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The potential of kelp holobionts and their microbiomes to provide resilience to their hosts under temperature stress

UNIVERSIDADE DO ALGARVE

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Master in Molecular and Microbial Biology

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Declaração de autoria de trabalho

The potential of kelp holobionts and their microbiomes to provide resilience to their hosts under temperature stress

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Assinatura

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Abstract

Marine ecosystems, particularly kelp forests, are critical habitats for diverse marine organisms and support productive fishing grounds. However, rapid ocean warming due to climate change has imposed physiological stress on kelp, threatening these forests. Heterotrophic bacteria on kelp surfaces play a crucial role in nitrogen fixation and growth promotion, and the microbiome's stability influences the kelp's recovery from environmental disturbances. This study aims to determine if introducing bacterial communities can improve the health and heatwave resistance of *Laminaria. ochroleuca*. Bacterial inoculation from *L. ochroleuca* sporophytes and gametophytes is used to manipulate the kelp's microbiome, potentially enhancing its resistance to temperature increases. We specifically examine the physiological responses of *L. ochroleuca* gametophytes under different temperature treatments (13°C, 23°C, 25°C, and 27°C) and their subsequent recovery capacity, including gametophyte density and reproductive performance. In the recovery period, results indicate that bacterial communities enhance gametophyte density, promoting kelp recovery under the 23°C heatwave treatment but not in the 25°C and 27°C treatments. There was a significant interaction between heat wave temperature on the density of female gametophytes and heat wave temperature x bacterial treatment on the density of male gametes. Bacterial inoculation increases female gametophyte density at 23°C and male gametophyte density at 25°C. The density of gametophytes decreased significantly with time at 27°C treatments compared to other temperatures. Furthermore, bacterial communities reduce male gametophytes in the 13ºC control, 23°C heatwave treatment, and 25°C heatwave treatment. Bacterial intervention facilitates the transition from the vegetative stage to egg production at 23°C treatment. In sporophyte development, bacteria slightly reduce normal sporophytes at 25°C while significantly increasing asexual sporophytes at 23°C.The primary findings of this study reveal that male gametophytes exhibit a higher sensitivity to temperature and are also more responsive to changes in bacterial communities compared to female gametophytes, which differs from previous research. This heightened sensitivity leads to an increase in asexual sporophytes under heatwave conditions. Additionally, it was observed that at the highest temperature allowing gametophyte survival, bacterial communities can, to some extent, aid in the development of female gametophytes. In conclusion, this study underscores the impact of temperature stress on the development and reproduction of *L. ochroleuca* and sheds light on the effects of bacterial communities as a treatment on the recovery of *L. ochroleuca* following temperature stress. It highlights the diverse effects of bacterial inoculation, providing crucial insights into complex interactions within kelp forests. These findings are essential for enhancing the resilience of this ecosystem-engineering species in the face of global ocean warming.

Keywords: brown alga, gametogenesis, thermal adaptation, bacteria, kelp

Resumo

As florestas de algas, ecossistemas marinhos altamente diversos, oferecem refúgio a várias formas de vida marinha, incluindo peixes, herbívoros e organismos bentônicas, em regiões marinhas temperadas e frias. Contudo, as mudanças climáticas, caracterizadas pelo aumento das temperaturas oceânicas, representam uma ameaça às florestas de algas e afetam os principais predadores dentro deste ecossistema. Quanto às algas, o microbioma presente em suas superfícies apresenta baixa diversidade, com algumas OTUs (unidades taxonômicas operacionais) bacterianas abundantes desempenhando papéis significativos. Essas comunidades bacterianas estão envolvidas na fixação de nitrogênio, na transferência de carbono e podem promover indiretamente a libertação de esporos nas algas. O aumento da temperatura também afeta a diversidade beta e as funções metabólicas das comunidades bacterianas epifíticas. A estabilidade e a composição do microbioma desempenham um papel crucial em auxiliar as algas na recuperação de distúrbios ambientais. Um cenário de elevação da temperatura é representado pelas ondas de calor marinhas. Esses eventos submetem as algas não apenas ao estresse térmico, mas também resultam em níveis elevados de dióxido de carbono, redução da disponibilidade de luz e limitações de nutrientes, entre outros estressores. As algas respondem a essas pressões com redução da pigmentação, diminuição das taxas de respiração e danos nos tecidos.

Laminaria ochroleuca, conhecida como alga-dourada, é uma alga castanha grande e perene e um dos principais componentes das florestas de algas, encontrada principalmente em águas temperadas. *L. ochroleuca* segue um ciclo de vida heteromórfico haplodiplofásico, alternando entre esporófitos macroscópicos e gametófitos microscópicos filamentosos. Esporófitos maduros libertam esporos, que se desenvolvem em gametófitos femininos e masculinos. A fertilização dos óvulos nos gametófitos femininos pelos espermatozoides dos gametófitos masculinos dá origem a uma nova geração de esporófitos. Os gametófitos podem entrar em dormência em condições adversas, e mesmo que os esporófitos desapareçam em climas extremos, os gametófitos podem se reproduzir. O ciclo de vida é sazonal, com pico de atividade reprodutiva ocorrendo no final do verão, por volta de agosto, e a formação de esporos influenciada pelo estresse ambiental.

O principal objetivo deste estudo é avaliar os potenciais benefícios da introdução de comunidades bacterianas em gametófitos de *L. ochroleuca* para melhorar a resistência e a adaptabilidade às temperaturas elevadas. Manipulamos o microbioma de *L. ochroleuca* introduzindo comunidades bacterianas isoladas de esporófitos e gametófitos. Essa manipulação visa aumentar a resistência das algas ao aumento de temperatura. Especificamente, avaliamos as respostas fisiológicas de gametófitos de *L.*

ochroleuca de uma população francesa submetidos a diferentes tratamentos de temperatura (13°C, 23°C, 25°C e 27°C) e um subsequente período de recuperação. Esta avaliação concentrou-se nas taxas de sobrevivência dos gametófitos e no desempenho reprodutivo.

A partir de 65 amostras bacterianas isoladas de esporófitos, foram selecionadas três espécies distintas para formar uma comunidade bacteriana composta por: *Sulfitobacter pseudonitzschiae, Hoeflea halophila* e *Rhodobacteraceae bacterium*. Essas espécies são conhecidas pelas suas contribuições para a fixação de nitrogênio, adaptação ao carbono e resistência à temperatura. O tratamento bacteriano foi preparado com um OD600 de 0,8. Os gametófitos receberam luz com um fotoperíodo de 16:8 horas, e água do mar artificial com 30% de PES e uma salinidade de 32 ppm serviu como meio de cultura. Os gametófitos de *L. ochroleuca* foram submetidos a quatro tratamentos de ondas de calor diferentes (13, 23, 25 e 27°C), com ou sem inoculação bacteriana. Cada tratamento tinha quatro frascos de replicação, resultando em um total de 32 frascos (2 tratamentos bacterianos × 4 tratamentos de temperatura × 4 repetições = 32). A densidade de gametófitos, as proporções de gênero dos gametófitos, a reprodução de gametófitos femininos e o desenvolvimento de esporófitos foram monitorados e analisados estatisticamente ao longo do experimento.

Os resultados da pesquisa indicam que, em termos de densidade de gametófitos, a adição de comunidades bacterianas promove a recuperação das algas sob o tratamento de ondas de calor de 23°C, enquanto esse efeito não é observado nas algas submetidas aos tratamentos de ondas de calor de 25°C e 27°C. Além disso, o estudo revela diferenças na proporção de gametófitos masculinos para femininos entre os tratamentos de ondas de calor. A inoculação bacteriana resulta em uma maior densidade de gametófitos femininos a 23°C, mas uma maior densidade de gametófitos masculinos a 25°C. No tratamento de ondas de calor a 27°C, as densidades de gametófitos masculinos e femininos são mais baixas em comparação com outros tratamentos de temperatura. Além disso, a adição de comunidades bacterianas reduz os gametófitos masculinos no tratamento controlo de 13ºC, no tratamento de ondas de calor de 23°C e 25°C. Quanto à taxa de sucesso da reprodução feminina, observou-se que a adição de bactérias facilita a transição da fase vegetativa para a produção de ovos nas algas sob o tratamento de ondas de calor a 23°C. Em termos de desenvolvimento de esporófitos, a adição de bactérias leva a uma leve redução nos esporófitos normais no tratamento de ondas de calor a 25°C, enquanto aumenta significativamente os esporófitos assexuados no tratamento de ondas de calor a 23°C.

As principais descobertas deste estudo indicam que, em contraste com os gametófitos femininos, os gametófitos masculinos apresentam uma maior sensibilidade à temperatura e são mais responsivos às mudanças na comunidade bacteriana, o que difere de pesquisas anteriores. Essa sensibilidade aumentada dos gametófitos

masculinos resulta em um aumento nos esporófitos assexuados em condições de ondas de calor. Além disso, o estudo revela que, na temperatura mais alta em que os gametófitos podem sobreviver, as comunidades bacterianas contribuem em certa medida para o desenvolvimento dos gametófitos femininos.

Em conclusão, esta pesquisa destaca o impacto do estresse térmico no crescimento e reprodução de gametófitos de *L. ochroleuca*. Além disso, realça as consequências multifacetadas da inoculação bacteriana, oferecendo informações cruciais sobre as dinâmicas existentes nas florestas de algas. No contexto do contínuo aquecimento global dos oceanos, essas descobertas possuem uma significância fundamental para melhorar a resiliência desta espécie de kelp que é um engenheiro do ecossistema marinho.

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1. Introduction

In temperate to cold regions of the ocean, high-density aggregations of kelp create extensive benthic forests, constituting a highly diverse and dynamic ecosystem recognized as kelp forests (Mann, 1973). Kelp forests are vital marine habitats, supporting diverse organisms and serving as productive fishing grounds (Lotze, Coll, Magera, Ward-Paige, & Airoldi, 2011). These ecosystems house various fish species like *Sebastes spp.* and *Brachyistius frenatus* in the understory and herbivorous species like abalones and sea urchins beneath the kelp canopy. The ocean floor also hosts a complex benthic network with benthic fish, starfish, and leeches (Foster & Schiel, 1985).

The phenomenon of rapidly increasing ocean temperatures, attributed to climate change, has induced widespread mortality among kelps and other macroalgae globally. These organisms are often unable to acclimate fast enough to cope with the abrupt temperature shifts (Pörtner et al., 2022). Consequently, researchers have intensified their scrutiny of the myriad threats encumbering kelp forests. Notably, around the year 2010, kelp forests in California, USA, experienced a drastic reduction to a mere 5% of their former extent due to an oceanic heatwave.

Like other keystone species in ecosystems, kelps have faced substantial pressure due to climate change. Despite kelp forests being considered one of the most resilient marine ecosystems (Dayton, Tegner, Parnell, & Edwards, 1992; Filbee-Dexter & Scheibling, 2014; Wernberg et al., 2010), there is direct evidence of a global decline in kelp abundance over the past few decades (Krumhansl et al., 2016). With global warming, the climate-related stress on kelps has garnered significant research attention, as kelps experience physiological stress with rising temperatures (Gerard, 1997; Tegner, Dayton, Edwards, & Riser, 1996). The decline of kelp forests resulting from the El Niño phenomenon has led to a significant reduction in keystone species

such as lobsters, abalones, and apex predators like torpedo rays that inhabit these ecosystems(Foster & Schiel, 2010).

Within kelp forests, heterotrophic bacteria associated with kelps play a pivotal role in the food web, acting as a crucial link between kelp forest consumers and primary producers. The biofilm communities on the surface of kelps have been observed to exhibit low diversity, with a dominance of a few highly abundant bacterial operational taxonomical units (Bengtsson, Sjøtun, Lanzén, & Øvreås, 2012). Remarkably, the bacterial communities on kelp surfaces exhibit consistency across large spatial scales and on different host kelps. Microbes on the kelp surface can directly influence algal morphology and growth through plant hormones (Alsufyani et al., 2020), indirectly facilitating kelp spore release (Singh, Baghel, Reddy, & Jha, 2015), and participating in host algae nitrogen fixation and carbon transfer (Hamersley, Sohm, Burns, & Capone, 2015; Singh & Reddy, 2014). Negative interactions can also occur between the microbiome and hosts, potentially leading to pathogenic effects on kelp hosts (Case et al., 2011). When kelp faces a series of stressors, the stability and composition of the microbiome play a significant role in the host's recovery from environmental disturbances (King, Moore, Thorpe, & Smale, 2023). Monitoring the structure and dynamics of microbial communities on kelp surfaces provides valuable evidence for assessing changes in the host kelp and the entire ecosystem (Phelps et al., 2021). In the past fifteen years, the exploration of the relationship between kelp and microbial communities, such as bacteria, has become increasingly elucidated, thanks to advancements in metagenomics. Through omics-based research, it has become relatively straightforward to identify "significant microbes" (DeWeese & Osborne, 2021). Elevated temperatures have been shown to impact the beta diversity and metabolic functions of epiphytic bacterial communities associated with kelp. This is hypothesized to be linked to molecular interactions within a network of surface bacteria and their host kelp mediated by changes in gene expression regulated by micro RNAs (Düsedau et al., 2023).

Marine Heatwaves (MHWs) are discrete, prolonged episodes of anomalously warm water in local oceanic regions, often driven by various climatic processes operating at different spatiotemporal scales, such as El Niño, oceanic heat advection, and vertical mixing (Hobday et al., 2016). Rapid localized warming of seawater can trigger a cascade of associated environmental changes, including alterations in ocean currents, intertidal drying stress, and wave patterns. It is evident that MHWs have had significant impacts on marine organisms, including kelps, affecting their abundance, ecological dynamics, and physiology (Straub et al., 2019).

MHWs impose more than just temperature stress on kelps; they are also associated with factors like increased carbon dioxide levels, reduced light availability, and nutrient limitations (Britton et al., 2020; Sánchez-Barredo et al., 2020; Umanzor et al., 2021). These stressors manifest in kelps as decreased pigment content, elevated respiration rates, heightened primary production pressure, and tissue damage and bleaching(Almeida, 2022).

