Pilar De Pablo Tobajas

Light dependence of seagrass upper thermal thresholds

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2023/2024

Pilar De Pablo Tobajas

Light dependence of seagrass upper thermal thresholds

Mestrado em Biologia Marinha

Supervisors:
Nuria Marbà Bordalba
Andrea Anton Gamazo

Co-supervisor
João Silva

UNIVERSIDADE DO ALGARVE

Faculdade de

Ciências e Tecnologia

2023/2024

Abstract

As ervas marinhas representam um dos ecossistemas bentónicos costeiros mais produtivos e diversos do mundo. O seu grande valor ecológico e económico está associado a numerosos serviços ecossistémicos, que incluem a proteção costeira contra a erosão, áreas de berçário para espécies de peixes comerciais, filtração da água, circulação de nutrientes e sumidouros globais de carbono. Apesar do reconhecimento da sua importância, as pradarias marinhas estão altamente ameaçadas, com taxas de declínio global anual estimadas entre 2% a 5%. Os principais fatores atuais que potenciam este declínio incluem as atividades antropogénicas e as alterações climáticas, particularmente as ondas de calor no verão.

As projeções globais sugerem que as temperaturas da superfície do mar podem aumentar até 2°C até 2100 em muitas regiões, acompanhadas por um aumento na frequência, intensidade e duração de eventos térmicos extremos. Este aquecimento da coluna de água pode resultar em mudanças na distribuição das espécies em direção aos polos, eventos massivos de mortalidade, fenómenos de tropicalização das regiões temperadas, assim como migrações verticais das espécies para alcançar ambientes mais profundos e frios, que estão mais próximos das suas temperaturas ótimas. No entanto, em habitats mais profundos, a disponibilidade de luz pode ser considerada um fator limitante que determina o limite inferior dos produtores primários. A intensidade da luz diminui exponencialmente com a profundidade devido aos processos de absorção e dispersão. No caso das ervas marinhas, os seus requisitos mínimos de luz são mais elevados em comparação com outras plantas com flores, devido à sua grande quantidade de biomassa subterrânea, que inclui os rizomas e as raízes. Portanto, a extensão de profundidade para o crescimento das ervas marinhas depende da interação entre a disponibilidade de luz e o stress térmico.

Ambos os fatores abióticos afetam a produtividade das plantas, uma vez que o balanço de carbono é determinado pelo carbono fixado durante a fotossíntese, pelo carbono consumido durante a respiração e pela alocação de carbono armazenado. Um balanço de carbono positivo permite às plantas responder ao stress e sustentar o seu crescimento, reprodução e sobrevivência. Por outro lado, sob temperaturas elevadas ou condições limitadas de luz, o carbono fotossintético fixado não consegue satisfazer as exigências respiratórias da planta, resultando num desequilíbrio metabólico que leva ao

esgotamento do carbono, à redução do crescimento e, em última instância, pode levar à morte da planta.

Os estudos que investigam a interação entre a luz e a temperatura são limitados, sendo ainda mais raros os que analisam os efeitos da disponibilidade de luz no desempenho térmico das plantas (TPC). As TPCs representam a sensibilidade térmica de um indivíduo, caracterizado pela taxa de alteração no desempenho fisiológico ao longo de um gradiente de temperaturas. As TPCs típicas caracterizam-se por um limite letal térmico inferior, seguido por um aumento exponencial até se atingir uma temperatura ótima, e finalmente por um declínio subsequente em direção ao limite letal térmico superior da espécie. A análise das TPCs permite a determinação de parâmetros térmicos, como as temperaturas ótimas e críticas superior e inferior, bem como a energia de activação e desativação das fases de aumento e de declínio da curva, respetivamente. No entanto, estes parâmetros térmicos podem variar entre populações devido a adaptações genéticas, plasticidade fenotípica ou regulações fisiológicas a curto prazo. Estes parâmetros são ecologicamente relevantes, pois permitem comparações da sensibilidade ao aquecimento entre espécies e populações sob diferentes condições ambientais, como a luz.

