



FACULTAD DE CIENCIAS NATURALES Y EXACTAS
DOCTORADO INTERDISCIPLINARIO EN CIENCIAS AMBIENTALES

***Lessonia spicata (Ochrophyta) como organismo modelo para el estudio de
respuestas integradas al cambio climático en holobiontes***

Tesis para optar al grado de Doctor en Ciencias Ambientales

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ÍNDICE DE CONTENIDOS

Tabla de contenido

ÍNDICE DE CONTENIDOS	vi
Resumen.....	x
ÍNDICE DE FIGURAS Y TABLAS	xiii
ABREVIATURAS.....	xvii
Capítulo I. 1. Introducción general	1
1.1. Cambio Climático y estresores ambientales.....	2
1.2. Ecosistemas costeros: Bosques de algas pardas como especies clave.....	7
1.3. Respuestas de las macroalgas al estrés ambiental.....	9
1.4. Microbioma de macroalgas pardas y la visión del holobionte	12
1.5. <i>L. spicata</i> como especie modelo en el estudio del CC	14
1.6. Pregunta de investigación.....	16
Capítulo II. Hipótesis y objetivos	17
2.2. Hipótesis.....	17
2.2. Objetivo general.....	17
2.2.1. Objetivos específicos	18
Capítulo III	19
3.1. Abstract	20
3.1. Introduction.....	21
3.2. Materials and methods.....	23
3.2.1. Sampling site	23
3.2.2. Experimental design.....	24
3.2.3. Photosynthetic performance.....	25
3.2.4. Photosynthetic pigments	26
3.2.5. Phenolic compounds (PC)	26

3.2.6. Total antioxidant capacity (DPPH)	27
3.2.7. Oxidative damage	27
3.2.8. Lipid peroxidation (TBARS/MDA)	27
3.2.9. RNA extraction and cDNA synthesis.....	28
3.2.10. qPCR conditions	28
3.2.11. Reference gene (normalizer)	28
3.2.12. Relative expression analysis	29
3.2.13. Statistical analysis.....	29
3.3. Results.....	31
3.3.1 Photosynthetic responses	31
3.3.2. Biochemical responses	32
3.3.3. Molecular responses	34
3.4. Discussion	36
3.5. Conclusions	40
Capitulo IV	41
4.1. Graphical Abstract	42
4.1. Abstract	42
4.2. Introduction	44
4.3. Materials and Methods	48
4.3.1. Algae collection and Experimental design.....	48
4.3.2 Photosynthetic performance.....	49
4.3.3 Biochemical variables	50
4.3.4 Statistical analysis.....	51
4.4. Results.....	52
4.4.1. Multivariate Analysis (PCO)	52
4.4.2. Photosynthetic responses	54
4.4.3. Biochemical response.....	55

4.5. Discussion	58
4.6. Conclusions	62
Capítulo V.....	64
5.1. Abstract	65
5.2. Introduction	66
5.3. Materials and Methods	69
5.3.1. Sample collection and experimental design	69
5.3.2. RNA Extraction, DNase Treatment, cDNA Synthesis and 16S rRNA Gene Amplification and Sequencing.....	69
5.3.3. Sequence Processing and Taxonomic Assignment (QIIME2)	70
5.3.4. Taxonomic Composition, Relative Abundance and Functional Inference.....	70
4.3.5. Statistical analysis.....	70
5.4. Results.....	72
5.4.1. Alpha and Beta Diversity Patterns Under Thermal Stress	72
5.4.2. Relative abundance of bacterial classes.....	74
5.4.3. Family-level heatmap of bacterial taxa.....	75
5.4.4. Differentially Abundant Taxa Between Control and Treatment	76
5.4.5. Predicted microbial functional profiles	77
5.5. Discussion	78
5.6. Conclusions	82
Capítulo VI.....	83
6. Discusión final.....	83
6.1 Radiación estacional (PAR+UVR): ajustes ecofisiológicos en L. spicata.....	83
6.2 Respuestas del holobionte de Lessonia spicata bajo escenarios de calentamiento oceánico	85
6.3 Interacción entre radiación y temperatura.....	88
Capítulo VII.....	92

7. Conclusiones generales.....	92
7.1. Radiación solar estacional.....	92
7.2. Temperatura (+3 °C).....	92
7.3. Interacción radiación–temperatura.....	93
7.4. Aportes al conocimiento científico y perspectivas	93
Capítulo VIII.....	94
8. References	94
9. SUPPLEMENTARY MATERIAL	115

Resumen

El cambio climático global, impulsado por el aumento sostenido de gases de efecto invernadero (GEI), está generando alteraciones profundas en los ecosistemas marinos costeros, especialmente en regiones templadas del hemisferio sur. Entre los estresores ambientales más relevantes se encuentran el incremento de la radiación solar, incluyendo tanto la radiación fotosintéticamente activa (PAR) como la ultravioleta (UV-A y UV-B), y el aumento progresivo de la temperatura oceánica, ambos con consecuencias directas sobre organismos sésiles como las macroalgas pardas. Estas especies, además de su relevancia ecológica como ingenieras de hábitat, representan excelentes modelos biológicos para estudiar respuestas al cambio climático. En este contexto, la presente tesis doctoral evaluó las respuestas ecofisiológicas, moleculares, bioquímicas y microbianas asociadas al alga *Lessonia spicata*, bajo escenarios controlados que simularon las condiciones proyectadas para las próximas décadas. El primer objetivo abordó el efecto estacional de la radiación solar, simulando en condiciones de laboratorio los niveles de PAR, UVA y UVB característicos de las cuatro estaciones del año. Se observó una clara capacidad de fotoaclimatación, destacándose la condición de primavera por presentar mayores tasas fotosintéticas (ETR_{max}), aumentos en la concentración de pigmentos clave (clorofila a, fucoxantina) y una menor acumulación de especies reactivas de oxígeno (ROS). A nivel molecular, esta condición también indujo una regulación positiva en la expresión de genes asociados a la fotosíntesis y el metabolismo energético, como *psaA*, *chlB*, *petF* y *rbcL*, indicando una respuesta integrada que le permite mantener su funcionalidad fotosintética ante distintos niveles de radiación. En base a estos resultados, el segundo objetivo evaluó el efecto de un aumento sostenido de temperatura (+3 °C) sobre individuos expuestos previamente a las condiciones lumínicas de primavera. Se compararon dos tratamientos térmicos (15 °C y 18 °C), representando condiciones actuales y proyectadas para fines de siglo según el escenario SSP2-4.5 del IPCC. Los resultados mostraron un deterioro significativo en el desempeño fisiológico de *L. spicata* a 18 °C, con disminución del rendimiento

fotosintético (F_v/F_m), reducción de pigmentos fotosintéticos, aumento del daño oxidativo y pérdida de capacidad antioxidante, lo que evidencia una baja tolerancia térmica sostenida de la especie. Finalmente, el tercer objetivo incorporó el análisis del microbioma epífito de *L. spicata*, utilizando el mismo diseño experimental de incremento de temperatura (*in situ*, 15 °C y 18 °C). La estructura microbiana cambió significativamente con la temperatura. Se observó una pérdida de diversidad y un recambio en la composición comunitaria, destacando la disminución de la actividad de bacterias epífitas con funciones beneficiosas y el aumento relativo de géneros oportunistas, como *Vibrio* sp., particularmente a 18 °C. Estos cambios sugieren una posible disbiosis inducida por el estrés térmico, que podría debilitar la interacción hospedador-microbiota y amplificar los efectos negativos del calentamiento sobre la especie. En conjunto, los resultados de esta tesis indican que *L. spicata* presenta una plasticidad fenotípica y fisiológica frente a los estresores derivados del cambio climático. Si bien logra aclimatarse a distintos regímenes de radiación estacional, su capacidad para tolerar un aumento térmico sostenido es limitada, tanto a nivel funcional como simbiótico. Esta plasticidad le permite ajustar ciertos procesos fotosintéticos y bioquímicos en el corto plazo, pero sus límites se hacen evidentes cuando el estrés se mantiene o intensifica, lo que sugiere que la resiliencia de la especie depende de la interacción entre la magnitud del estresor y la duración de la exposición. Dado su rol ecológico central en los ecosistemas de bosques de macroalgas, cualquier debilitamiento fisiológico o ecológico de esta especie podría desencadenar efectos negativos en cascada sobre la biodiversidad marina. Finalmente, los indicadores analizados eficiencia fotosintética, expresión génica, acumulación de ROS, concentración de pigmentos y estructura del microbioma epífito se proponen como herramientas relevantes para el monitoreo ambiental y la gestión adaptativa de ecosistemas marinos templados, posicionando a *L. spicata* como un modelo clave para el estudio de holobiontes frente al cambio climático.

Palabras clave:

Lessonia spicata; cambio climático, antioxidantes; resiliencia ecológica; holobionte.

ÍNDICE DE FIGURAS Y TABLAS

Figura I.1. Esquema con las formas en que la radiación solar llega al planeta, y sus efectos en el sistema climático, en donde, se muestran los forzantes físicos que resultan como consecuencias de la perturbación en la estructura de la atmósfera producto de la actividad humana, como el aumento de la radiación UV que ingresa al planeta y el incremento de la temperatura global del planeta.

Figura I.2. Mapa resumen de la energía solar que ingresa al planeta Tierra representada con los promedios diarios/anuales totales de irradiación normal directa (DNI), que abarca el período comprendido entre 1994/1999/2007 y 2019. Fuente: World Bank Group, 2019.

Figura I.3. Presentación de los 5 escenarios (SSP1-1.9, SSP1-2.3, SSP2-4.5, SSP3-7.0 y SSP5-8.5), propuestos hasta final de siglo por el IPCC en distintas variables ambientales, los cuales son usados como guía para este estudio, en particular el escenario intermedio SSP2-4.5. A) Forzamiento radiactivo global medio entre 1983 y 2100 estimado por un conjunto de métodos y modelos predictivos normalizados por datos tomados desde 1850, B) Proyecciones de aumento de temperatura global hasta el 2100 respecto de los 5 escenarios propuestos, y C) Proyección de las emanaciones de CO₂ hasta finales del siglo. Fuente: informe IPCC 2023.

Figura I.4. Diagrama explicativo del problema de investigación propuesto

Figure III. 1. Geographic location of the sampling site in the Valparaíso Region, central Chile. The red dot indicates the coastal collection area where algal samples were obtained. The regional map (left) shows the sampling point within Valparaíso, and the national inset map (right) highlights the position of the region along the Chilean coast.

Figure III. 2.. A) F_v/F_m - Maximal quantum yield, B) α_{ETR} - Photosynthetic efficiency, C) ETR_{max} - Maximal electron transport rate, and D) NPQ_{max} - Maximal non-photochemical quenching in *L. spicata* during the daily cycle experiments under PAB-autumn, PAB-winter, PAB-spring, and PAB-summer treatments.

Figure III. 3. Photosynthetic pigments A) Chlorophyll *a*, B) Chlorophyll *c1+c2*, and C) Photoprotective compounds as Phenolics, quantified in *L. spicata* during the

daily cycle experiments in laboratory under PAB-autumn, PAB-winter, PAB-spring, and PAB-summer treatments, values correspond to the average of the three sampling times (10:00, 14:00, and 18:00 h).

Figure III. 4. A) Hydrogen peroxide (H_2O_2) and B) Thiobarbituric acid-reactive substance (TBARS) accumulation in *L. spicata* exposed to daily cycle experiments under PAB- autumn, PAB-winter, PAB-spring, and PAB-summer treatments, values correspond to the average of the three sampling times (10:00, 14:00, and 18:00 h).

Figure III. 5. Relative expression of genes from the chloroplast of *L. spicata* subjected to different radiation treatments (PAB). A) Genes of photosystem II, B) photosystem I, C) photosynthetic pigments and (D) electron transport chain. different letters denote significant differences (SNK, $p < 0.05$).

Figure IV. 1. Explanatory diagram of the steps to follow during the experiment, from the sampling site to the collection of material for analysis.

Figure IV. 2. Principal Coordinates Ordination (PCO) of all photophysiological and biochemical variables. The ordination shows the separation between the 15 °C (green triangles) and 18 °C (blue inverted triangles) treatments. Vectors indicate the contribution and direction of each variable. PCO1 and PCO2 explain 25.9% and 17.9% of the total variance, respectively.

Figure IV. 3. Photophysiological responses: A) Maximal quantum yield (F_v/F_m), B) maximal electron transport rate (ETR_{max}). C) Photosynthetic efficiency (α ETR) and D) Maximal non-photochemical quenching (NPQ_{max}).

Figure IV. 4. Photosynthetic pigments: A) Chlorophyll *a* and B) Chlorophyll *c* (c_1+c_2) and C) Fucoxanthin pigment content.

Figure IV. 5. Phenolic compounds: A) Insoluble phenolic content, B) Soluble phenolic content, and C) Total antioxidant capacity, measured by the DPPH assay and expressed as Trolox equivalents.

Figure IV. 6. Oxidative stress markers: A) Total Reactive Oxygen Species (ROS) and B) lipid peroxidation (TBARS).

Figure V.1: Taxonomic composition and relative abundance of bacterial families associated with *L. spicata* under different thermal conditions. Barplots represent the 10 most abundant families across *in situ*, Control, and treatment samples.

Rare families were grouped under “Others.” The color gradient from green to purple indicates increasing thermal stress.

Figure V.2: Heatmap showing the relative abundance of dominant bacterial classes in the epibiotic microbiome of *L. spicata*. Samples are clustered by condition: *in situ*, Control (15 °C), and Treatment (18 °C). Only classes with relative abundance >1% in at least one sample are shown. Color intensity reflects abundance (pastel green to purple scale).

Figure V. 3: Functional annotation of epibiotic bacterial communities of *L. spicata* inferred using the FAPROTAX database. The heatmap shows the relative abundance of predicted microbial functions across *in situ*, Control, and Treatment groups. Clustering is based on Bray–Curtis dissimilarity, with stress levels indicated by a green–purple gradient.

Figure V.4: Alpha diversity metrics of the active epibiotic bacterial community associated with *L. spicata* under three thermal conditions: *in situ* (natural environment), Control (15 °C), and Treatment (18 °C). Boxplots represent Observed Richness (a), Shannon Index (b), and Pielou’s Evenness (c). Significant differences between treatments are indicated by different letters (ANOVA or Kruskal–Wallis, $p < 0.05$).

Figure V.5: Redundancy Analysis (RDA) of Bray–Curtis dissimilarities illustrating the separation of microbial communities associated with *L. spicata* under different thermal conditions. Arrows represent the influence of temperature gradients. Ellipses denote 95% confidence intervals for each group.

Figure V.6: Volcano plots displaying differentially abundant ASVs between experimental conditions: Control vs Treatment. Significant ASVs were defined by adjusted p-value (FDR) < 0.05 and $|\log_2 \text{Foldchange}| > 1$. Labeled genera highlights ASVs with the highest effect sizes

Table III. 1. The irradiance of UVB (280–315 nm), UVA (315–400 nm), PAR (400–700), and Temperature (°C) of seawater at each seasonal time.

Table III. 2. Genes analyzed by RT-qPCR in *L. spicata*

Supplementary Figure III. 1. The average dose by month between 2021 and 2023 measured in Viña del Mar Chile is shown.

Supplementary Table III. 1. Statistic result of test ANOVA for any indicator.

ABREVIATURAS

Abreviatura	Significado
α_{ETR}	Pendiente inicial de la curva ETR (eficiencia cuántica aparente)
A	Absortancia
ANOVA	Analysis of Variance (análisis de varianza)
ASV(s)	Amplicon Sequence Variants (variantes de secuencia de amplicón)
CC	Cambio Climático
CFC	Clorofluorocarbonos
Chl- <i>a</i>	Clorofila <i>a</i>
Chl- <i>c</i>	Clorofila <i>c</i>
CO ₂	Dióxido de carbono
DNA	Ácido desoxirribonucleico
DPPH	2,2-difenil-1-picrilhidracilo
DW	Dry Weight (peso seco)
E	Irradiancia
E _{KETR}	Irradiancia de saturación para ETR
ETR	Electron Transport Rate (tasa de transporte de electrones)
ETR _{max}	Máxima tasa de transporte de electrones
FAPROTAX	Functional Annotation of Prokaryotic Taxa
Fv/Fm	Rendimiento cuántico máximo del fotosistema II
GEI	Gases de efecto invernadero
HSP	Heat Shock Proteins (proteínas de choque térmico)
IPCC	Intergovernmental Panel on Climate Change (Panel Intergubernamental sobre Cambio Climático)
MDA	Malondialdehído (biomarcador de peroxidación lipídica)
NPQ	Non-Photochemical Quenching (desactivación no fotoquímica)
OTU(s)	Operational Taxonomic Units (unidades taxonómicas operacionales)
PAM	Pulse Amplitude Modulated fluorometry (fluorometría PAM)
PAR	Photosynthetically Active Radiation (radiación fotosintéticamente activa)
PC(o)A	Principal Coordinate Analysis (análisis de coordenadas principales)
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Analysis of Multivariate Dispersions
PSII	Photosystem II (fotosistema II)
qP	Coeficiente de disipación fotoquímica (photochemical quenching)
RDA	Redundancy Analysis (análisis de redundancia)
RNA	Ácido ribonucleico
rRNA	Ribosomal RNA (ARN ribosomal)
ROS	Reactive Oxygen Species (especies reactivas de oxígeno)
SNK	Student-Newman-Keuls (test post hoc)

Abreviatura	Significado
SSP2-4.5	Shared Socioeconomic Pathway 2-4.5 (escenario del IPCC)
TEAC	Trolox Equivalent Antioxidant Capacity (capacidad antioxidante total)

Capítulo I. 1. Introducción general

1.1. Cambio Climático y estresores ambientales

El clima terrestre funciona como un complejo sistema de retroalimentaciones de procesos interconectados físicos, químicos y biológicos, impulsada primariamente por la radiación solar, donde el balance energético entre la energía entrante y saliente regula las condiciones ambientales del planeta (Singh & O'Neill, 2022). Aproximadamente un 30 % de la radiación solar incidente es reflejada por nubes, aerosoles, hielo y otras superficies de alto albedo, mientras que el resto de la radiación es absorbida principalmente por los océanos y la superficie continental, que luego liberan parte de esa energía como radiación infrarroja hacia el espacio (Loeb et al., 2021; NASA Earth Observatory, 2023). Este equilibrio se ve modulado por gases atmosféricos como el dióxido de carbono (CO₂), metano (CH₄) y óxidos de nitrógeno (NO_x), que retienen parte de esa radiación infrarroja, generando el denominado efecto invernadero. Si bien este fenómeno es esencial para mantener condiciones habitables, su intensificación antropogénica ha generado un **desequilibrio energético positivo estimado en +0,8 W/m²**, lo que ha impulsado el calentamiento global observado en las últimas décadas (IPCC, 2023; Li et al., 2024).

Históricamente, las concentraciones de CO₂ atmosférico han fluctuado en escalas geológicas, pero desde la Revolución Industrial han aumentado abruptamente debido a la quema de combustibles fósiles, deforestación y procesos industriales (Mikhaylov et al., 2020). Este aumento ha sido acompañado por emisiones de otros GEI como el CH₄ y el N₂O, que provienen de la agricultura intensiva, la ganadería y el uso de fertilizantes nitrogenados (Forster et al., 2024). Como resultado, se ha registrado un calentamiento global cercano a +1,5 °C respecto a niveles preindustriales (IPCC, 2023), lo que ha inducido alteraciones significativas en la dinámica climática global.

En paralelo, la liberación masiva de compuestos clorofluorocarbonados (CFCs) a lo largo del siglo XX principalmente en refrigeración y aerosoles ha deteriorado la capa de ozono estratosférico, disminuyendo su capacidad de filtrar la radiación ultravioleta (UV) (Molina & Rowland, 1974; Barnes et al., 2019). A pesar de los

esfuerzos del Protocolo de Montreal, la recuperación de esta capa ha sido parcial y desigual, en parte por nuevas emisiones de sustancias agotadoras de ozono y retroalimentaciones asociadas al deshielo polar (Ball et al., 2020). Como consecuencia, regiones del hemisferio sur, incluyendo Chile central, experimentan **altos niveles de radiación UV-B**, especialmente durante primavera y verano austral (Magnan et al., 2023), coincidiendo además con los máximos estacionales de radiación PAR, caracterizados para el hemisferio sur en estudios de variabilidad de la radiación solar (Hernández et al., 2012; Cordero et al., 2023).

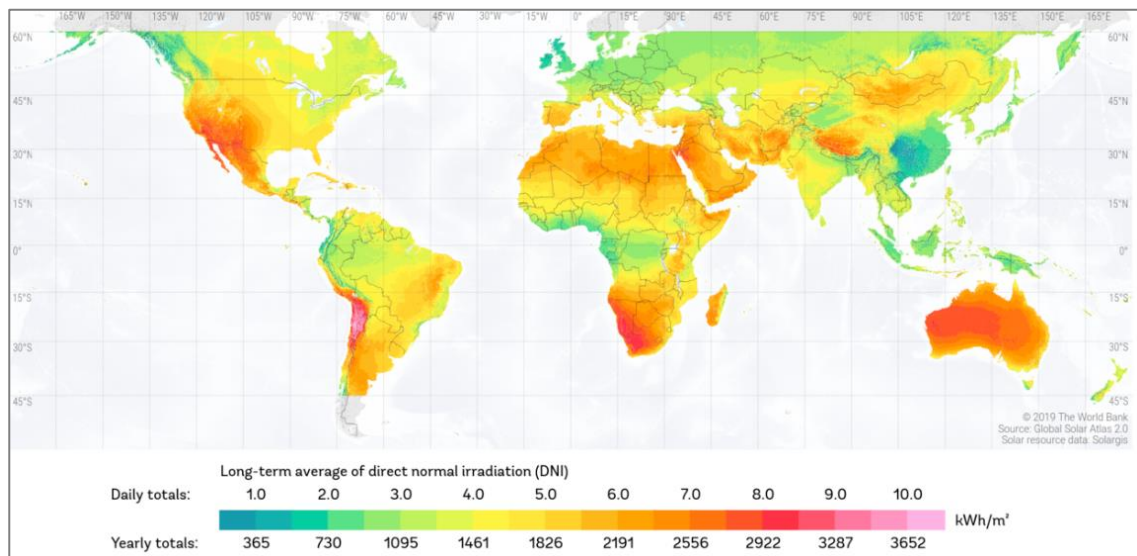


Figura I.2. Mapa resumen de la energía solar que ingresa al planeta Tierra representada con los promedios diarios/anuales totales de irradiación normal directa (DNI), que abarca el período comprendido entre 1994/1999/2007 y 2019. Fuente: World Bank Group, 2019.

Estas transformaciones en la atmósfera y en el balance térmico global han intensificado la exposición a **estresores ambientales** en ecosistemas marinos, particularmente en zonas costeras someras. En estas áreas, los organismos bentónicos incluidas las macroalgas enfrentan condiciones extremas caracterizadas por aumentos en la temperatura superficial del mar (TSM), alta radiación solar (PAR y UVR), y una elevada variabilidad estacional y diaria (Stillman et al., 2025). Estas condiciones pueden inducir efectos sinérgicos sobre las comunidades biológicas, comprometiendo su fisiología, su distribución geográfica y su capacidad adaptativa (Wernberg et al., 2016).

El Panel Intergubernamental sobre el Cambio Climático (IPCC) ha desarrollado una serie de trayectorias proyectivas denominadas **Shared Socioeconomic Pathways (SSP)**, las cuales exploran posibles escenarios futuros climáticos en función de los niveles de emisiones de gases de efecto invernadero y las decisiones políticas y tecnológicas adoptadas por la sociedad (IPCC, 2023). Entre estas trayectorias se incluyen:

- **SSP1-1.9**, un escenario muy optimista con transformaciones sostenibles profundas y una meta de calentamiento inferior a 1,5 °C;
- **SSP1-2.6**, de desarrollo verde con altas medidas de mitigación;
- **SSP2-4.5**, considerado intermedio, con compromisos moderados y políticas climáticas parciales;
- **SSP3-7.0**, basado en rivalidades regionales y escasa cooperación internacional; y
- **SSP5-8.5**, un escenario extremo de altas emisiones y continuidad del modelo fósil.

En particular, el **SSP2-4.5** ha sido ampliamente utilizado como referencia realista para evaluar impactos ecológicos bajo un futuro de mitigación parcial. Este escenario proyecta un incremento promedio global de **+2,7 °C hacia fines del siglo XXI**, acompañado de aumentos en la frecuencia, duración e intensidad de **olas de calor terrestres y marinas**, con consecuencias severas para la biodiversidad y los servicios ecosistémicos (Arafteh-Dalmau et al., 2020; Stillman et al., 2025). Entre los ecosistemas más vulnerables se encuentran los sistemas costeros templados, donde la exposición simultánea a múltiples estresores ambientales pone en riesgo la estabilidad de comunidades biológicas clave.

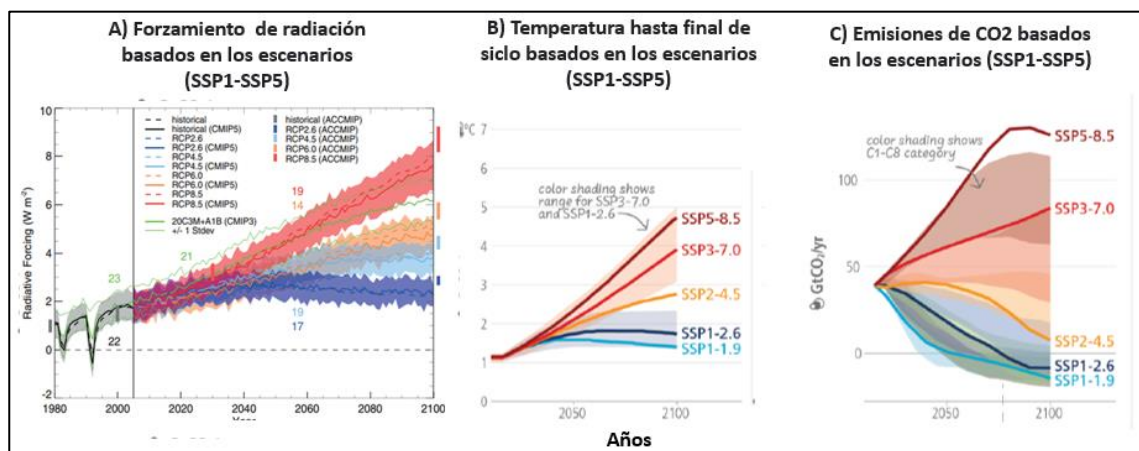


Figura 1.3. Presentación de los 5 escenarios (SSP1-1.9, SSP1-2.3, SSP2-4.5, SSP3-7.0 y SSP5-8.5), propuestos hasta final de siglo por el IPCC en distintas variables ambientales, los cuales son usados como guía para este estudio, en particular el escenario intermedio SSP2-4.5. A) Forzamiento radiactivo global medio entre 1983 y 2100 estimado por un conjunto de métodos y modelos predictivos normalizados por datos tomados desde 1850, B) Proyecciones de aumento de temperatura global hasta el 2100 respecto de los 5 escenarios propuestos, y C) Proyección de las emanaciones de CO₂ hasta finales del siglo. Fuente: informe IPCC 2023.

En el caso de las macroalgas, se ha observado que el exceso de radiación solar y el aumento térmico pueden afectar procesos fundamentales como la fotosíntesis, el crecimiento y la regeneración, reduciendo su desempeño fisiológico y limitando su distribución geográfica (Wernberg et al., 2016; Zúñiga et al., 2021; Celis-Plá et al., 2022). La exposición prolongada a radiación UV puede dañar estructuras celulares y comprometer el reclutamiento y la viabilidad de las poblaciones, mientras que temperaturas elevadas pueden llevar a pérdidas de cobertura, desplazamientos hacia latitudes más frías o incluso mortalidad masiva en eventos extremos (Figueroa et al., 2019; Smale et al., 2020). Si bien estos efectos varían según la especie y la intensidad del estrés, existe consenso en que el calentamiento y la radiación representan amenazas serias para la persistencia de macroalgas estructurantes en zonas templadas y frías.