Laminaria ochroleuca, commonly known as the golden kelp, is a large brown seaweed species that typically reaches a length of approximately 1.5 meters when mature. The blade is notably broad and flat, characterized by numerous ribbon-like fronds, while the stipe is cylindrical and robust (Smirthwaite, 2007). *L. ochroleuca* is primarily distributed in temperate waters and represents a perennial kelp species. It has been documented along the coastlines of Portugal, northwestern Spain, the Strait of Gibraltar, the Brittany coast of France, the Bristol Channel of the United Kingdom, Morocco, and the Azores archipelago (Bartsch et al., 2008; Birkett, Maggs, Dring, Boaden, & Seed, 1998; Braud, 1974; Drew, 1974; Hoek, 1982; Tittley & Neto, 2000). It thrives at depths ranging from the intertidal zone to as deep as 30 meters, with its survival in deeper waters being contingent on light availability (Birkett et al., 1998). Notably, climate change has led to the poleward expansion of *L. ochroleuca*, potentially displacing other kelp species in the future (Franco et al., 2018; Franco et al., 2015; Franco et al., 2017; Smale, Wernberg, Yunnie, & Vance, 2015).

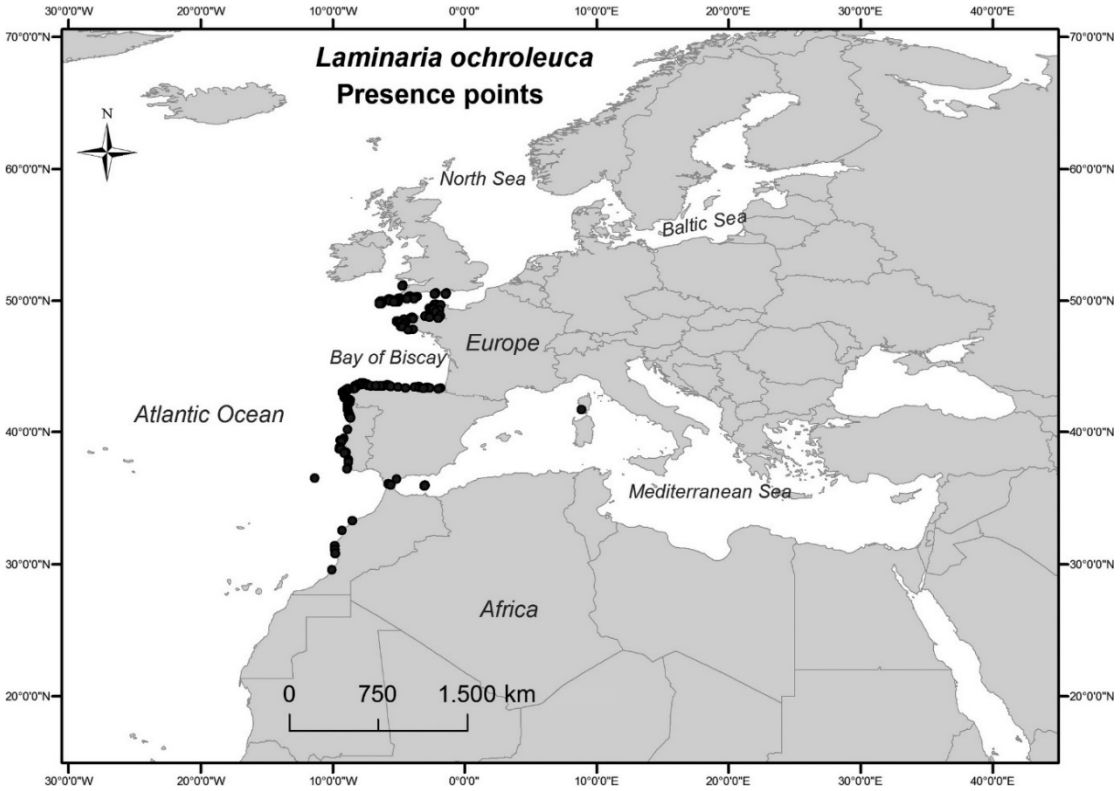

Figure 1.1. Records of the kelp, *L. ochroleuca*, across its distributional range (n = 511)(Franco et al., 2015).

Within the intricate web of *L. ochroleuca* kelp forests, this species plays a pivotal ecological role. It exhibits a heteromorphic haplodiplophasic life cycle, alternating between macroscopic sporophytes and microscopic filamentous gametophytes. Mature sporophytes release the spores that develop into female and male gametophytes. Fertilization of eggs in female gametophytes by sperm from male gametophytes gives rise to a new generation of sporophytes (Bertocci, Araújo, Oliveira, & Sousa-Pinto, 2015; Colin et al., 2003; Mineur et al., 2015; Teagle, Hawkins, Moore, & Smale, 2017). The gametophytes can remain dormant in unfavorable environmental conditions, therefore recruitment can be initiated even in populations where sporophytes died due to extreme climatic events (Graham, Vasquez, & Buschmann, 2007). Its life cycle exhibits seasonality across growth, reproduction, and recruitment phases. While reproductive individuals can be found year-round in coastal regions, the peak of reproductive activity occurs in late summer, typically around August, and the formation of spores may be influenced by environmental

stressors (Pereira, Azevedo, Oliveira, Silva, & Sousa-Pinto, 2019)

Figure 1.2. *L. ochroleuca*, photographed in Muxía (Costa da Morte, Province of A Coruña, Galicia (Spain)); 1988-06-01; cubeta de litoral inferior. Luis Fernández García photographed.

The primary objective of this study is to assess the potential benefits of introducing a bacterial consortium to enhance the health and temperature resilience of kelp. In this experiment, we manipulated the microbial community of *L. ochroleuca* by introducing a consortium of bacteria isolated from *L. ochroleuca* sporophytes and gametophytes. These bacteria are hypothesized to bolster the kelp's resistance to temperature increase. Specifically, we evaluated the physiological responses of *L. ochroleuca* gametophytes sourced from France when subjected to different temperature treatments (13ºC, 23ºC, 25ºC, and 27ºC) and subsequent recovery periods

following bacterial inoculation. This assessment aimed to elucidate the species' functional responses, focusing on gametophyte survival and reproductive performance.

2. Materials and methods

2.1 Bacterial Consortium Preparation and Cultivation Strategy

From a pool of 65 bacterial samples isolated from sporophyte tissues, three distinct species, were meticulously selected to compose a composite bacterial consortium intended for a specific treatment application. These species include *Sulfitobacter pseudonitzschiae*, which exhibits a positively correlated not only with temperature but also significant negative correlation with nitrogen concentration and may assist kelp in nitrogen fixation under conditions of nutrient scarcity (Florez, Camus, Hengst, Marchant, & Buschmann, 2019); *Hoeflea halophila*, which shows a positive correlation between population abundance and elevated carbon dioxide levels in the water where kelp grows (Minich et al., 2018); and *Rhodobacteraceae bacterium*, known to thrive on the surface of the brown macroalga *Fucus vesiculosus*, with community abundance positively associated with rising temperatures (Stratil, Neulinger, Knecht, Friedrichs, & Wahl, 2013). The designated bacterial strains, consisting of two replicates for each of the three species, thus totaling six cultures, were individually cultivated in separate volumes of 75 ml marine broth (MB) medium. These cultures were diligently maintained within a climatically controlled chamber at a constant temperature of 21°C. A regular medium renewal schedule, occurring every three weeks, was followed, and an additional medium replacement was performed on the day immediately preceding the intended utilization of the bacterial consortium. The quantification of cellular concentrations was achieved through the utilization of a photometric approach employing optical density measurements at a wavelength of 600 nm (OD600), thereby ensuring an accurate assessment of bacterial growth. Cultures were selectively sampled in proportion to their corresponding solution

volumes, aiming to attain a desired target OD of 0.8. Following centrifugation at a speed of 10,000 revolutions per minute for a duration of one minute, the resultant supernatant was meticulously decanted. Subsequently, the bacterial pellets obtained from the six centrifuge tubes were individually and meticulously resuspended in 3 ml of sterile artificial seawater. These resuspended bacterial entities were meticulously combined in a calculated ratio, ultimately yielding an 18 ml composite bacterial consortium. This consortium achieved a final concentration characterized by an OD600 value of 0.8.

2.2 Effects of bacterial consortiums on gametophyte thermal resistance and subsequent recovery capacity

2.2.1 Algal material

Eleven mature sporophytes of *L. ochroleuca* were collected by divers in Roscoff, France (48°41'35.90"N, 03°56'28.53"W) in November 2018. There, sea surface temperatures are around 13ºC (http://www.bio-oracle). Reproductive tissue (sorus) of each mature sporophyte was cleaned with dry paper towel and left overnight in dark conditions in sterile seawater to induce meiospore release. The male and female gametophytes developed from each sporophyte were maintained together in a vegetative state in sterile half-strength Provasoli enriched seawater (PES; Provasoli 1968, modifications: HEPES buffer instead of TRIS, double concentration of Na2-glycerophosphate). The cultures were kept in climate-controlled chambers (Fitoclima, S600, Aralab, Lisboa, Portugal) at 13°C under 3-6 µmol photons m^{-2} s^{-1} of fluorescent red light to prevent gametogenesis under a 16:8 h light: dark photoperiod. The culture medium was changed monthly, until the start of the experiment (ca. 4 y). Sterile artificial seawater (Tropic Marin Sea Salt, Dr. Biener, GmbH, Wartenberg, Germany) with 32 ppm salinity (hand refractometer ATAGO Co., Ltd) was used for culture

maintenance and the thermal experiment.

2.2.2 Experimental setup

An equivalent amount (approximately 0.5 mL) of female and male gametophyte cultures derived from the eleven *L. ochroleuca* sporophytes were mixed and fragmented using a mortar and pestle. The resulting mixture underwent sieving (stainless stell sieve with 100 µm mesh) to retain gametophytes with a length not exceeding or equal to 100 µm. These gametophytes were then suitably diluted in 30% PES with a salinity of 32 ppm. An inverted microscope (100 × magnification, Zeiss Axio Observer D1, Carl Zeiss MicroImaging GmbH, Göttingen, Germany) was employed to determine the gametophyte density in the stock solution. The requisite volume needed to achieve a density of 400 gametophytes cm^{-2} was calculated and subsequently added to glass beakers (5.5 cm diameter, 5.5 cm height) filled with 80 ml of 30% PES. The bacterial consortium was inoculated in half of the beakers and a 3-day period for gametophyte recovery from fragmentation and for bacteria symbiosis was established under 3 µmol photons m^{-2} s^{-1} of red light at a temperature of 13°C and a photoperiod of 16:8 hours light-dark cycle.

The thermal tolerance of *L. ochroleuca* gametophytes to four distinct temperature treatments (13, 23, 25, and 27°C) was investigated in the presence or absence of an inoculated bacterial consortium. Four replicate beakers were used per treatment, resulting in a total of 32 beakers (2 bacterial treatments × 4 temperature treatments × 4 replicates = 32). After the recovery period, the gametophytes were transferred to the heat wave treatments (23, 25, and 27°C) or left under control conditions at 13ºC. The temperatures were gradually increased from 13ºC to each target temperature at a warming rate of 2-3°C day^{-1}. The target temperature of 23ºC was kept for 8 days, while 25°C and 27°C temperatures were kept for 6 days. Following this, the temperatures were gradually decreased again at a cooling rate of 2-3°C day^{-1} until it reaches 13°C, starting the recovery phase for the subsequent 25 days. The temperature

of 13°C was established as the optimal temperature for *L. ochroleuca* gametogenesis (I Tom Dieck, 1992), whereas temperatures of 23, 25, and 27°C were designated as heat stress conditions to assess the influence of bacterial inoculation on the thermal resilience of *L. ochroleuca* gametophytes. Experiments were conducted in temperature-controlled waterbaths (one waterbath per thermal treatment; Huber Variostat with Pilot ONE, Offenburg, Germany), provided with 20 µmol photons m^{-2} s^{-1} white LED light in a 16:8 h light: dark photoperiod. The experiment spanned a duration of 40 days. Culture medium was 50% changed on the 18th and 39th days post-transition to white light conditions.

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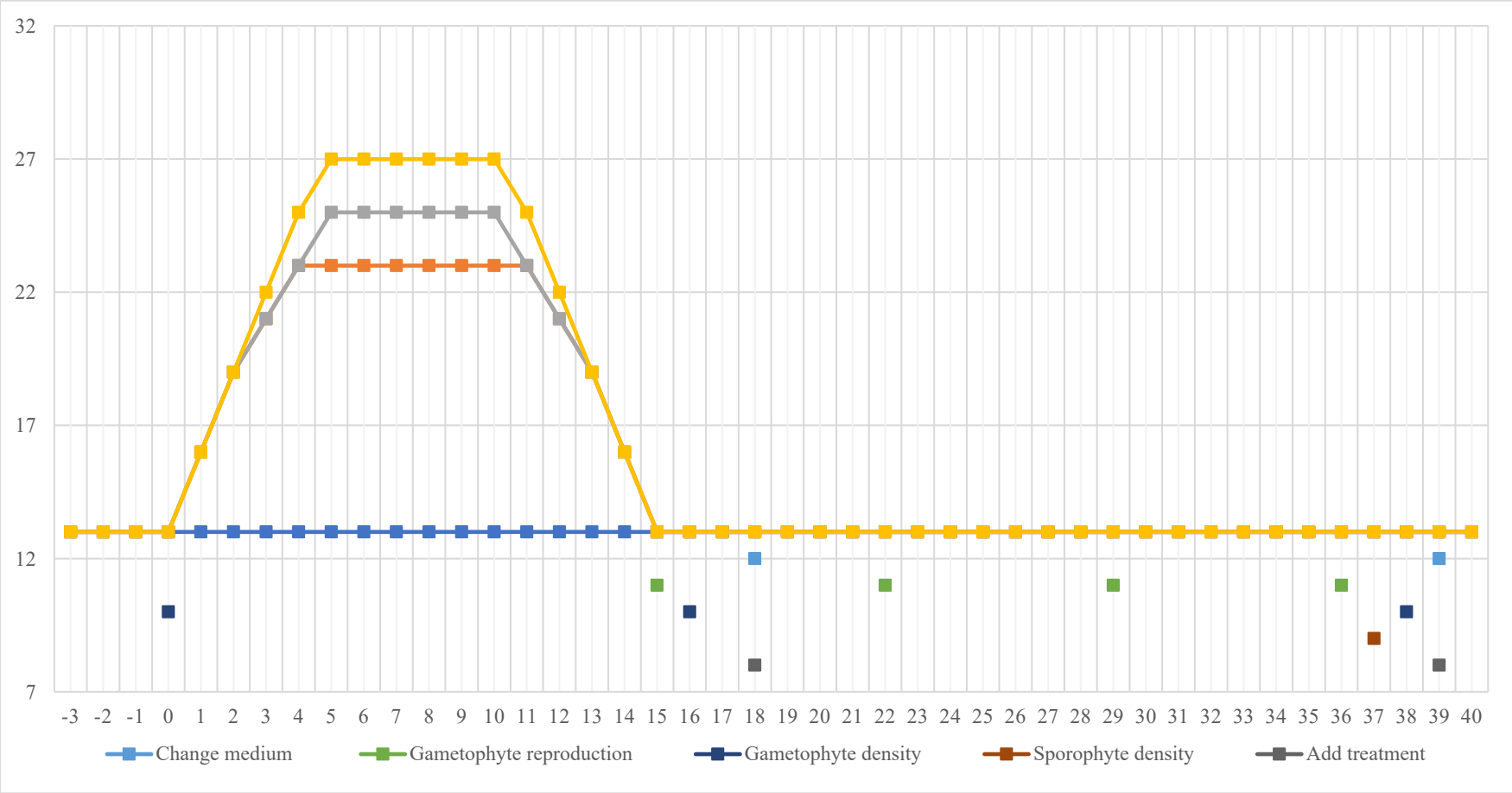

Figure 2.1. The experimental design for assessing the impact of bacterial consortia on gametophyte thermal resilience and subsequent recovery capacity. The X-axis of the graph represents the date, and the Y-axis represents the temperature.

