UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA

FACULTAD DE CIENCIAS MARINAS

INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS

POSGRADO EN OCEANOGRAFÍA COSTERA

"RESPUESTAS ECOFISIOLÓGICAS DE JUVENILES DEL ALGA PARDA *Macrocystis pyrifera* FRENTE A LA VARIACIÓN DE FACTORES ABIÓTICOS: TEMPERATURA, LUZ Y NITRATO"

TESIS

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS EN OCEANÓGRAFIA COSTERA

PRESENTA

MARIANA SÁNCHEZ BARREDO

ENSENADA, B.C., MÉXICO. SEPTIEMBRE, 2020.

FACULTAD DE CIENCIAS MARINAS

INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS

POSGRADO EN OCEANOGRAFÍA COSTERA

RESPUESTAS ECOFISIOLÓGICAS DE JUVENILES DEL ALGA PARDA *MACROCYSTIS PYRIFERA* FRENTE A LA VARIACIÓN DE FACTORES ABIÓTICOS: TEMPERATURA, LUZ Y NITRATO.

TESIS
QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS EN OCEANOGRAFÍA COSTERA

PRESENTA

MARIANA SÁNCHEZ BARREDO

APROBADO POR:

Dr. José Miguel Sandoval Gil
Director de tesis

Dr. José Antonio Zertuche González
Codirector de tesis

Dra. Lydia Betty Ladah
Sinodal

Dr. Rodrigo Beas Luna
Sinodal

Dr. Luis Walter Daessié Heuser
Sinodal

INTRODUCCIÓN

Las olas de calor marinas son eventos que se caracterizan por la temperatura superficial del mar extremadamente alta, que puede afectar miles de kilómetros, cuya duración puede ser de días a meses (Frölicher et al. 2018, Smale et al. 2019) y se prevé que sean cada vez frecuentes e intensos bajo el efecto del cambio climático (Schär y Jendritzky 2004). Los cambios en la temperatura, así como en la química del agua de mar, asociados con las olas de calor marino, están induciendo variaciones extendidas en sistemas biológicos, generando preocupación por el futuro de las comunidades biológicas costeras, tales como los mantos de macroalgas. Estos hábitats proveen, a escala mundial, funciones críticas, tanto ecológicas como para el hombre (Hurd et al. 2014). Por esta razón es importante predecir su respuesta (tolerancia, resiliencia) ante el estrés térmico y el impacto en los patrones de distribución específicos según la especie.

La exposición a temperaturas altas puede causar estrés disruptivo en las macroalgas, provocando daño metabólico (Davison y Pearson, 1996). Así mismo, pueden alterar la fisiología de los macrófitos marinos como se ha visto en observaciones in situ, asi como mediante aproximaciones experimentales (Bruhn y Gerard 1996, Short et al. 2015, Marín-Guirao et al. 2017). El estrés térmico puede, a grandes rasgos, alterar los procesos enzimáticos y la composición de la membrana celular (Machalek et al. 1996, Los y Murata 2004), aumentar o reducir la fotosíntesis y el crecimiento, dependiendo de la duración e intensitad del estrés térmico y su interacción con otros factores ambientales como: luz, nutrientes, exposición a radiación UVB entre otros (Kübler y Davison 1993, Bruhn y Gerard 1996, Brown et al. 2014, Xiao et al. 2015, Mabin et al. 2019) y conducir a la fotoinhibición, aumento en la respiración y cambión en la composición pigmentaria (Kübler y Davison 1993, Bruhn y Gerard 1996, et al. 2007, Andersen et al. 2013). El estrés térmico también se ha correlacionado con la producción de especies reactivas del oxígeno y el incremento de la capacidad antioxidante de las macroalgas intermareales (Collen y Davison 1999, Cruces et al. 2012).

La evidencia apunta a que los eventos cortos (días) de anomalías térmicas de alta intensidad pueden resulta más perjudiciales para la vegetación sumergida, que aquellos

que son persistentes pero de menor intensidad (Thompson et al. 2013, Wilson et al. 2015, Guerrero-Meseguer et al. 2017). La exposición a temperaturas máximas cada vez mayores, la influencia de olas de calor marinas o de fenómenos naturales episódicos o estacionales, como El Niño, afectan a las macroalgas a escalas locales y regionales (Pacheco-Ruíz et al. 2003, Jordá et al. 2012, Schiel y Foster 2015). Aún si dichas exposiciones son breves, el patrón de distribución, crecimiento y reclutamiento de las macroalgas pueden alterarse (Andrews et al. 2014, Smale et al. 2019). En particular, los bosques de sargazos se han visto afectados en su abundancia y patrón biogeográfico (Krumhansl et al. 2016) y las poblaciones más cercanas al límite de su distribución han sido perjudicadas en forma más severa por estos procesos (Wernberg et al. 2011).

Macrocystis pyrifera

Generalidades

El sargazo gigante, *Macrocystis pyrifera* es un alga parda, del orden de las laminariales. característica de mares templados, ubicada en un rango semi-continuo en el hemisferio Norte, formado por poblaciones aisladas en Alaska, Oregón y Washington y por poblaciones grandes desde el Norte de California hasta Baja California Sur. Crece en la zona submareal, con individuos en promedio de 30 m de longitud, que forman los bosques icónicos de sargazo (Abbott y Hollemberg, 1976) que son de importancia ecológica. A estos bosques están asociadas especies numerosas de otras algas, invertebrados, peces, aves y mamíferos marinos a quienes provee sustrato, refugio o alimento. *M. pyrifera* contribuye en el ciclo del carbono, debido a su alta producción de biomasa y tiempo de recambio (un año aproximadamente) se considera como un sumidero de carbono eficiente (Chung et al. 2018). También reduce la acidificación del océano en escala local (Chan et al. 2016). Comercialmente se ha explotado desde principios del siglo pasado, primero para la obtención de potasa (Cameron, 1913), posteriormente para la extracción de alginatos, un polisacárido usado extensamente en la industria farmacéutica y alimentaria FAO, 2002, Steinbüchel y Rhee, 2005). Sin embargo su uso principal hoy en día es como forraje de

herbívoros marinos como el erizo o el abulón (Gordon y Cook, 2011, Zertuche *et al*., 2014).

Relación con factores abióticos

El estado de los bosques de *Macrocystis pyrifera* está estrechamente relacionada con las características abióticas del medio, entre las que destacan con las condiciones de temperatura, nitrato y luz adecuadas para el crecimiento, desarrollo y reproducción de los individuos. Los cambios extremos en estos factores pueden llevar incluso a la pérdida de poblaciones enteras. La costa de Baja California se considera particularmente vulnerable a las olas de calor marinas, debido a la prevalencia de *M. pyrifera* y otros sargazos en su límite de distribución. En la península de Baja California, la influencia de la corriente de California provee los requerimientos básicos para la el desarrollo de *M. pyrifera*. Por otro lado, los eventos episódicos en la región, como El Niño o las olas de calor marinas se han correlacionado con la reducción o desaparición de bosques enteros, probablemente correlacionadas con el alza de temperatura y otros factores ambientales, como el hundimiento de la termoclina y la reducción de nutrientes (North y Zimmerman 1984, Zimmerman y Robertson 1985, Ladah 2003, Schiel y Foster 2015, Arafeh-Dalmau et al. 2019). Esta situación se observó en Baja California Sur, donde desaparecieron algunas poblaciones en forma permanente, después de El Niño 1982-1983 (Hernández-Carmona et al. 2000). Más recientemente, la ola de calor de larga duración conocida como La Mancha (The Blob) provocó la pérdida de hasta un 50 % de los mantos de Baja California 2014 y 2015, (Cavanaugh et al, 2019). Es común observar en *M. pyrifera* durante los meses de verano, reducción en su crecimiento, blanqueamiento y degradación tisular, mayor epifitación y muerte (North 1987) sin embargo esos efectos pueden aminorarse por el aporte de nitrato causado por el aporte de agua de abajo de la termoclina, generado por ondas internas y mezcla (Zimmerman y Robertson 1985, Ladah et al. 2012).

A pesar de que los efectos del estrés térmico en los mantos de *Macrocystis pyrifera* se han descrito ampliamente (North 1987, Ladah et al. 1999, Cavanaugh et al. 2019), los mecanismos fisiológicos subyacentes tras su vulnerabilidad y/o resiliencia aún son

limitados (Brown et al. 2014), principalmente aquellos enfocados en el estadio juvenil. Por otro lado, la historia de vida de *M. pyrifera* incluye la alternancia generacional de un estado macroscópico conspicuo a uno microscópico. Los estados tempranos (Gametofitos o esporofitos juveniles) pueden tener tolerancias diferentes ante estresores ambientales (Manley y North 1984, Dean y Jacobsen 1986, Muñoz et al. 2004, Ladah y Zertuche-González 2007, Mabin et al. 2019) que pueden resultar cruciales en la supervivencia de una población (Graham 1996, Clarke 2003) puesto que el metabolismo de *M. pyrifera* varía entre estados ontogénicos, por ejemplo en su fotosíntesis o requerimientos de luz (Dean y Jacobsen 1986, Xu et al. 2015). Así mismo, los esporofitos juveniles de *M. pyrifera* carecen de la complejidad estructural de los adultos y no cuentan con mecanismos como la translocación que les permita hacer frente a condiciones estresantes (Graham et al. 2007). Por consiguiente se espera que las respuestas aclimatativas y la resistencia al estrés térmico sean diferentes entre adultos y juveniles.

Otro factor de importancia en las respuestas fisiológicas de *Macrocystis pyrifera* ante el aumento de temepratura, es la concentración de nitrato en el medio, que puede causar en estrés (Dayton y Tegner, 1984, Ladah et al. 1999, Steneck et al. 2002, Ladah 2003). En las regiones templadas, la presencia de nitrato en las capas superficiales de la columna de agua tiene un patrón estacional marcado, que depende de las termoclinas estacionales (Roleda and Hurd, 2019). En el Pacífico Noroeste, la influencia del sistema de surgencias provoca que la concentración de nitrato tienda a ser menor en otoño y mayor en primavera (Zimmermann and Kremer, 1986). En estos sistemas, el agua proveniente de debajo de la termoclina, tica en nitrientes, emerge a la superficie favoreciendo la producción primaria (Jacox et al. 2015; Schiel and Foster 2015. En la península de Baja California, la influencia de la corriente de California provee los requerimientos básicos para la el desarrollo de *M. pyrifera*. Por otro lado, los eventos episódicos en la región, como El Niño o las olas de calor marinas se han correlacionado con la reducción o desaparición de bosques enteros, probablemente correlacionadas con el alza de temperatura y otros factores ambientales, como el hundimiento de la termoclina y la reducción de nutrientes (North y Zimmerman 1984, Zimmerman y Robertson 1985, Ladah 2003, Schiel y Foster 2015, Arafeh-Dalmau et al. 2019). Esta situación se observó en Baja California Sur, donde desaparecieron

algunas poblaciones en forma permanente, después de El Niño 1982-1983 (Hernández-Carmona et al. 2000). Más recientemente, la ola de calor de larga duración conocida como La Mancha (The Blob) provocó la pérdida de hasta un 50 % de los mantos de Baja California 2014 y 2015, (Cavanaugh et al, 2019). Es común observar en *M. pyrifera* durante los meses de verano, reducción en su crecimiento, blanqueamiento y degradación tisular, mayor epifitación y muerte (North 1987) sin embargo esos efectos pueden aminorarse por el aporte de nitrato causado por el aporte de agua proveniente por debajo de la termoclina, generado por ondas internas y mezcla (Zimmerman y Robertson 1985, Ladah et al. 2012).

Los ajustes fisiológicos que *Macrocystis pyrifera* lleva a cabo bajo condiciones de estrés térmico en combinación con la escasez de nitrato, están descritos ampliamente en la literatura, (Wheeler 1980; Wheeler y North 1980; Gerard 1982, 1986; Colombo-Pallotta et al. 2006; Edwards y Kim 2010; Kopczak et al, 1991; Stephens y Hepburn; 2016). *M. pyrifera* puede aclimatarse a la exposición breve a un rango de temperaturas, ya sea con disponibilidad o ausencia de nutrientes, ajustando su composición de ácidos grasos (Schmid et al. 2020). Otros ajustes incluyen cambios en la fluorescencia de la clorofila a, composición pigmentaria, translocación de fotosintatos y cambios en la composición química del tejido (Fox, 2013; Schiel y Foster, 2015; Rothäusler et al. 2018).

La diferencia en la interacción de juveniles o adultos con cambios ambientales que pueden ocurrir simultáneamente con el alza de temperatura, tal como la disponibilidad de luz o nitrato, que pueden representar otra fuente potencial de variación en la respuesta ante el estrés térmico. Por ejemplo la mayor turbidez del agua provocada por tormentas o incluso los florecimientos fitoplanctónicos pueden reducir drásticamente la luz incidente, hasta valores cercanos a cero, durante CUÑA A MP semanas en el bentos benthos (Dean y Jacobsen 1984, Dean 1985, Cabello-Pasini et al. 2002, Schiel y Foster 2015). El dosel formado en los bordes de los mantos de *M. pyrifera*, por macroalgas de crecimiento rápido, como las especies invasivas *Sargassum horneri*, *Sargassum muticum* y *Undaria pinnatifida* que han colonizado progresivamente áreas en las costas del Pacífico

Norteamericano, puede disminuir la luz disponible para el reclutamiento desarrollo de los juveniles de *M. pyrifera* (Ambrose y Nelson 1982, Schiel y Foster 2015, South et al. 2017). El dosel de los mantos de *M. pyrifera* y otros sargazos también pueden sombrear a los juveniles de *M. pyrifera*. Mientras los adultos de *M. pyrifera* pueden contrarrestar la limitación por luz tanto por fotoaclimatación de las láminas presentes en las zonas más profundas, como por translocación de recursos desde las láminas más superficiales (Manley y North 1984, Colombo-Pallota et al. 2006), los juveniles menos desarrollados tienen que depender exclusivamente de su plasticidad fotosintética para sobrevivir. La escasez de luz trae como consecuencia menor disponibilidad de energía metabólica, necesaria para asimilar e incorporar carbono inorgánico, causando el decremento de la producción de fotosintatos. (Hanlet y López-Figueroa 2012) debido a que la incorporación y asimilación de nitrógeno inorgánico necesita energía y esqueletos hidrocarbonados, la limitación por luz puede alterar el metabolismo del nitrógeno, incluyendo las tasas de incorporación (Hurd et al 2014). Para optimizar la captura de luz, cuando ésta es limitante, las macroalgas, incluida *M. pyifera* (Umanzor et al. 2019) pueden activar mecanismos de fotoaclimatación que incluyen el incremento de pigmentos y cambios en las propiedades bio-ópticas del tejido como la absorptancia. Sin embargo, el conocimiento de las estrategias fotoaclimatativas de *M. pyrifera* aún está en ciernes (Mabin et al.2019, Umanzor et al. 2019).

Las predicciones que indiquen como responderán los ecosistemas de los mantos de sargazo gigante, ante los efectos del cambio climático global y la incidencia de olas de calor marinas, más largas e intensas

J. Phycol. *, ***–*** (2020)

DOI: 10.1111/jpy.13000

EFFECTS OF HEAT WAVES AND LIGHT DEPRIVATION ON GIANT KELP JUVENILES (*MACROCYSTIS PYRIFERA*, LAMINARIALES, PHAEOPHYCEAE)[1]

Mariana Sánchez-Barredo[#], *Jose Miguel Sandoval-Gil*[2,#], *Jose Antonio Zertuche-González*

Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Baja California, México

Lydia B. Ladah

Department of Biological Oceanography, CICESE, Ensenada, Baja California, México

María Dolores Belando-Torrentes

Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Baja California, México

Rodrigo Beas-Luna

Facultad de Ciencias Marinas, Universidad Autónoma de Baja California, Ensenada, Baja California, México

and Alejandro Cabello-Pasini

Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Baja California, México

Due to climate change, the incidence of marine heat waves (MHWs) has increased, yet their effects on seaweeds are still not well understood. Adult sporophytes of *Macrocystis pyrifera*, the species forming the iconic giant kelp forests, can be negatively affected by thermal stress and associated environmental factors (e.g., nutrient depletion, light deprivation); however, little is known about the tolerance/vulnerability of juvenile sporophytes. Simultaneously to MHWs, juveniles can be subjected to light limitation for extended periods of time (days–weeks) due to factors causing turbidity, or even because of shading by understory canopy-forming seaweeds. This study evaluated the effects of a simulated MHW (24°C, 7 d) in combination (or not) with light deprivation, on the photosynthetic capacities, nutrient uptake, and tissue composition, as well as oxidative stress descriptors of *M. pyrifera* juvenile sporophytes (single blade stage, up to 20 cm length). Maximum quantum yield (F_v/F_m) decreased in juveniles under light at 24°C, likely reflecting some damage on the photosynthetic apparatus or dynamic photoinhibition; however, no other sign of physiological alteration was found in this treatment (i.e., pigments, nutrient reserves and uptake, oxidative stress). Photosynthetic capacities were maintained or even enhanced in plants under light deprivation, likely supported by photoacclimation (pigments increment); by contrast, nitrate uptake and internal storage of carbohydrates were strongly reduced, regardless of temperature. This study indicated that light limitation can be more detrimental to juvenile survival, and therefore recruitment success of *M. pyrifera* forests, than episodic thermal stress from MHWs.

Key index words: **heat waves; Juveniles; *Macrocystis pyrifera*; photoacclimation; physiology**

***Abbreviations*: D, darkness; ETR, electron transport rate; L, Light; MHW, marine heat wave; NPQ, non-photochemical quenching; RLC, rapid light curve**

In the framework of global climate change, a rise of seawater temperature (up to 3–4°C) is predicted by the end of this century (IPCC 2013), and thus, there is an increasing concern about the effects of thermal stress on coastal biological communities, such as seaweed beds (Smale et al. 2019). Macroalgae-dominated habitats provide critical ecological and human services worldwide (Hurd et al. 2014). Therefore, it is important to predict their response (tolerance, resilience) to thermal stress and the subsequent impact on species-specific distribution patterns (Andrews et al. 2014, Smale et al. 2019, Straub et al. 2019).

Exposure to high temperatures can lead to "disruptive stress" in seaweeds, resulting from metabolic damage (Davison and Pearson 1996). Both experimental approaches and in situ observations have shown that the physiology of marine macrophytes can be altered by exposure to high temperatures (Bruhn and Gerard 1996, Short et al. 2015, Marín-Guirao et al. 2017). Generally, thermal stress can (i) alter enzymatic-mediated processes and cell membrane composition (Machalek et al. 1996, Los and Murata 2004), (ii) increase or decrease photosynthesis and growth depending on the duration and

[1]Received 28 October 2019. Accepted 15 March 2020.
[2]Author for correspondence: e-mail jmsandovalgil@gmail.com.
[#]These authors contributed equally to the manuscript.
Editorial Responsibility: M. Edwards (Associate Editor)

intensity of heat stress and its interaction with other environmental factors (e.g., light, nutrients, UVB radiation; Kübler and Davison 1993, Bruhn and Gerard 1996, Brown et al. 2014, Xiao et al. 2015, Mabin et al. 2019), and (iii) result in photoinhibition, increased respiration, and changes in pigment composition (Kübler and Davison 1993, Bruhn and Gerard 1996, Koch et al. 2007, Andersen et al. 2013). Heat stress has also been correlated with the production of reactive oxygen species and increased antioxidant capacity of intertidal seaweeds (Collén and Davison 1999, Cruces et al. 2012). Populations near their distribution boundaries will most likely be more affected by thermal stress (Wernberg et al. 2011).

There is evidence that short-term (days) thermal anomalies of high intensity can be more harmful for submerged vegetation than persistent (but moderate) warming (Thompson et al. 2013, Wilson et al. 2015, Guerrero-Meseguer et al. 2017). The exposure to increasing annual maximum temperatures, the influence of marine heat waves (MHWs), or even the influence of episodic/seasonal natural events (e.g., ENSO) strongly affect macroalgae at regional and local scales (Pacheco-Ruíz et al. 2003, Jordà et al. 2012, Schiel and Foster 2015). Even short-term exposure to thermal stress may affect distribution patterns, growth, and recruitment of seaweeds (Andrews et al. 2014, Smale et al. 2019).

As a result of climate change, short-term periods of extreme temperature anomalies, such as longer and more severe summer MHWs, have increased (Schär and Jendritzky 2004, Perkins et al. 2012). MHWs, often of short duration, can reduce reproduction and alter distribution of some seaweeds (Andrews et al. 2014), and can promote mortality of crustose coralline algae and local extinction of kelp communities (Short et al. 2015, Thomsen et al. 2019). The impact of MHWs on kelp forests may depend on the influence of latitudinal patterns of genetic diversity, physiological versatility, and ecological resilience (Wernberg et al. 2018). Although understanding the effects of weather extremes (including MHWs) on submerged vegetation has been highlighted (Wernberg et al. 2012, 2016), seaweed responses to thermal stress, such as physiological plasticity, tolerance, and resilience, remain poorly addressed to date in juvenile stages.