Estos impactos no se limitan a los individuos, sino que afectan profundamente la estructura, dinámica y funcionalidad de los **ecosistemas costeros**, especialmente en regiones intermareales donde la exposición a radiación y cambios térmicos es particularmente intensa. Las macroalgas pardas, por los múltiples **servicios ecosistémicos** que proveen, son especialmente vulnerables y relevantes como indicadores del cambio ambiental. Comprender cómo

responden a estos estresores es fundamental para proyectar el destino de las comunidades costeras frente al cambio climático.

En este contexto, se vuelve prioritario investigar los efectos de estos factores sobre especies clave que estructuran hábitats costeros templados del hemisferio sur, como aquellas presentes en el litoral central de Chile. Esta región, se ubica en un sistema de borde oriental del Pacífico Sudoriental, caracterizada por la surgencia costera de aguas frías ricas en nutrientes (Pinochet et al., 2019; Muñoz et al., 2023), y una elevada radiación estacional, representa un laboratorio natural ideal para evaluar la sensibilidad biológica frente a la intensificación de estresores climáticos. El presente trabajo de tesis se centró en estudiar los efectos del cambio climático sobre una de las especies dominantes del intermareal rocoso templado del Pacífico suroriental.

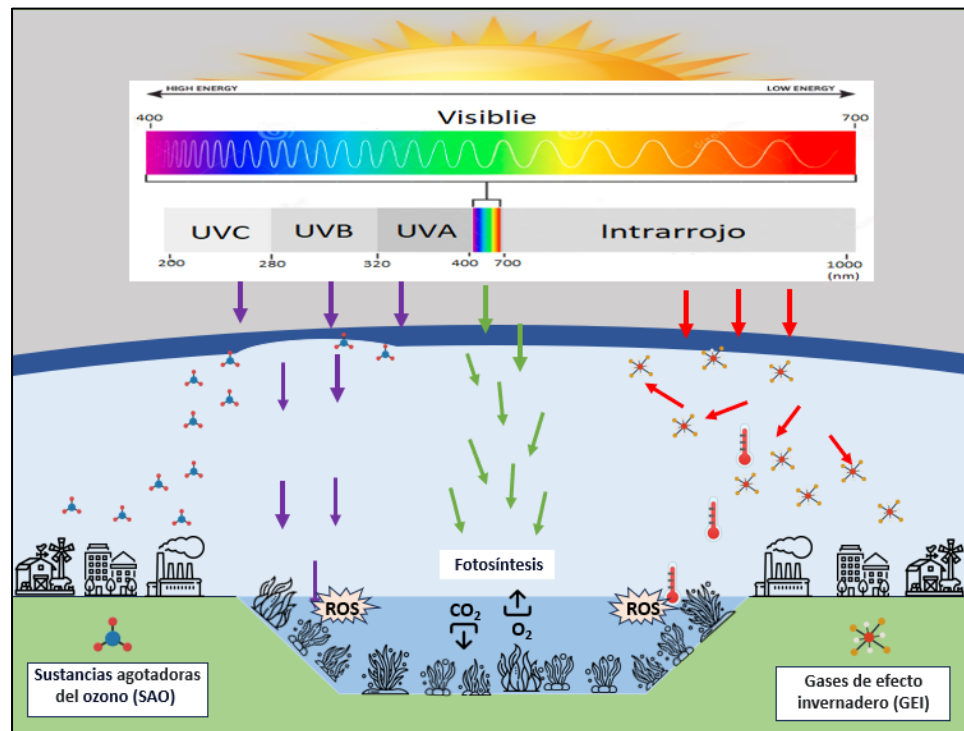


Figura I.1. Esquema con las formas en que la radiación solar llega al planeta, y sus efectos en el sistema climático, en donde, se muestran los forzantes físicos

1.2. Ecosistemas costeros: Bosques de algas pardas como especies clave

Las zonas intermareales son un ecotono donde se superponen sistemas marino y continental, caracterizado por condiciones ambientales variables y desafiantes para los organismos que lo habitan. La oscilación diaria del nivel del mar, junto con la radiación solar, las variaciones térmicas, la salinidad y la exposición al aire durante la emersión, impone una serie de presiones físicas y fisiológicas sobre los organismos que allí habitan (Wolfe et al., 2020; Schoeman et al., 2023). En este contexto, las **macroalgas pardas**, especialmente aquellas del grupo conocido como **Kelps**, dominan los ambientes rocosos de zonas templadas y frías, donde forman **bosques submarinos densos y estructuralmente complejos** (Filbee-Dexter & Wernberg, 2018; van Son et al., 2020).

Estas especies prosperan en aguas frías, ricas en nutrientes, y con irradiancia moderada, típicamente entre 6 y 20 °C, condiciones favorecidas por procesos oceanográficos como la surgencia costera característica del sistema de Humboldt en el Pacífico suroriental (Neale et al., 2023; Aguilera et al., 2025). Su distribución geográfica abarca principalmente las costas orientales de los océanos Atlántico y Pacífico, así como algunas regiones del Océano Índico, donde establecen praderas intermareales o bosques submareales con alta capacidad de cobertura (van Hees et al., 2017; Davis et al., 2021). Al crecer en agregación, estas macroalgas generan un dosel que regula las condiciones lumínicas, térmicas y químicas del microhábitat, proporcionando refugio, alimento y estabilidad estructural para diversas comunidades bentónicas (Teagle et al., 2018; Carneiro et al., 2023).

El rol ecológico de estos organismos se manifiesta en al menos tres dimensiones fundamentales que aportan al funcionamiento del ecosistema:

Primero, como **bioestructuradores del hábitat**, los bosques de macroalgas pardas proporcionan superficies de asentamiento, zonas de desove y refugio frente a depredadores o condiciones físicas adversas, facilitando el establecimiento de una alta diversidad de especies en diferentes etapas de su ciclo de vida (Eger et al., 2023; Gibbons and Quijón, 2023). Esta función de

"ingenieros ecosistémicos" es clave para mantener la complejidad y estabilidad de las redes tróficas costeras (Teagle et al., 2017).

Segundo, actúan como **principales productores primarios** en ambientes litorales templados. A través de la fotosíntesis, capturan energía solar y la convierten en biomasa que alimenta directamente a herbívoros y, de forma indirecta, a niveles tróficos superiores, incluyendo peces comerciales y mamíferos marinos (Ricciardelli et al., 2020; Carneiro et al., 2023). Además, su biomasa en descomposición y los exudados disueltos contribuyen al reciclaje de nutrientes y sostienen procesos de remineralización en la zona fótica.

Tercero, tienen un papel clave como **agentes mitigadores del cambio climático**. Su elevada tasa de fijación de carbono y capacidad para almacenar biomasa en tejidos o transportarla hacia sedimentos profundos las posiciona como potenciales sumideros de carbono azul (Krause-Jensen & Duarte 2016; Pessarrodona et al., 2019). También pueden modular el pH del agua local y contribuir a la resiliencia de ecosistemas frente a la acidificación oceánica.

A pesar de su importancia ecológica, los bosques de macroalgas están siendo afectados por **estresores ambientales cada vez más intensos**, particularmente el aumento de la temperatura y la radiación solar (Cruces et al., 2013; Britton et al., 2024). Estas presiones pueden alterar la productividad fotosintética, el crecimiento, la reproducción y la regeneración de tejidos, incrementando la vulnerabilidad a enfermedades o eventos extremos. De hecho, estudios han reportado **eventos de mortalidad masiva**, pérdidas de cobertura y desplazamientos de especies hacia latitudes más altas como respuesta al cambio climático (Wernberg et al., 2016; Smale et al., 2020; Davis et al., 2021). Por ejemplo, en Australia occidental, el 43 % de la cobertura de algas pardas se perdió entre 2011 y 2015, debido a una ola de calor marina excepcionalmente intensa (Arafeh-Dalmau et al., 2020).

Las zonas intermareales, debido a su alta exposición y fluctuaciones diarias, son especialmente sensibles. La variabilidad térmica, salina y lumínica en estas áreas impone límites fisiológicos a muchas especies, y pequeñas variaciones en estos parámetros pueden generar impactos ecológicos amplificadas (Fukumoto et al., 2019; Stillman et al., 2025). **A ello se suma la presión antrópica derivada de**

la intensa extracción de macroalgas pardas en Chile. En la última década, los desembarques han bordeado las 400 mil toneladas anuales, con un máximo histórico en 2013 y predominio de huiro negro (*Lessonia berteroana/spicata*), especialmente en la macrozona norte (Oceana Chile, 2021). Más recientemente, evaluaciones nacionales de las Áreas de Manejo (AMERB) muestran que, si bien el régimen presenta un desempeño general aceptable, el componente biológico ha disminuido sostenidamente desde 2005, con riesgo de caer bajo niveles adecuados si no se intensifica el control de extracción y la fiscalización de actividades ilegales (IFOP, 2024). A nivel de praderas, estudios en la Región de Tarapacá han evidenciado biomasa cosechable inferior al 10 % bajo criterios tradicionales de talla mínima, situando a varias praderas “al borde de la explotación plena” y con riesgo de sobreexplotación (IFOP, 2017). En conjunto, estos antecedentes reflejan cómo la presión humana se suma a los estresores ambientales del intermareal, intensificando la vulnerabilidad de los bosques de macroalgas y la biodiversidad asociada.

En este contexto, la conservación y estudio de los bosques de macroalgas pardas resulta esencial para comprender las dinámicas costeras y las respuestas de estas especies frente a variables ambientales. En Chile, donde estos ecosistemas dominan gran parte del litoral, su protección es clave no solo por su relevancia ecológica, sino también por su importancia en la pesca artesanal y en la provisión de recursos marinos. En un escenario marcado por el cambio climático y la presión antrópica por extracción, contar con información científica sólida resulta fundamental para proyectar su persistencia en el futuro.

1.3. Respuestas de las macroalgas al estrés ambiental

Las macroalgas pardas responden a los estresores asociados al cambio climático, como el aumento de radiación solar (PAR y UVR) y la elevación de la temperatura superficial del mar, mediante una serie de **mecanismos de aclimatación** que se manifiestan a distintos niveles de organización biológica. Estos niveles incluyen respuestas **fisiológicas, bioquímicas y moleculares**, que operan de forma jerárquica e interconectada para mantener la funcionalidad

del organismo frente a condiciones adversas (Narain et al., 2023). Comprender estos mecanismos integrados resulta clave para anticipar la resiliencia de especies clave bajo escenarios de cambio global.

A nivel **fisiológico**, una de las primeras respuestas observables frente al estrés ambiental es la alteración de la capacidad fotosintética. Esta puede evaluarse mediante técnicas de fluorescencia de clorofila *a*, que permiten estimar el rendimiento cuántico máximo del fotosistema II (F_v/F_m), la tasa de transporte electrónico y la eficiencia de uso de la luz (Celis-Plá et al., 2018, 2022; Figueroa et al., 2019). Bajo condiciones de exceso de radiación o temperatura elevada, estos parámetros suelen disminuir, indicando fotoinhibición, mientras que la activación de mecanismos no fotoquímicos de disipación de energía (NPQ) actúa como una estrategia de protección frente al daño lumínico. Además, variaciones en la tasa de crecimiento o en la eficiencia fotosintética pueden revelar límites de tolerancia fisiológica, los cuales difieren entre especies y están influenciados por la historia ambiental reciente del organismo (Burdett et al., 2019; Paix et al., 2021).

En cuanto a las respuestas **bioquímicas**, las macroalgas activan rutas metabólicas orientadas a la fotoprotección y al control del estrés oxidativo. Se ha documentado una modificación en la concentración de pigmentos fotosintéticos (como clorofila *a*, *c* y fucoxantina), los cuales pueden reducirse por daño estructural del aparato fotosintético o aumentar como parte de un proceso de fotoaclimatación (Álvarez-Gómez et al., 2017; Mabin, 2019). Además, especies como *Macrocystis pyrifera* y *Ecklonia radiata* incrementan su producción de compuestos fenólicos como polifenoles y flavonoides frente a altos niveles de radiación UV o temperaturas elevadas, actuando como antioxidantes y fotoprotectores (Zúñiga et al., 2021; Celis-Plá et al., 2022). Paralelamente, se genera un aumento en la producción de especies reactivas de oxígeno (ROS), y si su acumulación sobrepasa la capacidad antioxidante celular, puede inducir daño estructural, como la peroxidación lipídica detectable mediante el biomarcador malondialdehído (MDA) (Rodríguez-Rojas et al., 2020; Muñoz et al., 2023). Estos indicadores bioquímicos permiten inferir el grado de vulnerabilidad de las macroalgas a condiciones de estrés agudo o crónico.

A nivel **molecular**, se activan mecanismos de regulación génica que permiten a las macroalgas responder a nivel transcripcional. Una respuesta ampliamente reportada es la sobreexpresión de genes que codifican para **proteínas de choque térmico (HSPs)**, como *dnaK*, que actúan estabilizando proteínas desnaturalizadas y favoreciendo su replegamiento bajo estrés térmico o lumínico (Collén et al., 2007; Gómez & Huovinen, 2011). También se observan cambios en genes relacionados con la **maquinaria fotosintética**, como *psbA*, que codifica la proteína D1 del fotosistema II, altamente sensible al fotodaño, así como *psbD* (D2), *CP47* y *CP43*, involucradas en la transferencia de electrones y captura de luz (Barber et al., 2000; Kato et al., 2024). En escenarios de alta irradiancia o temperatura, estos genes pueden presentar una regulación positiva como parte del proceso de reparación del complejo PSII. Otros genes clave incluyen *chlI* y *chlB* (síntesis de clorofila), *petF* (ferredoxina) y *rbcL* (RuBisCO), cuya expresión se ve modulada por condiciones ambientales adversas (Smolina et al., 2016; Rodríguez-Rojas et al., 2020). Este conjunto de respuestas moleculares refleja la activación de rutas metabólicas complejas orientadas a la protección del aparato fotosintético, la homeostasis redox y la eficiencia energética celular.

Junto con estas dimensiones, se ha reconocido la importancia del **microbioma del alga**, el cual incluye principalmente bacterias y otros microorganismos epibiontes o epífitos que habitan sobre la superficie del talo o endófitos que son parte interna del alga, en conjunto el alga y su microbioma se denomina holobionte (Vila Dupla, 2025). Este microbioma, formado por comunidades bacterianas estrechamente asociadas al alga, puede influir en su nutrición, defensa química y tolerancia al estrés (Egan et al., 2013; Aires et al., 2015). Cambios ambientales como el aumento de temperatura pueden inducir disbiosis, afectando negativamente la estabilidad del holobionte algal y aumentando la susceptibilidad a patógenos o condiciones desfavorables (Marzinelli et al., 2015; Manca et al., 2023). En este contexto, la dimensión microbiana se convierte en una pieza clave para comprender la resiliencia del sistema alga-microbioma bajo escenarios de cambio climático, abriendo paso a una mirada más holística del funcionamiento ecológico de estas especies estructuradoras.

1.4. Microbioma de macroalgas pardas y la visión del holobionte

El estudio de las interacciones entre macroalgas y microorganismos ha cobrado creciente relevancia en los últimos años, a partir del reconocimiento de que las algas no existen como entidades aisladas, sino como holobiontes, es decir, sistemas funcionales compuestos por el hospedador macroscópico y sus comunidades microbianas asociadas (Egan et al., 2013; Aires et al., 2015). Estas comunidades que incluyen bacterias, arqueas, hongos y virus colonizan la superficie del talo formando el microbioma, y cumplen funciones esenciales para la salud, el desarrollo y la respuesta al estrés del alga.

En especies de macroalgas pardas de zonas templadas, como *M. pyrifera* y *Durvillaea antarctica*, se han realizado estudios detallados que describen la estructura y variabilidad del microbioma bajo diferentes condiciones ambientales, revelando una alta especificidad hospedador-dependiente, así como patrones temporales y espaciales de ensamblaje microbiano (Tujula et al., 2010; Marzinelli et al., 2015). Estas especies, ampliamente distribuidas en las costas templadas y frías de Chile, comparten hábitat con algas del género *Lessonia*. Sin embargo, en contraste con *Macrocystis* y *Durvillaea*, el conocimiento sobre el microbioma asociado a *L. spicata* es aún escaso, lo que restringe la posibilidad de entender de manera integrativa su funcionamiento ecológico y las sinergias entre el alga y sus comunidades microbianas.

La mayoría de los estudios sobre microbiomas de macroalgas se concentran en especies del hemisferio norte, mientras que en el hemisferio sur y en particular en Chile aún persiste una carencia de investigaciones que aborden la diversidad, estructura y funcionalidad del microbioma en especies locales. Esta brecha es relevante si se considera que estas comunidades bacterianas cumplen funciones críticas para el metabolismo, la defensa química y la tolerancia al estrés ambiental (Singh & Reddy, 2014; Flores et al., 2017).

Los factores ambientales, como la temperatura, la radiación, la salinidad y la disponibilidad de nutrientes, afectan de forma significativa la composición del microbioma algal. Entre ellos, el aumento de temperatura ha sido identificado como uno de los principales causantes de disbiosis, generando alteraciones en

la estructura microbiana que pueden comprometer la funcionalidad del holobionte. Estudios recientes han evidenciado que el calentamiento oceánico puede favorecer la proliferación de taxones oportunistas o patógenos y reducir la abundancia relativa de simbiontes beneficiosos, alterando procesos clave como la defensa, la adquisición de nutrientes o la regulación del estrés oxidativo (Manca et al., 2023).

En este escenario, evaluar cómo responde el microbioma y en particular el epífito de especies estructuradoras frente a un incremento térmico moderado se vuelve especialmente pertinente. Analizar la composición, estabilidad y diversidad funcional del microbioma asociado a *L. spicata* ofrece la oportunidad de identificar señales tempranas de vulnerabilidad ecológica, así como de proyectar su capacidad adaptativa frente a presiones ambientales futuras. Esta dimensión microbiana, integrada a los indicadores ecofisiológicos, bioquímicos y moleculares, proporciona un enfoque robusto para entender la respuesta de los holobiontes algales al cambio climático en ecosistemas costeros del hemisferio sur.

En las frondas, los epibiontes suelen organizarse en biopelículas inmersas en una matriz de sustancias exopoliméricas (EPS). Estas biopelículas no son pasivas: pueden proteger al alga frente a la colonización de organismos incrustantes y regular el microambiente superficial, al mismo tiempo que liberan compuestos señalizadores que influyen en la fisiología del hospedador (Ren et al., 2022; Nahor et al., 2024). El microbioma de kelps incluye bacterias capaces de degradar polisacáridos, metabolizar carbono disuelto, transformar nitrógeno y sintetizar vitaminas esenciales como la B12, contribuyendo así a la nutrición y metabolismo del alga (Weigel et al., 2022). Además, procesos de señalización microbiana y metabolitos producidos en la biopelícula pueden modular sus defensas químicas y la interacción con otros organismos (Cooney et al., 2024; Minich et al., 2018; Mancuso et al., 2023). Sin embargo, la composición de estas comunidades es altamente sensible a estresores como el aumento de temperatura, lo que implica que cambios en la estructura del biofilm y en la calidad de los compuestos exopoliméricos pueden debilitar las defensas químicas y

fisiológicas del alga, comprometiendo la estabilidad del holobionte bajo escenarios de cambio climático (Cooney et al., 2024; Minich et al., 2018).

1.5. *L. spicata* como especie modelo en el estudio del CC

L. spicata es una de las macroalgas más abundantes y relevantes de la zona intermareal rocosa de Chile central, donde forma densas praderas que proveen hábitat, refugio y alimento a una amplia diversidad de organismos, desempeñando un papel clave en la organización y estabilidad de comunidades costeras (Thiel et al., 2007; Vásquez et al., 2012). Además de sustentar biodiversidad, participa en procesos esenciales como el flujo de energía, el ciclado de nutrientes y la captura de carbono, contribuyendo al funcionamiento de los ecosistemas templados del Pacífico suroriental. Su importancia trasciende lo ecológico, ya que constituye un recurso de gran valor socioeconómico por la intensa explotación que sostienen comunidades costeras para la obtención de compuestos como alginatos (González et al., 2016; Ramírez et al., 2023). La especie se distribuye a lo largo de un amplio rango geográfico y recientemente fue registrada en los canales subantárticos de Magallanes (Reserva Nacional Katalalixar, ~47° S), lo que representa un corrimiento de su límite histórico más allá del Golfo de Penas y sugiere dinámicas de expansión posiblemente asociadas a variaciones ambientales y al cambio climático (Rosenfeld et al., 2019). A pesar de esta capacidad de expansión, sus esporas presentan dispersión limitada, lo que hace a las poblaciones dependientes de bancos parentales locales y, en consecuencia, más vulnerables a perturbaciones antrópicas o climáticas. En este contexto, *L. spicata* se presenta como un modelo ecológico idóneo para estudiar cómo estresores como la radiación y la temperatura afectan a especies formadoras de hábitat del hemisferio sur, aportando información crítica para comprender su resiliencia y la de los ecosistemas que sustenta (Zúñiga et al., 2021; Celis-Plá et al., 2022).

Sin embargo, los mecanismos subyacentes que regulan su tolerancia o vulnerabilidad siguen siendo poco comprendidos. La mayoría de las investigaciones disponibles han abordado respuestas específicas a nivel

fisiológico o bioquímico, pero aún son escasos los estudios que integren múltiples escalas biológicas desde la fisiología hasta la interacción con su microbioma en condiciones ambientalmente controladas. Esta limitación impide generar una visión holística sobre su capacidad de aclimatación y su potencial resiliencia ante escenarios proyectados de cambio climático, como el SSP2-4.5 propuesto por el IPCC (2023), que anticipa un aumento moderado de temperatura y mayor radiación solar en zonas como el litoral chileno (Arafeh-Dalmau et al., 2020; Stillman et al., 2025).

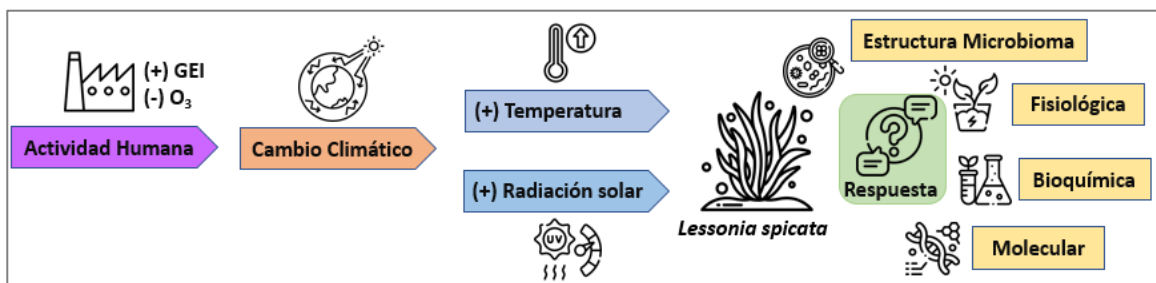


Figura I.4. Diagrama explicativo del problema de investigación propuesto.

Asimismo, aún no se ha caracterizado en profundidad la estructura y función del microbioma epibionte activo de *L. spicata*, a pesar de que estudios en otras especies han demostrado que este componente microbiano puede modificar sustancialmente la respuesta del hospedador frente al estrés (Egan et al., 2013; Marzinelli et al., 2015; Manca et al., 2023). La ausencia de investigaciones sobre esta dimensión simbiótica en *L. spicata* limita la posibilidad de entender su comportamiento como holobionte y, por ende, de predecir adecuadamente su destino ecológico bajo condiciones cambiantes. Más ampliamente, persiste un vacío de conocimiento en torno a cómo se integran las respuestas ecofisiológicas, bioquímicas, moleculares y microbiológicas en esta especie clave frente a escenarios de cambio climático. Esta brecha del conocimiento impide evaluar de manera robusta cómo de *L. spicata* responde frente a las variaciones ambientales en sus diferentes niveles de organización, y con ello entender las consecuencias potenciales de estas respuestas sobre su rango de distribución y las transformaciones en la estructura de sus poblaciones en Chile central en contexto de cambio climático.

1.6. Pregunta de investigación

¿Cómo responde *L. spicata* a nivel ecofisiológico, considerando distintos niveles de organización, frente al aumento de radiación PAR+UV y temperatura bajo escenarios de cambio climático en el litoral templado del centro-sur de Chile, y cómo el calentamiento moderado altera la estructura del microbioma epífito activo, con posibles consecuencias para la resiliencia del holobionte?

Capítulo II. Hipótesis y objetivos

2.2. Hipótesis

H1: Las funciones ecofisiológicas y de respuesta al estrés en el alga parda *L. spicata* se verán afectadas disminuyendo su rendimiento, en respuesta a una alta exposición a radiación PAR+UV producto de la variación estacional y a incrementos de temperatura proyectado por el IPCC (4.5 +2.7 °C), en la zona de Chile Central, en escenario de CC.

H2: El microbioma epibionte del alga parda *L. spicata* presentará una disminución en su riqueza y actividad de bacterias clave, frente a una alta exposición a incrementos de temperatura proyectado por el IPCC (4.5 +2.7 °C), en la zona de Chile Central, en escenario de CC.

2.2. Objetivo general

Evaluar y cuantificar las respuestas ecofisiológicas, del alga parda *L. spicata* frente al incrementos de radiación PAR+UV - temperatura y determinar cambios en la riqueza específica de su microbioma epibionte.

2.2.1. Objetivos específicos

1.-Evaluar y cuantificar las respuestas ecofisiológicas de *L. spicata* sometida a variaciones de radiación PAR+UV en condiciones controladas de laboratorio, de acuerdo con la variación estacional en la zona de Chile Central.

2.-Evaluar y cuantificar las respuestas ecofisiológicas de *L. spicata* sometida a incrementos de temperatura, de acuerdo con el escenario SSP2-4,5 del IPCC (+2,7 °C), en la zona de Chile Central.

3.-Describir la estructura de la comunidad microbiana activa, asociada al alga *L. spicata* en la zona de Chile Central y determinar cambios en la riqueza al ser sometida a aumento de temperatura proyectadas por el IPCC (SSP2-4.5).

Capítulo III

ENERGY STRATEGIES OF BROWN ALGAE TO COUNTER EXCESS SOLAR

RADIATION IN THE INTERTIDAL ZONE

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3.1. Abstract

Climate change is intensifying variability in coastal light regimes, exposing intertidal macroalgae to rapid shifts in photosynthetically active radiation (PAR) and ultraviolet radiation (UVR). *Lessonia spicata* (Ochrophyta), a canopy-forming kelp of ecological relevance, must adjust its light-harvesting and protective machinery to persist under such conditions. We experimentally simulated seasonal PAR+UVR (PAB) exposures and quantified ecophysiological and molecular responses across a diurnal cycle. Photosynthetic productivity (ETR_{max}) was higher in PAB-spring than in winter, and chlorophyll *a* and *c* generally increased under PAB spring/summer relative to -autumn. Under higher irradiance, *L. spicata* exhibited greater non-photochemical energy dissipation (NPQ) and concurrent shifts in expression of photosynthesis-related genes, including photosystem subunits (*Psa/Psb*) and carbon fixation (*rbcL*). These coordinated, short-term adjustments across photophysiological, pigmentary, and transcriptional levels are consistent with rapid acclimation to fluctuating light. Our inferences are limited to controlled, short-term conditions and do not address demographic outcomes; future work should couple these responses to growth, survival, and enzyme level antioxidant dynamics under longer exposures and combined stressors (e.g., warming) typical of climate-change scenarios.

