UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA

INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS

RESPUESTAS ECOFISIOLÓGICAS Y DESARROLLO DE DIFERENTES ESTADOS ONTOGÉNICOS DE MACROALGAS LAMINARIALES FRENTE A CONDICIONES DE ESTRÉS TÉRMICO

TESIS

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS PARA OBTERNER EL GRADO DE

DOCTORA EN MEDIO AMBIENTE Y DESARROLLO

PRESENTA

ANTONELLA CAROLINA ALMEIDA SAA

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PRESENTA

Antonella Carolina Almeida Saa

APROBADA POR:

Dr. Jose Antonio Zertuche

Dra. Schery Umanzor

Dra. Raquel Muñiz

Dr. Ricardo Cruz

Dr. Jose Miguel Sandoval-Gil
Director de Tesis

RESUMEN

Las macroalgas pardas del orden Laminariales (conocidas como “kelps” en inglés), pueden conformar auténticos bosques marinos que ofrecen distintos servicios ecológicos y económicos. A pesar de su importancia, los bosques de Laminariales enfrentan importantes amenazas asociadas a actividades humanas y eventos extremos naturales. La eutrofización, la pérdida de la calidad del agua, las obras costeras o los factores asociados al cambio climático, se encuentran entre los estresores que pueden impactar negativamente a estas macroalgas. En particular, y en contexto del cambio climático, existen robustas evidencias del deterioro de bosques del Laminariales asociado a la incidencia de olas de calor marinas (OCM). Al igual que en otras partes del mundo, en el Pacífico Norteamericano se han documentado alteraciones en la estructura y distribución de bosques de Laminariales relacionadas con el paso de OCMs. Sin embargo, el conocimiento acerca de las respuestas eco-fisiológicas de estas macroalgas frente a incrementos de temperatura es aún muy deficiente, más aún en las fases tempranas de desarrollo.

Esta tesis tuvo el objetivo de evaluar las respuestas fisiológicas de macroalgas Laminariales de Baja California frente a OCMs. Especies importantes como *Macrocystis pyrifera*, *Pterygophora californica* y *Eisenia arborea* tienen su extremo de distribución en aguas costeras de esta península. Esta es además una zona oceanográfica de transición al extremo meridional de la Corriente de California, y en donde las comunidades biológicas marinas son consideradas vulnerables en un escenario de cambio climático. La tesis presenta dos trabajos experimentales en dos capítulos. El primer experimento tuvo como objetivo examinar la fisiología y el crecimiento de esporofitos adultos de *Pterygophora californica* frente a una OCM simulada en mesocosmos. En este trabajo se compararon las respuestas de esporofitos colectados de los extremos batimétricos de la población de estudio (8–10 *vs.* 25–27 m), con la

finalidad de probar la hipótesis de que su adaptación a las diferentes profundidades puede condicionar su resistencia a OCMs. En el segundo experimento se estudió también el efecto de una OCM, pero esta vez sobre la fotobiología de gametofitos de *P. californica*, *M. pyrifera* y *E. arborea*. Dado que estudios previos indican las especies presentan distintas capacidades de termorresistencia, la hipótesis de partida fue que sus gametofitos presentarían también estas diferencias. En ambas aproximaciones experimentales, las variables fisiológicas se evaluaron en dos fases de las OCMs, una fase de calentamiento y otra de retorno a temperatura control. Algunas conclusiones relevantes de ambos estudios fueron que i) los esporofitos adultos *de P. californica* del extremo batimétrico profundo mostraron mayor tolerancia a la OCM aplicada (i.e. anomalía térmica de +6° C durante 6 días) que los esporofitos del extremo somero, que, ii) los en esporofitos someros mostraron signos de estrés oxidativo y fisiológico, y un escaso crecimiento de sus láminas, que iii), mientras que las variables de fotobiología no se vieron afectadas por una OCM experimental en gametofitos de *E. arborea*, el calentamiento sí indujo alteraciones de las tasas fotosintéticas y respiratorias en gametofitos de *M. pyrifera* y *P. californica*; esta distinta termo-tolerancia entre gametofitos coincide con la que se atribuye a esporofitos adultos. Por último, cabe destacar que nuestros resultados revelaron que muchas de las respuestas fisiológicos inducidas por el calentamiento en ambos experimentos se encontraron durante la fase experimental de retorno a temperatura control, y no durante la exposición a la OCM. Esto demuestra que la inclusión de estas fases experimentales es crucial para la obtención de resultados concluyentes en relación a las respuestas de estrés térmico.

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ÍNDICE

INTRODUCCIÓN

1. Algas pardas Laminariales y estrés por temperatura

Los bosques de algas pardas del orden Laminariales, también conocidas como "kelp" por su nombre común en inglés, son ecosistemas marinos vitales que brindan importantes servicios ecosistémicos y beneficios directos e indirectos a la sociedad (Smale et al., 2013; Eger et al., 2023). Entre estos, se incluyen la formación de hábitats y refugio para gran variedad de especies desde pequeños invertebrados hasta peces de importancia comercial (Bologna et al., 1993; Steneck et al., 2002). Además, los bosques de algas pardas ayudan a mitigar el cambio climático al absorber dióxido de carbono del agua y liberar oxígeno a través de la fotosíntesis (Dayton, 1985; Krumhansl & Scheibling, 2012). Asimismo, protegen las costas de la erosión al disminuir la fuerza de las olas y actúan como amortiguadores frente a eventos extremos como tormentas y marejadas (Lovas & Torum 2001; Türker et al. 2006). En México, por ejemplo, algunas especies de macroalgas Laminariales tienen un papel fundamental en la acuicultura y en industrias como la alimentaria, farmacéutica y cosmética (Zertuche, 1993; Vázquez-Delfín et al., 2019).

Las macroalgas Laminariales están sujetas a cambios continuos en las condiciones ambientales a diferentes escalas espacio-temporales, como fluctuaciones en la temperatura, nutrientes, y la cantidad/calidad de irradiancia, y que determinan su productividad y distribución (Dayton et al., 1999; Matson, 2007). La resistencia y resiliencia de las macroalgas a estos cambios ambientales dependerá, entre otros factores, de las capacidades adaptativas/aclimatativas interespecíficas y entre ecotipos y genotipos intra-específicos, o de su estado fisiológico/ontogénico (Muth et al., 2019; Butler et al., 2020; Liesner et al., 2020; Saada et al., 2020). Sin embargo, cuando los cambios ambientales son más severos, en términos de intensidad o duración, pueden desencadenar condiciones de estrés disruptivo y limitante (*sensu* Davison & Pearson, 1996) que exceden los límites de tolerancia fisiológica de la macroalga,

generando daños metabólicos, y en último término, reducciones del crecimiento y supervivencia. Los incrementos de temperatura y su combinación con otros factores (limitación de luz o de nutrientes) pueden ser condiciones causales de este tipo de estrés severo en macroalgas Laminariales (Brown et al., 2014; Xiao et al., 2015; Mabin et al., 2019).

Autores como Eggert (2012) y Koch et al. (2013) han revisado los efectos del estrés en macrófitos marinos por efecto del incremento de la temperatura (Fig. 1). De forma general, el calentamiento del agua puede impactar a nivel fisiológico y metabólico, modificando los sistemas enzimáticos, la composición de pigmentos (Bruhn & Gerard, 1996; Anderson, 2006) y la producción de carbohidratos (Machalek et al., 1996). Asimismo, la temperatura puede alterar el el rendimiento fotosintético y las tasas de respiración (Los y Murata, 2004; Cruces et al., 2012; Andersen et al., 2013). Estas alteraciones pueden tener un efecto negativo en el desarrollo reproductivo y vegetativo de las macroalgas, y a largo plazo, pueden incidir en la estructura de la comunidad (Rinde & Sjøtun, 2005; Matson & Edward, 2007; Bartsch et al., 2012; Eggert, 2012; Andrews et al., 2014; Smale et al., 2019). A pesar de esto, las macroalgas desarrollan mecanismos de aclimatación a corto (p.e., procesos metabólicos) y largo plazo (p.e., crecimiento y balance de carbono). A nivel fisiológico, las algas pueden manifestar diferentes respuestas, que incluyen cambios en la composición de ácidos grasos, lípidos totales, ajustes en el aparato fotosintético, en la translocación de fotosintatos, o cambios en la composición química del tejido, p.e. florotaninos y compuestos antioxidantes (Koch et al., 2016; Schmid et al., 2020). Como se ha comentado previamente en relación a las respuestas de macroalgas frente a otros cambios ambientales, éstas variarán dependiendo de distintos factores, incluyendo la etapa específica en la historia de vida en la que se encuentre el alga. Las algas pardas exhiben una historia de vida heteromórfica, que alterna entre una fase haploide y otra diploide (Fig. 2; Fritsch, 1942; Schiel & Foster, 2015). Ya que los gametofitos y esporofitos exhiben diferentes tasas metabólicas y de desarrollo, además de notables

diferencias en sus estructuras vegetativas, sus respuestas frente a condiciones de incremento de temperatura también pueden ser muy distintas. En comparación con la etapa macroscópica de esporofito, los estudios con etapas microscópicas haploides son mucho más escasos (rev.en Veenhof et al, 2023). Resulta interesante que algunos estudios sugieren que estas etapas (esporas, gametofitos) podrían mostrar una mayor tolerancia a condiciones ambientales desfavorables, tales como limitación de luz, cambios de temperatura y escasez de nutrientes (Nakahara, 1984; Wienke & Dieck, 1989, 1990; Hoffmann & Santelices, 1991). De hecho, existe evidencia de que pueden permanecer en estado latente hasta que las condiciones ambientales mejoren (Kain, 1964; Dayton, 1984; Hoffmann & Santelices, 1991; Edwards, 2000). En la actualidad, la pregunta de cómo el calentamiento del agua de mar y la incidencia de olas de calor marinas asociados al cambio climático pueden afectar a los bosques de Laminariales suscita especial interés en la comunidad científica, ya que existe sobrada evidencia de su impacto negativo en distintas especies en distintas regiones del mundo, revisados en Filbee-Dexter & Wernberg (2018), e.g. disminución de *M. pyrifera* en Australia (Butler et al., 2020), de *Ecklonia spp* en Japón (Tanaka et al., 2012), *S. latissima* en Noruega (Bennett et al. 2015, Wernberg et al. 2013);

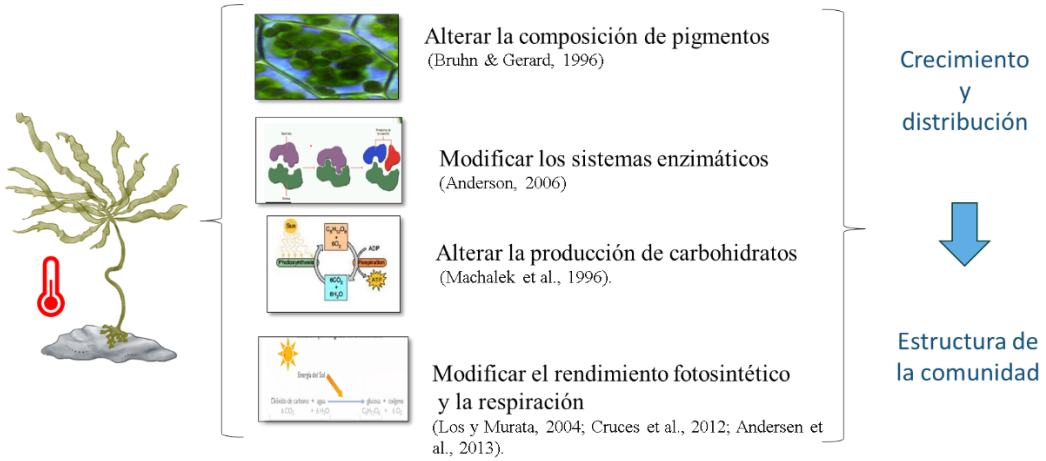

Figura 1. Efectos del incremento de la temperatura en macrófitos marinos.

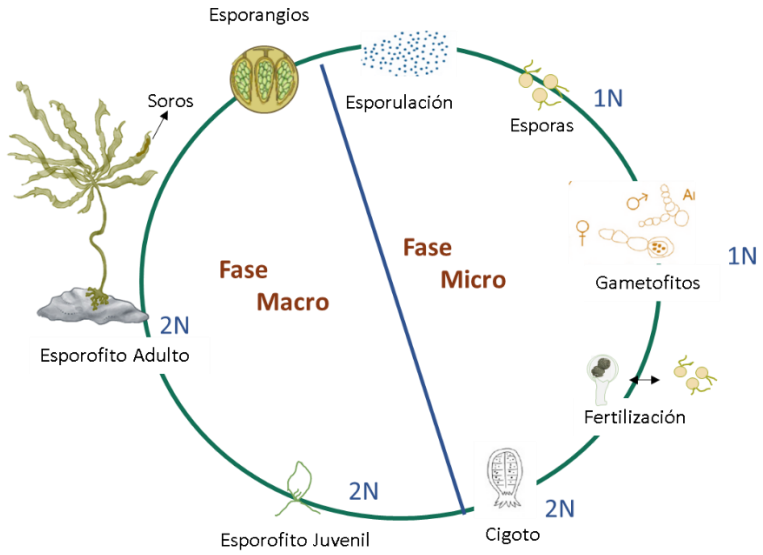

Figura 2. Historia de vida de algas pardas Laminariales.

2. Efectos de olas de calor marinas en bosques de Laminariales del Pacífico Norteamericano

La región del Pacífico Norteamericano es interesante para el estudio de los efectos del calentamiento y las olas de calor marinas (en adelante, OCMs) en los bosques de Laminariales. En esta región ocurren eventos térmicos anómalos recurrentes como OCMs y El Niño que han tenido un impacto significativo en la fisiología, metabolismo, productividad y reproducción de macroalgas de la región (Ladah et al. 1999; Cavanaugh et al., 2019). Las OCMs se caracterizan por incrementos en la temperatura en el agua del mar durante mínimo 5 días consecutivos, en los cuales la temperatura supera el percentil 90 en relación a las condiciones locales promedio de las últimas décadas (Hobday et al. 2016). Las proyecciones indican un aumento en la frecuencia, duración e intensidad de las OCMs en las próximas décadas (Oliver et al., 2018). Uno de estos eventos más extremos acaeció entre 2013-2016, y fue el conocido como 'el blob'. El Blob ocasionó un aumento drástico de las temperaturas del agua del mar (6° C por encima del promedio) durante aproximadamente 200 días consecutivos, con una profundización de la termoclina de hasta 50 m de profundidad (Zaba & Rudnick, 2016; Sen Gupta et al., 2020).

Estas condiciones han tenido consecuencias significativas para la población de algas marinas (Bond et al., 2015; Hu et al., 2017; Cavanaugh et al., 2019), impactando drásticamente su dinámica poblacional, productividad y reproducción, e incrementado la mortalidad a nivel regional y local en algunas poblaciones (Ladah et al., 1999; Scrosati, 2001; Schiel & Foster, 2015; Smale et al., 2019; Cavanaugh et al., 2019). La mayor presencia de anomalías térmicas profundas, también llamadas OCMs de fondo (o bottom marine heatwaves; Amaya et al., 2023), puede ser un factor de riesgo grave para los bosques de Laminariales, ya que suponen la exposición de macroalgas que crecen en aguas profundas a temperaturas inusualmente elevadas para su ambiente. Sin embargo, hasta la fecha no se han documentado sus impactos en dichas estas comunidades.

Especies importantes de algas Laminariales del Pacífico Nororiental encuentran su límite de distribución meridional en la península de Baja California (México). De hecho, Baja California, ubicada en el extremo sur de la Corriente de California (CdC), representa una zona de transición oceanográfica considerada prioritaria para el estudio de los efectos del cambio climático en las comunidades biológicas marinas (Smale et al. 2019). El aparente debilitamiento de la CdC, con el consiguiente calentamiento de aguas superficiales y profundización de termoclinas (Bograd et al. 2023), son factores añadidos que estimulan este hecho. Por su distribución y productividad, entre las macroalgas Laminariales más representativas de la región se incluyen *Macrocystis pyrifera (*Linnaeus) C. Agardh, *Pterygophora californica Ruprecht*, y *Eisenia arborea* Areschoug (Abbott & Hollenberg, 1976; Pedroche et al. 2008; Fig. 3). Cada una de estas especies de kelp provee beneficios socio-ecológicos únicos, siendo componentes integrales del ecosistema marino costero. Por tanto, se considera imperativo el conocer los efectos de factores de cambio climático (como el calentamiento y OCMs) en estas especies.

Los estudios acerca de la tolerancia y resiliencia al estrés por temperatura de *M. pyrifera*, *P. californica* y *E. arborea* son aún escasos, sobre todo para el caso de estas dos últimas. Los límites de distribución de *M. pyrifera* y *P. californica* alcanzan aproximadamente la mitad de la península, en Bahía Asunción y el Rosario, respectivamente (Abbott & Hollenberg, 1976; Pedroche et al., 2008), donde experimentan temperaturas máximas entre 20 y 23 °C (NOAA, 2022). Por su parte, *E. arborea* es el único kelp que sobrevive en aguas subtropicales de Bahía Magdalena en Baja California Sur (Abbott & Hollenberg, 1976; Pedroche et al., 2008), donde la temperatura máxima en verano oscila entre 20 y 26 °C y la disponibilidad de nutrientes es muy baja (< 1µM nitrato) (Dayton et al., 1992; 1999; Edwards & Hernández-Carmona, 2005; Arafeh-Dalmau et al., 2019). Al contrario que para el caso de *E. arborea*, se considera que, *M. pyrifera* tiene una aparente menor resistencia térmica (North & Zimmerman, 1984; Hernández-Carmona et al., 2001; Edwards & Hernández-Carmona et al., 2005) lo que se relaciona con el reciente retroceso en su distribución meridional (Arafeh-Dalmau et al., 2019). De hecho, Arafeh-Dalmau et al. (2019) documentaron que, tras el evento del Blob, la desaparición de bosques de *M. pyrifera* en algunas islas del pacífico mexicano propició un mayor reclutamiento y crecimiento de bosques de *E. arborea*. Estudios recientes como los de Sánchez-Barredo et al. (2020) y Umanzor et al. (2021) también han puesto de manifiesto la sensibilidad de esporofitos juveniles de *M. pyrifera* frente a OCMs, también en interacción con otros estresores como la limitación por luz o de disponibilidad de nutrientes. Estudios realizados en el sur de California (EUA) y Baja California (México), también han documentado una recuperación más rápida de *P. californica* y *E. arborea* tras el paso del Niño, en comparación con *M. pyrifera* (Dayton, 1984; 1992; 1999; Edwards & Hernández-Carmona, 2005).

Si la información sobre los efectos del calentamiento y OCMs es limitada para el caso de esporofitos de *M. pyrifera*, *P. californica* y *E. arborea*, aún es más escasa en relación con sus fases de desarrollo tempranas, p.e. gametofitos. Algunos trabajos han examinado cómo factores

abióticos tales como la luz (Lunning & Neushul, 1978; Reed, 1990a; 1990b; Reed et al., 1991), la temperatura (Dieck, 1993; Howard, 2014) y nutrientes (Graham, 1999; Ladah & Zertuche-González, 2007), pueden afectar a su supervivencia, asentamiento y crecimiento de esporas y gametofitos. Sin embargo, la carencia de información sobre la fisiología de estas fases microscópicas genera vacíos significativos en la comprensión de los posibles efectos de los cambios extremos de temperatura, como los generados por OCMs (Veenhof, et al 2023). Se necesita recabar información acerca de la tolerancia y resiliencia al estrés por temperatura de estos estados tempranos de desarrollo, ya que representan etapas claves para el mantenimiento y recuperación de a través del reclutamiento (Reed, 1990a, Coelho et al., 2000).

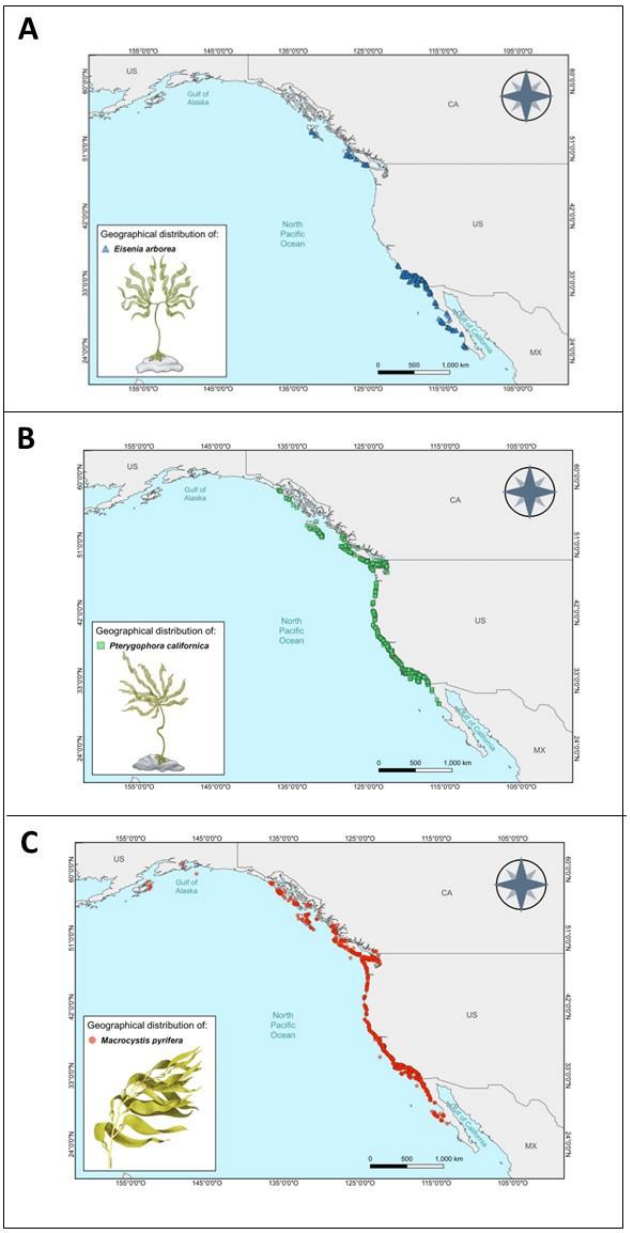

Figura 3. Distribución en el Pacífico Nororiental de (A) *Pterygophora californica*, (B) *Eisenia arborea* y (C) *Macrocystis pyrifera.* Datos obtenidos de Global Biodiversity Information Facility- GBIF.

3. Objetivos de la tesis

El **objetivo general** de esta tesis fue evaluar las respuestas ecofiológicas de esporofitos adultos y gametofitos de macroalgas Laminariales que tienen su límite de distribución sur en la península de Baja California, frente a ondas de calor marinas (OCMs) simuladas en condiciones experimentales.

La **estructura de esta tesis** consta de dos capítulos.

En el **Capítulo 1,** el objetivo fue evaluar los efectos de una OCM simulada en mesocosmos en la fisiología de los esporofitos adultos de *Pterygophora californica* provenientes de los extremos batimétricos (10 y 27 metros) de una población cercana a su límite de distribución sur en Baja California.