2.2.3 Gametophyte density

The procedure for quantifying gametophyte density involved the use of an inverted microscope (Zeiss Axio Observer D1, Carl Zeiss MicroImaging GmbH, Göttingen, Germany) with a magnification of 100×. Gametophyte density (combined female and male) assessments were conducted at the beginning (day 0) and at the end of the thermal treatments (day 16) for all replicates. On the 38th day (15 days of heat treatment + 23 days of recovery), the density of male and female gametophytes was evaluated separately. For each beaker, a minimum of 40 fields of view were selected and the total number of gametophytes was counted. The gametophyte density per square centimeter was calculated using the following formula:

Gametophytes per cm^2 = (gametophytes per field) / 0.0096 cm^2

2.2.4 Female reproductive success

Utilizing an inverted microscope (Zeiss Axio Observer D1, Carl Zeiss MicroImaging GmbH, Göttingen, Germany) set at 100× magnification, female gametogenesis was assessed every 7 days (15th, 22nd, 29th, and 36th days) during the recovery phase, by evaluating the relative occurrence of three distinct ontogenetic stages (vegetative, eggs released, gametophytes and sporophytes attached). A minimum of 45 fields of view were evaluated per replicate.
Reproductive success was also assessed as the absolute numbers of normal sporophytes and asexual sporophytes (I Tom Dieck, 1992) at the end of the recovery phase (day 37). The absolute number of normal and parthenogenetic sporophytes per cm^2 was counted in a minimum of 27 fields of view per replicate and using the following formula:

Sporophytes per cm^2 = (Sporophytes per field) / 0.0096 cm^2

2.2.5 Statistics

Data was analysed with permutation-based multivariate analysis of variance (PERMANOVA). The analysis of gametophyte density was performed with temperature, treatment, and time as the selected factors, while the female reproductive success rate was performed with temperature and treatment as the selected factors. Data analysis was performed with Euclidian distances, 9999 permutations and the Monte Carlo test was used. Differences were considered significant at $p<0.05$. The analysis of all statistical analyses was carried out using PRIMER 6 version 6.1.11 & PERMANOVA+ version 1.0.1 from PRIMER-E.

3. Result

3.1 Gametophyte density.

The examination of gametophyte density on both day 16 and day 38 revealed significant differences among the various temperature groups simulating heatwaves ($p=0.001$). Furthermore, significant differences were observed between results obtained on different dates ($p=0.0251$). Within the same temperature group simulating a heatwave, a significant interaction was detected between treatment and date ($p=0.0173$). Similarly, among the kelp samples subjected to the same treatment, a significant interaction was found between temperature and date ($p=0.0001$). Additionally, a significant three-way interaction was observed among temperature, treatment, and date ($P=0.0286$).

In the 23°C heatwave treatment group, both on day 16 and day 38, the addition of bacteria was found to significantly increase gametophyte density. In the 25°C heatwave treatment group, on day 38, the addition of bacteria led to a decrease in gametophyte density. In the 27°C heatwave treatment group, not adding bacterial groups resulted in a decrease in gametophyte density (see appendix Table 3.5.3.6, 3.7). When gametophyte density was assessed on day 16, a significant interaction was observed between heatwave and bacteria treatment ($p=0.0032$, see Supplementary Table x). Specifically, at a temperature of 23°C, the addition of bacterial groups significantly decreased gametophyte density, resulting in an average decrease of 173.14/cm², whereas at 27°C heatwave treatment, the addition of bacterial groups significantly increased gametophyte density, resulting in an average increase of 56.13/cm². In kelp without the addition of bacterial groups, the gametophyte density in the 23ºC heatwave treatment was significantly higher than that in the no heatwave simulation group and the 27ºC heatwave treatment, with differences of 125.78/cm²

and 150.45/cm², respectively. In kelp with the addition of the bacterial consortia, the gametophyte density in the 23ºC heatwave treatment was significantly lower than that in the 27ºC heatwave treatment, with a difference of 78.83/cm² (see appendix Table 3.8, 3.9).

When gametophyte density was assessed on day 38, a significant effect of temperature on the results was observed (p=0.0001, see Supplementary Table x). All the different temperature groups were significantly different from each other, except for no significant difference between 13ºC control treatment and 23ºC heatwave treatment (see appendix Table 3.10).

In day 38, the density of female gametophytes was significantly influenced solely by the heatwave temperature (p=0.0001). Within the 27ºC heatwave group, regardless of the presence of bacterial communities, female gametophytes exhibited significantly lower densities compared to other temperature groups. In fact, at 27ºC heatwave group, female gametophyte density did not exceed 40/cm^2, whereas all other temperature groups exceeded 210/cm^2. On the other hand, male gametophyte density exhibited a significant interaction between heatwave temperature and bacterial presence (p=0.0481). Male gametophytes were absent in the 27°C heatwave group. In the remaining temperature groups, the addition of bacterial communities led to varying degrees of reduction in male gametophyte density. In the control temperature group, it decreased to 34.5% of the level without bacteria, in the 23°C heatwave group, it decreased to 42.1% of the level without bacteria, and in the 25°C heatwave group, it decreased to 1.7% of the level without bacteria. At the beginning of the experiment, due to the difficulty in clearly distinguishing between male and female gametophytes, only the combined density of male and female gametophytes was measured. Consequently, it is impossible to ascertain whether population differences in male gametophyte density existed from the outset of the experiment. (Figure 3.2., Table 3.2, appendix Table 3.11, 3.12, 3.13.)

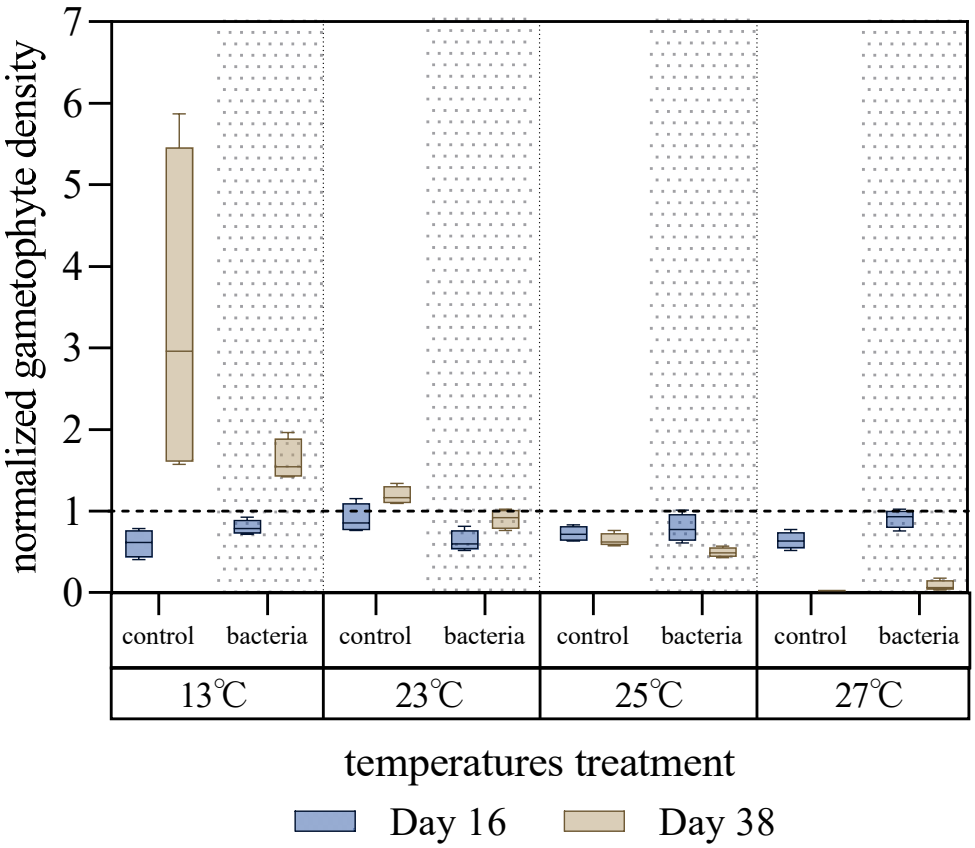

Figure 3.1. Influence of temperature and bacterial consortia on gametophyte density of *L. ochroleuca* after the heatwave treatments (day 16) and the recovery period at 13 °C (day 38). Box plots are presented, displaying the median, 25th and 75th percentiles as boxes, and whiskers representing the minimum and maximum values (n=4). Density values were standardized relative to the gametophyte density on the first day of the simulated heatwave (Day 0) (adjusted mean = 1). See Tables 3.1 and appendix Table 3.5, 3.6, 3.7, 3.8, 3.9, 3.10 for statistics.

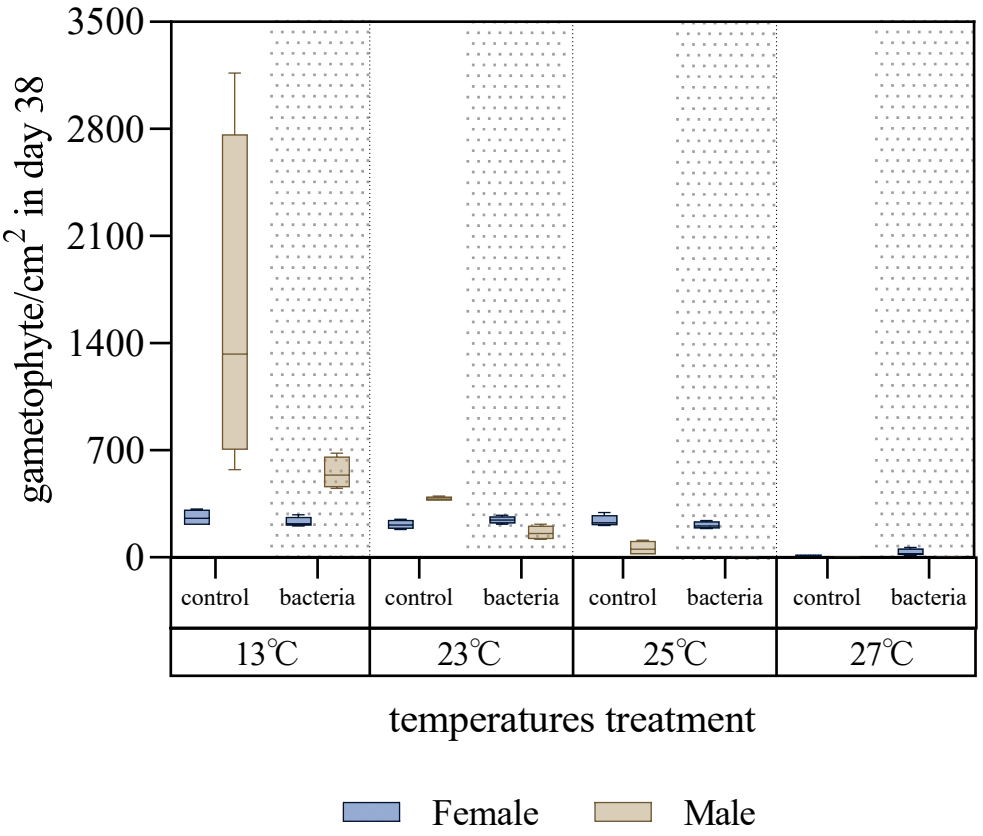

Figure 3.2. Influence of temperature and bacterial consortia on the female and male gametophyte density of *L. ochroleuca* at the end of the 13°C recovery period (day 38). Box plots are presented, displaying the median, 25th and 75th percentiles as boxes, and whiskers representing the minimum and maximum values (n=4). Statistical analyses were conducted for intra-group variations. See Tables 3.2 and appendix Table 3.11, 3.12, 3.13 for statistics.

Table 3.1. PERMANOVA analysis for the effects of different heatwave treatments (13, 23, 25, 27 °C) and bacterial consortia on the normalized density of *L. ochroleuca* gametophytes at the end of the simulated heatwave (day 16) and recovery period at 13 °C (day 38).

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Temperature	3	12.332	4.1108	14.402	**0.0001**	9945
Treatment	1	0.84363	0.84363	2.9556	0.0914	9859
Date	1	1.3089	1.3089	4.5856	**0.0251**	9864
Temperature x Treatment	3	1.9177	0.63924	2.2395	0.0668	9943
Temperature x Date	3	13.855	4.6182	16.179	**0.0001**	9948
Treatment x Date	1	1.3955	1.3955	4.8888	**0.0173**	9860
Temperature x Treatment x Date	3	2.3911	0.79703	2.7923	**0.0286**	9960

Table 3.2. PERMANOVA analysis for the effects of different heatwave treatments (13, 23, 25, 27 °C) and bacterial consortia on the normalized density of gametophytes of different sexes at the recovery period at 13 °C (day 38).