Keywords: *Climate Change, acclimation, Brown algae, Lessonia spicata, photoprotection, Solar radiation*

3.1. Introduction

Global climate change has driven environmental shifts beyond rising atmospheric CO₂, including changes in surface solar radiation linked to ozone depletion by chlorofluorocarbons and other ozone-depleting substances¹. Resulting variability spans photosynthetically active radiation (PAR) and ultraviolet radiation (UVR), posing challenges for aquatic primary producers that depend on light for photosynthesis, growth, and survival (Barnes et al., 2019; Ji and Gao, 2021).

In macroalgae, high irradiance perturbs chloroplast electron transport, enhancing electron leakage to oxygen, overproduction of reactive oxygen species (ROS), and oxidative stress; early responses include shifts in photosynthetic performance and pigment pools (chlorophyll *a*, chlorophyll *c*, carotenoids), together with upregulation of photoprotective and antioxidant pathways that support photoacclimation (Heinrich et al 2012; Cruces et al., 2013; Celis-Plá et al., 2016; Zuñiga et al., 2020; Kaur et al., 2023).

Transcriptional regulation complements these physiological adjustments. Variation in relative gene expression under controlled stress gradients provides sensitive readouts of macroalgal stress physiology (Rodríguez-Rojas et al., 2020). Recent work highlights a substantive role for chloroplast-level transcriptional control in responses to environmental change, offering a window into organelle processes that directly affect photosystem II (PSII) under elevated UVR (Zhang et al., 2023 Kato et al., 2024). However, expression dynamics of many chloroplast-encoded genes central to photosynthetic machinery remain sparsely characterized in brown algae, and their linkage to organism-level photophysiology and antioxidant status is still poorly resolved.

In coastal central Chile, PAR increases from winter to summer and wind-driven upwelling dominates the spring–summer period (Zúñiga et al., 2020; Bustos et al., 2024). Within this context, *Lessonia spicata* (Ochrophyta) is widely distributed along Chile’s temperate coast (~29°–41° S) (González et al., 2012), with recent records to ~47° S in Sub-Antarctic channels. As a canopy-forming kelp, it contributes to nearshore primary production and nutrient dynamics and supports economically relevant harvests for alginate; it is included in national fisheries management plans (Gómez et al., 2016; Rosenfeld et al., 2019; Celis- Plá et al., 2022, 2025).

Although multiple studies have documented photophysiological responses of *L. spicata* to changes in irradiance (PAR and UVR) (Gómez et al., 2016; Cruces et al., 2017; Zúñiga et al., 2020; Celis-Pla et al., 2022), integration with molecular endpoints such as expression of genes encoding photosystem components, xanthophyll-cycle enzymes, and antioxidant pathways remains limited. Such integration is needed to clarify acclimation mechanisms under the highly variable light regimes characteristic of central Chile. Together with evidence of rapid responses to sudden darkening (Celis-Plá et al., 2025), testing *L. spicata* under excess irradiance can reveal whether common acclimation motifs emerge across opposite ends of the light spectrum, linking photophysiology with gene-expression dynamics. Here, we hypothesized that *L. spicata* tolerates shifts under isolated PAR and UVR across multiple levels of biological organization. Using field-collected thalli exposed to a controlled diurnal cycle, we evaluated photophysiological, antioxidant, and molecular responses as potential markers of seasonal acclimation strategies under contemporary light-stress conditions

3.2. Materials and methods

3.2.1. Sampling site

Fronds of *L. spicata* (Surh) Santelices (Phaeophyceae, Laminariales) were collected in the intertidal area of Playa Cochoa, Montemar Marine Protected Area, Valparaíso, central Chile ($32^{\circ}57'19''\text{S}$, $71^{\circ}32'52''\text{W}$) (Fig. III. 1). Samples were taken randomly at 6 separate points parallel to the coastline along a 70 m transect. Samples were transported at low temperatures in plastic containers to the laboratory and were acclimatized for 48 h with a 12:12 photoperiod, and constant aeration and water exchange to achieve a constant temperature until the experiments began.

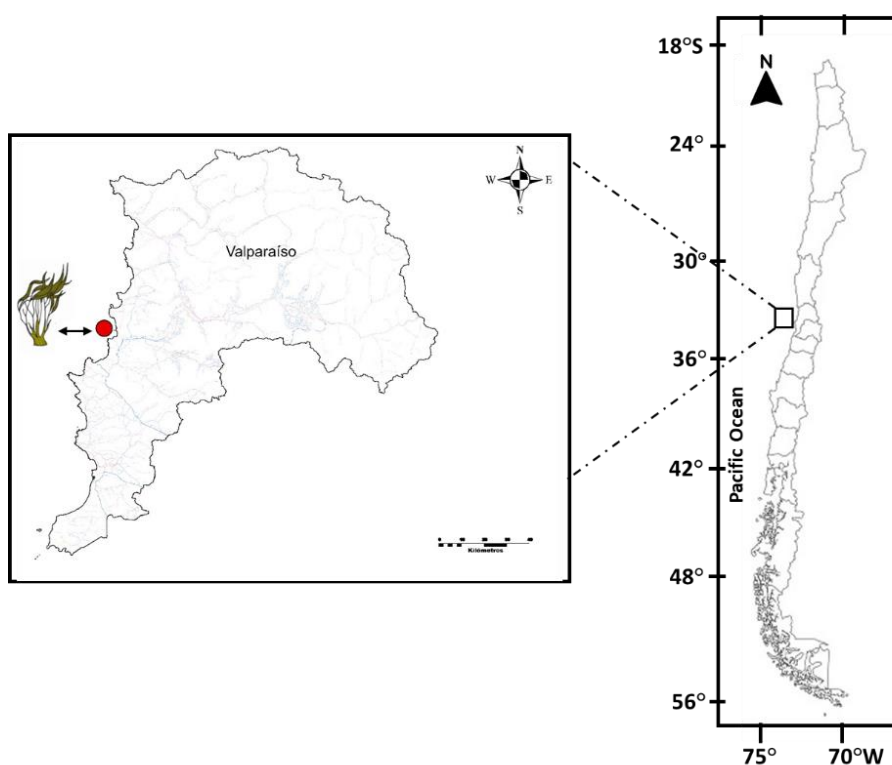


Figure III. 1. Geographic location of the sampling site in the Valparaíso Region, central Chile. The red dot indicates the coastal collection area where algal samples were obtained. The regional map (left) shows the sampling point within Valparaíso, and the national inset map (right) highlights the position of the region along the Chilean coast.

3.2.2. Experimental design

The algae were maintained in trays with seawater filtered sequentially through a natural sand bed and four sand columns with nominal pore sizes of 20, 10, 5, and 1 μm . Seasonal solar-irradiance regimes were simulated as PAB (PAR + UVA + UVB) using fluorescent PAR tubes (Philips TL-D® 36 W/54-765; Philips, Netherlands) for PAR ($\lambda = 400\text{--}700\text{ nm}$) and two Q-Panel® 340 tubes for UVR (Q-Panel Co., Ohio, USA) covering UVA–UVB ($\lambda = 315\text{--}400\text{ nm}$). Total spectral irradiance was verified with a spectroradiometer (Apogee SS-110; Apogee Instruments, Utah, USA). In the same context, the temperature was held at $15 \pm 0.9\text{ }^{\circ}\text{C}$ across treatments using a recirculating chiller (Sensen HYH-05D; China). Target the intensities for each season (PAB-Autumn, PAB-Winter, PAB-Spring, PAB-Summer) followed natural conditions (Celis-Plá et al., 2022) (see Table III. 1).

For each seasonal time, thalli underwent a 48-h acclimation under a 12:12 h light: dark cycle. Sampling for ecophysiological, biochemical, and molecular analyses was conducted during the subsequent exposure day at 10:00, 14:00, and 18:00 h, during dayle cycle (Celis-Plá et al., 2022). Tissue was immediately flash-frozen in liquid nitrogen to minimize handling stress and stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

Table III. 1. The irradiance of UVB (280–315 nm), UVA (315–400 nm), PAR (400–700), and Temperature ($^{\circ}\text{C}$) of seawater at each seasonal time.

	PAB Autumn	PAB Winter	PAB Spring	PAB Summer
PAR daily dose (kJ m^{-2})	922	827	953	1017
UVA daily dose (kJ m^{-2})	63.6	57.2	76.3	89.8
UVB daily dose (kJ m^{-2})	3.8	2.5	4.7	5.0
Sea water temperature ($^{\circ}\text{C}$)	15	15	15	15

3.2.3. Photosynthetic performance

Photosynthetic responses were determined by measuring *in vivo* chlorophyll *a* fluorescence associated with Photosystem II using a pulse amplitude modulated (PAM) fluorometer Junior-PAM (blue light) (Walz GmbH, Germany). Basal fluorescence (F_o) and maximum fluorescence (F_m') were measured under light conditions to obtain the effective quantum yield ($\Delta F/F_m'$), where $\Delta F/F_m' = F_m' - F_o/F_m'$.

To determine the maximum quantum yield (F_v/F_m) and perform rapid light curves, algal fronds were incubated in a dark chamber with fresh seawater for 15 min to fully photoreduce all involved reaction centers (Figuerola et al., 2014; Celis-Plá et al., 2016, 2018, 2022). The maximum ETR was determined by exposing the tissue for 20s to twelve irradiances of actinic light in the rapid light curve (RLC), using the following Equation 1 (Schreiber et al., 1995).

$$\text{ETR (mmol electron m}^{-2} \text{ s}^{-1}) = \Delta F/F_m' * E_{\text{PAR}} * A * F_{\text{II}} \quad (1)$$

Where $\Delta F/F_m'$ is the effective quantum yield, E_{PAR} is the irradiance of PAR in each light pulse ($\text{mmol m}^{-2} \text{ s}^{-1}$), A is the absorptance of the thalli equivalent to the fraction of the incident irradiance that is absorbed by algae (Figuerola et al., 2003), and F_{II} is the portion of chlorophyll *a* associated to PSII (400–700 nm), which is 0.8 in brown macroalga (Figuerola et al., 2014). ETR parameters such as maximum electron transport rate (ETR_{max}) and the initial slope of ETR versus irradiance function (α_{ETR}) as an index of photosynthetic efficiency were calculated from the tangential model (Eilers and Peeters 1998).

Non-photochemical quenching (NPQ) was calculated according to Equation 2, and the maximum non-photochemical quenching (NPQ_{max}) was obtained by the tangential model (Eilers and Peeters 1998).

$$\text{NPQ} = (F_m - F_m') / F_m' \quad (2)$$

The maximum non-photochemical quenching (NPQ_{max}) was obtained by the tangential model contained in (Eilers and Peeters, 1988).

3.2.4. Photosynthetic pigments

Pigments were quantified from ~20 mg fresh mass (FM) of thallus tissue. Samples were flash-frozen and cryo-ground in liquid nitrogen, then extracted in 1.5 mL of 90% (v/v) acetone. Extracts were kept 30 min at 4 °C in the dark and centrifuged at 16,200 g for 10 min at 4 °C (NU-C200R, NuWind, Plymouth, MN, USA). Supernatants were read at 630, 664, and 750 nm using a microplate spectrophotometer (SPECTROstar Nano, BMG Labtech, Offenburg, Germany); A750 was used as a turbidity blank. Chlorophyll concentrations (Chla, Chlc) were calculated with the equations 2 and 3 and expressed as µg g⁻¹ FM. Replicate blanks (90% acetone) and extraction duplicates were included for quality control (Ritchie 2006, 2008).

$$\text{Chla} = 11.47 * (A_{664} - A_{750}) - 0,45 * (A_{630} - A_{750}) \quad (3)$$

$$\text{Chlc}_{1+c_2} = 22.679 * (A_{630} - A_{750}) - 3,404 * (A_{664} - A_{750}) \quad (4)$$

3.2.5. Phenolic compounds (PC)

Approximately 0.25 g fresh, frozen thallus was mixed with 2.5 mL 80% (v/v) methanol in 15 mL conical tubes, then incubated overnight at 4 °C in the dark on a shaker (4,600 rpm) (Celis-Pla et al., 2016; Zúñiga et al., 2020). Extracts were centrifuged at 2,253 g for 30 min at 4 °C, and supernatants were assayed colorimetrically using Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) with phloroglucinol (1,3,5-trihydroxybenzene; P-3502; Sigma) as the standard. Absorbance was read at 760 nm on a microplate spectrophotometer (SPECTROstar Nano, BMG Labtech, Offenburg, Germany). Results are expressed as mg phloroglucinol equivalents (PGE) g⁻¹ DW.

3.2.6. Total antioxidant capacity (DPPH)

Total radical-scavenging capacity was estimated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay using the same methanolic extracts (Celis-Plá et al., 2022). Aliquots (150 μL) were combined with DPPH stock (1.27 mM in 90% MeOH), incubated 30 min in the dark at room temperature, and read at 517 nm (SPECTROstar Nano). A Trolox calibration curve was used to compute Trolox-equivalent antioxidant capacity (TEAC), reported as $\mu\text{mol TEAC g}^{-1}\text{ DW}$ (Álvarez-Gómez et al., 2017). Reagent blanks and extract blanks (without DPPH) were included for background correction.

3.2.7. Oxidative damage

ROS-related stress was quantified as hydrogen peroxide equivalents (Sáez et al., 2015). Briefly, 100 mg cryo-macerated biomass was extracted with 100 μL 10% (w/v) trichloroacetic acid (TCA), 100 μL 10 mM potassium phosphate buffer (pH 7.0), 100 μL FAPRB lysis buffer (Favorgen), and 500 μL 1 M KI. Absorbance was measured immediately at 350 nm on a UV-transparent plate (SPECTROstar Nano). Sample-specific controls (ultrapure water in place of KI) were run in parallel. A commercial H_2O_2 standard (Merck) provided the calibration; data are expressed as H_2O_2 equivalents per g DW.

3.2.8. Lipid peroxidation (TBARS/MDA)

Oxidative damage was assessed by thiobarbituric-acid reactive substances (TBARS) as malondialdehyde (MDA) (Sáez et al., 2015). Frozen powder (100 mg) was lysed with 300 μL 10% TCA plus four 3 mm glass beads and shaken 15 min at room temperature. After clearing, supernatants were mixed with 200 μL 0.5% (w/v) TBA, incubated at 95 °C for 45 min, cooled, and read at 532 nm (200 μL per well). A commercial MDA standard (Merck) was used for calibration. Results are expressed as $\text{ng MDA g}^{-1}\text{ DW}$. (If desired, nonspecific turbidity can be corrected by subtracting A_{600} .)

All biochemical variables were normalized using a fresh-to-dry mass conversion factor of 2.8 for *L. spicata* (Celis-Plá et al., 2022).

3.2.9. RNA extraction and cDNA synthesis

Total RNA was extracted from ~100 mg of thallus (cryo-ground in liquid nitrogen) using the Plant Total RNA Kit (Favorgen, Austria), following the manufacturer's instructions. A fixed input of 300 ng RNA was reverse transcribed with the ProtoScript First Strand cDNA Synthesis Kit (New England Biolabs, USA).

3.2.10. qPCR conditions

Reactions (20 µL) contained 2 µL cDNA (50 ng), 0.25 µM of each primer, Brilliant II SYBR Green qPCR Master Mix (Agilent Technologies, USA), and nuclear free water. Amplifications were run on a MIC magnetic-induction thermocycler (Biomolecular Systems, Australia) with the following program: 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s (with plate read); final extension at 72 °C. Primers were designed with GenScript's online tool (<https://www.genscript.com/tools/pcr-primers-designer>) (table III. 2)

3.2.11. Reference gene (normalizer)

Expression values were normalized to 18S rRNA. This choice was supported by (i) prior evidence of low variability in brown algae (e.g., *Ectocarpus*, *Adenocystis*) (Rodríguez-Rojas et al., 2020) and (ii) the lowest Ct dispersion for 18S across our treatments, indicating stable performance as a single reference.

3.2.12. Relative expression analysis

Relative expression was calculated with the $2^{-\Delta\Delta Ct}$ method (Livak et al., 2001). The calibrator was the mean expression at 10:00 h pooled across seasons; each seasonal 14:00 h condition was compared to this calibrator. Results are reported as $\log_2$ fold change ($\log_2$ FC): positive values indicate higher expression at 14:00 h relative to the 10:00 h calibrator, and negative values indicate lower expression. We interpret these values as relative transcriptional shifts rather than definitive activation/repression.

3.2.13. Statistical analysis

Interactive effects of treatments on ecophysiological and molecular responses of *L. spicata* were tested by analysis of variance (ANOVA) at $\alpha = 0.05$ (Underwood et al., 1997). Prior to ANOVA, data were evaluated for normality and homoscedasticity via residual diagnostics (Q–Q plots, residuals vs. fitted) and Cochran's C test for variance homogeneity; variables violating assumptions were re-checked after appropriate transformations or analysed with the stated caution.

To physiological parameter ETR_{max} , a two-way ANOVA was applied with fixed factors Time (10:00, 14:00 h) and Season (Autumn, Winter, Spring, Summer). For F_v/F_m , α_{ETR} , NPQ_{max} , and all biochemical variables, a one-way ANOVA was run on a combined factor Season - Time (levels = each Season–Time combination). For molecular responses (gene expression), relative expression values were analysed as $\log_2$ -transformed $2^{-\Delta\Delta Ct}$. A one-way ANOVA included five levels: the 10:00 h calibrator (pooled across seasons) and the four seasonal conditions at 14:00 h (PAB-Autumn, -Winter, -Spring, -Summer). When ANOVA tests were significant, pairwise comparisons used the student–Newman–Keuls (SNK) procedure ($p > 0.05$). All analyses were performed in R (R Core Team, 2024) and SNK tests were implemented with the appropriate R package.

Table III. 2. Genes analyzed by RT-qPCR in *L. spicata*.

Gene	Coding protein	NCBI ID	Primer séquence (Forward/reverse)	Amplicon size (bp)
<i>psbA</i>	Photosystem II reaction center protein D1	YP_009684102.1	CCATATGGCTGGTGTGCGGTTGT TCCCAACTACAGGCCATGCTGCAA	257
<i>psbB</i>	Photosystem II chlorophyll A core antenna apoprotein	YP_009684151.1	TGCAGGATGGGCTGGTTCAATGGC AGGCTCACCTGTTCTTGGATCACGGA	307
<i>psbC</i>	Photosystem II chlorophyll A core antenna apoprotein CP43	YP_009684166.1	TGGGCACCCGGTGGTGGAGA TGCTCTACGTGCCCATGCAAATGGC	225
<i>psbD</i>	Photosystem II D2 protein	YP_009684165.1	TCACAAGAGCTTCGTGCGGCTGA GGCGTTTCCACGTGGTAGAACCTCCT	156
<i>psaA</i>	Photosystem I P700 apoprotein A1	YP_009684093.1	GGGCCCTTTACTGGTGCTGGCCATA CCCATGTGCTGCACCACCGACA	237
<i>psaB</i>	Photosystem I P700 apoprotein A2	YP_009684083.1	CGCACAATTTATCCAAGCGGCTTCAGG ACCACCACGACCTGGACCATCACA	322
<i>psaL</i>	Photosystem I protein L	YP_009684112.1	ACGATGATCCTTCGGTTGGG AGTCCCTTTCTATAAGCTGGTAAACT	97
<i>chlB</i>	Photo-chlorophyllide reductase subunit B	YP_009684054.1	AATGGAAAGACATGTTGCTAAACG TGGTTTGACCTTCATAACCAAG	120
<i>chlI</i>	Magnesium-chelatase subunit I	YP_009684038.1	ATTAGATTCTGCAGCGTCAGG TCCGGATTACCTGAACCAACT	99
<i>chlN</i>	Photo-chlorophyllide reductase subunit N	YP_009684089.1	TGTAAATTAATTGGTGCTCCATTCCC TCTCTCTGTAACCCTTTGGG	108
<i>petF</i>	Ferredoxin	YP_009684152.1	AGCAGCTGAAGAGCAAGACT GCTCTGTTTGATCCACTTCCC	104
<i>rbcl</i>	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	YP_009684080.1	AACTCCACAACCAGGCGTAG GTCTGTCCAAACTACCGTCCA	91
<i>dnaK</i>	Hsp70-type chaperone	YP_009684063.1	CACAACGCCAAGCAACAAGA AAGCTAGTGAAGCCGCTGTT	90
<i>atpB</i>	ATP synthase CF1 beta chain	YP_009684096.1	TGCTAAAGCTCATGGTGGTGT ACACCGGATTCTTTCATCTCCA	96
<i>18S</i>	18S rRNA Subunit		AAT TTG ACT CAA CAC GGG TAC AAA GGG CAG GGA CG	448

3.3. Results

3.3.1 Photosynthetic responses

The maximum quantum yield of photosynthesis (F_v/F_m), a variable related to photoinhibition and physiological status, did not present significant changes between treatments ($p > 0.05$; Figure III. 2A) but in the case of PAB-autumn and PAB-spring the values were higher at 10:00 hrs (after the dark period) than at 14:00 hrs, which could be associated with photoinhibition. The α_{ETR} presented the same trend as the ETR_{max} , with magnitudes significantly higher for PAB-spring compared to PAB-winter ($p < 0.05$; Figure III. 2B). The ETR_{max} indicator of productivity and photosynthetic capacity showed values that were significantly higher for the PAB-spring treatment compared to PAB-winter ($p < 0.05$), but ETR_{max} values during PAB-autumn and PAB-summer did not present significant changes (Figure III. 2C). Finally, the NPQ_{max} was different between the treatments, with significantly higher values in PAB-spring, and with significantly lower values for PAB-autumn treatments ($p < 0.05$; Figure III. 2D).

The α_{ETR} presented the same trend as the ETR_{max} , with magnitudes significantly higher for PAB spring compared to PAB winter ($p < 0.05$; Fig III. 2B). The NPQ_{max} was different between the treatments, with significantly higher values in spring and summer PAB compared to winter PAB, and with significantly lower values for autumn PAB compared to the other treatments ($p < 0.05$; Fig. III. D).

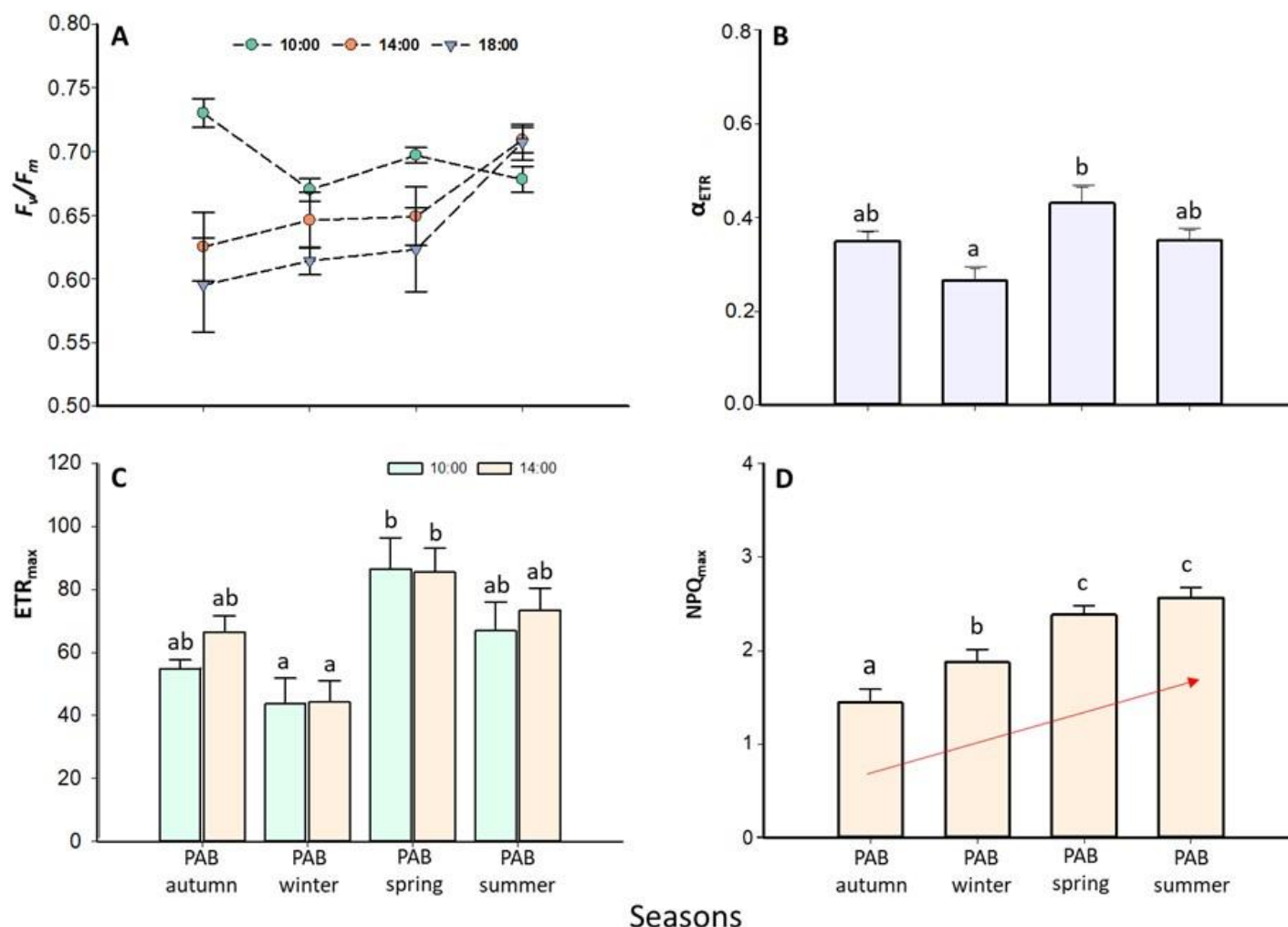


Figure III. 2. A) A) F_v/F_m - Maximal quantum yield, B) α_{ETR} - Photosynthetic efficiency, C) ETR_{max} - Maximal electron transport rate, and D) NPQ_{max} - Maximal non-photochemical quenching in *L. spicata* during the daily cycle experiments under PAB-autumn, PAB-winter, PAB-spring, and PAB-summer treatments.

3.3.2. Biochemical responses

Chla concentration showed a significant increase under PAB-spring and PAB-summer ($p < 0.05$; Figure III. 3A). In the same context, Chl $c1+c2$ concentrations were the highest under the PAB-spring conditions ($p < 0.05$) with decreased values under PAB-summer conditions (Figure III. 3B). Photoprotection through to phenolic compounds concentrations, showed significantly higher

values under PAB-autumn treatments compared to -winter, -spring, and -summer ($p < 0.05$; Fig. III. 3C), and the positive correlations with the antioxidant capacity, and it not present significant changes among treatments (Data do not show).