En el **Capítulo 2**, el objetivo fue evaluar los efectos de una OCM simulada en laboratorio en la fotobiología y respiración de los gametofitos de tres especies de macroalgas laminariales (*M. pyrifera, E. arborea y P. californica*).

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RESEARCH ARTICLE

Bathymetric origin shapes the physiological responses of *Pterygophora californica* (Laminariales, Phaeophyceae) to deep marine heatwaves

Antonella C. Almeida-Saá[1] | **Schery Umanzor**[2] | **Jose Antonio Zertuche-González**[1] | **Ricardo Cruz-López**[1] | **Raquel Muñiz-Salazar**[3] | **Alejandra Ferreira-Arrieta**[1] | **Paula Bonet Melià**[1] | **Jessica Anayansi García-Pantoja**[1] | **Laura K. Rangel-Mendoza**[4] | **Manuel Vivanco-Bercovich**[1] | **Leonardo Ruiz-Montoya**[1] | **Jose Manuel Guzmán-Calderón**[1] | **Jose Miguel Sandoval-Gil**[1]

[1]Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Mexico

[2]College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Juneau, Alaska, USA

[3]Escuela de Ciencias de la Salud, Universidad Autónoma de Baja California, Ensenada, Mexico

[4]Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico

Correspondence
Jose Miguel Sandoval-Gil, Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Baja California, México.
Email: jmsandovalgil@gmail.com

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Abstract

Kelp communities are experiencing exacerbated heat-related impacts from more intense, frequent, and deeper marine heatwaves (MHWs), imperiling the long-term survival of kelp forests in the climate change scenario. The occurrence of deep thermal anomalies is of critical importance, as elevated temperatures can impact kelp populations across their entire bathymetric range. This study evaluates the impact of MHWs on mature sporophytes of *Pterygophora californica* (walking kelp) from the bathymetric extremes (8–10 vs. 25–27 m) of a population situated in Baja California (Mexico). The location is near the southernmost point of the species's broad distribution (from Alaska to Mexico). The study investigated the ecophysiological responses (e.g., photobiology, nitrate uptake, oxidative stress) and growth of adult sporophytes through a two-phase experiment: warming simulating a MHW and a post-MHW phase without warming. Generally, the effects of warming differed depending on the bathymetric origin of the sporophytes. The MHW facilitated essential metabolic functions of deep-water sporophytes, including photosynthesis, and promoted their growth. In contrast, shallow-water sporophytes displayed metabolic stress, reduced growth, and oxidative damage. Upon the cessation of warming, certain responses, such as a decline in nitrate uptake and net productivity, became evident in shallow-water sporophytes, implying a delay in heat-stress response. This indicates that variation in temperatures can result in more prominent effects than warming alone. The greater heat tolerance of sporophytes in deeper waters shows convincing evidence that deep portions of *P. californica* populations have the potential to serve as refuges from the harmful impacts of MHWs on shallow reefs.

Abbreviations: A, absorptance; Chl *a*, chlorophyll *a*; DC, deep control; DH, Deep heat; DIN, dissolved inorganic nitrogen; DMSO, dimethylsulfoxide; DP, daily productivity; DW, dry weight; E_c, compensation irradiance; E_k, saturation irradiance; ENSO, El Niño-Southern Oscillation; ETR, electron transport rate; EU, experimental unit; F_0, minimum fluorescence; F_m, maximum fluorescence; F_v/F_m, maximum quantum yield; FW, fresh weight; FX, fucoxanthin; G, growth; gross-P_{max}, maximum gross photosynthesis; H, hole; MDS, non-metric multidimensional scaling; MH, movement of the hole; MHW, Marine heatwaves; N, nitrate; net-P_{max}, maximum net photosynthesis; NPQ, non-photochemical quenching; OD, optical density; PAM, pulse amplitude modulated fluorometer; P-E, photosynthesis-irradiance; PERMANOVA, permutational multivariate analysis of variance; PPFD, photosynthetic photon flux density; PSII, photosystem II; R, respiration; ROS, reactive oxygen species; SC, shallow control; SH, shallow heat; T, time; TBA, thiobarbituric acid; TCA, trichloroacetic acid; α, photosynthetic efficiency; Φ_{PSII}, effective quantum yield.

Antonella C. Almeida-Saá and Jose Miguel Sandoval-Gil contributed equally to this study.

KEYWORDS
climate change, depth, heat stress, kelp ecophysiology, photobiology, thermal stress responses

INTRODUCTION

Kelps form diverse and productive underwater forests with significant socio-economic and ecological benefits (Eger et al., 2023; Smale et al., 2013). However, global kelp forests face threats from both human-induced and natural factors, often acting synergistically (Krumhansl et al., 2016; Smale, 2019; Straub et al., 2019). Climate change-related ocean warming, particularly marine heatwaves (MHWs), is a key driver of these impacts (Smale, 2019; Smale et al., 2019). Therefore, understanding the effects of warming and MHWs on kelp communities is essential for developing management strategies to safeguard, recover, or facilitate artificial adaptation of these iconic macroalgae (Coleman et al., 2020; Wernberg et al., 2012).

The future of kelp forests in warmer oceans hinges on factors like genetic diversity, phenotypic plasticity, and ecological resilience (Wernberg et al., 2018). These responses, integrated with physiological and morphological variables across biological structures and life stages, play critical roles in determining their reproductive success, vegetative development, and community structure (Andrews et al., 2014; Arafeh-Dalmau et al., 2019; Liesner et al., 2020; Mabin et al., 2019a; Michaud et al., 2022). Additionally, the combination of MHWs with other stressors, such as low light (Bass et al., 2023; Xiao et al., 2015), nitrate scarcity (Fernández et al., 2020; Mabin et al., 2019b; Umanzor et al., 2021), and hyposalinity (Diehl et al., 2020), may surpass kelps' physiological tolerance thresholds, potentially overwhelming their acclimatory capacity.

Deeper, longer, and more severe MHWs are anticipated in the coming decades (Frölicher & Laufkötter, 2018; Oliver et al., 2018). This specific type of extreme MHW raises concerns due to its potential impact on kelp species with broad latitudinal and bathymetric distribution ranges. In the North American Pacific region, frequent extreme MHWs and episodic temperature anomalies, like the El Niño Southern Oscillation (ENSO), have adversely affected kelp ecosystems like those in Baja California, MX (Arafeh-Dalmau et al., 2019; Hernández-Carmona et al., 2011; Ladah & Zertuche-González, 2007). Notably, the recent extreme MHW known as "the Blob" (2013–2016) elevated sea surface temperatures by up to 3.5°C above normal, causing persistent changes in California and Baja California giant kelp forests (Bond et al., 2015; Cavanaugh et al., 2019; Hu et al., 2017). "The Blob" also triggered significant shifts in sub-canopy and invasive seaweed compositions (Arafeh-Dalmau et al., 2019; Felix-Loaiza et al., 2022; Michaud et al., 2022). This event induced strong downwelling anomalies, impacting oceanographic conditions, with potential implications for deep kelp communities (Sen Gupta et al., 2020; Zaba & Rudnick, 2016). The significance of deep kelp reefs as global climate refuges is vital but remains unexplored in the context of warming impacts (Assis et al., 2016; Davis et al., 2021; Giraldo-Ospina et al., 2020, 2023; Ladah et al., 1999).

The Baja California coastline, marking the end of the California Current System, is a crucial oceanographic transition region susceptible to climate change, carrying substantial ecological and economic implications (Smale et al., 2019; Smith et al., 2021). Anticipated warming anomalies associated with the apparent weakening of the California Current System pose significant threats to vital kelp species thriving at their southernmost distribution limit in this area. These threats include increased sea surface temperature and deepening mixing layers, as well as heightened synchronization between bottom and surface MHWs (Amaya et al., 2023; Schiel & Foster, 2015). Although studies have explored MHW impacts on the well-known giant kelp (*Macrocystis pyrifera*; Arafeh-Dalmau et al., 2019; Sánchez-Barredo et al., 2020; Umanzor et al., 2021), limited attention has been given to other species such as *Pterygophora californica*.

Pterygophora californica is long-lived stipitate kelp located from Alaska to the Baja California peninsula (Abbott & Hollenberg, 1976). This species grows under contrasting sea surface temperatures at its distribution extremes, from 2–11°C at the northernmost sites to 14–21°C at the southernmost region (according to data obtained from marineheatwaves.org). In the latter characterized by warmer waters (see Appendix S1 in the Supporting Information: Figure S1), it is likely that the persistence of the species is linked to an elevated thermotolerance (Hernández-Carmona et al., 2011; Matson & Edwards, 2007; Muth et al., 2019) and its resistance and recovery capabilities during ENSO events (Dayton et al., 1999; Edwards & Hernández-Carmona, 2005; Hernández-Carmona et al., 2001). However, there are no existing records on the physiological responses of *P. californica* to warming. Moreover, given that sporophytes can grow in a wide range of depths (from shallow subtidal areas to 35 m depth; Spalding et al., 2003), the ability of *P. californica* to acclimate to MHWs may be indirectly influenced by its adaptation to depth-related factors such as light and temperature. The interplay between depth adaptation and heat-stress responses in marine macrophytes requires further investigation, as

this aspect has been inadequately addressed to date (Giraldo-Ospina et al., 2020; Marín-Guirao et al., 2016).

This study aimed to investigate the impact of a MHW on the physiology and growth of adult sporophytes of *Pterygophora californica* at different bathymetric extremes (8–10 vs. 25–27 m) within a population in Baja California, Mexico. Using a mesocosm experiment, we simulated a MHW and a subsequent warming-cessation phase. This research holds particular significance as it marks the initial exploration of MHW consequences on *P. californica*, providing insight into various physiological characteristics previously unknown in this species.

MATERIALS AND METHODS

Sampling site and collection of sporophytes

In spring 2021, mature sporophytes of *Pterygophora californica* were collected by scuba diving in Campo Kennedy, Baja California, Mexico (31°42′2.394″ N, 116°41′1.467″ W). In this location, a healthy population of the species was observed that extended from 8–10 to 25–27 m in depth. The average densities in the shallow and deep areas were 3.7 ± 1.2 and 1.7 ± 0.9 sporophytes per square meter, respectively. Twenty-four adult sporophytes ($N = 24$) were collected from each bathymetric limit, shallow and deep. All the sporophytes had a similar structure and size, measuring around 110 cm from the holdfast to the tip of the vegetative blade tip, with eight to 11 blades, a maximum blade length of 80–100 cm, and stipe length ranging from 40 to 60 cm. The holdfast of each sporophyte was growing naturally attached to a stone, so it was carefully preserved to avoid any damage. Additional blades were randomly collected from 12 sporophytes at each depth to evaluate their natural biological status. Black fiber meshes were applied to cover the sporophytes and blades during the collection to minimize sunlight exposure. The samples were then placed carefully in large coolers filled with seawater. Within an hour, the sporophytes were transferred to an outdoor mesocosm system situated in the Marine Botany Laboratory at the Instituto de Investigaciones Oceanológicas (Universidad Autónoma de Baja California).

Irradiance (PAR) was monitored at the bottom of both bathymetric extremes for 25 days prior to the experiment, using submersible sensors (Onset-Hobo MX2202) and excluding shaded portions by the canopy (Figure 1c). At a depth of 8–10 m, the daily light dose ranged from 13.9 to 2.2 mol photons · m^{-2} · d^{-1}, with irradiance peaks between 150 and 800 μmol photons · m^{-2} · s^{-1} occurring at noon. At a depth of 25–27 m, the daily light dose ranged from 3.7 to 0.2 mol photons · m^{-2} · d^{-1}, while with irradiance peaks at noon were 20 to 180 μmol photons · m^{-2} · s^{-1}. The lowest recorded irradiance values were attributed to either cloudy days or reduced transparency in the water column. To obtain photon flux density measurements in μmol photons · m^{-2} · s^{-1}, our submersible sensor's lux unit readings were calibrated against a cosine-corrected quantum sensor (Li-COR Biosciences, LI-192) in the laboratory. Seawater temperature throughout the year was also recorded by the same submersible sensors (Figure 1a,b). The average temperature, maximum, and minimum values at 25–27 m depth were 13.7 ± 0.01°C, 18.3°C, and 10°C, respectively. At 8–10 m, the corresponding values were 14.4 ± 0.01°C, 19.6°C, and 9.9°C.

Mesocosm system and experimental design

The outdoor mesocosm system for experimentation consisted of 16 fiberglass tanks (1 m^3) and closed water circulation. By using self-priming pumps with a capacity of 10,000 liters per hour, seawater was circulated and completely replaced in each tank a total of 124 times per day. The temperature of the seawater was precisely regulated through chillers (Aqua Logic MT-8, Inc., San Diego, CA) and titanium heaters. The tanks were filled with filtered (100 μm and UV) seawater with low dissolved inorganic nitrogen (DIN) concentration (1–3 μM NO_3^-, <1 μM NH_4^+) collected in an open sea area adjacent to the university. To prevent thermal gradients and improve blade movement, aeration was provided by the bottom of each tank. The temperature was set to 14 ± 0.2°C, based on average values recorded at both deep and shallow collection sites during spring (Figure 1a,b). Additionally, fiber meshes were placed on top of the tanks to simulate the mean irradiance measured in the field (Figure 1c). The average daily light dose for shallow and deep (sporophytes was adjusted at 6.9 ± 0.8 mol photons · m^{-2} · d^{-1} (~350 μmol photons · m^{-2} · s^{-1} at noon) and 1.3 ± 0.2 mol photons · m^{-2} · d^{-1} (~60 μmol photons · m^{-2} · s^{-1} at noon), respectively. To maintain the optimum nutritional condition of the sporophytes throughout the experiment, pulsed fertilization with a commercial fertilizer (Allganic Nitrogen®) was carried out every 3 days, using $NaNO_3$ (2.9 g · m^{-3}) to achieve a final concentration of 20 μM. Salinity and pH were monitored using a submersible multiparameter probe (YSI Pro Plus, USA), and the values of seawater ranged from 32.5 to 33.5 and 7.9 to 8.2, respectively. Filtered fresh water was added continuously to balance evaporation and maintain constant salinity.

After a 5-day acclimation period, four tanks were assigned to each experimental treatment. Each tank was designated as an experimental unit (EU). The experiment included two phases, each lasting 6 days. The first phase involved *Pterygophora californica* and a simulate MHW (i.e., MHW phase). The second phase consisted of sporophyte recovery to a control

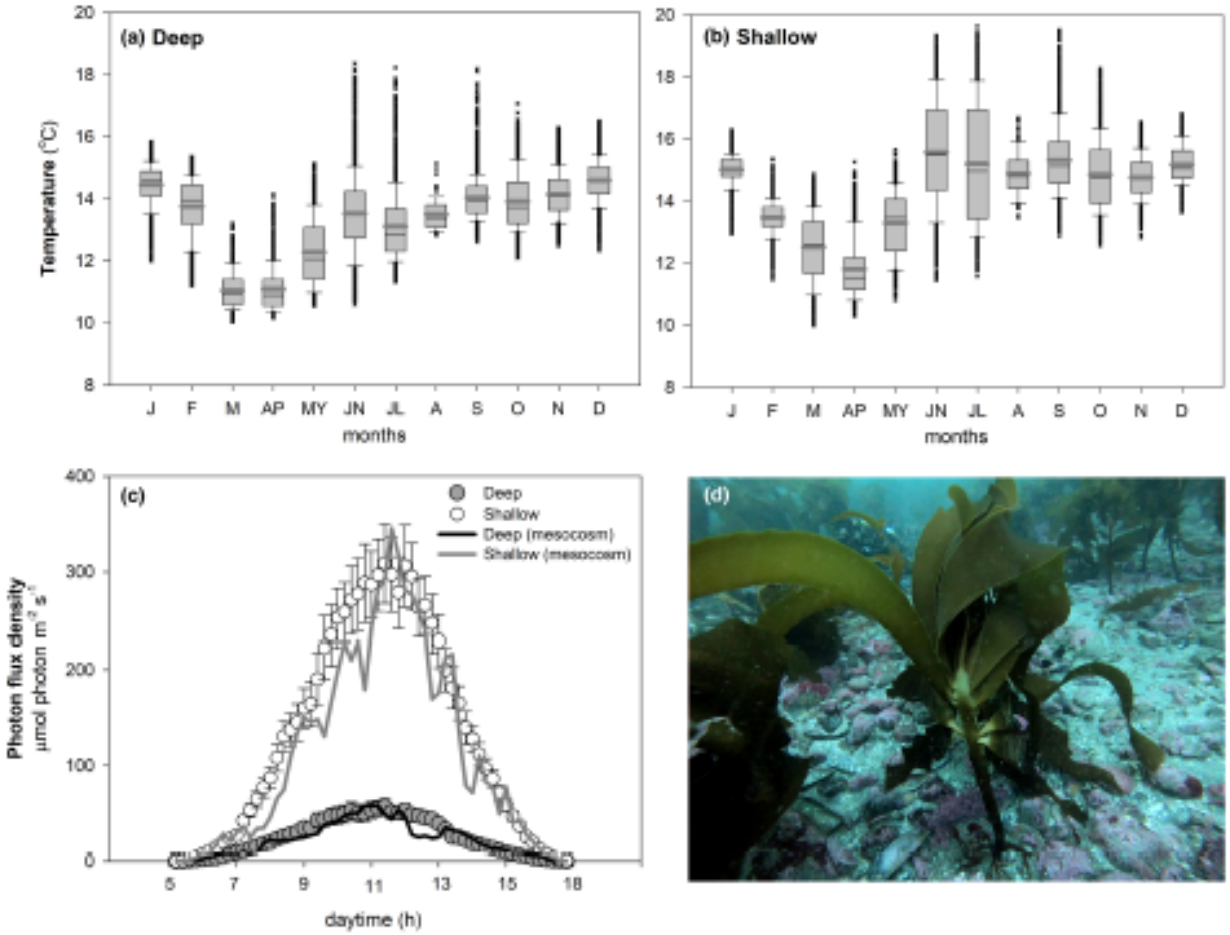

FIGURE 1 Boxplots of the seasonal temperature changes recorded in the bathymetric extremes for the experimental *Pterygophora californica* population (a) Deep: 25–27 m, (b) Shallow: 9–10 m. Boxplots with median, mean (thick gray line), boxes for 25th and 75th percentiles, and whiskers indicating 10th and 90th percentiles. (c) Daily variation of Irradiance (PAR) measured at both depths. Symbols represent the mean and standard errors, $N = 25$, and the lines represent the average Irradiances measured in the mesocosm systems for the deep and shallow treatments. (d) Adult sporophyte of *Pterygophora californica* at the collection site (Campo Kennedy, Baja California, Mexico). [Color figure can be viewed at wileyonlinelibrary.com]

temperature (i.e., post-MHW phase). The MHW phase comprised of four treatments: (1) shallow sporophytes under a control temperature of 14°C (SC), (2) shallow sporophytes exposed to a high temperature of 20°C (SH), (3) deep sporophytes under a control temperature (DC), and (4) deep sporophytes exposed to high temperature (DH). The temperature was raised gradually from 14 to 20°C by 2°C each day. This simulated MHW was based on thermal anomalies recorded in the region utilizing an online MHW Tracker (Schlegel, 2020). In the post-MHW phase, the temperature in SH and DH steadily decreased by 2°C per day, reaching 14°C from 20°C. The temperature in control treatments (SC, DC) remained constant at 14°C throughout the experiment.

The biological characteristics of the sporophytes (see below) were examined at the conclusion of each experimental phase (MHW and post-MHW). At each sampling time, only one sporophyte per EU ($n = 4$) was used. All the physiological descriptors were measured from the first two fully mature blades beneath the vegetative (terminal) blade to minimize a possible variability between tissues in biological traits. For each physiological variable, three pseudo-replicates (blade pieces) were obtained in each sporophyte per tank. Values of pseudo-replicates were averaged per tank.

Photosynthesis and respiration (P-E curves)

A blade segment (20 cm^2) was incubated inside a jacketed borosilicate chamber (200 mL) filled with filtered (1 µm) seawater. Magnetic stirring was continuously applied during the incubation period. Oxygen evolution was measured by optodes (dipping probe DP-PSt3, PreSens, Germany) connected to a fiber optic oxygen meter (OXY 4 SMA, PreSens, Germany) controlled by the software (Measurement Studio 2,

PreSens, Germany). A tissue biomass of approximately 0.12 g of dry weight (DW) was purposely chosen to prevent an underestimation of photosynthesis caused by oxygen supersaturation and carbon limitation. First, the blade segment was kept in the dark for 15 min to calculate its dark respiration rate. Afterward, it underwent exposure to nine gradually increasing photon flux densities within the PAR range of 5, 19, 32, 60, 88, 100, 200, 400, and 800 µmol photons · m^{-2} · s^{-1} to measure its net photosynthetic rate. Light intensities were achieved using four LED light sources (10 W) positioned around the incubation chamber. The temperature of the incubations was adjusted according to the temperature of each treatment. The gross and net-photosynthetic rates (gross-P_{max} and net-P_{max}; µmol O_2 · g^{-1} DW · h^{-1}), the dark respiration (R; µmol O_2 · g^{-1} DW · h^{-1}), the saturation and compensation irradiances (E_k and E_c; µmol photons · m^{-2} · s^{-1}), and the photosynthetic efficiency (α; µmol O_2 · g^{-1} DW · h^{-1}/µmol photons · m^{-2} · s^{-1}), were calculated according to Sandoval-Gil et al. (2023). Daily net productivity (DP; mmol O_2 · g^{-1} DW · d^{-1}) was calculated following the equation:

$$DP = (\Sigma \text{ net} - Pi) - (\Sigma R)$$

where ∑ net-Pi is the sum of net-photosynthetic rates at each irradiance level during daylight and ∑ R is the sum of respiration rates during the night (i.e., assuming the mean R obtained from P-E curves). Values of Pi were calculated by the model of P-I following Jassby and Platt (1976) [$Pi = P_{max} \times \tanh(\alpha \times I \times P_{max})$], where I is the changing irradiance during the daylight.

Blade absorption spectra

Blade absorptance represents the efficiency of photosynthetic tissues (pigments) to harvest light for photosynthesis. Blade absorbance (OD, optical density) was measured spectrophotometrically by utilizing the opal glass technique developed by Shibata (1959) and following the guidelines outlined in Vásquez-Elizondo et al. (2017). To conduct the measurements, an intact blade piece (~2 cm^2) was placed between two microscope glass slides. Additionally, another blade piece was treated with commercial bleach diluted in seawater and used as a white reference. Optical opal glasses and glass slides containing pigmented and bleached tissues were positioned near to the photomultipliers (detectors) of the spectrophotometer. Absorbance was measured in the PAR range (400–700 nm), and the resulting spectra were adjusted for residual scattering and non-photosynthetic absorption by subtracting the absorbance at 725 nm. The proportion of light captured by blade tissues was expressed as absorptance ($A = 1–10^{-OD}$), assuming a negligible tissue reflectance. Values of A_{PAR} and A_{680} corresponded to the average absorptance in the PAR region and at the peak of chlorophyll *a* (chl *a*).

