S. M., Bogaert, K. A., Blomme, J., Foflonker, F., Kwantes, M., Vancaester, E., Vanderstraeten, L., Aydogdu, E., Boesger, J., Califano, G., Charrier, B., Clewes, R., Del Cortona, A., D'Hondt, S., Fernandez-Pozo, N., Gachon, C. M., Hanikenne, M., Lattermann, L., . . . Bothwell, J. H. (2018). Insights into the Evolution of Multicellularity from the Sea Lettuce Genome. *CB/Current Biology*, *28*(18), 2921-2933.e5. https://doi.org/10.1016/j.cub.2018.08.015

Dieck, I. T. (1992). North Pacific and North Atlantic digitate Laminaria species (Phaeophyta): hybridization experiments and temperature responses. *Phycologia*, *31*(2), 147–163. https://doi.org/10.2216/i0031-8884-31-2-147.1

Dittami, S. M., Arboleda, E., Auguet, J. C., Bigalke, A., Briand, E., Cárdenas, P., Cardini, U., Decelle, J., Engelen, A. H., Eveillard, D., Gachon, C. M., Griffiths, S. M., Harder, T., Kayal, E., Kazamia, E., Lallier, F. H., Medina, M., Marzinelli, E. M., Morganti, T. M., . . . Not, F. (2021). A community perspective on the concept of marine holobionts: current status, challenges, and future directions. *PeerJ*, *9*, e10911. https://doi.org/10.7717/peerj.10911

Dittami, S. M., Duboscq-Bidot, L., Perennou, M., Gobet, A., Corre, E., Boyen, C., & Tonon, T. (2016). Host–microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *the ISME Journal*, *10*(1), 51–63. https://doi.org/10.1038/ismej.2015.104

Ebert, D. (2013). The Epidemiology and Evolution of Symbionts with Mixed-Mode Transmission. *Annual Review of Ecology Evolution and Systematics*, *44*(1), 623–643. https://doi.org/10.1146/annurev-ecolsys-032513-100555

Edwards, M. S., & Connell, S. D. (2012). Competition, a Major Factor Structuring Seaweed Communities. In *Ecological studies* (pp. 135–156). https://doi.org/10.1007/978-3-642-28451-9_7

Egan, S., & Gardiner, M. (2016). Microbial Dysbiosis: Rethinking disease in marine ecosystems. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00991

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., & Thomas, T. (2013). The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiology Reviews*, *37*(3), 462–476. https://doi.org/10.1111/1574-6976.12011

Eger, A. M., Marzinelli, E. M., Beas-Luna, R., Blain, C. O., Blamey, L. K., Byrnes, J. E. K., Carnell, P. E., Choi, C. G., Hessing-Lewis, M., Kim, K. Y., Kumagai, N. H., Lorda, J., Moore, P., Nakamura, Y., Pérez-Matus, A., Pontier, O., Smale, D., Steinberg, P. D., & Vergés, A. (2023). The value of ecosystem services in global marine kelp forests. *Nature Communications*, *14*(1). https://doi.org/10.1038/s41467-023-37385-0

Eger, A. M., Marzinelli, E. M., Christie, H., Fagerli, C. W., Fujita, D., Gonzalez, A. P., Hong, S. W., Kim, J. H., Lee, L. C., McHugh, T. A., Nishihara, G. N., Tatsumi, M., Steinberg, P. D., & Vergés, A. (2022). Global kelp forest restoration: past lessons, present status, and future directions. *Biological Reviews/Biological Reviews of the Cambridge Philosophical Society*, *97*(4), 1449–1475. https://doi.org/10.1111/brv.12850

Erwin, P. M., Pita, L., López-Legentil, S., & Turon, X. (2012). Stability of Sponge-Associated Bacteria over Large Seasonal Shifts in Temperature and Irradiance. *Applied and Environmental Microbiology*, *78*(20), 7358–7368. https://doi.org/10.1128/aem.02035-12

Fernandes, N., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2012). Community Structure and Functional Gene Profile of Bacteria on Healthy and Diseased Thalli of the Red Seaweed Delisea pulchra. *PLoS ONE*, *7*(12), e50854. https://doi.org/10.1371/journal.pone.0050854

Fernández, C. (2011). The retreat of large brown seaweeds on the north coast of Spain: the case ofSaccorhiza polyschides. *European Journal of Phycology*, *46*(4), 352–360. https://doi.org/10.1080/09670262.2011.617840

Filbee-Dexter, K., Feehan, C. J., & Scheibling, R. E. (2016). Large-scale degradation of a kelp ecosystem in an ocean warming hotspot. *Marine Ecology. Progress Series*, *543*, 141–152. https://doi.org/10.3354/meps11554

Franco, J. N., Tuya, F., Bertocci, I., Rodríguez, L., Martínez, B., Sousa-Pinto, I., & Arenas, F. (2017). The ‘golden kelp’ Laminaria ochroleuca under global change: Integrating multiple eco-physiological responses with species distribution models. *Journal of Ecology*, *106*(1), 47–58. https://doi.org/10.1111/1365-2745.12810

Freitas, S., Hatosy, S., Fuhrman, J. A., Huse, S. M., Welch, D. B. M., Sogin, M. L., & Martiny, A. C. (2012). Global distribution and diversity of marine Verrucomicrobia. *The ISME Journal*, *6*(8), 1499–1505. https://doi.org/10.1038/ismej.2012.3

Frenkel, J., Vyverman, W., & Pohnert, G. (2014). Pheromone signaling during sexual reproduction in algae. *The Plant Journal*, *79*(4), 632–644. https://doi.org/10.1111/tpj.12496

Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, *560*(7718), 360–364. https://doi.org/10.1038/s41586-018-0383-9

Ghaderiardakani, F., Coates, J. C., & Wichard, T. (2017). Bacteria-induced morphogenesis of Ulva intestinalis and Ulva mutabilis (Chlorophyta): a contribution to the lottery theory. *FEMS Microbiology Ecology/FEMS Microbiology, Ecology*, *93*(8). https://doi.org/10.1093/femsec/fix094

Girão, M., Ribeiro, I., Ribeiro, T., Azevedo, I. C., Pereira, F., Urbatzka, R., Leão, P. N., & Carvalho, M. F. (2019). Actinobacteria Isolated From Laminaria ochroleuca: A Source of New Bioactive Compounds. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00683

Goecke, F., Labes, A., Wiese, J., & Imhoff, J. (2010). Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, *409*, 267–299. https://doi.org/10.3354/meps08607

Graham, M. H., Kinlan, B. P., Druehl, L. D., Garske, L. E., & Banks, S. (2007). Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. *Proceedings of the National Academy of Sciences*, *104*(42), 16576–16580. https://doi.org/10.1073/pnas.0704778104

Grueneberg, J., Engelen, A. H., Costa, R., & Wichard, T. (2016). Macroalgal morphogenesis induced by waterborne compounds and bacteria in coastal seawater. *PloS One*, *11*(1), e0146307. https://doi.org/10.1371/journal.pone.0146307

Gundlapally, S. R., Ara, S., & Sisinthy, S. (2015). Draft genome of Kocuria polaris CMS 76orT isolated from cyanobacterial mats, McMurdo Dry Valley, Antarctica: an insight into CspA family of proteins from Kocuria polaris CMS 76orT. *Archives of Microbiology*, *197*(8), 1019–1026. https://doi.org/10.1007/s00203-015-1138-8

Hamdeno, M., & Alvera-Azcaráte, A. (2023). Marine heatwaves characteristics in the Mediterranean Sea: Case study the 2019 heatwave events. *Frontiers in Marine Science*, *10*. https://doi.org/10.3389/fmars.2023.1093760

Hobday, A. J., Alexander, L. V., Perkins, S. E., Smale, D. A., Straub, S. C., Oliver, E. C., Benthuysen, J. A., Burrows, M. T., Donat, M. G., Feng, M., Holbrook, N. J., Moore, P. J., Scannell, H. A., Gupta, A. S., & Wernberg, T. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography/Progress in Oceanography*, *141*, 227–238. https://doi.org/10.1016/j.pocean.2015.12.014

Hollarsmith, J. A., Buschmann, A. H., Camus, C., & Grosholz, E. D. (2019). Varying reproductive success under ocean warming and acidification across giant kelp (Macrocystis pyrifera) populations. *Journal of Experimental Marine Biology and Ecology*, *522*, 151247. https://doi.org/10.1016/j.jembe.2019.151247

Hördt, A., López, M. G., Meier-Kolthoff, J. P., Schleuning, M., Weinhold, L., Tindall, B. J., Gronow, S., Kyrpides, N. C., Woyke, T., & Göker, M. (2020). Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00468

Hu, T., Wang, S., Shan, Y., Zhang, Y., Zhu, Y., & Zheng, L. (2021). Complete Genome of Marine Microalgae Associated Algicidal Bacterium Sulfitobacter pseudonitzschiae H46 with Quorum Sensing System. *Current Microbiology*, *78*(10), 3741–3750. https://doi.org/10.1007/s00284-021-02632-4

Huber, J. A., Welch, D. B. M., Morrison, H. G., Huse, S. M., Neal, P. R., Butterfield, D. A., & Sogin, M. L. (2007). Microbial Population Structures in the Deep Marine Biosphere. *Science*, *318*(5847), 97–100. https://doi.org/10.1126/science.1146689

Ihua, M. W., FitzGerald, J. A., Guihéneuf, F., Jackson, S. A., Claesson, M. J., Stengel, D. B., & Dobson, A. D. W. (2020). Diversity of bacteria populations associated with different thallus regions of the brown alga Laminaria digitata. *PLoS ONE*, *15*(11), e0242675. https://doi.org/10.1371/journal.pone.0242675

Inderjit, N., Wardle, D. A., Karban, R., & Callaway, R. M. (2011). The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology & Evolution*, *26*(12), 655–662. https://doi.org/10.1016/j.tree.2011.08.003

IPCC, 2019: IPCC Special Report on the Ocean and Cryosphere in a Changing Climate [H.-O. Pörtner, D.C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, A. Alegría, M. Nicolai, A. Okem, J. Petzold, B. Rama, N.M. Weyer (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, 755 pp. https://doi.org/10.1017/9781009157964.