The giant kelp, *Macrocystis pyrifera,* is a large kelp forming some of the biggest and most productive temperate submerged vegetated ecosystems of the world, with relevant ecological and economic values (e.g., aquaculture, maintenance of water quality, nursery and shelter for invertebrates, fishes, mammals, and other algae; Reed and Brzezinski 2009, Gordon and Cook 2013). The coastline of Baja California, Mexico, is considered among the regions particularly vulnerable to MHWs, due to the high levels of biodiversity, the prevalence of important kelp species at their warm boundaries (such as *M. pyrifera*), and the influence of anthropogenic impacts (Holbrook et al. 2019, Smale et al. 2019). In Baja California, the influence of the California Current and upwelling events provides the basic abiotic requirements (e.g., lower temperatures, nutrients) for the development of *M. pyrifera* (Tegner and Dayton 1987). Conversely, thermal anomalies as a consequence of episodic events such as ENSO, MHWs, or prolonged events such as the "Blob" (see Hu et al. 2017) can be correlated with damage or even complete disappearance of Giant Kelp forests, likely due to the cross-correlation of temperature and other environmental factors, such as the deepening of the thermocline and associated reduction of nutrients (North and Zimmerman 1984, Zimmerman and Robertson 1985, Ladah 2003, Schiel and Foster 2015, Arafeh-Dalmau et al. 2019). Normal summer warming causes reduced growth, bleaching and softening of tissues, increased epiphytation and death (North 1987); however, these effects can be ameliorated by sub-thermocline nitrate influx fueled by internal waves and mixing (Zimmerman and Robertson 1985, Ladah et al. 2012). Ocean warming and linked factors such as nutrient limitation can restrict the distribution boundaries of kelp forests, as particularly observed along the coastline of Baja California Peninsula (Ladah et al. 1999, Graham et al. 2007, Arafeh-Dalmau et al. 2019) and other coastal systems.

Despite the well-described negative effects of thermal stress on *Macrocystis pyrifera* forests (e.g., population dynamics, loss of canopy; North 1987, Ladah et al. 1999, Cavanaugh et al. 2019), knowledge about the physiological mechanisms governing their vulnerability and/or resilience is still limited (Brown et al. 2014), particularly at the juvenile stages. Moreover, *M. pyrifera* has a complicated alternation of generations in its life history, and juvenile stages such as gametophytes and juvenile sporophytes may exhibit different resistance to environmental stressors (Manley and North 1984, Dean and Jacobsen 1986, Muñoz et al. 2004, Ladah and Zertuche-González 2007, Mabin et al. 2019). Differences in the thermal tolerance of the various life history stages of kelps can be decisive for the survival of a population (Graham 1996, Clarke 2003). Metabolism differs between the ontogenic stages of *M. pyrifera* (i.e., photosynthesis, light requirements; Dean and Jacobsen 1986, Xu et al. 2015), similar to that indicated for other seaweeds (Xu et al. 2017). Also, juvenile sporophytes of *M. pyrifera* lack the structural complexity of adults, and consequently, they cannot rely on translocation of resources to cope with stressful conditions (Graham et al. 2007). Therefore, acclimation responses and resistance to thermal stress would be expected to differ between adults and juveniles.

The differential interaction of adults and juveniles with changing environmental factors that can occur simultaneously with thermal stress, such as light

availability, can represent another potential source of variation of the responses to heat stress. For instance, increased turbidity due to storm waves, coastal runoff, or even phytoplankton blooms can dramatically reduce incident irradiance (near darkness) for weeks on the benthos (Dean and Jacobsen 1984, Dean 1985, Cabello-Pasini et al. 2002, Schiel and Foster 2015). Phytoplankton blooms commonly resulted for the combination of rising of seawater temperature and upwelled nutrients in spring–summer, and can lead to drastic reductions of irradiance during weeks (less than 0.5 mol photons $\cdot$ m^{-2} $\cdot$ d^{-1}) in shallow bottoms near giant kelp forests (Dean and Jacobsen 1984). Also overgrowth of canopy-forming seaweeds in later-spring/early-summer at the edges of *Macrocystis pyrifera* populations, like the invasive *Sargassum horneri*, *Sargassum muticum*, and *Undaria pinnatifida* that have colonized progressively new coastal areas on the Pacific coast of North America, can comprise light availability for the development of juvenile stages and recruitment (Ambrose and Nelson 1982, Schiel and Foster 2015, South et al. 2017). The canopy of *M. pyrifera* adults and understory canopies of other seaweeds (e.g., kelps species such as *Laminaria* and *Pterygophora*) can also shade juveniles of *M. pyrifera* (North 1994). While adult plants can counterbalance light limitation by both photoacclimation of deeper blades and translocation of resources from apical parts of the fronds reaching the sea surface (Manley and North 1984, Colombo-Pallota et al. 2006), young juveniles must depend on their photosynthetic plasticity to survive. Light deprivation results in lower availability to metabolic energy needed to incorporate and assimilate inorganic carbon, and thus, in a drop in photosynthate production (Hanelt and Lopez-Figueroa 2012). Since the assimilation of inorganic nitrogen into organic compounds requires energy and carbon skeletons from photoassimilates, light limitation can lead to the alteration of the plant N-metabolism, including N-uptake rates (Hurd et al. 2014). Changes in the bio-optical properties of photosynthetic tissues (e.g., absorptance) and pigments content are among the photoacclimation mechanisms which can be activated to optimize light harvesting in seaweeds, including *M. pyrifera* juveniles (Umanzor et al. 2019). Compared to adult sporophytes, photoacclimation strategies of juveniles are still poorly understood (Mabin et al. 2019, Umanzor et al. 2019).

The purpose of the present study was to evaluate the combined effects of a simulated MHW and light deprivation on juvenile sporophytes of *Macrocystis pyrifera*. An orthogonal design was used, based on the exposure of juveniles to two temperatures (16 and 24°C) and two light conditions (saturating irradiance ~180 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}, and a light below compensation irradiance for photosynthesis, 5–10 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}). The main hypothesis of this study was that MHWs and light deprivation can induce physiological changes in juvenile sporophytes, and the metabolic integration of these responses can be decisive to their tolerance and/or vulnerability to those combined stressors. Photosynthesis, bio-optical properties, nutritional status, oxidative stress descriptors, and nitrate uptake rates were evaluated. As far as we know, there is no background information about the integration of such physiological descriptors of *M. pyrifera* juveniles under the combination of disruptive (heat) and limitation (light deprivation) stressful conditions.

MATERIALS AND METHODS

Collection of juvenile sporophytes. In September 2017, juvenile sporophytes (single blade stage, up to 20 cm length) were collected along the edges of a healthy and dense (~0.3 stipes $\cdot$ m^{-2}) *Macrocystis pyrifera* forest growing at 7–10 m depth in Bahía Todos Santos, Baja California, Mexico (35°54′21.90″ N, 116°45′12.05″ W). Surface seawater temperature in this region ranges between 12 and 20°C, although it can increase up to 4°C above the long-term mean during ENSO and MHWs (Hernández de la Torre et al. 2004, Leising et al. 2015).

During collection, algae were kept in black plastic bags to avoid exposure to excessive sunlight. Within 2 h after collection, juveniles were transported in large coolers filled with seawater to the Marine Botany Lab at the University of Baja California. Juveniles were kept in 1-m^3 outdoor tanks until the beginning of the experiment, at averaged values of field temperature and light, 16°C and 6.6 mol photons $\cdot$ m^{-2} $\cdot$ d^{-1}, respectively. Temperature was adjusted by using chillers, while the tanks were covered with plastic meshes to adjust light to field values. Temperature was recorded at the collection site during the previous weeks using HOBO MX2202 (ONSET, Bourne, MA, USA) data loggers. Light values were obtained by using a spherical 4π underwater quantum sensor attached to a data logger (LI-192 and LI-1500; LI-COR Biosciences, Lincoln, NE, USA), and HOBO MX2202 data loggers calibrated against 2π quantum sensor LI-190R (LI-COR Biosciences).

Experimental setup and design. After a 4 d acclimation period, *Macrocystis pyrifera* juveniles were transplanted to 10-L plastic transparent containers (nine juveniles per container) filled with filtered (1 μm) UV-irradiated seawater and aerated. Each container was considered as an experimental unit (EU), and all the EUs were placed within large incubators (VWR, model 2015 2015-2), which allowed for the control of light and temperature. Within the incubators, temperature was adjusted to 16 and 24°C, corresponding to the averaged field values at the collection site and maximum values recorded during MHWs, respectively. A control light treatment was also adjusted at 180 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}, photoperiod 12:12 h light:dark; this light corresponded to the averaged maximum irradiances measured at the collection site at midday (from 90 to 300 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}), and can be saturating for the photosynthesis of juvenile sporophytes (Umanzor et al. 2019). Other juveniles were kept in almost darkness (5–10 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) by covering the EUs with black plastic mesh. This low light condition, which is below compensation irradiance for photosynthesis of juveniles (Umanzor et al. 2019), was selected following Dean and Jacobsen (1984) and Cabello-Pasini et al. (2002) who reported light values close to zero (0–0.5 mol

photons · m^{-2} · d^{-1}) near giant kelp forests in northern Baja California and southern California during prolonged periods (weeks) of phytoplanktonic blooms and storms. Four EUs were used for each treatment ($N = 4$): 16L, 24L, 16D, 24D (L = light treatment, D = darkness treatment). To adjust the simulated MHW, temperature was gradually increased by 2°C per day, which is within the increment rates measured during these events in the northern Pacific of Baja California (Arafeh-Dalmau et al. 2019). Seawater was replaced every day in each container and water quality was checked by using a submersible multiparameter probe (YSI Pro Plus, USA); nitrate (1–2 μM), salinity (33.5), and pH (7.9–8.1) were maintained almost constant during the experiment. Juveniles were kept under the experimental treatments for 7 d.

Physiological descriptors. All the physiological descriptors were measured for two juveniles per EU (i.e., eight plants), and values per container were averaged to obtain the true experimental replication ($n = 4$). Reference field values of physiological traits were also measured in juveniles of *Macrocystis pyrifera* ($n = 6$) prior to acclimation (Table 1).

Chlorophyll-a fluorescence. Chlorophyll-*a* fluorescence emission of PSII was measured using a Diving-PAM portable fluorometer (Walz, Germany) following Schreiber (2004) and Larkum et al. (2006). In order to standardize measurements among juveniles, measurements were performed in the middle section of the blade, where maximum values of maximum quantum yield, F_v/F_m, were found in previous trials. The blade surface was carefully cleaned of epiphytes and blade tissue was held in the fluorometer DCL-8 leaf clip holder to assure a constant distance between the tissue and the fluorometer fiber optics. Rapid Light Curves, RLCs, were obtained from juveniles kept in darkness overnight. The clipped segment was exposed to nine increasing actinic light intensities during 30 s, ranging from 4 to 300 μmol photons · m^{-2} · s^{-1}. Saturating pulses (~5,000 μmol photons · m^{-2} · s^{-1}, 0.8 s) were applied at the beginning of the RLC and after each actinic light intensity. Values of F_v/F_m corresponded to the first quantum yield after the first saturating pulse. Electron Transport Rate, ETR, Effective Quantum Yields, Φ_{PSII}, and Non-Photochemical Quenching, NPQ, were also calculated. Absolute ETR was calculated as

$$ETR = \Phi_{PSII} \cdot Ii \cdot A \cdot 0.5 \quad (1)$$

where Ii is each actinic light intensity, A is the blade absorptance (see below), and 0.5 is a correction factor of 50:50 percentage distribution of absorbed photons between the two photosystems (Schreiber 2004, Beer et al. 2014). Alpha (α) was calculated as the initial linear slope of the ETR curve (Saroussi and Beer 2007). Values of NPQ were calculated using the equation

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

where F_m is the maximum fluorescence and $F_{m'}$ the fluorescence measured under each actinic light intensity.

Blade bio-optical properties. Blade spectral absorption in the PAR range (400–700 nm) was determined by using a Taylor-type integrating sphere (Li-Cor 1800-12, USA) attached via fiber optic cable to a portable spectroradiometer (Fieldspec, ASD, Boulder, CO, USA). A blade section of ~2 cm was placed between two glass slides then attached to the sample holder of the integrating sphere, covering the entire light path. The spectroradiometer recorded Reflectance (R) and Transmittance (T) at 1 nm intervals, by using barium sulfate as the reference material. Absorptance was determined as 1-T-R following Krause and Weis (1991). Absorption not related to pigments was corrected by subtracting the averaged absorbance between 725 and 750 nm.

Pigment content. Approximately 0.02 g of fresh tissue was ground twice with 0.5 mL of 1% aqueous magnesium carbonate, 4 mL of acetone, and 2 mL distilled water; volume was completed with acetone up to 10 mL. Homogenates were kept in the dark at −18°C for 24 h and centrifuged for 5 min at 3,000 rpm. Supernatants were collected in a glass cuvette (1 cm length) and the concentration of chlorophyll *a*, *c* and total carotenoids was determined by spectrophotometry using the equations described by Parsons et al. (1984) and Seely et al. (1972).

Total soluble carbohydrates. Soluble carbohydrates were measured using the colorimetric phenol-sulfuric acid method using glucose as standard (Dubois et al. 1956). Blade tissue was dried, ground, and then digested with 2 mL of 0.2 M HCl at 60°C during 3 h, and centrifuged for 5 min at 1,000*g*. A mixture of 0.025 mL of supernatant, 1 mL of distilled water, and 0.25 mL of 3% phenol was cooled in an ice bath; then, 2.5 mL of concentrated sulfuric acid was added directly into the solution and mixed vigorously. After 30 min, absorbance was read at 490 nm.

Total nitrogen content. Total nitrogen was quantified by elemental analysis. Blade surfaces were rinsed with distilled water and dried at 60°C for 72 h. Samples were then ground into fine powder and encapsulated in tins. Analyses were carried out at UC Davis Stable Isotope Facility using an elemental analyzer (EA) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS).

Nitrate uptake rates. At the end of the experimental period, juvenile sporophytes were incubated separately for 30 min with 15 μM of labeled nitrate ($K^{15}NO_3$ at. % = 99, Cambridge Isotope Laboratories) dissolved in artificial seawater with low (<1 μM) nitrate concentration (Instant Ocean). Incubations were carried out in 1-L Plexiglas transparent chambers, under the experimental conditions of each treatment. Seawater was mixed continuously to reduce the blade boundary layer, and to homogenize the tracer during the incubation. Following previous trials, a plant biomass per seawater volume of about 0.7 g DW. L^{-1} was selected to avoid substantial changes in seawater nitrate concentration, as well as carbon limitation. At the end of incubations, the entire plant was removed from the chamber, rinsed with deionized water to remove the remains of the tracer adsorbed to the tissue surface, and dried at 60°C until constant weight. Samples were ground to a fine powder for isotope analysis. Isotopic analyses were carried out at UC Davis Stable Isotope Facility using an EA interfaced to a continuous flow IRMS. Nitrate uptake

TABLE 1. Field reference values of physiological descriptors measured in *Macrocystis pyrifera* juvenile sporophytes.

	Mean (SE)	Min–max
Photochemistry		
F_v/F_m	0.763 (0.002)	0.757–0.769
ETRμmol photons · m^{-2} · s^{-1}	7.5 (0.2)	7.0–8.2
Φ_{PSII}	0.150 (0.002)	0.140–0.163
NPQ	0.067 (0.004)	0.053–0.075
α	0.166 (0.003)	0.155-0.176
Bio-optical properties		
$A_{400\text{-}700}$	0.629 (0.017)	0.577–0.667
A_{680}	0.779 (0.003)	0.768–0.785
Pigmentsμg · g FW^{-1}		
Chl *a*	766 (22)	722–789
Chl *c*	139 (18)	106–168
Carotenoids	346 (41)	272–414
Soluble NSCs % DW	3.55 (0.18)	3.13–3.87
Nitrogen % DW	1.85 (0.09)	1.73–2.03

rates (V, expressed as μmol N1 g DW1 $1h^{-1}$; DW = dry weight) were calculated as:

$$V = [(^{15}N_{exp} -^{1\ 5}N_{back}) \times Nc]/(M_N \times t) \quad (3)$$

where the difference ($^{15}N_{exp}$-$^{15}N_{back}$, at. %) is the ^{15}N enrichment relative to natural ^{15}N sporophyte levels (i.e., background), Nc is the nitrogen content (g N · g DW^{-1}), M_N is the molar mass of the labeled nitrogen (15 g · mol^{-1}), and t is the incubation period.

Lipid peroxidation. Lipid peroxidation was measured following the thiobarbituric acid reactive substances (TBARS) assay described by Hodges et al. (1999) and Correia et al. (2006). Ultrafrozen blade tissues were ground in liquid nitrogen using a mechanical grinder, and homogenized (dilution 1:10) in trichloroacetic acid (TCA, 20%). Tissue homogenates were then centrifuged for 10 min (3,000*g*, 4°C) and supernatants were mixed with a solution of TCA (20%) and thiobarbituric acid (0.5% v/v). These solutions were heated at 90°C for 30 min, and then centrifuged again (10,000*g*) for 10 min. The supernatants were extracted and their absorbances (440, 532, and 600 nm) were determined spectrophotometrically. Lipid peroxidation was expressed as equivalents of malonil-dialdehyde (Eq MDA; molar extinction coefficient 155 · mM^{-1} · cm^{-1}) using the equations in Hodges et al. (1999).

Total phenolic content and antioxidant capacity. Dried and ground blade tissue (0.03 g DW) was extracted in 0.75 mL 80% methanol in darkness for 24 h, and the extract was then centrifuged at 10 rpm for 10 min. The phenolic compounds and antioxidant capacity were quantified in the methanolic supernatant. The phenolic compounds were measured with a modification of the Folin–Ciocalteu assay using gallic acid as standard (Singleton and Rossi 1965). A volume of 0.025 mL of methanolic extract was diluted in 1 mL of distilled water. Then, 0.1 mL of Folin–Ciocalteu reagent and 0.3 mL of distilled water saturated in $NaCO_3$ were added. The mixture was homogenized, heated (40°C for 3 min), and the absorbance determined at 765 nm. The radical scavenging activity of the same methanolic extracts was also determined (Farvin and Jacobsen 2013); the reaction mixture was prepared with 0.1 mL of diluted extract (1:4 with 80% methanol) and 1 mL of DPPH 30 μM dissolved in 90% methanol. Absorbance at 517 nm was measured after 30 min of DPPH addition. The total antioxidant capacity of algal extract was expressed as ascorbic acid equivalents.

Statistical analysis. Multivariate analyses were performed with the normalized data of all biological variables, and a ranked triangular similarity matrix was constructed using Euclidean distances. Then, a two-way PERMANOVA crossed design was performed (9999 permutations) to test the factors of temperature, light, and their interactive effects on the physiological status of juvenile sporophytes. Significant differences in the pairwise posteriori comparisons were checked using Monte Carlo P-values, P (MC), due to the restricted number of possible permutations. SIMPER analysis was applied to explore similarities among treatments (average distance). These statistical procedures were implemented using PRIMER 6 & PERMANOVA+ v.1.0.2 software package (Anderson et al. 2008). Principal components analysis (PCA) was performed using PAST software (Hammer et al. 2001) to display multivariate patterns of treatments, and to explore their relationship with the different biological descriptors.

A two-way analysis of variance (ANOVA) was used to test the effect of temperature and light on each response variable. A post hoc Student Newman–Keuls (SNK; Zar 1984) was performed when significant differences were found ($P < 0.05$). Prior to the analysis, Shapiro–Wilk and Levene tests were used to check the assumptions of normality and homocedasticity, and data were transformed when necessary. A non-parametric Kruskal–Wallis test was applied if assumptions were not met. All analyses and graphs were done using the statistical package STATISTICA (StatSoft INC, version 8.0).

RESULTS

The initial physiological status was similar in all the studied plants (Table 1), therefore, physiological changes observed at the end of the experimental period were due to the treatment conditions.

The two-way PERMANOVA indicated that both temperature and light had significant effects on physiological traits of kelp juveniles after the 7 d treatment, and that temperature effects differed between light or dark conditions (i.e., significant interaction Temp. × Light; Table 2). The two-dimensional PCA plot (Fig. 1; Table S1 in the Supporting Information) showed two clear eigenvectors: the component 2, which explained the highest percentage of variance (45.4%) and mainly represented differences due to the light treatments; and the second component 1, which explained the variance of 17.4%, which separates both temperature treatments. The component 2 was highly and positively correlated (correlation coefficient ~0.8) with ETR_{max}, effective quantum yield, NPQ, α, F_v/F_m, and pigment (Chl *a*, Chl *c*, and carotenoids); on the contrary, component 2 was negatively correlated with nitrate uptake rates (−0.8) and soluble carbohydrates content (−0.7), which diminished in juvenile sporophytes subjected to light deprivation. Component 1 was positively correlated (0.85) with absorptance values ($A_{400\text{-}700}$ and A_{680}). SIMPER analysis (Table S2 in the Supporting Information) also indicated that light treatments (16D, 24D vs. 16L, 24L) had higher dissimilarity (average distance = 42.9) and were more separately in the biplot than temperature treatments (16D, 16L vs. 24L, 24D; average distance = 24.3). Moreover, the change in temperature promoted more pronounced changes on the biological traits of *Macrocystis pyrifera* under light limitation (average distance = 28.5, 16D vs. 24D) than light conditions (16L vs. 24L, average distance = 20.5).