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Female gametophyte density						
Temperature	3	2.7059E5	90198	93.641	**0.0001**	9958
Treatment	1	13.261	13.261	1.3767E-2	0.908	9842
Temperature x Treatment	3	6295.7	2098.6	2.1787	0.1115	9952
Male gametophyte density						
Temperature	3	6.0504E6	2.0168E6	12.728	**0.0001**	9953
Treatment	1	8.8317E5	8.8317E5	5.5736	**0.0054**	9883
Temperature x Treatment	3	1.4181E6	4.7268E5	2.9831	**0.0089**	9956

3.2 Female reproductive success

The transformation rate of gametophytes varied among different heatwave treatments.

In the control heatwave group, gametophytes exhibited the fastest overall transformation rate, predominantly transitioning into sporophytes. Within the 23°C heatwave treatment, the majority of gametophytes transitioned into sporophytes, with a small fraction developing into eggs. The 25°C heatwave treatment showed a partial transformation into eggs, along with some transitioning into sporophytes. In contrast, within the 27°C heatwave treatment, the majority of gametophytes remained in the vegetative stage.

At day 15, only vegetative gametophytes were observed at 23ºC, 25ºC, and 27ºC.

In the control group without simulated heatwaves, a substantial number of sporophytes had already emerged by day 15.

In the control heatwave group, on day 15, whether or not bacterial groups were added, the proportion of female gametophytes transitioning into sporophytes remained consistently above 85%. This trend persisted until day 22, and by day 29, all gametophytes, regardless of the treatment, had successfully developed into sporophytes.

In the 23°C heatwave treatment, by day 22, it was observed that the addition of bacterial consortia led to a higher proportion of female gametophytes undergoing this transition (8% < 44%). By day 29, a substantial proportion of female gametophytes had developed into sporophytes in both treatment groups. By day 36, all female gametophytes with added bacterial consortia had developed into sporophytes, while even in the group without added consortia, a high proportion (93%) had undergone this transition.

In the 25ºC heatwave treatment, by days 22 and 29, a high proportion of female gametophytes in the group with added bacterial consortia had not undergone transition (day 22: 62% > 46%, day 29: 17% > 5%). By day 36, all female gametophytes in the group with added bacterial consortia had completed the transition into sporophytes, while in the group without added consortia, 97% of female gametophytes had developed sporophytes.

In the 27ºC heatwave treatment, no transitions were observed until day 29. By day 36, despite the transition occurring in the presence of bacterial consortia and at a higher proportion compared to the group without added consortia (98% > 90%), it represented only a tenth of all female gametophytes.

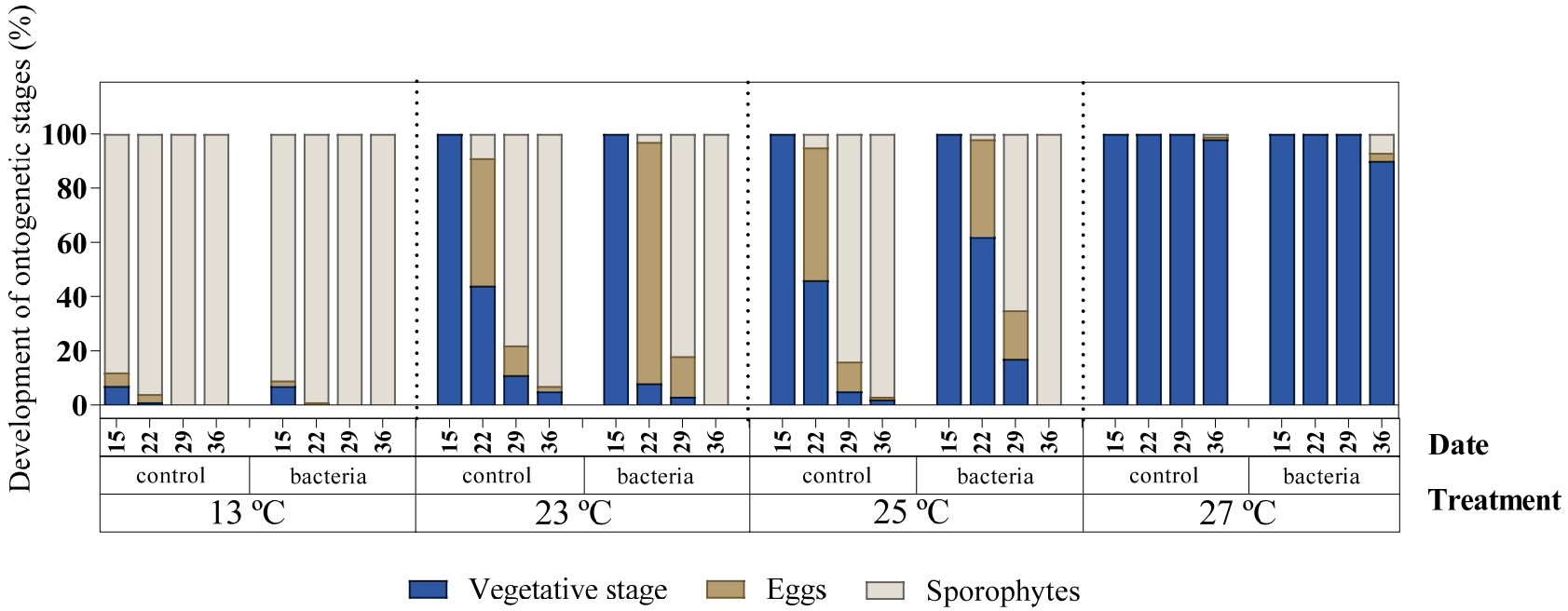

Figure 3.3. Temperature and bacterial consortia effects on the different ontogenetic stages of *L. ochroleuca* over time (mean, n=4). counts were taken at intervals of 7 days within a 22-day period. See Tables 3.3 and appendix Table 3.14, 3.15, 3.16 for statistics.

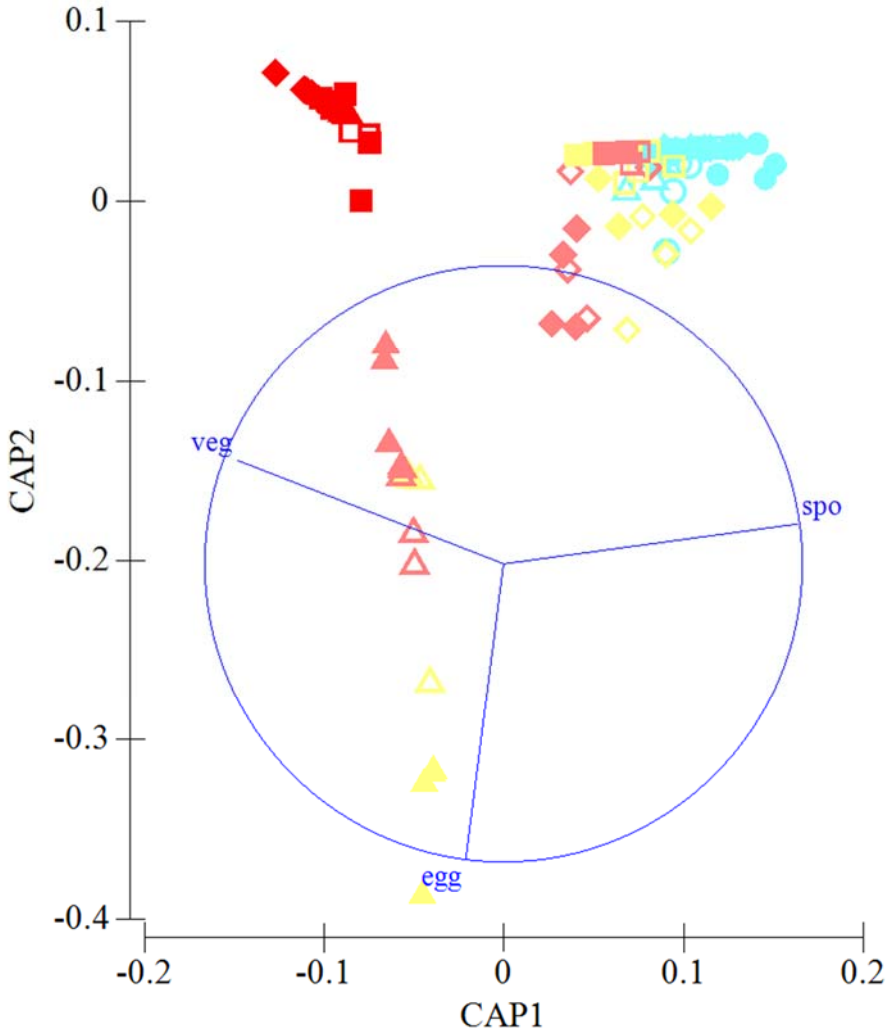

Figure 3.4. Canonical Analysis of Principal Coordinates results of ontogenetic stages in different heatwave treatments with or without the addition of bacterial consortia. In the plot, light blue represents the 13°C control treatment, yellow represents the 23°C heatwave treatment, light red represents the 25 °C heatwave treatment, and red represents the 27°C heatwave treatment. Circular markers represent results from day 15, triangular markers represent results from day 22, diamond markers represent results from day 29, and square markers represent results from day 36. Hollow markers indicate the absence of bacterial consortia, while solid markers indicate their presence. Vectors are drawn for three different ontogenetic stages, with a correlation set at 0.2. See Tables 3.3 for statistics.

Table 3.3. PERMANOVA analysis for the effects of different heatwave treatments (13, 23, 25, 27 °C) and bacterial consortia on the normalized density of gametophytes of different sexes at the recovery period at 13 °C (day 15,22,29,36).

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Temperature	3	2.54E+06	8.48E+05	545.1	**0.0001**	9953
Treatment	1	2744.1	2744.1	1.7636	0.1731	9953
Date	3	8.08E+05	2.69E+05	173.03	**0.0001**	9941
Temperature x Treatment	3	21191	7063.6	4.5396	**0.0002**	9947
Temperature x Date	9	8.30E+05	92176	59.239	**0.0001**	9919
Treatment x Date	3	12410	4136.7	2.6586	**0.0261**	9946
Temperature x Treatment x Date	9	39889	4432.1	2.8484	**0.0003**	9914

3.3 Thermal tolerance of microscopic sporophytes

On day 37, density estimations were conducted separately for normal and asexual spores. Statistical analysis revealed a significant interaction between temperature and spore type (p=0.0001). In the case of normal spores, a significant interaction was observed between heatwave temperature (p=0.0001), while for asexual spores, there was a significant interaction between heatwave temperature and bacterial treatment (p=0.0068) (Table 3.4).

In the control heatwave treatment, the addition of bacterial communities did not have a significant impact on spore density. In the 23-degree heatwave treatment, the addition of bacterial communities significantly increased the density of asexual spores, reaching 2.9 times that of the no-bacteria treatment. In the 25-degree heatwave treatment, the addition of bacterial communities slightly reduced the density of normal spores, decreasing to 76.9% of the no-bacteria treatment, while it slightly increased the density of asexual spores, resulting in a 1.6-fold increase compared to the no-bacteria treatment. In the 27-degree heatwave treatment, the addition of bacteria did not significantly affect the density of normal or asexual spores, and the

combined density of both spore types remained below 200/cm^2.

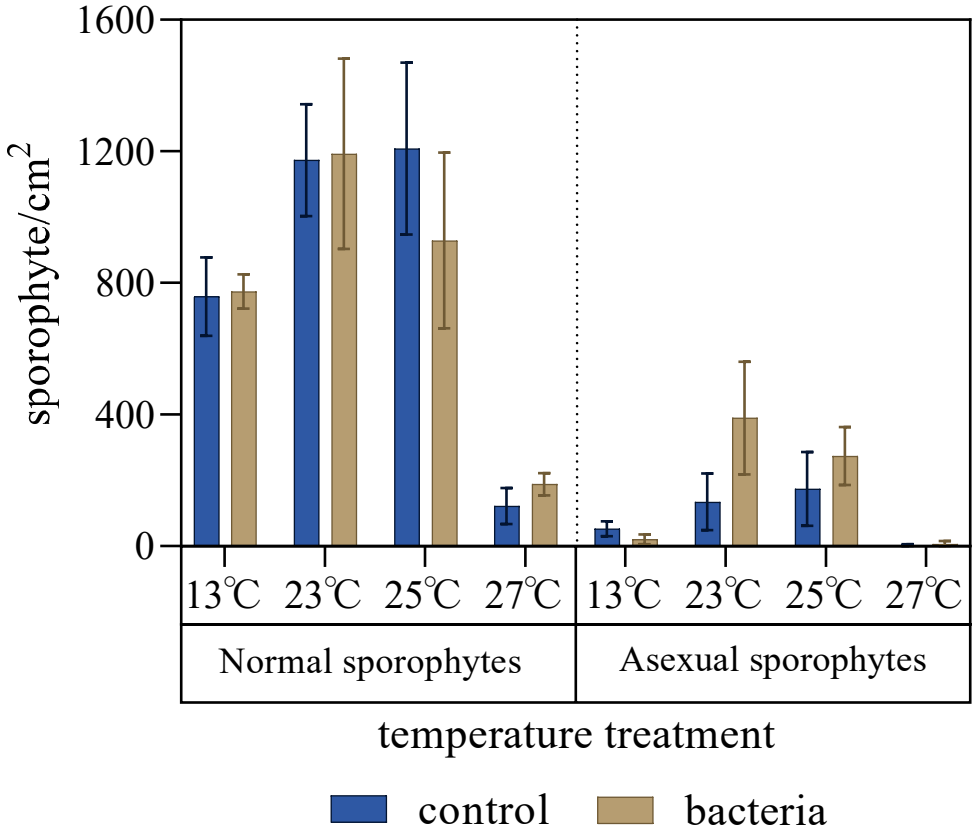

Figure 3.5. Influence of temperature and bacterial consortia on sporophytes development of *L. ochroleuca* at the end of the 13°c recovery period (day 37). Statistical analyses were conducted for both inter-group and intra-group variations. See Tables 3.4 and appendix Table 3.17, 3.18, 3.19, 3.20 for statistics.