Isolauri, E., Sütas, Y., Kankaanpää, P., Arvilommi, H., & Salminen, S. (2001). Probiotics: effects on immunity. *the American Journal of Clinical Nutrition*, *73*(2), 444s–450s. https://doi.org/10.1093/ajcn/73.2.444s

Ivanova, E. P., Christen, R., Sawabe, T., Alexeeva, Y. V., Lysenko, A. M., Chelomin, V. P., & Mikhailov, V. V. (2005). Presence of Ecophysiologically Diverse Populations within Cobetia marina Strains Isolated from Marine Invertebrate, Algae and the Environments. *Microbes and Environments*, *20*(4), 200–207. https://doi.org/10.1264/jsme2.20.200

Izquierdo, J., Pérez-Ruzafa, I. M., & Gallardo, T. (2001). Effect of temperature and photon fluence rate on gametophytes and young sporophytes of Laminaria ochroleuca Pylaie. *Helgoland Marine Research*, *55*(4), 285–292. https://doi.org/10.1007/s10152-001-0087-6

Kessler, R. W., Crecelius, A. C., Schubert, U. S., & Wichard, T. (2017). In situ monitoring of molecular changes during cell differentiation processes in marine macroalgae through mass spectrometric imaging. *Analytical and Bioanalytical Chemistry/Analytical & Bioanalytical Chemistry*, *409*(20), 4893–4903. https://doi.org/10.1007/s00216-017-0430-7

Kim, S., & Bhatnagar, I. (2011). Physical, chemical, and biological properties of wonder Kelp—Laminaria. In *Advances in food and nutrition research* (pp. 85–96). https://doi.org/10.1016/b978-0-12-387669-0.00007-7

Krause-Jensen, D., Lavery, P., Serrano, O., Marbà, N., Masque, P., & Duarte, C. M. (2018). Sequestration of macroalgal carbon: the elephant in the Blue Carbon room. *Biology Letters*, *14*(6), 20180236. https://doi.org/10.1098/rsbl.2018.0236

Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., Connell, S. D., Johnson, C. R., Konar, B., Ling, S. D., Micheli, F., Norderhaug, K. M., Pérez-Matus, A., Sousa-Pinto, I., Reed, D. C., Salomon, A. K., Shears, N. T., Wernberg, T., Anderson, R. J., . . . Byrnes, J. E. K. (2016). Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(48), 13785–13790. https://doi.org/10.1073/pnas.1606102113

Lachnit, T., Blümel, M., Imhoff, J., & Wahl, M. (2009). Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. *Sexuality and Early Development in Aquatic Organism/Aquatic Biology/Sexuality and Early Development in Aquatic Organisms*, *5*(2), 181–186. https://doi.org/10.3354/ab00149

Layton, C., Coleman, M. A., Marzinelli, E. M., Steinberg, P. D., Swearer, S. E., Vergés, A., Wernberg, T., & Johnson, C. R. (2020). Kelp Forest Restoration in Australia. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00074

Lemay, M. A., Davis, K. M., Martone, P. T., & Parfrey, L. W. (2021). Kelp-associated Microbiota are Structured by Host Anatomy1. *Journal of Phycology*, *57*(4), 1119–1130. https://doi.org/10.1111/jpy.13169

Li, J., Majzoub, M. E., Marzinelli, E. M., Dai, Z., Thomas, T., & Egan, S. (2022). Bacterial controlled mitigation of dysbiosis in a seaweed disease. *The ISME Journal*, *16*(2), 378–387. https://doi.org/10.1038/s41396-021-01070-1

Li, J., Pang, S., Liu, F., Shan, T., & Gao, S. (2013). Spermatozoid life-span of two brown seaweeds, Saccharina japonica and Undaria pinnatifida, as measured by fertilization efficiency. *Chinese Journal of Oceanology and Limnology*, *31*(4), 774–781. https://doi.org/10.1007/s00343-013-2207-y

Li, N., He, X., Liu, N., Gu, T., Li, J., Geng, Y., Zhang, S., Wang, P., Fu, H., Shi, M., Chen, X., Zhang, Y., Zhang, X., & Qin, Q. (2021). Tritonibacter aquimaris sp. nov. and Tritonibacter litoralis sp. nov., two novel members of the Roseobacter group isolated from coastal seawater. *Antonie Van Leeuwenhoek*, *114*(6), 787–798. https://doi.org/10.1007/s10482-021-01558-y

Liang, K. Y. H., Orata, F. D., Boucher, Y. F., & Case, R. J. (2021). Roseobacters in a Sea of Poly- and Paraphyly: Whole Genome-Based Taxonomy of the Family Rhodobacteraceae and the Proposal for the Split of the “Roseobacter Clade” Into a Novel Family, Roseobacteraceae fam. nov. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.683109

Lüning, K. (1981). Egg release in gametophytes ofLaminaria saccharina: Induction by darkness and inhibition by blue light and u.v. *British Phycological Journal*, *16*(4), 379–393. https://doi.org/10.1080/00071618100650441

Mabeau, S., & Kloareg, B. (1987). Isolation and Analysis of the Cell Walls of Brown Algae:Fucus spiralis, F. ceranoides, F. vesiculosus, F. serratus, Bifurcaria bifurcataandLaminaria digitata. *Journal of Experimental Botany*, *38*(9), 1573–1580. https://doi.org/10.1093/jxb/38.9.1573

Macreadie, P. I., Anton, A., Raven, J. A., Beaumont, N., Connolly, R. M., Friess, D. A., Kelleway, J. J., Kennedy, H., Kuwae, T., Lavery, P. S., Lovelock, C. E., Smale, D. A., Apostolaki, E. T., Atwood, T. B., Baldock, J., Bianchi, T. S., Chmura, G. L., Eyre, B. D., Fourqurean, J. W., . . . Duarte, C. M. (2019). The future of Blue Carbon science. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-11693-w

Maestre, F. T., Callaway, R. M., Valladares, F., & Lortie, C. J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, *97*(2), 199–205. https://doi.org/10.1111/j.1365-2745.2008.01476.x

Maier, I., Hertweck, C., & Boland, W. (2001). Stereochemical Specificity of Lamoxirene, the Sperm-Releasing Pheromone in Kelp (Laminariales, Phaeophyceae). *Biological Bulletin*, *201*(2), 121–125. https://doi.org/10.2307/1543327

Mamer, F. (1984). Lamoxirene. Structural Proof of the Spermatozoid Releasing and Attracting Pheromone of Laminariales. *Zeitschrift Für Naturforschung C*, *39*(6), 689–691. https://doi.org/10.1515/znc-1984-0629

Mann, K. H. (1973). Seaweeds: Their Productivity and Strategy for Growth. *Science*, *182*(4116), 975–981. https://doi.org/10.1126/science.182.4116.975

Marín-Guirao, L., Entrambasaguas, L., Ruiz, J. M., & Procaccini, G. (2019). Heat-stress induced flowering can be a potential adaptive response to ocean warming for the iconic seagrass Posidonia oceanica. *Molecular Ecology*, *28*(10), 2486–2501. https://doi.org/10.1111/mec.15089

Martin-Platero, A. M., Cleary, B., Kauffman, K., Preheim, S. P., McGillicuddy, D. J., Alm, E. J., & Polz, M. F. (2018). High resolution time series reveals cohesive but short-lived communities in coastal plankton. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-017-02571-4

Martins, N., Pearson, G. A., Bernard, J., Serrão, E. A., & Bartsch, I. (2020). Thermal traits for reproduction and recruitment differ between Arctic and Atlantic kelp Laminaria digitata. *PloS One*, *15*(6), e0235388. https://doi.org/10.1371/journal.pone.0235388

Martins, N., Pearson, G. A., Gouveia, L., Tavares, A. I., Serrão, E. A., & Bartsch, I. (2019). Hybrid vigour for thermal tolerance in hybrids between the allopatric kelpsLaminaria digitataandL. pallida(Laminariales, Phaeophyceae) with contrasting thermal affinities. *European Journal of Phycology*, *54*(4), 548–561. https://doi.org/10.1080/09670262.2019.1613571

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Morris, R. L., Graham, T. D. J., Kelvin, J., Ghisalberti, M., & Swearer, S. E. (2019). Kelp beds as coastal protection: wave attenuation of Ecklonia radiata in a shallow coastal bay. *Annals of Botany*. https://doi.org/10.1093/aob/mcz127

Morrissey, K. L., Çavaş, L., Willems, A., & De Clerck, O. (2019). Disentangling the Influence of Environment, Host Specificity and Thallus Differentiation on Bacterial Communities in Siphonous Green Seaweeds. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00717

Müller, R., Wiencke, C., & Bischof, K. (2008). Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Climate Research*, *37*(2–3), 203–213. https://doi.org/10.3354/cr00762

Noskova, Y., Nedashkovskaya, O., & Balabanova, L. (2025). Production, Purification, and Biochemical Characterization of a Novel ATP-Dependent Caseinolytic Protease from the Marine Bacterium Cobetia amphilecti KMM 296. *Microorganisms*, *13*(2), 307. https://doi.org/10.3390/microorganisms13020307

Oliver, E. C. J., Benthuysen, J. A., Darmaraki, S., Donat, M. G., Hobday, A. J., Holbrook, N. J., Schlegel, R. W., & Gupta, A. S. (2021). Marine heatwaves. *Annual Review of Marine Science*, *13*(1), 313–342. https://doi.org/10.1146/annurev-marine-032720-095144

Pastor, F., & Khodayar, S. (2022). Marine heat waves: Characterizing a major climate impact in the Mediterranean. *The Science of the Total Environment*, *861*, 160621. https://doi.org/10.1016/j.scitotenv.2022.160621

Peixoto, R. S., Rosado, P. M., De Assis Leite, D. C., Rosado, A. S., & Bourne, D. G. (2017). Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00341

Pereira, T., Engelen, A., Pearson, G., Serrao, E., Destombe, C., & Valero, M. (2011). Temperature effects on the microscopic haploid stage development of L. ochroleuca and S. polyschides, kelps with contrasting life histories. *Cahiers De Biologie Marine*, *52*, 395–403.