Electron transport rate (ETR), Φ_{PSII}, and α values were 2- to 3-fold greater in juveniles exposed to the 16D treatment than control plants (16L; Fig. 2, A, C, and D). Also, these values corresponded to the maximum values measured among treatments. Juveniles at 24L and 24D also showed increased ETR (two-way ANOVA, $F_{1,12} = 9.78$, $P = 0.0087$) and Φ_{PSII} (two-way ANOVA, $F_{1,12} = 3.5$, $P = 0.08$) with respect to 16L sporophytes, while higher maximum values were found at 16D. Non-photochemical quenching (NPQ) significantly increased with the increasing temperature in dark conditions (two-way ANOVA, $F_{1,12} = 5.63$, $P = 0.035$), 2-fold higher at 24D

TABLE 2. Statistical two-way PERMANOVA results showing the interactive effects of temperature (16 and 24°C) and light treatments (L: saturating irradiance, D: light below compensation irradiance) on physiological variables of *Macrocystis pyrifera*. Bold numbers indicate significant differences.

Main test	SS	MS	Pseudo-F	*P* (perm)
Temp.	22.489	22.489	2.9159	**0.0161**
Light	123.26	123.26	15.983	**0.0001**
Temp. × Light	31.698	31.698	4.11	**0.0007**
Pairwise	(L) Light		(D) Dark	
	t	*P* (MC)	*t*	*P* (MC)
16 × 24	1.7677	**0.0341**	1.9535	**0.03**
Pairwise	16°C		24°C	
	t	*P* (MC)	*t*	*P* (MC)
L × D	3.2902	**0.0023**	3.0442	**0.0024**

compared to 16L (Fig. 2E). Values of F_v/F_m were slightly higher (but significant) in 16D plants with respect to control (two-way ANOVA, $F_{1,12} = 76.74$, $P < 0.001$), while F_v/F_m was reduced by 17% in juveniles exposed to the 24L treatment (Fig. 2E). In general, all photochemical descriptors showed significant effects caused independently by light and temperature.

Chlorophyll *a* (two-way ANOVA, $F_{1,12} = 61.79$, $P < 0.001$) and total carotenoids (Kruskal–Wallis ANOVA, $H_{3,16} = 11.67$; $P = 0.087$) increased 2-fold in juveniles exposed to darkness relative to the controls (Fig. 3, A and E), regardless of the temperature level. A slight (but significant) increase in Chl *c* was detected in plants at 24D with respect to the control treatment, and it was significantly reduced in 24L plants (Fig. 3C; two-way ANOVA, $F_{1,12} = 29.79$, $P < 0.001$). Blade absorptance ($A_{400\text{-}700}$, A_{680}; Fig. 3, B, D, and F) increased by ~10% in juveniles at 16D compared to the other treatments, including the control; however, these differences were only near significant for A_{680} (two-way ANOVA, $F_{1,12} = 4.91$, $P = 0.057$).

Light condition (regardless of temperature) had significant effects on soluble carbohydrates, nitrogen concentration, and nitrate uptake rates (Figs. 4 and 5). Soluble carbohydrates were about 40% lower in juveniles growing under dark conditions (16D, 24D; Fig. 4A; two-way ANOVA, $F_{1,12} = 11.76$, $P = 0.004$), with the opposite pattern found for N content (Fig. 4B; two-way ANOVA, $F_{1,12} = 7.24$, $P = 0.019$). Similar to carbohydrate concentration, nitrate uptake rates measured in juveniles from 16D and 24D were up to 7.7 times lower than in

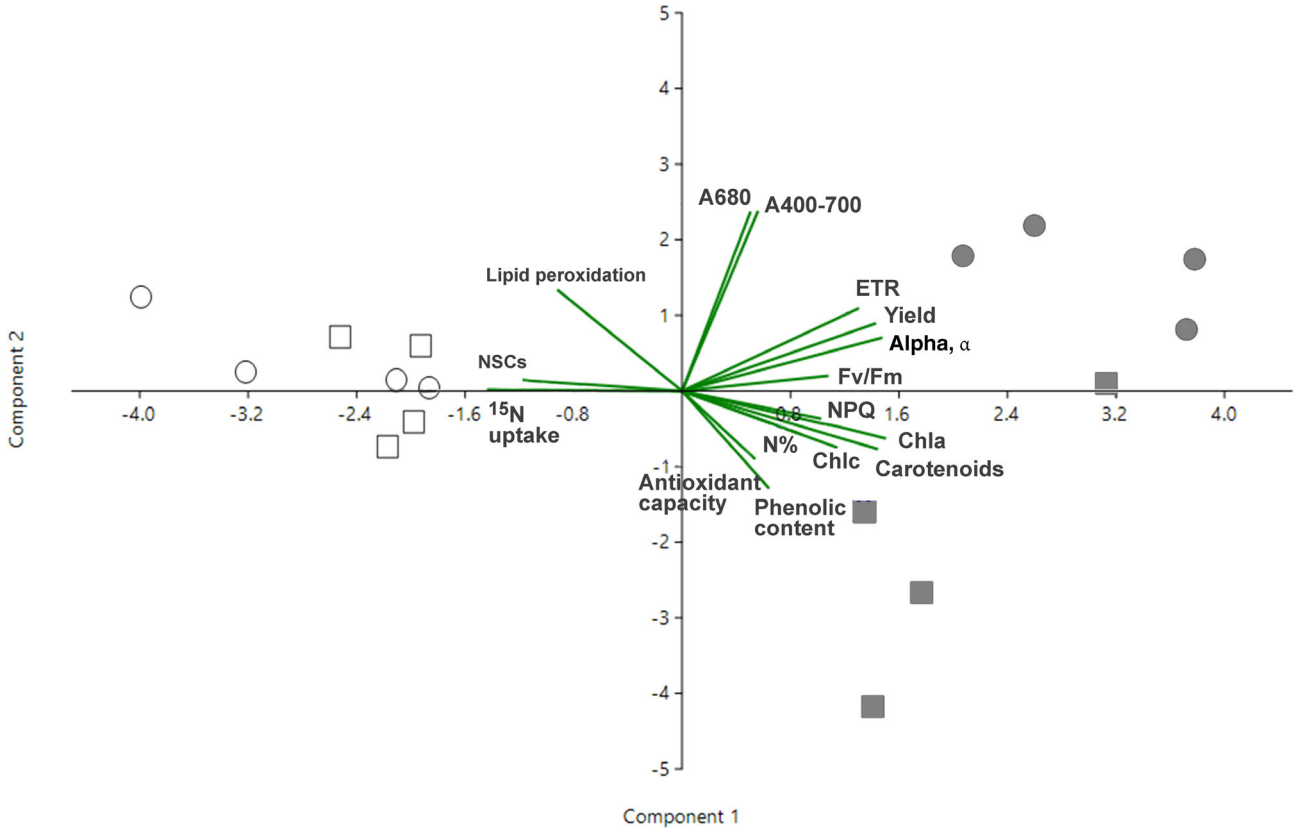

FIG. 1. Ordination diagram of principal component analysis (PCA) performed with physiological variables measured in *Macrocystis pyrifera* juveniles after the exposition to the experimental treatments of temperature (16°C, circles; 24°C, squares) and light (saturating irradiance—open symbols; below compensation irradiance—filled symbols).

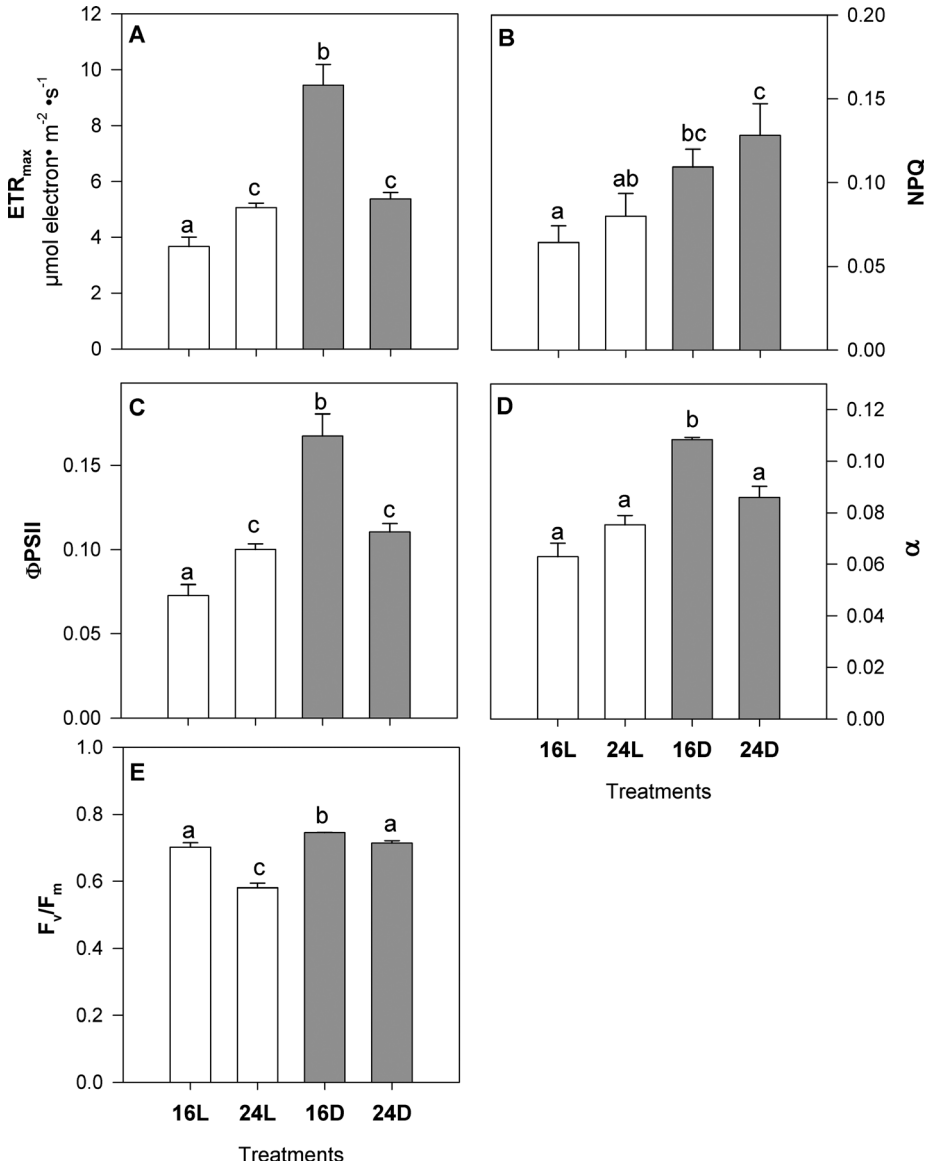

FIG. 2. Photochemical descriptors (mean ± SE, N = 4) measured in *Macrocystis pyrifera* juveniles after the exposition to the experimental treatments of temperature (16 and 24°C) and light (L: saturating irradiance, white bars; D: light below compensation irradiance, gray bars). (A) ETR = Electron transport rate; (B) NPQ = Non-photochemical quenching; (C) Φ_{PSII} = Effective quantum yield; (D) α = photosynthetic efficiency; (E) F_v/F_m = Maximum quantum yield. Different letters denote significant differences between treatments.

illuminated juveniles (Fig. 5; two-way ANOVA, $F_{1,12} = 73.03$, $P < 0.001$).

Changes in total phenolic content, antioxidant capacity, and lipid peroxidation were not statistically significant among treatments (Table 3); uniquely the effect of light was close to significance (two-way ANOVA, $F_{1,12} = 3.82$, $P = 0.074$) in the case of lipid peroxidation.

The two-way ANOVA (Table 4) revealed that effects of temperature significantly differed between light and dark treatments for all photochemical descriptors, except for NPQ.

DISCUSSION

Giant kelp populations can be negatively impacted by thermal stress and/or its associated environmental factors, for example, nutrient limitation (North 1987). Both seasonal-dependent temperature increments and thermal anomalies associated with ENSO and MHWs can cause deterioration and loss of populations (Edwards and Estes 2006, Graham et al. 2007). Available knowledge about the effects of thermal stress, in combination or not with other factors, is typically restricted to adult sporophytes of *Macrocystis pyrifera*, while little

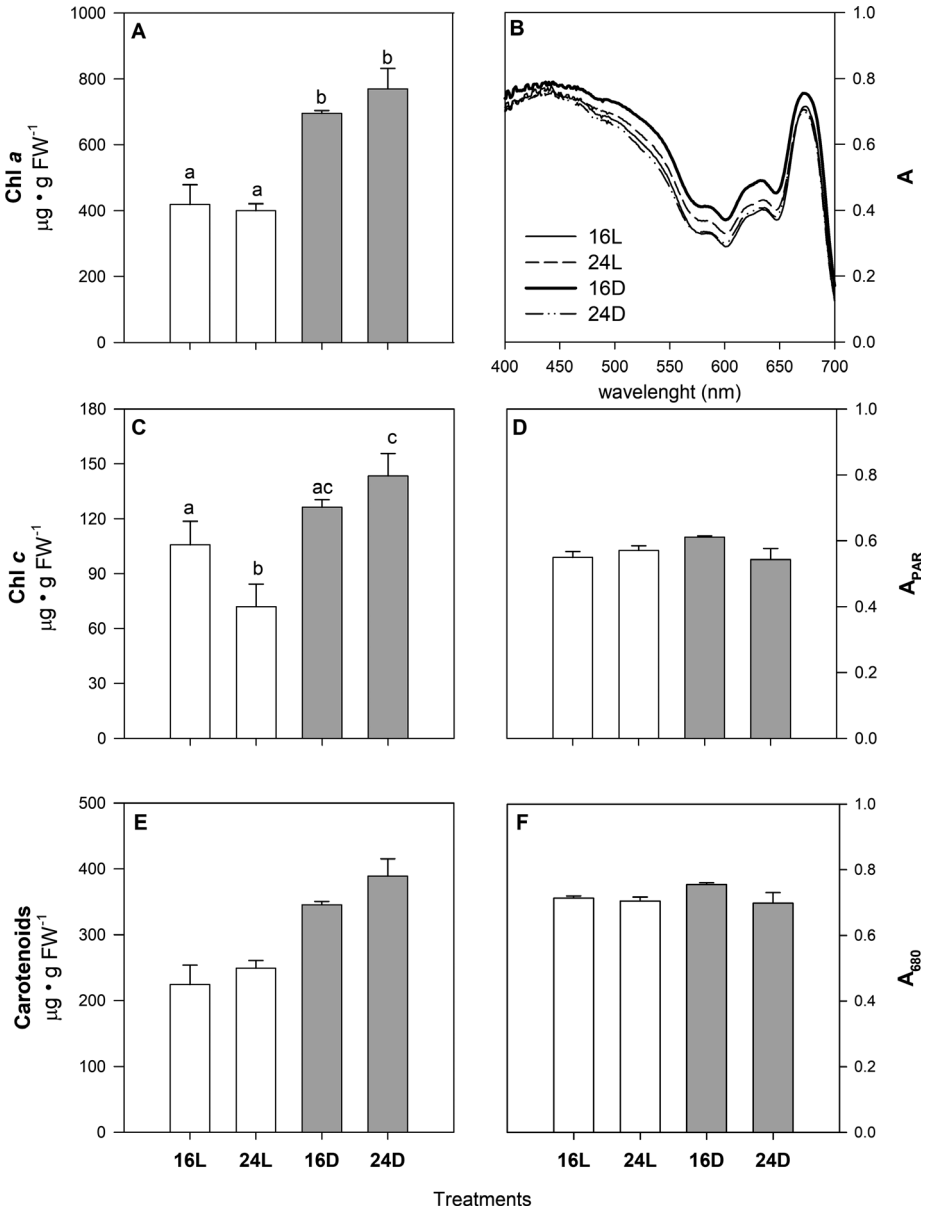

FIG. 3. Pigment content and bio-optical properties measured in *Macrocystis pyrifera* juveniles (mean ± SE, N = 4) after the exposition to the experimental treatments of temperature (16 and 24°C) and light (L: saturating irradiance, white bars; D: light below compensation irradiance, gray bars). (A) chlorophyll *a*, (B) light absorptance spectra in the visible PAR range, (C) chlorophyll *c*, (D) integrated absorptance in the PAR range, (E) carotenoids, (F) absorptance at 680 nm. Different letters denote significant differences between treatments.

is known about the resistance/vulnerability of juvenile stages. This study represents one of the few works (see Mabin et al. 2019) which assess the physiological plasticity of juvenile sporophytes. Juvenile stages, including microscopic phases, can be decisive for the population resilience after extreme events causing loss of kelp forest, such as MHWs (Cavanaugh et al. 2019).

This study examined the responses of juvenile sporophytes to a simulated MHW in the laboratory, similar in duration and intensity (7 d, 24°C) to those that occur in the Mexican North Pacific during summer and early-autumn (Hernández de la Torre et al. 2004, Leising et al. 2015, Arafeh-Dalmau et al. 2019). These responses were compared to plants maintained under control temperature (16°C). Also, some sporophytes were also subjected to light values below compensation irradiance for photosynthesis (5–10 µmol photons · m^{-2} · s^{-1}) to explore potential interactive effects between the experimental factors. Generally, our results indicated that both high temperature and light deprivation caused significant biological changes in juvenile sporophytes (Fig. 1, Table 2). A notable reduction in F_v/F_m (until values below 0.6) was detected in juvenile sporophytes when exposed to a high temperature and control saturating light (180 µmol photons · m^{-2} · s^{-1}; i.e., 24L treatment; Fig. 2E), but no other evidence of physiological damage associated with thermal stress was found. Although juvenile sporophytes maintained photosynthetic functionality under light deprivation, regardless the temperature level, this imposed experimental condition induced stronger

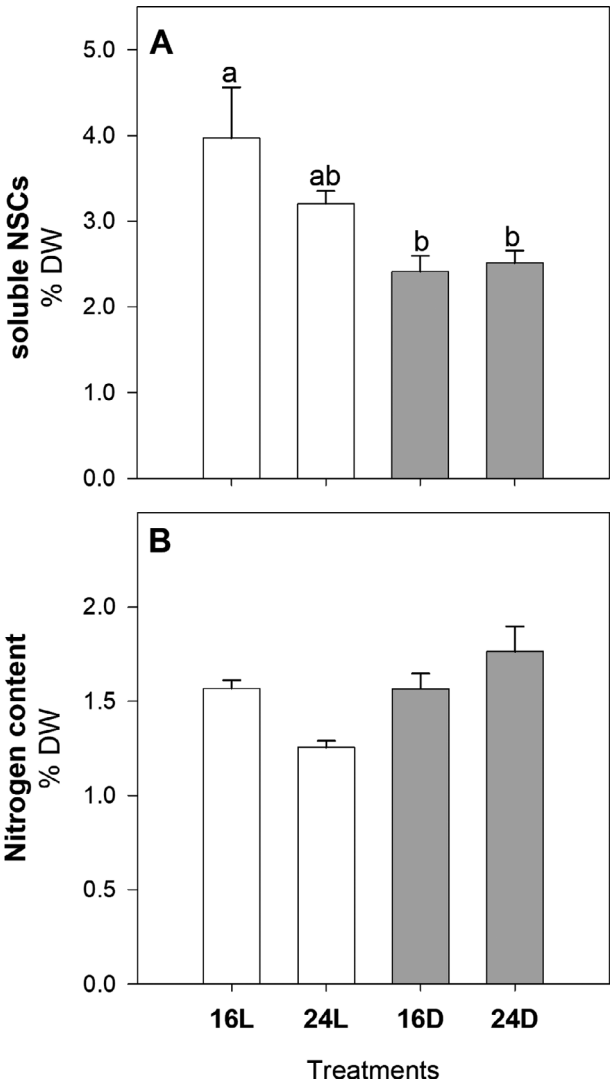

FIG. 4. (A) Total non-structural carbohydrates and (B) total nitrogen content measured in *Macrocystis pyrifera* juveniles (mean ± SE, N = 4) after the exposition to the experimental treatments of temperature (16 and 24°C) and light (L: saturating irradiance —white bars, D: light below compensation irradiance—gray bars). Different letters denote significant differences between treatments.

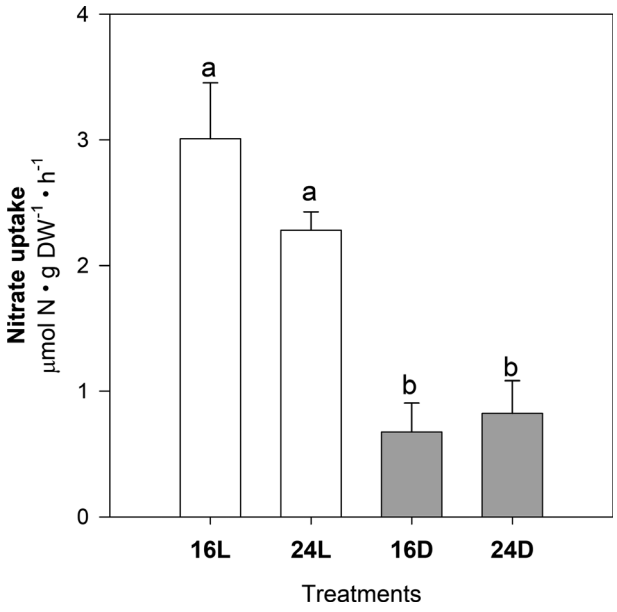

FIG. 5. Nitrate uptake rate measured in *Macrocystis pyrifera* juveniles (mean ± SE, N = 3) after the exposition to the experimental treatments of temperature (16 and 24°C) and light (L: saturating irradiance, white bars; D: light below compensation irradiance, gray bars). Different letters denote significant differences between treatments.