O presente estudo examina três espécies de ervas marinhas do Mar Mediterrâneo (*Cymodocea nodosa*, *Posidonia oceanica* e *Zostera noltei*). Foi conduzido um modelo experimental de mesocosmo, onde as plantas foram expostas a diferentes tratamentos de temperatura, entre 16ºC e 40ºC, dependendo da espécie estudada. A intensidade da luz foi usada como um indicador de profundidade, aplicando-se dois tratamentos de luz: Alta Luz (191 μmol m^{-2} s^{-1}) e Baixa Luz (55 μmol m^{-2} s^{-1}), que simulam as condições ambientais próximas ao limite superior e inferior de profundidade dos prados. No final do plano experimental, as taxas de crescimento foram medidas para estabelecer um TPCs, com o objetivo de calcular os parâmetros térmicos teóricos (temperatura ótima e crítica superior, bem como a energia de desativação), e as taxas de sobrevivência foram medidas para determinar o limite térmico letal da população (L_{T50}). Adicionalmente, as taxas metabólicas foram medidas para uma das espécies (*Z. noltei*) para se compreenderem respostas fisiológicas adicionais das ervas marinhas, incluindo a Produção Primária Líquida (NPP), Produção Primária Bruta (GPP) e respectivas taxas de Respiração. Este passo permitiu-nos avaliar se as ervas marinhas sob diferentes condições de luz e, por conseguinte, a diferentes profundidades, podem tolerar limites térmicos semelhantes e apresentar sensibilidades térmicas comparáveis, bem como respostas de crescimento e metabólicas face ao aumento das temperaturas.

Os nossos resultados demonstraram limites térmicos superiores semelhantes, incluindo temperaturas ótimas, críticas superiores e letais, bem como energias de desativação entre os tratamentos de luz para as três espécies. No entanto, observaram-se diferenças significativas nas curvas de desempenho de crescimento e nas respostas metabólicas, com valores mais baixos sob condições de baixa luz. O NPP apresentou um padrão semelhante às curvas de crescimento, enquanto o GPP demonstrou uma correlação negativa com o aumento da temperatura, enquanto as taxas de respiração não apresentaram nenhuma tendência clara. Adicionalmente, a temperatura à qual o sistema de ervas marinhas passa de autotrófico para heterotrófico foi significativamente superior sob condições de alta luz. Entre as espécies estudadas, *C. nodosa* exibiu limites térmicos mais elevados e uma capacidade de manter a taxa de crescimento sob condições de menor luz, sugerindo que esta espécie poderia ser uma boa candidata para ocupar o Mar Mediterrâneo, enquanto outras espécies de zonas temperadas já sofrem um declínio progressivo e poderão migrar para habitats mais profundos em cenários de aquecimento. Foram também obtidos limites térmicos inesperados para *P. oceanica* em comparação com pesquisas anteriores. Os limites térmicos mais elevados podem ser atribuídos à adaptação térmica dos clones para mitigar os efeitos do aumento das temperaturas. No caso de *C. nodosa*, observaram-se diferentes limites térmicos devido à possível diferenciação genética entre as populações do Mar Mediterrâneo e do Oceano Atlântico, facilitando a existência de ecótipos locais.

Em conclusão, os nossos resultados sugerem que as pradarias marinhas profundas também podem ser vulneráveis ao aquecimento. Estas pradarias tendem a crescer em densidades mais baixas, apresentando menor capacidade de recuperação em condições desfavoráveis e podendo tornar-se em sistemas heterotróficos a temperaturas mais baixas. Como resultado, prevê-se uma contração da gama de profundidade adequada, em que as populações de águas rasas serão expostas a temperaturas acima dos seus limites térmicos, enquanto as populações profundas lutarão para manter a viabilidade do sistema. Estes resultados podem ser extrapolados para outros produtores primários, como corais e comunidades de macroalgas. É necessário reforçar a necessidade para estudos de campo de modo validar os limites térmicos obtidos e fornecer previsões mais precisas sobre a

distribuição vertical futura das pradarias de ervas marinhas em cenários de alterações climáticas.