Pita, L., Rix, L., Slaby, B. M., Franke, A., & Hentschel, U. (2018). The sponge holobiont in a changing ocean: from microbes to ecosystems. *Microbiome*, *6*(1). https://doi.org/10.1186/s40168-018-0428-1

Rao, D., Webb, J. S., & Kjelleberg, S. (2005). Competitive Interactions in Mixed-Species Biofilms Containing the Marine Bacterium Pseudoalteromonas tunicata. *Applied and Environmental Microbiology*, *71*(4), 1729–1736. https://doi.org/10.1128/aem.71.4.1729-1736.2005

Roohi, N., Zaheer, M. R., & Kuddus, M. (2017). PHB (poly-β-hydroxybutyrate) and its enzymatic degradation. *Polymers for Advanced Technologies*, *29*(1), 30–40. https://doi.org/10.1002/pat.4126

Rosenberg, E., & Zilber-Rosenberg, I. (2016). Microbes Drive Evolution of Animals and Plants: the Hologenome Concept. *MBio*, *7*(2). https://doi.org/10.1128/mbio.01395-15

Saha, M., Berdalet, E., Carotenuto, Y., Fink, P., Harder, T., John, U., Not, F., Pohnert, G., Potin, P., Selander, E., Vyverman, W., Wichard, T., Zupo, V., & Steinke, M. (2019). Using chemical language to shape future marine health. *Frontiers in Ecology and the Environment*, *17*(9), 530–537. https://doi.org/10.1002/fee.2113

Saha, M., Dove, S., & Weinberger, F. (2020). Chemically Mediated Microbial “Gardening” Capacity of a Seaweed Holobiont Is Dynamic. *Microorganisms*, *8*(12), 1893. https://doi.org/10.3390/microorganisms8121893

Saha, M., Rempt, M., Gebser, B., Grueneberg, J., Pohnert, G., & Weinberger, F. (2012). Dimethylsulphopropionate (DMSP) and proline from the surface of the brown alga Fucus vesiculosus inhibit bacterial attachment. *Biofouling*, *28*(6), 593–604. https://doi.org/10.1080/08927014.2012.698615

Schiel, D. R., & Foster, M. S. (2006). The Population Biology of Large Brown Seaweeds: Ecological Consequences of Multiphase Life Histories in Dynamic Coastal Environments. *Annual Review of Ecology Evolution and Systematics*, *37*(1), 343–372. https://doi.org/10.1146/annurev.ecolsys.37.091305.110251

Seyedsayamdost, M. R., Case, R. J., Kolter, R., & Clardy, J. (2011). The Jekyll-and-Hyde chemistry of Phaeobacter gallaeciensis. *Nature Chemistry*, *3*(4), 331–335. https://doi.org/10.1038/nchem.1002

Si, X., Liu, Z., Cheng, S., Xi, J., Zeng, B., Li, M., Zhu, L., Yan, S., & Zhang, N. (2024). Genomic analysis of Marinobacter sp. M5B reveals its role in alginate biosynthesis. *Marine Genomics*, *79*, 101163. https://doi.org/10.1016/j.margen.2024.101163

Singh, R. P., & Reddy, C. (2014). Seaweed-microbial interactions: key functions of seaweed-associated bacteria. *FEMS Microbiology Ecology*, *88*(2), 213–230. https://doi.org/10.1111/1574-6941.12297

Smale, D. A. (2019). Impacts of ocean warming on kelp forest ecosystems. *New Phytologist*, *225*(4), 1447–1454. https://doi.org/10.1111/nph.16107

Smale, D. A., Pessarrodona, A., King, N., & Moore, P. J. (2021). Examining the production, export, and immediate fate of kelp detritus on open-coast subtidal reefs in the Northeast Atlantic. *Limnology and Oceanography*, *67*(S2). https://doi.org/10.1002/lno.11970

Smale, D. A., & Wernberg, T. (2013). Extreme climatic event drives range contraction of a habitat-forming species. *Proceedings - Royal Society. Biological Sciences/Proceedings - Royal Society. Biological Sciences*, *280*(1754), 20122829. https://doi.org/10.1098/rspb.2012.2829

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., Burrows, M. T., Alexander, L. V., Benthuysen, J. A., Donat, M. G., Feng, M., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Gupta, A. S., Payne, B.

L., & Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, *9*(4), 306–312. https://doi.org/10.1038/s41558-019-0412-1

Smale, D. A., Wernberg, T., Yunnie, A. L. E., & Vance, T. (2014). The rise of Laminaria ochroleuca in the Western English Channel (UK) and comparisons with its competitor and assemblage dominant Laminaria hyperborea. *Marine Ecology*, *36*(4), 1033–1044. https://doi.org/10.1111/maec.12199

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., Arrieta, J. M., & Herndl, G. J. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere." *Proceedings of the National Academy of Sciences*, *103*(32), 12115–12120. https://doi.org/10.1073/pnas.0605127103

Spoerner, M., Wichard, T., Bachhuber, T., Stratmann, J., & Oertel, W. (2012). Growth and Thallus Morphogenesis of Ulva mutabilis (Chlorophyta) Depends on A Combination of Two Bacterial Species Excreting Regulatory Factors. *Journal of Phycology*, *48*(6), 1433–1447. https://doi.org/10.1111/j.1529-8817.2012.01231.x

Steneck, R. S., Graham, M. H., Bourque, B. J., Corbett, D., Erlandson, J. M., Estes, J. A., & Tegner, M. J. (2002). Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, *29*(4), 436–459. https://doi.org/10.1017/s0376892902000322

Strasser, F., Barreto, L. M., Kaidi, S., Sabour, B., Serrão, E. A., Pearson, G. A., & Martins, N. (2022). Population level variation in reproductive development and output in the golden kelp Laminaria ochroleuca under marine heat wave scenarios. *Frontiers in Marine Science*, *9*. https://doi.org/10.3389/fmars.2022.943511

Tapia, J. E., González, B., Goulitquer, S., Potin, P., & Correa, J. A. (2016). Microbiota Influences Morphology and Reproduction of the Brown Alga Ectocarpus sp. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00197

Teagle, H., Hawkins, S. J., Moore, P. J., & Smale, D. A. (2017). The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology*, *492*, 81–98. https://doi.org/10.1016/j.jembe.2017.01.017

Thøgersen, M. S., Melchiorsen, J., Ingham, C., & Gram, L. (2018a). A Novel Microbial Culture Chamber Co-cultivation System to Study Algal-Bacteria Interactions Using Emiliania huxleyi and Phaeobacter inhibens as Model Organisms. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01705

Thøgersen, M. S., Melchiorsen, J., Ingham, C., & Gram, L. (2018b). A Novel Microbial Culture Chamber Co-cultivation System to Study Algal-Bacteria Interactions Using Emiliania huxleyi and Phaeobacter inhibens as Model Organisms. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01705

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., Prill, R. J., Tripathi, A., Gibbons, S. M., Ackermann, G., Navas-Molina, J. A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J. T., Mirarab, S., Xu, Z. Z., Jiang, L., . . . Zhao, H. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*(7681), 457–463. https://doi.org/10.1038/nature24621

Tujula, N. A., Crocetti, G. R., Burke, C., Thomas, T., Holmström, C., & Kjelleberg, S. (2009). Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. *The ISME Journal*, *4*(2), 301–311. https://doi.org/10.1038/ismej.2009.107

Van Den Hoek, C., Mann, D. G., & Jahns, H. M. (1998). Algae. An Introduction to Phycology. *Photosynthetica*, *35*(4), 506. https://doi.org/10.1023/a:1006955729654

Van Der Loos, L. M., Eriksson, B. K., & Salles, J. F. (2019). The macroalgal holobiont in a changing sea. *Trends in Microbiology*, *27*(7), 635–650. https://doi.org/10.1016/j.tim.2019.03.002

Van Der Loos, L. M., Steinhagen, S., Stock, W., Weinberger, F., D'hondt, S., Willems, A., & De Clerck, O. (2025). Low functional change despite high taxonomic turnover characterizes the Ulva microbiome across a 2000-km salinity gradient. *Science Advances*, *11*(3). https://doi.org/10.1126/sciadv.adr6070

Vannini, A. (2002). The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. *The EMBO Journal*, *21*(17), 4393–4401. https://doi.org/10.1093/emboj/cdf459

Veenhof, R. J., Champion, C., Dworjanyn, S. A., Wernberg, T., Minne, A. J., Layton, C., Bolton, J. J., Reed, D. C., & Coleman, M. A. (2022). Kelp Gametophytes in Changing Oceans. In *CRC Press eBooks* (pp. 335–371). https://doi.org/10.1201/9781003288602-7

Veenhof, R. J., McGrath, A. H., Champion, C., Dworjanyn, S. A., Marzinelli, E. M., & Coleman, M. A. (2025). The role of microbiota in kelp gametophyte development and resilience to thermal stress. *Journal of Phycology*. https://doi.org/10.1111/jpy.70018

Verdura, J., Sales, M., Ballesteros, E., Cefalì, M. E., & Cebrian, E. (2018). Restoration of a Canopy-Forming Alga Based on Recruitment Enhancement: Methods and Long-Term Success Assessment. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01832

Wahl, M., Goecke, F., Labes, A., Dobretsov, S., & Weinberger, F. (2012). The Second Skin: Ecological Role of Epibiotic Biofilms on Marine Organisms. *Frontiers in Microbiology*, *3*. https://doi.org/10.3389/fmicb.2012.00292

Wernberg, T., Coleman, M. A., Bennett, S., Thomsen, M. S., Tuya, F., & Kelaher, B. P. (2018). Genetic diversity and kelp forest vulnerability to climatic stress. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-20009-9

Wernberg, T., Krumhansl, K., Filbee-Dexter, K., & Pedersen, M. F. (2019). Status and Trends for the World's Kelp Forests. In *Elsevier eBooks* (pp. 57–78). https://doi.org/10.1016/b978-0-12-805052-1.00003-6

Wernberg, T., Smale, D. A., Tuya, F., Thomsen, M. S., Langlois, T. J., De Bettignies, T., Bennett, S., & Rousseaux, C. S. (2012). An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. *Nature Climate Change*, *3*(1), 78–82. https://doi.org/10.1038/nclimate1627

Wichard, T. (2015a). Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta). *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00086

Wichard, T. (2015b). Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta). *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00086

Wichard, T. (2023). From model organism to application: Bacteria-induced growth and development of the green seaweed Ulva and the potential of microbe leveraging in algal aquaculture. *Seminars in Cell & Developmental Biology*, *134*, 69–78. https://doi.org/10.1016/j.semcdb.2022.04.007