Table 3.4. PERMANOVA analysis of experimental sporophyte development: effects of different heatwave treatments at different temperature treatments (13, 23, 25, 27°C) and bacterial consortia on *L. ochroleuca* sporophytes at the end of the recovery period (day 37).

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Normal and asexual						
Temperature	3	5.49E+06	1.83E+06	44.172	**0.0001**	9947
Treatment	1	69568	69568	1.6791	0.1926	9943
Temperature x Treatment	3	2.49E+05	8.29E+04	1.9998	0.1069	9937
Normal						
Temperature	3	5.08E+06	1.69E+06	49.523	**0.0001**	9959
Treatment	1	16272	16272	0.47556	0.4984	9851
Temperature x Treatment	3	1.49E+05	49796	1.4553	0.2463	9959
Asexual						
Temperature	3	4.07E+05	1.36E+05	18.798	**0.0001**	9947
Treatment	1	53296	53296	7.3869	**0.0107**	9835
Temperature x Treatment	3	99172	33057	4.5818	**0.0068**	9958

4. Discussion

The main findings of this study include: (1) male gametophytes are more temperature-sensitive than female gametophytes and are also sensitive to bacterial communities. This sensitivity leads to an increase in asexual sporophytes in the presence of under the heatwave treatment;(2) At the highest temperature that allows gametophyte survival, bacterial communities assist to some extent in the development of female gametophytes.

4.1 Effect of bacterial consortia and heatwave treatment on the gametophyte's density

In the experiment, the addition of bacteria facilitated a better recovery of gamete densities in both the control heatwave group and the 23 °C simulated heatwave group. This was in contrast to the use of sporophytes, where a negative effect of the bacterial consortium on the growth of sporophytes was found in the simulated heat wave group at greater than or equal to 22 °C (Cojoc, 2022). Probably because gametophytes remain dormant under unfavorable environmental conditions, whereas sporophytes are more susceptible to environmental extremes (Graham, Vasquez, & Buschmann, 2007). However, in the 25 °C and 27 °C simulated heatwave groups, this intervention did not yield beneficial effects and even led to adverse outcomes. Notably, 23 °C represents the upper survival threshold for *L. ochroleuca* gametophytes (Inka Tom Dieck, 1993). It is postulated that when gametophytes were exposed to constant temperatures at 23 °C, they barely survived, allowing for sufficient gamete presence to interact with the introduced bacterial consortium during the subsequent recovery period (I Tom Dieck, 1992).

On day 16, it was noted that in the 23 °C simulated heatwave group, the addition of bacteria (control: appendix Figure 4.1, bacteria: appendix Figure 4.2) led to the formation of a significantly larger aggregate of gametophytes, compared to the control group. Gametophytes in clusters were considered to be a single gametophyte when counting gametophyte densities, so the larger the clusters the smaller the actual counts, which is consistent with the results of gametophyte densities at day 16, which were significantly smaller for the added bacteria treatment than for the control group. This suggests that the reduced configuration density observed in the bacterial treatments does not mean that gametophytes are dying, but rather that clusters are being formed. In red algae, heat stress has been known to stimulate gametophytes to produce abnormal callus tissue, initiating a mechanism for asexual reproduction(Suda & Mikami, 2020). The abundance of selected bacteria correlated positively with temperature increase, the genus *Rhodobacteraceae* increased in relative abundance from 20% to 50% with increasing temperature (Stratil et al., 2013), *S. pseudonitzschiae*, was not only positively correlated with temperature but also

significantly negatively correlated with nitrogen concentration(Florez et al., 2019) ,thus leading to an enrichment of bacterial populations during the simulated heatwave. As these bacteria are surface-associated microbes on kelp, they effectively acted as a "glue," causing gametes to aggregate into larger clusters(Hughes, 2014). In the 25 °C simulated heatwave group, gametes did not take on their typical shape, with dark, filamentous structures, which further coalesced into clusters. Some gametes at the periphery of these aggregates displayed smooth edges (control: appendix Figure 4.3, bacteria: appendix Figure 4.4). Conversely, in the 27 °C simulated heatwave group, gametes did not exhibit smooth edges (control: appendix Figure 4.5, bacteria: appendix Figure 4.6). Due to the clustering of gametes and their adhesion to one another, many gametes remained internally within these clusters, possibly limiting direct contact with the bacterial consortium and the surrounding environment, which likely reduced their performance during the recovery period. Considering the results observed at day 38, it can be concluded that the addition of bacterial communities does not significantly impact the health of kelp at temperatures conducive to its growth. Furthermore, while bacterial assistance aids in the recovery period of gametophytes following a heatwave at 23°C, their maximum survival temperature, it does not improve overall development compared to the no-addition group. This suggests that even at the high stressor level of 25 °C, gametophytes can recover without a significant enhancement from bacterial communities.

However, the 27ºC simulated heatwave profoundly impaired the recovery capacity of most gametophytes. Although the gametophytes survive, there are only a few of them. This situation may be attributed to a disruption in the balance of the kelp's surface microbiome under the extreme conditions of the 27 °C heatwave treatment (Minich et al., 2018). High temperatures can act as a signal inducing the expression of pathogenic genes in some members of the entire microbiome (Case et al., 2011). Thus, it is highly likely that certain bacteria within the microbiome become opportunistic pathogens under these high-temperature conditions, leading to the poor recovery observed in the 27 °Cheatwave treatment. Diversity analysis of microorganisms on the surface of kelp in heat waves should be added in such cases and structural changes in the microbiota should be analyzed against control group.

4.2 Effect of bacterial consortia and heatwave treatment on the sex of gametophytes

When observing the gender ratio of gametophytes on day 38, it was noted that in the 13ºC control group, the proportion of male gametophytes exceeded that of female gametophytes. There was no significantly increase in the number of male gametophytes compared to day 0 .This observation aligns with similar patterns observed in *L. digitata*, where male and female gametophytes exhibit distinct strategies: Males grew vegetatively and reproduced at the same time, while females stopped growing after reproduction (Destombe & Valeria Oppliger, 2011). Moreover, male gametophytes exhibited higher temperature sensitivity than their female counterparts, with reduced adaptive capacity under elevated temperature stress, as indicated by experimental findings. This contrast with previous studies showing that male gametophytes are more heat-tolerant than female gametophytes and suggested to be a common trend in kelp， like different species from the same genus such as *L. digitata* (Bolton & Lüning, 1982) and *L. pallida* [= *L. schinzii*](Inka Tom Dieck, 1993). These discrepancies may be attributed to species-specific variations. The varying sensitivity of *L. ochroleuca* populations to heat stress may account for the differences observed(Gauci, Bartsch, Martins, & Liesner, 2022). The population used in this experiment, originating from France, appears to be highly sensitive to heat stress(Strasser et al., 2022), which could be one of the contributing factors to the results described above.

4.3 Effect of bacterial consortia and heatwave treatment on the development of ontogeny stage

Temperature has a significant impact on the development and reproduction of seaweed cells(Eggert, 2012). For instance, in *L. digitata*, gametogenesis is entirely suppressed at temperatures between >17°C and 21°C(I Tom Dieck, 1992). For *L. ochroleuca*, 23°C is already at the upper limit of its growth range. When observing the ontogenetic stages of *L. ochroleuca* in the experiment, the addition of the bacterial

consortium appeared to enhance the transition from the vegetative stage to the egg stage in the 23°C simulated heatwave group on day 22. Therefore, the bacterial consortium could potentially aid in the survival of gametes in the 23°C simulated heatwave group. *S. pseudonitzschiae* has been shown to assist kelp in nitrogen fixation during periods of nutrient starvation(Florez et al., 2019). Nitrogen, as a crucial driving factor, can modulate kelp's response to temperature stress, ameliorating its physiological adversities(Fernández et al., 2020). Female gametophytes of kelp exhibit a transcriptional preference towards metabolism and energy-related processes(Monteiro et al., 2019). Therefore, it is plausible to hypothesize that bacterial assistance could enhance the metabolic activity of female gametophytes, consequently promoting their development.

4.4 Effect of bacterial consortia and heatwave treatment on the development of sporophyte

When assessing the impact of bacteria on gametophyte development, it was observed that in the 23°C simulated heatwave group, the added bacterial community led to a higher proportion of spores developing into asexual sporophytes, which typically lack roots(Lüning & Tom Dieck, 1989; 本村泰三 & 阪井與志雄, 1981). This phenomenon might occur due to the release of eggs from female gametophytes over several days(Lüning, 1981), while the lifespan of sperm from male gametophytes usually does not exceed 12 hours(Li, Pang, Liu, Shan, & Gao, 2013). As a result, a large-scale release of eggs occurred without a sufficient number of sperm for fertilization(Hsiao & Druehl, 1971). Consequently, some eggs remained unfertilized, leading to asexual reproduction and development.

In summary, the findings of this study underscore the impact of temperature stress on kelp growth and reproduction, as well as the effectiveness of the bacterial consortium as a treatment in assisting kelp in responding to temperature stress. This research contributes to a better understanding of the potential applications and performance of bacterial consortia in the context of kelp's response to global environmental changes. There is an urgent need for further research and related experiments on bacterial

consortia to explore and validate additional strategies that can enhance the resilience of this keystone species in the face of global marine heatwaves.

5. Reference

Almeida, D. A. L. R. (2022). *Assessing the potential for resilience towards marine heatwaves and artificial light at night in kelp species.*

Alsufyani, T., Califano, G., Deicke, M., Grueneberg, J., Weiss, A., Engelen, A. H., . . . Wichard, T. (2020). Macroalgal–bacterial interactions: identification and role of thallusin in morphogenesis of the seaweed Ulva (Chlorophyta). *Journal of experimental botany, 71*(11), 3340-3349.

Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C. M., Buck, B. H., Eggert, A., . . . Karez, R. (2008). The genus Laminaria sensu lato: recent insights and developments. *European journal of phycology, 43*(1), 1-86.

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.

Bertocci, I., Araújo, R., Oliveira, P., & Sousa-Pinto, I. (2015). Potential effects of kelp species on local fisheries. *Journal of Applied Ecology, 52*(5), 1216-1226.

Birkett, D., Maggs, C., Dring, M., Boaden, P., & Seed, R. (1998). Infralittoral reef biotopes with kelp species. *An overview of dynamic and sensitivity characteristics for conservation management of marine SACs, 7*.

Bolton, J., & Lüning, K. (1982). Optimal growth and maximal survival temperatures of Atlantic Laminaria species (Phaeophyta) in culture. *Marine Biology, 66*, 89-94.

Braud, J.-P. (1974). Etude de quelques parametres écologiques, biologiques et biochimiques chez une phéophycée des côtes bretonnes *Laminaria ochroleuca*. *Revue des Travaux de l'Institut des Pêches Maritimes, 38*(2).

Britton, D., Schmid, M., Noisette, F., Havenhand, J. N., Paine, E. R., McGraw, C. M., . . . Mundy, C. N. (2020). Adjustments in fatty acid composition is a mechanism that can explain resilience to marine heatwaves and future ocean conditions in the habitat-forming seaweed Phyllospora comosa (Labillardière) C. Agardh. *Global change biology, 26*(6), 3512-3524.

Case, R. J., Longford, S. R., Campbell, A. H., Low, A., Tujula, N., Steinberg, P. D., & Kjelleberg, S. (2011). Temperature induced bacterial virulence and bleaching disease in a chemically defended marine macroalga. *Environmental Microbiology, 13*(2), 529-537.

Colin, C., Leblanc, C., Wagner, E., Delage, L., Leize-Wagner, E., Van Dorsselaer, A., . . . Potin, P. (2003). The brown algal kelp Laminaria digitata features distinct bromoperoxidase and iodoperoxidase activities. *Journal of Biological Chemistry, 278*(26), 23545-23552.

Dayton, P. K., Tegner, M. J., Parnell, P. E., & Edwards, P. B. (1992). Temporal and spatial patterns of disturbance and recovery in a kelp forest community. *Ecological Monographs, 62*(3), 421-445.

Destombe, C., & Valeria Oppliger, L. (2011). Male gametophyte fragmentation in Laminaria digitata: a life history strategy to enhance reproductive success. *CBM-Cahiers de Biologie Marine, 52*(4), 385.