TABLE 3. Total phenolic compounds, total antioxidant capacity, and lipid peroxidation measured in *Macrocystis pyrifera* juveniles (mean [SE], $N = 4$) after the exposure to the experimental treatments of temperature (16 and 24°C) and light (L: saturating irradiance; D: light below compensation irradiance).

	Experimental treatments			
	16L	24L	16D	24D
Total phenolic content (Eq. mg GA · g DW^{-1})	3.215 (0.936)	5.035 (0.441)	4.232 (0.783)	5.136 (0.832)
Antioxidant capacity (Eq. mg AA · g DW^{-1})	0.778 (0.058)	0.878 (0.065)	0.946 (0.112)	0.909 (0.078)
Lipid peroxidation (Eq. nmol MDA · g FW^{-1})	21.22 (1.20)	17.48 (2.92)	16.99 (1.25)	16.35 (2.81)

physiological changes than thermal stress, including photoacclimation responses and nutrient physiology alteration (Fig. 1, Table 2).

Maximum quantum yield (F_v/F_m) decreased drastically in illuminated juveniles exposed to heat stress. Recently, Mabin et al. (2019) documented a similar reduction of F_v/F_m when juvenile sporophytes of *Macrocystis pyrifera* were exposed to experimental temperatures of 22°C, although the extent depended on the combination of temperature with light and nutrient (nitrate) availability. In addition, thermal stress has been correlated with decreasing F_v/F_m in other seaweeds, likely associated with the enhancement of energy dissipation mechanisms (e.g., NPQ) or the impairment of PSII and related downstream metabolic processes (e.g., carbon fixation; Davison and Davison 1987, Sharkey 2005, Fernández-Marín et al. 2011, Pereira et al. 2015, Mabin et al. 2019). In adult *M. pyrifera* sporophytes, quantum efficiency was also shown to be negatively affected by light overexposure in surface blades (Cabello-Pasini et al. 2000, Colombo-Pallotta et al. 2006). Super-optimal temperature also appears to enhance photoinhibition in *Saccharina latissima* when exposed to saturating irradiances (Bruhn and Gerard 1996). This effect might be responsible for the decline in populations of this species along the south coast of Norway during high temperatures (Andersen et al. 2013). In our study, despite the

TABLE 4. Two-way ANOVA analysis performed to test the interactive effects of temperature (*T*; 16 and 24°C) and light treatments (L; saturating irradiance and light below compensation irradiance) on physiological variables of *Macrocystis pyrifera*. Bold numbers indicate significant differences. Cases in which a non-parametric Kruskal–Wallis was used (i.e., total carotenoids and photosynthetic efficiency) are shown as footnotes (a, b).

	Temperature				Light			T × L		
	df	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*
Photochemistry										
F_v/F_m	1	0.02	57.19	**<0.001**	0.03	76.74	**<0.001**	0.01	19.48	**<0.001**
ETR	1	7.21	9.79	**0.009**	36.92	50.15	**<0.001**	29.92	40.64	**<0.001**
Φ_{PSII}	1	0.0008	3.50	0.086	0.01	44.59	**<0.001**	0.01	28.74	**<0.001**
NPQ	1	0.0001	0.0001	0.998	0.01	5.64	**0.035**	0.00	0.31	0.586
Bio-optics										
A_{PAR}	1	0.002	2.25	0.172	0.0004	0.39	0.550	0.005	4.38	0.070
A_{680}	1	0.004	4.91	0.051	0.0004	0.43	0.53	0.001	1.11	0.32
Pigments										
Chl *a*	1	2947.46	0.44	0.522	417958	61.79	**<0.001**	8535.64	1.26	0.283
Chl *c*	1	265.39	0.95	0.350	8366.58	29.79	**<0.001**	2642.52	9.41	**0.010**
Nutrient content										
Soluble NSCs	1	0.44	1.03	0.331	5.05	11.76	**0.005**	0.75	1.75	0.211
Nitrogen	1	0.01	0.11	0.743	0.50	7.25	**0.020**	0.09	1.36	0.266
Nitrate uptake	1	0.57	1.64	0.225	25.40	73.03	**0.0001**	1.04	2.99	0.109
Lipid peroxidation	1	1176.52	1.13	0.310	3992.76	3.82	0.074	458.80	0.44	0.520
Total phenols	1	7.42	3.12	0.103	1.25	0.53	0.482	0.84	0.35	0.564
Antioxidant capacity	1	0.00	0.15	0.704	0.04	1.51	0.242	0.02	0.71	0.415

[a]Total carotenoids ($df = 3$; $H = 11.67$; $P = 0.087$).
[b]Photosynthetic efficiency-Alpha ($df = 3$; $H = 12.79$; $P = 0.005$).

reduction in F_v/F_m, juveniles at 24L showed similar or higher values of ETR_{max}, photosynthetic efficiency-α, and Φ_{PSII} than juveniles growing at optimum conditions of temperature and light, 16L. Although these responses seem contradictory, these might be explained by the activation of alternative electron sinks which, under diminished photosynthetic capacities, help to dissipate excess photons and avoid over-reduction of electron transport carriers (Niyogi 2000). One of these mechanisms is the thermal dissipation of energy operated by the xanthophylls cycle, expressed as an increase in NPQ. This photoprotective mechanism has been demonstrated in adults and juveniles of *M. pyrifera* mainly in the framework of light acclimation (Colombo-Pallotta et al. 2006, García-Mendoza and Colombo-Pallotta 2007, Umanzor et al. 2019). Because our results did not show a significant increase in NPQ in 24L juveniles, other mechanisms could likely act as safety valves for photosynthesis (e.g., photoreduction of oxygen by PS I or the water–water cycle; Niyogi 2000). Contrary to that found in 24L, F_v/F_m was not reduced in juveniles maintained in darkness and higher temperatures (i.e., 24D). Also, values of ETR_{max}, quantum yield, and α were greater in 24D plants than those at optimum conditions (16L). This indicated that light can critically contribute to the damage of the photosynthetic apparatus in combination with temperature. Because of the absence of photonic energy in 24D, photodamage cannot occur and costly photo repair processes (e.g., D1 protein replacement) are unnecessary (Demmig-Adams and Adams 2006, Raven 2011). Previous works indicated that factors associated with higher temperatures (such as nutrient depletion, pathogenic biota, or epibionts), instead of thermal stress itself, can be responsible for seaweed deterioration. Moreover, *M. pyrifera* can maintain or even enhance its physiological performance (e.g., photosynthesis, nutrient uptake) when exposed solely to increasing temperatures (Clendenning 1971, Dean and Jacobsen 1984, Arnold and Manley 1985, North 1994). The maximum values of ETR_{max}, α, and Φ_{PSII} corresponded to juveniles exposed to 16°C and darkness, and indicated the optimum status of their photosynthetic machinery. Particularly, the increase in photosynthetic efficiency, α, is a typical photoacclimative response which helps to cope with light scarcity in *M. pyrifera* and other seaweeds (Fairhead and Chesire 2004, Sampath-Wiley et al. 2008). Higher α can reflect an increase in the number and/or size of photosynthetic units (Colombo-Pallotta et al. 2006, Falkowski and Raven 2007). Our results are also consistent with the general increase in pigment concentration (Chl *a*, Chl *c*, and carotenoids) in juveniles kept in darkness, regardless of temperature, as a widely recognized response within the photoacclimation strategies of this and other seaweeds (Schiel and Foster 2015, Mabin et al. 2019). This increase in blade pigmentation was uniquely reflected in a slight rise in leaf absorptance in 16D juveniles, but not in 24D, likely due to a pigment packaging phenomenon (Enríquez et al. 1994). We did not find evidence of pigment degradation in juveniles at 24L, which reinforced the idea that tissue bleaching can be a consequence of environmental factors related to higher temperatures (e.g., nutrient depletion) than

from thermal stress itself (Gerard 1984, North 1994, Schiel and Foster 2015).

Values of nitrogen and soluble non-structural carbohydrates content were similar to the levels reported previously for juvenile and adults of *Macrocystis pyrifera* (North and Zimmerman 1984, Davison and Davison 1987, Kopczak 1994). Also, experimental sporophytes showed values of N-content close 1.5% DW (or even higher) than the assumed critical level of 1% DW known to represent N-limitation to support plant growth (Gerard 1982, Zimmerman and Kremer 1984). Internal concentration of nitrogen (free amino acids, proteins, internal nitrate) and soluble sugars (e.g., mannitol) can vary among *M. pyrifera* tissues and are strongly influenced by seasonal-dependent factors, such as temperature, light, and nutrient availability (Wheeler and Srivastava 1984, North 1994). In our study, carbohydrate content decayed in dark treatments (16D, 24D). Under light limitation, the reduction of sugars is caused by metabolic carbon imbalance reflecting both photoassimilate synthesis reduction and/or their consumption by respiratory processes (Hurd et al. 2014). An interesting example of a similar (but much more extreme) condition can be observed in Arctic kelps, which maintain growth during the Arctic winter (i.e., 6 months of darkness) at the expense of the use of carbohydrates previously accumulated during the warmer season when sea ice breaks up (Young et al. 2007). Nitrogen content slightly increased in juveniles kept under light deprivation, following the opposite pattern of sugars. This inverse correlation has been documented in *M. pyrifera*, primarily related to nitrate availability and the use of internal carbon for its assimilation (Zimmerman and Kremer 1986, North 1994).

Nitrate uptake rates measured in experimental juveniles (1–3 μmol N · g DW^{-1} · h^{-1}) were within the range measured for adult blades of *Macrocystis pyrifera*, both in situ and in the laboratory (Gerard 1982, Druehl 1984, Kopczak 1994). Kopczak (1994) showed that rates can be near saturation (i.e., near to V_{max}) at the concentrations used in this study (15 μM), which is similar to the nitrate measured in kelp stands during upwelling (Reed et al. 2011). Nitrate uptake rates were strongly reduced in juveniles subjected to light-limiting conditions, regardless of temperature, similar to that found for carbohydrate content. There is contrasting literature on the effect of light on nitrate uptake in *M. pyrifera*. For example, Gerard (1982) and Wheeler (1982) reported that light limitation can suppress nitrate uptake, while the opposite trend was reported for this and other seaweeds (Wheeler and Srivastava 1984, Philips and Hurd 2003, Young et al. 2007). Furthermore, Kopczak (1994) did not find any correlation between irradiance and nitrate uptake. In our study, the decrease of nitrate uptake can be linked to the lack of metabolic energy and carbon skeletons required to incorporate and assimilate this nutrient, and the activity and/or synthesis of involved enzymes (e.g., nitrate reductase; Berges 1997, Hurd et al. 2014). Therefore, nutrition of juvenile sporophytes can be comprised in nature under limiting irradiance as nitrate is the nitrogen form most acquired by giant kelp, although urea and ammonium associated with biota excretion and sediment efflux, respectively, can contribute to the plant N budget (Brzezinski et al. 2013, Schiel and Foster 2015). Besides light, the incorporation of nitrate by *M. pyrifera* (and other species) can also be modulated by both the surrounding environment and internal physiological and morphological factors (e.g., tissue type, blade morphology, N-status; North 1994).

Diminished nitrate uptake rates in juveniles under darkness were not reflected in a decrease in tissue N-content. This apparent discrepancy may be explained by the metabolic decoupling between nitrate incorporation and the consumption of internal non-structural N, due to a potential decrease in plant growth. Nitrogen uptake and utilization can be temporally uncoupled due to light availability, as in the strategies developed by Arctic kelps among seasons, or even other seaweeds during diurnal cycles (Wiencke and Amsler 2012). There are other examples of this temporal partitioning between N-acquisition, internal N-metabolic processes, and *Macrocystis pyrifera* growth. This species can maintain growth for days to weeks at the expense of internal non-structural N compounds (including nitrate) accumulated during "luxury consumption" when N concentration is high in the surrounding water (Chapman and Craigie 1977, Schiel and Foster 2015).

Our results indicated that higher temperatures did not trigger stimulation of non-enzymatic antioxidative capacities and oxidative damage (i.e., lipid peroxidation) in juvenile sporophytes. This suggested that the generation of reactive oxygen species (ROS) was restricted by the activation of photoprotective mechanism. For instance, the decrease in F_v/F_m and Chl *c* in 24L sporophytes can reflect a "dynamic photoinhibition" strategy, either to reduce light harvesting or to induce photochemical quench (Franklin and Forster 1998). The production of ROS is typically associated with disruptive environmental stressors, including temperature and light overexposure (Collén and Davison 1999, Bischof and Rautenberger 2012, Cruces et al. 2012). In intertidal seaweeds, for example, exposure to extreme temperatures and light lead to the over-energization (or over-reduction) of the photosynthetic apparatus at the thylakoid level, and the production of ROS by the activation of alternative electron sinks (Niyogi 2000, Bischof and Rautenberger 2012). To avoid oxidative damage, seaweeds are able to activate a variety of antioxidant mechanisms to scavenge ROS species, including antioxidant enzymes (e.g., catalase, superoxide dismutase)

and compounds (e.g., amino acids, carotenoids, phenols such as phlorotannins; Bischof and Rautenberger 2012, Cruces et al. 2012). However, and reinforcing the neutral response of antioxidant response in our study, total phenols did not change in plants subjected to thermal stress.

In conclusion, our results supported that light limitation can be more harmful to juvenile sporophytes of *Macrocystis pyrifera* than high temperatures similar to that experienced by this species in Baja California during MHWs. Although light deprivation can induce some photoacclimation responses, it can lead to severe reduction in plant carbon balance and diminished capacities to acquire nitrate from the surrounding seawater. These effects can obviously comprise growth and survival of juvenile sporophytes, and thus regeneration and recruitment of giant kelp forest. Simultaneously to MHWs, several causes can lead to a reduction of incident light on *M. pyrifera* juveniles, including hydrodynamic forces causing resuspension and turbidity, phytoplanktonic blooms, and the overgrowth of canopy-forming seaweeds. The effects of light deprivation on juvenile stages (up to 20 cm length) by the presence of invasive canopy-forming species are particularly interesting, because some introduced species are spreading prolifically in coastal areas on the Pacific coast of North America, including Baja California. For instance, when the invasive brown algae *Undaria pinnatifida* and *Sargassum* spp. reach their maximum size in spring–summer, they can overshadow *M. pyrifera* juveniles growing at the external edges of the understory (Schiel and Foster 2015, South et al. 2017). The particular interaction between *M. pyrifera* and invasive species is an issue of special concern (Raffo et al. 2009, Schiel and Foster 2015), since warming trends of seawater (tropicalization of temperate systems) can promote the survival and spread of exotic seaweeds in temperate regions (Bartsch et al. 2012). Finally, although juveniles did not exhibit apparent vulnerability under simulated MHWs in this study, it must be highlighted that more specific research is needed to explore the implication of other external (e.g., duration and intensity of heat stress, incidence of fluctuating temperature) and internal (e.g., physiological status, ecotypic/genotypic divergences) limiting factors on their response.

This work was funded by NPTC-PRODEP project granted to JMSG, as well as by the companies PROMAC (Productos Marinos de las Californias) and Blue Evolution under the project "*Ulva* sp cultivation in land-based ponds" to JAZG. A doctoral CONACYT (Consejo Nacional de Ciencia y Tecnología) grant was awarded to MSB. A postdoctoral PRODEP grant was awarded to MDBT. Authors are deeply grateful for all the support and insights provided by the people of Marine Botany Lab at UABC: Laura Karina Rangel-Mendoza, Schery Umanzor, Víctor Macías-Carranza.

Ambrose, R. F. & Nelson, B. V. 1982. Inhibition of giant kelp recruitment by an introduced brown alga. *Bot. Mar.* 25:265–7.

Andersen, G. S., Pedersen, M. F. & Nielsen, S. L. 2013. Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *J. Phycol.* 49:689–700.

Anderson, M. J., Gorley, R. N. & Clarke, K. R. 2008. *Guide to Software and Statistical Methods. PERMANOVA+ for PRIMER.* University of Auckland and PRIMER-E, Plymouth, UK, pp. 15–84.

Andrews, S., Bennett, S. & Wernberg, T. 2014. Reproductive seasonality and early life temperature sensitivity reflect vulnerability of a seaweed undergoing range reduction. *Mar. Ecol. Prog. Ser.* 495:119–29.

Arafeh-Dalmau, N., Montaño-Moctezuma, G., Martinez, J. A., Beas-Luna, R., Schoeman, D. S. & Torres-Moye, G. 2019. Extreme marine heatwaves alter kelp forest community near its equatorward distribution limit. *Front. Mar. Sci.* 6:499.

Arnold, K. E. & Manley, S. L. 1985. Carbon allocation in *Macrocystis pyrifera* (Phaeophyta): intrinsic variability in photosynthesis and respiration. *J. Phycol.* 21:154–67.

Bartsch, I., Wiencke, C. & Laepple, T. 2012. Global seaweed biogeography under a changing climate: the prospected effects of temperature. *In* Wiencke, C. & Bischof, K. [Eds.] *Seaweed Biology. Ecological Studies (Analysis and Synthesis).* Vol 219. Springer, Berlin, Heidelberg, pp. 383–406.

Beer, S., Björk, M. & Beardall, J. 2014. *Photosynthesis in the Marine Environment.* John Wiley & Sons, Hoboken, NJ, USA, 141 pp.

Berges, J. 1997. Miniview: Algal nitrate reductases. *Eur. J. Phycol.* 32:3–8.

Bischof, K. & Rautenberger, R. 2012. Seaweed responses to environmental stress: reactive oxygen and antioxidative strategies. *In* Wiencke, C. & Bischof, K. [Eds.] *Seaweed Biology. Ecological Studies (Analysis and Synthesis).* Vol 219. Springer, Berlin, Heidelberg, pp. 109–32.

Brown, M. B., Edwards, M. S. & Kim, K. Y. 2014. Effects of climate change on the physiology of giant kelp, *Macrocystis pyrifera*, and grazing by purple urchin, *Strongylocentrotus purpuratus*. *Algae* 29:203–15.

Bruhn, J. & Gerard, V. A. 1996. Photoinhibition and recovery of the kelp *Laminaria saccharina* at optimal and superoptimal temperatures. *Mar. Biol.* 125:639–48.

Brzezinski, M. A., Reed, D. C., Harrer, S., Rassweiler, A., Melack, J. M. & Goodridge, B. M. 2013. Multiple sources and forms of nitrogen sustain year-round kelp growth on the inner continental shelf of the Santa Barbara channel. *Oceanography* 26:114–23.

Cabello-Pasini, A., Aguirre-Von-Wobeser, E. & Figueroa, F. L. 2000. Photoinhibition of photosynthesis in *Macrocystis pyrifera* (Phaeophyceae), *Chondrus crispus* (Rhodophyceae) and *Ulva lactuca* (Chlorophyceae) in outdoor culture systems. *J. Photochem. Photobiol. B Biol.* 57:169–78.

Cabello-Pasini, A., Lara-Turrent, C. & Zimmerman, R. C. 2002. Effect of storms on photosynthesis, carbohydrate content and survival of eelgrass populations from a coastal lagoon and the adjacent open ocean. *Aquat. Bot.* 74:149–64.

Cavanaugh, K. C., Reed, D. C., Bell, T. W., Castorani, M. C. N. & Beas-Luna, R. 2019. Spatial variability in the resistance and resilience of giant kelp in southern and Baja California to a multiyear heatwave. *Front. Mar. Sci.* 6:1–14.

Chapman, A. R. O. & Craigie, J. S. 1977. Seasonal growth in *Laminaria longicruris*: Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40:197–205.

Clarke, A. 2003. Costs and consequences of evolutionary temperature adaptation. *Trends Ecol. Evol.* 18:573–81.

Clendenning, K. A. 1971. Organic productivity in kelp areas. *Nova Hedwigia* 32:259–63.

Collén, J. & Davison, I. R. 1999. Production and damage of reactive oxygen in intertidal *Fucus* spp. (Phaeophyceae). *J. Phycol.* 61:54–61.