Wiese, J., Thiel, V., Nagel, K., Staufenberger, T., & Imhoff, J. F. (2008). Diversity of Antibiotic-Active Bacteria Associated with the Brown Alga Laminaria saccharina from the Baltic Sea. *Marine Biotechnology*, *11*(2), 287–300. https://doi.org/10.1007/s10126-008-9143-4

Wilkins, L. G. E., Leray, M., O'Dea, A., Yuen, B., Peixoto, R. S., Pereira, T. J., Bik, H. M., Coil, D. A., Duffy, J. E., Herre, E. A., Lessios, H. A., Lucey, N. M., Mejia, L. C., Rasher, D. B., Sharp, K. H., Sogin, E. M., Thacker, R. W., Thurber, R. V., Wcislo, W. T., . . . Eisen, J. A. (2019). Host-associated microbiomes drive structure and function of marine ecosystems. *PLoS Biology*, *17*(11), e3000533. https://doi.org/10.1371/journal.pbio.3000533

Yumoto, I., Hirota, K., Iwata, H., Akutsu, M., Kusumoto, K., Morita, N., Ezura, Y., Okuyama, H., & Matsuyama, H. (2004). Temperature and nutrient availability control growth rate and fatty acid composition of facultatively psychrophilic Cobetia marina strain L-2. *Archives of Microbiology*, *181*(5), 345–351. https://doi.org/10.1007/s00203-004-0662-8

Zhou, J., Lyu, Y., Richlen, M. L., Anderson, D. M., & Cai, Z. (2016). Quorum Sensing Is a Language of Chemical Signals and Plays an Ecological Role in Algal-Bacterial Interactions. *Critical Reviews in Plant Sciences*, *35*(2), 81–105. https://doi.org/10.1080/07352689.2016.1172461

7. Appendix

7.1. Supplementary

7.1.1. External stressor

Considering the consistently lower abundances of reproductive structures in the lab of uGent compared to the outcomes of Faro, the presence of an unknown external stressor is suggested. This is supported by the higher absolute counts of abnormal sporophyte morphology for both populations (Figure A - 13). Additionally, bleaching of the sporophytes also occurred more frequently in the last experiment (3.3.). It is important to highlight that these are personal observations (Figure A - 12).

Although every effort was made to replicate the experiment accurately, subtle differences may have contributed to these discrepancies.

- **Tropical Marine**: In Belgium, the salt was added to the distilled water before autoclaving. Although the salinity concentrations were checked prior to autoclaving, the concentrations were lower than expected. Therefore, salts were added again after autoclaving, followed by the addition of PES. It appeared that both PES and salt did not dissolve properly, something not observed in Faro.

- **PES:** This solution is made, together with vitamins, as a nutrient boost for the kelp. PES is home-made and therefor differences might have accounted for our different outcomes. To explore this, the methodology of both labs should be checked.

- **Settlement:** Gametophytes in Gent tended not settle properly, but rather cluster together. This could – verry locally – cause nutrient depletion or competition in general. Due to their suspended state and continuous growth, it was nearly unavoidable that some gametophytes were removed during medium refreshment. Densities dropped at DAY 38 (Figure A - 9) which may confirm the non-settlement.

- **Different light:dark cycle:** The light:dark cycle at UGent was 12:12 h, compared to 14:10 h at CCMAR. Although previous studies by the Phycology Lab did not report such low abundances, Martins et al. (2021) demonstrated that variations in the light:dark cycle can influence outcomes.

- **Climate room**: The largest discrepancies were seen at 15ºC. These samples were incubated in a climate room rather than a climate chamber. Given that multiple users share this room, the temperatures might have fluctuated. Additionally, no dataloggers were available to verify temperature stability during the experiment.

7.1.2. Sperm-release experiment

To assess whether *K. polaris* inhibited sperm release, an experiment was conducted where the suspension of female gametophytes was added to a petri dish with only containing males, either treated with *K. polaris* or without. This setup was observed under the microscope to detect sperm-release. Although the use of lamoxirene was considered, it was not used due to its volatile character and its high cost. It is important to recognize the lack of knowledge and experience to recognize antheridia. As a result, it could not be determined whether sperm release was absent, not observed, or male gametophytes were affected by the bacteria.

7.2. Supporting figures and tables

7.2.1. Functional traits of bacteria

Table A - 1. Functional traits of different bacteria genera and their corresponding phylum. (1) Wienhausen et al. (2017), (2) Croft et al. (2005), (3) Dogs et al. (2017), (4) Amin et al. (2009), (5) Amin et al. (2015), (6) Spoerner et al. (2012), (7) Johnson et al. (2016), (8) Singh and Reddy (2014), (9) Holmström and Kjelleberg (2006), (10) Bengtsson et al. (2012), (11) Wichard (2022), (12) Si et al. (2024), (13) Freitas et al. (2012), (14) Girão et al. (2019), and (15) Beiralas et al. (2023).

Genus	**Phylum**	**Role in Algal Interaction**	**Citation**
1. Vitamin and Nutrient Exchange			
Phaeobacter inhibens	α-Proteobacteria	Exchanges vitamins/precursors with auxotrophic algae	[1,2]
Dinoroseobacter shibae	α-Proteobacteria	Same as above	[1]
Halomonas spp.	γ-Proteobacteria	Supplies vitamins to algae	[2]
Sulfitobacter	α-Proteobacteria	Supplies vitamin B12	[3]
Marinobacter	γ-Proteobacteria	Provides bioavailable Fe^{2+}	[4]
2. Auxin and Growth Hormone Production			
Phaeobacter inhibens	α-Proteobacteria	Produces auxins and ammonium in exchange for tryptophan	[5]
Roseobacter	α-Proteobacteria	Produces cytokinins that promote cell division and morphogenesis.	[6]
Halomonas spp.	γ-Proteobacteria	Produces cytokinins that promote cell division and morphogenesis.	[6]
Sulfitobacter	α-Proteobacteria	Produces auxins and ammonium in exchange for tryptophan, quorum sensing (QS) facilitates communication	[5,6]

Ruegeria pomeroyi	α-Proteobacteria	Produces auxins and quorum-sensing molecules in response to algal sulfuric signals	[7]
3. Morphogenesis Induction			
Bacillus	Firmicutes	Promotes structural development	[8]
Cytophaga	Bacteroidetes	Produces auxins promoting structural development	[6,8]
Pseudomonas	γ-Proteobacteria	Induces morphogenesis	[8]
Caulobacter	α-Proteobacteria	Induces morphogenesis	[8]
Halomonas spp.	γ-Proteobacteria	Produces cytokinins that promote algal cell division and morphogenesis.	[6]
Sulfitobacter	α-Proteobacteria	Produces cytokinins that promote algal cell division and morphogenesis.	[6]
Roseobacter	α-Proteobacteria	Produces cytokinins that promote algal cell division and morphogenesis.	[6]
Maribacter	Bacteroidetes	Promotes morphogenesis and formation cell wall of rhizoid cells and	[11]
4. Biofilm Formation			
Planctomycetes	Planctomycetota	Supports microbial community structuring on algal surfaces	[10]
Pseudoalteromonas	γ-Proteobacteria	Employs EPS production	[9]
Marinobacter	γ-Proteobacteria	Synthesizes alginate (a kind of extracellular polysaccharide)	[12]
5. Nitrogen Fixation and Uptake			
Azotobacter	γ-Proteobacteria	Fixes nitrogen for algal use	[8]
Rhizobium	α-Proteobacteria	Same as above	[8]
Marinomonas	γ-Proteobacteria	Nitrogen-fixing inducing morphogenesis and algal growth	[8]
Agrobacterium	α-Proteobacteria	Same as above	[8]
6. Polymer Degradation & Antibacterial Activity			
Pseudoalteromonas	γ-Proteobacteria	Degrades polysaccharides; produces catalase	[9]
unspecified genus	Planctomycetes	Degrades polymers or anammox	[13]
unspecified genus	Bacteriodetes	Degrades polymers or anammox	[13]

unspecified genus	Actinobacteria	Produces antimicrobial compounds; Degrades polymers or anammox	[13,14]
7. Protection from pathogens			
Sulfitobacter	α-Proteobacteria	Protection from pathogens (mechanism unrevealed)	[15]