DeWeese, K. J., & Osborne, M. G. (2021). Understanding the metabolome and metagenome as extended phenotypes: The next frontier in macroalgae

domestication and improvement. *Journal of the World Aquaculture Society, 52*(5), 1009-1030.
Drew, E. A. (1974). An ecological study of *Laminaria ochroleuca* Pyl. growing below 50 metres in the Straits of Messina. *Journal of Experimental Marine Biology and Ecology, 15*(1), 11-24.
Düsedau, L., Ren, Y., Hou, M., Wahl, M., Hu, Z.-M., Wang, G., & Weinberger, F. (2023). Elevated Temperature-Induced Epimicrobiome Shifts in an Invasive Seaweed Gracilaria vermiculophylla. *Microorganisms, 11*(3), 599.
Eggert, A. (2012). Seaweed responses to temperature. *Seaweed biology: Novel insights into ecophysiology, ecology and utilization*, 47-66.
Fernández, P. A., Gaitán-Espitia, J. D., Leal, P. P., Schmid, M., Revill, A. T., & Hurd, C. L. (2020). Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: implications for acclimation under thermal stress. *Scientific Reports, 10*(1), 3186.
Filbee-Dexter, K., & Scheibling, R. E. (2014). Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Marine Ecology Progress Series, 495*, 1-25.
Florez, J. Z., Camus, C., Hengst, M. B., Marchant, F., & Buschmann, A. H. (2019). Structure of the epiphytic bacterial communities of Macrocystis pyrifera in localities with contrasting nitrogen concentrations and temperature. *Algal Research, 44*, 101706.
Foster, M. S., & Schiel, D. R. (1985). *The ecology of giant kelp forests in California: a community profile* (Vol. 85): US Fish and Wildlife Service.
Foster, M. S., & Schiel, D. R. (2010). Loss of predators and the collapse of southern California kelp forests (?): Alternatives, explanations and generalizations. *Journal of Experimental Marine Biology and Ecology, 393*(1-2), 59-70.
Franco, J. N., Tuya, F., Bertocci, I., Rodríguez, L., Martínez, B., Sousa-Pinto, I., & Arenas, F. (2018). The 'golden kelp'*Laminaria ochroleuca* under global change: Integrating multiple eco-physiological responses with species distribution models. *Journal of Ecology, 106*(1), 47-58.
Franco, J. N., Wernberg, T., Bertocci, I., Duarte, P., Jacinto, D., Vasco-Rodrigues, N., & Tuya, F. (2015). Herbivory drives kelp recruits into 'hiding'in a warm ocean climate. *Marine Ecology Progress Series, 536*, 1-9.
Franco, J. N., Wernberg, T., Bertocci, I., Jacinto, D., Maranhão, P., Pereira, T., . . . Tuya, F. (2017). Modulation of different kelp life stages by herbivory: compensatory growth versus population decimation. *Marine Biology, 164*, 1-10.
Gauci, C., Bartsch, I., Martins, N., & Liesner, D. (2022). Cold thermal priming of Laminaria digitata (Laminariales, Phaeophyceae) gametophytes enhances gametogenesis and thermal performance of sporophytes. *Frontiers in Marine Science, 9*, 862923.
Gerard, V. A. (1997). The Role of Nitrogen Nutrition in High-Temperature Tolerance of the Kelp, Laminaria Saccharina (Chromophyta) 1. *Journal of Phycology, 33*(5), 800-810.
Graham, M. H., Vasquez, J. A., & Buschmann, A. H. (2007). Global ecology of the giant kelp Macrocystis: from ecotypes to ecosystems. *Oceanography and Marine Biology, 45*, 39.
Hamersley, M. R., Sohm, J. A., Burns, J. A., & Capone, D. G. (2015). Nitrogen fixation associated with the decomposition of the giant kelp Macrocystis

pyrifera. *Aquatic botany, 125*, 57-63.
Hobday, A. J., Alexander, L. V., Perkins, S. E., Smale, D. A., Straub, S. C., Oliver, E. C., . . . Feng, M. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography, 141*, 227-238.
Hoek, C. v. d. (1982). The distribution of benthic marine algae in relation to the temperature regulation of their life histories. *Biological journal of the Linnean Society, 18*(2), 81-144.
Hsiao, S. I., & Druehl, L. D. (1971). Environmental control of gametogenesis in Laminaria saccharina. I. The effects of light and culture media. *Canadian Journal of Botany, 49*(8), 1503-1508.
Hughes, P. (2014). *An investigation into marine biofouling and its influence on the durability of concrete sea defences.* University of Central Lancashire,
King, N. G., Moore, P. J., Thorpe, J. M., & Smale, D. A. (2023). Consistency and variation in the kelp microbiota: patterns of bacterial community structure across spatial scales. *Microbial ecology, 85*(4), 1265-1275.
Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., . . . Ling, S. D. (2016). Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences, 113*(48), 13785-13790.
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.
Lotze, H. K., Coll, M., Magera, A. M., Ward-Paige, C., & Airoldi, L. (2011). Recovery of marine animal populations and ecosystems. *Trends in ecology & evolution, 26*(11), 595-605.
Lüning, K. (1981). Egg release in gametophytes of Laminaria saccharina: induction by darkness and inhibition by blue light and UV. *British Phycological Journal, 16*(4), 379-393.
Lüning, K., & Tom Dieck, I. (1989). Environmental triggers in algal seasonality.
Mann, K. (1973). Seaweeds: Their Productivity and Strategy for Growth: The role of large marine algae in coastal productivity is far more important than has been suspected. *Science, 182*(4116), 975-981.
Mineur, F., Arenas, F., Assis, J., Davies, A. J., Engelen, A. H., Fernandes, F., . . . Vaz-Pinto, F. (2015). European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of sea research, 98*, 91-108.
Minich, J. J., Morris, M. M., Brown, M., Doane, M., Edwards, M. S., Michael, T. P., & Dinsdale, E. A. (2018). Elevated temperature drives kelp microbiome dysbiosis, while elevated carbon dioxide induces water microbiome disruption. *PLoS One, 13*(2), e0192772.
Monteiro, C., Heinrich, S., Bartsch, I., Valentin, K., Corre, E., Collén, J., . . . Bischof, K. (2019). Temperature modulates sex-biased gene expression in the gametophytes of the kelp Saccharina latissima. *Frontiers in Marine Science, 6*, 769.
Pereira, T. R., Azevedo, I. C., Oliveira, P., Silva, D. M., & Sousa-Pinto, I. (2019). Life history traits of *Laminaria ochroleuca* in Portugal: The range-center of its geographical distribution. *Aquatic botany, 152*, 1-9.
Phelps, C. M., McMahon, K., Bissett, A., Bernasconi, R., Steinberg, P. D., Thomas, T., . . . Huggett, M. J. (2021). The surface bacterial community of an

Australian kelp shows cross-continental variation and relative stability within regions. *FEMS microbiology ecology, 97*(7), fiab089.
Pörtner, H. O., Roberts, D. C., Adams, H., Adler, C., Aldunce, P., Ali, E., . . . Biesbroek, R. (2022). *Climate change 2022: impacts, adaptation and vulnerability*. Retrieved from
Sánchez-Barredo, M., Sandoval-Gil, J. M., Zertuche-González, J. A., Ladah, L. B., Belando-Torrentes, M. D., Beas-Luna, R., & Cabello-Pasini, A. (2020). Effects of heat waves and light deprivation on Giant kelp juveniles (Macrocystis pyrifera, Laminariales, Phaeophyceae). *Journal of Phycology, 56*(4), 880-894.
Singh, R. P., Baghel, R. S., Reddy, C., & Jha, B. (2015). Effect of quorum sensing signals produced by seaweed-associated bacteria on carpospore liberation from Gracilaria dura. *Frontiers in plant science, 6*, 117.
Singh, R. P., & Reddy, C. (2014). Seaweed–microbial interactions: key functions of seaweed-associated bacteria. *FEMS microbiology ecology, 88*(2), 213-230.
Smale, D. A., Wernberg, T., Yunnie, A. L., & Vance, T. (2015). 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.
Smirthwaite, J. (2007). *Laminaria ochroleuca* Golden kelp. *Biology and Sensitivity Key Information Reviews.* Retrieved from https://www.marlin.ac.uk/species/detail/1838
Strasser, F.-E., 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*, 943511.
Stratil, S. B., Neulinger, S. C., Knecht, H., Friedrichs, A. K., & Wahl, M. (2013). Temperature-driven shifts in the epibiotic bacterial community composition of the brown macroalga F ucus vesiculosus. *Microbiologyopen, 2*(2), 338-349.
Straub, S. C., Wernberg, T., Thomsen, M. S., Moore, P. J., Burrows, M. T., Harvey, B. P., & Smale, D. A. (2019). Resistance, extinction, and everything in between–The diverse responses of seaweeds to marine heatwaves. *Frontiers in Marine Science*, 763.
Suda, M., & Mikami, K. (2020). Reproductive responses to wounding and heat stress in gametophytic thalli of the red alga Pyropia yezoensis. *Frontiers in Marine Science, 7*, 394.
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.
Tegner, M., Dayton, P. K., Edwards, P. B., & Riser, K. L. (1996). Is there evidence for long-term climatic change in southern California kelp forests? *California Cooperative Oceanic Fisheries Investigations Report*, 111-126.
Tittley, I., & Neto, A. I. (2000). A provisional classification of algal-characterised rocky shore biotopes in the Azores. *Hydrobiologia, 440*(1-3), 19-25.
Tom Dieck, I. (1992). North Pacific and North Atlantic digitate Laminaria species (Phaeophyta): hybridization experiments and temperature responses. *Phycologia, 31*(2), 147-163.
Tom Dieck, I. (1993). Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. *MARINE ECOLOGY-PROGRESS SERIES, 100*, 253-253.

Umanzor, S., Sandoval-Gil, J., Sánchez-Barredo, M., Ladah, L. B., Ramírez-García, M. M., & Zertuche-González, J. A. (2021). Short-term stress responses and recovery of giant kelp (Macrocystis pyrifera, Laminariales, Phaeophyceae) juvenile sporophytes to a simulated marine heatwave and nitrate scarcity1. *Journal of Phycology, 57*(5), 1604-1618.

Wernberg, T., Thomsen, M. S., Tuya, F., Kendrick, G. A., Staehr, P. A., & Toohey, B. D. (2010). Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. *Ecology letters, 13*(6), 685-694.

本村泰三, & 阪井與志雄. (1981). Effect of chelated iron in culture media on oogenesis in Laminaria angustata. *日本水産学会誌, 47*(12), 1535-1540.

Appendix

Table 3.5. PERMANOVA multiple pairwise comparisons between the different bacteria treatment groups (control and bacteria) organized by date (day 16 and 38) and temperature (13ºC, 22 ºC, 23 ºC and 25ºC) on normalized gametophytes density of *L. ochroleuca.*

Factor	Groups	t	P(perm)	Unique perms	P(MC)
13 16	Control, Bacteria	2.0676	0.0906	35	0.0847
13 38	Control, Bacteria	1.6478	0.1991	35	0.1546
23 16	Control, Bacteria	2.5095	0.0826	35	**0.0444**
23 38	Control, Bacteria	3.4857	0.0281	35	**0.0132**
25 16	Control, Bacteria	0.6993	0.565	35	0.5169
25 38	Control, Bacteria	3.0427	0.0265	35	**0.0268**
27 16	Control, Bacteria	3.5077	0.0551	35	**0.015**
27 38	Control, Bacteria	1.8564	0.0545	35	0.1174

Table 3.6. PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) organized by date (day 16 and 38) and temperature different bacteria treatment groups (control and bacteria) on normalized gametophytes density of *L. ochroleuca.*

Factor	Groups	t	P(perm)	Unique perms	P(MC)
16 Control	13, 23	2.4641	0.0554	35	**0.0479**
	13, 25	1.2218	0.3166	35	0.2644
	13, 27	0.3466	0.7662	35	0.7459
	23, 25	1.8318	0.1376	35	0.1183
	23, 27	2.592	0.0565	35	**0.041**
	25, 27	1.1845	0.2521	35	0.2859
38 Control	13, 23	2.0686	0.0269	35	0.0861
	13, 25	2.5944	0.0286	35	**0.0412**
	13, 27	3.1963	0.0295	35	**0.0195**
	23, 25	8.0002	0.0286	35	**0.0003**
	23, 27	21.271	0.0305	35	**0.0001**
	25, 27	15.178	0.0295	35	**0.0001**
16 bacteria	13, 23	2.1839	0.1146	35	0.0673
	13, 25	0.11865	0.942	35	0.9177
	13, 27	1.518	0.2213	35	0.1777
	23, 25	1.4911	0.199	35	0.1876
	23, 27	3.2603	0.0553	35	**0.0169**
	25, 27	1.1806	0.2826	35	0.2734
38 bacteria	13, 23	5.0547	0.0289	35	**0.0023**
	13, 25	8.6264	0.0283	35	**0.0003**
	13, 27	11.701	0.0295	35	**0.0001**
	23, 25	6.1542	0.0259	35	**0.0006**
	23, 27	11.993	0.0301	35	**0.0001**
	25, 27	9.4317	0.0317	35	**0.0001**

Table 3.7. PERMANOVA multiple pairwise comparisons between the different date (day 16 and 38) organized by temperature (13ºC, 22 ºC, 23 ºC and 25ºC) and temperature different bacteria treatment groups (control and bacteria) on normalized gametophytes density of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
13 Control	16, 38	2.6266	0.0275	35	**0.0422**
13 bacteria	16, 38	6.0611	0.0241	35	**0.0007**
23 Control	16, 38	2.7186	0.0891	35	**0.0363**
23 bacteria	16, 38	3.1065	0.0615	35	**0.0202**
25 Control	16, 38	1.2539	0.1657	35	0.2581
25 bacteria	16, 38	3.3237	0.0289	35	**0.0153**
27 Control	16, 38	11.619	0.029	35	**0.0002**
27 bacteria	16, 38	12.68	0.027	35	**0.0001**

Table 3.8. PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) organized by different bacteria treatment groups (control and bacteria) and day 16 on normalized gametophytes density of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
Control	13, 23	2.4641	0.0562	35	**0.0497**
	13, 25	1.2218	0.3157	35	0.2643
	13, 27	0.3466	0.7721	35	0.7434
	23, 25	1.8318	0.146	35	0.1183
	23, 27	2.592	0.0567	35	**0.0416**
	25, 27	1.1845	0.2608	35	0.2791
bacteria	13, 23	2.1839	0.113	35	0.0704
	13, 25	0.11865	0.9423	35	0.9103
	13, 27	1.518	0.232	35	0.1763
	23, 25	1.4911	0.1957	35	0.1818
	23, 27	3.2603	0.0587	35	**0.0171**
	25, 27	1.1806	0.2838	35	0.275

Table 3.9. PERMANOVA multiple pairwise comparisons between the different bacteria treatment groups (control and bacteria) organized by different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) and day 16 on normalized gametophytes density of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
13	Control, Bacteria	2.0676	0.0891	35	0.0806
23	Control, Bacteria	2.5095	0.0846	35	**0.0454**
25	Control, Bacteria	0.6993	0.5771	35	0.5116
27	Control, Bacteria	3.5077	0.0556	35	**0.0113**

Table 3.10. PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) organized by day 38 on normalized gametophytes density of *L. ochroleuca*.