Pigment content

Chlorophyll *a* and Fucoxanthin were analyzed through sequential extractions following the method outlined by Seely et al. (1972) with modifications by Wheeler (1980). Initially, dimethylsulfoxide (DMSO) was used to extract fucoxanthin and chl *a* from 0.02 g FW of blade tissue. Subsequently, absorbance was determined at 665, 631, 582, and 480 nm. A second 24-h extraction using 100% acetone was conducted on the same tissue sample, under 4°C and darkness. The resulting solution was diluted 3:1:1 with methanol and distilled water. The pigment content was then measured by its absorbance wavelengths at 470, 581, 631, and 664 nm. The total pigment content was calculated as the sum of the pigment concentration from both sequential extractions.

Photosynthesis based on chlorophyll *a*-fluorescence (PSII)

Photochemical capacities were assessed by measuring changes in the fluorescence emission of the chl *a* of PSII using a portable Pulse Amplitude Modulated Fluorometer (Diving-PAM, Walz, Germany). Maximum Quantum Yield [$(F_m - F_0)/F_m$ or (F_v/F_m)] was measured in sporophytes dark-adapted overnight by applying a saturating pulse (0.8 s, ~5000 µmol photons · m^{-2} · s^{-1}). The minimum fluorescence (F_0) was measured was measured under a "measuring light" before the actinic light pulse, while F_m was the maximum fluorescence induced by the pulse. At midday, fluorescence measurements were repeated on illuminated sporophytes in the same precise blade pieces. The blades were illuminated with the actinic light of the fluorometer through the fiber optic at a saturating intensity of 300 µmol photons · m^{-2} · s^{-1}. Three minutes of illumination were adequate to establish a steady state of photosynthesis after induction curve trials. Next, a saturating pulse was applied to measure F and F_m and, thus, the Effective Quantum Yield [i.e., Φ_{PSII}, $(F_{m'} - F)/F_{m'}$, or $(\Delta F)/F_{m'}$] and Non-Photochemical Quenching [NPQ, $(F_m - F_{m'})/F_{m'}$]. In addition, the Electron Transport Rate was determined using the formula [$ETR = \Phi_{PSII} \times E \times A \times 0.5$]. Here, E represents the actinic light applied, A denotes tissue absorptance (refers to methods above), and 0.5 is a correction factor applied for the assumed equal distribution of photons between photosystems.

Blade growth rates

Blade growth was measured in two sporophytes per EU and treatment, using Parke's (1948) method. Growth was measured for the vegetative blade and first blade, which were approximately 20 cm lengths. A 2-mm hole was punched in the midrib of the chosen blades, 10 cm from the base of the blade (above the meristem). After exposure to the experimental treatments (6 days in each phase), the distances between the base of the meristem and the hole were re-measured, allowing for the determination of the longitudinal growth per day. Blade growth (G, cm per day) was calculated using the formula [G = MH – H/*T*], where MH is the hole's movement distance from the base of the blade, H (hole) is the punching's initial distance from blade's base (i.e., 10 cm), and *T* represents the days of the experimental phase. Blade growth was also measured in situ in six sporophytes per depth at the collection site.

Nitrate uptake kinetics and total N

Nitrate uptake rates and N content were examined to assess the effects of warming on the incorporation and assimilation of external N. Blade pieces were incubated in transparent plastic Ziploc bags containing artificial seawater with various nitrate concentrations ($^{15}KNO_3$ at. % = 99, Cambridge Isotope Laboratories), including 5, 10, 20, and 30 μM. Each plastic bag (2.5 L) contained four blade pieces (7 × 7 cm, ~0.15 DW). This seaweed biomass-to-volume ratio was utilized in previous trials to prevent a substantial decrease in the external nitrate concentration during the 30-min incubation period. To prevent a decrease in external nitrate concentration, it was important to avoid a concomitant decrease in nitrate uptake rates and thus an underestimation of nitrate uptake by tissues. Four bags were used for each nitrate concentration and treatment ($n = 4$). The bags were freely suspended within the mesocosms tanks under corresponding temperature and irradiance conditions for each treatment. All incubations were conducted at noon to avoid changes in irradiance during the day, which can affect nitrate uptake. Upon completing incubations, blade tissues were washed with deionized water to remove surface nutrients and subsequently dried at 60°C for 48 h. Samples were ground for isotope enrichment analysis. Isotopic determinations were carried out at the UC-Davis Stable Isotope Facility, using an elemental analyzer (EA) connected to a continuous flow isotope ratio mass spectrometer (IRMS). Nitrate uptake rates (V, expressed as μmol N · g^{-1} DW · h^{-1}) were calculated as $V = [(^{15}N_{exp} - {}^{15}N_{back}) \times N_c]/(M_N \times t)$, where the difference ($^{15}N_{exp} - {}^{15}N_{back}$, at. %) is the ^{15}N enrichment relative to natural ^{15}N (background signal), N_c is the nitrogen content (g N·g^{-1} DW), M_N is the molar mass of nitrogen (15 g · mol^{-1}), and t is the duration of the incubation. Nitrogen content was also analyzed from the samples used as background (i.e., those not exposed to the $^{15}NO_3^-$ tracer) for isotope analyses.

Total soluble carbohydrates

Total intracellular carbohydrate content was analyzed to assess the internal C-reserves in the blades. Carbohydrates were measured using the phenol-sulfuric acid colorimetric method (Dubois et al., 1956). Approximately, 0.02 g FW of tissue was dried, ground, and then digested in 0.2 M HCl at 60°C for 3 h. After centrifugation (5 min, 1000 *g*), the supernatant was mixed with 3% phenol and sulfuric acid (95%). Absorbances were read at 490 nm, using glucose as a standard.

Lipid peroxidation

Lipid peroxidation (i.e., a variable that represents the oxidative damage of reactive oxygen species over biological membranes) was determined using the thiobarbituric acid reactive substances (TBARS) assay as described by Hodges et al. (1999) and Correia et al. (2006). Blade fresh tissue (~0.5 g) was homogenized with trichloroacetic acid (TCA, 20%). Next, the homogenates were centrifuged (10 min, 3000 *g*, 4°C), and the resulting supernatants were mixed with a solution of TCA (20%) and thiobarbituric acid (TBA, 0.5%). The solutions were heated at 90°C for 30 min, followed by another round of centrifugation (10 min, 10,000 *g*). The supernatants were extracted, and their absorbances (440, 532, and 600 nm) were measured.

Total phenolic content and antioxidant capacity

The antioxidant capacity and total phenols were measured as a proxy of the general antioxidant response under a potential oxidative stress caused by warming. Approximately 0.02 g DW were ground and mixed with 80% methanol. The mixture was left in darkness for 24 h, and then centrifuged (10 rpm for 10 min). The resulting supernatant was used to measure phenolic compound content through a modified Folin–Ciocalteu assay, with gallic acid as a reference (Singleton & Rossi, 1965). To briefly summarize, an aliquot of the methanolic extract was diluted in 1 mL of distilled water. Then, 0.1 mL of Folin–Ciocalteu reagent and 0.3 mL of saturated $NaCO_3$ were added, homogenized, and heated at 40°C for 3 min.

Subsequently, absorbance was read at 765 nm. The radical scavenging activity of the same methanolic extracts was determined over the stable free radical, DPPH, using ascorbic acid as the standard (Sabeena Farvin & Jacobsen, 2013). The reactive solution was prepared by diluting 0.1 mL of diluted extract in aqueous methanol (80% concentration) in a 1:4 ratio and mixing it with 1 mL of 30 μM DPPH freshly prepared in aqueous methanol (90% concentration). After precisely 30 min, the DPPH was added, and the absorbance was measured at 517 nm. A blank control was also prepared with the same proportions of DPPH and aqueous methanol, but without the algal extract. The absorbance of the blank solution was used as a reference.

Statistical analysis

A *t*-test was conducted to compare the averages of the different biological variables measured in *Pterygophora terygophora* sporophytes growing at both depths in the natural population (Table 1). For each experimental phase (MHW and post-MHW), a two-way ANOVA (posthoc Student–Newman–Keuls) was applied with temperature (two levels; control and high) and depth (two levels: shallow and deep) as fixed and independent factors. Significant differences were identified at $p < 0.05$. Data were tested for the assumptions of normality and homoscedasticity through the Shapiro–Wilk and Levene tests, respectively. A permutation ANOVA test (10,000 permutations) was performed when the assumptions of normality and homoscedasticity were not met even after transformations. Differences in nitrate uptake rates were statistically analyzed using a three-way ANOVA for each experimental phase, with temperature, depth, and nitrate concentration as fixed factors. Rstudio software and Sigmaplot 14.5 were applied for statistical analyses. Supplementary material (Appendix S1: Tables S1–S4) includes the provided statistical outcomes.

Multivariate analyses were conducted using normalized data from all response variables, and a ranked triangular similarity matrix was created based on Euclidean distances. A two-way PERMANOVA crossed design (9999 permutations) was completed to assess the interactive effects of the factors Depth and Temperature. A Monte Carlo *p*-values, P(MC), was used to verify significant differences in pair-wise posterior comparisons, as the number of possible permutations was limited. A non-metric multidimensional scaling (MDS) ordination was utilized to visualize multivariate patterns (Appendix S1: Figure S2). The statistical procedures were carried out with the PRIMER 6 & PERMANOVA+ v.1.0.2 software package (Anderson et al., 2008).

RESULTS

Comparison of deep and shallow *Pterygophora californica* from the natural population

The following result corresponds to the biological descriptors measured in sporophytes just after collection, before the beginning of the experiment. Values of net-P_{max}, respiration, and E_c were significantly higher (10%, 60%, and 140%, respectively) in shallow-water sporophytes compared to deep-water sporophytes (Table 1). Similarly, values of lipid peroxidation, antioxidant capacity, chl *a* content, and Φ_{PSII} were higher in shallow-water individuals. Blade growth was three-fold higher in shallow *Pterygophora californica* (Table 1). Conversely, deep-water sporophytes showed higher F_v/F_m, soluble carbohydrates concentration, and blade absortance, A_{PAR} (Table 1). Values of gross-P_{max}, E_k, α, A_{680}, fucoxanthin, NPQ, ETR, total phenols, $\delta^{15}N$, and total N were similar in sporophytes collected from depths (Table 1).

Biological responses in the MHW and post-MHW phases

The two-way PERMANOVA (Table 2) revealed significant differences in the analyzed biological characteristics between sporophytes from both depths ($p = 0.001$). Furthermore, this multivariate analysis demonstrated the significant impact of the MHW ($p = 0.001$) on the physiology of *Pterygophora californica* during both experimental phases (MHW and post-MHW), and these varied significantly between deep-water and shallow-water individuals (i.e., significant interaction depth x temperature, $p < 0.001$). The non-metric multidimensional scaling analysis (MDS, Figure S2) supports the PERMANOVA findings. The MDS also demonstrated greater 2D distance differences between control and heated deep-water sporophytes (DC vs. DH).

The P-E curves (Figure 2a,b) were fitted to tangential hyperbolic adjustments and showed a strong correlation ($R^2 > 0.97$, $p < 0.05$). The values of E_c (Figure 2c) decreased by 50% in DH relative to control sporophytes during the post-MHW phase (Table S2). Gross-P_{max} (Figure 2d) significantly increased by –25%–50% in DH and SH sporophytes exposed to high temperature (Table S1). In the post-MHW phase, DH sporophytes exhibited a similar trend, but their values decreased by 20% compared to their control in SH (Table S2). Warming exposure induced a 40%–60% drop in respiration (R, Figure 2e) in both SH and DH sporophytes, but significant differences were found only for DH (Table S1). During the post-MHW phase, the interaction between factors (D×T) was found to be statistically significant (Table S2). The decrease of R by 40% in SH (Table S2)

TABLE 1 Field reference values of biological descriptors measured in *Pterygophora californica* from the deep (25–27 m) and shallow (8–10 m) population limits.

Biological variables	*Pterygophora californica* (Deep) Mean ± *SE*	*Pterygophora californica* (Shallow) Mean ± *SE*	*p*
Net-P_{max} µmol O_2 · g^{-1} DW · h^{-1}	232 ± 1	257 ± 10	**0.04**
Gross-P_{max} µmol O_2 · g^{-1} DW · h^{-1}	266 ± 5	278 ± 10	0.339
Respiration µmol O_2 · g^{-1} DW · h^{-1}	14 ± 1	22 ± 1	**<0.001**
α	1.5 ± 0.1	1.3 ± 0.1	0.099
E_c µmol photons · m^{-2} · s^{-1}		19 ± 1	**<0.001**
E_k	174 ± 9	195 ± 6	0.092
Total soluble carbohydrates % DW	3.27 ± 0.06	2.98 ± 0.04	**0.003**
A_{PAR}	0.73 ± 0.003	0.71 ± 0.003	**<0.001**
A_{680}	0.94 ± 0.002	0.93 ± 0.002	0.224
Chl *a* µg · g^{-1} FW	470 ± 41	693 ± 32	**0.001**
Fucoxanthin µg · g^{-1} FW	1211 ± 97	1151 ± 52	0.588
F_v/F_m	0.77 ± 0.01	0.70 ± 0.01	**0.001**
Φ_{PSII}	0.33 ± 0.01	0.37 ± 0.01	**0.038**
NPQ	0.41 ± 0.04	0.53 ± 0.05	0.094
ETR µmol e^- · m^{-2} · s^{-1}	25 ± 0.4	27 ± 1	0.116
$\delta^{15}N$	6.74 ± 0.26	6.29 ± 0.29	0.271
N % DW	1.60 ± 0.1	1.62 ± 0.07	0.907
Lipid peroxidation nmol equation AA · g^{-1} DW		38.16 ± 3.03	**0.009**
Total phenols mg equation AA · g^{-1} DW	2.69 ± 0.21	3.13 ± 0.16	0.119
Antioxidant capacity mg equation AA · g^{-1} DW	0.71 ± 0.1	1.58 ± 0.04	**<0.001**
Growth (first blade) cm · d^{-1}	0.35 ± 0.05	0.98 ± 0.05	**<0.001**

Note: Measurements were taken immediately after collection (i.e., before the experiment). Bold values denote significant values ($p < 0.05$, *t*-test).

and the increase of R by 10% in DH (Table S2) further supported these results. Photosynthetic efficiency (α, Figure 2f) did not vary in either SH or DH sporophytes. However, the D×T interaction was significant during the post-MHW phase (Table S2) due to a 60% increase in values observed in DH, while decreasing by 40% in SH. In shallow sporophytes, daily net productivity (Figure 2g) increased by ~15% when exposed to warming (Table S1), while decreasing by 40% during the post-MHW phase. Deep sporophytes (DH) exhibited a notable rise in their daily productivity (by ~50%) during the post-MHW phase (Figure 2i; Table S2). During the post-MHW phase, saturation irradiance (E_k, Figure 2h) uniquely decreased in DH sporophytes compared to the control (DC; Table S2).

Blade absorptance in the PAR range (A_{PAR}; Figure 3c) increased in sporophytes that were exposed to high temperatures (SH) in the post-MHW phase (Table S2). Nonetheless, temperature did not significantly impact A_{680} in DH and SH sporophytes during any phase. The contents of chl *a* and Fx did not undergo changes in deep and shallow sporophytes upon warming (Figure 3e,f; Table S1). However, both pigments exhibited a decrease in DH sporophytes during the post-MHW phase (Figure 3e,f; Table S2).

The F_v/F_m values (Figure 4a) were similar across treatments in both experimental phases. During the exposure phase, there was an increase in Φ_{PSII} in DH and SH sporophytes, but the difference was statistically significant only for DH (Figure 4b; Table S1). On

TABLE 2 PERMANOVA outputs of the effects of the temperature treatments on the physiology of shallow-water and deep-water *Pterygophora californica* after the experimental phases of marine heatwave (MHW) and post-MHW.

Experimental phase	Main test	*df*	SS	Pseudo-*F*	*p* (Perm)
MHW	Depth	1	119.65	15.837	**0.001**
	Temperature	1	39.575	5.238	**0.001**
	D×T	1	35.109	4.647	**0.001**
	Residuals	12	7.555		
Post-MHW	Depth	1	104.72	18.252	**0.001**
	Temperature	1	51.677	9.007	**0.001**
	D×T	1	59.749	10.414	**0.001**
	Residuals	12	68.851		

Note: Bold numbers indicate significant differences.

the contrary, DH sporophytes displayed lower Φ_{PSII} compared to their respective controls in the post-MHW phase (Figure 4c; Table S2). Non-photochemical quenching (Figure 4c) exhibited no significant variation in *Pterygophora californica* between treatments across both experimental phases, with the exception of a decline in SH sporophytes observed in the post-MHW phase (Table S2). Exposure to high temperature elevated the ETR of SH sporophytes (Figure 4d; Table S1).

The statistical significance of the interaction between the factors (D×T, depth) was observed for blade growth in both phases (Figure 5a,b; Tables S1 and S2). The growth of the first blade (Figure 5a) and the vegetative blade increased by 140%–471% in DH sporophytes and decreased by 32%–72% in SH sporophytes. Although statistically significant changes were observed in the growth of the first blade (Tables S1 and S2), only significant differences were found between SC and SH during the post-MHW phase (Table S2).

Nitrate uptake rates (Figure 6a,b) of both shallow and deep sporophytes did not reach saturation at the tested nitrate concentrations (up to 30 μM). Linear regression adjustments indicated a good fit for all the treatments ($R^2 > 0.92$; $p < 0.05$). There was a significant decrease in nitrate uptake rates for both deep and shallow sporophytes in the post-MHW phase compared to control values (Table S4), with differences being higher for the former.

Nitrogen content (Table 3) was significantly greater in sporophytes from deeper waters than those from shallower water in both experimental stages, regardless of temperature treatment (Table S1 and S2). Total soluble carbohydrates (Table 3) decreased by −30% in deep sporophytes exposed to warm temperatures compared to DC individuals (Table S1). In the post-MHW phase, total carbohydrates decreased in both deep and shallow sporophytes compared to their respective controls (Table S2).

During the exposure phase, there was a significant interaction of factors for lipid peroxidation (D×T; Figure 7a; Table S1). Specifically, this variable decreased by −30% in DH sporophytes but increased by −30% in SH (Table S1). In the post-MHW phase, lower values of lipid peroxidation continued to persist in DH sporophytes (Table S2), whereas SH and SC sporophytes displayed comparable lipid peroxidation. The total amount of phenolic compounds (Figure 7b) rose by −35% in DH sporophytes following exposure to warming conditions (Table S1). However, DH sporophytes experienced a decline in total phenols while SH sporophytes showed an increase after the occurrence of MHW (i.e., the interaction of factors D×T was significative; Table S2). Only SH sporophytes demonstrated an increase in antioxidant capacity during the post-MHW phase (Figure 7c; Table S2).

DISCUSSION

Ecophysiology of *Pterygophora californica* in its bathymetric extremes

Pterygophora californica undergoes significant variations in lighting throughout its bathymetric distribution. In the collection site during spring, the shallow portion of the population (8–10 m depth) receives a higher light dose of approximately 7 mol photons · m^{-2} · d^{-1}. Maximum irradiance at midday reached around 350 μmol photons · m^{-2} · s^{-1}. In contrast, the deeper sporophytes (25–27 m) are exposed to lower light levels, receiving approximately 1 mol photons · m^{-2} · d^{-1} with irradiance peaks of around 60 μmol photons · m^{-2} · s^{-1}. These differences in light exposure significantly impact the photo-acclimation and photo-adaptation of *P. californica* (Table 1). Shallow-water sporophytes showed higher maximum photosynthetic rates (gross-P_{max}), electron transport rate (ETR), and saturation irradiance (E_k). Blades of these sporophytes also contain more chl *a*, indicating more photosystem reaction centers (Beer et al., 2014; Davison et al., 1991). These photobiological properties enable more efficient use of light above saturating irradiances and are typically associated with seaweeds acclimated to high light conditions (Hurd et al., 2014). As the light intensity increases, the photosynthetic apparatus at the thylakoid level becomes more excited and undergoes greater reduction (Beer

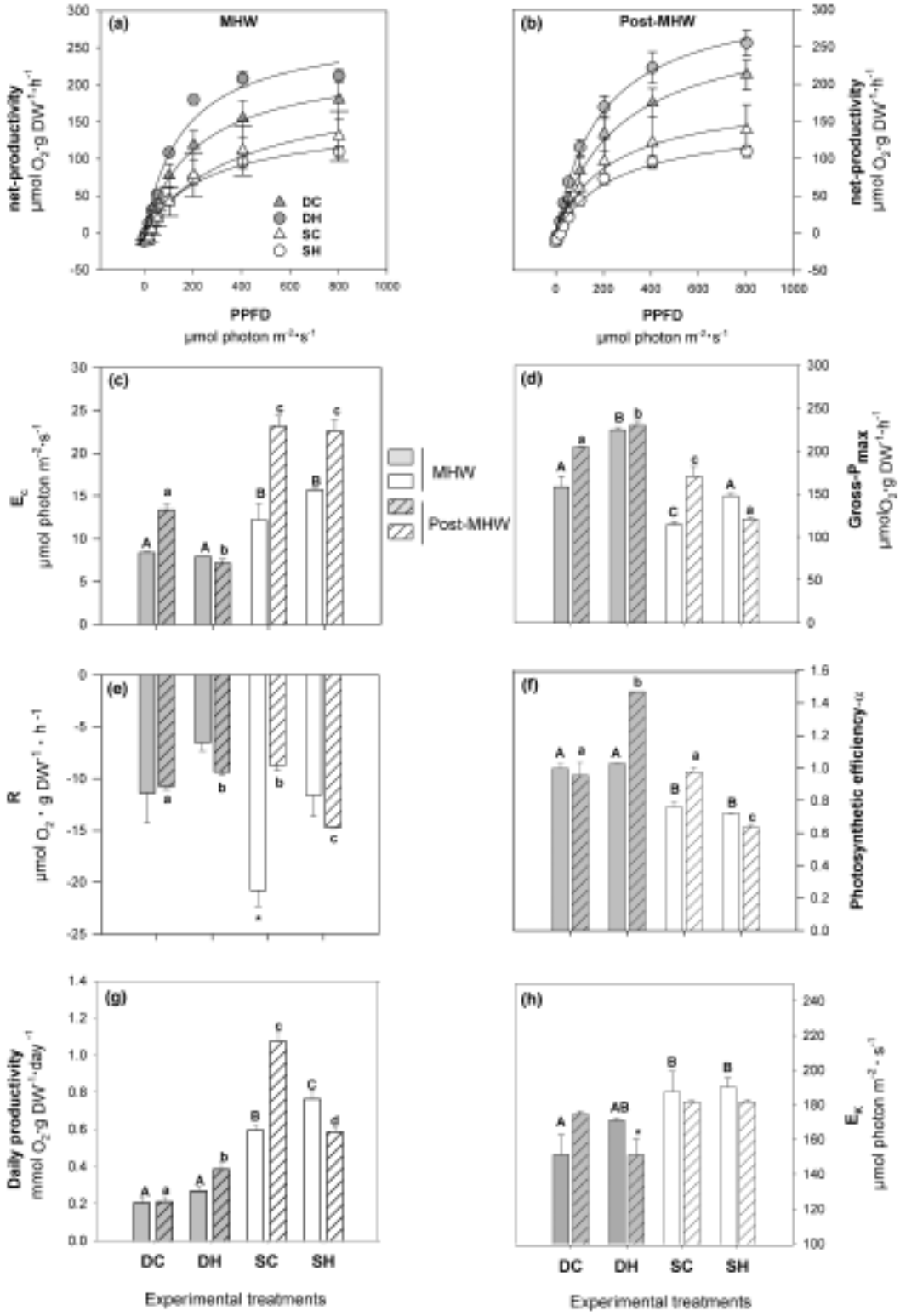

FIGURE 2 Photosynthetic parameters from P-E curves measured in deep water (25–27 m; gray bars) and shallow water (8–10 m; white bars) *Pterygophora californica* sporophytes under the different temperature treatments during both experimental phases. In the MHW phase, DC = deep sporophytes under control temperature (14°C), DH = deep sporophytes under heatwave (20°C), SC = shallow sporophytes under control temperature (14°C), SH = shallow plants under heatwave (20°C). In the post-MHW phase, 14°C was adjusted for all the treatments. Statistical differences among treatments in MHW and post-MHW phases are indicated by different uppercase and lowercase letters, respectively (two-way ANOVA, post hoc SNK). (a) MHW Net productivity, (b) Post-MHW Net productivity, (c) Compensation Irradiance, (d) Gross maximum photosynthetic rates, (e) Respiration, (f) Photosynthetic efficiency, (g) Daily productivity, (h) Saturation Irradiance. Bars are means and standard errors ($n = 4$).