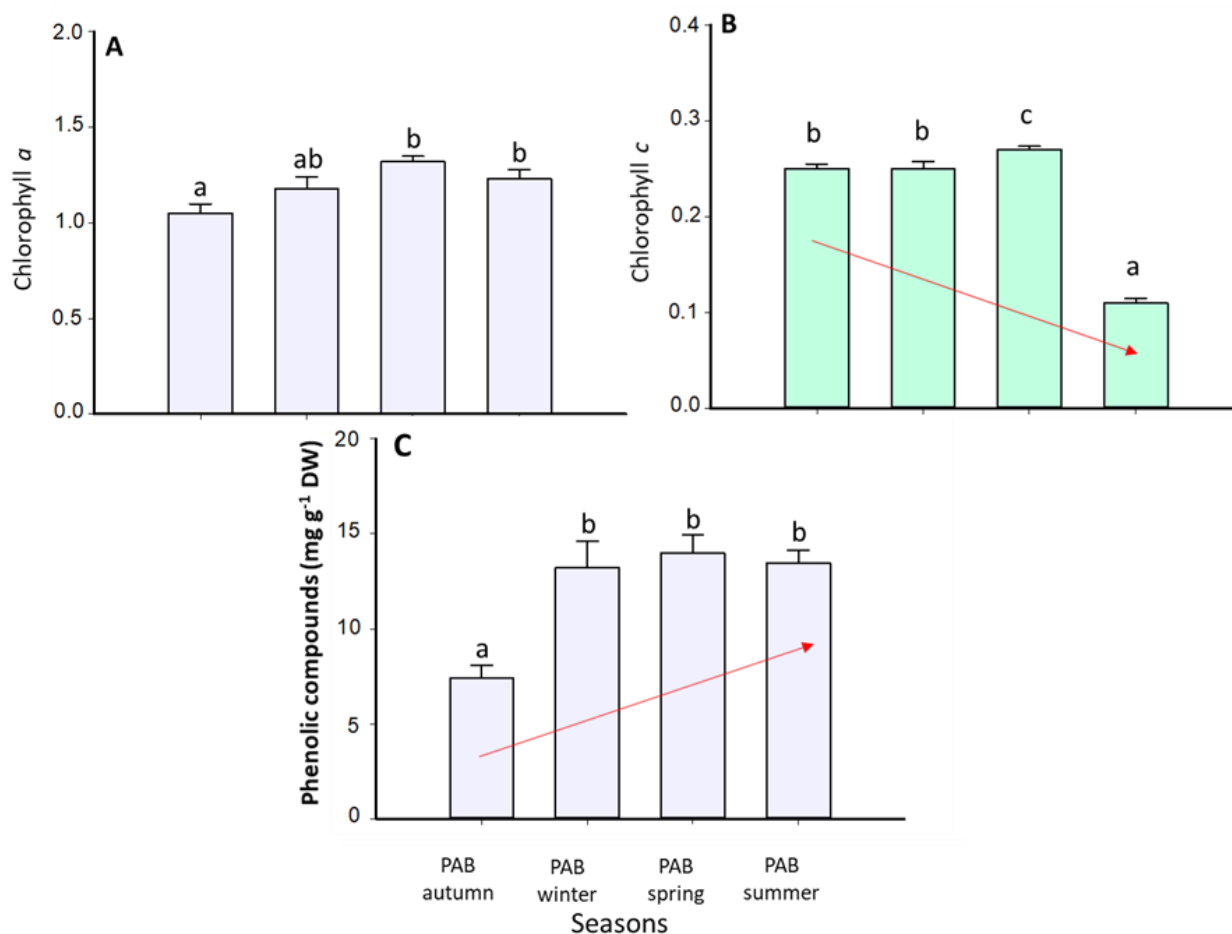


Figure III. 3. Photosynthetic pigments A) Chlorophyll *a*, B) Chlorophyll *c*1+*c*2, and C) Photoprotective compounds as Phenolics, quantified in *L. spicata* during the daily cycle experiments in laboratory under PAB-autumn, PAB-winter, PAB-spring, and PAB-summer treatments, values correspond to the average of the three sampling times (10:00, 14:00, and 18:00 h).

H₂O₂ levels showed significant differences for all treatments ($p < 0.05$; Figure III 4A). H₂O₂ had the highest values in PAB-autumn compared to -spring and -summer. Finally, TBARS (MDA) levels showed significant differences ($p < 0.05$; Figure III 4B), with the highest values to the PAB-summer treatments compared to the other treatments.

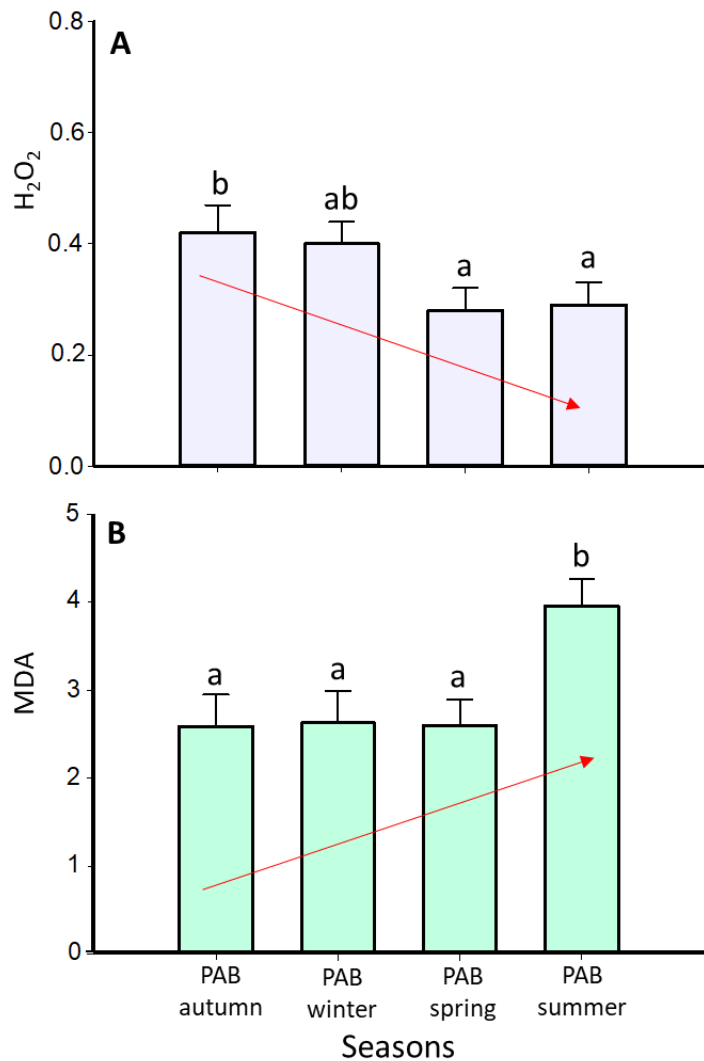


Figure III. 4. A) Hydrogen peroxide (H₂O₂) and B) Thiobarbituric acid-reactive substance (TBARS) accumulation in *L. spicata* exposed to daily cycle experiments under PAB- autumn, PAB-winter, PAB-spring, and PAB-summer treatments, values correspond to the average of the three sampling times (10:00, 14:00, and 18:00 h).

3.3.3. Molecular responses

Seasonal PAB exposure elicited distinct transcriptional patterns in photosynthesis- and pigment-related genes. Photosystem II (PSII) core genes (*psbA*, *psbB*, *psbC*, *psbD*) were generally downregulated in PAB-winter, PAB-spring, and PAB-summer—most notably *psbA* and *psbC*—whereas PAB-autumn

showed stable or slightly elevated expression (Figure III 5). In Photosystem I (PSI), *psaA* decreased in PAB-winter and PAB-summer, while *psaL* increased in PAB-autumn and PAB-spring, indicating subunit-specific seasonal modulation. Chlorophyll-biosynthesis genes (*chlB*, *chlI*, *chlN*) rose in PAB-autumn and declined in PAB-winter–spring–summer, with *chlN* showing the largest seasonal amplitude. Genes linked to electron transport and carbon metabolism (*petF*, *rbcL*, *dnaK*, *atpB*) increased in autumn and spring—especially *petF* and *dnaK*—but were reduced in winter and summer, with *rbcL* and *atpB* most suppressed in PAB-summer. Collectively, these profiles are consistent with enhanced transcriptional investment during milder periods (autumn/spring) and reduced expression during the more demanding seasonal regimes (winter/summer) under our PAB treatments (see Figure 5; Table 2 for targets and statistics).

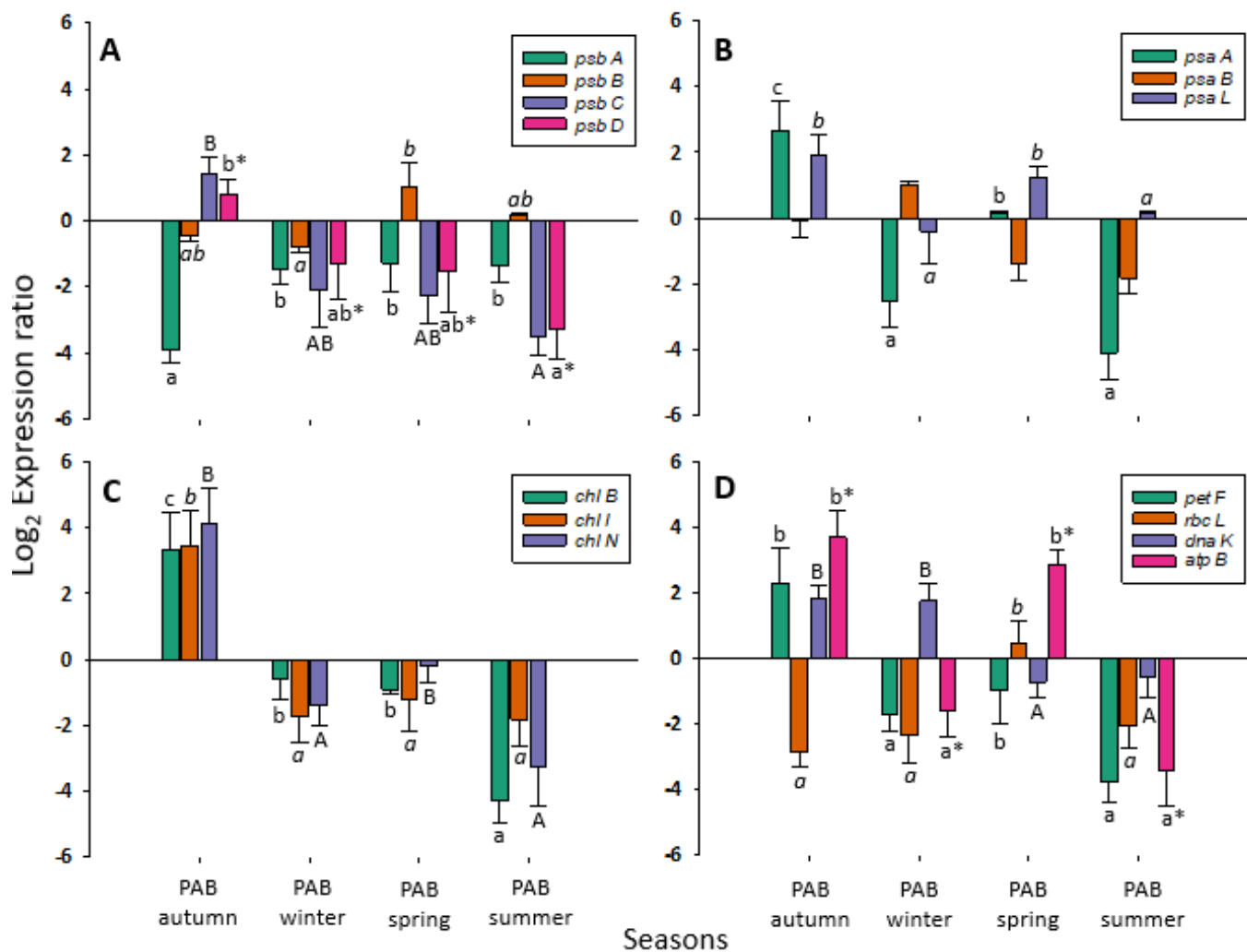


Figure III. 5. Relative expression of genes from the chloroplast of *L. spicata* subjected to different radiation treatments (PAB). A) Genes of photosystem II, B) photosystem I, C) photosynthetic pigments and (D) electron transport chain. different letters denote significant differences (SNK, $p < 0.05$).

3.4. Discussion

Our results showed significant differences in the physiological, biochemical and molecular responses of *L. spicata* to seasonal PAB regimes. Physiological responses as ETR_{max} and light-use efficiency (α_{ETR}) were highest under PAB-spring and significantly exceeded -winter, whereas the higher total irradiance of -

summer did not translate into greater photosynthetic performance. This decoupling is consistent with the marked increases in NPQ_{max} during PAB-spring and -summer, indicating engagement of non-photochemical energy dissipation and a shift toward photoprotection rather than enhanced photochemistry (Zúñiga et al., 2020; Celis-Plá et al., 2022).

Across treatments, F_v/F_m remained within ranges commonly reported for brown algae acclimated to high irradiance environments (Cruces et al., 2017; Rosenfeld et al., 2019), suggesting that PSII maximum efficiency was maintained despite variation in excitation pressure. Pigment responses were selective: chlorophyll *a* was broadly stable across seasonal PAB, whereas chlorophyll *c* declined under summer conditions. The resulting reduction in the Chl *c*/Chl *a* ratio is consistent with a contraction of the peripheral antenna/harvesting capacity under elevated irradiance—a well-described photoacclimation motif in algae (Raven et al., 2004; Charan et al., 2022; Endo et al., 2023). These patterns support a mechanistic interpretation in which *L. spicata* under higher seasonal irradiance prioritizes excess-energy dissipation (NPQ_{max}) and antenna adjustment (Chl*c*/Chl*a*), thereby stabilizing PSII efficiency (F_v/F_m) while limiting gains in electron transport (ETR_{max}) during high light periods. Complementing these adjustments of the light-harvesting apparatus, many intertidal brown algae deploy biochemical defences under high PAR/UVR, notably the synthesis and accumulation of phenolic compounds that act as UV screens and antioxidants (Flores-Mollina et al., 2016; Gómez et al., 2016, 2020; Celis-Plá et al., 2022). *L. spicata* shows such inducible phenolics after ~48 h of UV + PAR exposure in laboratory assays (Cruces et al. 2013; Gómez et al., 2016), with similar patterns reported for the cosmopolitan kelp *Macrocystis pyrifera*.

Here, the phenolic levels were broadly comparable under PAB-winter, -spring, and -summer, but were significantly lower in -autumn. This seasonal contrast suggests that phenolic investment did not rise above baseline during winter–summer PAB, whereas the lower -autumn values may reflect reduced

demand for screening/antioxidant capacity under milder irradiance. In this sense, the excess irradiance can also drive oxidative chemistry by promoting electron leakage to oxygen, overproduction of reactive oxygen species, and downstream lipid peroxidation that damages chloroplasts and mitochondria (Agati et al., 2013). Consistent with this, we observed higher malondialdehyde (MDA; TBARS) in PAB-summer relative to other seasons. Together with the strong NPQ_{max} engagement in summer/spring (previous section), the MDA increase indicates that, under high seasonal irradiance, photochemical dissipation (NPQ) and constitutive phenolics were not sufficient to prevent measurable oxidative strain. These outcomes align with prior reports for *L. spicata* under natural and experimental light stress (Cruces et al., 2017; Zúñiga et al., 2020) and reinforce a mechanistic picture in which *L. spicata* modulates both antenna size (Chl *c*/Chl *a*) and antioxidant/photo-screening defences to manage excess energy, with oxidative markers rising when exposure exceeds those protective capacities.

PAR and UVR can affect macroalgae across physiological, biochemical, and molecular levels. Although wild populations of *L. spicata* experience irradiances higher than our laboratory PAB simulations, the seasonal treatments elicited rapid responses consistent with intertidal acclimation capacity (Celis-Plá et al., 2022). A primary target is the photosynthetic apparatus: at midday (14:00 h), we observed inhibition of transcripts encoding PSII core subunits (*psbA*, *psbC*) and selected PSI components (e.g., *psaA/psaL*) under the more demanding seasonal regimes, paralleling reports in *Saccharina latissima* and *Macrocystis pyrifera* where high radiation downregulates photosystem-structural genes (Heinrich et al., 2012; Buschmann et al 2014), and in higher plants (An et al., 2022). Such downregulation is often interpreted as an energy-allocation strategy: limiting de novo synthesis of large membrane complexes while routing resources to protective processes (e.g., NPQ engagement and phenolic/antioxidant pathways). This interpretation aligns with our physiology, where PSII maximum efficiency (F_v/F_m) remained stable, NPQ_{max} increased in spring/summer, and oxidative markers (MDA) rose in summer.

At the pigment/gene interface, chlorophyll *a* remained broadly stable whereas chlorophyll *c* decreased under PAB-summer, consistent with antenna contraction; concordantly, chlorophyll-biosynthesis genes (*chlB*, *chlI*) were strongly inhibited in -summer, suggesting transcriptional modulation of pigment supply under peak irradiance. Evidence for such regulation is sparse in macroalgae but well described in terrestrial plants (Li et al., 2022; Su et al., 2022). Midday repression of *rbcL* (autumn, winter, summer) points to a potential coupling between irradiance peaks and carbon-fixation gene expression; contrasting responses reported in *Euglena gracilis* under high UVR (Lv et al 2024), underscore that taxon-specific regulation is likely. Notably, *dnaK* (Hsp70) was not inhibited—and in some cases increased—indicating activation of protein-homeostasis/stress-response machinery under elevated radiation, consistent with patterns in *Fucus vesiculosus* and microalgae (Creis et al., 2015; Cao et al., 2019)

Our results support a mechanistic model in which *L. spicata* manages seasonal irradiance in Climate change conditions, by (i) shifting excitation handling toward dissipation (NPQ_{max}), (ii) reducing antenna size (Chl *c*/Chl *a*) and pigment-biosynthesis transcription during peak exposure, (iii) mobilizing phenolic/antioxidant defences, and (iv) transiently downregulating photosystem and carbon-fixation transcripts at midday while maintaining F_v/F_m . These coordinated, short-term adjustments are consistent with rapid acclimation in an intertidal kelp; however, they do not, by themselves, imply demographic or ecosystem outcomes and should be linked to growth/survival and enzyme-level assays in longer-term studies.

In summary, *L. spicata* showed rapid, season-dependent responses to contrasting PAB intensities across molecular, biochemical, and photophysiological levels. Under the highest irradiance (PAB-summer), transcripts encoding photosynthetic machinery were repressed at midday, while PSII maximum efficiency (F_v/F_m) remained stable and excess-energy dissipation (NPQ_{max}) increased—consistent with a shift toward photoprotection rather than enhanced photochemistry. Phenolic/antioxidant metrics were broadly maintained across treatments, suggesting a sustained protective investment that may contribute to the observed decoupling between gene expression and

instantaneous performance. Together with recent evidence of fast ecophysiological and biochemical shifts under eclipse-induced darkening in *L. spicata* (Celis-Plá et al., 2025), these patterns support a model of short-term acclimation to abrupt light fluctuations. Future work should link these short-term responses to performance metrics (growth, survival) and enzyme-level antioxidant dynamics over longer exposures, and test interactions with temperature in the seasonally upwelling coastal system of central Chile.

3.5. Conclusions

This study shows that *Lessonia spicata* mounts rapid, short-term acclimation responses to contrasting PAR and UVR. Under higher irradiance, transcripts associated with the photosynthetic machinery were reduced at midday, while photophysiological performance (e.g., F_v/F_m) remained stable and non-photochemical dissipation (NPQ) increased. Together with broadly maintained phenolic/antioxidant metrics, these patterns are consistent with a shift toward protective energy management rather than enhanced photochemistry. Our findings are limited to short-term, controlled conditions and do not address growth, survival, or population-level outcomes. Future work should couple these molecular and photophysiological responses with performance metrics and enzyme-level antioxidant assays over longer exposures, and test interactions with temperature typical of seasonally upwelling coasts.

Thermal stress on *Lessonia spicata*: physiological and biochemical responses to climate change



Thermal stress impairs photophysiology and redox balance in the kelp *Lessonia spicata*

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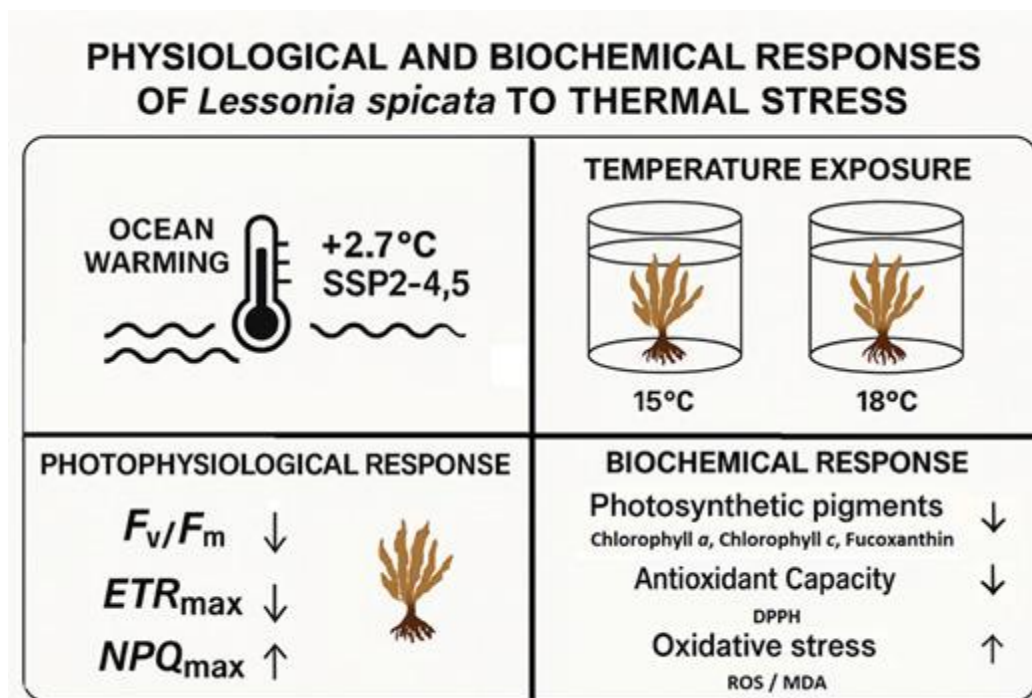
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4.1. Graphical Abstract



4.1. Abstract

Climate change is driving significant warming in marine ecosystems, with profound consequences for macroalgae, which are key primary producers in temperate coastal habitats. Under the IPCC's moderate emission scenario SSP2-4.5, sea surface temperatures are expected to rise by ~2.7 °C by the end of the century, potentially altering the physiology, abundance, and distribution of habitat-forming species, such as *Lessonia spicata* (Ochrophyta). In this study, we experimentally assessed the physiological and biochemical responses of *L. spicata* to short-term thermal stress under laboratory-controlled conditions that simulate near-future ocean warming. Macroalgae were exposed for 14 days to two temperatures regimes: a control at 15 °C and a treatment at 18 °C (+3 °C), consistent with IPCC projections. Elevated temperature reduced maximum quantum yield of photosystem II (F_v/F_m) and the maximum electron transport rate (ETR_{max}) and increased non-photochemical quenching (NPQ_{max}), consistent with photochemical impairment and enhanced energy dissipation. Pigments declined while ROS and MDA increased and antioxidant capacity decreased, indicating

redox imbalance. Together, these results reveal the rapid onset of photophysiological inhibition and oxidative stress in *L. spicata* under moderate warming, providing an ecophysiological baseline for understanding its thermal tolerance and resilient to climate change.

Keywords: *Brown algae, Lessonia spicata, photoprotection, thermal stress, antioxidant responses.*

4.2. Introduction

Canopy-forming brown macroalgae (kelp), such as *L. spicata* (Suhr) (resilient Phaeophyceae, Ochrophyta), are foundational species and among the most ecologically important primary producers in temperate coastal habitats worldwide. They play a critical role in structuring intertidal and subtidal habitats, contributing substantially to nutrient cycling, primary production, and providing essential habitat complexity (Buschmann et al., 2014; Gómez et al., 2016; Rosenfeld et al., 2019). Climatic change (CC) is one of the most urgent environmental threats of the 21st century. Its impacts are particularly severe in marine ecosystems, where rising greenhouse gas emissions elevate sea surface temperatures (SST), leading to widespread ecological disruption (Dubash et al., 2020; IPCC 2023). In temperate coastal regions, increasing SST imposes multiple stressors on kelp species, affecting key physiological processes such as photosynthesis, respiration, nutrient uptake, and life-cycle dynamics (Harley et al., 2012; Smale, 2020; Gundersen et al., 2021; Tregarot et al., 2024). These physiological disturbances can compromise not only individual performance but also the persistence of entire populations of primary producers over time. Moreover, warming is causing unprecedented shifts in species distributions, triggering biodiversity loss, habitat degradation, and reorganization of biological communities, and the emergence of novel species assemblages, which may alter ecosystem services in ways we are only beginning to understand (Wernberg et al., 2019; Duarte et al., 2022). Such distributional shifts have already been predicted for canopy-forming macroalgae under climate warming scenarios; similar patterns have been predicted for other canopy-forming species using ecological niche models under warming conditions (Carneiro et al., 2023).

Projections under SSP2–4.5 scenarios, estimate a rise of +2.7 °C in SST by 2100 (IPCC, 2023). While moderate, this warming still poses critical risks to macroalgae, particularly foundational species in temperate marine ecosystems, which may exceed their physiological thermal limits. This is consistent with findings in other cold-adapted macroalgae exhibiting reduced thermal tolerance

when exposed to increased irradiance and temperature (Marambio et al., 2021). When such thresholds are surpassed, abrupt changes in abundance and geographical range can occur, with cascading consequences for ecosystem structure and function (Smale, 2020). The loss of kelp forests, for example, has already been documented in several regions, including a 43% decline in Western Australia between 2011 and 2015 because of extreme marine heatwaves (Coelman & Wernberg, 2017).

Their productivity and resilience strongly depend on efficient photosynthetic and photoprotective mechanisms, which are sensitive to temperature and irradiance. Photochemical indicators such as the maximum quantum yield of Photosystem II (F_v/F_m), the maximum electron transport rate ETR_{max} and non-photochemical quenching NPQ are widely used to quantify photosynthetic performance and stress tolerance in kelps. F_v/F_m is the primary measure of photosynthetic efficiency and is highly sensitive to stress; its decline indicates photoinhibition and cellular damage to the photosystem II reaction centers. ETR_{max} represents the maximum rate of light-driven electron flow through the thylakoid membranes, serving as an indicator of maximum photosynthetic capacity. Finally, NPQ quantifies the dissipation of excess light energy as heat, acting as a crucial photoprotective mechanism under stress conditions (Figueroa et al., 2019). Integrating these metrics provides a robust understanding of the physiological status of macroalgae.

Multiple studies have documented strong physiological and biochemical impacts of elevated temperature on brown macroalgae. The decline in the maximum quantum yield of PSII (F_v/F_m) is a consistent indicator of thermal damage. For instance, *Cladosiphon okamuranus*, *Nemacystus decipiens*, and *Turbinaria ornata* exhibited significant drops in F_v/F_m when exposed to elevated temperatures (Fukumoto et al., 2019; Narrain et al., 2023; Bell et al., 2024). Observed seasonal variation in F_v/F_m values has been documented in *L. spicata*, highlighting its ecophysiological plasticity and capacity for photoacclimation to predictable environmental shifts (Zúñiga et al., 2021; Celis-Plá et al., 2022). However, this acclimation capacity is finite, and vulnerability increases when

physiological thresholds are exceeded by acute events. Reductions in the maximum electron transport rate (ETR_{max}) have been observed in *Fucus serratus* under elevated temperature, indicating impaired capacity for electron flow a distribution ranges and energy production (Figueroa et al., 2019). Similarly, elevated temperature exposure in other brown algae led to lower ETR, signaling compromised photochemical efficiency (Fukumoto et al., 2019). Additional impacts on photosynthetic performance have been reported in *Lessonia corrugata*, which exhibited reduced net productivity and increased erosion near its upper thermal threshold (James et al., 2023). Meanwhile, non-photochemical quenching (NPQ) often increases as a compensatory response, as in *Turbinaria ornata*, which raised NPQ to dissipate excess excitation energy under stress (Narain et al., 2023).