Groups	t	P(perm)	Unique perms	P(MC)
13, 23	2.7279	0.0127	9897	**0.0182**

13, 25	3.6478	0.0025	9875	**0.0035**
13, 27	4.637	0.001	9852	**0.0009**
23, 25	10.018	0.0004	9784	**0.0001**
23, 27	22.655	0.0003	9480	**0.0001**
25, 27	17.278	0.0003	9630	**0.0001**

Table 3.11. PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) on female gametophytes density of *L. ochroleuca.*

Groups	t	P(perm)	Unique perms	P(MC)
13, 23	0.8926	0.3888	9834	0.3986
13, 25	1.1072	0.2891	9828	0.2919
13, 27	13.452	0.0001	9725	**0.0001**
23, 25	0.33395	0.7455	9784	0.7396
23, 27	18.651	0.0001	9414	**0.0001**
25, 27	16.369	0.0002	9637	**0.0001**

Table 3.12. PERMANOVA multiple pairwise comparisons between the different bacteria treatment groups (control and bacteria) organized by different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) on male gametophytes density of *L. ochroleuca.*

Factor	Groups	t	P(perm)	Unique perms	P(MC)
13	Control, Bacteria	1.8643	0.0874	35	0.1082
23	Control, Bacteria	9.712	0.0283	31	**0.0002**
25	Control, Bacteria	2.6395	0.0301	12	**0.0377**
27	Control, Bacteria	0	0	0	0

Table 3.13. PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) organized by different bacteria treatment groups (control and bacteria) on male gametophytes density of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
control	13, 23	2.1726	0.0268	35	0.0718
	13, 25	2.7476	0.0289	35	**0.0333**
	13, 27	2.8578	0.0298	8	**0.0274**
	23, 25	13.864	0.0277	30	**0.0001**
	23, 27	62.502	0.0287	8	**0.0001**
	25, 27	2.6887	0.0293	8	**0.0362**
	Groups	**t**	**P(perm)**	**Unique perms**	**P(MC)**
bacteria	13, 23	6.8412	0.0313	35	**0.0005**
	13, 25	10.466	0.0307	15	**0.0002**
	13, 27	10.488	0.0316	8	**0.0001**
	23, 25	7.2812	0.0288	15	**0.0001**
	23, 27	7.3367	0.0292	8	**0.0001**
	25, 27	1	1	1	0.3576

Table 3.14. PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) on gametophytes organized by different bacteria treatment groups (control and bacteria) and date (day 16 and 38) on the development of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
15 control	13, 23	33.39	0.0305	35	**0.0001**
	13, 25	26.151	0.0296	35	**0.0001**
	13, 27	27.834	0.0279	35	**0.0001**
	23, 25	1.2157	0.2881	35	0.2762
	23, 27	2.6237	0.0873	35	**0.0395**
	25, 27	0.93226	0.371	35	0.3933
22 control	13, 23	11.422	0.0298	35	**0.0001**
	13, 25	14.816	0.0288	35	**0.0001**
	13, 27	32.606	0.0292	35	**0.0001**
	23, 25	0.43334	0.8767	35	0.8317
	23, 27	10.159	0.0273	35	**0.0001**
	25, 27	12.955	0.0269	35	**0.0001**
29 control	13, 23	2.8343	0.0286	32	**0.0132**
	13, 25	4.1267	0.0304	35	**0.0039**
	13, 27	22.124	0.0297	26	**0.0001**
	23, 25	2.701	0.0567	35	**0.0154**
	23, 27	16.633	0.0304	35	**0.0001**
	25, 27	13.743	0.024	35	**0.0001**
36 control	13, 23	3.6129	0.0288	34	**0.0075**
	13, 25	5.8407	0.0294	35	**0.0009**
	13, 27	8.8656	0.0265	35	**0.0001**
	23, 25	1.3599	0.2248	35	0.1964
	23, 27	7.8524	0.0286	35	**0.0001**
	25, 27	7.8369	0.0304	34	**0.0002**
15 bacteria	13, 23	22.421	0.0283	35	**0.0001**
	13, 25	24.597	0.0301	35	**0.0001**
	13, 27	20.636	0.0265	25	**0.0001**
	23, 25	0.94668	0.3744	35	0.392
	23, 27	6.06E-03	1	15	0.9965
	25, 27	0.88007	0.3686	25	0.4127
22 bacteria	13, 23	23.051	0.0274	35	**0.0001**
	13, 25	15.271	0.0304	35	**0.0001**
	13, 27	39.445	0.03	26	**0.0001**
	23, 25	8.3435	0.0281	35	**0.0001**
	23, 27	26.722	0.0273	35	**0.0001**
	25, 27	8.9326	0.0269	35	**0.0001**
29 bacteria	13, 23	2.7576	0.0561	35	**0.0296**
	13, 25	6.4529	0.0258	35	**0.0001**
	13, 27	11.304	0.0284	26	**0.0001**
	23, 25	2.4035	0.0292	35	**0.0209**
	23, 27	8.998	0.0264	35	**0.0002**
	25, 27	8.0229	0.0283	35	**0.0002**
36 bacteria	13, 23	4.2899	0.0303	24	**0.0052**
	13, 25	3.5836	0.0284	21	**0.0126**
	13, 27	5.691	0.0317	35	**0.0008**
	23, 25	1.8014	0.1402	16	0.1231
	23, 27	5.3853	0.0243	35	**0.0009**

	25, 27	5.5165	0.0283	35	**0.0009**

Table 3.15 PERMANOVA multiple pairwise comparisons between the different bacteria treatment groups (control and bacteria) on gametophytes organized by different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) and date (day 16 and 38) on the development of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
13 15	Control, Bacteria	4.4693	0.0295	35	**0.0012**
13 22	Control, Bacteria	1.8143	0.0898	35	0.1096
13 29	Control, Bacteria	2.8726	0.0809	35	**0.0285**
13 36	Control, Bacteria	3.9418	0.0283	25	**0.0076**
23 15	Control, Bacteria	2.6435	0.0562	35	**0.041**
23 22	Control, Bacteria	5.2256	0.0267	35	**0.0005**
23 29	Control, Bacteria	1.1507	0.2864	35	0.2868
23 36	Control, Bacteria	3.5355	0.0273	33	**0.0061**
25 15	Control, Bacteria	2.8572	0.0564	35	**0.0278**
25 22	Control, Bacteria	2.1234	0.0577	35	0.0672
25 29	Control, Bacteria	1.268	0.1649	34	0.2245
25 36	Control, Bacteria	1.6095	0.1405	15	0.122
27 15	Control, Bacteria	0.81515	0.5421	25	0.4443
27 22	Control, Bacteria	0.32552	0.6869	35	0.7602
27 29	Control, Bacteria	1.0482	0.3979	35	0.3364
27 36	Control, Bacteria	0.88197	0.4265	35	0.4089

Table 3.16 PERMANOVA multiple pairwise comparisons between the different date (day 16 and 38) on gametophytes organized by different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) and bacteria treatment groups (control and bacteria) on the development of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
13 control	15, 22	2.8981	0.0306	35	**0.0083**
	15, 29	1.9491	0.0647	35	**0.0412**
	15, 36	2.2414	0.0598	35	**0.0223**
	22, 29	3.3905	0.0317	35	**0.017**
	22, 36	4.3132	0.0285	35	**0.0041**
	29, 36	0.76595	0.5375	16	0.4757
13 bacteria	15, 22	6.1776	0.0306	35	**0.0007**
	15, 29	2.7983	0.0279	35	**0.0125**
	15, 36	6.8922	0.0285	35	**0.0003**
	22, 29	5.3416	0.0312	35	**0.0013**
	22, 36	1.4269	0.2571	35	0.2036
	29, 36	6.2764	0.0281	35	**0.0012**
23 control	15, 22	11.307	0.0307	35	**0.0001**
	15, 29	21.471	0.0281	35	**0.0001**
	15, 36	29.946	0.0312	35	**0.0001**
	22, 29	9.3114	0.0271	35	**0.0001**
	22, 36	10.575	0.0304	35	**0.0001**
	29, 36	1.9365	0.0275	34	**0.0387**
23 bacteria	15, 22	20.052	0.0265	35	**0.0001**

	15, 29	13.267	0.0312	35	**0.0001**
	15, 36	21.136	0.0283	35	**0.0001**
	22, 29	9.7976	0.0298	35	**0.0001**
	22, 36	17.739	0.0293	35	**0.0001**
	29, 36	2.0215	0.0609	35	0.079
25 control	15, 22	13.244	0.0279	35	**0.0001**
	15, 29	14.492	0.0324	35	**0.0001**
	15, 36	28.041	0.0252	29	**0.0001**
	22, 29	7.6282	0.03	35	**0.0001**
	22, 36	14.031	0.0273	35	**0.0001**
	29, 36	1.7549	0.1448	35	0.0926
25 bacteria	15, 22	10.685	0.0288	35	**0.0002**
	15, 29	13.285	0.0295	35	**0.0001**
	15, 36	27.846	0.0305	26	**0.0001**
	22, 29	6.2928	0.0243	35	**0.0002**
	22, 36	13.728	0.027	35	**0.0001**
	29, 36	2.9659	0.029	32	**0.0053**
27 control	15, 22	3.6515	0.0555	35	**0.0118**
	15, 29	1.5977	0.2549	35	0.1586
	15, 36	0.54659	0.6333	35	0.5998
	22, 29	3.8629	0.026	35	**0.0076**
	22, 36	0.41864	0.7084	35	0.6916
	29, 36	1.1785	0.3167	35	0.2786
27 bacteria	15, 22	3.2619	0.0309	25	**0.0163**
	15, 29	1.3817	0.2832	25	0.2212
	15, 36	0.61566	0.6055	25	0.5738
	22, 29	2.8556	0.0564	35	**0.0337**
	22, 36	1.3244	0.3185	35	0.2362
	29, 36	0.6076	0.5915	35	0.5927

Table 3.17 PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) organized by normal and asexual sporophytes of *L. ochroleuca*.

Groups	t	P(perm)	Unique perms	P(MC)
13, 23	4.6355	0.0006	9933	**0.0002**
13, 25	3.3743	0.0013	9935	**0.0024**
13, 27	16.519	0.0004	9742	**0.0001**
23, 25	0.86362	0.4319	9937	0.4385
23, 27	10.814	0.0002	9884	**0.0001**
25, 27	9.2688	0.0005	9881	**0.0001**

Table 3.18. PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) organized by normal sporophytes of *L. ochroleuca.*

Groups	t	P(perm)	Unique perms	P(MC)
13, 23	4.6274	0.0015	9821	**0.0013**
13, 25	3.0557	0.0104	9847	**0.0102**

13, 27	16.821	0.0003	9585	**0.0001**
23, 25	0.90591	0.375	9823	0.3802
23, 27	12.025	0.0004	9735	**0.0001**
25, 27	9.6257	0.0001	9802	**0.0001**

Table 3.19 PERMANOVA multiple pairwise comparisons between the different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) organized by different bacteria treatment groups (control and bacteria) on asexual sporophytes of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
Control	13, 23	1.8358	0.1109	32	0.1163
	13, 25	2.1106	0.0554	33	0.0828
	13, 27	4.4218	0.0286	14	**0.0057**
	23, 25	0.55386	0.6004	29	0.606
	23, 27	3.0878	0.0291	15	**0.0215**
	25, 27	3.0653	0.0279	15	**0.0221**
Bacteria	13, 23	4.3005	0.0265	25	**0.0056**
	13, 25	5.7035	0.0312	25	**0.0019**
	13, 27	1.6531	0.1404	10	0.148
	23, 25	1.2037	0.3194	31	0.2797
	23, 27	4.4794	0.0291	23	**0.0046**
	25, 27	6.0819	0.0272	25	**0.0006**

Table 3.20. PERMANOVA multiple pairwise comparisons between the different bacteria treatment groups (control and bacteria) organized by different temperature (13ºC, 22 ºC, 23 ºC and 25ºC) on asexual sporophytes of *L. ochroleuca*.

Factor	Groups	t	P(perm)	Unique perms	P(MC)
13	Control, Bacteria	2.4082	0.0619	16	0.0524
23	Control, Bacteria	2.6649	0.0557	35	**0.0382**
25	Control, Bacteria	1.4107	0.2581	22	0.2123
27	Control, Bacteria	0.7746	0.7175	4	0.4597

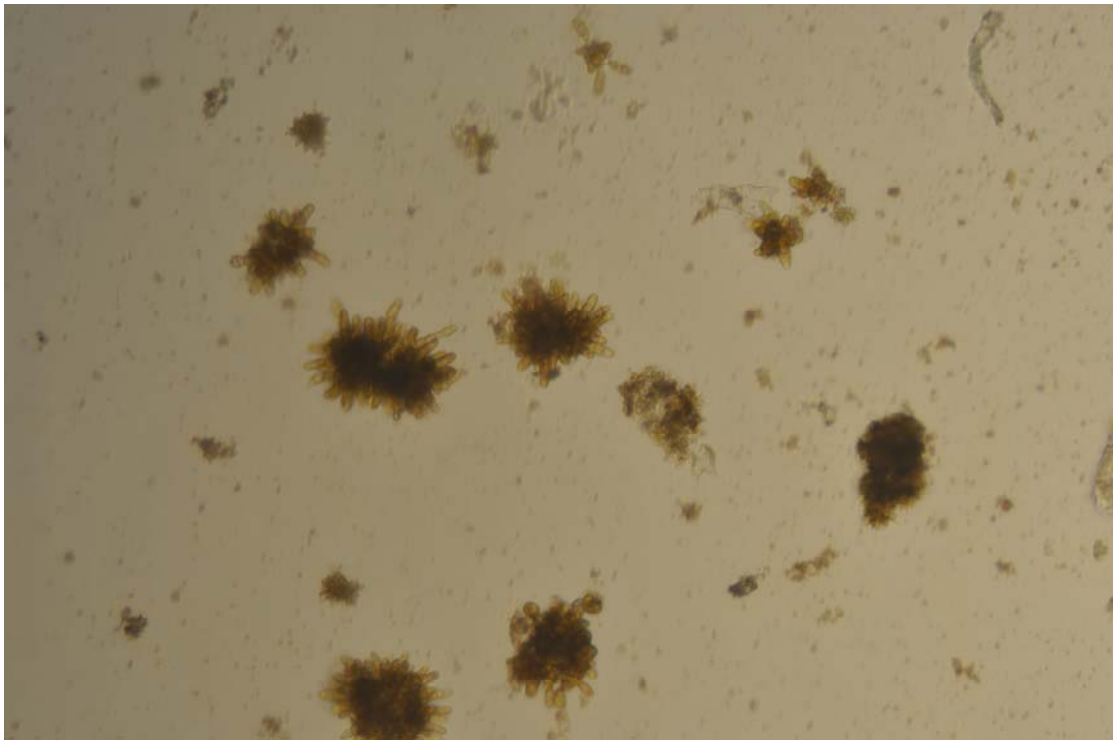

Figure 4.1. Photography of gametophytes at 23°C heatwave treatment without bacteria treatment on day 16, captured with Nikon D90, f/0, 1/20 second, ISO 200, exposure bias 0, 100 × magnification.