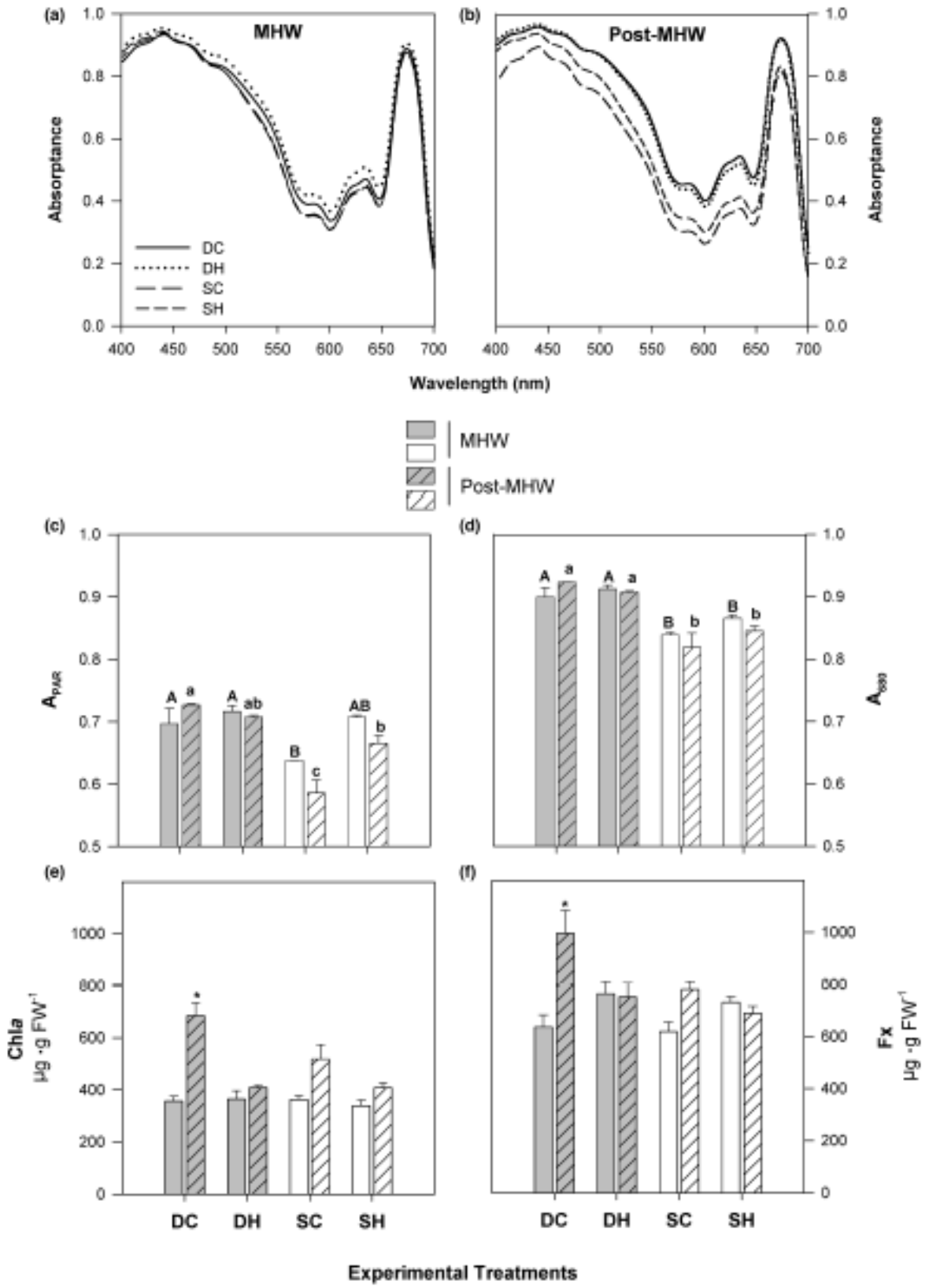

FIGURE 3 Blade tissue absorptance and pigment content measured in deep-water (gray bars) and shallow-water (white bars) *Pterygophora californica* sporophytes under the different temperature treatments during both experimental phases. See Figure 2 for further details on symbols, *x*-axis labels, and statistical analysis. (a) Absorptance spectrum in MHW-phase, (b) Absorptance spectrum in post-MHW-phase, (c) Integrated absorptance in the PAR range, (d) absorptance at 680 nm (chl *a* peak), (e) chlorophyll *a* content and (f) fucoxanthin. Bars are means and standard errors ($n = 4$).

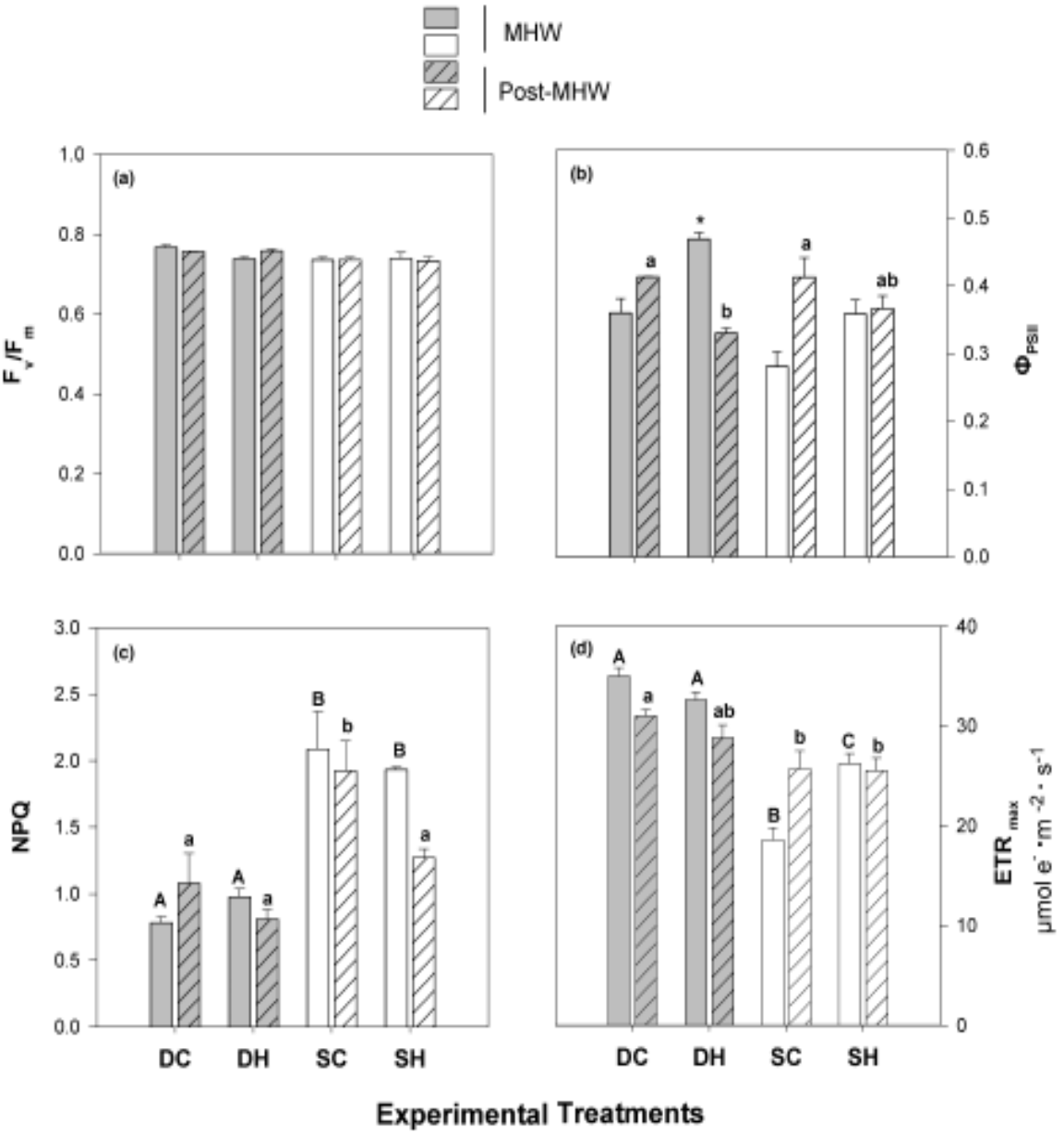

FIGURE 4 Photochemical descriptors based on chl a fluorescence measured in deep-water (gray bars) and shallow-water (white bars) *Pterygophora californica* sporophytes under the different temperature treatments during both experimental phases. See Figure 2 for further details on symbols, *x*-axis labels, and statistical analysis. (a) Maximum quantum yield, (b) effective quantum yield, (c) non-photochemical quenching, and (d) electron transport rate. Bars are means and standard error ($n = 4$).

et al., 2014; Lobban & Harrison, 1994). The increased values of NPQ observed in shallow sporophytes were consistent with this state, potentially functioning as a mechanism to dissipate excitation energy as heat through the xanthophylls cycle (XC; Beer et al., 2014; García-Mendoza & Colombo-Pallotta, 2007).

Shallow-water sporophytes exhibit higher respiration rates than those growing in deeper waters, likely due to a stimulated carbon metabolism or other energy-demanding processes such as growth (Eggert, 2012; Markager & Sand-Jensen, 1994). Stimulated respiration and photosynthesis inevitably produce more reactive oxygen species (ROS) via redox reactions in thylakoids and mitochondria (Beer et al., 2014). This is likely responsible for the higher lipid peroxidation levels (as a sign of oxidative damage) in these individuals compared to deep-water sporophytes (Umanzor et al., 2020).

Deep-water *Pterygophora californica* demonstrated photoacclimation to low-light conditions. These sporophytes showed greater photosynthetic efficiency (α, and, thus, greater F_v/F_m) and blade absorptance values, indicating their enhanced capacity to harvest light

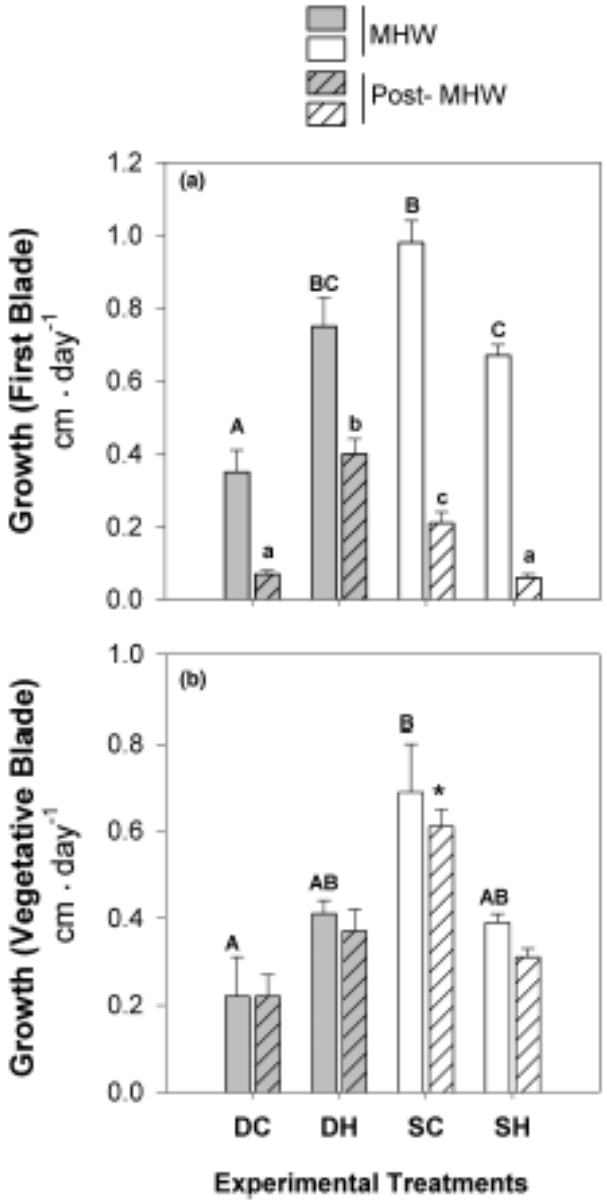

FIGURE 5 Growth of the blade measured in deep-water (D, gray bars) and shallow-water (S, white bars) *Pterygophora californica* sporophytes under the different temperature treatments during both experimental phases. (a) first blade and (b) vegetative blade. See Figure 2 for further details on symbols, *x* axis labels, and statistical analysis. Bars are means and standard error ($n = 4$).

in the PAR range. Therefore, photosynthesis becomes more efficient at sub-saturating irradiances in low-light regimes (Hanelt & Figueroa, 2012; Hurd et al., 2014; Tait & Schiel, 2013). As a result of a higher α, the compensation irradiance (E_c) is reduced, requiring less light for photosynthesis to balance respiration and achieve positive net metabolic productivity. The sporophytes' low respiratory activity optimizes carbon balance under light limitation (Bernardeau-Esteller et al., 2011, 2015; Sandoval-Gil et al., 2014; Umanzor et al., 2020).

Several biological differences between the natural deep-water and shallow-water *Pterygophora californica* were also observed when comparing control individuals from both depths, referred to as DC and SC, respectively. These differences included physiological descriptors such as respiration, α, E_c, NPQ, antioxidant capacity, blade absorptance, and growth rate. This suggests that the collection and transplantation of these sporophytes had no significant impact on their physiological status and that the mesocosm system maintained the biological differences attributed to adaptation to depth. It additionally confirmed that the effects and responses observed in *P. californica* were due to the implemented experimental treatments rather than any confounding effects associated with manipulating sporophytes. Furthermore, and regarding thermal conditions, deep and shallow sporophytes encountered analogous thermal regimes in the studied population (see Figure 1a,b), despite growing in the bathymetric extremes. Thus, rather than thermo-adaptative mechanisms, photo-acclimation to light availability and other adaptive processes related to environmental conditions not evaluated in this study (such as nutrients and hydrodynamic) may significantly impact the distinct thermo-tolerance of *P. californica* at different depths (see below), rather than thermo-adaptive mechanisms.

Deep and shallow *Pterygophora californica* under MWHs

Our study demonstrated that exposure of sporophytes of *Pterygophora californica* to heatwave-like warming had varying impacts on their physiology. These effects were dependent on the bathymetric origin of the tested sporophytes.

Blade growth of deep-water sporophytes increased during both the exposure to warming and post-MHW phase. Overall, this can be explained by the high thermo-tolerance of the photobiological processes. Specifically, the warming applied during the MHW phase did not affect most of the photobiological variables studied in the deep-water sporophytes, except for a rise in gross-P_{max} and Φ_{PSII}. However, in the post-MHW phase, these sporophytes displayed enhanced photosynthetic capability and daily productivity due to increased values of gross-P_{max}, α, and E_c. This stimulation of photosynthesis may result from positive warming effects that are delayed and impact various related processes, including the activity of enzymes involved in carbon assimilation and the mobility of proteins in the thylakoid membranes (Andersen et al., 2013; Shindo et al., 2022; Vivanco-Bercovich et al., 2022; Wernberg et al., 2016). The unaltered values of F_v/F_m, ETR, and NPQ recorded during both the experimental periods (MHW and post-MHW) point toward the functional and structural integrity of the photosynthetic apparatus (Murchie & Lawson, 2013; Sánchez-Barredo et al., 2020; Umanzor et al., 2021), thereby sustaining

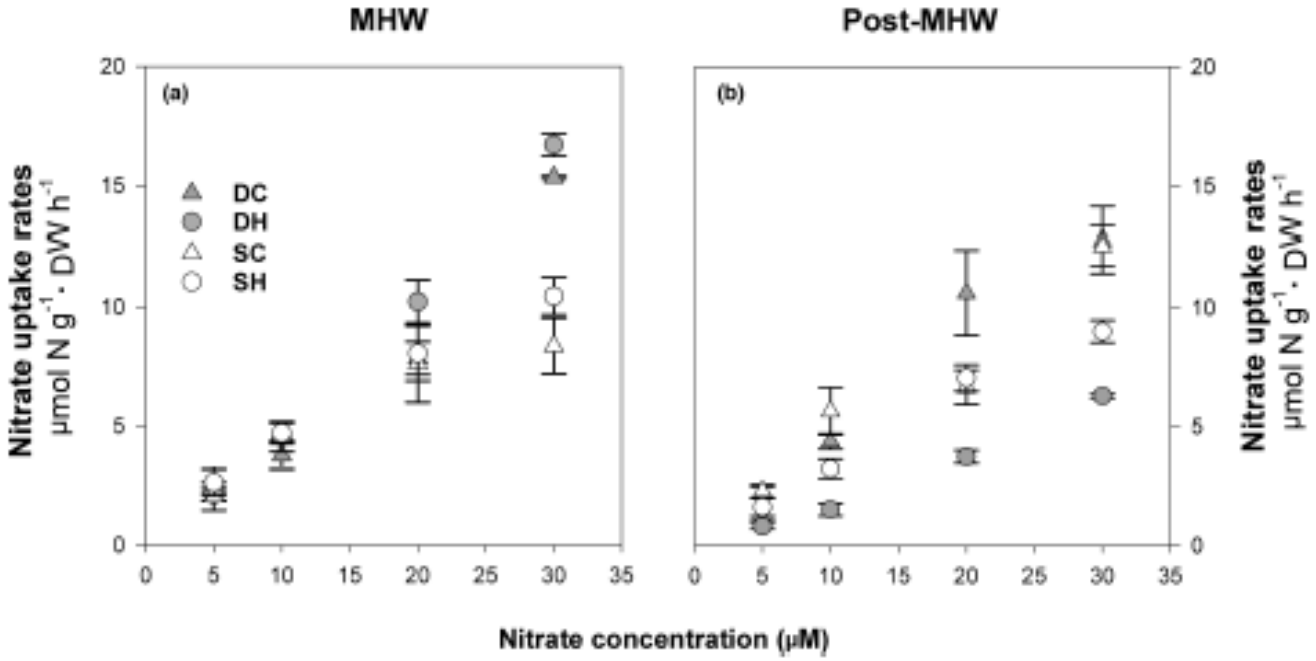

FIGURE 6 Nitrate uptake rates of deep-water (25–27 m; gray) and shallow-water (8–10 m; white) *Prerygophora californica* sporophytes under the different temperature treatments in the (a) MHW and (b) post-MHW phases. See Figure 2 for further details on the meaning of treatment abbreviations. Bars are means and standard errors ($n = 4$).

	Exp. phase	DC	DH	SC	SH
% N	MHW	1.37 ± 0.13a	1.49 ± 0.09a	1.23 ± 0.05b	1.21 ± 0.05b
	Post-MHW	1.44 ± 0.07a	1.56 ± 0.06a	1.22 ± 0.05b	1.37 ± 0.06b
TSC	MHW	3.20 ± 0.06a	2.45 ± 0.05b	2.99 ± 0.03ac	2.89 ± 0.09c
	Post-MHW	2.97 ± 0.16a	2.63 ± 0.1b	2.89 ± 0.12a	2.61 ± 0.07b

Note: Values show means and standard errors ($n = 4$). Different letters on the bars indicate statistical differences among treatments (two-way ANOVA, post hoc SNK; Tables S1 and S2). See Figure 1 for further details on the meaning of treatment abbreviations.

Abbreviations: DC, deep control; DH, deep heat; SC, shallow control; SH, shallow heat.

TABLE 3 Total nitrogen content (% N) and total soluble carbohydrates (TSC) measured in shallow-water and deep-water *Prerygophora californica* sporophytes after the experimental phases of marine heatwave (MHW) and post-MHW.

the positive photosynthetic response of deep-water sporophytes. The lower pigment concentration (chl *a* and Fx) and the similar blade absorptance (A_{PAR}, A_{680}) measured in these sporophytes appear to contradict this trend. Many studies (e.g., Sandoval-Gil et al., 2023) have documented a similar lack of correlation between light-harvesting and photosynthetic abilities. Processes not measured in this study are likely to be responsible for the increase in photosynthesis, rather than light capture capacities.