Palavras-chave: ervas marinhas – profundidade – ondas de calor marinhas – crescimento – sobrevivência – metabolismo

Abstract:

In warming scenarios, many primary producers tend to migrate vertically to cooler habitats. However, light-limited conditions in these deeper ecosystems can impact on the thermal tolerance of species. This study focuses on three key seagrass species in the Mediterranean Sea (*Cymodocea nodosa*, *Posidonia oceanica* and *Zostera noltei*). Three mesocosm experiments were conducted, exposing the plants to various temperature treatments (ranging from 16ºC to 40ºC, depending on the species) for 10 to 20 days. Light intensity was used as a proxy of depth, applying two light treatments, which were archived though shading: High Light (191 μmol m^{-2} s^{-1}) and Low Light (55 μmol m^{-2} s^{-1}), simulating environmental conditions near their upper and lower depth limits. We measured growth and survival rates to calculate thermal thresholds (optimum, upper critical and lethal temperatures), and deactivation energy from a thermal performance curve. Additionally, metabolic rates (Net Primary Production, Gross Primary Production and Respiration) were measured for *Z. noltei*. We tested whether the plants at different light intensities, and consequently at varying depths, have similar upper thermal thresholds and optimal temperatures, as well as exhibit similar growth and metabolic responses throughout a thermal gradient. Upper thermal thresholds and deactivation energies were unaffected by light intensity; however, significant differences were observed in growth rates and metabolic responses, particularly in the transition to heterotrophic systems, with lower performance under low light conditions. Among the three species, *C. nodosa* demonstrated a higher capacity to thrive under warmer conditions and could survive in deeper environments. Moreover, unexpectedly high upper thermal limits were observed for *P. oceanica* when compared to previous studies, suggesting a possible thermal adaptation of clones to recent rising temperatures. Our findings indicate a habitat contraction of seagrass species is expected under future climate change scenarios, in which deep meadows may also be vulnerable to warming conditions.

Key-words: seagrass – depth - marine heat waves – growth – survival - metabolism

Acknowledgements

I would like to take this opportunity to express my deepest gratitude to everyone who has contributed, in one way or another, to the successful completion of this thesis.

I would like to extend my sincere thanks to my external supervisors, Dr. Núria Marbà and Dr. Andrea Anton, for giving me the opportunity to be part of this project. Your constructive feedback, patience, and insightful suggestions have been invaluable throughout the research process. Your attention to detail, guidance, and wealth of knowledge have not only helped shape this thesis but also fostered my personal growth as a researcher. I truly appreciate the time and effort you have invested in me.

I am also grateful to my internal supervisor, Dr. João Silva, for offering his guidance and expertise throughout this study.

To all the members of the Global Change Research Group, thank you for your warm welcome, kindness, and for creating a supportive environment in which I could develop as a scientist. Your shared advice and insightful discussions have significantly contributed to my progress. A special mention goes to the technicians—Lidia, Peru, Laura, Coral and Alex —whose assistance during the experiments was crucial. Without your help, the completion of this thesis would not have been possible.

Lastly, I want to express my heartfelt appreciation to my family and friends, whose unwavering moral support, patience, and encouragement carried me through the more challenging moments of this journey. Your faith in me, along with your advice and understanding, have been a constant source of strength.

The research of this master thesis has been funded by the projects OCEAN CITIZEN (Marine forest coastal restoration: an underwater gardening socio-ecological plan; European Union under the Horizon Europe program (grant agreement no. 101093910) and CYCLE (Complex DYnamics of CoastaL Ecosystems: Resilience to Climate Change; Spanish Research Agency, PID2021-123723OB-C21). The present research was carried out within the framework of the activities of the Spanish Government through the "Maria de Maeztu Centre of Excellence" accreditation to IMEDEA (CSIC-UIB) (CEX2021-001198).