7.2.2. Results of Bacteria treatment

7.2.2.1. Reproductive structures

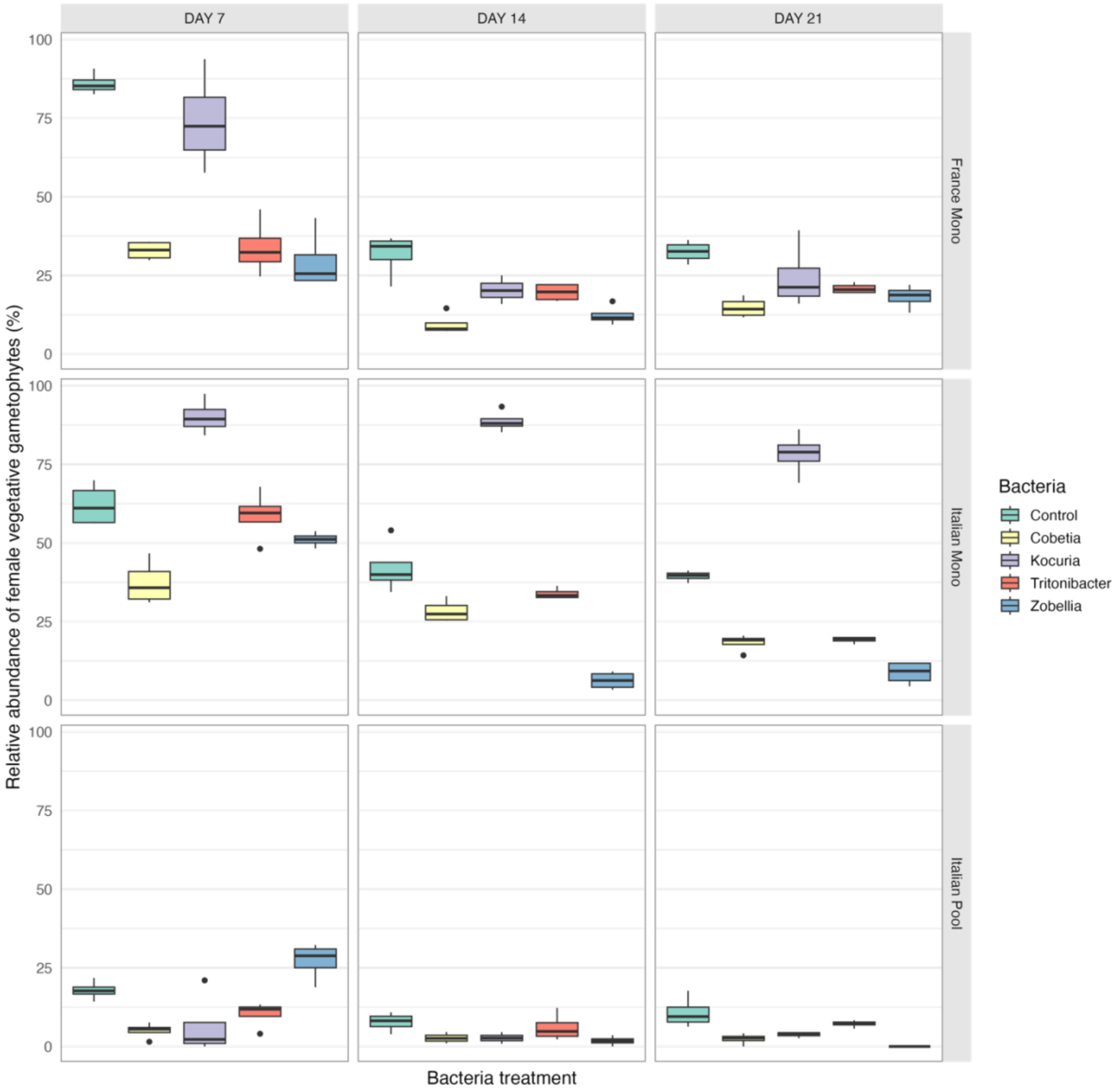

Figure A - 1 Boxplot showing the relative abundance of vegetative gametophytes in percentages across different bacteria treatments. The plot is faceted by the three populations and different counting days. Boxes represent median and interquartile range; whiskers indicate 1.5× IQR; points show outliers.

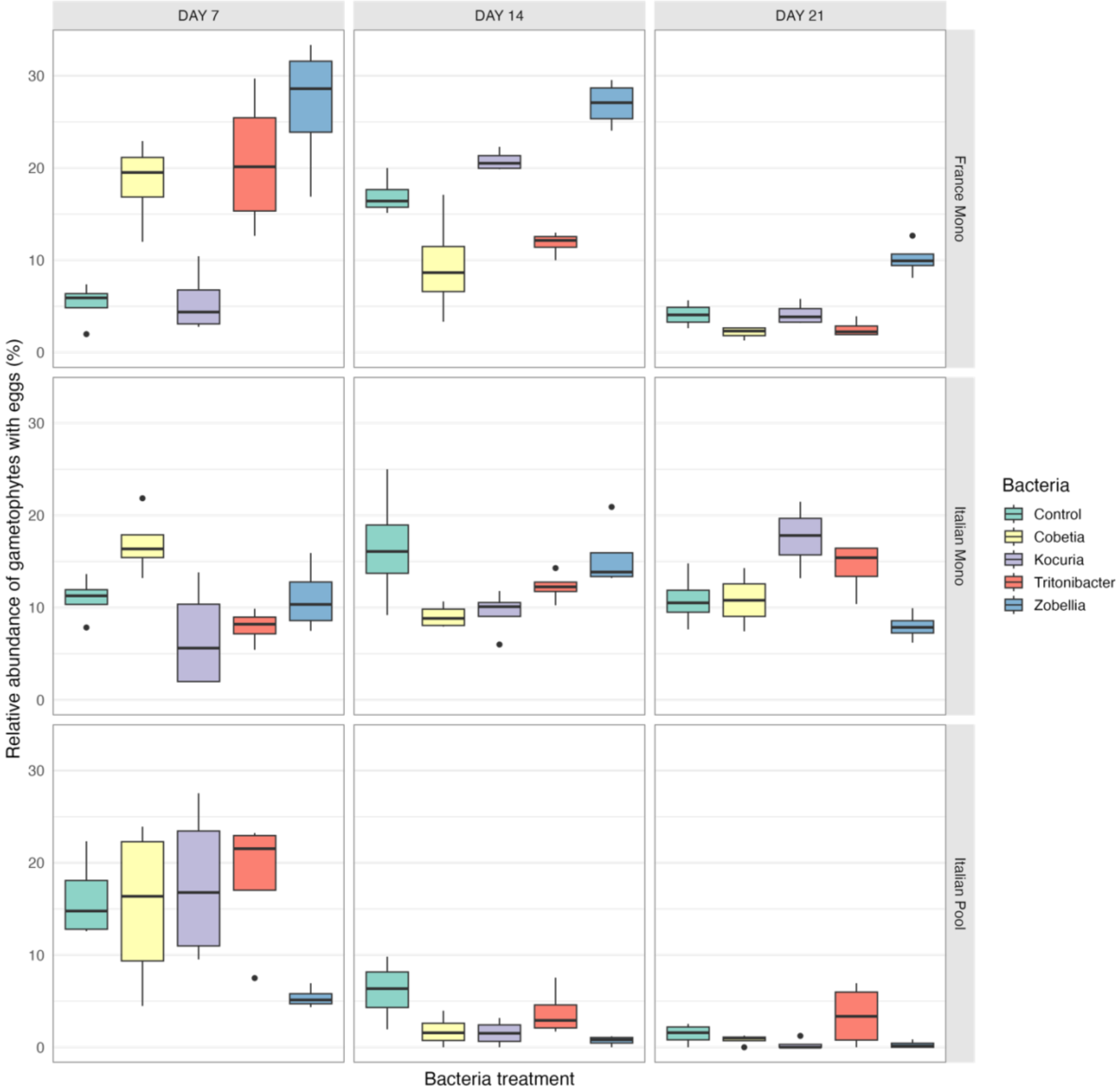

Figure A - 2 Boxplot showing the relative abundance of gametophytes with sporophytes in percentages across different bacteria treatments. The plot is faceted by the three populations and different counting days. Boxes represent median and interquartile range; whiskers indicate 1.5× IQR; points show outliers

7.2.2.2. Pictures of reproductive stages

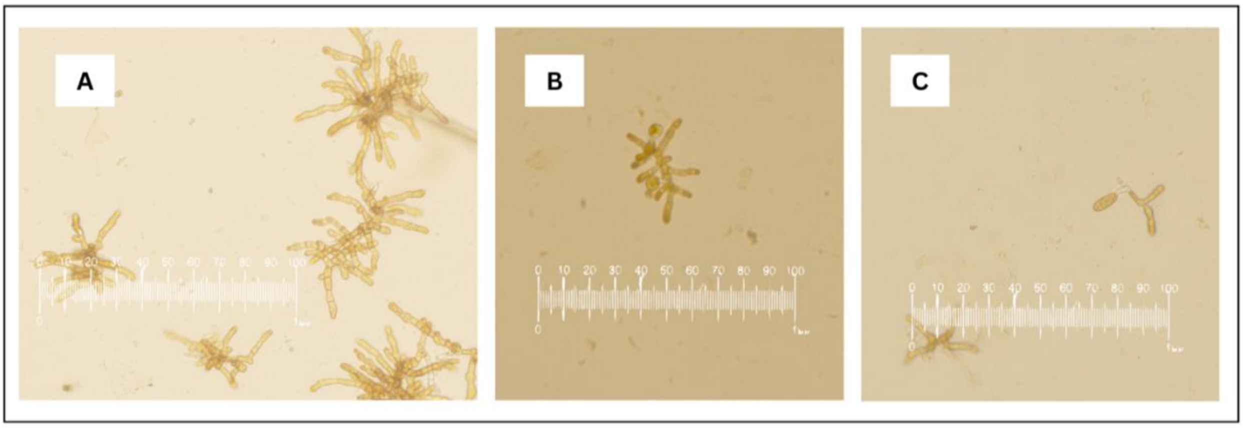

Figure A - 3. Pictures of the three different reproductive stages from IT MONO population on DAY7. A = vegetative, B = gametophyte with eggs, C = gametophyte with a sporophyte showing first cell divisions. Pictures were taken on 100x magnification. Scale is added for reference (ruler = 1mm)

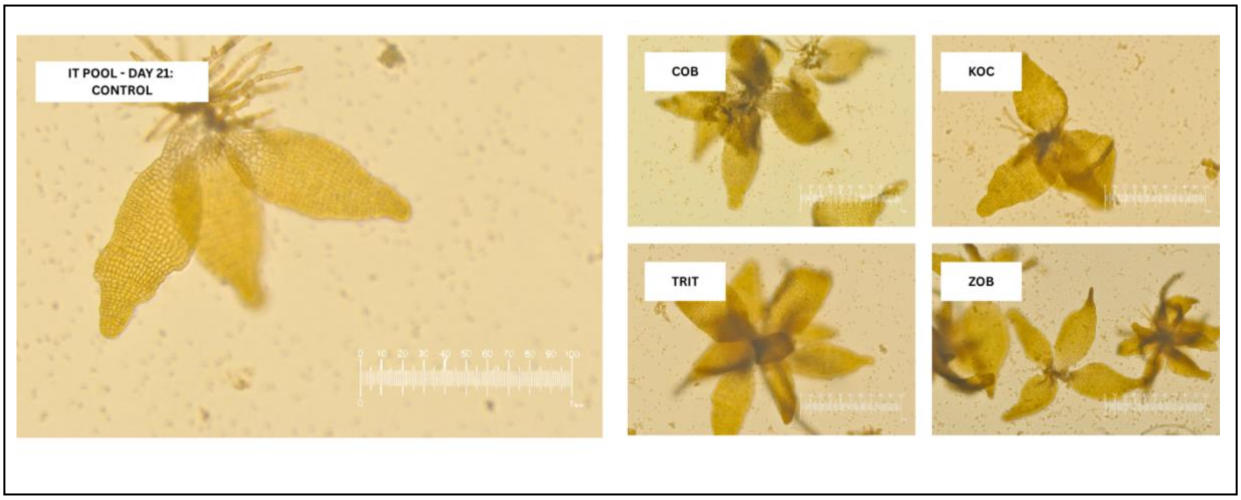

Figure A - 4. Pictures of sporophyte growth for the IT POOL for all treatments at DAY 21. Scale is added for reference (ruler = 1mm). Pictures are taken at 100x magnification. Scale is added for reference (ruler = 1mm). Labels on the pictures: COB = Cobetia; KOC = Kocuria; TRIT = Tritonibacter; ZOB = Zobellia.