During the period of warming, shallow-water sporophytes of *Prerygophora californica* exhibited a slight increase in gross-P_{max} and a decrease in respiratory activity and showed a higher daily productivity. An increase in gross-P_{max} was also observed in deep-water individuals. However, the results observed during the post-MHW period differed significantly between deep-water and shallow-water *P. californica*, since gross-P_{max} and α of shallow-water sporophytes drastically decreased. This delayed stress induced by heat is likely a result of multiple alterations affecting both the light-dependent reactions of photosynthesis and subsequent photo-assimilation processes, such as carbon fixation/assimilation. These changes may encompass modifications in the structure and functionality of the PSII and thylakoid membranes, dissociation of the water-splitting complex, or the deceleration of electron transport between photosystems (Heinrich et al., 2012; Sandoval-Gil et al., 2014; Shindo et al., 2022). Despite a decline in gross-P_{max} and α, F_v/F_m, Φ_{PSII}, and ETR values remained similar to control levels. Discrepancies in photobiological parameters obtained from P-E curves and chl *a*-fluorescence can be attributed to the activation of mechanisms providing alternative electron sinks beyond photosynthesis, including the water–water cycle, photorespiration, and cyclic electron transport of PSI (Niyogi, 2000; Voss et al., 2013), as documented in other marine macrophytes (Marin-Guirao et al., 2016). Furthermore, the induction of these electron-draining processes likely contributed to reactive oxygen species (ROS) production in shallow *P. californica*, triggering antioxidant and free-radical scavenging responses,

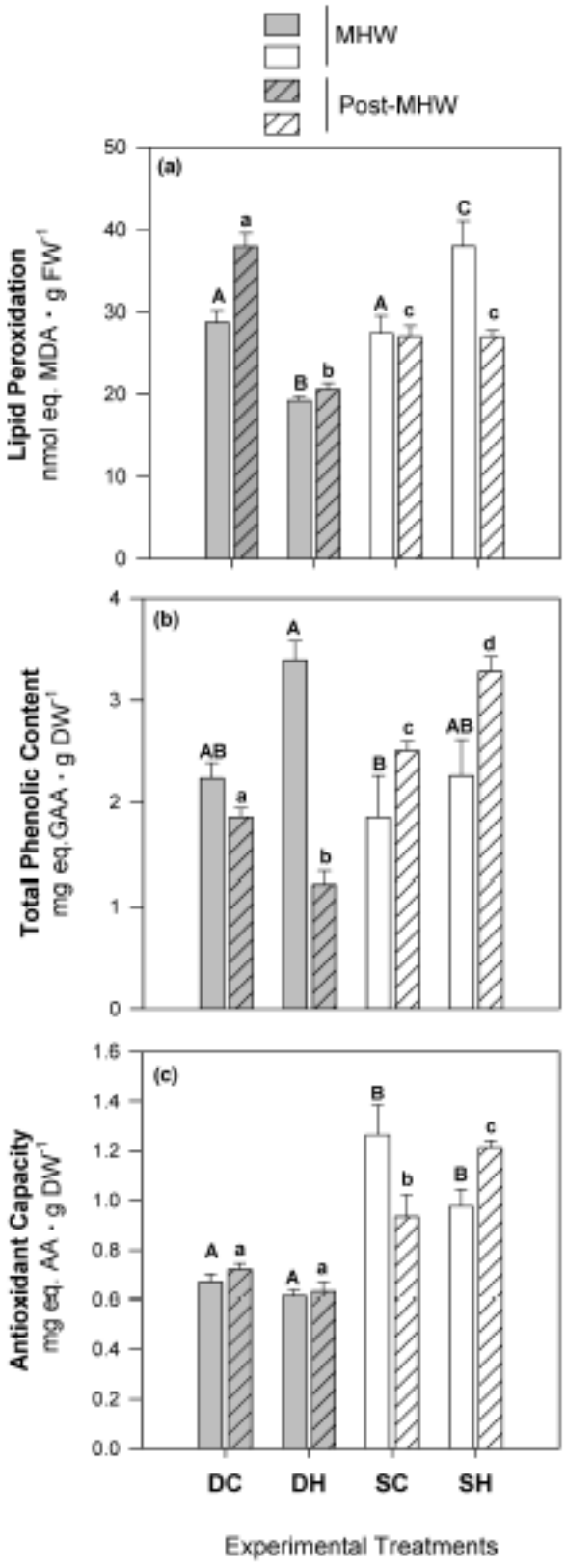

FIGURE 7 (a) Lipid peroxidation, (b) total phenolic content, and (c) total antioxidant capacity measured in deep-water (gray bars) and shallow-water (white bars) *Pterygophora californica* sporophytes under the different temperature treatments during both experimental phases. See Figure 2 for further details on symbols, *x* axis labels, and statistical analysis. Bars are means and standard error ($n = 4$).

leading to a higher phenolic content and antioxidant capacity. Although warming enhanced certain photosynthetic capabilities and daily net productivity during the MHW phase, shallow-water *P. californica* exhibited reduced blade growth rates and increased lipid peroxidation (i.e., oxidative damage), indicating unassessed heat-related metabolic stress in our study.

The reduced photosynthetic capacity of shallow *Pterygophora californica* in the post-MHW phase led to a severe decline in daily net productivity and internal carbon reserves. Respiration also increased in these sporophytes, further contributing to these metabolic carbon imbalances. Increased respiration to sustain high energy-demanding stress responses has been described in seaweeds under warming and other stressors (Anderson, 2006; Andersen et al., 2013; Britton et al., 2020). However, the opposite was observed for deep-water sporophytes, in which stimulated photosynthesis and reduced respiration likely fueled blade growth. Down-regulation of respiration not only reduces internal carbon losses but also allows optimization of net productivity at low (sub-saturating) irradiances near the compensation point, E_c (Blain & Shears, 2020; Davison et al., 1991; Fairhead & Chesire, 2004; Hurd et al., 2014; Rodrigues et al., 2000).

Nitrate uptake rate was another physiological trait affected in the post-MHW phase instead of during the warming period. Both deep-water and shallow-water *Pterygophora californica* sporophytes significantly declined their nitrate acquisition capacities. Evidence of the effects of warming on the N-uptake kinetics of seaweeds is limited (e.g., Fernández et al., 2023; Gerard, 1997; Sánchez-Barredo et al., 2020). Warming can directly affect the transmembrane transportation of nitrate and indirectly modify processes that determine its incorporation (e.g., Hurd et al., 2014; Sánchez-Barredo et al., 2020; Umanzor et al., 2023). In our study, for example, exposure to warming could have resulted in metabolic energy depletion of shallow-water sporophytes, which can potentially hinder the activity of N-assimilatory enzymes. A decrease in nitrate uptake rates was observed for *P. californica* for common external nitrate concentrations in the water column of the region (Camacho-Ibar et al., 2003; Hernández-Ayón et al., 2004) and was more pronounced at levels linked to upwelling events (~20 μM; Camacho-Ibar et al., 2003). This hinders *P. californica*'s ability to utilize this primary source of DIN that is necessary for the growth and survival of this and other foundation kelp species (Fernández et al., 2020; Hurd et al., 2014; Sandoval-Gil et al., 2023; Zimmerman & Kremer, 1986). Despite the decreased uptake of nitrate, the total N content in blade tissues did not decay, indicating that internal N-storage

may have been sustained by N mobilization and reabsorption from other vegetative compartments (Forbord et al., 2021; Gerard, 1982; Young et al., 2007). This decoupling between nitrate uptake and total N content in blade tissues has also been described for juvenile sporophytes of giant kelp exposed to warming and light limitation (Sandoval-Gil et al., 2023; Umanzor et al., 2021).

Overall, our study reveals that MHWs can impact the physiology and growth of the kelp *Pterygophora californica* in the North American Pacific, particularly near the southern limit of its distribution. The effects of warming vary based on the bathymetric origin of sporophytes. While MHWs may benefit deeper sporophytes (25–27 m), sporophytes in shallow waters (8–10 m) exhibit signs of metabolic stress, diminished growth, and oxidative damage. Like other kelp species, *P. californica* is expected to face more intense and deep MHWs. Shallow-water sporophytes may be significantly affected, while deeper ones may benefit. However, species responses could differ due to local adaptation and thermal niches (Andersen et al., 2013; Delebecq et al., 2013; Eggert, 2012; King et al., 2018). The potential within-species variability and unexplored factors affecting *P. californica*'s thermo-tolerance, such as early-life stage resistance and seaweed-microbiome interaction, require further investigation.

Our findings support the concept that deeper kelp populations can serve as refuges from MHWs (Giraldo-Ospina et al., 2020). This outcome presents an opportunity for future restoration strategies, such as transplanting deep-water sporophytes to revitalize damaged shallow *Pterygophora californica* beds or exploring their potential as a source of reproductive propagules for natural repopulation, as seen in other kelp species (e.g., *Ecklonia radiata*; Giraldo-Ospina et al., 2023). Notably, most effects and responses were observed after MHW cessation, aligning with studies on other macrophytes (Marín-Guirao et al., 2016; Umanzor et al., 2021; Vivanco-Bercovich et al., 2022) and indicating a delayed response to heat stress. Our results emphasize the need for extended experimental periods to comprehend seaweed's physiological tolerance and resilience capacities within the context of MHWs.

AUTHOR CONTRIBUTIONS

Antonella C. Almeida-Saá: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Schery Umanzor:** Investigation (equal); writing – original draft (supporting); writing – review and editing (supporting). **Jose Antonio Zertuche-González:** Investigation (equal); writing – review and editing (supporting). **Ricardo Cruz-López:** Investigation (equal); writing – review and editing (supporting). **Raquel Muñiz-Salazar:** Investigation (equal); writing – review and editing (supporting). **Alejandra Ferreira-Arrieta:** Methodology (equal); supervision (equal). **Paula Bonet Melià:** Methodology (equal). **Jessica Anayansi García-Pantoja:** Methodology (equal). **Laura K. Rangel-Mendoza:** Methodology (equal). **Manuel Vivanco-Bercovich:** Methodology (equal). **Leonardo Ruiz-Montoya:** Methodology (equal). **Jose Manuel Guzmán-Calderón:** Methodology (equal). **Jose Miguel Sandoval-Gil:** Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); supervision (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead).

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ORCID

Jose Miguel Sandoval-Gil https://orcid.org/0000-0001-8973-0306

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Containing Figures S1 and S2 and Tables S1-S4.

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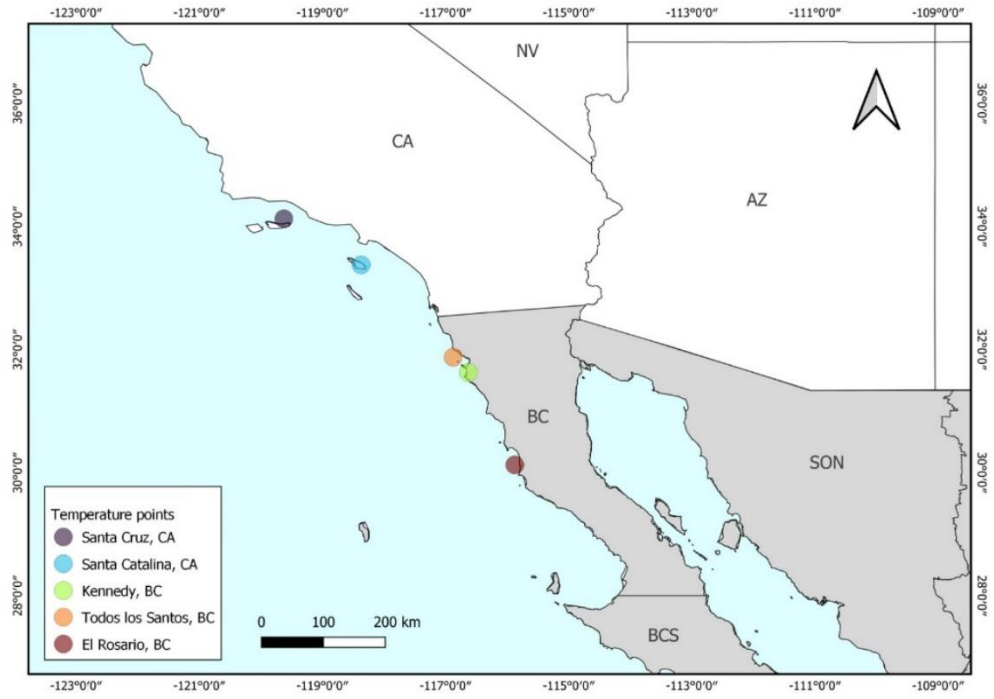
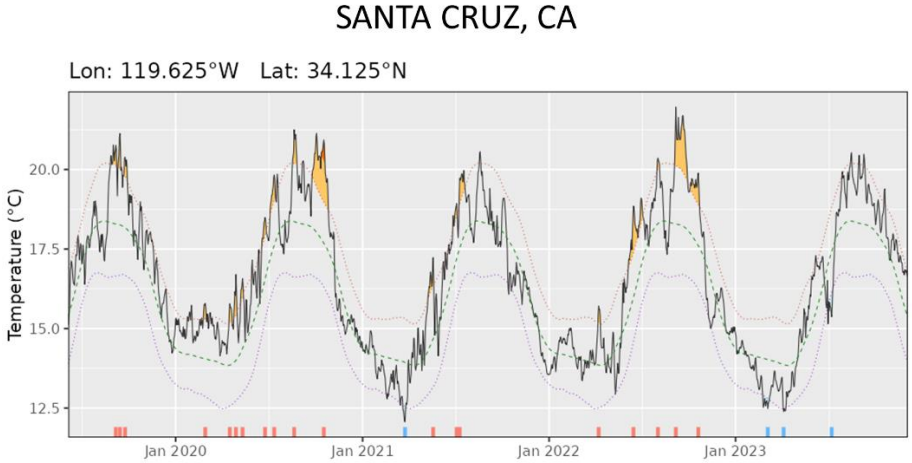
Supplementary Material

Figure S1. Seawater surface temperature measured at different regions where *Pterygophora californica* populations have been registered near the species' southern distribution limit. Data were obtained from http://www.marineheatwaves.org/tracker.html





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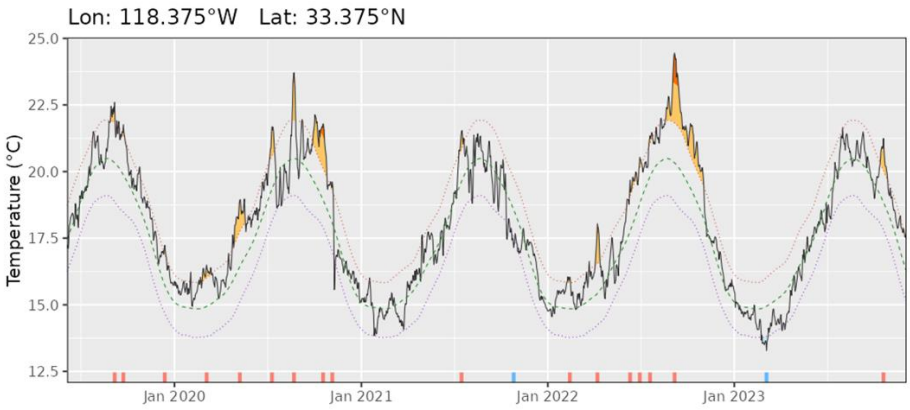

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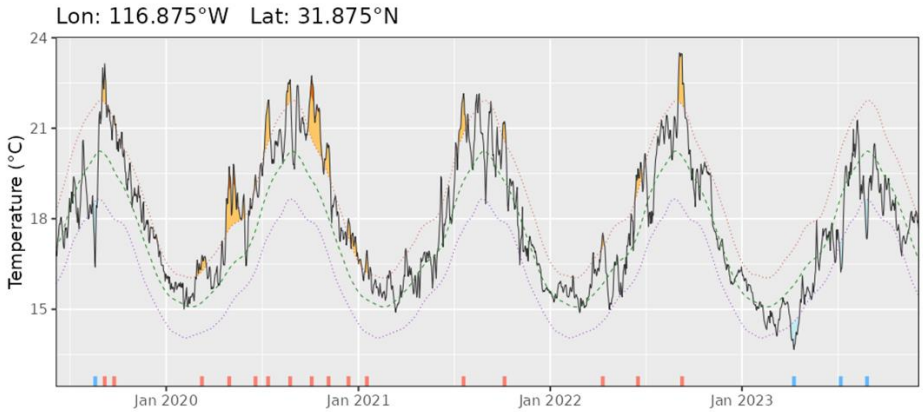

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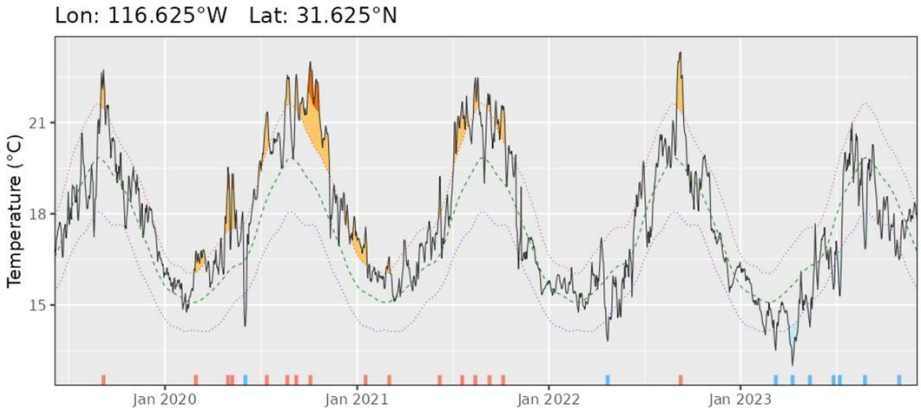

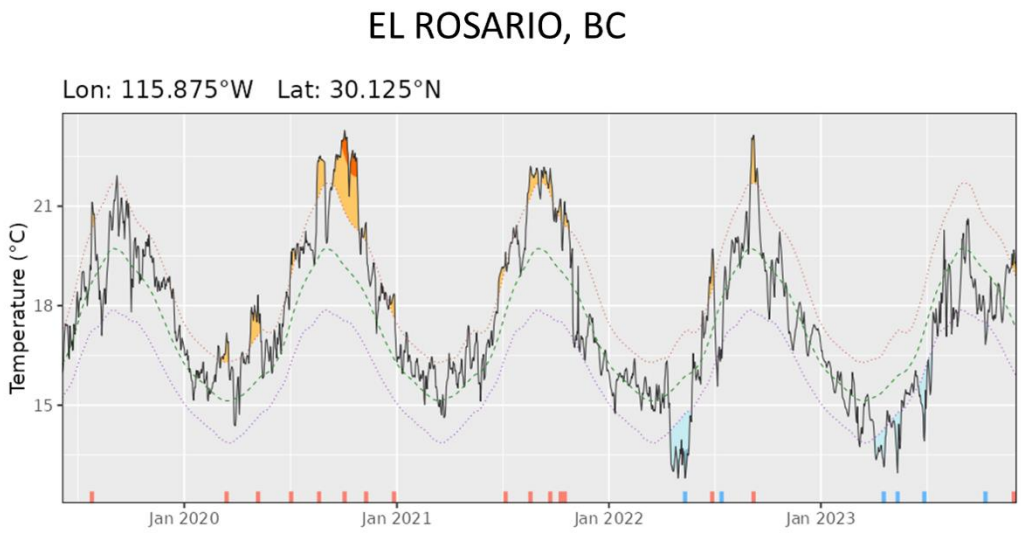

Figure S2. Non-metrics Multidimensional Scaling (MDS) for the biological responses of deep (D) and shallow (S) *Pterygophora californica* sporophytes under the different temperature treatments (C = control; H = heatwave). Each symbol represents an experimental unit (EU).

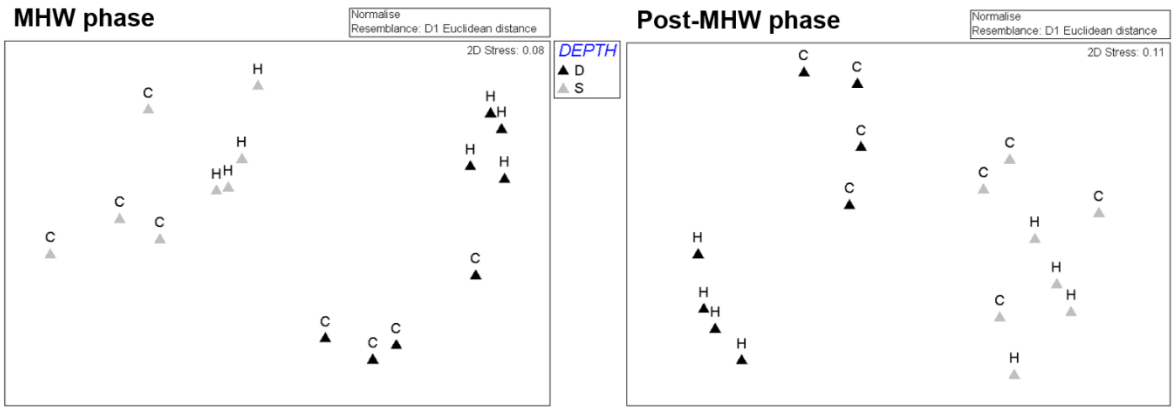

TABLE S1. Two-way ANOVA analyses applied to the different biological descriptors of deep and shallow *Pterygophora californica* sporophytes in the experimental MHW phase. Significant values ($p < 0.05$) are indicated in bold.

Biological descriptors	**Depth (D)**				**Temperature (T)**				**D x T**			
	df	**MS**	***F***	***p***	**df**	**MS**	***F***	***p***	**df**	**MS**	***F***	***p***
Net-P_{max} µmol $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$	1	0.12	65.14	**<0.001**	1	0.09	49.65	**<0.001**	1	0.004	2.07	0.18
Gross-P_{max} µmol $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$	1	0.10	67.61	**<0.001**	1	0.07	47.07	**<0.001**	1	0.002	1.35	0.27
R µmol $O_2 \cdot g^{-1}$ DS $\cdot h^{-1}$	1	208.27	13.41	**0.003**	1	196.18	12.63	**0.004**	1	18.69	1.20	0.29
α	1	0.29	159.16	**<0.001**	1	0.00004	0.25	0.878	1	0.004	2.38	0.15
E_k µmol quanta $\cdot m^{-2} \cdot s^{-1}$	1	3060.88	10.59	**0.007**	1	505.92	1.75	0.210	1	281.12	0.97	0.34
Daily net-productivity mmol $O_2 \cdot g^{-1}$ DW $\cdot d^{-1}$	1	4.90	242.98	**<0.001**	1	0.34	16.75	**<0.001**	1	0.068	3.41	0.07
E_c µmol $O_2 \cdot g^{-1}$ DW $\cdot h^{-1}$	1	134.75	34.03	**<0.001**	1	9.39	2.37	0.149	1	15.86	4.01	0.07
Chl *a* µg $\cdot g^{-1}$ FW	1	406.22	0.2	0.66	1	292.24	0.14	0.71	1	1000.62	0.49	0.50
Fucoxanthin µg $\cdot g^{-1}$ FW	1	2455.9	0.40	0.54	1	57944	9.46	**0.01**	1	226.69	0.04	0.85
A_{680}	1	0.01	38.09	**<0.001**	1	0.001	5.04	**0.04**	1	0.0001	0.63	0.44
F_v/F_m	1	0.0009	2.28	0.16	1	0.0006	1.59	0.23	1	0.001	2.66	0.13
Φ_{PSII}	1	0.04	24.48	**<0.001**	1	0.04	23.84	**<0.001**	1	0.0009	0.66	0.43
NPQ	1	2.716	112.24	**<0.001**	1	0.0278	1.148	0.305	1	0.0724	2.989	0.109
ETR µmol $e^- \cdot m^{-2} \cdot s^{-1}$	1	521.83	134.35	**<0.001**	1	27.41	7.06	**0.021**	1	101.87	26.23	**<0.001**

Total soluble carbohydrates % DW	1	0.03	2.10	0.17	1	0.65	40.89	**<0.001**	1	0.49	30.91	**<0.001**
Growth (first blade) $cm \cdot d^{-1}$	1	0.30	21.80	**<0.001**	1	0.008	0.61	0.45	1	0.50	35.75	**<0.001**
Growth (vegetative blade) $cm \cdot d^{-1}$	1	0.20	9.95	**0.008**	1	0.01	0.50	0.49	1	0.25	11.76	**0.005**
N % DW	1	0.260	5.83	**0.025**	1	0.16	0.36	0.55	1	0.03	0.68	0.42
Lipid Peroxidation nmol eq. AA $\cdot g^{-1}$ DW	1	305.54	22.92	**<0.001**	1	1.16	0.09	0.77	1	403.3	30.27	**<0.001**
Total Phenols mg eq. AA $\cdot g^{-1}$ DW	1	2.31	6.57	**0.025**	1	2.44	6.94	**0.02**	1	0.57	1.61	0.23
Antioxidant Capacity mg eq. AA $\cdot g^{-1}$ DW	1	0.66	49.07	**<0.001**	1	0.04	3.13	0.10	1	0.02	1.28	0.28

TABLE S2. Two-way ANOVA analyses applied to the different biological descriptors of deep and shallow *Pterygophora californica* sporophytes in the experimental post-MHW phase. Significant values ($p < 0.05$) are indicated in bold.