Thermal stress also alters key biochemical traits in brown algae. For instance, *Sargassum horneri* exhibited significant reductions in chlorophylls above 30° C, indicating pigment degradation (Yong et al., 2021), while *Sargassum stenophyllum* showed declines in chlorophyll *a* after 5° C increase in temperature (Urrea-Victoria et al., 2020; Schmid et al., 2021) reported carotenoid adjustments and reductions in total pigment content in temperate kelps *Laminaria digitata* under heat stress, and *Macrocystis pyrifera* experienced losses in chlorophyll *c* and fucoxanthin under elevated temperatures (Mabin et al., 2019). Furthermore, recent work demonstrated that *L. spicata* exposed to thermal and light stress accumulates hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), indicating oxidative damage and membrane destabilization (Celis-Plá et al., 2025). *L. spicata* (Suhr) (Laminariales, Phaeophyceae, Ochrophyta) is distributed along the temperate coast of Chile (29° to 41°S) and is one of the most ecologically important brown macroalgae in the Pacific Ocean (Buschmann et al., 2014; Gómez et al., 2016). It plays a foundational role in structuring intertidal and subtidal habitats, contributing to nutrient cycling, primary production, and habitat complexity.

In central Chile, where *L. spicata* is ecologically dominant, water temperatures can vary throughout the year, peaking during the summer months

(December, January, and February) when temperatures range between 15–22 °C. However, significant increases in maximum temperatures have been recorded in recent years, attributable to global warming (Pujol et al., 2022; IPCC, 2023; Rojo-Garibaldi et al., 2023). These thermal anomalies such as the negative impacts observed on kelp beds in northern Chile (Tarapacá) during the recent El Niño event (Avilés, 2024), are altering distribution patterns. Notably, while *L. spicata* has historically dominated central Chile, recent findings document its expansion poleward (southward), reporting the first record of the species in the Sub-Antarctic Magellan Channels (Rosenfeld et al., 2019). This raises concerns about its long-term ecological viability and the potential loss of ecosystem services it supports. In this context, in vivo chlorophyll *a* fluorescence remains a valuable tool for detecting physiological impairment, as reductions in F_v/F_m and ETR_{max} are widely used as indicators of photoinhibition and stress in kelps (Abdala-Dias et al., 2006; Figueroa et al., 2014; Celis-Plá et al., 2016). These measures offer rapid, non-invasive insights into the photosynthetic status of macroalgae exposed to changing environments. Integrating these photophysiological metrics with biochemical traits such as pigment degradation, antioxidant capacity, and oxidative markers provides a comprehensive perspective on algal stress physiology.

Given regional warming trends and the increasing frequency of marine heat anomalies along central Chile, we tested a +3 °C increment relative to 15 °C to represent a realistic, short-term thermal stressor. This short-term stress is relevant because the degree of cellular damage and redox imbalance measured here is the mechanistic foundation that determines longer-term organismal fitness and demographic performance under chronic or repeated stress, as physiological responses underpin individual life history traits and, consequently, population dynamics (Coleman et al., 2024). Our objective was strictly mechanistic: to quantify short-term photophysiological and biochemical responses of *L. spicata* to moderate warming under controlled conditions.

4.3. Materials and Methods

4.3.1. Algae collection and Experimental design

Thalli of *L. spicata* were collected in September 2023 from the rocky intertidal zone (upper intertidal) at Cochoa Beach, located north of Valparaíso Bay, Chile (32°57'19" S; 71°32'52" W). To avoid pseudoreplication, each replicate corresponded to a distinct thallus of *L. spicata* collected from independent holdfasts at least 1 m within the sampling site. From each thallus, only one healthy blade segment was used per experimental unit, ensuring that all replicates represented independent biological individuals rather than subsamples from the same organism. A total of approximately 50 individuals were collected. The approximate sporophyte size ranged from 20 to 50 cm in length. During the sampling the sea surface temperature (SST) was $15 \pm 1^\circ\text{C}$ under low tide.

The samples were transported in plastic containers under cooled (13-15 °C) conditions to the Laboratory of Environmental Research (LACER) at the HUB Ambiental UPLA research center of Universidad de Playa Ancha, Chile. Upon arrival, thalli were maintained with constant aeration and periodic seawater exchange to stabilize temperature to (15 °C) until the experiment began. Fronds from 10 to 11 different individuals were placed in each experimental unit (tank). During the entire experimental period, algae were exposed to a natural daily light regime simulating local conditions (PAR: 953 kJ m^{-2} , UVA: 76.3 kJ m^{-2} , UVB: 4.7 kJ m^{-2}), under a 12:12 h light: dark photoperiod.

Two temperature treatments were applied: a control at (15 °C) and a warming treatment of (18 °C), as projected by the IPCC 2023 scenario SSP2-4.5. The experimental unit for the temperature factor was the tank ($n=3$ per treatment), totaling 6 experimental units ($n = 2 \times 3$). The temperature was regulated using a chiller system (Sunsun Chiller Mod. HYH 05D, Sensen, China). The tanks were assigned randomly to the two temperature treatments, and the temperature regulation was performed via an external water bath, ensuring no water circulation or exchange between the temperature treatments. Fronds were repositioned

regularly to prevent self-shading, and fresh filtered seawater was changed every three days to avoid nutrient depletion and microbial overgrowth.

Sampling was performed at 10:00 h (local time) on day-1, day-7, and day-14. The selection of these time points was designed to capture both the immediate, acute physiological response (day-1) and the potential long-term response or compensatory acclimation (day-7; day-14). Photosynthetic parameters were measured in situ, on different adults' fronds at each time point (days-1-7-14) to avoid time-series pseudo-replication and the influence of the measurement process. Algal tissue for biochemical and molecular analyses was collected from adult fronds using sterile scissors and immediately froze in liquid nitrogen to prevent degradation due to manipulation (Figure IV. 1).

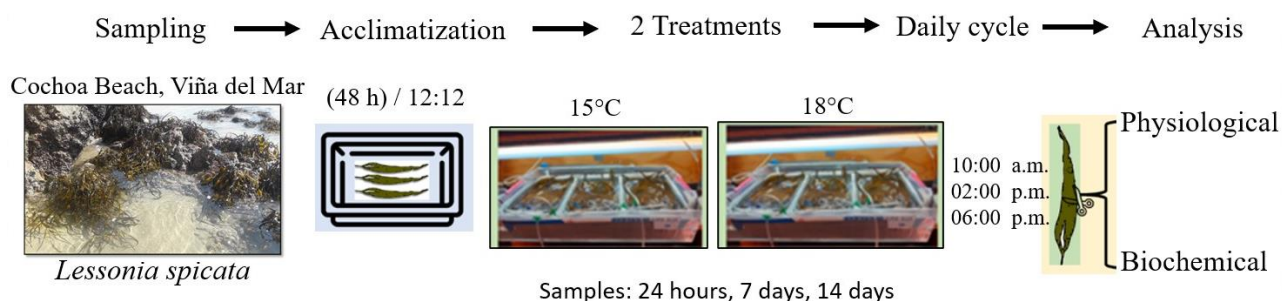


Figure IV. 1. Explanatory diagram of the steps to follow during the experiment, from the sampling site to the collection of material for analysis.

4.3.2 Photosynthetic performance

In vivo chlorophyll *a* fluorescence was measured using a pulse amplitude modulated fluorometer (Junior-PAM, Walz GmbH, Germany).

The effective quantum yield ($\Delta F/F_m'$) was calculated from initial and maximum fluorescence under illuminated conditions, and maximum quantum yield (F_v/F_m) was determined after 15 minutes of dark adaptation (Figueroa et al., 2019; Celis- Plá et al., 2018, 2022, 2025).

The maximal electron transport rate (ETR_{max}) was estimated by exposing algal tissue to twelve different intensities of actinic light for 20 seconds during the rapid light curve (Schreiber et al., 1995), according to Equation 1:

$$ETR \text{ (mmol electron m}^{-2} \text{ s}^{-1}) = \Delta F / F_m' * EPAR * A * FII \quad (1)$$

ETR parameters, including ETR_{max} and the initial slope of the ETR versus irradiance relationship (α_{ETR}), were determined using the tangential model (Eilers and Peeters, 1988).

Non-photochemical quenching was calculated as follows (Celis-Plá et al., 2016), according to Equation 2:

$$NPQ = (F_m - F_m') / F_m' \quad (2)$$

The maximum non-photochemical quenching (NPQ_{max}) was obtained by the tangential model contained in (Eilers and Peeters, 1988).

4.3.3 Biochemical variables

All spectrophotometric measurements, including pigments, phenolics, antioxidant capacity, and malondialdehyde (MDA), were conducted using a microplate reader (SPECTRO-star Nano, BMG Labtech, Offenburg, Germany), except for ROS fluorescence quantification, which was performed with a Cytation 5 instrument (Agilent BioTek, Santa Clara, USA).

Chlorophyll *a* and *c* were extracted from 20 mg of fresh algal tissue using 90% acetone, following (Ritchie, 2008). After 30 minutes of dark incubation at 4 °C, samples were centrifuged at 16,200 g for 10 minutes. Absorbance was measured in microplates, and concentrations were calculated using the following equations 3 and 4:

$$Chl \ a = 11.47 \times (A_{664} - A_{750}) - 0.45 \times (A_{630} - A_{750}) \quad (3)$$

$$Chl \ c = 22.679 \times (A_{630} - A_{750}) - 3.404 \times (A_{664} - A_{750}) \quad (4)$$

Fucoxanthin concentration was determined from 25 mg of algal tissue using an extract in dimethyl sulfoxide (DMSO), following the modified protocol of (Seely et al., 1972) according to (Celis-Plá et al., 2018). Absorbance was read at 480 nm and fucoxanthin concentration calculated using Equation 5:

$$F_x = [A_{480} - 0.722(A_{631} + A_{582} - 0.297 \times A_{665}) - 0.049 \times A_{665}] / 130 \quad (5)$$

Total phenolic compounds were extracted from 250 mg of fresh-frozen algal tissue using 80% methanol and quantified using the Folin–Ciocalteu method with phloroglucinol as a standard, following (Celis-Plá et al., 2022, 2025). The antioxidant capacity was assessed via the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay using 150 µL aliquots of the ex-tract prepared from 250 mg of biomass, and results were expressed as µmol TEAC g⁻¹ DW using Trolox as a standard (Alvares-Gomez et al., 2017). Reactive oxygen species (ROS) production was measured from 30 mg of frozen algal tissue using a commercial detection kit (Sigma-Aldrich), following the manufacturer's instructions. The tissue was ground in liquid nitrogen, extracted with HCl, and reacted with the fluorophore probe. Fluorescence was read at 540/570 nm using a Cytation 5 reader, and values were expressed as mmol g⁻¹ DW. Lipid peroxidation was estimated from 50 mg of algal biomass via the quantification of malondialdehyde (MDA) using the Thiobarbituric acid reactive substances (TBARS) method, following protocols described in Celis-Plá et al., (2025) and Zuñiga et al., (2021), with absorbance measured at 532 nm. Results were expressed as ng MDA g⁻¹ DW.

4.3.4 Statistical analysis

We analyzed temperature (2 levels) × time (3 levels) with two-way ANOVA. For each response variable we report F, p, and partial η² with 95% CIs. Where interactions were significant, we provide simple effects with Hedges' g and 95% CIs. Previously, the data were assessed for homogeneity and homoscedasticity of variance using Bartlett's test, and student-Newman-Keuls post hoc tests were

applied. All diagnostics and code are in Supplementary Information. The experimental unit and replication ($n = \dots$ per treatment $\times$ time) are stated in each figure caption. Where significant interactions were observed, the. All statistical analyses were performed using R version 3.3.0 (R Core Team).

Principal Coordinates Ordination (PCO) was conducted to integrate all photophysiological and biochemical variables and to visualize multivariate similarities among treatments. This complementary analysis allowed the identification of coordinated response patterns of *L. spicata* under control (15 °C) and warming (+3 °C) conditions. All variables—maximum quantum yield (F_v/F_m), maximum electron transport rate (ETR_{max}), photosynthetic efficiency (α_{ETR}), photosynthetic saturation irradiance ($E_{K_{ETR}}$), non-photochemical quenching (NPQ_{max}), chlorophyll *a* (Chl *a*), chlorophyll *c* (Chl *c*), fucoxanthin (FX), total phenolic compounds, antioxidant capacity (DPPH), reactive oxygen species (ROS), and malondialdehyde (MDA), Insoluble Phenolics compounds (PCI) and Soluble Phenolics Compounds (PCS) —were standardized by their maximum values to ensure comparability. The analysis was executed using the PRIMER v7 software package, following standard procedures for ecological datasets.

4.4. Results

4.4.1. Multivariate Analysis (PCO)

The multivariate Principal Coordinates Ordination (PCO) analysis revealed a clear separation between treatments along the primary axis (PCO1), which explained 25.9 % of the total variance (Figure IV. 2). Samples exposed to 18 °C clustered towards the negative side of PCO1, associated with higher levels of non-photochemical quenching (NPQ), reactive oxygen species (ROS), and malondialdehyde (MDA). In contrast, control samples at 15 °C grouped on the positive side, characterized by higher values of maximum quantum yield (F_v/F_m), maximum electron transport rate (ETR_{max}), photosynthetic efficiency (α_{ETR}), and pigment contents (Chl *a*, Chl *c*, fucoxanthin), as well as greater antioxidant capacity (DPPH). The second axis (PCO2; 17.9 % of total variance) captured minor within-treatment variability. Overall, this ordination underscores the consistent multivariate distinction between control and warming conditions,

indicating that short-term exposure to +3 °C induces a coordinated decline in photochemical performance and redox homeostasis in *L. spicata*.

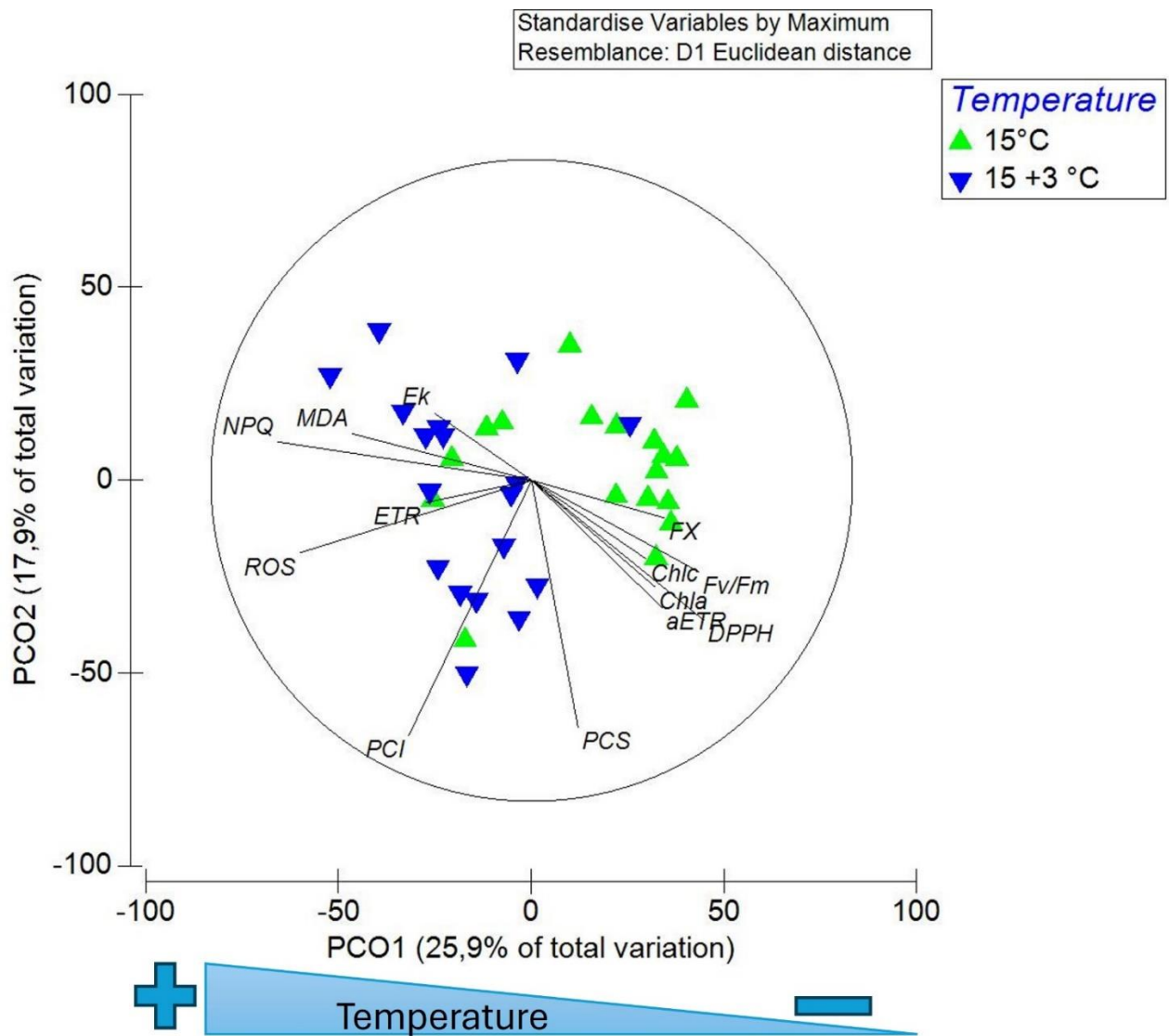


Figure IV. 2. Principal Coordinates Ordination (PCO) of all photophysiological and biochemical variables. The ordination shows the separation between the 15 °C (green triangles) and 18 °C (blue inverted triangles) treatments. Vectors indicate the contribution and direction of each variable. PCO1 and PCO2 explain 25.9% and 17.9% of the total variance, respectively.

4.4.2. Photosynthetic responses

Thermal stress produced consistent and statistically significant changes in the photophysiological performance of *L. spicata* (Table IV. S1). F_v/F_m decreased progressively under 18 °C treatment, with significant differences at days 7 and 14 relative to the control ($F(2, 30) = 18.9$, $p < 0.05$, $\eta^2 = 0.46$).

(Figure IV. 3A). The corresponding Hedges' g effect size for day-14 (-1.27 , 95 % CI = -1.98 to -0.56) confirms a strong impairment of PSII efficiency.

Similarly, ETR_{max} was markedly lower at 18 °C by day 14 ($F(2, 30) = 0.07$, $p < 0.05$, $\eta^2 = 0.39$), whereas control individuals exhibited a recovery trend (Figure IV. 3B). The photosynthetic efficiency (α_{ETR}) also declined at 18° C, indicating reduced light-use capacity ($F(2, 30) = 5.66$, $p < 0.05$) (Figure IV. 3C). Although the photosynthetic saturation irradiance (E_{kETR}) has no significant differences (data not shown). In contrast, maximum non-photochemical quenching (NPQ_{max}) increased significantly at 18° C from day 14 onward ($F(2, 30) = 25.8$, $p < 0.05$, $\eta^2 = 0.51$) (Figure IV. 3D), consistent with enhanced energy dissipation as a protective but transient mechanism. Correlation analysis revealed strong negative relationships between F_v/F_m and NPQ_{max} ($r = -0.78$, $p < 0.01$) and between ETR_{max} and ROS ($r = -0.71$, $p < 0.05$), highlighting the functional coupling between photochemical inhibition and oxidative stress (Figure IV. 3).

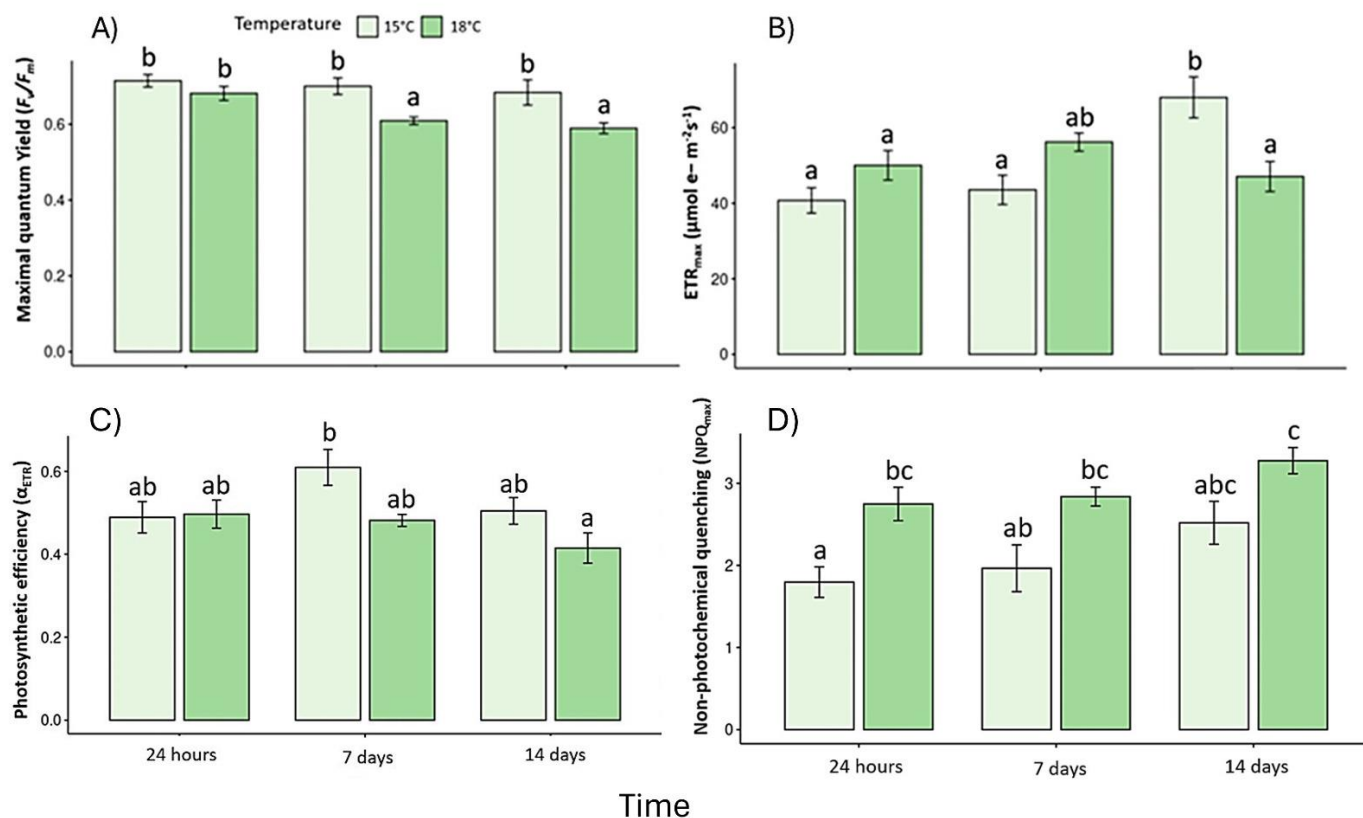


Figure IV. 3. Photophysiological responses: A) Maximal quantum yield (F_v/F_m), B) maximal electron transport rate (ETR_{max}). C) Photosynthetic efficiency (α_{ETR}) and D) Maximal non-photochemical quenching (NPQ_{max}).

4.4.3. Biochemical response

Pigment content declined under elevated temperature (Table S2). Chlorophyll *a* decreased by 32 % and chlorophyll *c* by 27% after 14 days at 18° C ($p < 0.05$) (Figure IV. 4A-B), whereas fucoxanthin declined by 23 %, confirming pigment degradation (Figure IV. 4C). In the same context, photoprotection as phenolic compounds exhibited a biphasic response: a transient rise at 24 h under 18 °C (early stress activation; $F(2, 30) = 13.7$, $p < 0.05$) (Figure IV. 5A-B), followed by a pronounced decrease on day 14. Antioxidant capacity (DPPH assay) dropped by 40 % relative to the control ($p < 0.05$) (Figure IV. 5C), while reactive oxygen species (ROS) (Figure IV. 6A) and malondialdehyde (MDA) (Figure IV. 6B) increased ~60 % and 45 %, respectively ($p < 0.05$) (Figure IV. 6).

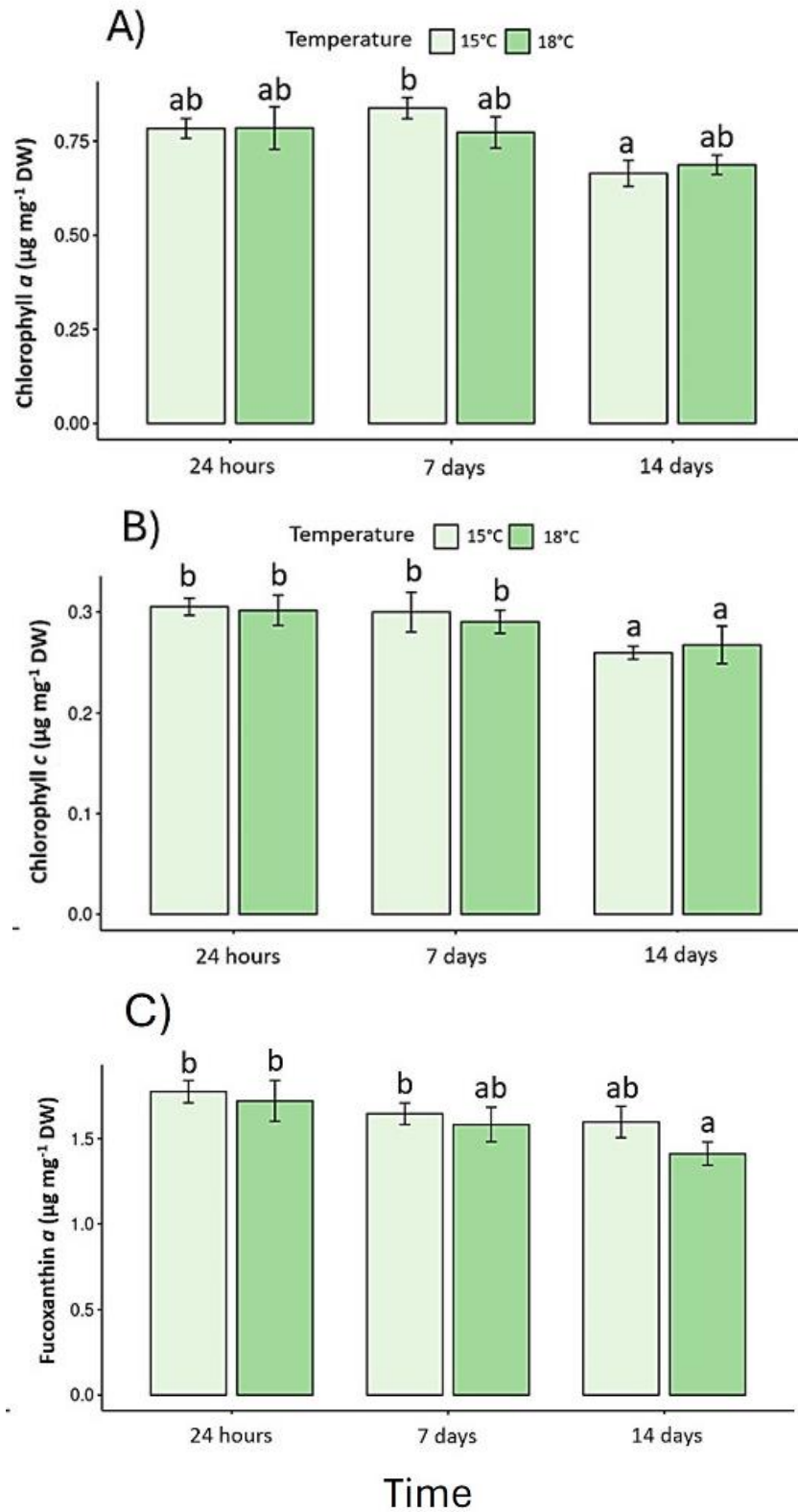


Figure IV. 4. Photosynthetic pigments: A) Chlorophyll *a* and B) Chlorophyll *c* (*c*₁+*c*₂) and C) Fucoxanthin pigment content.