Table of contents

Index of Figures

Chapter 1: General Introduction

Chapter 2: Light dependence of seagrass thermal thresholds

Chapter 3: Supplementary Materials

Index of Tables

Chapter 2: Light dependence of seagrass thermal thresholds

Chapter 3: Supplementary Materials

List of abbreviations

CI: Confidence Intervals

DO: Dissolved O_2

Eh: deactivation energy

GPP: Gross Primary Production

HL: High Light

MLR: Minimum Light Requirement

NPP: Net Primary Production

LL: Low Light

L_{T50}: Lethal Thermal Threshold

SI: Surface Irradiance

Tmax: Upper Critical Temperature

Topt: Optimal Temperature

TPC: Thermal Performance Curve

Chapter 1:

General Introduction

Seagrasses are aquatic angiosperms that complete their entire life-cycle while submerged in seawater (1). In order to survive to these environmental conditions, they have developed unique ecological, physiological, and morphological adaptations, such as internal gas transport, epidermal chloroplasts, submarine pollination, and marine dispersal (2,3). Despite these unique characteristics and a relatively low species diversity (60 species), seagrass have managed to colonize most of the planet (4).

Seagrasses are clonal plants composed of physiologically independent units, the ramets, but genetically identical to the parent, the genet. These plants can reproduce sexually and asexually, generating ramets though horizontal rhizome growth (4).

Ecosystem services

Seagrass meadows are recognized as some of the most productive coastal benthic ecosystems (5). Those species hold significant ecological and economic value due to the ecosystem services they provide (6). They function as bioengineers, modifying the environment in ways that positively influence the fitness of other species (7). Other services include protecting coastlines from erosion, trapping particles and water filtration, providing nursery areas for juvenile stages of commercially important species, supporting fisheries, playing a key role in nutrient cycle, enhancing oxygenation in marine sediments, being recognized as an important global sink of carbon, and providing physicalstructures that promote diversity and contribute primary and secondary production (8– 10).

Problems

Seagrass meadows are recognized as important ecosystems, however, they are among the most threatened ecosystems worldwide (11). Between 1879 and 2009, approximately 29% of the global seagrass ecosystem has been lost, and annual decline rates are estimated between 2% and 5% (12,13).

This decline can be attributed to an interaction between multiple stresses, including sediment and nutrient runoff, physical disturbance, invasive species, diseases, commercial fishing practices, aquaculture, overgrazing, algal blooms, and global warming (2). Nowadays, the rate of change observed in coastal waters is significantly faster than those experienced in the previous 100 million years of evolutionary history, leaving insufficient time for species to adapt to new environmental conditions (2). Therefore, the principal factors are anthropogenic activities and climate change.

The ocean possesses a high heat capacity, absorbing over 90% of excess heat. Global projections indicate that sea surface temperatures could increase by 2ºC by 2100 in many regions (14), as well as the increase of frequency, intensity and duration of extreme thermal events, such as heatwaves (15). Specifically, the Mediterranean Sea is particularly vulnerable due to its semi enclosed structure, resulting in a faster warming that the global average (16).

This warming influences the seagrass distribution, resulting in massive die-offs events, tropicalization and migration of species in some regions (15). For instance, the decline of *P. oceanica* meadows creates opportunities for other species, such as *C. nodosa* and the invasive *Halophila stipulacea* to settle down (17–19). However, these changes in biodiversity have impacts for ecosystem services, particularly the loss of the higher capacity of *P. oceanica* to sequester carbon in the marine sediments (12). Conversely, there are areas where the seagrass meadows would not be affected by climate change, whose increase of temperature is included within their thermal tolerance. For example, the subarctic seagrass species *Zostera marina*, which has an upper thermal limit of 25ºC, significantly higher than the seawater temperatures (9ºC to 15ºC) found in its habitat in in western Greenland (20).