	Depth (D)				**Temperature (T)**				**D x T**			
	df	**MS**	***F***	***p***	***df***	**MS**	***F***	***p***	**d.f.**	**MS**	***F***	***p***
Net-P_{max} µmol $O_2 \cdot g^{-1}$ DW · h^{-1}	1	19051	55.31	**<0.001**	1	2555	6.55	**0.025**	1	8723	25.33	**<0.001**
Gross-P_{max} µmol $O_2 \cdot g^{-1}$ DW · h^{-1}	1	20723	131.38	**<0.001**	1	633.64	4.02	0.068	1	5672	35.95	**<0.001**
R µmol $O_2 \cdot g^{-1}$ DW · h^{-1}	1	11.11	26.11	**<0.001**	1	21.23	49.88	**<0.001**	1	54.04	126.99	**<0.001**
α	1	0.65	88.98	**<0.001**	1	0.03	3.96	0.070	1	0.73	98.89	**<0.001**
E_k µmol quanta · $m^{-2} \cdot s^{-1}$	1	1407.1	16.58	**0.002**	1	547.9	6.46	**0.026**	1	538.5	6.3	**0.027**
E_c µmol $O_2 \cdot g^{-1}$ DW · h^{-1}	1	634.58	161.10	**<0.001**	1	46.07	11.7	**0.005**	1	32.41	8.23	**0.014**
Daily net-productivity mmol $O_2 \cdot g^{-1}$ DW · d^{-1} µmol $O_2 \cdot g^{-1}$ DW · h^{-1}	1	7.06	241.15	**<0.001**	1	0.62	21.15	**<0.001**	1	2.76	94.22	**<0.001**
Chl *a* µg · g^{-1} FW	1	27869.9	4.53	0.06	1	149202.38	24.28	**<0.001**	1	27518.08	4.48	0.06
Fucoxanthin µg · g^{-1} FW	1	77348.8	6.41	**0.03**	1	112888.27	9.36	**0.010**	1	23301.26	1.93	0.19
A_{PAR}	1	0.03	56.90	**<0.001**	1	0.003	65.98	**0.031**	1	0.009	16.78	**0.001**

F_v/F_m	1	0.002	11.35	**0.006**	1	0.000001	0.008	0.93	1	0.00001	0.10	0.76
Φ_{PSII}	1	0.009	1.10	0.314	1	0.12	14.48	**0.003**	1	0.012	1.51	0.24
NPQ	1	1.71	10.67	**0.007**	1	0.85	5.28	**0.040**	1	0.145	0.903	0.361
ETR $\mu mol\ e^- \cdot m^{-2} \cdot s^{-1}$	1	35.19	6.36	**0.027**	1	25.62	4.63	0.05	1	21.67	83.92	0.07
Total non-structural carbohydrates % DW	1	0.007	0.18	0.679	1	0.27	6.29	**0.027**	1	0.02	0.46	0.51
Growth (first blade) $cm \cdot d^{-1}$	1	0.03	14.06	**0.003**	1	0.03	11.98	**0.005**	1	0.22	83.43	**<0.001**
Growth (vegetative blade) $cm \cdot d^{-1}$	1	0.10	13.82	**0.003**	1	0.02	2.85	0.117	1	0.19	24.58	**<0.001**
N % DW	1	0.25	11.32	**0.003**	1	0.104	4.630	**0.044**	1	0.000452	0.0200	0.889
Lipid Peroxidation nmol eq. AA· g^{-1} DW	1	21.53	2.88	0.115	1	303.20	40.55	**<0.001**	1	300	40.12	**<0.001**
Total Phenols mg eq. AA· g^{-1} DW	1	7.41	138.84	**<0.001**	1	0.01	0.35	0.57	1	2.02	37.99	**<0.001**
Antioxidant Capacity mg eq. AA· g^{-1} DW	1	0.59	197.66	**<0.001**	1	0.05	16.009	**0.002**	1	0.11	37.13	**<0.001**

TABLE S3. Permutational ANOVA analyses performed on the different biological descriptors of sporophytes of *Pterygophora californica* after the experimental phases of MHW and post-MHW. Bold numbers indicate significant differences.

Exp. phase	Biological variables	Main Test	*df*	SS	*F* value	Pr(>*F*)	p (perm)
MHW	A_{PAR}	**Depth**	1	0.0138	20.0129	0.0008	**0.0022**
		Temperature	1	0.0015	2.1585	0.1675	0.168
		D x T	1	0.00000	0.0002	0.9888	0.9811
		Residuals	12	0.0083			
Post-MHW	A_{680}	**Depth**	1	0.0276	49.6157	0	**0.0001**
		Temperature	1	0.0001	0.1886	0.6718	0.6656
		D x T	1	0.0017	3.129	0.1023	0.0954
		Residuals	12	0.0006			

TABLE S4. Three-way ANOVA analyses applied to the nitrate uptake rates of deep and shallow *Pterygophora californica* sporophytes after the experimental phases of MHW and post-MHW. Significant values ($p < 0.05$) are indicated in bold.

MHW phase				
	df	**MS**	***F***	***p***
Depth	1	0.145	1.019	0.320
Temp	1	0.405	2.849	0.101
Nitrate concentration	3	6.651	46.735	**<0.001**
Depth x Temp	1	0.00821	0.0577	0.812
Depth x N concentration	3	0.248	1.745	0.177
Temp x N concentration	3	0.0260	0.183	0.907
Depth x Temp x N concentration	3	0.0712	0.500	0.685

Residual	32	0.555		

Post-MHW phase				
	df	**MS**	***F***	***p***
Depth	1	2.372	20.317	**<0.001**
Temp	1	5.162	44.213	**<0.001**
Nitrate concentration	3	7.544	64.610	**<0.001**
Depth x Temp	1	1.481	12.684	**0.001**
Depth x N concentration	3	0.0607	0.520	0.672
Temp x N concentration	3	0.0851	0.729	0.542
Depth x Temp x N concentration	3	5.162	3.304	**0.033**
Residual	32	0.787		

CAPÍTULO 2.

Efectos de las olas de calor marinas en la fotobiología de tres especies clave de algas pardas cerca de su límite más meridional en el Pacífico Nororiental.

Effects of marine heatwaves on the photobiology of three kelp foundation species near their southernmost limit in the Northeastern Pacific

ABSTRACT

Kelp forests are threatened by the effects of ocean warming and marine heatwaves (MHWs) associated with climate change. Although there is considerable evidence for the effects of warming on kelp sporophytes, the thermotolerance of microscopic stages remains a black box. In this study, we compared the photophysiology of *Eisenia arborea*, *Macrocystis pyrifera*, and *Pterygophora californica* gametophytes when exposed to warm conditions simulating a MHW in the laboratory. These are foundation species in the North American Pacific and are the kelps that extend farthest south, along the entire coast of the Baja California peninsula in Mexico. Our results showed that *E. arborea* gametophytes exhibited greater thermal tolerance under simulated MHW compared to *P. californica* and *M. pyrifera*. In general, descriptors of photosynthetic rates, compensatory irradiance, and respiration were affected in these latter species when exposed to warming or after warming ceased. Therefore, our results helped to explain the apparently higher thermal resistance of *E. arborea*, which was able to survive and recruit in sites where *M. pyrifera* was affected or even disappeared the impact of MHWs, as well as the southernmost distribution of *E. arborea* in the Baja California peninsula.

1. Introduction

Kelps, brown algae belonging to the Order Laminariales, establish extensive underwater forests along cold-water and temperate coastlines (Schiel & Foster, 1986; Steneck et al., 2002; Teagle et al., 2017), playing vital roles as primary producers and habitats for diverse marine species (Metzger et al., 2019; Duarte et al., 2022; Eger et al., 2023). Kelp forests provide essential

ecosystem services and can have high direct and indirect economic value (Smale et al., 2013; Eger et al., 2023). However, kelps seem to be extremely sensitive to environmental a number of environmental stressors like light limitation, and nutrient scarcity (Bartsch et al., 2008; Schiel & Foster, 2015), and can be especially vulnerable to climate change-related factors (Wernberg et al., 2012; Teagle et al., 2018; McPherson et al., 2021; Smith et al. 2023). A worldwide reduction in kelp populations has been attributed to oceans warming and the higher incidence of anomalous warming events, such as marine heatwaves (Krumhansl et al., 2016; Thomsen et al., 2018; Wernberg et al., 2021).

Marine heatwaves (hereafter MHWs) are defined as a prolonged discrete anomalously warm water (Hobday et al., 2016), which can severely alter marine ecosystems and the services they provide (Smale et al., 2019; Smith et al., 2021, 2023; Wernberg et al., 2012, 2016). Projected trends suggest an escalation in the severity, duration, and frequency of MHWs in forthcoming years and decades (Frölicher et al., 2018; Oliver et al., 2018). These thermal events can have deleterious impacts on critical marine habitats such as kelp forests, perturbing their ecological dynamics and precipitating shifts in species distribution and abundance (Wernberg et al., 2016; Arafeh-Dalmau, et al 2019; Smale et al., 2019; Michaud et al., 2022; Bass et al., 2023), even facilitating the habitat colonization by exotic seaweeds (Félix -Loaiza et al., 2022).

The vulnerability of kelp species to warming and MHWs can be highly variable among species, as well as between ecotypes/genotypes within them (Gerard & Du Bois, 1988; Alsuwaiyan et al., 2021).Their physiological status at the time of the exposure to warming and their previous thermal history (Mohring et al., 2014; Muth et al., 2019), their phenotypic plasticity (Gerard, 1997; Liesner et al., 2022), their adaptive properties to local thermal regimes (Barreto, 2017; Saada et al., 2020; Schwoerbel et al., 2023), o their responses to the interaction of temperature with other environmental variables (Mohring et al., 2014; Umanzor et al., 2021; Bass et al.,

2023), are part of the mosaic of conditioning factors responsible for the thermal resistance and resilience of kelp to warming. The recruitment capabilities under warming conditions is also a critical (and understudied) aspect determining the stability and recovery of kelp population. Kelps exhibit complex heteromorphic life cycles, and the thermo-resistance of species and populations will be also defined by the heat tolerance of the different ontogenic stages, from the haploid microscopic spores and gametophytes to the diploid sporophytes (Fredersdorf et al., 2009; Alsuwaiyan et al., 2021). While there is a significant knowledge on the effects of warming on the sporophyte phase (Andersen et al., 2013; Wernberg et al., 2016; Straub et al., 2019), contributions about the response of early microscopic stages are comparatively much scarce (Veenhof et al., 2021 and references therein). Some studies have shown that high temperatures can decrease the gametophytes´ physiological performance (e.g., photosynthesis) and growth (Müller et al., 2008; Delebecq et al., 2016; Shukla & Edwards, 2017; Borlongan et al., 2018a). Also, processes such as sporogenesis and gametogenesis seem to be thermo-sensitive (Martins et al., 2017; de Bettignies et al., 2018). Interestingly, previous studies found that gametophytes can face warming in a vegetative (non-reproductive) stage, and they can recover following the thermal stressor (Ladah & Zertuche-González, 2007; Becheler et al., 2022; Alsuwaiyan et al., 2021). In fact, the permanence of gametophytes as vegetative is considered a survival strategy among kelps under environmental unfavorable conditions (Carney & Edwards, 2010; Schoenrock et al., 2020; Edwards, 2022). However, resistance and resilience of gametophytes to elevated temperatures can vary depending to their adaptive properties to local thermal regimes (Mohring et al., 2014; Hollarsmith et al., 2020; Camus et al., 2021), their evolutionary history (Muth et al., 2019) and genotypic variability (Alsuwaiyan et al., 2021). Microscopic stages are essential to ensuring the maintenance and persistence of kelp forests through recruitment (Carney & Edwards, 2006; Barradas et al., 2011; Becheler et

al., 2022; Edwards, 2022), making it critical to determine their thermal vulnerability (Veenhof et al., 2021).

At their latitudinal distribution boundaries, kelp populations can be particularly susceptible to temperature increase (Matson, 2007; Arafeh-Dalmau et al., 2019; Butler et al., 2020; Nauer et al., 2022). In the North American Pacific, *Pterygophora californica* Ruprecht, the giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh, and *Eisenia arborea* J.E. Areschoug are the stipitate kelps that reach more southerly regions, throughout the coast of the Baja California peninsula in Mexico (Stephens et al., 2006; Pedroche et al., 2008; Foster & Schiel, 2015). Among them, *E. arborea* extends furthest to the south where seawater temperature in summer can reach up to 26 °C (Bahía Magdalena, Baja California Sur; Abbott & Hollenberg, 1976; Pedroche et al., 2008), while *P. californica* and *M. pyrifera* experienced maximum temperatures up to 18.5-20 °C and 20-23 ºC in their respective southernmost limits at El Rosario (Baja California) and Bahía Asunción (Baja California Sur) (Pedroche et al., 2008; Schlegel, 2020). In this region, these species play important ecological roles and are essential components of the marine socio-ecosystem (Schiel & Foster, 2015). Though among other kelp, these species seem to be more adapted to high seawater temperatures, thermal anomalies fueled by MHWs and El Niño Southern Oscillation (ENSO) can threat these submerged forests and thus, their associated socio-ecosystem-services (Edwards & Hernández-Carmona, 2005; Hernández-Carmona et al., 2011; Arafeh-Dalmau et al., 2019). Indeed, Baja California, located at the end of the California Current System, is considered as an oceanographic transition zone where a higher incidence of extreme MHWs is expected (Amaya et al., 2023). For instance, the extreme MHW known as "the Blob" involved prolonged and deep thermal anomalies up to 6 °C, causing severe alterations in giant kelp populations, and drifts between these forests and subcanopy kelps and invasive seaweeds (Bond et al., 2015; Hu et al., 2017; Arafeh-Dalmau et

al., 2019; Cavanaugh et al., 2019; Félix-Loaiza et al., 2022; Michaud et al. 2022). Laboratory experiments have also demonstrated that the metabolism and growth of *M. pyrifera* juvenile sporophytes can be affected by high temperatures simulating a MHW (Sánchez-Barredo et al. 2020; Umanzor et al. 2021). In contrast to the apparent thermo-sensitivity of the giant kelp, *E. arborea* and *P. californica* seem to be more resistant to warming (Dayton et al., 1999; Edwards & Hernández-Carmona, 2005; Matson & Edward, 2007), making this species potential winners in future scenarios of ocean warming. In this sense, it is noteworthy that *E. arborea* populations can persist and even growth in locations where *M. pyrifera* decreased or completely disappear after the impact of El Niño (Edwards & Hernández-Carmona, 2005) and MHWs (Arafeh-Dalmau et al., 2019). Despite there is some evidence of their different resistance to warming, knowledge on the responses of microscopic stages is almost absent (Ladah & Zertuche-González, 2007; Matson & Edwards, 2007; Muth et al. 2019).

This study assesses the photobiological responses of gametophytes of *Pterygophora californica*, *Macrocystis pyrifera*, and *Eisenia arborea* during a simulated MHW in controlled laboratory conditions. The variables of photosynthesis-irradiance curves, photochemical descriptors obtained from Chl*a* fluorescence of photosystem II, and pigment content, were analyzed during both a warming phase and after exposure to warming ceases. The main objective was to investigate whether the varying thermo-resistance of each species can be applied to their microscopic stages.

2. Materials and Methods

Sampling site and collection

Fertile blades from different individuals of *Eisenia arborea, Macrocystis pyrifera* and *Pterygophora californica* were collected by scuba diving or snorkeling during May-June 2021. Reproductive blades of *E. arborea* and *P. californica* were collected at Campo Kennedy, Baja California, Mexico (31° 42' 2.394" N, 116° 41' 1.467" W), while samples of *Macrocystis pyrifera* were collected at Isla Todos Santos, Baja California, Mexico (32° 00' 09.0" N, 117° 15' 14.0" W). After collection, the samples were rapidly (within 2 hours) transported in coolers (between wet papers in darkness) to the Marine Botany Laboratory at the Universidad Autónoma de Baja California.

Spore release and culture conditions

The reproductive tissues (sori) were separated from the rest of the blade tissue, and washed with naturally filtered (1 µm) sterile seawater. The soris´ surfaces were cleaned with soaked paper to remove impurities and epiphytes. To prevent as possible contamination of the cultures, the tissue was immersed in a solution of iodine/povidone Betadine Antiseptic (5% in UV-sterilized, filtered seawater), then rinsed with deionized water. Finally, the sori were placed between moist paper towels and foil, and stored in darkness at 4°C for 24 hours. To induce the release of zoospores, the sori were submerged in Erlenmeyer flasks containing 500 mL of filtered (0.45 µm) and sterilized seawater. The flasks were then gently stirred for one hour at room light/temperature conditions. The zoospores suspension was collected from the flasks using a Pasteur pipette, and carefully examined under microscope (Axio Observer.A1, Zeiss, Germany). The spore concentration was quantified using a standard hemocytometer, until a final density of 10,000 spores mL^{-1}. The spore suspensions of each species were then inoculated

onto different Erlenmeyer flask (400 mL) filled with filtered (0.45 µm) seawater, and kept in large incubators at a constant temperature of 14 °C.

The culture medium consisted of 400 mL of sterilized seawater enriched with 4 mL of Provasoli without iron to suppress gametogenesis. To prevent diatoms growth, a saturated germanium dioxide (GeO_2) solution was added at a concentration of 0.5 mL L^{-1} (Shea & Chopin, 2007). Initially, the spores were kept in darkness for one day. Thereafter, irradiance was adjusted at 30 µmol photon m^{-2} s^{-1} for two days (12:12, L:D). For the subsequent six days, the light intensity was raised to 50 µmol photon m^{-2} s^{-1} (12:12, L:D), and 80 µmol photon m^{-2} s^{-1} (16:8, L:D). Cool-white LED lamps were the light sources, but red-light filters (LEE-filters) were used to prevent gametogenesis during this first growth phase of the culture (Lüning & Neushul, 1978). The light intensity was measured using a quantum scalar laboratory radiometer (QSL-2100, Biospherical Instruments Inc.). After 15 days, gametophytes showed clear sexual dimorphism. Both males and females were maintained together and these were not separated in the culture. The culture was maintained for six months until gametophytes reached a diameter of ~2-3 mm (Fig.1). Throughout this period, pulsed fertilization using a 10 ml L^{-1} Provasoli solution was administered every three days to ensure optimal growth and development of the gametophytes.

Experimental setup

The gametophytes of each species were randomly separated into sixteen Petri dishes (35 mm diameter) containing 10 mL of filtered (0.45 µm) seawater. Approximately, a concentration of 300 gametophyte per milliliter (300 gametophytes mL^{-1}) was used. Eight dishes were used for the control treatment, and eight for the warming treatment. Control gametophytes (C) were kept at temperature culture conditions of 14 °C, while in the warming treatment the gametophytes were heated to a temperature of 20 °C. In the MHW phase, the temperature was

gradually elevated +2 ºC per day. The warming was maintained for six days, and then it was gradually decreased (-2°C per day) until 14 °C. Gametophytes were kept at this temperature for six days during this post-MHW phase. The thermal anomaly of +6 ºC above control and its duration, were selected according to the characteristics of usual marine heatwaves previously recorded in the region. These parameters were determined using the online MHW Tracker tool (Schlegel, 2020). In both treatments, light (80 µmol photon m^{-2} s^{-1}; 16:8 L:D) was provided by cool-white LED lamps (without red filters). At the end of each experimental phase (MHW and post-MHW), all gametophytes from four of the ten Petri dishes (N = 4) were used for the analyses of the biological variables.

Chlorophyll a fluorescence of PSII

The Chl*a* fluorescence of PSII was measured in the gametophytes at each treatment and sampling time using a portable Pulse Amplitude Modulated Fluorometer (Diving-PAM, Walz, Germany). To ensure uniform measurements of chlorophyll a fluorescence across the entire sample, gametophyte subsamples (~50 tufts) were taken from each Petri dish, and transferred into sixteen (four per treatment, N = 4) culture wells of 200 µL. Each well was maintained under the experimental conditions of each treatment. To measure minimum fluorescence (F_0), maximum fluorescence (F_m), and maximum quantum yield (F_v/F_m), a saturating light pulse (0.8 s, ~5000 µmol photon m^{-2} s^{-1}) was applied to gametophytes previously dark-adapted overnight. These measurements were repeated at midday on gametophytes illuminated by the culture light (80 µmol photon m^{-2} s^{-1}). For the fluorescence determination, the gametophytes were illuminated with the actinic light through the fiber optic of the fluorometer at a saturating light intensity of 300 µmol photon m^{-2} s^{-1} for three minutes. According to previous trials, three minutes of illumination were sufficient for the chlorophyll-a fluorescence to reach its steady-state, indicating photosynthetic activity. A saturating pulse was then applied to obtain F and

$F_{m'}$, the effective quantum yield [Φ_{PSII}, ($F_{m'}$ -F)/$F_{m'}$], and the non-photochemical quenching [NPQ, (F_m-$F_{m'}$)/$F_{m'}$]. Additionally, the relative electron transport rate was calculated as ETRr = Φ_{PSII} x E x 0.5, where E was the actinic light applied of 213 µmol photon m^{-2} s^{-1}, and 0.5 is a correction factor used for the assumed equal distribution of photons between photosystems.

Photosynthesis and Respiration (P vs. -E curves)

Approximately 200 tufts of gametophyte (30-40 mg FW^{-1}) were incubated in a jacketed borosilicate chamber (4 mL) filled with filtered (1 µm) seawater. The gametophytes were continuously stirred during incubations to homogenize the light. Oxygen evolution was measured by optodes (dipping probe DP-PSt3, PreSens, Germany) connected to a fiber optic oxygen meter (OXY 4 SMA, PreSens, Germany) controlled by the software (Measurement Studio 2, PreSens, Germany). The temperature was adjusted according to the temperature of each treatment and experimental phase by a cooling circulator.