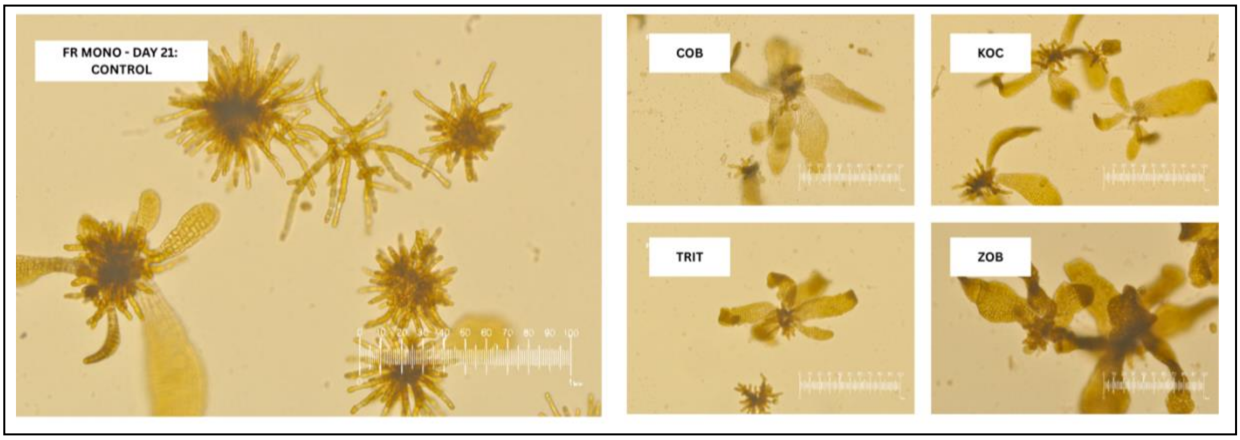

Figure A - 5. Pictures of sporophyte growth for the FR MONO for all treatments at DAY 21. Scale is added for reference (ruler = 1mm). Pictures are taken at 100x magnification. Scale is added for reference (ruler = 1mm). Labels on the pictures: COB = Cobetia; KOC = Kocuria; TRIT = Tritonibacter; ZOB = Zobellia.

7.2.2.3. PAM results

Table A - 2. Results of a three-way GLM for the effects of Bacteria, Population and Day on the photosynthetic yields of female gametophytes of L. ochroleuca with likelihood ratio test. Significance is highlighted.

Term	**Chisq**	**Df**	**p-value**	**Significance**
Bacteria	19.64	4	0.0006	***
Population	13.51	2	0.0012	**
Day	77.86	3	< 2.2e-16	***
Bacteria × Population	92.15	8	< 2.2e-16	***
Bacteria × Day	21.97	12	0.0378	*
Population × Day	7.61	6	0.2682	
Bacteria × Population × Day	157.43	24	< 2.2e-16	***

7.2.3. Microbiome overview

7.2.3.1. Different phyla of populations

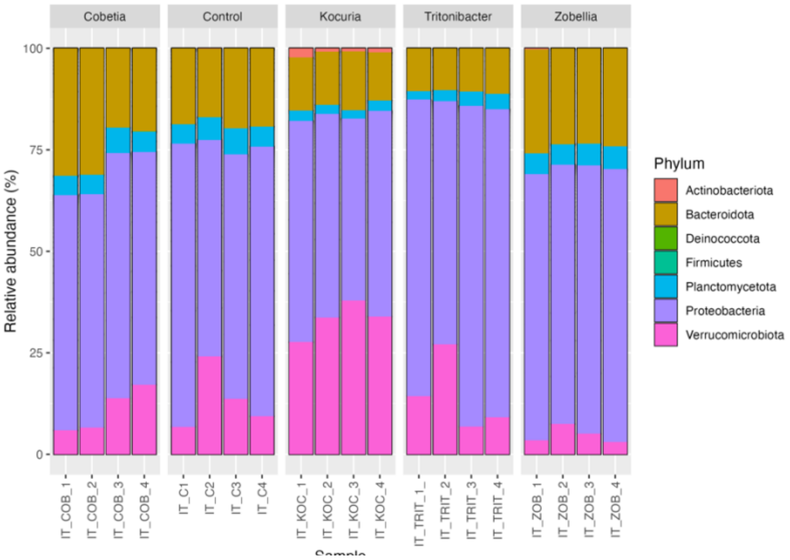

Figure A - 6. Relative abundance (%) of the different bacteria phyla across treatments for Italian Mono.

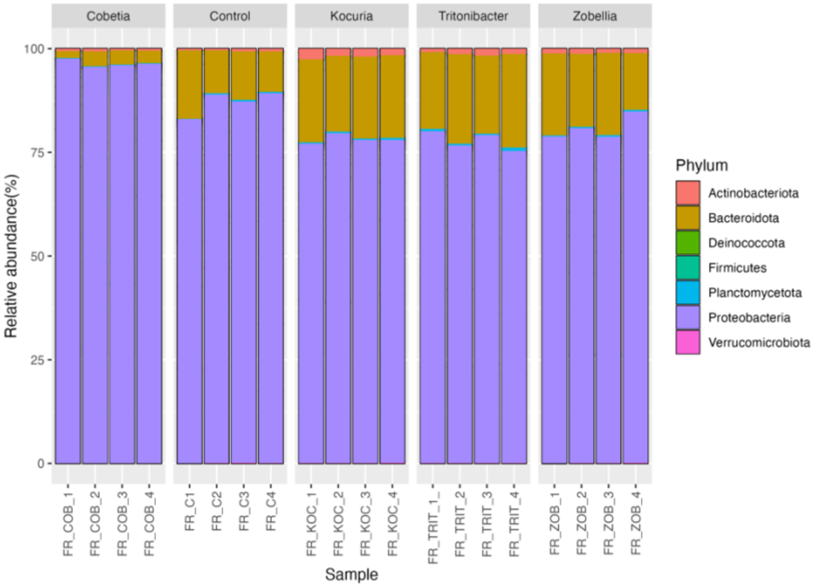

Figure A - 7. Relative abundance (%) of the different bacteria phyla across treatments for FR MONO.

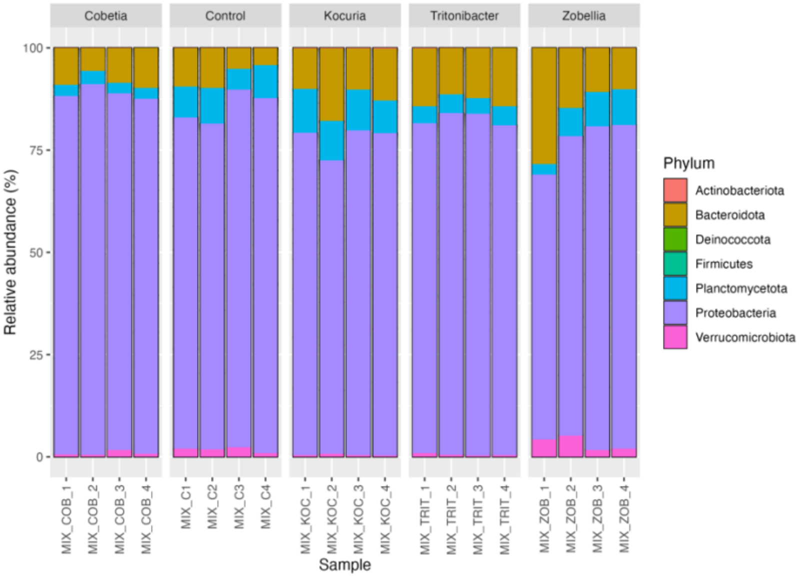

Figure A - 8. Relative abundance (%) of the different bacteria phyla across treatments for Italian Pool. MIX refers to the labelling used during the experiments to mark the Italian pool samples.

7.2.3.2. Composition of the core microbiome of each population shared across all treatments

Table A - 3. The core microbiome of all the bacteria treatments of FR MONO. The presence or absence of a genus is represented by 1-0. Coding refers to CONT = Control; COB = Cobetia; KOC = Kocuria; TRIT = Tritonibacter; ZOB = Zobellia.

Genus	**Control**	**KOC**	**COB**	**ZOB**	**TRIT**	**Phylum**	**Class**	**Order**
Aliiglaciecola	1	1	1	1	1	Proteobacteria	Gammaproteobacteria	Enterobacterales
Owenweeksia	1	1	1	1	1	Bacteroidota	Bacteroidia	Flavobacteriales
Pelagibius	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Kiloniellales
Nisaea	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Thalassobaculales
Marinobacter	1	1	1	1	1	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Alteromonas	1	1	1	1	1	Proteobacteria	Gammaproteobacteria	Enterobacterales
Fabibacter	1	1	0	1	1	Bacteroidota	Bacteroidia	Cytophagales
C1-B045	1	1	0	1	1	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Marinomonas	1	0	1	0	0	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Antarctobacter	1	0	0	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Kocuria	0	1	0	0	0	Actinobacteriota	Actinobacteria	Micrococcales
Kordiimonas	0	0	0	1	1	Proteobacteria	Alphaproteobacteria	Kordiimonadales
Ilumatobacter	0	0	0	1	1	Actinobacteriota	Acidimicrobiia	Microtrichales
Epibacterium	0	0	0	0	1	Proteobacteria	Alphaproteobacteria	Rhodobacterales

Table A - 4. The core microbiome of all the bacteria treatments of Italian Mono. The presence or absence of a genus is represented by 1-0. Coding refers to CONT = Control; COB = Cobetia; KOC = Kocuria; TRIT = Tritonibacter; ZOB = Zobellia.