Colombo-Pallotta, M. F., García-Mendoza, E. & Ladah, L. B. 2006. Photosynthetic performance, light absorption, and pigment composition of *Macrocystis pyrifera* (Laminariales,

Phaeophyceae) blades from different depths. *J. Phycol.* 42: 1225–34.
Correia, M. J., Osório, M. L., Osório, J., Barrote, I., Martins, M. & David, M. M. 2006. Influence of transient shade periods on the effects of drought on photosynthesis, carbohydrate accumulation and lipid peroxidation in sunflower leaves. *Environ. Exp. Bot.* 58:75–84.
Cruces, E., Huovinen, P. & Gomez, I. 2012. Phlorotannin and antioxidant responses upon short-term exposure to UV radiation and elevated temperature in three South Pacific kelps. *Photochem. Photobiol.* 88:58–66.
Davison, I. R. & Davison, J. O. 1987. The effect of growth temperature on enzyme activities in the brown alga *Laminaria saccharina*. *Br. Phycol. J.* 22:77–87.
Davison, I. R. & Pearson, G. A. 1996. Stress tolerance in intertidal seaweeds. *J. Phycol.* 32:197–211.
Dean, T. A. 1985. The temporal and spatial distribution of underwater quantum irradiation in a southern California kelp forest. *Estuar. Coast. Shelf Sci.* 21:835–44.
Dean, T. A. & Jacobsen, F. R. 1984. Growth of juvenile *Macrocystis pyrifera* (Laminariales) in relation to environmental factors. *Mar. Biol.* 83:301–11.
Dean, T. A. & Jacobsen, F. R. 1986. Nutrient-limited growth of juvenile kelp, *Macrocystis pyrifera*, during the 1982–1984 "El Niño" in southern California. *Mar. Biol.* 90:597–601.
Demmig-Adams, B. & Adams, W. W. 2006. Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. *New Phytol.* 172:11–21.
Druehl, L. D. 1984. The integrated productivity of a *Macrocystis integrifolia* plant. *Can. J. Bot.* 62:230–5.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebus, P. A. & Fred, S. 1956. Colorimetric method for the determination of sugars and related substances. *Anal. Chem.* 28:350–6.
Edwards, M. S. & Estes, J. A. 2006. Catastrophe, recovery and range limitation in NE Pacific kelp forests: A large-scale perspective. *Mar. Ecol. Prog. Ser.* 320:79–87.
Enríquez, S., Agustí, S. & Duarte, C. M. 1994. Light absorption by marine macrophytes. *Oecologia* 98:121–9.
Fairhead, V. A. & Chesire, A. C. 2004. Seasonal and depth related variation in the photosynthesis–irradiance response of *Ecklonia radiata* (Phaeophyta, Laminariales) at West Island, South Australia. *Mar. Biol.* 145:415–26.
Falkowski, P. G. & Raven, J. A. 2007. *Aquatic Photosynthesis*, 2nd edn. Princeton University Press, New Jersey, USA, pp. 247.
Farvin, K. S. & Jacobsen, C. 2013. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chem.* 138:1670–81.
Fernández-Marín, B., Míguez, F., Becerril, J. M. & García-Plazaola, J. I. 2011. Activation of violaxanthin cycle in darkness is a common response to different abiotic stresses: A case study in *Pelvetia canaliculata*. *BMC Plant Biol.* 11:181.
Franklin, L. A. & Forster, R. M. 1998. The changing irradiance environment: Consequences for marine macrophyte physiology, productivity and ecology. *Ocean. Lit. Rev.* 3:522.
García-Mendoza, E. & Colombo-Pallotta, M. F. 2007. The giant kelp *Macrocystis pyrifera* presents a different nonphotochemical quenching control than higher plants. *New Phytol.* 173:526–36.
Gerard, V. A. 1982. In situ water motion and nutrient uptake by the giant kelp *Macrocystis pyrifera*. *Mar. Biol.* 69:51–4.
Gerard, V. A. 1984. Physiological effects of El Niño on giant kelp in Southern California. *Marine Biol. Lett.* 5:317–22.
Gordon, H. R. & Cook, P. A. 2013. World abalone supply, markets, and pricing: 2011 Update. *J. Shellfish Res.* 32:5–7.
Graham, M. H. 1996. Effect of high irradiance on recruitment of the giant kelp *Macrocystis* (Phaeophyta) in shallow water. *J. Phycol.* 32:903–6.
Graham, M. H., Vasquez, J. A. & Buschmann, A. H.. 2007. Global ecology of the giant kelp *Macrocystis*: from ecotypes to ecosystems. *In* Gibson, R. N., Atkinson, R. J. A. & Gordon, J. D. [Eds.] *Oceanography and Marine Biology. An Annual Review.* Vol. 45. CRC Press, Boca Raton, USA, pp. 39–88.
Guerrero-Meseguer, L., Marín, A. & Sanz-Lázaro, C. 2017. Future heat waves due to climate change threaten the survival of *P. oceanica* seedlings. *Environ. Pollut.* 230:40–5.
Hammer, Ø., Harper, D. A. & Ryan, P. D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4:9.
Hanelt, D. & Lopez-Figueroa, F. 2012. Physiological and photomorphogenic effects of light on marine macrophytes. *In* Wiencke, C. & Bischof, K. [Eds.] *Seaweeds Biology: Novel Insights Into Ecophysiology, Ecology and Utilization.* Springer, Heilderberg, Germany, pp. 3–23.
Hernández de la Torre, B., Gaxiola-Castro, G. & Nájera-Martínez, S. 2004. Efectos del ENSO en la producción primaria frente a Baja California. *Ciencias Mar.* 30:427–41.
Hodges, D. M., Delong, J. M., Forney, C. F. & Prange, R. K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 1:604–11.
Holbrook, N. J., Scannell, H. A., Sen Gupta, A., Benthuysen, J. A., Feng, M., Oliver, E. C. J. & Alexander, L. V. 2019. A global assessment of marine heatwaves and their drivers. *Nat. Commun.* 10:1–13.
Hu, Z. Z., Kumar, A., Jha, B., Zhu, J. & Huang, B. 2017. Persistence and predictions of the remarkable warm anomaly in the northeastern Pacific Ocean during 2014-16. *J. Clim.* 30:689–702.
Hurd, C. L., Harrison, P. J., Bischof, K. & Lobban, C. S. 2014. *Seaweed Ecology and Physiology.* Cambridge University Press, Cambridge, UK, pp. 202–9.
IPCC. 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, UK.
Jordà, G., Marbà, N. & Duarte, C. M. 2012. Mediterranean seagrass vulnerable to regional climate warming. *Nat. Clim. Chang.* 2:821–4.
Koch, M. S., Schopmeyer, S. A., Kyhn-Hansen, C., Madden, C. J. & Peters, J. S. 2007. Tropical seagrass species tolerance to hypersalinity stress. *Aquat. Bot.* 86:14–24.
Kopczak, C. D. 1994. Variability of nitrate uptake capacity in *Macrocystis pyrifera* (Laminariales, Phaeophyta) with nitrate and light availability. *J. Phycol.* 30:573–80.
Krause, G. H. & Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Annu. Rev. Plant Biol.* 42:313–49.
Kübler, J. E. & Davison, I. R. 1993. High-temperature tolerance of photosynthesis in the red alga Chondrus crispus. *Marine Biology* 117:327–335.
Ladah, L. B. 2003. The shoaling of nutrient-enriched subsurface waters as a mechanism to sustain primary productivity off central Baja California during El Niño winters. *J. Mar. Syst.* 42:145–52.
Ladah, L. B., Filonov, A., Lavín, M. F., Leichter, J. J., Zertuche-González, J. A. & Pérez-Mayorga, D. M. 2012. Cross-shelf transport of sub-thermocline nitrate by the internal tide and rapid (3-6 h) incorporation by an inshore macroalga. *Cont. Shelf Res.* 42:10–9.
Ladah, L. B. & Zertuche-González, J. A. 2007. Survival of microscopic stages of a perennial kelp (*Macrocystis pyrifera*) from the center and the southern extreme of its range in the Northern Hemisphere after exposure to simulated El Niño stress. *Mar. Biol.* 152:677–86.
Ladah, L. B., Zertuche-González, J. A. & Hernandez-Carmona, G. 1999. Giant kelp (*Macrocystis pyrifera*, Phaeophyceae) recruitment near its southern limit in Baja California after mass disappearance during ENSO 1997-1998. *J. Phycol.* 35:1106–12.
Larkum, A. W. D., Drew, E. A. & Ralph, P. J. 2006. Photosynthesis and metabolisms in seagrasses at the cellular level. *In* Larkum, A. W. D., Orth, R. J. & Duarte, C. M. [Eds.] *Seagrasses: Biology, Ecology and Conservation.* Springer, Dordrecht, The Netherlands, pp. 323–45.
Leising, A. W., Schroeder, I. D., Bograd, S. J., Abell, J., Durazo, R., Gaxiola-Castro, G. & Bjorkstedt, E. P. 2015. State of the

California Current 2014–15: Impacts of the warm-water "Blob". *CalCOFI Rep.* 56:31–68.

Los, D. A. & Murata, N. 2004. Membrane fluidity and its roles in the perception of environmental signals. *Biochim. Biophys. Acta - Biomembr.* 1666:142–57.

Mabin, C. J. T., Johnson, C. R. & Wright, J. T. 2019. Physiological response to temperature, light, and nitrates in the giant kelp *Macrocystis pyrifera* from Tasmania, Australia. *Mar. Ecol. Prog. Ser.* 614:1–19.

Machalek, K. M., Davison, I. R. & Falkowski, P. G. 1996. Thermal acclimation and photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*. *Plant Cell Environ.* 19:1005–16.

Manley, S. L. & North, W. J. 1984. Phosphorus and the growth of juvenile *Macrocystis pyrifera* (Phaeophyta) sporophytes. *J. Phycol.* 20:389–93.

Marín-Guirao, L., Entrambasaguas, L., Dattolo, E., Ruiz, J. M. & Procaccini, G. 2017. Molecular mechanisms behind the physiological resistance to intense transient warming in an iconic marine plant. *Front. Plant Sci.* 8:1142.

Muñoz, V., Hernández-González, M. C., Buschmann, A. H., Graham, M. H. & Vásquez, J. A. 2004. Variability in per capita oogonia and sporophyte production from giant kelp gametophytes (*Macrocystis pyrifera*, Phaeophyceae). *Rev. Chil. Hist. Nat.* 77:639–47.

Niyogi, K. K. 2000. Safety valves for photosynthesis. *Curr. Opin. Plant Biol.* 3:455–60.

North, W. J. 1987. Biology of the *Macrocystis* resource in North America. *In* Doty, M. S., Caddy, J. F., Santelices, B. & Santelices, B. [Eds.] *Case Studies of Seven Commercial Seaweed Resources.* FAO Fish, Tech. Pap, Rome, Italy, pp. 265–311.

North, W. J. 1994. Review of *Macrocystis* biology. *In* Akatsuka, I. [Ed.] *Biology of Economic Algae.* SPB Academic Publishing, Amsterdam, The Netherlands, pp. 447–527.

North, W. J. & Zimmerman, R. C. 1984. Influences of macronutrients and water temperatures on summertime survival of *Macrocystis* canopies. *Hydrobiologia* 116–117:419–24.

Pacheco-Ruíz, I., Becerril-Bobadilla, F., Zertuche-González, J. A., Chee Barragán, A., Gálvez-Telles, A. & Blanco-Betancourt, R. 2003. Effects of El Niño on beds of *Ulva lactuca* along the northwest coast of the Gulf of California. *Mexico. Geofis. Int.* 42:447–53.

Parsons, T. R., Maita, Y. & Lalli, C. M. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis.* Pergamon Press, Oxford, UK, 173 pp.

Pereira, T. R., Engelen, A. H., Pearson, G. A., Valero, M. & Serrão, E. A. 2015. Response of kelps from different latitudes to consecutive heat shock. *J. Exp. Mar. Bio. Ecol.* 463:57–62.

Perkins, S. E., Alexander, L. V. & Nairn, J. R. 2012. Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophys. Res. Lett.* 39:1–5.

Phillips, J. C. & Hurd, C. L. 2003. Nitrogen ecophysiology of intertidal seaweeds from New Zealand: N uptake, storage and utilisation in relation to shore position and season. *Mar. Ecol. Prog. Ser.* 264:31–48.

Raffo, M. P., Eyras, M. C. & Iribarne, O. O. 2009. The invasion of *Undaria pinnatifida* to a Macrocystis pyrifera kelp in Patagonia (Argentina, south-west Atlantic). *J. Mar. Biol. Assoc. UK* 89:1571–80.

Raven, J. A. 2011. The cost of photoinhibition. *Physiol. Plant.* 142:87–104.

Reed, D. C. & Brzezinski, M. A. 2009. Kelp forests. *In* Laffoley, D. & Grimsditch, G. D. [Eds.] *The Management of Natural Coastal Carbon Sinks.* IUCN, Gland, Switzerland, pp. 31–8.

Reed, D. C., Rassweiler, A., Carr, M. H., Cavanaugh, K. C., Malone, D. P. & Siegel, D. A. 2011. Wave disturbance overwhelms top-down and bottom-up control of primary production in California kelp forests. *Ecology* 92:2108–16.

Sampath-Wiley, P., Neefus, C. D. & Jahnke, L. S. 2008. Seasonal effects of sun exposure and emersion on intertidal seaweed physiology: Fluctuations in antioxidant contents, photosynthetic pigments and photosynthetic efficiency in the red alga *Porphyra umbilicalis* Kützing (Rhodophyta, Bangiales). *J. Exp. Mar. Bio. Ecol.* 361:83–91.

Saroussi, S. & Beer, S. 2007. Alpha and quantum yield of aquatic plants derived from PAM fluorometry: uses and misuses. *Aquat. Bot.* 86:89–92.

Schär, C. & Jendritzky, G. 2004. Hot news from summer 2003. *Nature* 432:559–60.

Schiel, D. R. & Foster, M. S. 2015. *The Biology and Ecology of Giant Kelp Forests.* Univ of California Press, Oakland, USA, 412 pp.

Schreiber, U. 2004. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. *In* Papageorgiou, G. C. [Ed.] *Chlorophyll a Fluorescence: A Signature of Photosynthesis.* Vol. 19. Springer, Dordrecht, The Netherlands, pp. 279–319.

Seely, G. R., Duncan, M. J. & Vidaver, W. E. 1972. Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. *Mar. Biol.* 12:184–8.

Sharkey, T. D. 2005. Effects of moderate heat stress on photosynthesis: Importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ.* 28:269–77.

Short, J., Foster, T., Falter, J., Kendrick, G. A. & McCulloch, M. T. 2015. Crustose coralline algal growth, calcification and mortality following a marine heatwave in Western Australia. *Cont. Shelf Res.* 106:38–44.

Singleton, V. L. & Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16:144–58.

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C. & Burrows, M. T. 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat. Clim. Chang.* 9:306–12.

South, P. M., Floerl, O., Forrest, B. M. & Thomsen, M. S. 2017. A review of three decades of research on the invasive kelp *Undaria pinnatifida* in Australasia: An assessment of its success, impacts and status as one of the world's worst invaders. *Mar. Environ. Res.* 131:243–57.

Straub, S. C., Wernberg, T., Thomsen, M. S., Moore, P. J., Burrows, M., Harvey, B. P. & Smale, D. A. 2019. Resistance to obliteration; responses of seaweeds to marine heatwaves. *Front. Mar. Sci.* 6:763.

Tegner, M. J. & Dayton, P. K. 1987. El Niño effects on southern California kelp forest communities. *Adv. Ecol. Res.* 17: 243–79.

Thompson, R. M., Beardall, J., Beringer, J., Grace, M. & Sardina, P. 2013. Means and extremes: Building variability into community-level climate change experiments. *Ecol. Lett.* 16:799–806.

Thomsen, M. S., Mondardini, L., Alestra, T., Gerrity, S., Tait, L., South, P. M. & Lilley, S. A. 2019. Local extinction of bull kelp (*Durvillaea* spp.) due to a marine heatwave. *Front. Mar. Sci.* 6:84.

Umanzor, S., Ramírez-García, M. M., Sandoval-Gil, J. M., Zertuche-González, J. A. & Yarish, C. 2020. Photoacclimation and photoprotection of juvenile sporophytes of *Macrocystis pyrifera* (Laminariales, Phaeophyceae) under high-light conditions during short-term shallow-water cultivation. *J. Phycol.*, 56, 380–92. https://doi.org/10.1111/jpy.12951

Wernberg, T., Coleman, M. A., Bennett, S., Thomsen, M. S., Tuya, F. & Kelaher, B. P. 2018. Genetic diversity and kelp forest vulnerability to climatic stress. *Sci. Rep.* 8:1851.

Wernberg, T., de Bettignies, T., Joy, B. A. & Finnegan, P. M. 2016. Physiological responses of habitat-forming seaweeds to increasing temperatures. *Limnol. Oceanogr.* 61:2180–90.

Wernberg, T., Russell, B. D., Thomsen, M. S., Gurgel, C. F. D., Bradshaw, C. J. A., Poloczanska, E. S. & Connell, S. D. 2011. Seaweed communities in retreat from ocean warming. *Curr. Biol.* 21:1828–32.

Wernberg, T., Smale, D. A. & Thomsen, M. S. 2012. A decade of climate change experiments on marine organisms: Procedures, patterns and problems. *Glob. Change Biol.* 18:1491–8.

Wheeler, W. N. 1982. Nitrogen nutrition of *Macrocystis*. *In* Srivastava, L. M. [Ed.] *Synthetic and Degradative Processes in Marine Macrophytes: Proceedings of a Conference Held at Bamfield Marine Station, Bamfield, Vancouver Island, British Columbia, May 16-18, 1980.* W. de Gruyter, Berlin, Germany, pp. 121–37.

Wheeler, W. N. & Srivastava, L. M. 1984. Seasonal nitrate physiology *of Macrocystis integrifolia* Bory. *J. Exp. Mar. Bio. Ecol.* 76:35–50.

Wiencke, C. & Amsler, D. A. 2012. Seaweeds and their communities in polar regions. *In* Wiencke, C. & Bischof, K. [Eds.] *Seaweed Biology. Ecological Studies (Analysis and Synthesis).* Vol 219. Springer, Berlin, Heidelberg, pp. 265–92.

Wilson, K. L., Kay, L. M., Schmidt, A. L. & Lotze, H. K. 2015. Effects of increasing water temperatures on survival and growth of ecologically and economically important seaweeds in Atlantic Canada: implications for climate change. *Mar. Biol.* 162:2431–44.

Xiao, X., Bettignies, T. De, Olsen, Y. S., Agusti, S., Duarte, C. M. & Wernberg, T. 2015. Sensitivity and acclimation of three canopy- forming seaweeds to UVB radiation and warming. *PLoS ONE* 10:e014303.

Xu, D., Fan, X., Zhang, X., Ye, N. & Zhuang, Z. 2017. Physiological differences between gametophytes and sporophytes of five cultivar strains of *Saccharina japonica*. *Aquac. Res.* 48:3091–101.

Xu, D., Ye, N., Cao, S., Wang, Y., Wang, D., Fan, X. & Zhang, X. 2015. Variation in morphology and PSII photosynthetic characteristics of *Macrocystis pyrifera* during development from gametophyte to juvenile sporophyte. *Aquac. Res.* 46:1699–706.

Young, E. B., Dring, M. J., Savidge, G., Birkett, D. A. & Berges, J. A. 2007. Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. *Plant Cell Environ.* 30:764–74.

Zar, J. H. 1984. *Biostatistical Analysis*. Prentice-Hall, New Jersey, USA, 192 pp.

Zimmerman, R. C. & Kremer, J. N. 1984. Episodic nutrient supply to a kelp forest ecosystem in southern California. *J. Mar. Res.* 42:591–604.

Zimmerman, R. C. & Kremer, J. N. 1986. In situ growth and chemical composition of the giant kelp, *Macrocystis pyrifera*: response to temporal changes in ambient nutrient availability. *Mar. Ecol. Prog. Ser.* 27:277–85.

Zimmerman, R. C. & Robertson, D. L. 1985. Effects of El Niño on local hydrography and growth of the giant kelp, *Macrocystis pyrifera*, at Santa Catalina Island, California. *Limnol. Oceanogr.* 30:1298–302.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. PCA scores for the first two principal components. Treatments correspond to those of temperature (16 and 24°C) and light (L: saturating irradiance, D: light below compensation irradiance).

Table S2. SIMPER results showing average squared distance (%) among the experimental treatments of temperature (16 and 24°C) and light (L: saturating irradiance, D: light below compensation irradiance).

STRESS RESPONSES AND RECOVERY OF JUVENILE SPOROPHYTES OF THE GIANT KELP (*MACROCYSTIS PYRIFERA*, LAMINARIALES, PHAEOPHYCEAE) SIMULATED UNDER MARINE HEAT WAVES AND NITRATE SCARCITY[1]

Schery Umanzor[‡]

Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford CT 06901-2315, USA

*José Miguel Sandoval-Gil**[‡]

Universidad Autónoma de Baja California, Instituto de Investigaciones Oceanológicas, Km 106 Carretera Tijuana-Ensenada, Ensenada, Baja California, México CP 22860

*Corresponding author: jmsandovalgil@gmail.com; phone: 00521-646-174-46-01

Mariana Sánchez-Barredo

Universidad Autónoma de Baja California, Instituto de Investigaciones Oceanológicas, Km 106 Carretera Tijuana-Ensenada, Ensenada, Baja California, México CP 22860

José Antonio Zertuche-González

Universidad Autónoma de Baja California, Instituto de Investigaciones Oceanológicas, Km 106 Carretera Tijuana-Ensenada, Ensenada, Baja California, México CP 22860

‡ These authors contributed equally to this manuscript. ‡

RUNNING TITLE: KELP, HEATWAVE, AND LOW NITRATE

ABSTRACT

The frequency of marine heat waves (MHWs) has is expected to increase due to climate change. Although seaweeds are resilient to some environmental change it is not clear how temperate seaweeds such as the kelp, *Macrocystis pyrifera*, will respond to extreme fluctuations in seawater temperature and nutrient availability. Moreover, little is known about the tolerance and vulnerability that juvenile sporophytes can show. Couple with MHWs, juvenile sporophytes can be subjected to limiting nutrient availability driven by the increase in seawater temperature. As such, sporophytes can be exposed to unfavorable environmental conditions for extended periods that can range from days to weeks. This study summarizes our finding on the ability of juvenile sporophytes of *M. pyrifera* to survive and recover from simulated MHW conditions (22°C, 5 d) in combination (or not) with nitrate limitation (<1 µM). We evaluated the photosynthetic capacities, nitrate uptake, tissue composition, bio-optical properties, in addition to oxidative stress descriptors of juvenile sporophytes with a single blade and not larger than 20 cm length. Results showed that that temperature, nitrate availability, and their interaction had significant effects on physiological status of juvenile sporophytes after the exposure and recovery phases. Results also showed that during the exposure phase, temperature was the main factor driving dissimilarities across sporophyte responses, contrarily to the recovery phase were nitrate availability was the main driver. Overall, our findings indicate that the photosynthetic

capacities of juvenile sporophytes decreased with increased temperatures and decreased in nitrate availability. On the contrary, stress responses increase on this scenario. Results highlighted that unfavorable temperature or nitrate availability did affect the performance of juvenile sporophytes. Measurements obtained after the recovery phase showed that sporophytes subject to 22 °C and limited nitrate increased their growth rate and reduced nitrate uptake rates, while those exposed to fertilized conditions showed higher nitrate content in their tissue. This study highlights that despite exposure to simulated MHW conditions with high temperature and low nitrate availability, juvenile sporophytes of *M. pyrifera* can recover within days if temperature and nutrient conditions return to a favorable state.