To deal with this increase in temperature, seagrasses can response in different ways. On one hand, species can shift their distribution to migrate toward more suitable habitats, such as higher latitudes, or vertically to deeper ecosystems (21). On the other hand, these organisms can compensate these environmental changes by local adaptation, which result in genetic differentiation among populations, or phenotypic plasticity, without altering their genotype (22).

In light of the documented losses of seagrass, it is imperative to prioritize the protection, monitoring, management, and restoration of these vital ecosystems (2).

The study area

The mediterranean bioregion is characterized by a relatively diverse mix of temperate and tropical seagrass flora, including communities in northwest Africa, the Black Sea Basin and the Caspian and Aral Seas (3). Within this region, nine seagrass species can be found: *C. nodosa*, *P. oceanica*, *Ruppia cirrhosa*, *R. maritima*, *Z. marina*, *Zostera noltei*, *Halodule wrightii*, *H. decipiens* and *H. stipulacea* (4). It is important to consider that *P. oceanica* is an endemic species, whereas *H. stipulacea* is recognized as a Lessepsian invasive species (9).

Particularly, the ocean islands are home to a significant number of endemic species and unique biological communities, with several areas recognized as biodiversity hotspots, such as the Mediterranean Sea and the Canary Islands (9,23). Focusing on our study area, in the western Mediterranean, there is a characteristic ecosystem transition from *Z. noltei* communities to deeper meadows dominated by *C. nodosa* and *P. oceanica*. In contrast, the Canarian habitat is composed by *C. nodosa* and *H. decipiens* meadows (4). Therefore, our study involves three seagrass species, *C. nodosa*, *P. oceanica* and *Z. noltei*, which play an important role on the Mediterranean ecosystems, including the Balearic (in the Mediterranean Sea) and the Canary Islands (in the Atlantic Ocean).

Cymodocea nodosa

C. nodosa is a seagrass distributed across the Mediterranean Sea and along the adjacent eastern Atlantic coasts, including the Macaronesia archipelagos of the Canary Islands and Madeira (24). In the Canary Islands, this species plays a key role on the marine ecosystem in sandy bottom areas, functioning as a habitat engineer (25). It can befound developing monospecific meadows or coexisting with *H. decipiens* in muddy bottoms and with the macroalga *Caulerpa prolifera* in sandy areas (26). Its vertical distribution ranges from 2 to 35 m depth (26).

C. nodosa is considered as a “warm-temperate” species, with a tropical origin (4,5), as well as a pioneer species with a fast growing, able to colonize degraded areas, and characteristics of a eurybiotic species (27,28). Additionally, this species can survive under different light conditions, attributable to its photo-acclimative responses (29).

Posidonia oceanica

P. oceanica is recognized as an endemic species, constituting one of the most productive and valuable ecosystems in the Mediterranean Sea (16). This species inhabits sandy or rocky substrata, at depths ranging from 0.5 to 40 m (9).

This species is considered as a temperate seagrass, with a high vulnerability to thermal stress (16). It occupies more stable environmental conditions, more adapted to low light conditions (30), and demonstrates stenobiotic behaviour (27).

Due to its slow growing and limited sexual reproduction, taking centuries to millennia to develop, and recover from decline, *P. oceanica* is exceptionally vulnerable and have suffered significant diebacks due to the impacts of anthropogenic activities and climate change (12).

Zostera noltei

Z. noltei plays a crucial role as a primary producer in Atlantic coastal ecosystems from Mauritania to southern Norway and the Mediterranean Sea (31). This species colonises muddy sand and mud bottoms, primarily within the intertidal region of bays and lagoons along the coastline, typically at depths ranging from 0 to 5 m (28,32). Its distribution enables it to resist extreme fluctuations in environmental conditions, including desiccations, photodamage events, and fluctuations in temperature and salinity conditions (1,33). In the Mediterranean Sea, *Z. noltei* can be found forming monospecific meadows or coexisting in mixed populations with *C. nodosa* and *Z. marina* (32).