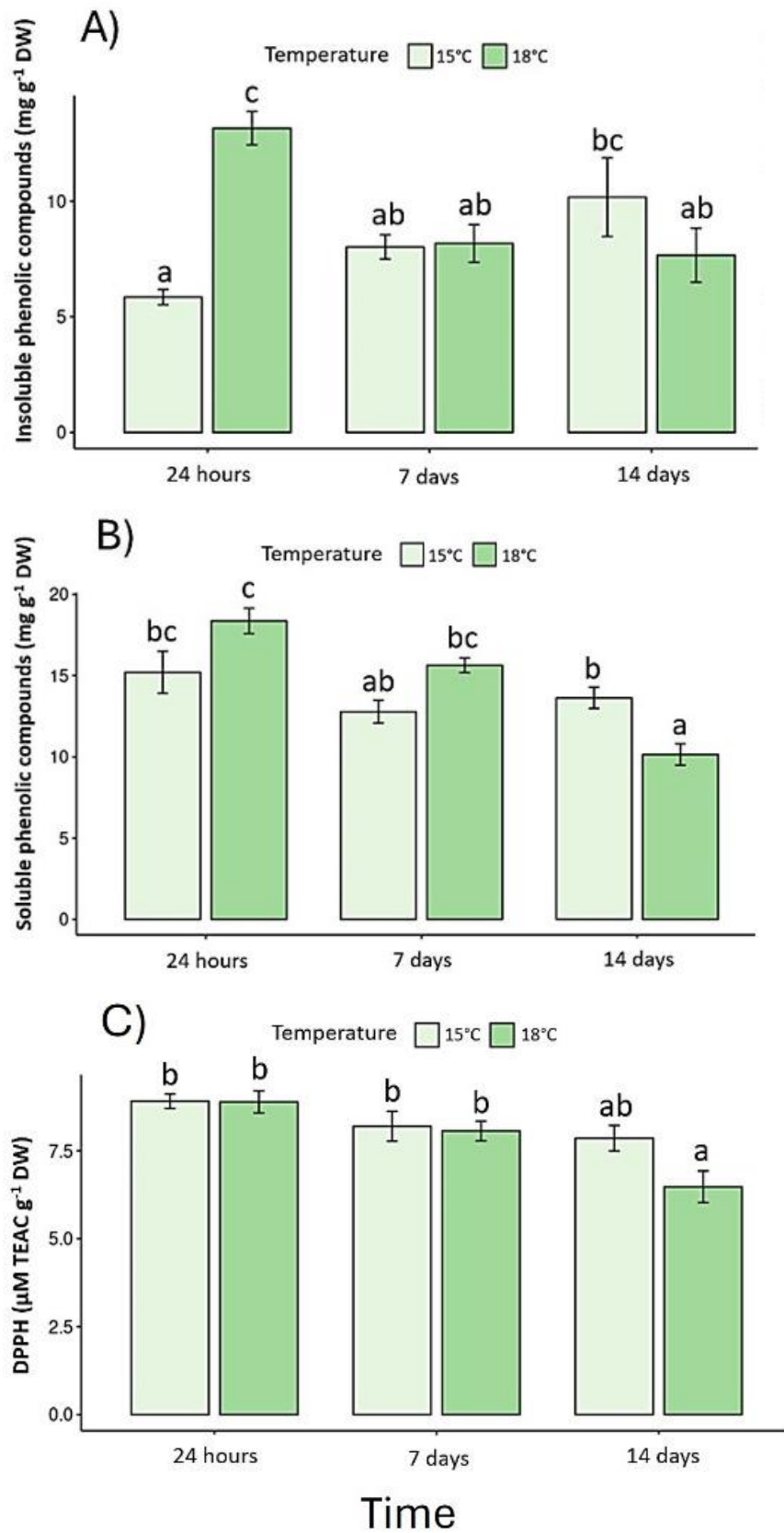


Figure IV. 5. Phenolic compounds: A) Insoluble phenolic content, B) Soluble phenolic content, and C) Total antioxidant capacity, measured by the DPPH assay and expressed as Trolox equivalents.

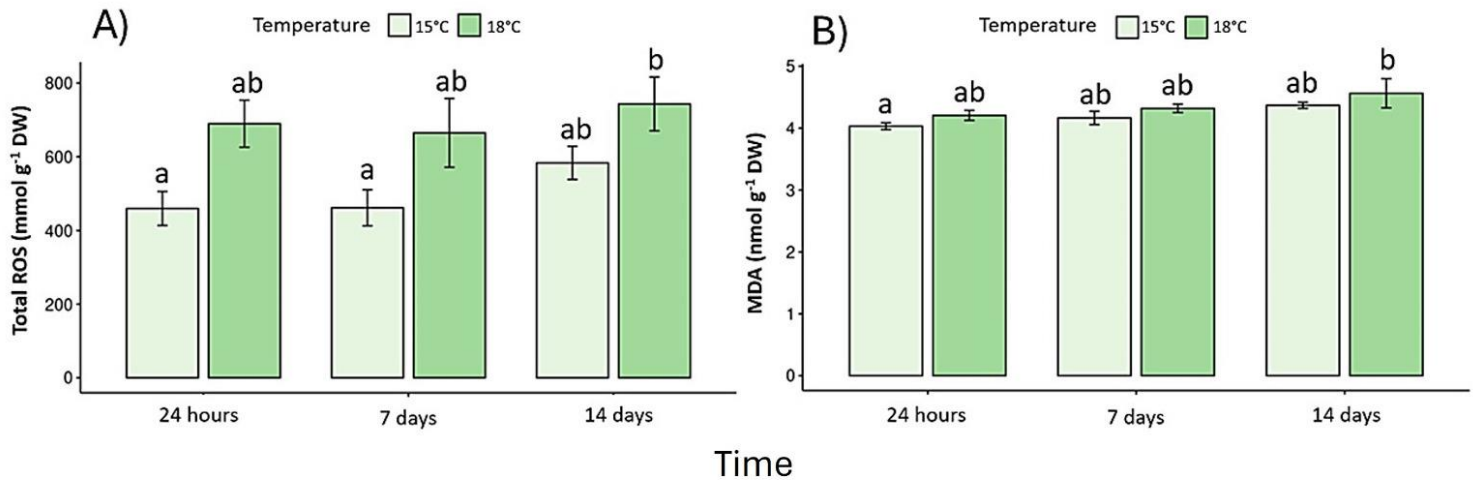


Figure IV. 6. Oxidative stress markers: A) Total Reactive Oxygen Species (ROS) and B) lipid peroxidation (TBARS).

4.5. Discussion

This study provides new experimental evidence of how *L. spicata* responds physiologically and biochemically to short-term thermal stress. Although integrative analyses of photophysiological and biochemical responses remain scarce for kelps from the southeastern Pacific, we simulated a realistic +3 °C increase relative to 15 °C – the mean summer temperature in central Chile – to quantify the immediate physiological and biochemical consequences of moderate warming under controlled laboratory conditions. The objective was explicitly mechanistic and time-bound, providing baseline data to interpret the cellular vulnerability of this species to near-future ocean warming. The multivariate analysis highlights the coordinated impairment of photosynthetic efficiency and redox balance in response to moderate warming. Here, the PCO analysis provides an integrated view of the multivariate stress response in *L. spicata* under

moderate thermal stress. The clear separation of treatments along the first ordination axis demonstrates that even a +3 °C increment triggers a coherent shift in the species' physiological state. The association of F_v/F_m , ETR_{max} , α_{ETR} , and pigment variables with the control treatment reflects efficient photochemical functioning and stable redox balance under ambient temperature. Conversely, the clustering of NPQ, ROS, and MDA vectors with the 18 °C samples highlight the convergence of photochemical down-regulation and oxidative stress as dominant processes under warming. This multivariate pattern supports the interpretation that short-term exposure to elevated temperature elicits a systemic response, coupling photophysiological impairment with biochemical redox disruption. Such integrative ordination evidence reinforces the notion that *L. spicata* exhibits limited short-term resilience to moderate thermal anomalies, aligning with experimental findings in other kelps subjected to comparable stress conditions (Li et al., 2020; Graiff et al., 2021; Narrain et al., 2023).

Our results show a significant impairment of photosynthetic performance in *L. spicata* under moderate thermal stress. The reduction of maximum quantum yield (F_v/F_m) and maximal electron transport rate (ETR_{max}) at 18° C on days 7 and 14, confirm sustained photoinhibition and damage to photosystem II. Such declines in photochemical efficiency have been widely observed in other brown macroalgae exposed to warming, including *Fucus serratus* (Figuerola et al., 2019), *Saccharina* spp. during summer anomalies (Li et al., 2020), and *Turbinaria ornata* under combined light-temperature stress (Narrain et al., 2023). The parallel reduction in α_{ETR} further indicates a decline in light-use efficiency, while the downward trend in E_{kETR} suggests constrained capacity to utilize high irradiance under heat exposure (Zúñiga et al., 2021). The marked increase in non-photochemical quenching (NPQ_{max}) after 14 days reflects an enhanced dissipation of excess excitation energy, which acts as a short-term photoprotective mechanism. Nevertheless, sustained NPQ upregulation typically signals stress compensation rather than successful acclimation (Fukumoto et al., 2019), a pattern consistent with the seasonal NPQ behavior previously described for *L. spicata* across natural gradients (Zúñiga et al., 2021). Altogether, these

physiological responses indicate that *L. spicata* operates near its upper thermal safety margin under moderate warm conditions. In its natural habitat, recurrent exposure to elevated temperatures and marine heatwaves could therefore push this species beyond its photophysiological compensation range, leading to declines in photosynthetic efficiency, carbon assimilation, and canopy recovery during summer peaks.

At the biochemical level, this study revealed consistent pigment degradation under warming. Chlorophyll *a* and *c* concentrations decreased significantly in 18 °C treatment, particularly by day 14. These reductions indicate compromised photosynthetic structures, consistent with those observed in *S. horneri* at >30 °C (Yonh et al., 2021) and *S. stenophyllum* following a 5 °C increase (Urrea-Victoria et al., 2020). The reduction of fucoxanthin key for light harvesting and photoprotection may further weaken the algae's capacity to manage high light under warming (Mabin et al., 2019; Schmid et al., 2021). In the same context, phenolic compounds, which act as non-enzymatic antioxidants, displayed a biphasic response, a transient early increase at 24 h, indicative of rapid activation of defense pathways, followed by a steep decline over time. This depletion coupled with the 40% reduction in overall antioxidant capacity (DPPH), suggests exhaustion of redox buffering mechanisms and the onset of oxidative stress (Cruces et al., 2013; Graiff & Karsten, 2021).

Comparable field patterns have been observed in *L. spicata*, where phenolic and antioxidant levels rise under moderate stress but collapse under extreme irradiance or warming (Celis-Plá et al., 2022). The increase in oxidative stress was evidenced by significantly elevated ROS and MDA levels in 18 °C treatment after 14 days, indicating lipid peroxidation and progressive membrane destabilization. These biochemical markers are reliable indicators of thermal damage in macroalgae, reflecting the breakdown of antioxidant defenses when stress thresholds are exceeded (Narain et al., 2023; Celis-Plá et al., 2025). Similar oxidative impairments have been documented in *S. latissima* under thermal and nutrient stress (LI et al., 2020), and comparable redox imbalances

have been reported in *S. horneri*, *S. stenophyllum*, and *L. digitata* under elevated temperatures, where disruptions in redox balance led to protein degradation, metabolic reallocation and compromised cellular integrity (Urrea-Victoria et al., 2020; Yong et al., 2021). In *S. mcclurei* and *F. vesiculosus*, even moderate warming has been shown to reduce antioxidant capacity and exacerbate ROS accumulation, particularly during prolonged exposures (Mabin et al., 2019; Graiff et al., 2021). Together, these patterns indicate that the antioxidant system of *L. spicata* can transiently buffer stress but becomes depleted under sustained warming, reducing its capacity to mitigate oxidative injury. The convergence of pigment loss, oxidative stress, and increased NPQ reveals a shift from transient photoprotection toward persistent oxidative strain, a process likely to define the upper thermal limits of *L. spicata* under projected warming scenarios (Teagle et al., 2018; Schmid et al., 2021; Ji et al., 2021). Overall, the convergent photophysiological (lower F_v/F_m , ETR_{max} , α_{ETR} ; higher NPQ_{max}) and biochemical signals (lower chlorophylls/fucoxanthin, reduced phenolics/antioxidant capacity, higher ROS and MDA) indicate that *L. spicata* experienced short-term redox and photochemical stress under a +3 °C increment in controlled treatment. In natural populations, similar physiological disruption could reduce productivity, tissue renewal, and overall canopy persistence during recurrent heat anomalies. These findings align with projections of increased frequency and duration of marine heatwaves in the southeastern Pacific, often exceeding +1 °C for > 40 days (Carrasco et al., 2023; Püjol et al., 2023), which may expose *L. spicata* to cumulative physiological damage.

The sustained decline in photochemical performance (F_v/F_m , ETR_{max}) and antioxidant capacity, alongside increased oxidative stress markers (ROS, MDA), suggest that the mechanisms normally used by this species to tolerate environmental variability are insufficient under prolonged moderate thermal stress. Future work should determine whether the short-term photophysiological and biochemical responses documented here persist over longer exposures and whether they co-vary with performance metrics measured independently (e.g., growth, survival, reproduction). Given that *L. spicata* spans ~29°S–48°S along the

Chilean coast (González et al., 2016; Ramírez et al., 2023), future studies comparing northern and southern populations could reveal differential thermal tolerance and local adaptation. Such latitudinal contrasts may also help predict range retractions or shifts under progressive ocean warming. Considering ongoing reports of intensified marine heatwaves and regional warming trends (Carrasco et al., 2023; Püjol et al., 2023), both short- and long-term *in situ* experiments are warranted to evaluate acclimation and adaptive capacity under natural environmental fluctuations. Related work on seasonal variability in photosynthetic quotients under warming scenarios suggests useful methodological avenues for future assessments of primary production, bridging mechanistic physiology with ecosystem-scale consequences (Piticar et al., 2018; Demortier et al., 2022; Franke et al., 2024).

Overall, our endpoints reveal rapid and coordinated cellular responses to moderate warming, establishing a mechanistic foundation for future long-term studies that integrate physiological, demographic, and ecological dimensions of thermal tolerance in *L. spicata*, and by extension, the resilience of kelp forests along the Chilean coast.

4.6. Conclusions

This study provides mechanistic and integrated evidence that a +3 °C thermal increment consistent with the IPCC SSP2-4.5 scenario induces rapid declines in photochemical efficiency, pigment content, and antioxidant capacity in *L. spicata*, leading to oxidative stress within two weeks. By combining effect-size metrics and multivariate analyses, the present revision enhances the mechanistic interpretation of these short-term responses. Although the experiment was limited to a controlled day 14 exposure, the results establish a quantitative physiological baseline from which future long-term and omics-level investigations can be designed. These findings reinforce the ecological concern that projected ocean warming may threaten the persistence and ecosystem functions of this foundational kelp species along the southeastern Pacific coast.

Capítulo V.

The epiphytic microbiome of *Lessonia spicata* responds to moderate ocean warming (+3°C): insights into holobiont sensitivity

Este capítulo se basa en:

Camilo Navarrete, Céline Lavergne, Verónica Molina, Polette Aguilar, Paula S.M. Celis-Plá. The epiphytic microbiome of *Lessonia spicata* responds to moderate ocean warming (+3°C): insights into holobiont sensitivity (en preparación).

5.1. Abstract

Macroalgae are holobionts whose associated microbiomes contribute to host fitness and ecological interactions. Yet, the impacts of ocean warming on the structure and functionality of brown algal epiphytic microbiomes remain poorly understood, particularly for foundational species in the Southern Hemisphere. Here, we assessed the response of the active epiphytic microbiome of the kelp *L. spicata* to a projected warming scenario (SSP2-4.5, +3 °C) under controlled laboratory conditions. Using 16S rRNA transcript sequencing and functional inference (FAPROTAX), we compared microbial communities with those associated with holobionts exposed to elevated temperature (18 °C) and control conditions (15 °C). Thermal stress significantly altered microbial community composition and alpha diversity, leading to shifts in dominant bacterial families and an increase in potentially opportunistic taxa such as Vibrionaceae. These taxonomic changes were accompanied by modifications in predicted functions related to nitrogen cycling and heterotrophic metabolism. Our findings suggest that moderate warming reshapes the ecological interplay within the algal holobiont, potentially compromising host–microbiome stability. Understanding these dynamics is essential for predicting the resilience of kelp holobionts to climate change in temperate coastal ecosystems.

Keywords: *epiphytic microbiome, Lessonia spicata, holobiont sensitivity, ocean warming, 16S rRNA.*

5.2. Introduction

Brown macroalgae of the order Laminariales are key foundational species in temperate coastal ecosystems, where they create three-dimensional habitats that support high biodiversity and numerous ecosystem functions (van der Loos et al., 2019). These ecosystems are increasingly threatened by climate change, particularly due to rising sea temperatures, ocean acidification, and alterations in radiation and nutrient availability, besides direct anthropogenic extractivistic pressure which can cascade through trophic levels and impact benthic productivity (Morrissey et al., 2021; Paix et al., 2022). Kelp forests have been recognized as climate sensitive habitats whose structural integrity and ecological performance are intimately linked to the environmental conditions that shape their physiology and symbiotic interactions (Qiu et al., 2019; Castro et al., 2024).

The surfaces of macroalgae host diverse microbial consortia—comprising bacteria, archaea, fungi, microalgae, and viruses forming a functional holobiont whose balance is essential for host health and physiological performance (Kaur et al., 2023; Egan et al., 2012). These microbiomes contribute to host function by supplying nutrients, defending against pathogens, producing secondary metabolites, and modulating responses to environmental stressors (Goecke et al., 2010; Zozaya-Valdés et al., 2021). Importantly, the holobiont perspective recognizes that macroalgae do not function in isolation, but as meta-organisms whose adaptability depends on both host and microbiome plasticity (Gang Ren et al., 2021; Schmidt & Ghaderiardakani, 2023).

Within this group, *L. spicata* stands out as a dominant species along the rocky intertidal zone of central-southern Chile. Its ecological significance lies not only in its structural contributions but also in its interactions with its complex epiphytic microbial community. However, the composition and functionality of these microbial communities are highly sensitive to environmental changes, particularly to ocean warming scenarios projected under climate change (Morrissey et al., 2021). Elevated temperatures have been shown to alter the composition, richness, and functional roles of epiphytic microbiota in several brown algae

species such as *E. radiata*, *F. vesiculosus*, and *M. pyrifera* (Mancuso et al., 2012; Minich et al., 2018; Qiu et al., 2019; Paix et al., 2022). These shifts may trigger dysbiosis, involving the loss of beneficial taxa and the proliferation of opportunistic bacteria, with deleterious effects on host photosynthesis, immunity, and resilience (Lemay et al., 2021; Delva et al., 2023).

Thermal stress has been linked to increases in Rhodobacteraceae, Flavobacteriaceae, Alteromonadaceae and Vibrionaceae, often associated with pathogenic or saprophytic behavior (Qui et al., 2019; Dobretsov et al., 2021; Wang et al., 2023). Moreover, some non-pathogenic bacteria may act as competitive displacers of beneficial microbes, reducing the abundance of key symbionts such as *Granulosicoccus* or *Loktanella*, thus impairing crucial functions such as secondary metabolite production or oxidative stress modulation (Florez et al., 2019; Ren et al., 2021). Interestingly, environmental stressors may also facilitate the expansion of rare bacterial taxa with previously cryptic roles, potentially reshaping microbiome-host interactions in unpredictable ways (Mensh et al., 2016; Morrissey et al., 2021).

These compositional shifts may not only reflect ecological imbalance but also trigger functional disruptions that compromise host development and resistance to stress. For instance, increased thermal stress has been associated with microbiome-mediated loss of photoprotection and nutrient exchange efficiency, as reported in *S. latissima* and *Cystoseira compressa* (Ghaderiardakani et al., 2020; Burgunter-Delamare et al., 2023). In some cases, the presence of stress-tolerant but functionally disruptive taxa may outcompete symbiotic bacteria, destabilizing the holobiont. This phenomenon has also been observed under synergistic stress scenarios involving temperature and high irradiance (Paix et al., 2022). Furthermore, studies suggest that the capacity for microbiome reassembly post-disturbance varies greatly among algal species and is influenced by factors such as life history traits, microbial recruitment specificity, and environmental context (Goecke et al., 2010; Comba-Gonzalez et al., 2017).

The adaptability of the algal holobiont to environmental stress is partially determined by the plasticity and stability of its associated microbiome. In species such as *S. latissima* and *Fucus distichus*, resilience to temperature stress has been linked to the conservation of core microbial taxa and functional redundancy within the epiphytic community (Singh & Reddy, 2010; Smolina et al., 2016). However, little is known about how the epibiotic microbiome of *L. spicata* responds to projected warming scenarios under moderate climate projections such as the SSP2-4.5 scenario (+3 °C).

Given its distribution in the intertidal zone of central Chile and its continuous exposure to environmental variability, understanding whether *L. spicata* harbors a thermally resilient microbiome is critical. Furthermore, characterizing potential changes in microbial community composition and diversity may provide early indicators of dysbiosis and offer insight into the mechanisms underpinning holobiont resilience to climate change (Lopez et al., 2018; Ramirez-Puebla et al., 2020). Previous studies in the region have demonstrated taxonomic and functional shifts in epibiotic bacterial assemblages of *Durvillaea* and *Macrocystis* spp., yet *Lessonia* remains largely underexplored in this context (Lata Singh & Reddy, 2010; Lopez et al., 2018).

In this study, we evaluated the effects of a +3 °C increase in seawater temperature (IPCC SSP2-4.5 scenario) on the structure of the active epibiotic microbiome of *L. spicata* using 16S rRNA transcripts metabarcoding. We aimed to identify potential changes in taxonomic richness, community composition, and the emergence of opportunistic or mutualistic bacterial groups under controlled laboratory conditions. Based on previous evidence of thermal sensitivity in macroalgal holobionts, we hypothesized that elevated temperature would alter the diversity and composition of the *L. spicata* microbiome, favoring the increase of stress-tolerant or opportunistic taxa while reducing mutualistic groups. This integrative approach provides critical insight into the role of microbial communities in modulating *L. spicata* responses to thermal stress and their potential as indicators of ecological change under future climate scenarios.

5.3. Materials and Methods

5.3.1. Sample collection and experimental design

To evaluate the effects of thermal stress on the epibiotic microbiome of the brown macroalga *L. spicata*, three experimental conditions were considered. *In situ* samples were collected directly from the intertidal zone at Playa Cochoa (32°57'19"S; 71°32'52"W, Valparaíso, Chile). The Control condition corresponded to individuals maintained under laboratory conditions at 15 °C for 14 days, representing the average ambient temperature. The Treatment condition consisted of individuals maintained for 14 days at 18 °C, following previous studies that used comparable incubation periods to detect thermal and microbiome responses in kelps (Minich et al., 2018; Qiu et al., 2019; Vadillo Gonzalez et al., 2024). After incubation, algal tissue and its epiphytic microbiome were carefully sampled, flash-frozen in liquid nitrogen, and stored at –80 °C until further RNA extraction and sequencing analyses.

5.3.2. RNA Extraction, DNase Treatment, cDNA Synthesis and 16S rRNA Gene Amplification and Sequencing

Total RNA was extracted using the mirVana™ miRNA Isolation Kit (Thermo Fisher Scientific), following a modified protocol optimized for microbial RNA recovery (Molina et al., 2016). RNA concentration was quantified using the Qubit RNA High Sensitivity Assay Kit (Thermo Fisher), and residual genomic DNA was removed with TURBO DNase (Thermo Fisher). RNA integrity was verified via agarose gel electrophoresis. First-strand cDNA synthesis was performed using the ImProm-II™ Reverse Transcription System (Promega) with random hexamer primers, following the manufacturer's instructions.

5.3.3. Sequence Processing and Taxonomic Assignment (QIIME2)

Raw reads were processed using QIIME2 v2024.2. Paired-end sequences were imported using the “qiime tools” import function with the “PairedEndFastqManifestPhred33V2” format. Quality filtering, denoising, chimera removal, and merging of paired reads were performed using the “dada2 denoise-paired” plugin (Callahan et al., 2016), yielding amplicon sequence variants (ASVs) with an average length of ~244 bp. Taxonomic classification was performed using a Naive Bayes classifier trained with the SILVA 138 reference database (SSURef NR99, full-length sequences), implemented through the “classify-consensus-vsearch” plugin in QIIME2 (Rognes et al., 2016).

5.3.4. Taxonomic Composition, Relative Abundance and Functional Inference

Relative abundance was calculated per sample and visualized using stacked bar plots (Family level) and heatmaps (Class level) using the ampvis2 package. Ten most abundant taxa were highlighted, while rare groups were grouped under “Others.” All plots were depicted using a green-to-purple pastel color gradient to reflect increasing thermal stress across treatments. Putative microbial metabolic functions were inferred using the “microeco” package in R, based on the FAPROTAX database. Functional annotations were assigned to ASVs using the “trans_func” object, and their relative proportions across thermal treatments were visualized as a clustered heatmap “plot_spe_func_perc”. This analysis aimed to detect functional shifts associated with experimental warming.

4.3.5. Statistical analysis

Statistical analyses were performed in R v4.2.2 using the packages phyloseq, ampvis2, vegan, DESeq2, and microeco. *Alpha and Beta Diversity*. The ASV table was rarefied (Schloss, 2024) to an even sequencing depth (50,000 reads per sample) using rarefy_even_depth (phyloseq). Alpha diversity metrics (Observed

Richness, Shannon Index, and Pielou's Evenness) were calculated and plotted using ggplot2. Differences between thermal treatments were tested using one-way ANOVA or Kruskal–Wallis tests, depending on data distribution (Shapiro–Wilk) and variance homogeneity (Levene's test). Beta diversity was assessed using Bray–Curtis dissimilarity and visualized through Redundancy Analysis (RDA) using ordinate and plot ordination (phyloseq). Finally, significantly differential analyses were run using DESeq2 to identify distriminant ASVs between the following: (i) *in situ* vs Control, (ii) *in situ* vs Treatment, and (iii) Control vs Treatment (Love et al., 2014). Significant ASVs were defined as those with FDR-adjusted p-values < 0.05 and $|\log_2\text{FoldChange}| > 1$. Results were visualized through volcano plots with taxonomic labels added using ggrepel.

5.4. Results

5.4.1. Alpha and Beta Diversity Patterns Under Thermal Stress

Alpha diversity indices (Figure V.1) showed that bacterial richness and diversity varied notably across thermal conditions. Observed richness index was significantly higher in the Treatment group (18 °C) compared to the *in-situ* samples, suggesting an enrichment of active microbial groups under thermal stress. Control samples at 15 °C displayed intermediate values that did not differ significantly from either the *in situ* or Treatment groups.

Shannon diversity and Pielou's evenness followed a similar pattern: both indices were significantly greater in the Control and Treatment groups compared to the *in-situ* condition, yet no differences were observed between the two laboratory conditions. These results imply that experimental thermal environments, even under mild warming, support more taxonomically rich and evenly distributed microbial communities than those found in the natural setting, potentially due to reduced environmental fluctuations or shifts in microbial colonization dynamics.