Key index words*:* electron transfer rate, heatwave, kelp, nitrate uptake, non-photochemical quenching, photosynthetic efficiency, pigment content.

Abbreviations*:* α, photosynthetic efficiency; Φ_{PSII}, effective quantum yield; A, absorptance; Chl *a*, chlorophyll *a*; Chl *c*, chlorophyll *c*; DW, dry weight; E_c, compensation irradiance; E_k, saturating irradiance; ETR, electron transport rate; FW, fresh weight; F_v/F_m, maximum quantum yield; gross-P_{max}, maximum gross photosynthesis; net-P_{max}, maximum net photosynthesis; NPQ, non-photochemical quenching; P vs E curve, photosynthesis *versus* irradiance curve; Rf, reflectance; R, respiration; RLC, rapid light curve; SGR, specific growth rate; T, transmittance.

INTRODUCTION

Marine heat waves (MHW) are global warming-driven events characterized by extreme warm sea surface temperatures, which can affect thousands of kilometers and persist from days to months (Frölicher et al. 2018, Smale et al. 2019). Changes in seawater temperature and ocean chemistry associated with the increasingly recurrent MHW are forcing widespread shifts in biological systems (Hollbrook et al. 2019, Oliver et al 2018, Straub et al 2019). It is known that exposure to elevated temperature beyond thermal tolerance can cause `disruptive stress´ in seaweeds, expressed at different biological levels (Davison and Pearson 1996, Koch et al. 2007, Hurd et al. 2014). Generally, thermal stress can alter enzymatic activity, cell-membrane composition, nutrient status and pigments content, as well as critical metabolic processes such as photosynthesis, respiration and antioxidant responses (Kübler and Davison 1993, Bruhn and Gerard 1996, Machalek 1996, Collén and Davison 1999, Los and Murata 2004, Cruces et al. 2012, Andersen et al. 2013). Consequently, seaweed growth, reproduction, and recruitment of juveniles may be severely affected (Andrews et al. 2014, Smale et al 2019). Though seaweeds are largely well adapted to their thermal environment, extreme changes can trigger disruptive stress in the form of cellular damage, hence influencing key vegetative and physiological processes (Wernberg et al. 2010; Harley et al. 2012; Mohring et al. 2013; Krumhansl et al. 2016 Eggert, 2012).

MHWs and ocean warming are already impacting seaweeds performance and their community-scale dynamics worldwide by breaking or redefining geographical boundaries (Wernberg et al. 2010; Vergés et al. 2014; Sunday et al. 2015; Pecl et al. 2017). A recent global analysis of kelp abundance showed that kelp forests have changed in many regions of the world (Krumhansl et al., 2016), with populations near their distribution limit

92 resulting severely affected by thermal stress associated to the ongoing increase in sea
93 surface temperature, as well as MHWs (Merzouk and Johnson 2011, Wernberg et al. 2010,
94 Mohring et al. 2013, Krumhansl et al. 2016). The impact of MHWs on kelp (and other
95 seaweeds) communities may be conditioned by their genetic diversity and their eco-
96 physiological versatility and resilience (Wernberg et al. 2018, Gurgel et al. 2020, Schimd et
97 al. 2020). Together with genetic and ecophysiological traits, MHWs properties such as with
98 their duration, intensity, as well as other abiotic factors (e.g., light, nutrients, water motion,
99 UVB radiation) can condition responses in kelp in different manners.
100 Differential responses driven by the interaction of different factors, for example
101 temperature and light, have been described for several intertidal, as well as subtidal species
102 including kelp (Bruhn and Gerard 1996, Kübler and Davison 1993, Brown et al., 2014,
103 Schiel and Foster 2015, Xiao et al. 2015). For instance, in *M. pyrifera*, increased sea
104 surface temperature coupled with nutrient limitation results in severe physiological stress
105 affecting the health and productivity of kelp forests (Dayton and Tegner, 1984, Ladah et al.
106 1999, Steneck et al. 2002, Ladah 2003). In many temperate regions, seawater nitrate
107 concentrations in the upper layers of the water column have a strong seasonal cycle driven
108 by seasonal thermoclines (Roleda and Hurd, 2019). In the NW Pacific, influenced by
109 upwelling systems, nitrate concentrations tend to decrease in fall compared to spring season
110 (Zimmermann and Kremer, 1986). In these systems, surface winds drive deep, nutrient-rich
111 water to the surface favoring primary productivity (Jacox et al. 2015; Schiel and Foster
112 2015). Conversely, increased sea surface temperature enhances the stratification of the
113 water column, inhibiting upwelling of deep water and reducing nutrient supply to the
114 surface (Jacox et al. 2015).

Specifically, in the Baja California Peninsula (Mexico), warm and nutrient-depleted waters caused by the El Niño (1982-83 and 2014-15) and The Blob (2014-17; Bond et al. 2015), lead to the disappearance of entire giant kelp forests, *Macrocystis pyrifera* (Ladah et al. 1999, Hernández-Carmona et al. 2000; Arafeh-Dalmau et al. 2019, Cavanaugh et al. 2019). Although *M. pyrifera* has been able to recover in many of its distributional range, it was not able to recover at its extreme southernmost limit, resulting in a loss of ca. 28,000 wet tons along a 50 km of coastline in the peninsula (Hernández-Carmona et al. 2000). Accurate predictions on how giant kelp ecosystems will respond to the increasing effects of global warming and the incidence of MHWs will depend on understanding how *M. pyrifera* will respond to multifactor abiotic stressors including elevated sea surface temperatures and nutrient scarcity (Harley et al. 2006; Gouvêa et al., 2017).

The physiological and morphological adjustments undergone by *M. pyrifera* are well described for adult sporophytes subjected to thermal stress, in combination (or not) with nitrate depletion (Wheeler 1980; Wheeler and North 1980; Gerard 1982, 1986; Colombo-Pallotta et al. 2006; Edwards and Kim 2010; Kopczak et al, 1991; Stephens and Hepburn; 2016). For instance, *M. pyrifera* can acclimate to short term exposure to a wide range of temperatures with either low to high nutrient availability by adjusting their fatty acid composition (Schmid et al. 2020). Other adjustments can include (but are not limited to) changes of variable Chl*a* fluorescence, pigment composition (Chl*c*, fucoxanthin), de-epoxidation state of the xanthophyll cycle pigments, translocation of photosynthates from one section of the fronds to another, and changes in the chemical composition of the tissue (Fox, 2013; Schiel and Foster, 2015; Rothäusler et al. 2018).

In contrast there is still a knowledge gap on how juvenile sporophytes may respond to multifactor stressors. Recently, Mabin et al. (2019) and Sánchez-Barredo et al. (2020) indicated that the effects of temperature on *M. pyrifera* juveniles can be conditioned to light and nitrate limitation. Different ontogenic stages of seaweeds, including *M. pyrifera*, can respond differently to changing environmental conditions (Dean and Jacobsen 1986, Muñoz et al. 2004, Ladah and Zertuche 2007, Xu et al. 2007). In addition to metabolic acclimation, integrative responses at vegetative level (e.g., translocation of resources between and within fronds) can be decisive in adult sporophytes of *M. pyrifera* to face stressful conditions (Fox 2014, 2016; Colombo-Pallota et al. 2006) Because of juvenile sporophytes lack the structural complexity of adults, they depend solely on their physiological plasticity (Umanzor et al. 2020). The susceptibility and resilience of juvenile individuals to thermal stress will ultimately affect the structure, abundance, and stability of *M. pyrifera* populations over time (Graham 1996; Gaillard et al. 2008), and hence the dynamics of associated ecosystem (O'Connor and Anderson 2010; Miller et al. 2018).

The stability of juvenile individuals is key to the persistence of populations and as such, we aim to contribute to the limited body of evidence examining the effect of increased sea surface temperature and its interaction with nutrient limitation on the performance of juvenile sporophytes of *M. pyrifera.* This study evaluated the stress responses and resilience capacity of juvenile individuals of *M. pyrifera* under simulated MHW and nitrate scarcity scenarios. The physiological plasticity of juveniles was examined through a wide range of physiological descriptors, including those related to photobiological adjustments, nutrient content, oxidative stress, and nitrate acquisition capacities. Integrating knowledge of juvenile physiological ecology to what is known

currently about adult response to environmental stressful conditions provides novel insights into factors shaping individuals of *M. pyrifera* along their macroscopic phase. Such understanding may contribute to predicting the functioning of future populations of *M. pyrifera.*

MATERIAL AND METHODS

Collection of juvenile sporophytes

Complete juvenile sporophytes of 10- 15 cm in length were collected in July 2018 by scuba-diving in a 10 m depth giant kelp bed (31°54'9.30" N, 116°44'55.83" W) in Bahía Todos Santos, Baja California (Mexico). One hundred and fourteen sporophytes were detached from the substrate, ensuring the collection of the entire holdfast and blade. Only healthy individuals, with a single blade, no pneumatophores, nor herbivory signals were collected. Individuals were kept inside black bags during collection to prevent overexposure to sunlight at emersion. Subsequently, they were promptly stored in coolers (100 L) filled with ambient seawater and transported to the Marine Botany Laboratory at the University of Baja California (Ensenada, B.C.). Upon arrival, all individuals were transferred to twelve 60 L aquaria (six juveniles per aquaria), filled with filtered (1 µm) and UV irradiated seawater, and provided with aeration. Sporophytes were acclimated for three days to reference field values of temperature (16 °C) and light (130 µmol quanta m^{-2} s^{-1}) with a 12:12 light-dark photoperiod. These parameters followed data of these variables monitored during previous weeks at the collection site using submersible sensors (HOBO MX2202; ONSET, Bourne, MA, USA). Submersible water pumps (Resun, SP980) ensured

continuous water circulation within aquarium. The initial physiological status of juvenile sporophytes was assessed by examining photobiological descriptors in six individuals randomly selected (Table 1). During acclimation, two nutrient enrichments were done by adding sodium nitrate (5 µM) to maintain sporophytes at an optimum nutritional state prior to applying the experimental treatments.

Experimental design

The treatments consisted of a combination of temperature (16.0 ± 0.5 °C or 22 ± 0.2 °C) and nitrate concentration (seawater fertilized with 15 µM nitrate or unfertilized <1 µM). Nitrate fertilization (hereafter 16N) resembled concentration of this nutrient in giant kelp forest during the influence of upwelling events in spring (Dayton et al. 1999; Hernandez-Carmona et al. 2001). As such, this treatment is considered as the control throughout the experiment. Treatment including the temperature of 22.0 °C without seawater fertilization (hereafter 22) reflected abnormal warming conditions (MHWs) such as those during the El Niño-The Blob event from 2014 to 2017 (Arafeh-Dalmau et al. 2019, Cavanagh et al 2019). Sporophytes assigned to the 22 °C treatments (i.e. 22N and 22) were exposed to 2 °C daily increments until the temperature was reached to avoid potential stress associated with the sudden exposure of sporophytes to high temperature, and to reproduce the thermal increment rates during these events in the northern Pacific of Baja California (Arafeh-Dalmau et al. 2019). Seawater for all aquaria was replaced every two days, with water quality checked daily using a submersible multi-parameter probe (YSI Pro Plus, USA). Salinity (33.5 ‰) and pH (7.9-8.1) were maintained constant during the experiment.

Each treatment, i.e., 16N, 16, 22N, and 22 considered three independent aquaria (n=3). Sporophytes remained subjected to their experimental treatment for five consecutive days. Immediately after this exposure period sporophytes were shifted to control conditions (hereafter recovery phase) where they remained for three consecutive days. In both experimental phases (i.e., exposure and recovery) all physiological descriptors were measured in two individuals per aquaria (i.e. six sporophytes per treatment), except P-E curves, which were obtained from one individual per treatment. Values per aquaria were averaged to obtain the true replicate (n = 3).

Photosynthesis and Respiration (P vs. E curves)

Photosynthetic and respiration rates were determined using a respirometer. The respirometer consisted of a 200 mL borosilicate jacketed chamber connected to a circulating bath with controlled temperature and surrounded by four light sources (LED 10 W) automatically controlled by a customized software. Oxygen evolution was measured using optodes (dipping probe DP-PSt3, PreSens, Germany) connected to a fiber-optic oxygen meter (OXY4 SMA, PreSens, Germany) also controlled by a software (Measurement Studio 2, PreSens, Germany). A biomass/volume ratio of 0.04-0.07 g DW L^{-1} was used based on preliminary trials to ensure accurate measurements of photosynthetic rates, avoiding the underestimation of photosynthesis due to oxygen oversaturation and carbon limitation. Blade segments were first incubated in darkness for 15 min to determine dark respiration rates. Then, they were exposed to eleven increasing light intensities (E) of 6, 22, 37, 51, 88, 119, 130, 156, 193, 251, and 327 µmol quanta m^{-2} s^{-1}. Light intensities within the chamber were calibrated using a spherical (4π) quantum sensor (Biospherical

Instruments; California, USA). The maximum net photosynthesis rate (net-P_{max}; µmol O_2 g^{-1} DW h^{-1}) was determined by averaging the maximum values above the saturating irradiance (E_k = net-P_{max}/ α; µmol quanta m^{-2} s^{-1}). Gross photosynthesis (gross-P_{max}; µmol O_2 g^{-1} DW h^{-1}) was calculated as the sum of net-P_{max} and R (respiration). Photosynthetic efficiency (α, µmol O_2 g^{-1} DW h^{-1}/µmol quanta m^{-2} s^{-1}) was the slope of the regression line fitted to the initial linear section of the P-E curve. The compensation irradiance (E_c; µmol quanta m^{-2} s^{-1}) was taken as the intercept of the initial linear part of the curve on the X-axis. A proxy of the seaweed carbon balance, P:R ratio, was calculated by dividing the net photosynthetic rate of P-E curves coinciding with the irradiance adjusted in the aquaria (~130 µmol quanta m^{-2} s^{-1}) and dark respiration.

Chlorophyll-a fluorescence

Chlorophyll-a fluorescence emissions of PSII were measured using a portable fluorometer (Diving-PAM; Walz, Germany). Photochemistry measurements were acquired from the mid-section of each blade to standardize measurements among individuals, avoiding within-tissue variability. This section showed the maximum values of F_v/F_m (maximum quantum yield) in previous trials. Each blade segment was held to the fluorometer using DCL-8 leaf clip holders to assure a constant distance between the tissue and fiber optic.

Rapid Light Curves, RLCs, were obtained from the blades after they remained in darkness overnight (Schreiber 2004, Larkum et al. 2006). The clipped segments of the blades were exposed for 30s each to eight increasing actinic light intensities, ranging from 4 up to 300 µmol quanta m^{-2} s^{-1}. After each irradiance, saturating pulses (~5000 µmol

quanta m^{-2} s^{-1}, 0.8 s) were applied to calculate the ETRs (Electron Transport Rates), Φ_{PSII} (Effective Quantum Yields), and NPQ (Non-Photochemical Quenching). The F_v/F_m, corresponded to the yield values obtained by exposing dark-adapted blades to the first saturating pulse. Absolute ETR were calculated as $ETR = \Phi_{PSII} \times E \times A \times 0.5$ (1)

where Φ_{PSII} is the quantum yield of photosynthetic electron transfer through photosystem II, E corresponded to each actinic light intensity, A is the blade absorptance (see below), and 0.5 is a correction factor used because absorption of quanta per photosystem (Schreiber and Neubauer 1990, Beer et al. 2014). Values of NPQ were calculated as

$NPQ = (F_m - F_{m'}) / F_{m'}$ (2)

where F_m is the maximum fluorescence and $F_{m'}$ the fluorescence measured when blades were exposed to each intensity of actinic light.

Blade bio-optical properties

Blade spectral absorption in the PAR range (400-700 nm) was determined using a Taylor-type integrating-sphere (Li-Cor 1800-12, USA) attached to a portable spectroradiometer (Fieldspec, ASD, Boulder, Colorado, USA) with a fiber optic cable. A blade segment of ~2 cm was placed between two microscope glass slides then attached to the sample holder of the integrating sphere covering the entire light path. The spectroradiometer recorded both Reflectance (Rf) and Transmittance (T) at 1 nm intervals using barium sulfate as the reference material. Absorptance (A) was determined as 1-T-Rf following Krause and Weis (1991). Absorption not related to pigments was corrected by subtracting the averaged absorbance between 725-750 nm.

Pigment content

Blade tissue was processed following the method described by Seely et al. (1972) and modified by Wheeler (1980). About 0.02 g of fresh blade tissue were placed into a tube containing dimethyl sulfoxide (DMSO). The solution of DMSO with extracted fucoxanthin (but also other pigments, including Chl *a* and Chl *c*) was diluted at a 4:1 ratio with distilled water, while the tissue was removed and stored at 4 °C. Pigments content from the dilution was measured using a spectrophotometer (Shimadzu UV-1800; Kyoto, Japan). The absorbance was calculated at wavelengths of 665, 631, 582 and 480 nm and using the equations and specific molar extinction coefficients from Seely et al. (1972). A second pigment extraction was conducted by placing the stored blade tissue in a tube with 100% acetone for 24 h at 4 °C. This solution was diluted at a 3:1:1 ratio with methanol and distilled water. Pigment content in the dilution was calculated by measuring the absorbance at wavelengths of 470, 581, 631, and 664 nm and using the same equations and specific molar extinction coefficients mentioned above. Final pigment content (Chl *a*, Chl *c* and Fucoxanthin) was calculated as the sum of all pigments obtained from both sequential extractions.

Specific growth rate

Two individuals per aquaria were randomly selected and marked by attaching a string to their stipe. Each of these individuals was measured from the apex to the distal-most section of the holdfast to obtain their total length at the beginning and end of each experimental period (i.e. exposure and recovery). Specific growth rates (SRG) were calculated as

297 $SGR = [(\ln (L_f) - \text{In } (L_i))/t] \times 100$ (3)

298 where L_f is the final length, L_i is initial length and t is the duration of either the
299 exposure or the recovery period in days.

300 *Soluble carbohydrates*

301 Soluble carbohydrates were measured using the colorimetric phenol-sulphuric acid
302 method, using glucose as standard (Dubois et al. 1956). Blade tissue was dried, ground, and
303 then digested with 2 mL of 0.2 M HCl at 60 °C for 3 h. After, each sample was centrifuged
304 for 5 min at 1000 x g. A mixture of 1 mL of distilled water, 0.025 mL of supernatant, and
305 0.25 mL of 3% phenol was cooled in an ice bath. Then 2.5 mL of concentrated sulphuric
306 acid were added directly into the mix and vortexed vigorously. After 30 min, absorbance
307 was measured at 490 nm.

308 *Nitrate uptake and total nitrogen content*

309 Entire juvenile sporophytes were incubated for 30 min in 1 L plexiglas transparent
310 chambers filled with artificial seawater (Instant Ocean®) enriched with 15 µM of labeled
311 nitrate $K^{15}NO_3$ (99 atoms % ^{15}N, Cambridge Isotope Laboratories). Preliminary trials
312 indicated that using a biomass: incubation volume ratio of 0.7 g DW L^{-1} prevented
313 substantial changes in the nitrate concentration of seawater, which could lead to
314 underestimation of uptake rates. During the incubations the chambers were submersed into
315 the experimental aquaria to maintain the temperature and light conditions of each treatment.
316 Chambers were agitated to ensure that seawater was mixed continuously to reduce the
317 boundary layer around the blades (Cornelisen and Thomas, 2004) and to homogenize the
318 tracer during incubation. Following the incubation period, sporophytes were rinsed with

deionized water to remove nutrients from the surface of the tissue and dried at 60 °C until constant weight. Samples were ground to a fine powder for isotope enrichment analyses. Isotopic determinations were carried out at UC-Davis Stable Isotope Facility using an elemental analyzer (EA) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS). Nitrate uptake rates (V, expressed as μmol N g^{-1} DW h^{-1}; DW = dry weight) were calculated as:

$$V = [(^{15}N_{exp}-^{15}N_{back}) \times Nc] / (M_N \times t) \quad (4)$$

where the difference ($^{15}N_{exp}-^{15}N_{back}$, at. %) is the ^{15}N enrichment relative to natural ^{15}N sporophyte levels (i.e., background), Nc is the nitrogen content (g N g^{-1} DW), M_N is the molar mass of the labeled nitrogen (15 g mol^{-1}), and t is the incubation period in hours. Total nitrogen content was measured in juveniles not exposed to the tracer.