Firstly, the gametophytes were incubated in complete darkness for 15 minutes to determine their respiration rates. Then, they were exposed to eight increasing irradiances of 5, 19, 32, 85, 140, 100, 200, 300 and 400 µmol photon m^{-2} s^{-1} for 5 minutes each to determine net photosynthetic rates. Light was provided by four LED light sources (10 W) that surrounded the chamber. Maximum net and gross photosynthetic rates (net-P_{max} and gross-P_{max}), photosynthetic efficiency (α), compensation irradiance (E_c) and saturation irradiance (E_k) were estimated from the P-E curve. The gross- P_{max} was calculated by the sum of the respiration plus the maximum net-P_{max}. Photosynthetic efficiency was calculated as the initial slope of the linear regression line adjusted to the net-P values. Values of E_c were calculated between the difference of respiration and photosynthetic efficiency (α), while E_k was calculated as net-P_{max}/α. The daily net productivity (a proxy of daily carbon balance) was calculated following the equation [(net-P80 x 16) - (R x 8)], being net-P80 the net photosynthesis from P-E curves at the

irradiance of 80 μmol photon m^{-2} s^{-1} (i.e., the culture irradiance), and R the respiration of P-E curves. Net-P80 and R were multiplied by the hours of daylight (16) darkness (8) of the culture conditions.

After the incubations, the gametophytes were filtered in 0.45 μm fiber glass filters using a hand pump. The fresh weight per incubation was calculated by subtracting the weight of a wet filter to the weight of the filters with gametophytes. Photosynthetic parameters were normalized by fresh weight (FW).

Pigments content

Chlorophylls *a* and *c* were extracted from all the gametophytes used for each incubation of P-E curves. These gametophytes were ground in Eppendorf tubes containing 4 mL of 90 % acetone solution. The samples were incubated in darkness at 4 °C for 24 hours. The extracts were then centrifuged at 5000 rpm for 10 minutes at 4 °C. The supernatant absorbances (730, 664, 647, and 630 nm) were measured in a spectrophotometer, and the pigment concentration was quantified using the equations of Jeffrey & Humphrey, (1975).

Statistical analyses

In each experimental phase, MHW and post-MHW, results were analyzed by applying two-way ANOVA, with temperature (two levels; control and high) and species (three levels: *E. arborea*, *M. pyrifera* and *P. californica*) as fixed factors. The *post hoc* Student-Newman-Keuls analysis was run to detect statistical differences ($P < 0.05$) among treatments. When the assumptions of normality and homoscedasticity were not met even after data transformation, a non-parametric Kruskal-Wallis test was used. Statistical results are provided as supplementary material (Tables 1SM and 2SM). These statistical analyses were performed using Rstudio software version 4.1.0 and the statistical package of Sigmaplot 14.5. Normal distribution of

standardized residuals was assessed using Shapiro-Wilk normality tests and homogeneity of variance was tested using Levene test before the statistical analyses.

The effects of temperature on all the photobiological variables among treatments and species were also analyzed by multivariate statistical approaches. These were performed with the normalized data of all response variables, and a ranked triangular similarity matrix was constructed using Euclidean distances. A two-way PERMANOVA crossed design (9999 permutations) was completed to test the interactive effects of the factors `Specie´ and `Temperature´. Significant differences in the pair-wise posterior comparisons were checked using Monte Carlo P-values, P(MC), due to the restricted number of possible permutations. A non-metric multidimensional scaling (MDS) ordination was also applied to visualize multivariate patterns (Fig. 6). These statistical procedures were implemented using PRIMER 6 & PERMANOVA+ v.1.0.2 software package (Anderson et al., 2008).

3. Results

The two-way PERMANOVA analysis (Table 1) demonstrated a significant difference in the response of biological descriptors among the three species when exposed to warming. These differences were observed between control gametophytes and those exposed to warming for the case of *M. pyrifera* (*t*=0.028, *P perm*=0.03) and *P. californica* (*t*=2.416, *P perm*=0.034). In the post-MHW phase, significant differences were found among species and in the interaction between temperature and species, but the factor temperature alone did not induce significant effects. Similar to the exposure phase, the differences in the gametophytes´ photo-physiology were significative for *M. pyrifera* (*t*=0.028, *P perm* =0.03) and *P. californica* (*t*=2.416, *P perm* =0.034). The photobiological responses of *E. arborea* gametophytes were similar between treatments in both experimental phases.

P-E curves obtained for the gametophytes exposed to the temperature treatments in both experimental phases (MHW and post-MHW) fitted well to tangential hyperbolic adjustments (R^2 >0.97, P <0.05; Fig. 2). Values of net and gross-P_{max} significantly increased (63-79 %) in *M. pyrifera* gametophytes exposed to warming compared to the control, but decreased by 22-27 % in the post-MHW phase (Fig. 3A-D; Table 1SM). In the MHW phase, the compensation irradiance E_c (Fig. 3E) increased (80-117 %) in the gametophytes of three species exposed to warming. In the post MHW phase, E_c values also increased in *P. californica*, but decreased in *M. pyrifera* (Fig. 3F; Table 1SM). Photosynthetic efficiency (α) did not show significative differences between treatments for each species (Fig. 3G, H; Table 1SM). In the post-MHW phase, α decreased by 46 % in the pre-heated *M. pyrifera* gametophytes compared to its control (Fig. 3H), but this difference was not statistically significative. The *M. pyrifera* and *P. californica* gametophytes exposed to warming exhibited a respiratory activity 3 to 4-fold higher than control gametophytes (Fig. 3I, Table 1SM). This pattern remained similar for *P. californica* in the post-MHW phase, but the R decreased by 25 % in the pre-heated *M. pyrifera* gametophytes (Fig. 3J). In both experimental phases, the saturation irradiance (E_k) did not vary significantly between treatments for each species (Fig. 3K, L, Table 1SM). However, E_k increased by 65.23 % in *M. pyrifera* gametophytes previously exposed to warming compared to its control (Fig. 3L).

The daily net productivity (Fig. 4) significantly increased in *M. pyrifera* during the MHW phase (Table 1SM). In the post-MHW phase, the values of daily net productivity were not statistically different between treatments for each species, although P. californica showed average negative values.

The values of maximum quantum yield F_v/F_m (Fig. 5A) increased in *M. pyrifera* gametophytes exposed to warming compared to its control (Table 1SM). However, in the post-MHW period

(Fig. 5B), the values did not exhibit a significant difference. In Φ_{PSII} values (Fig. 5C) *E. arborea* gametophytes exposed to warming compared to the control showed temperature effects (Table 1SM). In the post-MHW phase (Fig. 5D), the values did not display a statistically significant difference. The values of non-photochemical quenching NPQ (Fig. 5E) decreased by 56 % in the *P. californica* gametophytes exposed to warming compared to their controls during MHW phase (Table 1SM). In the post-MHW phase, NPQ did not show statistically significant differences between treatments for each species (Fig. 5F). In both experimental phases, the relative electron transport rate ETRr did not vary significantly between treatments for each species (Fig. 5G, H, Table 1SM).

The content in chlorophyll *a* (Chl*a*) and chlorophyll *c* (Chl*c*) did not display statistically differences in both experimental phases for each species, except for a reduction of 55 % in Chl*a* found in the heated *M. pyrifera* gametophytes compared to its control (Table 2, Table 2SM).

4. Discussion

In the Northeast Pacific, the kelp species *Macrocystis pyrifera*, *Pterygophora californica*, and *Eisenia arborea* have their warm-edge distribution in the Baja California peninsula (Mexico). Previous studies have documented that thermal anomalies associated with ENSO and marine heatwaves (MHWs) can affect the species differently, particularly at the forest structure level (Edwards & Hernández-Carmona, 2005; Arafeh-Dalmau et al., 2019; Cavanaugh et al., 2019). This suggests that the three species exhibit varying tolerance to warm oceanographic anomalies, and likely, to other associated factors such as nutrient scarcity, water transparency, and warm-associated diseases (Dayton et al., 1999; Dring, 2005; Matson & Edwards, 2007). Our results revealed that exposure to a MHW can lead to different effects on the photo-physiology of the gametophytes of the three species (Table 1, Fig. 5). Thus, the varying

physiological tolerance of early-life history stages to elevated temperatures may contribute to differences in the species' thermo-tolerance.

In our study, exposure to warming did not negatively impact the photosynthetic performance of the gametophytes of *E. arborea* and *P. californica*, indicating that their photosynthetic apparatus remained unaltered under the temperature treatment. For *M. pyrifera*, warming even improved the photosynthetic activity of gametophytes. When exposed to high temperatures, *M. pyrifera* gametophytes showed an increase in their maximum photosynthetic capacities (net and gross-P_{max}), photochemical quantum efficiency of the photosystem II (Φ_{PSII}, F_v/F_m), and electron transport rate between photosystems (ETRr). In other seaweeds, photosynthesis can be stimulated by rising temperatures until signs of metabolic stress appear when warming exceeds physiological tolerance thresholds (Eggert, 2012; Koch et al., 2013). Within the photosynthetic machinery, some thermally sensitive reactions from light-harvesting to carbon fixation (e.g., electron transport, photophosphorylation, Rubisco activity) can be accelerated by warming (Collén & Davison, 1999; Bischof & Rautenberger, 2012; Müller et al., 2012). However, a thorough evaluation of these key processes involved in metabolic acclimation to warming in kelp gametophytes, including heat-stress responsive genes (Heinrich et al., 2012; Marín-Guirao et al., 2016), is still pending. Our results were also quite unexpected, since in previous studies, high temperatures led to a decrease in the photosynthetic capabilities in kelp gametophytes. For instance, photosynthesis rates decreased in gametophytes of *Costaria costata* and *Alaria crassifolia* when temperatures reached 23° C within an experimental temperature range of 8 to 36° C (Borlongan et al., 2018a, 2018b). Henkel et al. (2008) also documented a reduction in the photosynthetic capacities of *Undaria pinnatifida* gametophytes when exposed to warming during a controlled laboratory experiment. Adverse effects of exposure to high temperatures were also reflected in the growth of *Laminaria digitata* (Martins

et al., 2020) and in *Ecklonia radiata* gametophytes exposed above 23° C (Alsuwaiyan et al., 2021).

The three species exhibited higher respiration rates when exposed to warming, although the highest values corresponded to *M. pyrifera*. As argued for other seaweeds (Andersen et al., 2013; Tait & Schiel, 2013; Wernberg et al., 2016), the stimulation of respiration may reflect a higher demand for metabolic energy to sustain heat-acclimation responses and counter balance physiological stress through repair processes, such as the activation of antioxidant responses (Bischof & Rautenberger, 2012; Voss et al., 2013; Hurd et al., 2014). This increase in respiration has been reported previously in kelp gametophytes subjected to warming, in species such as *M. pyrifera, A. crassifolia, C. costata,* and *L. digitata* (Fain & Murray, 1982; Delebecq et al., 2016; Borlongan et al., 2018a, 2018b). Moreover, in our study we observed an increase in respiration without changes in photosynthetic efficiency (α), which resulted in higher compensation irradiance (E_c) in all the three species. Higher E_c values indicate that more light is required for photosynthesis to compensate for respiration and achieve positive net productivity (Beer et al., 2014; Hurd et al., 2014). Although the daily productivity (calculated as a proxy of carbon balance) was similar between heated and control gametophytes in this study, the prevalence of carbon losses by respiration and high E_c values could lead to metabolic C-imbalances in the long-term. Indeed, this effect might be dramatic for the gametophytes performance under low-light conditions, such as those prevailing in the understory of kelp canopies where seed banks are usually established (Novaczek, 1984; Zacher et al., 2016; Ebbing et al., 2020).

After the warming period ended, in the post-MHW phase none of the photo-physiological descriptors or the respiration of the pre-heated gametophytes of *E. arborea* differed from the control. However, there were differences for the case of *M. pyrifera* and *P. californica*. While

P. californica showed a noticeable stimulation of respiratory activity (up to 4-fold higher than the control) which caused a rise in E_c, *M. pyrifera* gametophytes exhibited a decrease in net/gross-P_{max} and α, as well as an increase in E_k. This can be interpreted as both delayed effects of warming and rapid effects of temperature change, as recently argued for kelp sporophytes (Umanzor et al., 2021; Almeida-Saá et al., 2023) and other marine macrophytes (e.g., seagrasses, Vivanco-Bercovich et al., 2024). At both saturating and sub-saturating irradiances, *M. pyrifera* gametophytes that were pre-exposed to high temperatures were unable to achieve the same photosynthetic rates as their control group. This reduction in photosynthesis could respond to heat-sensitive processes, such as protein denaturation and damage to temperature-sensitive enzymes or membranes, as documented on other seaweeds (Los & Murata, 2004; Eggert, 2012). The negative effects observed in the photosynthetic parameters obtained by oxygen evolution (P-E curves) were not found in those from Chl*a* fluorescence emission in the PSII. Indeed, values of F_v/F_m, Φ_{PSII}, and ETRr were similar between control and pre-heated gametophytes. Similar results have been documented in juvenile sporophytes of this species (Sánchez-Barredo et al., 2020; Umanzor et al., 2021), other seaweeds (Bruhn & Gerard, 1996; Almeida-Saá et al., 2023) and seagrasses (Marín-Guirao et al., 2016; Vivanco-Bercovich et al., 2022), likely due to the activation of electron sinks alternative to photosynthetic processes, such as photorespiration and the Mehler cycle (Niyogi, 2000; Voss et al., 2013).

During the post-MHW phase, *M. pyrifera* gametophytes also showed a decrease in respiration and E_c. These physiological adjustments may help to maintain a positive carbon balance despite reduced photosynthetic capabilities (Davison et al., 1991; Fairhead & Chesire, 2004; Hurd et al., 2014). However, prolonged low respiration may lead to metabolic deterioration (Beer et al., 2014). Similarly to the observations during the warming (MHW) phase, respiration and E_c were higher in the pre-heated *P. californica* gametophytes compared to the control. Moreover,

E_c reached ~59 µmol photon m^{-2} s^{-1}, a value three to four times higher than that of the other species, and importantly, a value similar to the E_k (~60 µmol photon m^{-2} s^{-1}) also measured in these gametophytes. Therefore, to compensate for respiratory carbon losses during photosynthesis, saturating irradiances are required. This condition can lead to severe metabolic C-imbalances in the *P. californica* gametophytes, which explains the average negative values in their daily productivity.

Based on photobiological descriptors, *E. arborea* gametophytes exhibited a greater thermal tolerance under a simulated MHW compared to *P. californica* and *M. pyrifera*. Descriptors of photosynthetic rates, compensation irradiance and respiration were affected in these species when exposed to warming or after warming ceased. The higher thermo-resistance of *E. arborea* gametophytes supports previous studies which documented the recruitment of this species in sites where *M. pyrifera* was affected or even disappeared following the passage of a marine heatwaves (Arafeh-Dalmau et al., 2019) and ENSO (Hernández-Carmona et al., 2001; Edwards & Hernández -Carmona, 2005). Additionally, our results agree with the higher resistance to warming of the microscopic stages of *E. arborea* compared to *P. californica* (Matson & Edwards, 2007; Muth et al., 2019), which contributed to explain the southernmost distribution of *E. arborea* in the Baja California peninsula. Further research efforts are needed to assess the basis of the apparent differences in the thermo-resistance of these kelps. For instance, applying -omics techniques and analyzing the interaction between the seaweed and its microbiome, can help to better understand the resistance and resilience different ontogenic stages as well as potential genotypic/ecotypic (including thermal niches) divergences in their responses to the warming associated to climate change.

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6. Tables

TABLE 1. PERMANOVA analysis on the effects of the temperature treatments on the photo physiology of the gametophytes of *Eisenia arborea*, *Macrocystis pyrifera* and *Pterygophora californica* in the experimental phases of MHW and post-MHW. Bold numbers indicate significant differences.

Experimental phase	**Main Test**	**d.f.**	**SS**	***Pseudo -F***	***P* (perm)**
MHW	**Species (S)**	2	116.77	11.011	**0.001**
	Temperature (T)	1	37.953	7.1574	**0.001**
	S x T	2	25.824	2.435	**0.01**
	Residuals	18	95.448		
Post-MHW	**Species (S)**	2	143.6	14.923	**0.001**
	Temperature (T)	1	7.473	1.5533	0.156
	S x T	2	38.316	3.9816	**0.001**
	Residuals	18	86.608		

TABLE 2. Pigments content measured in gametophytes of *Eisenia arborea*, *Macrocystis pyrifera* and *Pterygophora californica* after the experimental phases of MHW and post-MHW. Values are means and standard errors (N = 4). Different letters indicate statistical differences among treatments (two-way ANOVA, *post hoc* SNK; Tables 1SM and 2SM, Supplementary material).

	Experimental phase	***E. arborea* C**	***E. arborea* MHW**	***M. pyrifera* C**	***M. pyrifera* MHW**	***P. californica* C**	***P. californica* MHW**
Chl *a*	**MHW**	0.67± 0.02 **a**	0.87± 0.13 **a**	1.42 ± 0.52 **b**	0.63 ± 0.19 **c**	0.24 ± 0.04 **a**	0.37 ± 0.07 **a**
	Post-MHW	0.87± 0.16 **a**	0.73± 0.11 **a**	0.45 ± 0.18 **a**	0.21 ± 0.03 **a**	0.21 ± 0.08 **a**	0.32 ± 0.07 **a**
Chl *c*	**MHW**	0.17± 0.02 **a**	0.22 ± 0.04 **a**	0.22 ± 0.05 **a**	0.09 ± 0.03 **a**	0.09 ± 0.01 **a**	0.14 ± 0.05 **a**
	Post-MHW	0.18 ± 0.04 **a**	0.16 ± 0.03 **a**	0.09 ± 0.04 **a**	0.04±0.01 **a**	0.08 ± 0.36 **a**	0.11 ±0.02 **a**

7. Figures

FIG. 1. Gametophytes of (A) *Eisenia arborea* (B) *Macrocystis pyrifera* and (C) *Pterygophora californica* prior to being exposed to MHW treatments.

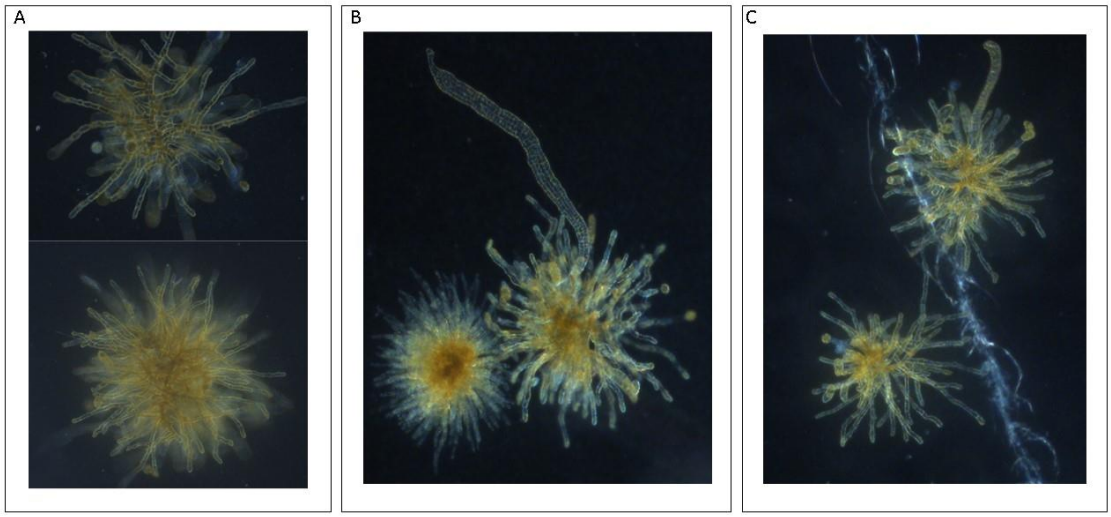


FIG. 2. P-E curves measured in (A) *Eisenia arborea* (B) *Macrocystis pyrifera* and (C) *Pterygophora californica* gametophytes under the different temperature treatments during both experimental phases. In the MHW and post-MHW phases

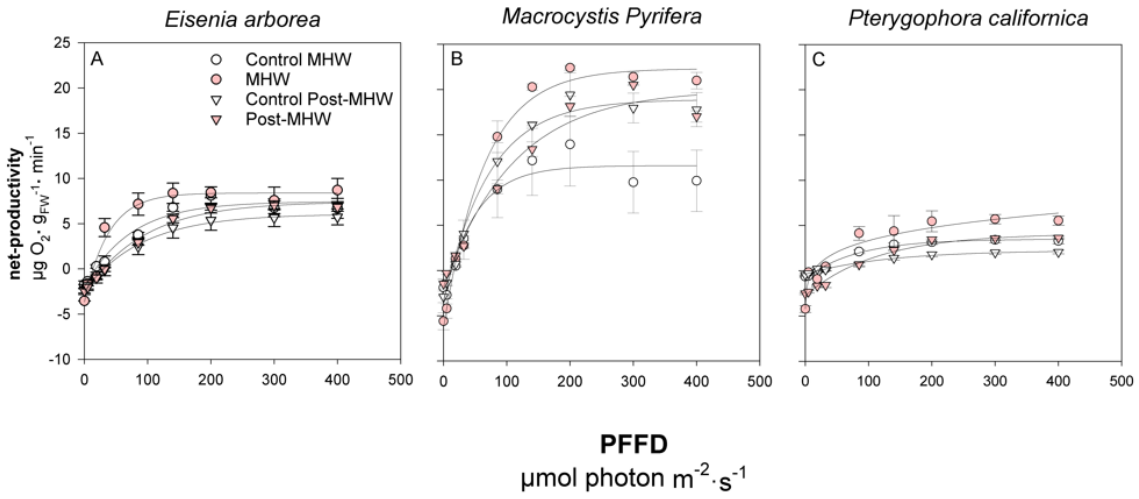


FIG. 3. Photosynthetic parameters from P-E curves measured in *E. a* (*Eisenia arborea*), *P.c* (*Pterygophora californica*), *M.p* (*Macrocystis pyrifera*). under the different temperature treatments during both experimental phases. In the MHW and the post-MHW phases. Statistical differences among treatments in MHW and post-MHW phases are indicated by lowercase letters (two-way ANOVA, *post hoc* SNK). (A) MHW Net-productivity, (B) Post-MHW Net-productivity, (C) MHW Gross maximum photosynthetic rates, (D) Post-MHW Gross maximum photosynthetic rates, (E) MHW Compensation irradiance, (F) Post-MHW Compensation irradiance, (G) MHW Photosynthetic efficiency, (H) Post-MHW Photosynthetic efficiency, (I) MHW Respiration, (J) Post-MHW Respiration, (K) MHW Saturation irradiance, (L) Post-MHW Saturation irradiance. Bars are means and standard errors (N = 4).