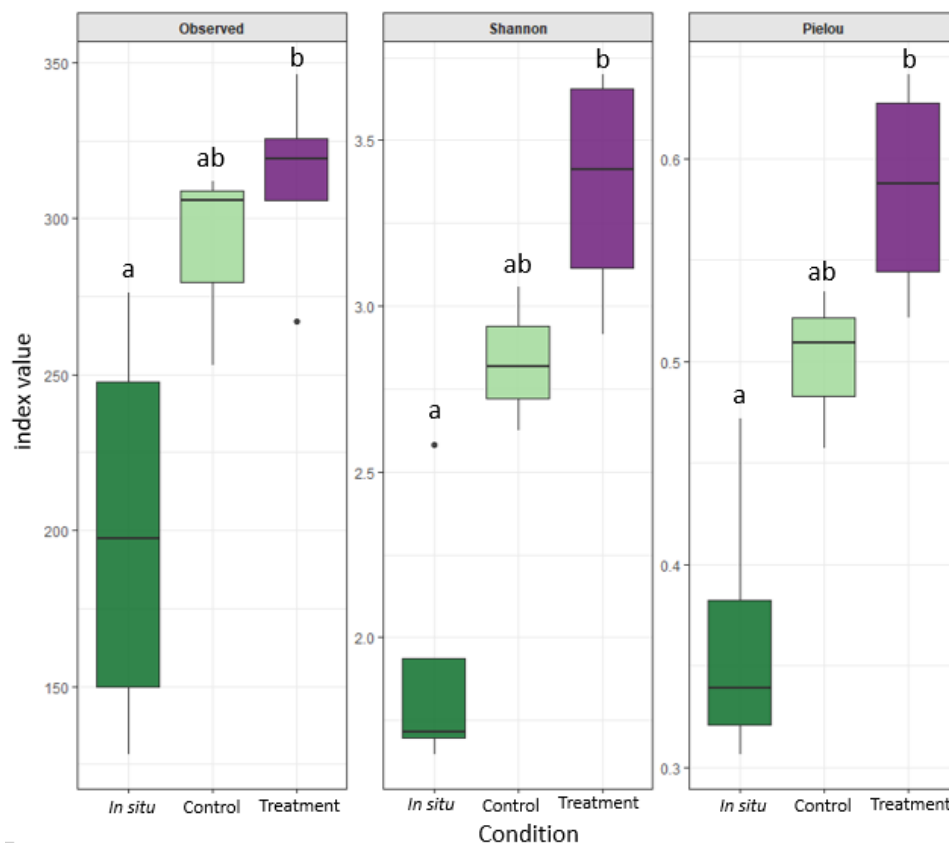


Figure V.1: Alpha diversity metrics of the active epibiotic bacterial community associated with *L. spicata* under three thermal conditions: *in situ* (natural environment), control (15 °C), and treatment (18 °C). Boxplots represent Observed Richness (a), Shannon Index (b), and Pielou's Evenness (c). Significant differences between treatments are indicated by different letters (ANOVA or Kruskal–Wallis, $p < 0.05$).

Beta diversity analysis based on Bray–Curtis distances (Figure V.2) revealed distinct microbial community clustering among the three temperature conditions. The *in-situ* samples clustered together indicate a relatively stable and homogeneous microbial composition in the natural environment. In contrast, both Control and Treatment samples exhibited wider dispersion, reflecting more heterogeneous microbial communities under laboratory conditions. PERMANOVA analysis confirmed that the differences in community composition between all three groups were statistically significant ($p < 0.05$), highlighting the role of thermal regimes in driving microbial community differentiation.

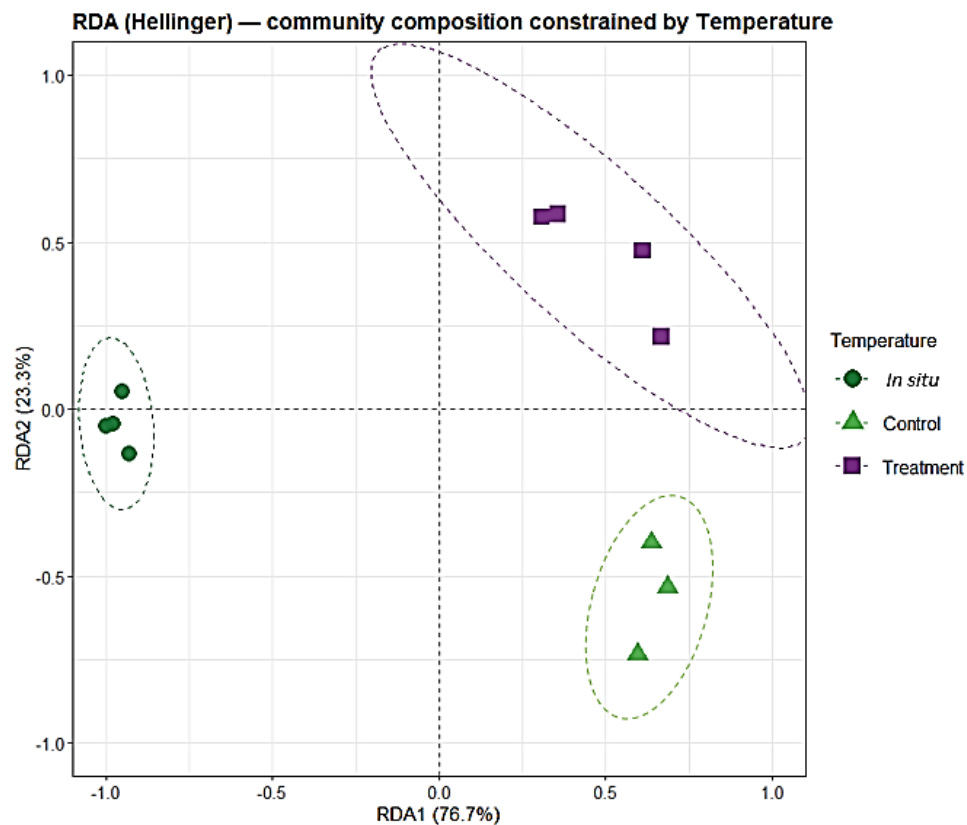


Figure V.2: Redundancy Analysis (RDA) of Bray–Curtis dissimilarities illustrating the separation of microbial communities associated with *L. spicata* under different thermal conditions. Arrows represent the influence of temperature gradients. Ellipses denote 95% confidence intervals for each group.

5.4.2. Relative abundance of bacterial classes

Across all conditions, including the *in-situ* samples, the epiphytic microbiome of *L. spicata* was consistently dominated by members of Gammaproteobacteria and Anaerolineae, indicating that these groups represent a stable core across treatments. Beyond this shared dominance, marked differences emerged between the Control and Treatment conditions (Figure V.3). In the Control group, communities were largely characterized by Anaerolineae and Gammaproteobacteria, with limited representation of other classes. In contrast, the Treatment condition exhibited higher relative abundance of Bacteroidia, Alphaproteobacteria, and Verrucomicrobiae. Additionally, classes such as Desulfurovibrionia and Planctomycetes were detected exclusively under elevated temperature, although at low relative abundance. While Control samples showed relatively uniform microbial profiles, greater compositional variability was observed among Treatment samples, suggesting that warming promotes less stable epiphytic microbiome structures.

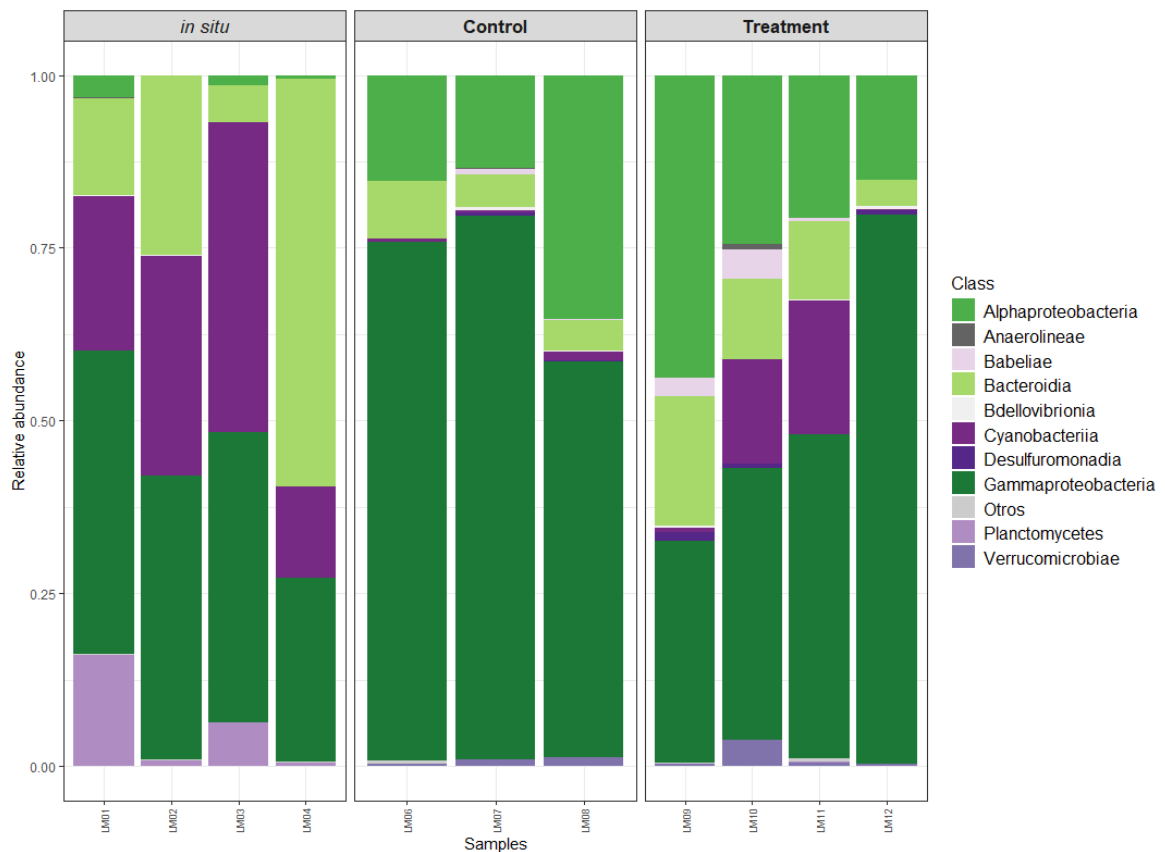


Figure V.3: Taxonomic composition and relative abundance of bacterial families associated with *L. spicata* under different thermal conditions. Barplots represent the 10 most abundant families across *in situ*, Control, and Treatment samples. Rare families were grouped under “Others.” The color gradient from green to purple indicates increasing thermal stress.

5.4.3. Family-level heatmap of bacterial taxa

The heatmap at the family level illustrates specific taxonomic shifts associated with thermal stress (Figure V.4). Alteromonadaceae remained highly abundant under both Control and Treatment conditions (45.9% and 47.1%, respectively). In contrast, Granulosicoccaceae, which accounted for 50.3% of relative abundance in the *in situ*, was almost absent under Treatment (0.7%). Other families such as Flavobacteriaceae, Micavibrionaceae, and Rhodobacteraceae increased their abundance under elevated temperature (12.4%, 12.7%, and 12.5%, respectively). Pirellulaceae was present in Control samples (13.9%) but nearly absent under Treatment (0.1%), whereas families like Hyphomonadaceae, Rubritaleaceae, and

Arenicellaceae increased slightly under warming. Most rare families remained below 1% relative abundance across both conditions.

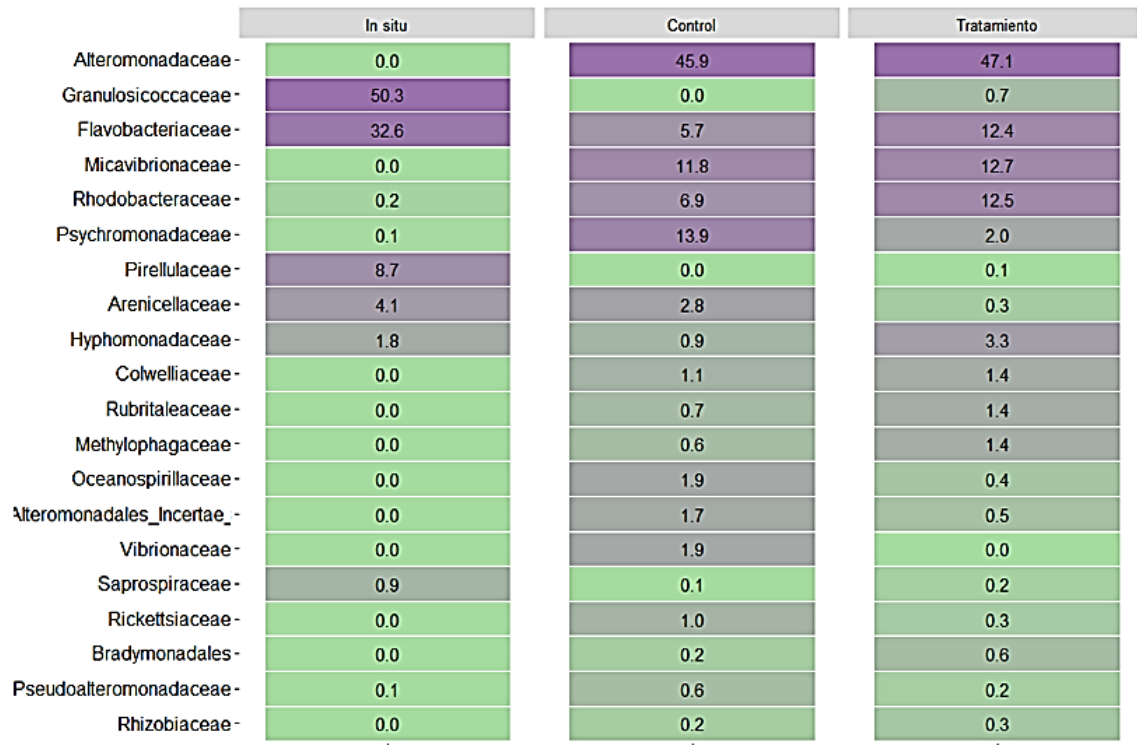


Figure V.4: Heatmap showing the relative abundance of dominant bacterial classes in the epibiotic microbiome of *L. spicata*. Samples are clustered by condition: *in situ*, Control (15 °C), and Treatment (18 °C). Only classes with relative abundance >1% in at least one sample are shown. Color intensity reflects abundance (pastel green to purple scale).

5.4.4. Differentially Abundant Taxa Between Control and Treatment

The differential abundance analysis based on DESeq2 (Figure V.5) identified specific bacterial genera whose relative abundance shifted significantly between the Control (15 °C) and Treatment (18 °C) groups. Among the taxa that increased under warming, *Vibrio* and *Aquimarina* showed the most pronounced responses, with elevated log2 fold changes in the Treatment group. In contrast, several genera decreased under thermal stress, including *Granulosicoccus*, *Sulfitobacter*, *Zobellia*, and *Maribacter*. These results highlight that even moderate warming (+3 °C) was sufficient to restructure the dominance patterns of multiple members of the epibiotic community on *L. spicata*.

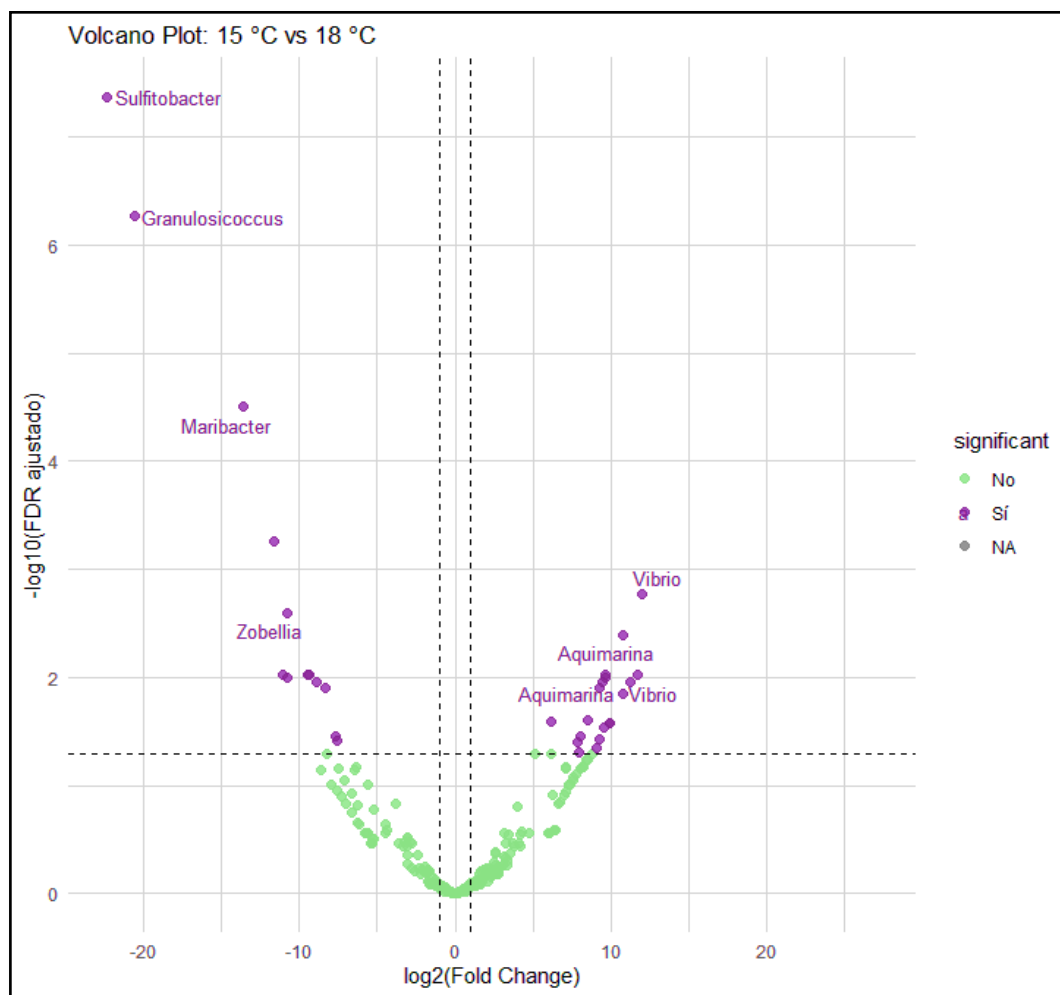


Figure V.5: Volcano plots displaying differentially abundant ASVs between experimental conditions: Control vs Treatment. Significant ASVs were defined by adjusted p-value (FDR) < 0.05 and $|\log_2 \text{Foldchange}| > 1$. Labeled genera highlights ASVs with the highest effect sizes

5.4.5. Predicted microbial functional profiles

The heatmap of predicted metabolic functions based on FAPROTAX annotations shows shifts in functional profiles between Control and Treatment (Figure V.6). In both conditions, photoheterotrophy and aerobic chemoheterotrophic were the most prominent functions. However, Treatment samples showed a general reduction in functions related to nitrogen cycling, such as nitrogen respiration and nitrate reduction. Minor differences were observed in other functions, including increased representation of intracellular parasitism and chemoheterotrophic

strategies under warming. Functions associated with oil bioremediation, methanotrophic, and sulfur metabolism were detected in both conditions but at low relative abundances.

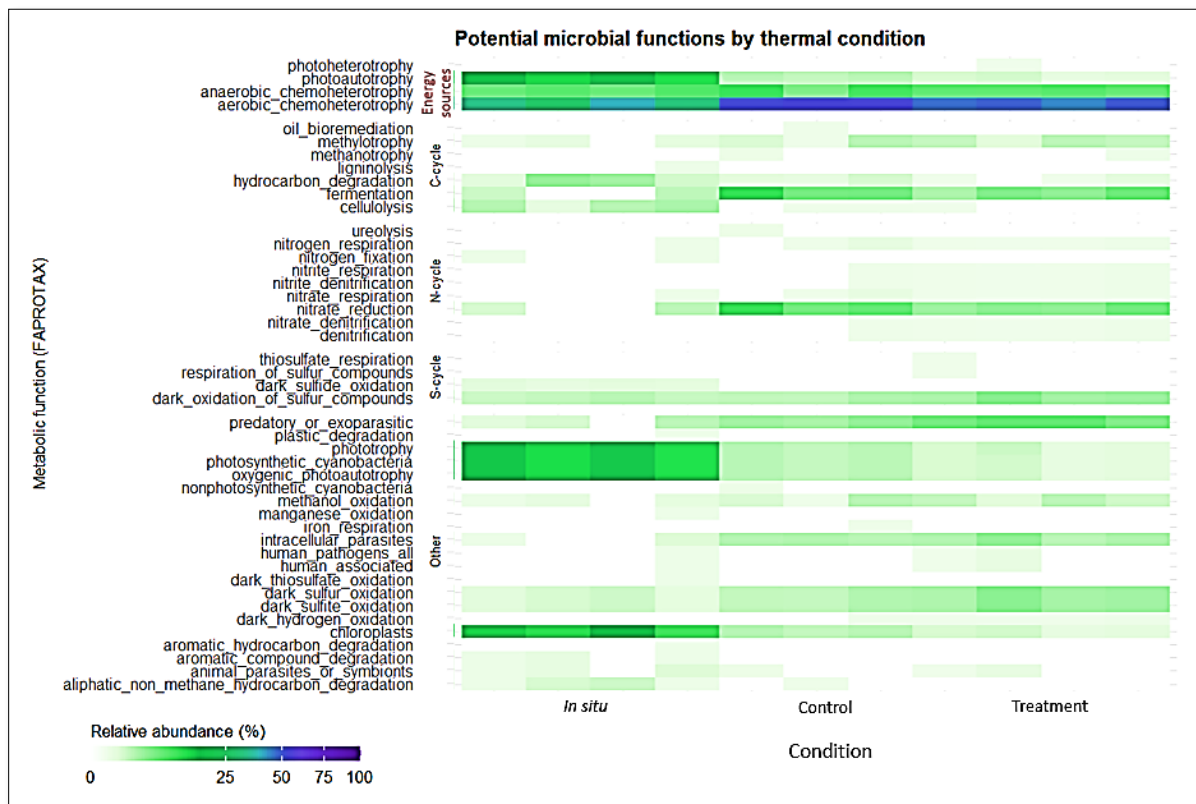


Figure V.6: Functional annotation of epibiotic bacterial communities of *L. spicata* inferred using the FAPROTAX database. The heatmap shows the relative abundance of predicted microbial functions across *in situ*, Control, and Treatment groups. Clustering is based on Bray–Curtis dissimilarity, with stress levels indicated by a green–purple gradient.

5.5. Discussion

The enrichment of *Vibrio* and *Aquimarina* under elevated temperature is consistent with previous research showing that warming and marine heatwaves promote opportunistic or stress-tolerant taxa in kelp-associated microbiomes (Qiu et al., 2019; Düsedau et al., 2023). In *E. radiata* and *Gracilaria vermiculophylla*, for example, elevated temperatures favored *Vibrio* and other potential pathogens,

leading to community-level dysbiosis (Minich et al., 2018; Düsedau et al., 2023). Conversely, the decline of *Granulosicoccus*, *Sulfitobacter*, *Zobellia*, and *Maribacter* may represent the loss of beneficial symbionts typically linked to vitamin synthesis, oxidative stress regulation, and polysaccharide degradation (Vila Duplá et al., 2025). Similar reductions of mutualistic taxa have been described in *Macrocystis* and *Durvillaea* under thermal stress (Vadillo González et al., 2024). Collectively, these shifts suggest that warming not only acts as a strong environmental filter shaping bacterial assemblages but may also compromise holobiont stability by eroding functional mutualisms while favoring opportunistic taxa.

Our findings reveal that an increment of +3 °C of sea surface temperature as predicted by the intermediate SSP2-4.5 scenario leads to noticeable shifts in community composition, diversity, and inferred microbial functions, aligning with growing evidence that kelp-associated microbiomes are highly responsive to climate-related stressors (Qiu et al., 2019; Paix et al., 2022). In this study, the results provide a novel insight into how moderate thermal stress, consistent with the IPCC's SSP2-4.5 scenario, can significantly alter the structure and functional potential of the epibiotic microbiome of *L. spicata*.

The taxonomic analyses showed that bacterial assemblages from fronds exposed to elevated temperatures were dominated by families such as Rhodobacteraceae, Alteromonadaceae, and Vibrionaceae, which have been previously associated with dysbiosis, pathogenicity, or opportunistic behaviors in marine holobionts (Dobretsov et al., 2021; Wang et al., 2023). Members of *Vibrio* sp. were more abundant at 18 °C, consistent with reports in other brown macroalgae under stress conditions (Qiu et al., 2019). These changes were accompanied by a reduction in beneficial or functionally relevant taxa such as *Granulosicoccus*, *Sulfitobacter*, *Zobellia*, and *Maribacter*. *Granulosicoccus* has been described as a core symbiont in kelps, contributing to vitamin B12 provision and oxidative stress regulation (Minich et al., 2018; Vila Duplá et al., 2025). *Sulfitobacter* plays a role in sulfur cycling and the production of bioactive compounds that can support algal health (Wang et al., 2023; Vila Duplá et al., 2025). *Zobellia* is specialized in the

degradation of algal polysaccharides and contributes to carbon turnover in the holobiont (Schmidt & Ghaderiardakani, 2023). Similarly, *Maribacter* spp. are commonly found in association with brown algal surfaces and are involved in polysaccharide metabolism and potential defense interactions (Burgunter-Delamare et al., 2023). Their decline under elevated temperature supports previous observations that warming can disrupt stable host–microbiome associations (Delva et al., 2023; Schmidt & Ghaderiardakani, 2023). Importantly, experimental studies have shown that pathogenic or opportunistic bacteria such as *Vibrio* may replace mutualistic groups involved in protective or metabolic functions when hosts are thermally stressed (Minich et al., 2018; Düsedau et al., 2023; Vila Duplá et al., 2025).

Alpha diversity patterns further highlighted the effect of thermal stress, with significantly higher richness and diversity in the treatment condition compared to *in situ* samples (Qiu et al., 2019; Düsedau et al., 2023). Rather than indicating recruitment of entirely new taxa, this pattern likely reflects the enrichment or increased activity of bacterial groups already present in the community (Minich et al., 2018; Vila Duplá et al., 2025). It is also important to note that some of the additional taxa detected in both Control and Treatment may reflect mesocosm-related effects, such as transport and seawater renewal, whereas the consistent increase of specific genera at 18 °C more reliably indicates a response to thermal stress. Similar patterns have been observed in *M. pyrifera* and *D. antarctica*, where warming has been linked to shifts from host-associated core microbiota to more variable assemblages (Pearman, 2023; Castro, 2024). While increased diversity is not inherently negative, under warming conditions it can indicate community destabilization or colonization by opportunistic taxa (Mensch et al., 2016; Morrissey et al., 2021).

Similar functional shifts have been observed in *S. latissima* exposed to projected ocean warming, where increases of just a few degrees above present conditions are expected to destabilize host–microbiome interactions (Burgunter-Delamare et al., 2023). Experimental work has shown that an increment of **+3 °C** can impair

the capacity of epiphytic bacteria to buffer thermal stress in brown seaweeds (Delva, 2023). Moreover, it has been proposed that, in addition to warming, other climate change drivers such as acidification could further modulate algal–microbiome associations, although temperature remains the primary factor influencing microbial reconfigurations (Ghaderiardakani et al., 2020). Collectively, these findings indicate that kelp-associated microbiomes undergo conserved functional reconfigurations, with potential increases in microbial processes related to nitrate reduction, sulfur metabolism, and fermentation under elevated temperature.

The implications of such changes for host performance remain unclear, particularly in terms of nutrient cycling and resistance to oxidative stress (Minich et al., 2018; Singh & Reddy, 2014; Vila Duplá et al., 2025). Although beta diversity patterns indicated distinct clustering among thermal treatments, suggesting compositional reorganization, the extent to which this reflects functional dysbiosis remains to be fully elucidated (Mensch et al., 2020). Previous research has shown that functional redundancy can buffer against taxonomic shifts, yet such resilience may be compromised in species with narrow microbial recruitment (Smolina et al., 2016; Comba-Gonzalez et al., 2017). In contrast to better-studied kelps, *L. spicata* occupies intertidal zones with fluctuating conditions, possibly preconditioning it for moderate stress. However, the emergence of *Vibrio* spp. and other opportunistic taxa at elevated temperatures suggests a shift in the balance of the microbiome, where stress-tolerant and potentially pathogenic bacteria may replace mutualistic groups involved in protective or metabolic functions (Düsedau et al., 2023; Vila Duplá et al., 2025). Such changes could compromise host stability in the long term, particularly if higher temperatures persist. Studies in *Durvillaea* and *Fucus* species have highlighted similar patterns, emphasizing the role of temperature in selecting microbial consortia with differing ecological roles (Lemay et al., 2021; Ren et al., 2021).