Genus	Control	KOC	COB	ZOB	TRIT	Phylum	Class	Order
C1-B045	1	1	1	1	1	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Marinobacter	1	1	1	1	1	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Tropicibacter	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Lentimonas	**1**	**1**	**1**	**1**	**1**	**Verrucomicrobiota**	**Verrucomicrobiae**	**Opitutales**
Phycisphaera	1	1	1	1	1	Planctomycetota	Phycisphaerae	Phycisphaerales
Reichenbachiella	1	1	1	1	1	Bacteroidota	Bacteroidia	Cytophagales
Fluviicola	1	1	1	1	1	Bacteroidota	Bacteroidia	Flavobacteriales
Sulfitobacter	1	1	1	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Erythrobacter	1	1	1	1	0	Proteobacteria	Alphaproteobacteria	Sphingomonadales
Marivirga	1	1	1	1	0	Bacteroidota	Bacteroidia	Cytophagales
Thalassospira	1	1	0	1	1	Proteobacteria	Alphaproteobacteria	Rhodospirillales
Alteromonas	1	1	0	1	1	Proteobacteria	Gammaproteobacteria	Enterobacterales
Marinoscillum	1	1	0	0	0	Bacteroidota	Bacteroidia	Cytophagales
Nisaea	1	0	1	1	0	Proteobacteria	Alphaproteobacteria	Thalassobaculales
Thalassococcus	1	0	0	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Pseudophaeobacter	1	0	0	0	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Phaeobacter	1	0	0	0	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Nioella	0	1	1	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Kocuria	0	1	0	0	0	Actinobacteriota	Actinobacteria	Micrococcales
Rhodopirellula	0	0	1	1	1	Planctomycetota	Planctomycetes	Pirellulales
Sneathiella	0	0	1	1	0	Proteobacteria	Alphaproteobacteria	Sneathiellales
Balneola	0	0	1	1	0	Bacteroidota	Rhodothermia	Balneolales
Oceanibulbus	0	0	1	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Cobetia	0	0	1	0	0	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Aestuariicoccus	0	0	1	0	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Ruegeria	0	0	0	1	1	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Altererythrobacter	0	0	0	1	0	Proteobacteria	Alphaproteobacteria	Sphingomonadales
Tateyamaria	0	0	0	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Octadecabacter	0	0	0	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Aliikangiella	0	0	0	1	0	Proteobacteria	Gammaproteobacteria	Enterobacterales
Epibacterium	0	0	0	0	1	Proteobacteria	Alphaproteobacteria	Rhodobacterales

Table A - 5. The core microbiome of all the bacteria treatments of Italian Pool. The presence or absence of a genus is represented by 1-0. Coding refers to CONT = Control; COB = Cobetia; KOC = Kocuria; TRIT = Tritonibacter; ZOB = Zobellia.

Genus	**Control**	**KOC**	**COB**	**ZOB**	**TRIT**	**Phylum**	**Class**	**Order**
Nisaea	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Thalassobaculales
Marinobacter	1	1	1	1	1	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Thalassospira	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Rhodospirillales
Paraglaciecola	1	1	1	1	1	Proteobacteria	Gammaproteobacteria	Enterobacterales
Labrenzia	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Rhizobiales
Sphingorhabdus	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Sphingomonadales
Fluviicola	1	1	1	1	1	Bacteroidota	Bacteroidia	Flavobacteriales
Sneathiella	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Sneathiellales
Thalassobaculum	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Thalassobaculales
Cohaesibacter	1	1	1	1	1	Proteobacteria	Alphaproteobacteria	Rhizobiales
Sulfitobacter	1	1	1	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Pelagibius	1	1	1	0	1	Proteobacteria	Alphaproteobacteria	Kiloniellales
Ekhidna	1	1	1	0	1	Bacteroidota	Bacteroidia	Cytophagales
SM1A02	1	1	1	0	1	Planctomycetota	Phycisphaerae	Phycisphaerales
Roseovarius	1	1	1	0	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Phycisphaera	1	1	0	1	0	Planctomycetota	Phycisphaerae	Phycisphaerales
Lentimonas	1	0	0	1	0	Verrucomicrobiota	Verrucomicrobiae	Opitutales
Limimaricola	1	0	0	1	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Gimesia	0	1	0	1	0	Planctomycetota	Planctomycetes	Planctomycetales
Marinoscillum	0	1	0	0	1	Bacteroidota	Bacteroidia	Cytophagales
Fabibacter	0	1	0	0	0	Bacteroidota	Bacteroidia	Cytophagales
Cobetia	0	0	1	0	0	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Aestuariicoccus	0	0	1	0	0	Proteobacteria	Alphaproteobacteria	Rhodobacterales
C1-B045	0	0	0	1	0	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Gaetbulibacter	0	0	0	1	0	Bacteroidota	Bacteroidia	Flavobacteriales
Crocinitomix	0	0	0	1	0	Bacteroidota	Bacteroidia	Flavobacteriales
Hoeflea	0	0	0	1	0	Proteobacteria	Alphaproteobacteria	Rhizobiales
Ruegeria	0	0	0	0	1	Proteobacteria	Alphaproteobacteria	Rhodobacterales
Vicingus	0	0	0	0	1	Bacteroidota	Bacteroidia	Flavobacteriales
Epibacterium	0	0	0	0	1	Proteobacteria	Alphaproteobacteria	Rhodobacterales

7.2.4. Results of ontogenetic experiment

Table A - 6. Results of a generalized linear model (GLM) testing the effects of Bacteria, Day, and Population on the relative abundance of gametophytes with eggs. Significant main effects ($p < 0.05$) are highlighted.

Predictor	**Estimate**	**Std. Error**	***t* value**	***p*-value**	**Significance**
(Intercept)	–2.948	0.316	–9.34	6.49×10^{-12}	***
Bacteria: Cobetia	0.214	0.182	1.18	0.246	
Bacteria: Kocuria	0.511	0.175	2.93	0.005	**
Day	–0.205	0.020	–10.13	5.90×10^{-13}	***
Population: IT MONO	2.759	0.254	10.88	6.37×10^{-14}	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

7.2.5. Results of heatwave experiment

7.2.5.1. Survival rate

Table A - 7. Analysis of deviance table from the four-way generalized linear model (GLM) assessing the effects of Day, Population, Temperature, and Bacteria treatment on total gametophyte survival. Significant main effects and interactions ($p < 0.05$) are highlighted.

Effect	**LR Chisq**	**Df**	**Pr(>Chisq)**
Population	1.717	1	0.1901
Day	27.757	1	< 0.001 ***
Bacteria	46.987	1	< 0.001 ***
Temperature	2.739	2	0.2543
Population:Day	1.823	1	0.1769
Population:Bacteria	13.648	1	0.0002 ***
Day:Bacteria	5.728	1	0.0167 *
Population:Temperature	4.042	2	0.1326
Day:Temperature	0.065	2	0.9682
Bacteria:Temperature	6.316	2	0.0425 *
Population:Day:Bacteria	5.106	1	0.0238 *
Population:Day:Temperature	5.638	2	0.0597 .
Population:Bacteria:Temperature	5.315	2	0.0701 .
Day:Bacteria:Temperature	3.866	2	0.1447
Population:Day:Bacteria:Temperature	2.479	2	0.2896

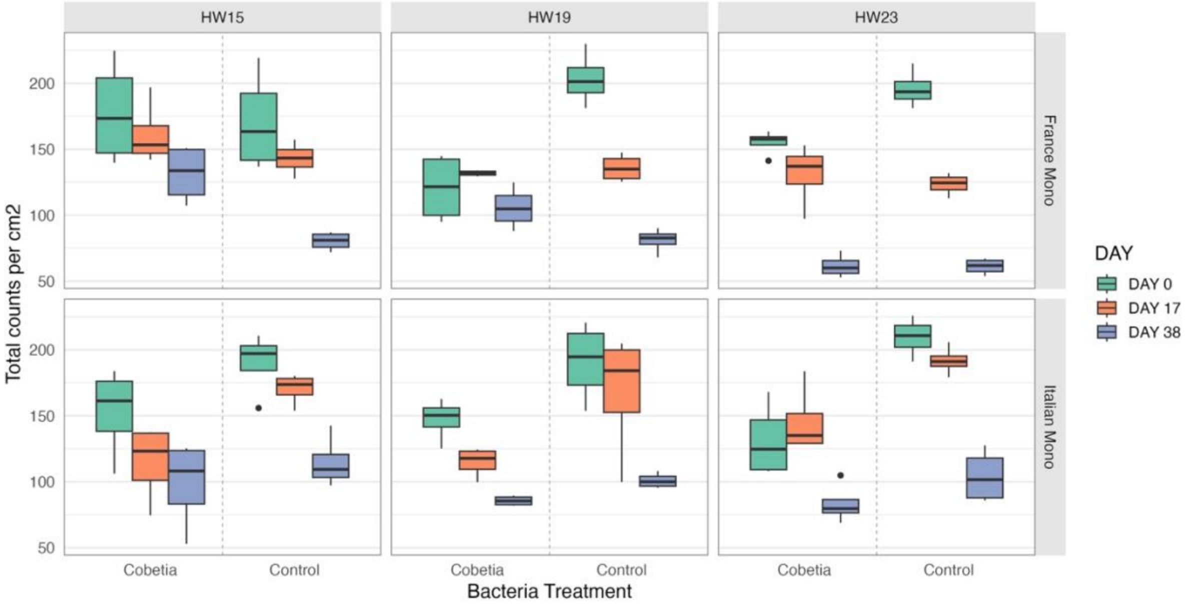

Figure A - 9. Overview of the densities per cm^2. Counted the first day, two days after the heatwave and the last day of the experiment.

7.2.5.2. Reproductive structures

Table A - 8. Results of a generalized linear model (GLM) testing the effects of Bacteria, Heatwave, Population and stage on the total abundance of gametophytes. Significant main effects and interactions ($p < 0.05$) are highlighted.