Furthermore, it is considered as a cold-temperate species (9), while its distribution also allows them to be adapted to high light availability (1).

Plant productivity

Seagrass productivity depends on the carbon balance of the plant, determined by the rate of photosynthesis, the availability of inorganic carbon, and the respiration rates of both photosynthetic and non-photosynthetic tissues. Therefore, the ratio between the carbon fixed in photosynthesis and the consumption of organic carbon plays an important role on the stress response of the plant (34). A positive carbon balance maintains growth, reproduction and survival (1,11).

Conversely, a negative carbon balance, caused by a reduction on the photosynthesis or rising temperatures, lead the remobilization of stored carbohydrates to maintain the respiratory and growth demands (35). Below-ground biomass, including the rhizomes and the roots, plays a crucial role in the accumulation of excess fixed carbon during the summer period (36,37). In response to unfavourable conditions, such as winter seasons, these reserves support the growth, and respiration demands until the next growing season (38). For instance, larger plants, such as *P. oceanica*, possess a high below-ground biomass, which enhances their storage capacity and enables them to survive periods of reduced light availability (37).

Productivity is influenced by both biotic and abiotic factors that affect the plant metabolism, including light availability, temperature and inorganic nutrients (5).

Effect of temperature

Temperature can influence metabolism and enzyme activity of seagrasses, including the diffuse transport of gases and solutes, the enzymatic capacity of RuBisCO, as well as the photosynthetic and respiration rates (39,40).

Both photosynthetic and respiration rates tend to rise with warming temperatures, up to a physiological optimum (5). However, it is important to note that the photosynthesis apparatus exhibits a high sensitivity to temperature, particularly in relation to photosystem II (41). Consequently, respiration rates continue to increase, even after the photosynthesis exhibits a sharp decline (42). This increase in respiration is attributable to the deactivation of metabolic processes, as well as the denaturation of crucial macromolecules, such as proteins, in response to warmer temperatures (40). As a result, the amount of fixed carbon produced through photosynthesis is not enough to balance the respiration demands, leading to reduced seagrass growth and remobilization of carbon reserves (42,43).

Additionally, this difference between photosynthesis and respiration contributes to a transition in the seagrass meadows from an autotrophic system (NPP > 1, P:R > 1) to a heterotrophic system (NPP < 0, P:R < 1). This shift result in the meadows to act as sources of CO_2 sources and sinks of O_2 and organic matter (44,45). Consequently, this lead to a reduction in oxygen concentration in the water column, which can promote the production of sulphide in the rhizosphere, and potentially increase the risk of seagrass mortality (46).

To reduce those heat stress impacts, the seagrasses have developed acclimatory responses of the photosynthesis and respiratory systems (27). The thermal sensitivity of an organism is defined by the rate of change in physiological performance in response to a temperature fluctuation (47). This biological performance is characterized by a lower lethal thermal limit, an exponential increase until reaching a maximum activity at the optimum temperature and a sharp decline upon reaching the upper thermal limit (48) (Figure 1.1).