5.6. Conclusions

In conclusion, our results support the hypothesis that rising temperatures may not only shift microbial community structure but also impact their functional contributions to the host. This reinforces the need to include microbiome assessments in vulnerability frameworks for kelp forest conservation. Moreover, our findings serve as a foundation for exploring microbial indicators of stress and resilience in *L. spicata*, contributing to a broader understanding of kelp holobiont adaptability under climate change scenarios. Indeed, this study provides new evidence that moderate thermal stress (+2.7 °C, SSP2-4.5 scenario) can induce notable shifts in the epibiotic microbiome of *L. spicata*, even over relatively short exposure periods. The observed enrichment of microbial groups, including *Vibrio*, suggests that warming can destabilize the microbial balance on algal surfaces, potentially leading to dysbiosis or altered holobiont function. These findings align with previous reports in other kelps and reinforce the notion that the microbiome is a dynamic and sensitive component of macroalgal responses to environmental change. As such, the microbial community may serve not only as a sensor of ecological stress but also as an active modulator of host resilience. Future research should explore the functional consequences of these taxonomic shifts, including metabolomic profiling and time-series analyses, to better understand the ecological trajectories of kelp holobionts under climate change scenarios.

Capítulo VI.

6. Discusión final

Esta tesis buscó responder cómo *L. spicata* enfrenta estresores asociados al cambio climático, considerando que sus respuestas no se limitan al nivel celular o fisiológico del alga, sino que también involucran a las comunidades microbianas asociadas. La hipótesis planteó que el hospedero exhibiría ajustes moleculares y fisiológicos frente a la radiación particularmente en procesos vinculados a la fotosíntesis y a la fotoprotección y respuestas bioquímicas bajo condiciones de estrés, mientras que un aumento moderado de la temperatura (+3 °C, escenario SSP2-4.5) afectaría tanto la fisiología del alga como la estructura de su microbioma epífito.

6.1 Radiación estacional (PAR+UVR): ajustes ecofisiológicos en *L. spicata*

L. spicata enfrenta en la zona intermareal fluctuaciones bruscas de radiación solar que se intensificarán bajo escenarios de Cambio Climático (Kataria et al., 2014; Morales & Kaiser, 2020). La radiación PAR y UVR constituye un factor clave en la regulación de su desempeño fotofisiológico, bioquímico y molecular, y a nivel global se ha reconocido que los efectos de la radiación UV sobre ecosistemas marinos interactúan fuertemente con el Cambio Climático, alterando la productividad, la composición y la resiliencia de las comunidades costeras (Neale et al., 2023).

En este contexto, se evaluó cómo varían las respuestas fotofisiológicas de *L. spicata* frente a irradiancia estacional. Los resultados muestran un patrón de tolerancia caracterizado por la estabilidad del rendimiento cuántico máximo (F_v/F_m), lo que confirma su capacidad para sostener la eficiencia fotosintética bajo condiciones de alta radiación, como se ha descrito previamente en gradientes naturales (Zúñiga et al., 2021; Celis-Plá et al., 2022). Sin embargo, la máxima productividad no se registró en verano, cuando la radiación fue más intensa, sino en primavera, lo que sugiere un ajuste estacional a la disponibilidad lumínica.

Durante el verano se observó un marcado incremento del NPQ, reflejando que el exceso de energía fue destinado a disipación térmica y a fotoinhibición reversible, un patrón concordante con mecanismos descritos en otras algas pardas intermareales (Cruces et al., 2017; Delebecq et al., 2015).

Las respuestas pigmentarias y de las antenas fotosintéticas evidenciaron modificaciones estructurales en el aparato fotosintético. Mientras la clorofila *a* se mantuvo relativamente constante, la clorofila *c* disminuyó en verano, lo que redujo la razón Chl *c*/Chl *a* y sugiere una reducción del tamaño de la antena colectora de luz. Este ajuste coincide con estrategias de aclimatación a irradiancia elevada descritas en macroalgas (Charan et al., 2022; Endo et al., 2017, 2023; Raven & Geider, 2003). En paralelo, el aumento de NPQ evidenció una mayor inversión en disipación no fotoquímica, lo que limita la sobreexcitación del PSII y refuerza la capacidad de estas algas para resistir pulsos de alta radiación, en concordancia con observaciones en gradientes latitudinales de algas pardas donde los compuestos fotoprotectores cumplen un rol central (Schmitz et al., 2018).

A nivel bioquímico, *L. spicata* activó defensas antioxidantes y fotoprotectoras, con acumulación de compuestos fenólicos bajo alta PAR+UV (Gómez et al., 2016; Gómez & Huovinen, 2020; Celis-Plá et al., 2022). Este patrón, que también se ha documentado en *M. pyrifera*, resalta la relevancia de metabolitos secundarios en la fotoprotección. Sin embargo, el aumento de MDA en verano sugiere que la carga oxidativa puede superar las defensas antioxidantes, conduciendo a peroxidación lipídica y daño celular, un efecto indirecto de las ROS descrito en *L. spicata* y en otras algas pardas expuestas a radiación UVB (Cruces et al., 2017; Zúñiga et al., 2021; Polo & Chow, 2022).

Las respuestas moleculares refuerzan esta visión de ajuste dinámico. Se observaron inhibiciones transitorias en genes de los fotosistemas I y II (*psa*, *psb*) y de biosíntesis de pigmentos (*chlB*, *chlI*, *chlN*) bajo irradiancia elevada, lo que refleja una estrategia de ahorro energético y protección de la maquinaria fotosintética, análoga a patrones descritos en Kelps y en plantas vasculares

sometidas a exceso de luz (Heinrich et al., 2012; Buschmann et al., 2014; An et al., 2022; Li et al., 2022; Su et al., 2022). La regulación diferencial de *psbA* (D1) y la sobreexpresión de *dnaK* (Hsp70) son consistentes con un estado de “stress-readiness” descrito en *F. vesiculosus* y en microalgas expuestas a UV (Creis et al., 2015; Cao et al., 2019). Estos ajustes se alinean con la evidencia transcriptómica en algas pardas y microalgas antárticas, donde la activación de rutas de reparación, peroxisomas y defensas antioxidantes sostiene la funcionalidad bajo irradiancia extrema (Gylle et al., 2013; Poong et al., 2018; Zhang et al., 2020; Zhang et al., 2023; Lv et al., 2024).

En conjunto, estos resultados muestran que *L. spicata* despliega una respuesta integrada frente a la radiación estacional: reorganiza su aparato fotosintético reduciendo el tamaño de la antena, activa metabolitos fenólicos y defensas antioxidantes, e implementa una regulación génica fina para balancear eficiencia y costos. Esta plasticidad permite mantener estable el Fv/Fm, incluso bajo irradiancia elevada. No obstante, la acumulación de biomarcadores de daño bajo condiciones extremas de verano y la evidencia de otras macroalgas pardas expuestas a UV (Roleda et al., 2006; Schmitz et al., 2018) sugieren que los márgenes de seguridad son estrechos. Ante la intensificación proyectada de episodios de radiación extrema en el marco del Cambio Climático, la eficacia de estas respuestas podría verse comprometida, subrayando la necesidad de integrar fotofisiología, bioquímica y regulación génica en escenarios naturales y de afloramiento estacional en la costa chilena.

6.2 Respuestas del holobionte de *Lessonia spicata* bajo escenarios de calentamiento oceánico

Como especie fundacional, *L. spicata* cumple un rol clave en la estructuración de comunidades costeras (Gómez et al., 2016; Zúñiga et al., 2021; Celis-Plá et al., 2022, 2025). Evaluar su sensibilidad frente al calentamiento constituye un paso necesario para comprender la estabilidad de los ecosistemas dominados por Kelps en escenarios de cambio climático. En este trabajo se abordó un vacío

relevante al analizar de manera integrada sus respuestas fisiológicas, bioquímicas y microbiológicas bajo un incremento térmico moderado de +3° C, en concordancia con el escenario SSP2-4.5 proyectado por el IPCC (2023). La vulnerabilidad de *L. spicata* a este nivel de calentamiento plantea preocupaciones respecto de la resiliencia futura de estos bosques marinos (Rosenfeld et al., 2019; Schmid et al., 2021; Onitsuka, 2024).

El desempeño fotofisiológico mostró un deterioro sostenido bajo estrés térmico. El declive progresivo de F_v/F_m en los días 7 y 14 a 18 °C reflejó una fotoinhibición persistente y un daño acumulado en PSII, indicando que los mecanismos de reparación no logran compensar el estrés prolongado. De forma paralela, ETR_{max} y α_{ETR} disminuyeron significativamente, revelando limitaciones en el transporte de electrones y en la eficiencia de uso de la luz. Aunque $E_{k_{ETR}}$ no mostró diferencias significativas, la tendencia a valores menores a 18 °C sugiere una menor capacidad para utilizar irradiancias altas, restringiendo la aclimatación a regímenes lumínicos fluctuantes. Este patrón coincide con lo descrito en *F. serratus* y *S. latissima* durante periodos de estrés térmico estival (Li et al., 2019; Narrain et al., 2023), y con lo observado en *Turbinaria ornata* y *Sargassum* spp., donde la combinación de calor e irradiancia reduce el rendimiento fotosintético y aumenta NPQ como mecanismo de amortiguación (Fukumoto et al., 2019; Terada et al., 2016; Yong et al., 2021). En *Cladosiphon okamuranus*, respuestas similares se interpretaron como ajustes fotoprotectores de corto plazo que se tornan insostenibles en condiciones prolongadas (Marambio et al., 2021). En concordancia, el aumento de NPQ_{max} tras 14 días a 18 °C en *L. spicata* evidenció una activación de la disipación térmica con un probable costo fisiológico y metabólico elevado.

Las respuestas bioquímicas reforzaron este perfil de vulnerabilidad. A 18 °C, las concentraciones de clorofila *a* y *c* disminuyeron hacia el día 14, junto con una reducción de fucoxantina, lo que sugiere un deterioro del aparato fotosintético. Estos cambios han sido descritos en *S. horneri* bajo combinaciones de luz y temperatura (Yong et al., 2021), en Kelps donde la fotoinhibición crónica limita la recuperación de F_v/F_m (Sato et al., 2020), y en especies de tolerancia estrecha

como *L. corrugata* (Paine et al., 2021). La reducción de pigmentos limita tanto la absorción de energía como la fotoprotección, amplificando el impacto del estrés lumínico. En paralelo, los compuestos fenólicos y la actividad antioxidante exhibieron una respuesta transitoria: aumentaron inicialmente tras 24 h, sugiriendo una defensa rápida, pero descendieron posteriormente, dejando los tejidos vulnerables a la acumulación de ROS. Este perfil de “respuesta inicial seguida de declive” ha sido reportado en macroalgas tropicales (Narrain et al., 2023) y en *Sargassum mcclurei*, donde la recuperación vespertina de Φ PSII tras depresión de mediodía refleja los límites de aclimatación bajo irradiancia y calor extremos (Terada et al., 2016). En *L. spicata*, el aumento de ROS y MDA tras 14 días a 18 °C confirmó procesos de peroxidación lipídica y daño progresivo en membranas, consistente con lo descrito en *S. latissima* y otras algas pardas bajo estrés térmico prolongado (Urrea-Victoria et al., 2020; James et al., 2023).

El deterioro del hospedero se amplificó al considerar la respuesta del microbioma epífita. El incremento de +3 °C desencadenó cambios marcados en la composición, diversidad y funciones de la comunidad bacteriana asociada a las frondas. Se observó una pérdida de mutualistas como *Granulosicoccus* y un aumento de oportunistas como *Vibrio* y *Sulfitobacter*, junto con la proliferación de familias como Rhodobacteraceae, Alteromonadaceae y Vibrionaceae, comúnmente asociadas a disbiosis en holobiontes marinos (Dobretsov et al., 2021; Wang et al., 2023). Estos cambios estructurales coincidieron con una menor diversidad alfa y un aumento en la riqueza relativa, lo que sugiere que el calentamiento promueve la proliferación de taxones transitorios o tolerantes al estrés en detrimento de mutualistas estables. Patrones semejantes se han descrito en *M. pyrifera* y *D. antarctica*, donde el calentamiento favorece la pérdida de microbiota núcleo y la colonización por comunidades variables (Pearman, 2023; Castro, 2024).

A nivel funcional, el microbioma mostró una reducción de rutas metabólicas claves vinculadas al ciclo del nitrógeno y del azufre, junto con un aumento de funciones heterótrofas generalistas. Estos cambios son comparables a los descritos en *S. latissima*, donde incrementos de pocos grados redujeron la

capacidad de las bacterias epífitas para mitigar el estrés térmico del hospedador (Burgunter-Delamare et al., 2023; Delva, 2023). Asimismo, se ha planteado que, además de la temperatura, factores como la acidificación y la irradiancia pueden modular estas interacciones (Ghaderiardakani et al., 2020). La mayor abundancia de *Vibrio* spp. a 18 °C coincide con reportes en otras macroalgas pardas sometidas a estrés (Qiu et al., 2019) y evidencia el riesgo de reemplazo de mutualistas funcionales por potenciales patógenos, comprometiendo la provisión de nutrientes y la resistencia frente a oxidantes. Este proceso de disbiosis caracterizado por la pérdida de mutualistas y el aumento de oportunistas ha sido documentado en macroalgas bajo cambio climático (Egan et al., 2013; Vila Duplá, 2025).

En conjunto, la integración de respuestas fisiológicas, bioquímicas y microbiológicas indica que *L. spicata* enfrenta un riesgo amplificado bajo calentamiento moderado. La conjunción de fotoinhibición sostenida, pérdida de pigmentos, colapso de defensas antioxidantes y disbiosis microbiana configura un escenario de vulnerabilidad creciente, en línea con el escenario SSP2-4.5. De mantenerse estas tendencias, podrían comprometerse el crecimiento, la reproducción y la integridad tisular, debilitando su rol como formadora de hábitat y afectando cascadas tróficas y servicios ecosistémicos en las costas de Chile central (Smale et al., 2020; Manca et al., 2024). La emergencia de microbiomas variables y dominados por oportunistas, sumada al deterioro fisiológico del alga, subraya la necesidad de considerar el holobionte como unidad de estudio para evaluar la vulnerabilidad climática. Futuras investigaciones deberían integrar monitoreos de campo estacionales y durante olas de calor marinas, con el fin de anticipar la resiliencia de los bosques de *L. spicata* en un océano cada vez más cálido.

6.3 Interacción entre radiación y temperatura

Aunque en este estudio los factores de radiación y temperatura fueron evaluados de manera independiente, la proyección hacia escenarios futuros requiere considerar que ambos estresores co-ocurren en ambientes intermareales. La radiación solar, si bien representa un desafío fisiológico estacional, no mostró

efectos tan drásticos como la temperatura sobre la estabilidad fotosintética y bioquímica de *L. spicata*. Sin embargo, nuestros resultados sugieren que un incremento térmico moderado podría exacerbar los daños asociados a irradiancia elevada, generando condiciones de vulnerabilidad reforzada.

Diversos estudios en macroalgas pardas respaldan esta interacción. Fukumoto et al. (2019) demostraron que *Turbinaria ornata* expuesta simultáneamente a irradiancia alta y temperaturas elevadas presentó caídas pronunciadas de F_v/F_m y una activación excesiva de NPQ, lo que indica que la capacidad fotoprotectora puede saturarse cuando ambos factores coinciden. De forma similar, se ha reportado en *S. horneri* que altas temperaturas combinadas con baja irradiancia reducen significativamente F_v/F_m , mientras que la combinación de alta luz y calor induce una fuerte activación de NPQ como amortiguador (Yong et al., 2021; Terada et al., 2016). En *Saccharina japonica*, la fotoinhibición crónica se intensifica cuando la irradiancia y la temperatura interactúan, mostrando fallas en la recuperación de F_v/F_m (Sato et al., 2020). Estas evidencias refuerzan que la interacción entre calor y radiación determina umbrales de tolerancia más estrictos que cada factor por separado.

Estudios en ambientes polares y templados también ilustran este patrón. Marambio et al. (2021) demostraron que especies del Ártico como *Desmarestia aculeata* y *Palmaria palmata* presentan respuestas diferenciales de fotoinhibición, pigmentos y antioxidantes frente a la interacción de irradiancia y temperatura, lo que evidencia que la magnitud del impacto depende del contexto ambiental y de la historia evolutiva de cada linaje. Asimismo, Schmid et al. (2021) señalaron que la combinación de alta luz y escenarios de temperatura futura en macroalgas templadas afecta la composición pigmentaria y el balance bioquímico, comprometiendo productividad y estabilidad fisiológica. Estos hallazgos sugieren que la interacción entre estresores físicos no solo es aditiva, sino que puede detonar efectos sinérgicos difíciles de predecir.

Más allá de la fotofisiología, los impactos combinados sobre el microbioma representan una línea crítica de vulnerabilidad. Estudios recientes han mostrado

que el calentamiento oceánico puede reconfigurar profundamente los microbiomas de algas pardas, reduciendo funciones clave y alterando la estabilidad de asociaciones mutualistas (Burgunter-Delamare et al., 2023; Castro et al., 2024). Estos efectos tienden a ser modulados por la estacionalidad y la severidad de los escenarios (Truong et al., 2024), lo que sugiere que los periodos de verano podrían ser los más críticos para el holobionte. En paralelo, la radiación UV puede modificar la composición bacteriana superficial al alterar los metabolitos exudados por el alga (Paix et al., 2022), generando un escenario de doble presión: mayor producción de ROS y peroxidación en el hospedador, junto con una microbiota menos estable y dominada por oportunistas. El review de Vila Duplá (2025) destaca que la disbiosis en macroalgas bajo condiciones de cambio climático sigue patrones similares a los descritos en corales y esponjas: aumento de *Vibrio* spp. y otras bacterias oportunistas, pérdida de microbiota núcleo y disminución de funciones metabólicas esenciales. Bajo un escenario de coocurrencia de calor y radiación, *L. spicata* podría enfrentar no solo limitaciones fisiológicas, sino también una inestabilidad microbiana que comprometa su resiliencia ecosistémica. Esto refuerza la necesidad de considerar al holobionte como la verdadera unidad de vulnerabilidad y de resiliencia frente al cambio climático (Neale et al., 2023).

Estos elementos permiten proyectar que, bajo escenarios de Cambio Climático donde el aumento térmico y la radiación coocuran, *L. spicata* podría enfrentar umbrales críticos de tolerancia más rápidamente de lo esperado a partir de un solo factor. Si bien la radiación parece ser un estresor más estacional y en cierto grado amortiguado por mecanismos fotoprotectores, la temperatura emerge como el principal modulador de la vulnerabilidad. La interacción de ambos, sin embargo, probablemente intensifique la presión selectiva sobre los bosques de *Lessonia*, afectando productividad, estructura poblacional y servicios ecosistémicos en la zona intermareal (Truong et al., 2024).

En conjunto, nuestros resultados muestran que *L. spicata* mantiene una tolerancia relativa frente a la variación estacional de PAR+UV gracias a ajustes coordinados redimensionamiento de antenas, aumento de NPQ, acumulación

fenólica y regulación génica, pero exhibe una vulnerabilidad marcada ante un calentamiento moderado (+3 °C), con deterioro fotofisiológico sostenido, colapso progresivo de defensas antioxidantes, pérdida de pigmentos y disbiosis del microbioma epífita. La coocurrencia de radiación elevada y aumento térmico, prevista para escenarios como SSP2-4.5, podría potenciar estos efectos mediante sinergias negativas, acelerando la superación de umbrales fisiológicos y ecológicos. Esto refuerza la necesidad de abordar la resiliencia de *L. spicata* desde la perspectiva del holobionte, mediante monitoreos estacionales en campo y durante olas de calor marinas, y de implementar ensayos factoriales que integren fotofisiología, bioquímica y multiómicas (incluida la función microbiana) para anticipar respuestas y orientar estrategias de conservación y manejo de los bosques de *Lessonia* en Chile central.

Capítulo VII.

7. Conclusiones generales

7.1. Radiación solar estacional

L. spicata mostró una alta tolerancia frente a la variación estacional de PAR+UVR. El F_v/F_m se mantuvo en valores en el rango de 0.64–0.72 y sin diferencias marcadas entre tratamientos, lo que evidencia estabilidad fotoquímica incluso bajo condiciones de máxima radiación. Las estrategias de amortiguación incluyeron un incremento del NPQ, ajustes pigmentarios particularmente la reducción de clorofila c en verano y la acumulación de metabolitos fenólicos con capacidad antioxidante. A nivel molecular, la inhibición transitoria de genes fotosintéticos y la sobreexpresión de chaperonas como *dnaK* (Hsp70) reflejaron un control fino de la asignación energética y de la protección celular. En conjunto, estas respuestas indican que *L. spicata* despliega mecanismos coordinados que permiten sostener la eficiencia fotosintética, aunque con un costo metabólico que podría volverse crítico bajo escenarios de mayor frecuencia e intensidad de radiación.

7.2. Temperatura (+3 °C)

El incremento térmico moderado tuvo efectos mucho más marcados. A 18 °C se observó un deterioro sostenido de la fotosíntesis, con disminuciones de F_v/F_m , ETR_{max} y α_{ETR} , lo que evidencia una fotoinhibición persistente y daño acumulado en PSII. Este deterioro se acompañó de reducciones en clorofilas y fucoxantina, junto con una respuesta transitoria de compuestos fenólicos y antioxidantes que declinaron con el tiempo, permitiendo la acumulación de ROS y MDA. A nivel microbiano, El calentamiento generó alteraciones en la actividad del microbioma epífita y una reconfiguración de la comunidad asociada, evidenciada por cambios direccionales en composición y función, caracterizada por la pérdida de mutualistas y la proliferación de oportunistas, además de una reducción en funciones asociadas al ciclo de nitrógeno y azufre. La conjunción de estos procesos refleja que *L. spicata* opera cerca de un umbral fisiológico, donde la

capacidad de compensación bioquímica y de sostener interacciones microbianas estables se ve rápidamente sobrepasada.

7.3. Interacción radiación–temperatura

Aunque evaluados de manera independiente, los resultados sugieren que la coocurrencia de alta radiación y calentamiento representa un escenario de mayor vulnerabilidad. La radiación, por sí sola, no comprometió de forma drástica la fotoquímica de *L. spicata*, pero el estrés térmico sí indujo un colapso fisiológico progresivo. La evidencia disponible indica que ambos factores en conjunto pueden saturar las defensas fotoprotectoras, incrementar la producción de ROS y reducir la estabilidad del microbioma, generando una presión sinérgica que acelera la superación de los umbrales de tolerancia. Esto confirma que la temperatura emerge como el modulador principal de la vulnerabilidad, mientras la radiación actúa como un factor coadyuvante que intensifica los riesgos.

7.4. Aportes al conocimiento científico y perspectivas

Esta tesis brinda un aporte novedoso a la ciencia al demostrar cómo un enfoque del alga como un holobionte permite comprender de manera integrada las respuestas de *L. spicata* frente a estresores asociados al cambio climático. Al articular niveles ecofisiológicos, bioquímicos, moleculares y microbiológicos, el trabajo contribuye a ampliar la comprensión de los mecanismos que sostienen y limitan la resiliencia de esta especie. En perspectiva, estos hallazgos abren camino a nuevas investigaciones que incorporen monitoreos de campo, la interacción de múltiples estresores y enfoques interdisciplinarios propios de las ciencias ambientales, necesarios para anticipar la estabilidad de los bosques de algas pardas en escenarios futuros de cambio global

Capítulo VIII

8. References

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9. SUPPLEMENTARY MATERIAL

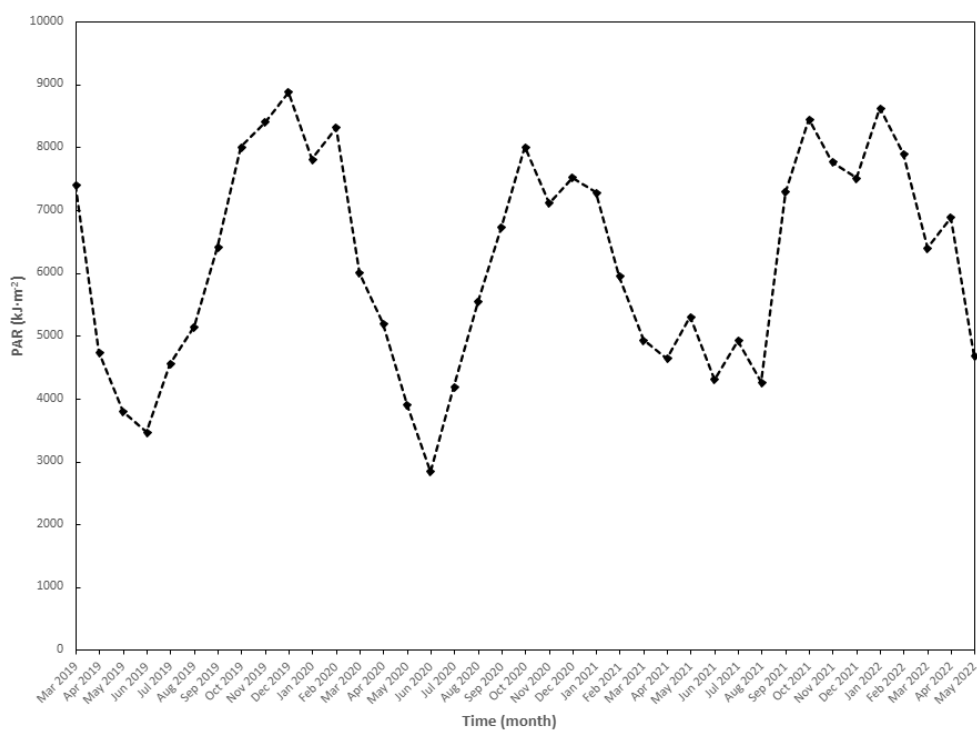


Figure III. 1. The average dose by month between 2021 and 2023 measured in Viña del Mar Chile is shown

Table III. S1. Results of one-way ANOVA for physiological analysis of *Lessonia spicata* during seasonal time in the laboratory culture (** $p < 0.05$)

		<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
ETR _{max}	Season	3	2000.7	3.4	**
	(s)				
	Res	32	594.8		
Fv/Fm	Season	3	0.007	2.240	
	(s)				
	Res	32	0.003		
αETR	Season	3	0.1378	6.5108	**
	(s)				
	Res	32	0.0021		
Ek ETR	Season	3	59371	5.4399	**
	(s)				
	Res	32	10914		
NPQ _{max}	Season	3	7.771	17.218	**
	(s)				
	Res	32	0.451		
Chlorophyll <i>a</i>	Season	3	0.108	4.774	**
	(s)				
	Res	32	0.022		
Chlorophyll <i>c</i>	Season	3	0.05012	200.88	**
	(s)				
	Res	32	0.00025		
PC	Season	3	85615.000	102642	**
	(s)				
	Res	32	8.341		
DPPH	Season	3	2.239	0.475	**
	(s)				
	Res	32	4.710		
H ₂ O ₂	Season	3	0.0523	3.0935	**
	(s)				
	Res	32	0.0168		
TBARS	Season	3	4.0495	4.0449	**
	(s)				
	Res	32	1.0011		

Res: Residual