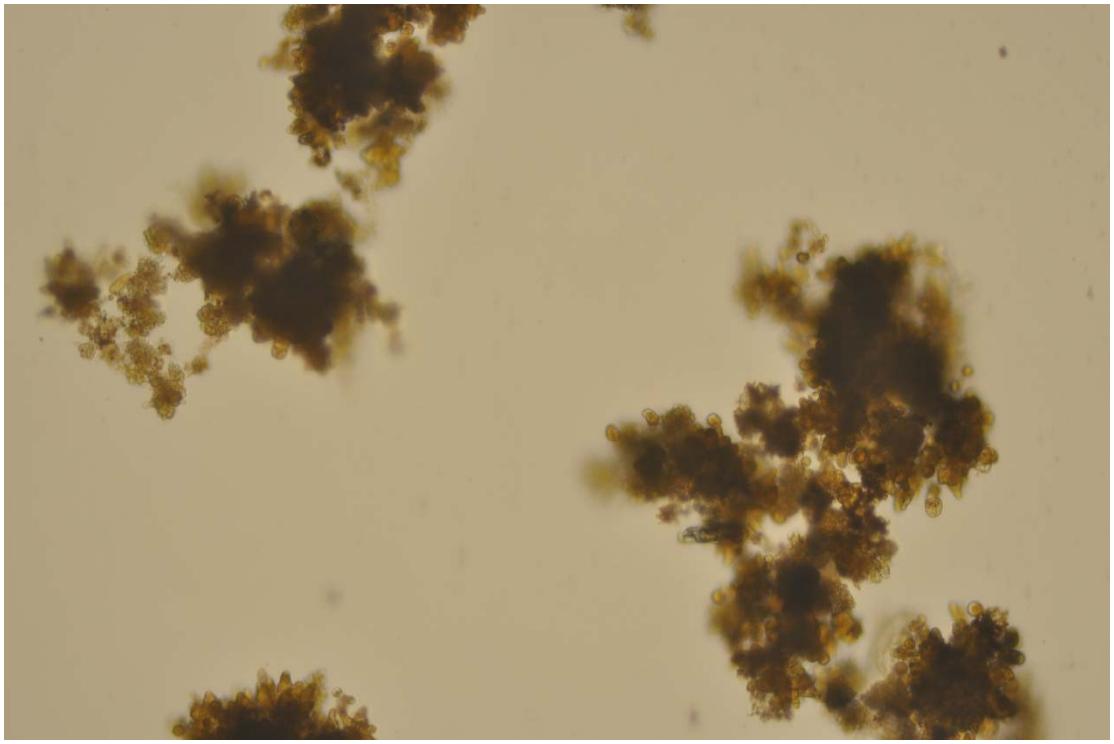

Figure 4.2, Photography of gametophytes at 23°C heatwave treatment with bacteria treatment on day 16, captured with Nikon D90, f/0, 1/20 second, ISO 200, exposure bias 0, 100 × magnification.

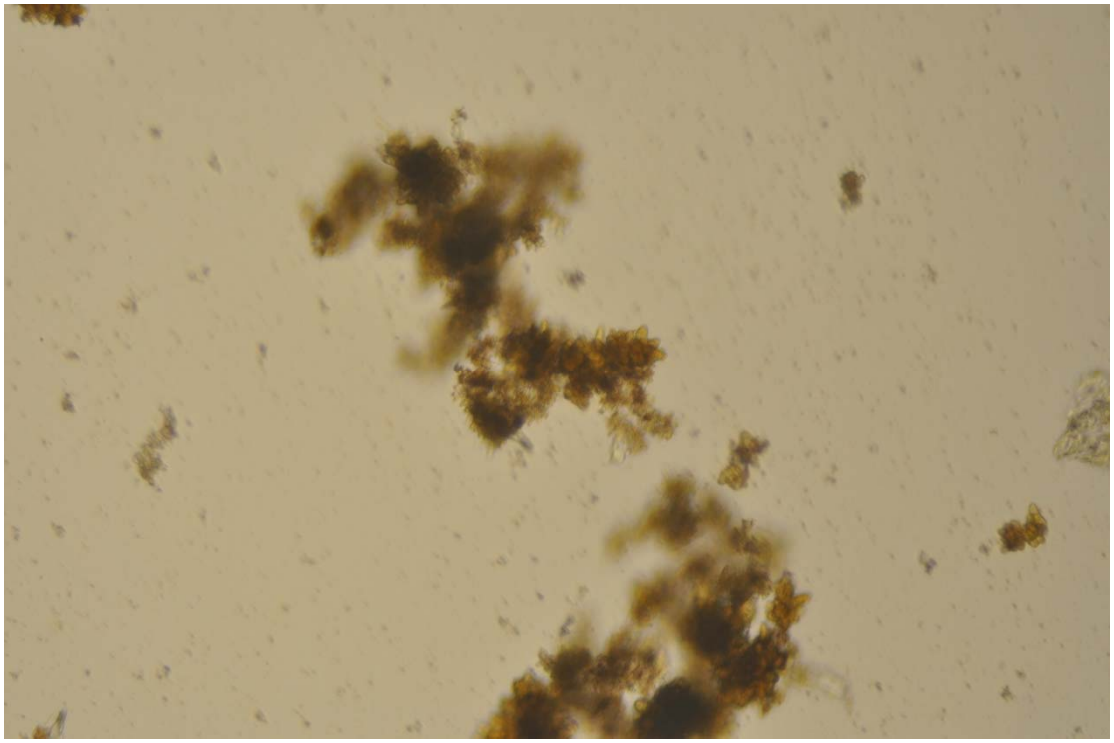

Figure 4.3. Photography of gametophytes at 25°C heatwave treatment without bacteria treatment on day 16, captured with Nikon D90, f/0, 1/20 second, ISO 200, exposure bias 0, 100 × magnification.

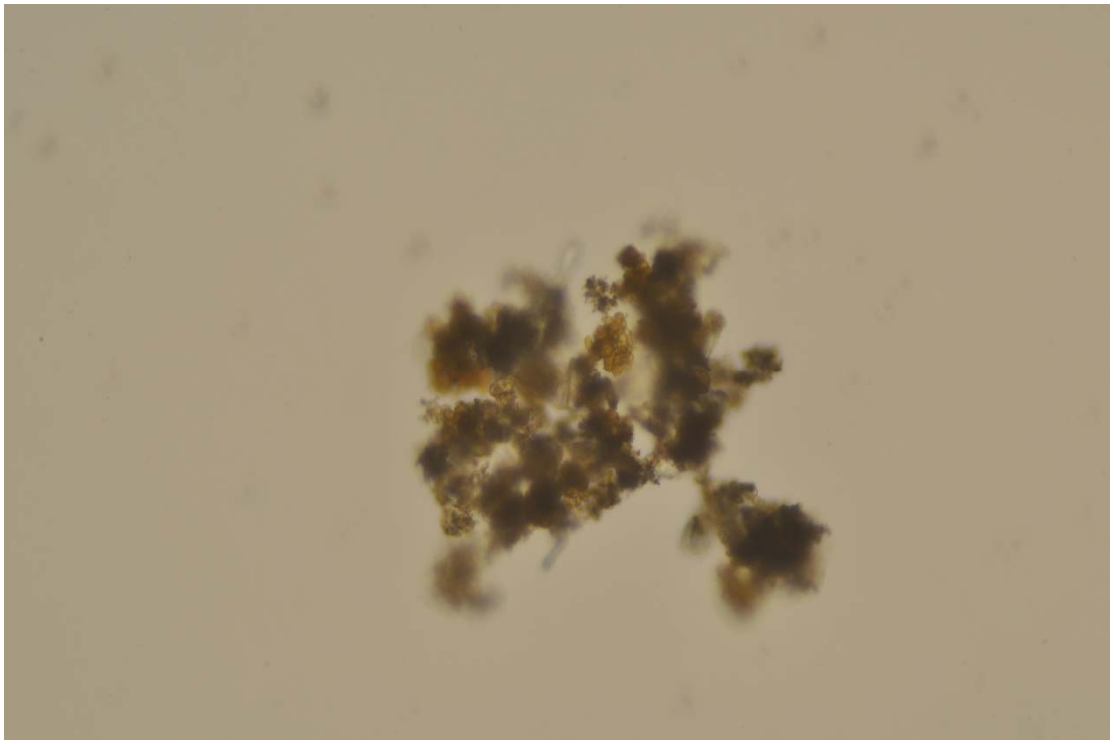

Figure 4.4. Photography of gametophytes at 25°C heatwave treatment with bacteria treatment on day 16, captured with Nikon D90, f/0, 1/20 second, ISO 200, exposure bias 0, 100 × magnification.

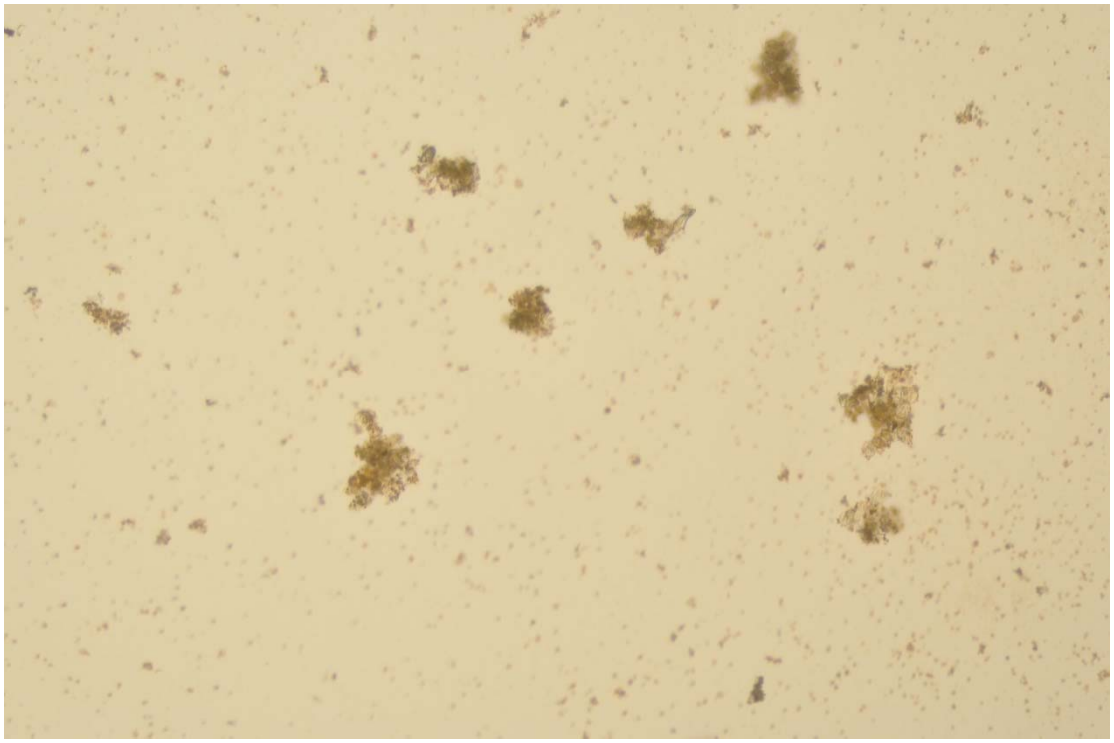

Figure 4.5. Photography of gametophytes at 27°C heatwave treatment without bacteria treatment on day 16, captured with Nikon D90, f/0, 1/20 second, ISO 200, exposure bias 0, 100 × magnification.

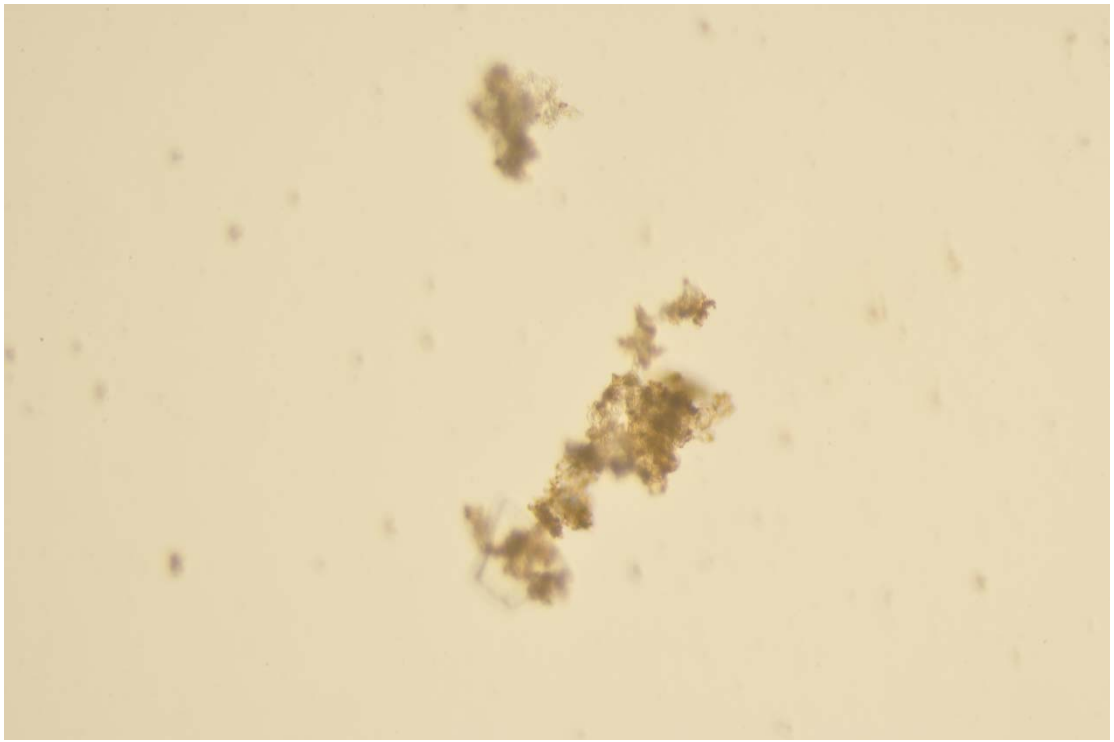

Figure 4.6. Photography of gametophytes at 27°C heatwave treatment with bacteria treatment on day 16, captured with Nikon D90, f/0, 1/20 second, ISO 200, exposure bias 0.