Lipid peroxidation

Membrane lipid peroxidation was measured following the thiobarbituric acid-reactive-substances assay, TBARS, described by Hodges et al. (1999) and Correia et al. (2006). Ultra-frozen blade tissues were ground in liquid nitrogen using a mechanical grinder and homogenized (dilution 1:10) in trichloroacetic acid (TCA, 20 %). Tissue homogenates were then centrifuged 10 min (3000 x g, 4°C) and supernatants were mixed with a solution of TCA (20 %) and TBA (thiobarbituric acid, 0.5 %). These solutions were heated at 90°C for 30 min and then centrifuged again (10000 x g) for 10 min. The supernatants were extracted, and their absorbances (440, 532 and 600nm) were measured by spectrophotometer. Equivalents of malonyl dialdehyde (Eq MDA; molar extinction coefficient 155 mM^{-1} cm^{-1}) was calculated following the equations in Hodges et al. (1999).

Total phenolic content and antioxidant capacity

Approximately 0.03 g DW of dried and ground up blade tissue were mixed with 0.75 mL 80% methanol in left darkness for 24 h. The extract was then centrifuged at 10 rpm for 10 min. The phenolic compound content was measured from the supernatant following a modified Folin–Ciocalteu assay with gallic acid as reference (Singleton and Rossi 1965). In brief, an aliquot of the methanolic extract was diluted in 1 mL 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. Finally, absorbance was read at 765 nm using a spectrophotometer. For its part, the radical scavenging activity of the same methanolic extracts was determined over the stable free radical, DPPH, using ascorbic acid as standard (Farvin and Jacobsen 2013). The reactive solution was prepared by mixing 0.1 mL of diluted extract (1:4 with aqueous methanol at 80 %) and 1 mL of 30 µM DPPH freshly prepared in aqueous methanol at 90 %. Exactly 30 min after, the DPPH was added, and the absorbance was measured at 517 nm. The absorbance was also measured to the same proportions of DPPH and aqueous methanol but without the algal extract. This solution was used as blank control.

Statistical analysis

Multivariate analyses were performed using normalized values for all biological variables to construct similarity matrices based on Euclidean distances for both the exposure and recovery phases. Principal coordinates analyses (PCO) were used to identify differential patterns of the ecophysiological responses by the juvenile sporophytes as a

function of temperature (16 and 22 °C) and nitrate availability (with or without fertilization) after both the exposure and recovery phases. SIMPER analyses were used to explore the percentage of dissimilarity between sporophytes across treatments. Two-way PERMANOVAs with crossed designs were performed applying 9999 permutations to test the factors of temperature, nitrate availability, and their interactive effects on the physiological status of juvenile sporophytes after both phases.

Furthermore, individual physiological responses sporophytes during the exposure and recovery phases were explored separately using temperature and nitrate availability as categorical and independent factors. The interactive effect of the two categorical factors on all physiological descriptors was analyzed using two-way ANOVA by least mean squares at an alpha value of 0.1. When significant differences were found, Student-Newman-Keuls tests were used as *post hoc* pairwise comparison. The non-parametric Kruskal-Wallis test was used to conduct comparisons when assumptions of homogeneity and homoscedasticity were not met despite data transformation. All statistical procedures were implemented using PRIMER 6 & PERMANOVA+ v.1.0.2 software package (Anderson et al. 2008), Statistica (StatSoft INC, v 8.0), and SigmaPLot (Systat, Inc).

RESULTS

Photobiological descriptors obtained from juveniles immediately after field collection showed low variability (Table 1), indicating that juvenile sporophytes started the experiment with a similar physiological status. As such, differences among treatments are considered to have resulted from the experimental treatments applied. Also, values of the photobiological descriptors obtained from the experimental sporophytes in the control

388 treatment (16N) throughout the experiment (i.e., exposure and recovery periods) were
389 generally within range of those obtained in field reference sporophytes. This indicated that
390 the experimental system and conditions did not lead to physiological stress in juvenile
391 sporophytes, which could potentially overlap their responses to the treatments proposed.
392 The two-dimensional PCO plot for the exposure phase showed two eigenvectors (Fig. 1)
393 explaining approximately 56 % of the total variance. Component 1 explained the highest
394 percentage of variance (40.8 %) and represented differences mainly as a function of
395 temperature treatments (Fig. 1A). The two-dimensional PCO plot for the recovery phase
396 (Fig. 2) also showed that the first two eigenvectors explained 51 % of the total variance.
397 Component 1, which also explained the highest percentage of variance (32 %) also
398 separated both temperature (Fig. 2A), in addition to fertilization treatments to some extent
399 (Fig. 2B)
400 In general, the two-way PERMANOVA indicated that temperature, nitrate availability and
401 their interaction had significant effects on physiological status of juvenile sporophytes after
402 the exposure and recovery phases (Table 2). The SIMPER analyses for the exposure phase
403 indicated that the temperature treatments (16N, 16 vs. 22N, 22) had higher dissimilarity
404 (average squared distance = 46.77) than the fertilization treatments (16N, 22N vs. 16, 22;
405 average squared distance = 35.47). On the contrary, in the exposure phase, temperature
406 treatments showed a lower dissimilarity (average squared distance = 37.63) than that of
407 fertilization treatments (average squared distance = 40.39).
408 Photosynthetic rates (gross-P, net-P) decreased significantly (~by 15%) in sporophytes at
409 the temperature of 22 °C without nitrate fertilization (Fig. 3A, B; Table S1). After recovery,
410 differences among treatments were much more extreme, being maximum and minimum

411 values those corresponded to plants from 16N (i.e., control) and 22, respectively. ($P \leq$
412 0.001; Table S2). During the exposure phase, sporophytes at 22 °C showed ~50% higher
413 respiration rates, R, than those at 16 °C (two-way ANOVA; $F_{1,8} = 20.70$, $P < 0.05$),
414 regardless of the nitrate treatment (Fig 3C; Table S1). Although this trend persisted after
415 recovery, differences among treatments were no longer significant. Similar to R,
416 compensation irradiance(E_c) also significantly increased by ~90%as a function of
417 temperature after the exposure phase (Fig.3E, Table S1; two-way ANOVA, $F_{1,8} = 12.68$, P
418 < 0.05) ; E_c values remained higher in recovered juveniles, although not significant
419 differences were detected. Values of P:R ratio (as a proxy of carbon balance) were
420 significantly lower in sporophytes exposed to heat stress after the exposure phase,
421 regardless nitrate availability (Fig. 3D, Table S1; two-way ANOVA, $F_{1,8} = 10.87$, $P < 0.05$).
422 Recovered sporophytes showed remarkable differences as a function of the previously
423 applied treatments of temperature (Table S2; two-way ANOVA, $F_{1,8} = 19.75$, $P < 0.05$),
424 nitrate (two-way ANOVA; $F_{1,8} = 24.32$, $P = 0.001$), and their interaction (two-way
425 ANOVA, $F_{1,8} = 10.87$, $P < 0.1$) , following the pattern described by net-P and gross-P.
426 Photosynthetic efficiency, α, did not change significantly after the exposure period, but
427 decreased in sporophytes recovered from the treatment with the temperature of 22°C and no
428 nitrate (Fig. 3F; Non-parametric Kruskal-Wallis test, $H_{3,12} = 7.51$, $P = 0.057$).
429 Blade absorptance ($A_{400\text{-}700}$, A_{680}; Fig. 2A-C) decreased in juveniles from all the treatments
430 respect to control plants (16N), but these differences were statistically undetectable (Table
431 S1). Similar to A, higher pigment content (Chl*a*, Chl*c*, Fx) was found in control juveniles,
432 and it significantly decreased in the rest of the treatments after exposure to heat stress and
433 and nitrate scarcity. (Fig. 4D-F, Table S1 and S2). Generally, Chl*a* and Fx decreased in

juveniles exposed the higher temperature, but this reduction was higher with the lack of nitrate (Fig. 4D, F; Table S1; two-way ANOVA, $P < 0.05$). For its part, content in Chl*c* was uniquely affected by the factor temperature, and it was reduced by ~60% at 22 °C (Fig. 4E, Table S1; two-way ANOVA, $F_{1,8} = 11.31$, $P < 0.05$). Pigments content were similar among recovered sporophytes (Fig 4D-F, Table S2).

The maximum electron transport rate, ETR, showed significant differences, particularly as a function of nitrate availability (Table S1; $F_{1,8} = 19.32$, $P < 0.1$), with unfertilized sporophytes having lower rates (Fig. 5C). Values of F_v/F_m, NPQ, and Φ_{PSII} (Fig. 5) did not show significant differences as a result of the exposure phase (Table S1). A similar trend was observed after the recovery phase, except for ETR and NPQ, which showed significant differences also as a function of nitrate availability (Table S2). However, significance for this group was no confirmed by the post-hoc analysis.

Specific growth rates of sporophytes at the temperature of 16 °C were approximately 2.5-fold greater compared to those at 22 °C (Fig. 6A, Table S1; $F_{1,8} = 8.56$, $P < 0.05$). After the recovery phase, sporophytes previously exposed to the temperature of 22 °C increased their growth rate and differences in growth across treatments were reduced. Significant differences were no longer detected (Fig. 6A; Table S2). Total carbohydrate content measured after the exposure phase differed as a function of nitrate availability (Figure 6B, Table S1; $F_{1,8} = 7.22$, $P < 0.05$) and the interaction of temperature and nitrate (Figure 6B, Table S1; $F_{1,8} = 3.69$, $P < 0.1$) On the other hand, after the recovery phase, carbohydrate content differed significantly as a function of temperature only (Figure 4B, Table S2; $F_{1,8} = 20.10$, $P < 0.05$).

Nitrate uptake rates showed significant differences as a function of temperature treatments and after recovery phase only (Fig. 7A, Table S2; $F_{1,8} = 20.10$, $P < 0.05$), with sporophytes at the temperature of 22 °C showing ~40% reduction (Table Fig. 7A). The total nitrogen content in sporophytes after the exposure phase was significantly higher (28-34%) in those growing in fertilized vs. unfertilized conditions (Fig. 7B, $F_{1,8} = 22.03$, $P < 0.05$). Temperature also showed a non- interactive significant effect (Table S1; $F_{1,8} = 6.32$, $P < 0.1$). In general, nitrogen content in sporophyte tissue increased significantly after de recovery phase due to the interactive effect of temperature and nitrate (Fig. 7B, Table S1, $F_{1,8} = 28.10$, $P < 0.05$).

Changes in total phenolic content, antioxidant capacity, and lipid peroxidation after the exposure period were statistically significant among treatments (Table S1), with temperature or the interaction of temperature and nitrate as driving factor. Differences were no longer detectable after the recovery phase (Table S1).

DISCUSSION

This study found that that juveniles sporophytes of *M. pyrifera* exposed to the unfavorable temperature of 22 °C with or without nitrate availability show significant acclimation responses in a variety of physiological parameters. Contrarily to Mariana Cap -1, which showed that temperature has a secondary role in contrast to light availability of juvenile sporophytes, here PERMANOVA outcomes indicate that temperature was the main factor promoting physiological alterations on juvenile sporophytes. Nitrate deprivation also showed to influence sporophytes only in an interactive effect with temperature. These

contrasting results highlight that the physiological responses of juvenile sporophytes driven by ocean warming events such as heatwaves may vary when there is a simultaneous change in other environmental factors such as light or nitrate in this study.

Juvenile sporophytes exposed to 22 °C without nitrate appeared to show lower photosynthetic rates with significant differences detected only after the recovery period. This outcome could suggest that photosynthetic processes of these sporophytes were affected negatively during the exposure period and these effects may be irreversible or require a longer recovery period.

Experiments assessing the effect of nitrogen availability in growth rates and thermal tolerance of adult sporophytes of *Macrocystis* and the sugar kelp *Saccharina* show that growth rates decline in the absence of an adequate source of N (Wheeler and North, 1980), while an optimal N supply can still support positive photosynthetic outcomes even when sporophytes are subject to unfavorable temperature conditions (Gerard, 1997). Metabolic processes such as respiration typically increases with temperature (Tait and Shield, 2013). In this experiment, sporophytes growing at 22 °C showed significant differences in respiration rates compared to those at 16°C, regardless of nitrate availability. Although other processes such as photosynthesis could also be affected by temperature changes, respiration rates often respond at a faster rate (Tait and shield, Wernberg, de bettignies 2016). As in other primary producers, dark respiration is key for growth and survival of macroalgae by modulating their carbon balance (Dinghui et al 2011). Increments in respiration rates with respect to gross photosynthesis reflect a decrease the

ratio of respiration to photosynthesis (P:R) of sporophytes exposed to the temperature of 22 °C. This C imbalance on both sporophytes at 22 and 22N coincides with the low SGR observed in these sporophytes during the exposure period. Sporophytes at 22 °C also showed a significant reduction in their photosynthetic efficiency, *alpha*, after the recovery period, coinciding with the low pigment content and high NPQ values measured of these sporophytes, particularly in sporophytes at 22. Moreover, increments in R during the exposure period of sporophytes at 22 °C, without any further adjustments in *alpha*, are responsible for the sudden increase observed in their Ec, particularly in sporophytes at 22. During the recovery period, the lower values of P:R were those of sporophytes previously at 22, coinciding with the reduction of gross- P observed in these sporophytes.

Pigment content in the experimental juvenile sporophytes decreased with increasing temperatures after the exposure period, regardless of nitrate availability. This highlights that temperature alone or the interaction between another factor rather than nitrate availability may be key drivers of tissue degradation or lack of pigment production and thus, bleaching of juvenile sporophytes of *M. pyrifera.* In adult sporophytes of *M. pyrifera,* pigments, particularly Chl-a, have shown temperature-dependent, with sporophytes in warm-water treatments showing significantly lower Chl-a content than sporophytes kept in the cool-water treatments (Rothausler et al, 2011). In general, primary producer exposed to unfavorable environmental conditions including heat can modify their pigment content to adjust their capacity to absorb and harvesting light (Lintenthaler et al. 2000; Camejo et al. 2005). However, in this study, the reduction in pigment content did not seem to cause a reduction in blade absorptance, A, perhaps because pigments could have had a less compact

package arrangement. The packaging effect, typical of primary producers, describes the decreased absorption by cell pigments compared with the potential absorption for the same amount of pigment in solution (Kirk,1994; Stuart, 1998). A lower packaging effect could have permitted a more flattened distribution of pigments, thus allowing blades to maximize light absorption despite the low pigment content. Nonetheless, lack of proper data to calculate the specific coefficient of absorption, a*, precludes further testing of this statement.

Regardless of the differences in the exposure treatments, the maximum quantum yield, Fv/Fm of sporophytes showed no significant differences, which may indicate absence of damage in the photosystem II activity of thylakoid membranes within sporophytes exposed to 22. Moreover, although not significant, sporophytes exposed to unfertilized conditions regardless of the temperature show a lower non-photochemical quenching, NPQ, and electron transfer rate, ETR, and effective quantum yield, *symbol*, than those exposed to fertilized conditions regardless of the temperature. This lack of significance may result from the limited number of replicates and therefore, differences should not be overlooked.

A study assessing the photosynthetic responses and recovery dynamics in prasinophytes (i.e. class of unicellular green algae) and diatoms exposed to nitrogen starvation showed that comparable to our results, individuals exposed to N-depleted conditions tend to reduce their ETR activity and effective quantum yield (liefer et al 2018). Another study measuring the effect of nitrate concentration on the relationship between photosynthetic oxygen evolution and electron transport rate in *Ulva rigida* also shows that ETR increases with nitrate concentration. Contrarily to the expected, in our study NPQ, which is typically

elevated under photosynthetic stress conditions (Harper, Mendoza-Garca) did not increase following nitrate deprivation, but did after the recovery period. Porqué baja el NPQ? discutir algo de los efectos de falta de N a estos niveles de metabolismo (p.e., ausencia de N provoca desaceleración en producción de proteínas/enzimas clave en estos procesos?). relacionar con que las menores tasas de fotosíntesis (y eficiencia y pigmentos) en recuperación se encuentran en esos juveniles; es como si, ante unas mermadas capacidades fotosintéticas a nivel de metabolismo de C (no en tilacoides), las plantas quisieran aliviar la presión fotónica/electrónica en los tilacoides para evitar fotodaño….ya sabéis de lo que va la historia porque hemos discutido estas cosas en diferentes ocasiones.

Despite results showing a C unbalance in sporophytes at 22, they did not show a reduction in the carbohydrates mencionar algo breve al respecto..

As expected, total nitrogen content was lower in sporophytes exposed to unfertilized conditions, showing values slightly higher than the assumed critical level of 1% DW indicative of severe N-depletion to support growth (Gerard, 1982; Hernandez Carmona, 2001). After the recovery period, total N-content in these sporophytes increased to 1.5 % DW or higher. This pattern of increase and decrease in total N-content based on environmental availability is well documented for adult sporophytes of *M. pyrifera* forming forests off Baja California. In 1997-98, El Niño caused sea surface temperature to reach 25.3 °C (i.e. + 5.7 °C anomaly) and nutrient levels to be below < 1.0 mM. During this warming event, N-content of sporophytes were depleted rapidly, reducing from 2.29% in

April to 1.45% in August, until all kelp forests at the southern end of the distribution disappeared (Hernandez carmona 2001).

Nitrate uptake rates measured in all sporophytes showed higher values than the reported (~3 µmol N g^{-1} DW h^{-1}) for adult sporophytes of *M. pyrifera* (Haines and Wheeler 1978, Gerard 1982. Although no significant differences were detected after the exposure period, sporophytes as 22 °C did show a lower uptake rate after the recovery period. esto además es significativo estadísticamente y hay que discutirlo porque es algo muy muy interesante.

Juvenile sporophytes exposed to 22 showed higher MDA and antioxidant activity indicating a high production of reactive oxygen species, ROS. The generation of ROS in primary producers is triggered by a variety of environmental stresses, such as high irradiance, high or low temperatures, and nutrient deficiency (Tripathy, Dring 1995, Collen and Davison 1999, another Collen and Davidson, 1999). Oxygen is continuously produced inside the chloroplast due to photosynthetic electron transport and simultaneously removed by reduction and assimilation. The reduction of this and other oxygen produces ROS, which if imbalance (i.e. generation vs. detoxification) generates oxidative stress (Tripathy, otras). A laboratory experiment assessing the effect of temperature on juvenile sporophytes of *M. pyrifera* showed that only 3h after a + 3 °C exposure individuals presented elevated values of MDA indicative of damage caused by excess of ROS (Cruces et al. 2012). High levels of ROS can result in irreparable damages that can, in turn, impede the proper functioning of PSII. The increased levels phenols resemble those of the antioxidant activity exhibited by the sporophytes exposed to 22. Seaweeds have several antioxidants including

592 a variety of phenols that are essential in preventing lipid peroxidation caused by ROS
593 (ramnadi 2007, wang et al 2007). Although not significant, the lower values of
594 photosynthesis were recorded in sporophytes at the temperature of 22 °C indicating a lower
595 capacity to transfer electrons. It is possible that these sporophytes also limited the
596 adquisicion of light by reducing their pigment content to limit the production of ROS. The
597 lack of significant differences in the Pmax and Fv/Fm values may suggest that regulatory
598 mechanisms against ROS were still effective after five days of exposure to the combined
599 temperature and nitrate stress treatment
600 *M. pyrifera* exhibits wide phenotypic plasticity that allows individuals to acclimate to a
601 range of shifting environmental conditions such as temperature, nutrient, and light
602 (Rothäusler et al. 2011; Buschmann et al. 2014). Here, we found that juvenile sporophytes
603 modify a variety of physiological processes that allowed them to survive short-term
604 simulated heatwave conditions. Nonetheless, despite the physiological adjustments,
605 sporophytes still showed stress signals. In a recent study, Cavanagh et al. (2019) indicate
606 that the resistance of *M. pyrifera* to heatwave events relates directly to a temperature
607 exceeding an absolute threshold more than to the magnitude of relative changes in
608 temperature. They also showed that resilience is spatially variable with local-scale
609 environmental and biotic processes playing a key role in determining the recovery of kelp
610 exposed to particular, extreme warming events. As such, the survival and recovery of these
611 populations will largely depend on the mechanisms of survival (i.e. protection and
612 acclimation) and recruitment of juvenile sporophytes (Edwards and Hernández-Carmona
613 2005). Our study suggests that juvenile sporophytes of *M. pyrifera,* at least from northern
614 Baja California, could recover after short-term heatwave conditions showing temperatures

of up to 22 °C with little to no nitrate availability. Nonetheless, they could still exhibit negative responses across several physiological processes and other stressors such as increased grazing, wave action because by storms or the joint effect all may drive juvenile sporophytes beyond their capacity for recovery.