Term	LR Chisq	Df	Pr(>Chisq)
Bacteria	0.1	1	0.709407
Temperature	5.3	2	0.068976 .
Stage	**3371.6**	**2**	**< 2.2e-16 *******
Population	0.3	1	0.568343
Day	**11.9**	**3**	**0.007868 ******
Bacteria:Temperature	**8.5**	**2**	**0.014046 ***
Bacteria:Stage	**123.4**	**2**	**< 2.2e-16 *******
Temperature:Stage	**152.2**	**4**	**< 2.2e-16 *******
Bacteria:Temperature:Stage	**17.9**	**4**	**0.001299 ******

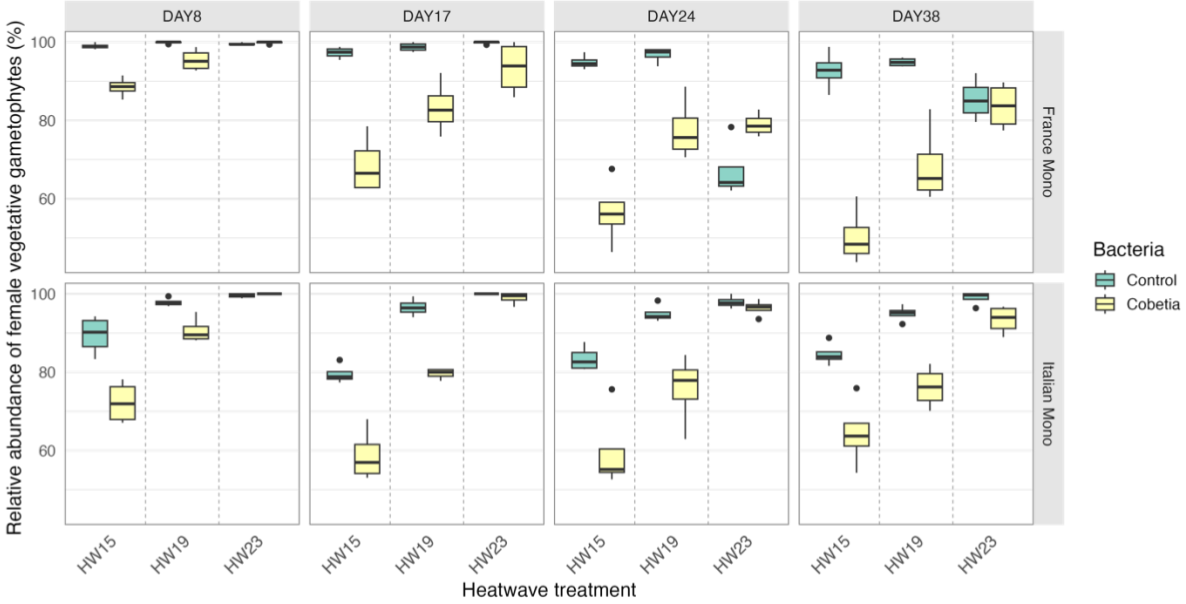

Figure A - 10. Boxplots of the relative abundance of female vegetative gametophytes. The plot is faceted per day and population.

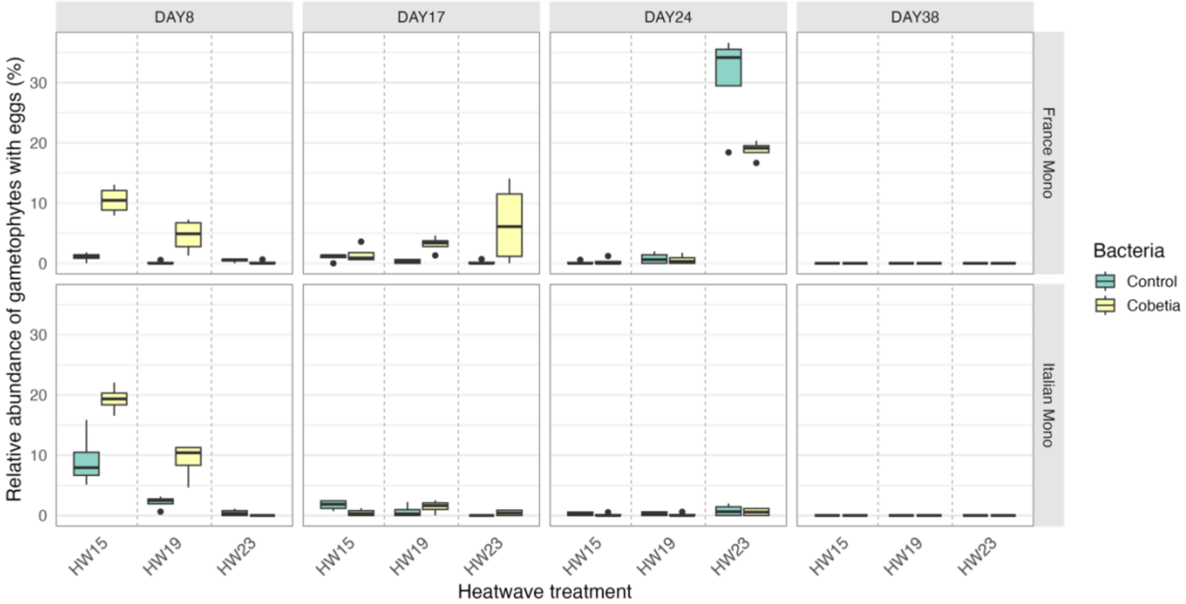

Figure A - 11. Boxplots of the relative abundance of gametophytes with eggs. The plot is faceted per day and population.

7.2.5.3. Recruitment success

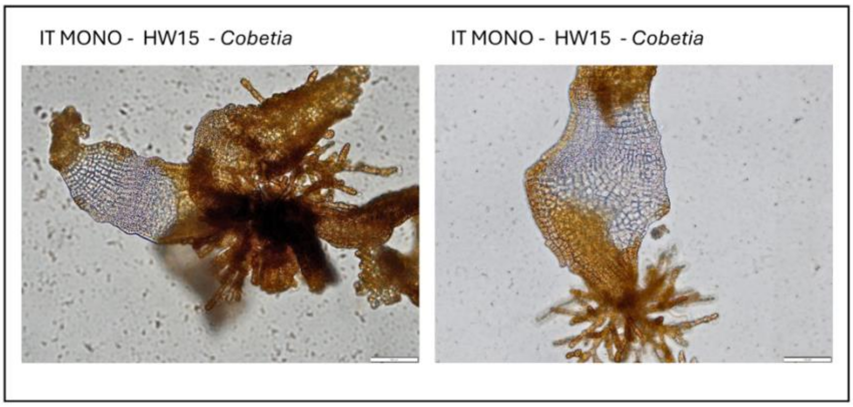

Figure A - 12. Pictures of bleaching sporophytes for IT MONO for both Cobetia HW15 on DAY 31, marking a 14-day recovery, taken at 100× magnification. Scale bar (0.1 mm) is shown in the bottom right corner.

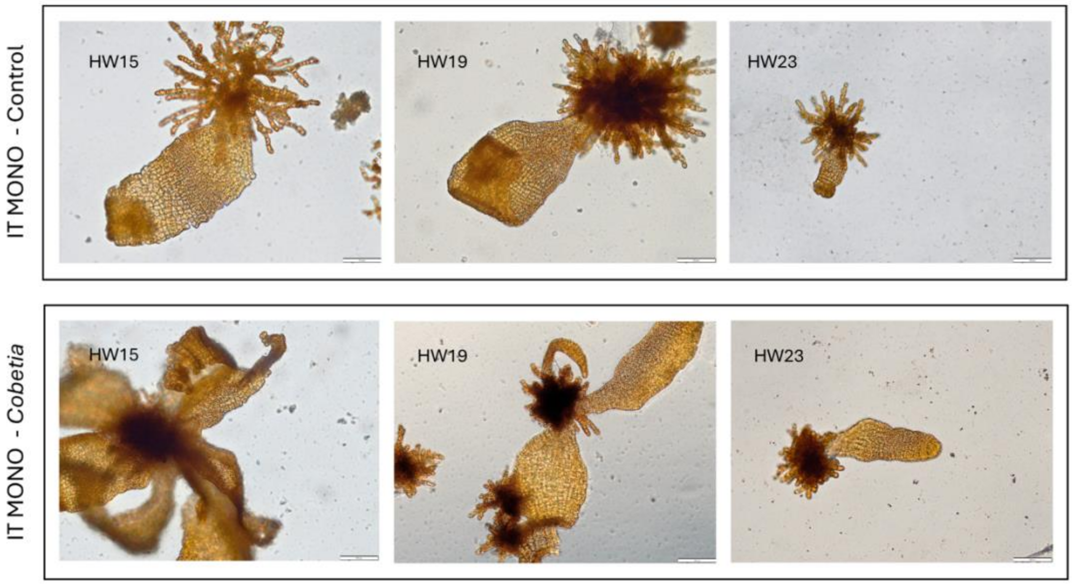

Figure A - 13. Pictures of gametophytes with sporophytes for IT MONO for both bacteria treatments and heatwave treatments on DAY 31, marking a 14-day recovery, taken at 100× magnification. Scale bar (0.1 mm) is shown in the bottom right corner.

Table A - 9. Results from the analysis of deviance for a generalized linear model (GLM) with a quasibinomial family, evaluating the effects of Bacteria, Population, and Treatment on gametophytes with sporophytes. Significant interactions and main effects are highlighted.

Effect	LR Chisq	Df	Pr(>Chisq)	Significance
Bacteria	171.758	1	< 2.2e-16	***
Population	2.074	1	0.1497949	
Treatment	152.226	2	< 2.2e-16	***
Bacteria:Population	5.462	1	0.0194327	*
Bacteria:Treatment	23.596	2	7.521e-06	***
Population:Treatment	7.399	2	0.0247346	*
Bacteria:Population:Treatment	18.233	2	0.0001099	***

Table A - 10. Results from the analysis of deviance for a generalized linear model (GLM) with a quasibinomial family, evaluating the effects of Bacteria, Population, and Treatment on healthy sporophytes per gametophyte. Significant interactions are highlighted.

Effect	LR Chi-Square	Df	Pr(>Chi)	Significance
Treatment	19.7563	2	5.13e-05	***
Bacteria	3.3563	1	0.0669	.
Population	1.7102	1	0.1910	
Treatment × Bacteria	15.1031	2	0.00053	***
Treatment × Population	3.7293	2	0.1550	
Bacteria × Population	4.8976	1	0.0269	*
Treatment × Bacteria × Population	1.8304	2	0.4004	

7.2.5.4. PAM values

Table A - 11. Results of three-way ANOVA testing the effects of Treatment, Population, and Bacteria and their interactions on the quantum yields (Fv/Fm). Significant main effects and interactions are highlighted.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Treatment	2	0.00797	0.00399	16.74	7.26e-06	***
Population	1	0.01083	0.01083	45.49	7.12e-08	***
Bacteria	1	0.01474	0.01474	61.89	2.48e-09	***
Treatment × Population	2	0.00464	0.00232	9.74	0.000416	***
Treatment × Bacteria	2	0.00470	0.00235	9.87	0.000384	***
Population × Bacteria	1	0.00062	0.00062	2.62	0.114	
Treatment × Population × Bacteria	2	0.00112	0.00056	2.35	0.110	
Residuals	36	0.00857	0.00024			