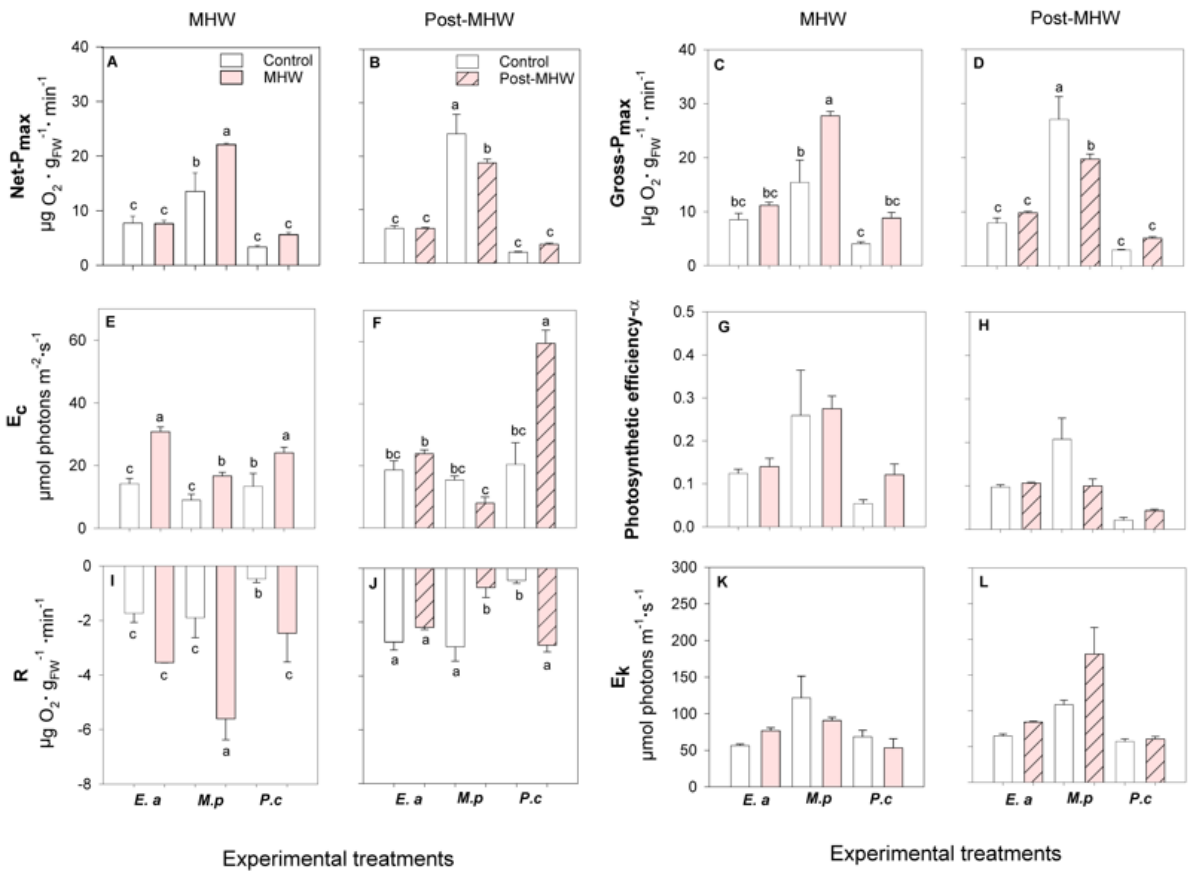

MHW
Post-MHW
MHW
Post-MHW
Control
MHW
Control
Post-MHW
A
B
C
D
Net-Pmax
μg O2 · gFW-1 · min-1
Gross-Pmax
μg O2 · gFW-1 · min-1
E
F
G
H
Ec
μmol photons m-2·s-1
Photosynthetic efficiency-α
I
J
K
L
R
μg O2 · gFW-1 ·min-1
Ek
μmol photons m-1·s-1
E. a
M.p
P.c
Experimental treatments
Experimental treatments

FIG.4. Daily productivity measured in *Eisenia arborea*, *Pterygophora californica*, *Macrocystis pyrifera* under the different temperature treatments during both experimental phases. (A) MHW and (B) post-MHW phases.

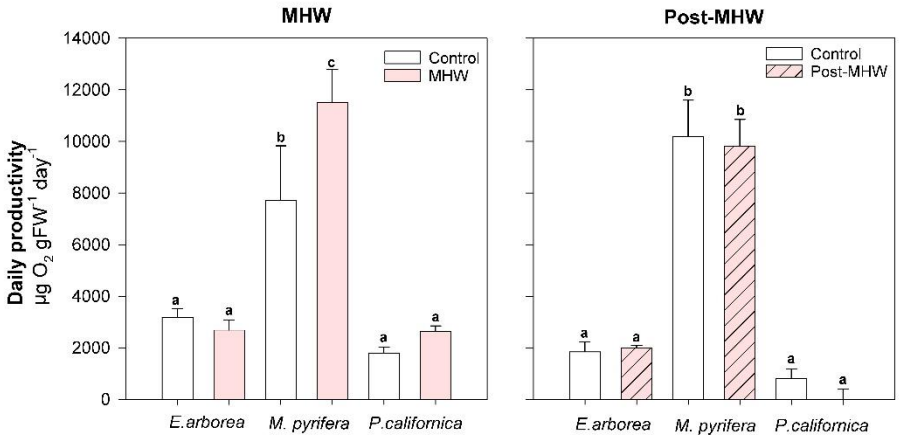


FIG. 5. Photochemical descriptors based on Chl*a* fluorescence measured in in *E. a* (*Eisenia arborea*), *P.c* (*Pterygophora californica*), *M.p* (*Macrocystis pyrifera*). under the different temperature treatments during both experimental phases. In the MHW and the post-MHW phases. Statistical differences among treatments in MHW and post-MHW phases are indicated by lowercase letters (two-way ANOVA, *post hoc* SNK). (A)MHW Maximum quantum yield, (B) Post- MHW Maximum quantum yield, (C) MHW effective quantum yield, (D) Post-MHW effective quantum yield, (E) MHW non-photochemical quenching, (F) Post-MHW non-photochemical quenching, (G) MHW electron transport rate and (G) Post-MHW electron transport rate. Bars are means and standard error (N = 4).

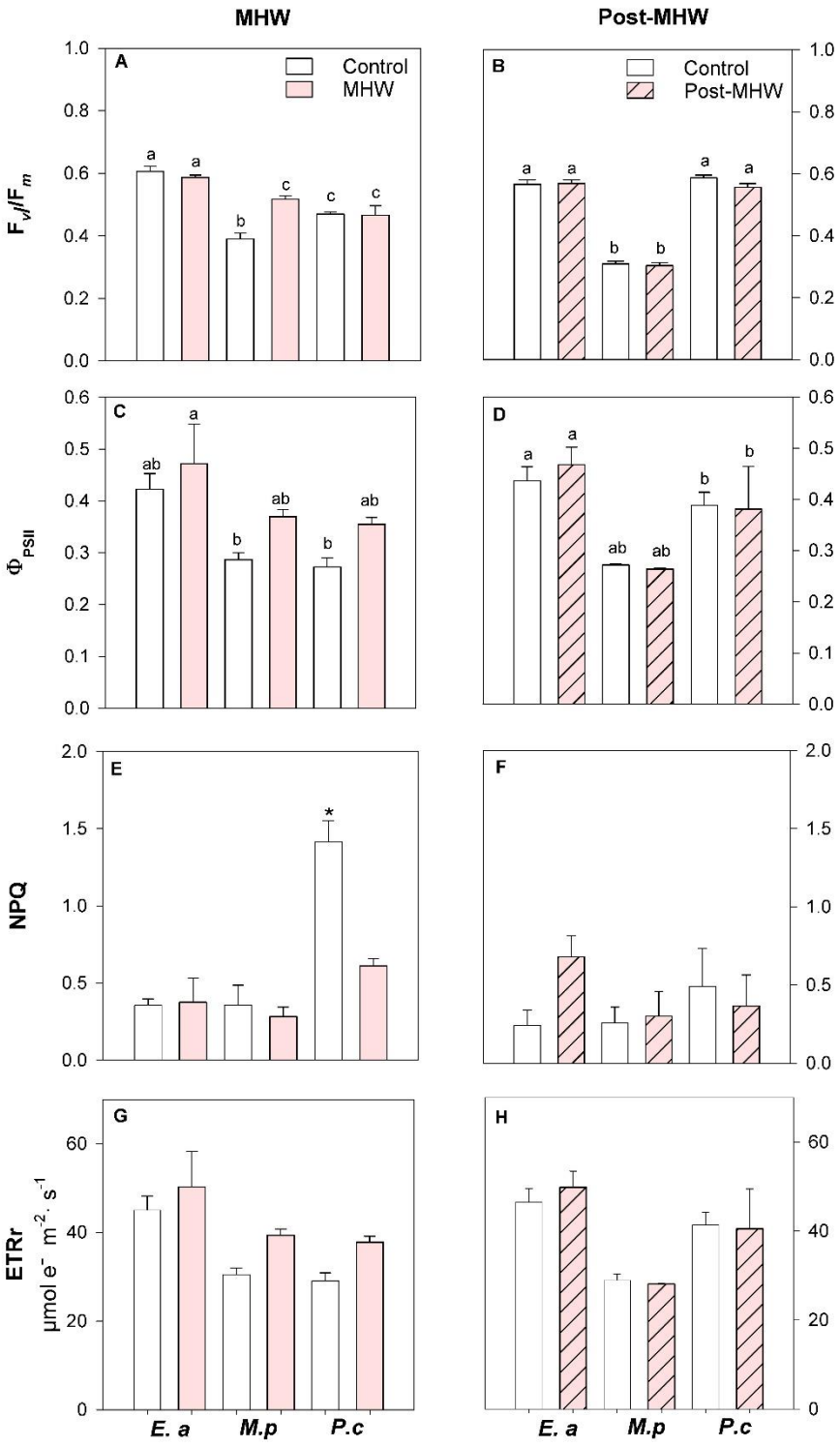
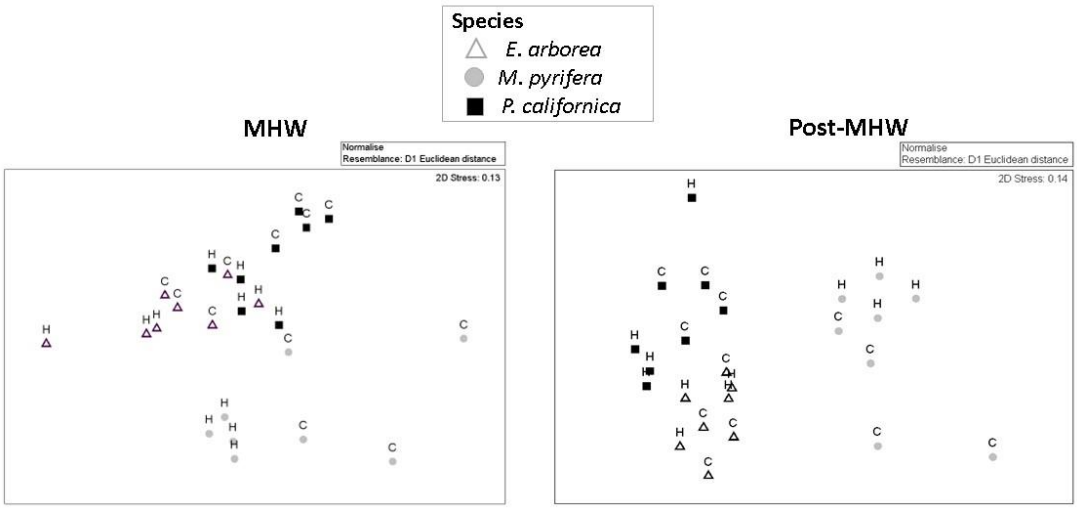

MHW
Post-MHW
A
B
C
D
E
F
G
H
Control
MHW
Post-MHW
F_v/F_m
Φ_{PSII}
NPQ
ETRr
µmol e⁻ m⁻². s⁻¹
E. a
M.p
P.c

FIG. 6. Non-metrics Multidimensional Scaling (MDS) for the biological responses of *Eisenia arborea*, *Pterygophora californica*, *Macrocystis pyrifera* under the different temperature treatments (C = control; H = heatwave). Each symbol represents an experimental unit (EU).



8. Supplementary Material

TABLE 1SM: Results of the two-way ANOVA analyses performed on the different biological descriptors measured in the gametophytes of *Eisenia arborea*, *Macrocystis pyrifera* and *Pterygophora californica* exposed to the different temperature treatments in the experimental MHW and Post-MHW phases. The significant values are indicated in bold **(*P*<0.05).** Variables are expressed in both fresh weight and chlorophyll *a* units, as indicated by the annotations (FW) and (Chl*a*).

		Specie (S)				**Temperature (T)**				**S x T**			
Exp. phase	Variable	**d.f**	**MS**	***F***	***P***	**d.f**	**MS**	***F***	***P***	**d.f**	**MS**	***F***	***P***
MHW	**Net-P_{max}** $\mu g\ O_2 \cdot g\ FW^{-1} \cdot min^{-1}$	2	3.637	44.935	**<0.001**	1	0.851	10.521	**0.005**	2	0.096	2.424	0.117
	Gross-P_{max} $\mu g\ O_2 \cdot g\ FW^{-1} \cdot min^{-1}$	2	505.9	37.224	**<0.001**	1	259.31	19.08	**<0.001**	2	51.37	3.78	**0.043**
	R $\mu g\ O_2 \cdot gFW^{-1} \cdot min^{-1}$	2	3.69	4.305	**0.030**	1	9.207	10.741	**0.004**	2	0.353	0.411	0.66
	E_c (FW)	2	190.765	9.496	**0.002**	1	816.585	40.650	**<0.001**	2	40.943	2.038	0.159

µmol quanta · m^{-2} · s^{-1}												
E_k (FW) µmol quanta · m^{-2} · s^{-1}	2	0.668	7.445	**0.004**	1	0.026	0.293	0.595	2	0.210	2.346	0.124
Daily productivity µg O_2·gFW^{-1} · min^{-1}	2	133165818.2	26.136	**<0.001**	1	11381860.9	2.234	0.152	2	9678146.54	1.899	0.17
Net-P_{max} mg O_2·mg^{-1}Chl*a* ·h^{-1}	2	12.84	164.57	**<0.001**	1	1.957	25.065	**<0.001**	2	0.911	11.669	**<0.001**
Gross-P_{max} mg O_2·mg^{-1}Chl*a* ·h^{-1}	2	23.023	79.005	**<0.001**	1	2.864	9.826	**0.006**	2	0.901	3.158	0.067
R mg O_2·mg^{-1}Chl*a* ·h^{-1}	2	0.306	21.847	**<0.001**	1	0.324	23.138	**<0.001**	2	0.089	6.422	**0.008**
E_c (Chl*a*) µmol quanta · m^{-2} · s^{-1}	2	234.148	6.667	**0.007**	1	641.825	18.275	**<0.001**	2	78.692	2.241	0.135
F_v/F_m	2	0.0495	41.243	**<0.001**	1	0.007	6.101	**0.024**	2	0.013	10.798	**<0.001**
F_0	2	0.047	1.369	0.280	1	0.0310	0.901	0.355	2	0.0236	0.687	0.516
Fm	2	159285.88	5.153	**0.017**	1	11440.67	0.370	0.551	2	12330.79	0.399	0.677

	F	2	93241.17	24.387	**<0.001**	1	1.042	0.0002	0.987	2	5253.167	1.374	0.278
	Fm'	2	279146	0.5672	**<0.001**	1	4760.167	0.267	0.611	2	24728.67	1.388	0.275
	NPQ	2	1.206	26.821	**<0.001**	1	0.494	10.984	**0.004**	2	0.409	9.092	**0.002**
	ETRr	2	5549.27	42.347	**<0.001**	1	580.11	4.427	0.05	2	63.856	0.487	0.622
	Chl *c* $\mu g \cdot gFW^{-1}$	2	0.0131	1.740	0.204	1	0.0016	0.212	0.651	2	0.00544	0.723	0.499
Post-MHW	**Net-P_{max}** $\mu g\ O_2 \cdot gFW^{-1} \cdot min^{-1}$	2	1.621	313.392	**<0.001**	1	0.0142	2.739	0.115	2	0.0592	11.456	**<0.001**
	Gross-P_{max} $\mu g\ O_2 \cdot gFW^{-1} \cdot min^{-1}$	2	1.173	224.130	**<0.001**	1	0.0292	5.572	**0.030**	2	0.0655	12.510	**<0.001**
	Photosynthetic efficiency -α (FW)	2	0.029	16.833	**<0.001**	1	0.004	2.193	0.156	2	0.010	5.794	**0.011**
	Daily productivity $\mu g\ O_2 \cdot gFW^{-1} \cdot min^{-1}$	2	213035580	90.568	**<0.001**	1	700598.5	0.298	0.592	2	434801.4	0.185	0.83
	R $\mu g\ O_2 \cdot gFW^{-1} \cdot min^{-1}$	2	1.206	3.278	0.061	1	0.006	0.016	0.900	2	12.087	32.848	**<0.001**

E_c (FW) µmol quanta · m^{-2} · s^{1}	2	1648.9	30.05	**<0.001**	1	900.9	16.42	**<0.001**	2	1149.4	20.95	**<0.001**
R mg O_2·mg^{-1}Chl*a* ·h^{-1}	2	0.211	21.232	**<0.001**	1	0.022	2.213	0.154	2	0.396	39.849	**<0.001**
Photosynthetic efficiency -α (Chl*a*)	2	0.003	150.131	**<0.001**	1	0.0002	10.166	**0.005**	2	0.0003	16.633	**<0.001**
E_c (Chl *a*) µmol quanta · m^{-2} · s^{1}	2	0686.958	30.844	**<0.001**	1	889.201	16.258	**<0.001**	2	1138.271	20.812	**<0.001**
F_v/F_m	2	0.184	384.087	**<0.001**	1	0.001	1.492	0.238	2	0.001	1.306	0.295
Φ_{PSII}	2	0.069	10.742	**<0.001**	1	0.0001	0.026	0.873	2	0.001	0.159	0.854
NPQ	2	0.103	0.708	0.506	1	0.07	0.482	0.497	2	0.311	2.139	0.147
ETRr µmol e^{-} · m^{-2} ·s^{-1}	2	787.117	10.742	**<0.001**	1	1.936	0.0264	0.873	2	11.651	0.1.59	0.854
Chl *a* µg · gFW^{-1}	2	0.683	12.214	**<0.001**	1	0.049	0.875	0.362	2	0.069	1.249	0.311
Chl *c*	2	0.020	5.365	**0.015**	1	0.001	0.298	0.592	2	0.004	1.212	0.321

$\mu g \cdot gFW^{-1}$

TABLE 2SM. Results of the non-parametric test of Kruskal-Wallis performed on the different biological descriptors measured in the gametophytes of *Eisenia arborea*, *Macrocystis pyrifera* and *Pterygophora californica* exposed to the different temperature treatments in the experimental MHW and Post-MHW phases. The significant values are indicated in bold **(*P*<0.05).** Variables are expressed in both fresh weight and chlorophyll *a* unit, as indicated by the annotations (FW) and (Chl*a*).

		Specie (S)			**Temperature (T)**			**Interaction S x T**		
Experimental Phase	**Variable**	**d.f**	**H**	***P***	**d.f**	**H**	***P***	**d.f**	**H**	***P***
MHW	**Photosynthetic efficiency -α (FW)**	2	9.74	**0.007**	1	2.644	0.106	5	12.86	**0.024**
	E_K (FW) $\mu mol\ quanta \cdot m^{-2} \cdot s^{1}$	2	8.385	**0.015**	1	0.213	0.644	5	11.62	**0.040**

	Photosynthetic efficiency -α (Chl *a*)	2	5.795	0.05	1	5.07	**0.024**	5	13.4	**0.019**
	E_K (Chl *a*) µmol quanta · m^{-2} · s^{1}	2	12.435	**0.001**	1	1.203	0.272	5	14.65	**0.011**
	Chl *a* µg · gFW^{-1}	2	12.666	**0.001**	1	0.053	0.817	5	13.806	**0.017**
	Φ_{PSII}	2	8.565	**0.014**	1	4.563	**0.03**	5	15.600	**0.008**
Post-MHW	**E_K (FW)** µmol quanta · m^{-2} · s^{1}	2	18.49	**<0.001**	1	1.471	0.225	5	20.299	**0.001**
	Net-P_{max} mg O_2· mgChl*a*-^{1} · h^{-1}	2	16.98	**<0.001**	1	1.08	0.298	5	19.4	**0.001**
	Gross-P_{max} mg O_2· mgChl*a*$^{-1}$ · h^{-1}	2	19.28	**<0.001**	1	0.75	0.386	5	20.29	**0.001**
	E_K (Chl *a*) µmol quanta · m^{-2} · s^{1}	2	13.745	**0.001**	1	0.403	‘0.525	5	17.96	**0.002**
	F	2	6.905	**0.031**	1	1.92	0.165	5	9.49	0.091

F0	2	10.545	**0.005**	1	0.333	0.564	5	15.91	0.007
Fm	2	10.955	**0.004**	1	0.333	0.564	5	14.45	**0.013**
Fm´	2	8.96	**0.011**	1	2.253	0.133	5	11.94	0.356

CONCLUSIONES

Capítulo 1

- Los esporofitos *Pterygophora californica* de los extremos batimétricos en la población de estudio mostraron signos claros de fotoadaptación a las distintas condiciones lumínicas en ambas profundidades.
- Los esporofitos adultos de *P. californica* provenientes de sus extremos batimétricos respondieron de manera distinta al calentamiento relacionado con la exposición a la onda de calor marina experimental.
- Los esporofitos de *P. californica* del extremo batimétrico somero exhibieron una evidente termo-sensibilidad al calentamiento. Esta termo-sensibilidad se vio reflejada en la disminución en tasas fotosintéticas y tasa de incorporación de nitrato, un desbalance en el metabolismo del carbono y una reducción clara del del crecimiento de las láminas, entre otros signos de estrés.
- Los esporofitos de *P. californica* del extremo batimétrico profundo mostraron una evidente termo-tolerancia durante su exposición a la OCM experimental. El calentamiento favoreció el rendimiento de distintos procesos fisiológicos (p.e. fotosíntesis) y sus tasas de crecimiento.
- Nuestros resultados revelaron que muchas de las respuestas fisiológicos inducidas por el calentamiento en ambos experimentos se encontraron durante la fase experimental de retorno a temperatura control, y no durante la exposición a la OCM. Esto demuestra que la inclusión de estas fases experimentales es crucial para la obtención de resultados concluyentes en relación a las respuestas de estrés térmico.
- La elevada resistencia térmica demostrada en los esporofitos profundos, abre la puerta a nuevas investigaciones en relación con el rol de las partes profundas de

esta especie como refugios frente al calentamiento oceánico y eventos térmicos extremos.

Capítulo 2

Los gametofitos de *E. arborea* exhibieron una mayor resistencia térmica frente a la OCM experimental en comparación con *M. pyrifera* y *P. californica*.

- De forma general, los gametofitos de *M. pyrifera* y *P. californica* presentaron alteraciones en sus tasas fotosintéticas y respiratorias, mientras que el calentamiento no indujo signos de estrés evidentes en *E. arborea*.

- Esta distinta termo-tolerancia entre gametofitos coincide con la que se atribuye a esporofitos adultos de las distintas especies. Por tanto, la termo-tolerancia de gametofitos de *E. arborea* contribuye a la resistencia térmica de la especie.

- Nuestros resultados revelaron que muchas de las respuestas fisiológicos inducidas por el calentamiento en ambos experimentos se encontraron durante la fase experimental de retorno a temperatura control, y no durante la exposición a la OCM. Esto demuestra que la inclusión de estas fases experimentales es crucial para la obtención de resultados concluyentes en relación a las respuestas de estrés térmico.