Reference

Allakhverdiev, S. I., Kreslavski, V. D., Klimov, V. V., Los, D. A., Carpentier, R., & Mohanty, P. (2008). Heat stress: an overview of molecular responses in photosynthesis. *Photosynthesis research*, *98*(1-3), 541.

Anderson MJ (2017) Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef Stat Ref Online 1–15. doi: 10.1002/9781118445112.stat07841

Arafeh-Dalmau N, Montaño-Moctezuma G, Martinez JA, Beas-Luna R, Schoeman DS, Torres-Moye G (2019) Extreme Marine Heatwaves Alter Kelp Forest Community Near Its Equatorward Distribution Limit. Front Environ Sci 6:499. doi: 10.3389/fmars.2019.00499

Breeman AM (1988) Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: Experimental and phenological evidence. Helgoländer Meeresuntersuchungen 42:199–241. doi: 10.1007/BF02366043

Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends Ecol Evol 18:119–125.

Buschmann AH, Pereda S V., Varela DA, Rodríguez-Maulén J, López A, González-Carvajal L, Schilling M, Henríquez-Tejo EA, Hernández-González MC (2014) Ecophysiological plasticity of annual populations of giant kelp (Macrocystis pyrifera)

638 in a seasonally variable coastal environment in the Northern Patagonian Inner Seas of
639 Southern Chile. J Appl Phycol 26:837–847. doi: 10.1007/s10811-013-0070-z
640 Camejo D, Rodríguez P, Angeles Morales M, Miguel Dell'Amico J, Torrecillas A, Alarcón
641 JJ (2005) High temperature effects on photosynthetic activity of two tomato cultivars
642 with different heat susceptibility. J Plant Physiol 162:281–289. doi:
643 10.1016/j.jplph.2004.07.014
644 Cavanaugh KC, Reed DC, Bell TW, Castorani MCN, Beas-Luna R (2020) Spatial
645 variability in the resistance and resilience of giant kelp in southern and Baja California
646 to a multiyear heatwave. Front Mar Sci 6:1–14. doi: 10.3389/fmars.2019.00413
647 Chapman ARO, Craigie JS (1977) Seasonal growth in Laminaria longicruris: Relations
648 with dissolved inorganic nutrients and internal reserves of nitrogen. Mar Biol 40:197–
649 205. doi: 10.1007/BF00390875
650 Colombo-Pallotta MF, García-Mendoza E, Ladah LB (2006) Photosynthetic performance,
651 light absorption, and pigment composition of Macrocystis pyrifera (Laminariales,
652 Phaeophyceae) blades from different depths. J Phycol 42:1225–1234. doi:
653 10.1111/j.1529-8817.2006.00287.x
654 Cornelisen, C.D., Thomas, F. I. M., 2004. Ammonium and nitrate uptake by leaves of the
655 seagrass *Thalassia testudinum*: impact of hydrodynamic regime and epiphyte cover on
656 uptake rates. *Journal Mar. Syst*. 49:177–94
657 Correia, M. J., Osório, M. L., Osório, J., Barrote, I., Martins, M., & David, M. M. 2006.
658 Influence of transient shade periods on the effects of drought on photosynthesis,
659 carbohydrate accumulation and lipid peroxidation in sunflower leaves. *Environ Exp*
660 *Bot*, *58*:75-84.

Davison, I. R. (1991). Environmental effects on algal photosynthesis: temperature. *Journal of phycology*, *27*(1), 2-8.

Dayton PK (1985) The structure and regulation of some south american kelp communities. Ecol Monogr 55:447–468.

Dean TA, Jacobsen FR (1986) Nutrient-limited growth of juvenile kelp, Macrocystis pyrifera, during the 1982-1984 El Nino in southern California. Mar Biol 90:597–601. doi: 10.1007/BF00409280

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem*. *28*:350-6.

Edwards MS, Hernández-Carmona G (2005) Delayed recovery of giant kelp near its southern range limit in the North Pacific following El Niño. Mar Biol 147:273–279. doi: 10.1007/s00227-004-1548-7

Edwards, M. S., & Estes, J. A. (2006). Catastrophe, recovery and range limitation in NE Pacific kelp forests: a large-scale perspective. *Marine Ecology Progress Series*, *320*, 79-87.

Edwards MS, Kim KY (2010) Diurnal variation in relative photosynthetic performance in giant kelp Macrocystis pyrifera (Phaeophyceae, Laminariales) at different depths as estimated using PAM fluorometry. Aquat Bot 92:119–128. doi: 10.1016/j.aquabot.2009.10.017

Edyvane K, Dunstan P, Johnson C (2004) Large-scale loss of giant kelp, Macrocystis pyrifera, forests off the east coast of Tasmania. Aust. Mar. Sci. Assoc.

684 Fox MD (2013) Resource translocation drives δ13C fractionation during recovery from
685 disturbance in giant kelp, Macrocystis pyrifera. J Phycol 49:811–815. doi:
686 10.1111/jpy.12099
687 Fox MD (2016) Biomass loss reduces growth and resource translocation in giant kelp
688 Macrocystis pyrifera. Mar Ecol Prog Ser 562:65–77. doi: 10.3354/meps11949
689
690 Foyer, C. H., & Shigeoka, S. (2011). Understanding oxidative stress and antioxidant functions
691 to enhance photosynthesis. *Plant physiology*, *155*(1), 93-100.
692 Gaillard J, Coulson T, FEsta-Bianchet M (2008) Recruitment. In: Jørgensen SE, Fath BD
693 (eds) Encyclopedia of Ecology. Elsevier Science, Copenhagen, pp 2982–2986
694 Geider RJ, Osborne BA (1992) Algal Photosynthesis: The Measurement of Algal Gas
695 Exchange. Chapmand and Hall, New York
696 Gerard VA (1982) Growth and Utilization of Internal Nitrogen Reserves by the Giant Kelp
697 Macrocystis pyrifera in a Low-Nitrogen Environment. Mar Biol 35:27–35.
698 Gerard VA (1986) Photosynthetic characteristics of giant kelp (Macrocystis pyrifera)
699 determined in situ. Mar Biol 90:473–482. doi: 10.1007/BF00428571
700 Graham MH (1996) Effect of high irradiance on recruitment of the giant kelp Macrocystis
701 (Phaeophyta) in shallow water. J Phycol 32:903–906. doi: 10.1111/j.0022-
702 3646.1996.00903.x
703 Gouvêa, LP., Schubert, N., Martins, C.D.L., Sissini, M., Ramlov, F., Rodrigues,
704 E.R.D.O.,Bastos, E.O., Freire, V.C., Maraschin, M., Simonassi, J.C., Varela, D.A., Franco,
705 D.,Cassano, V., Fonseca, A.L., Barufi, J.B., Horta, P.A., 2017. Interactive effects of marine

706 heatwaves and eutrophication on the ecophysiology of a widespread and ecologically
707 important macroalga. Limnol. Oceanogr. 62, 2056–2075.
708 Gutiérrez JL, Jones CG, Byers JE, Arkema KK, Berkenbusch K, Commito JA, Duarte CM,
709 Hacker SD, Lambrinos JG, Hendriks IE, Hogarth PJ, Palomo MG, Wild C (2011)
710 Physical Ecosystem Engineers and the Functioning of Estuaries and Coasts. In:
711 Wolanski E, McLusky DS (eds) Treatise on Estuarine and Coastal Science. Academic
712 Press, Waltham, pp 53–82
713 Hargrave, M. S., Foggo, A., Pessarrodona, A., & Smale, D. A. (2017). The effects of
714 warming on the ecophysiology of two co-existing kelp species with contrasting
715 distributions. *Oecologia*, *183*(2), 531-543.
716 Harley CDG, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH
717 (2012) Effects of climate change on global seaweed communities. J Phycol 48:1064–
718 1078. doi: 10.1111/j.1529-8817.2012.01224.x
719 Hernádez-Carmona G (1989) Evaluation of Macrocystis pyrifera (Phaeophyta,
720 Laminariales) kelp beds in Baja California, Mexico. II. Spring 1986. Ciencias Mar
721 15:117–140. doi: 10.7773/cm.v15i4.665
722 Hernandez-Carmona G, Garcia O, Robledo D, Foster M (2000) Restoration techniques for
723 Macrocystis pyrifera (Phaeophyceae) populations at the southern limit of their
724 distribution in Mexico. Bot Mar 43:273–284. doi: 10.1515/BOT.2000.029
725 Holbrook NJ, Scannell HA, Sen Gupta A, Benthuysen JA, Feng M, Oliver ECJ, Alexander
726 L V., Burrows MT, Donat MG, Hobday AJ, Moore PJ, Perkins-Kirkpatrick SE, Smale
727 DA, Straub SC, Wernberg T (2019) A global assessment of marine heatwaves and
728 their drivers. Nat Commun 10:1–13. doi: 10.1038/s41467-019-10206-z

Hodges, D. M., DeLong, J. M., Forney, C. F. & Prange, R. K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, *207*: 604-11.

Huppe, H.C., Turpin, D.H., 1994. Integration of carbon and nitrogen metabolism in plant and algal cells. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 45:577–607.

Jackson GA (1977) Nutrients and production of giant kelp off southern california.pdf. Limnol Oceanogr 22:979–995.

Jueterbock, A., Kollias, S., Smolina, I., Fernandes, J. M., & Coyer, J. A. (2014). Thermal stress resistance of the brown alga Fucus serratus along the North-Atlantic coast: Acclimatization potential to climate change. *Marine Genomics*, *13*, 27-36.

Kopczak, C. D., Zimmerman, R. C., & Kremer, J. N. (1991). Variation in nitrogen physiology and growth among geographically isolated populations of the giant kelp, macrocystis pyrifera (phaeophyta) 1. *Journal of phycology*, *27*(2), 149-158.

Krumhansl KA, Okamoto DK, Rassweiler A, Novak M, Bolton JJ, Cavanaugh KC, Connell SD, Johnson CR, Konar B, Ling SD, Micheli F, Norderhaug KM, Pérez-Matus A, Sousa-Pinto I, Reed DC, Salomon AK, Shears NT, Wernberg T, Anderson RJ, Barrett NS, Buschmann AH, Carr MH, Caselle JE, Derrien-Courtel S, Edgar GJ, Edwards M, Estes JA, Goodwin C, Kenner MC, Kushner DJ, Moy FE, Nunn J, Steneck RS, Vásquez J, Watson J, Witman JD, Byrnes JEK (2016) Global patterns of kelp forest change over the past half-century. Proc Natl Acad Sci 113:13785–13790. doi: 10.1073/pnas.1606102113

752 Ladah LB, Filonov A, Lavín MF, Leichter JJ, Zertuche-González JA, Pérez-Mayorga DM
753 (2012) Cross-shelf transport of sub-thermocline nitrate by the internal tide and rapid
754 (3–6h) incorporation by an inshore macroalga. Cont Shelf Res 42:10–19. doi:
755 10.1016/j.csr.2012.03.010
756 Larkum, A.W.D., Drew, E.A., Ralph, P.J., 2006. Photosynthesis and metabolisms in
757 seagrasses at the cellular level. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.),
758 Seagrasses: Biology, Ecology and Conservation, pp. 323-345. The Netherlands.
759 Lintenthaler H, Babani F, Langdorf G, Buschmann A (2000) Measurement of differences in
760 red chlorophyll fluorescence and photosynthetic activity between sun and shade leaves
761 by fluorescence imaging. Photosynthetica 38:521–529.
762 Lonhart SI, Jeppesen R, Beas-luna R, Crooks JA, Lorda J (2019) Shifts in the distribution
763 and abundance of coastal marine species along the eastern Pacific Ocean during
764 marine heatwaves from 2013 to 2018. 8:1–15.
765 Lohrmann, N. L., Logan, B. A., & Johnson, A. S. (2004). Seasonal acclimatization of
766 antioxidants and photosynthesis in Chondrus crispus and Mastocarpus stellatus, two co-
767 occurring red algae with differing stress tolerances. *The Biological Bulletin*, *207*(3), 225-
768 232.
769 Mabin CJT, Johnson CR, Wright JT (2019) Physiological response to temperature, light,
770 and nitrates in the giant kelp Macrocystis pyrifera from Tasmania, Australia. Mar Ecol
771 Prog Ser 614:1–19. doi: 10.3354/meps12900
772 Marba N, Duarte CM (2009) Mediterranean warming triggers seagrass (Posidonia
773 oceanica) shoot mortality. Glob Chang Biol 16:2366–2375. doi: 10.1111/j.1365-
774 2486.2009.02130.x

775 Marín-Guirao, L., Ruiz, J. M., Dattolo, E., Garcia-Munoz, R., & Procaccini, G. (2016).
776 Physiological and molecular evidence of differential short-term heat tolerance in
777 Mediterranean seagrasses. *Scientific reports*, *6*, 28615.
778 Martínez B, Arenas F, Rubal M, Burgués S, Esteban R, García-Plazaola I, Figueroa FL,
779 Pereira R, Saldaña L, Sousa-Pinto I, Trilla A, Viejo RM (2012) Physical factors
780 driving intertidal macroalgae distribution: physiological stress of a dominant fucoid at
781 its southern limit. Oecologia 170:341–353. doi: 10.1007/s00442-012-2324-x
782 Martínez, B., Viejo, R. M., Carreño, F., & Aranda, S. C. (2012). Habitat distribution
783 models for intertidal seaweeds: responses to climatic and non- climatic drivers. *Journal of*
784 *biogeography*, *39*(10), 1877-1890.
785 Merzouk A, Johnson LE (2011) Kelp distribution in the northwest Atlantic Ocean under a
786 changing climate. J Exp Mar Bio Ecol. doi: 10.1016/j.jembe.2011.02.020
787 Miller RJ, Lafferty KD, Lamy T, Kui L, Rassweiler A, Reed DC (2018) Giant kelp,
788 Macrocystis pyrifera , increases faunal diversity through physical engineering. Proc R
789 Soc B Biol Sci 285:20172571. doi: 10.1098/rspb.2017.2571
790 Mohring MB, Kendrick GA, Wernberg T, Rule MJ, Vanderklift MA (2013) Environmental
791 Influences on Kelp Performance across the Reproductive Period: An Ecological
792 Trade-Off between Gametophyte Survival and Growth? PLoS One. doi:
793 10.1371/journal.pone.0065310
794 O'Connor KC, Anderson TW (2010) Consequences of habitat disturbance and recovery to
795 recruitment and the abundance of kelp forest fishes. J Exp Mar Bio Ecol 386:1–10.
796 doi: 10.1016/j.jembe.2010.01.016
797 Pecl GT, Araújo MB, Bell JD, Blanchard J, Bonebrake TC, Chen IC, Clark TD, Colwell

798 RK, Danielsen F, Evengård B, Falconi L, Ferrier S, Frusher S, Garcia RA, Griffis RB,
799 Hobday AJ, Janion-Scheepers C, Jarzyna MA, Jennings S, Lenoir J, Linnetved HI,
800 Martin VY, McCormack PC, McDonald J, Mitchell NJ, Mustonen T, Pandolfi JM,
801 Pettorelli N, Popova E, Robinson SA, Scheffers BR, Shaw JD, Sorte CJB, Strugnell
802 JM, Sunday JM, Tuanmu MN, Vergés A, Villanueva C, Wernberg T, Wapstra E,
803 Williams SE (2017) Biodiversity redistribution under climate change: Impacts on
804 ecosystems and human well-being. Science (80-). doi: 10.1126/science.aai9214
805 Rasheed M, Unsworth R (2011) Long-term climate-associated dynamics of a tropical
806 seagrass meadow: implications for the future. Mar Ecol Prog Ser 422:93–103. doi:
807 10.3354/meps08925
808 Rivers, j. s., & Peckol, p. (1995). Summer decline of ulva lactuca (chlorophyta) in a
809 eutrophic embayment: interactive effects of temperature and nitrogen availability
810 1. *journal of phycology*, *31*(2), 223-228.
811 Rothäusler E, Gómez I, Hinojosa IA, Karsten U, Miranda L, Tala F, Thiela M (2011) Kelp
812 rafts in the humboldt current: Interplay of abiotic and biotic factors limit their floating
813 persistence and dispersal potential. Limnol Oceanogr 56:1751–1763. doi:
814 10.4319/lo.2011.56.5.1751
815 Schreiber, U. (2004). Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse
816 method: an overview. In *Chlorophyll a fluorescence* (pp. 279-319). Springer,
817 Dordrecht.
818 Seely, G. R., Duncan, M. J. & Vidaver, W. E. 1972. Preparative and analytical extraction of
819 pigments from brown algae with dimethyl sulfoxide. *Mar Biol*: *12*:184-8.

Sharkey, T. D. (2005). Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant, Cell & Environment*, *28*(3), 269-277.

Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ (2013) Threats and knowledge gaps for ecosystem services provided by kelp forests: A northeast Atlantic perspective. Ecol Evol 3:4016–4038. doi: 10.1002/ece3.774

Smale DA, Wernberg T, Oliver ECJ, Thomsen M, Harvey BP, Straub SC, Burrows MT, Alexander L V, Benthuysen JA, Donat MG, Feng M, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Sen Gupta A, Payne BL, Moore PJ (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat Clim Chang 9:306–312. doi: 10.1038/s41558-019-0412-1

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ Conserv 29:436–459. doi: 10.1017/S0376892902000322

Stephens, T. A., & Hepburn, C. D. (2016). A kelp with integrity: Macrocystis pyrifera prioritises tissue maintenance in response to nitrogen fertilisation. *Oecologia*, *182*(1), 71-84.

Sunday JM, Pecl GT, Frusher S, Hobday AJ, Hill N, Holbrook NJ, Edgar GJ, Stuart-Smith R, Barrett N, Wernberg T, Watson RA, Smale DA, Fulton EA, Slawinski D, Feng M, Radford BT, Thompson PA, Bates AE (2015) Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. Ecol Lett 18:944–953. doi: 10.1111/ele.12474

Tait LW, Schiel DR (2013) Impacts of Temperature on Primary Productivity and Respiration in Naturally Structured Macroalgal Assemblages. PLoS One 8:1–10. doi: 10.1371/journal.pone.0074413

Umanzor S, Ramirez M mar, Sandoval-Gil JM, Zertuche-González JA (2019a) Photoacclimation and photoprotection of juvenile sporophytes of Macrocystis pyrifera (Laminariales, Phaeophyceae) under high-light conditions during short-term shallow-water cultivation.

Umanzor S, Ladah L, Calderon-Aguilera LE, Zertuche-González JA (2019b) Testing the relative importance of intertidal seaweeds as ecosystem engineers across tidal heights. J Exp Mar Bio Ecol 511:100–107. doi: 10.1016/j.jembe.2018.11.008

Valdez, M. C., Zaragoza, E. S., Belda, D. L., Marcos, R., & Ramírez, R. A. (2003). Effect of climatic change on the harvest of the kelp Macrocystis pyrifera on the Mexican Pacific Coast. *Bulletin of Marine Science*, *73*(3), 545-556.

Vásquez JA, Zuñiga S, Tala F, Piaget N, Rodríguez DC, Vega JMA (2013) Economic valuation of kelp forests in northern Chile: values of goods and services of the ecosystem. J Appl Phycol 26:1081–1088. doi: 10.1007/s10811-013-0173-6

Vergés A, Steinberg PD, Hay ME, Poore AGB, Campbell AH, Ballesteros E, Heck KL, Booth DJ, Coleman MA, Feary DA, Figueira W, Langlois T, Marzinelli EM, Mizerek T, Mumby PJ, Nakamura Y, Roughan M, van Sebille E, Gupta A Sen, Smale DA, Tomas F, Wernberg T, Wilson SK, Soc PR, Roughan SKW, van Sebille E, Gupta A Sen, Smale DA, Tomas F, Wernberg T, Langlois T, Marzinelli EM, Mizerek T, Mumby PJ, Nakamura Y, Ballesteros M, Heck KL, Booth DJ, Coleman MA, Feary DA, Figueira W, Vergés A, Steinberg PD, Hay ME, Poore AGB, Campbell AH,

Ballesteros E, Heck Jr KL, Roughan M (2014) The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. Proc R Soc B 281:1–10. doi: 10.1098/rspb.2014.0846

Wernberg T, Thomsen MS, Tuya F, Kendrick GA, Staehr PA, Toohey BD (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: Potential implications for a warmer future. Ecol Lett 13:685–694. doi: 10.1111/j.1461-0248.2010.01466.x

Wheeler PA, North WJ (1980) Effect of Nitrogen Supply on Nitrogen Content and Growth Rate of Juvenile Macrocystis Pyrifera (Phaeophyta) Sporophytes. J. Phycol. 16:577–582.

Wheeler WN (1980) Pigment content and photosynthetic rate of the fronds of Macrocystis pyrifera. Mar Biol 56:97–102. doi: 10.1007/BF00397127

Zimmerman R, Kremer J (1986) In situ growth and chemical composition of the giant kelp, Macrocystis pyrifera: response to temporal changes in ambient nutrient availability. Mar Ecol Prog Ser 27:277–285. doi: 10.3354/meps027277

Zimmerman RC, Robertson DL (1985) ODU Digital Commons Effects of El Nino on Local Hydrography and Growth of the Giant Kelp, Macrocystis Pyrifera, at Santa Catalina Island, California Repository Citation. Limnol Oceanogr 30:1298–1302. doi: 10.4319/lo.1985.30.6.1298