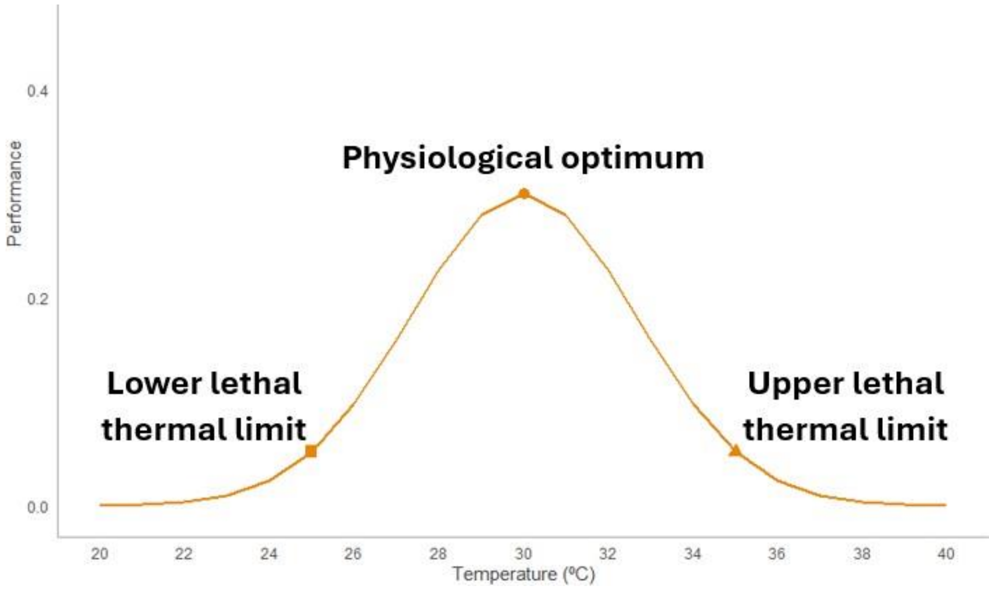

Figure 1.1. Performance curve along a range of temperatures, in which the lower lethal thermal limit, the physiological optimum and the upper lethal thermal limit are indicated.

The responses to warming depend on the thermal tolerance range of the species and populations (49), which is particularly important for those that inhabit environments near their thermal limits (50). Moreover, this thermal tolerance is linked to a species' biogeographical distribution (48). For temperate species, the optimal growth temperature ranges from 11.5ºC to 26ºC, while tropical/subtropical species exhibit an optimal temperature range of 23ºC to 32ºC (5). Consequently, tropical species appear to be better adapted to high temperatures (30).

Effect of light

The abundance, growth and distribution of seagrass exhibit a high sensitive to the light availability (38). The minimum light requirements (MLR) determine the critical light availability necessary for survival, whose values are calculated at the maximum colonization depth (51). Compared to other flowering plants, seagrasses have a relatively high MLR due to the respiratory demand associated with their high amount of below-ground biomass (11). The MLR varies among species due to unique adaptations, as well as within species, as a result of photo-acclimation responses (5).

This photo-acclimation adaptations allow the regulation of the efficiency of the photosynthetic activity, as well as the respiratory demands to maintain a positive carbon balance under a wide range of light habitat (22,52,53). Under adverse conditions, such as light reduction, plants initially respond through physiological adjustment; however, if the stress event persists or intensifies, morphological changes may occur (54). Key adjustments include the improvement of light harvesting capabilities by increasing chlorophyll concentration, improving photosynthesis efficiency, modifying leaf morphology to maximize the light capture, and reducing shoot size and density to minimize the respiratory demands, as well as mitigate self-shading. Additionally, stored carbohydrates serve as an alternative source of fixed carbon to support growth and survival (52, 55, 56).

Consequently, the light availability represents a crucial limiting factor for plant distribution (57). A reduction in light or variations in water optical properties can compromise seagrass survival, particularly under eutrophication events (58), as well as plants that inhabit at the lower limit of the meadow (37). Underwater light intensity is attenuated exponentially with water depth due to absorption and scattering processes that involve dissolved substrates, phytoplankton, particulate matter and water itself (5). The seagrass vertical limit depends on the light penetration and its extinction coefficient (57), ranging from intertidal zones to depths of 90 m, depending on the availability of enough light to support seagrass metabolism (50).

Therefore, plants have developed adaptations to cope specific light conditions at different depths. On one hand, shallow plants suffer warmer and more unstable thermal environments alongside higher light availability. As a result, these plants demonstrate photo-acclimative, photoprotective and reparative responses, as well as enhanced physiological heat acclimation capacities compared to deeper individuals of the same meadow. On other hand, deep plants are adapted to low light environments (59, 60).

References

1. Hemminga MA, Duarte C M. 2009 Seagrass Ecology. *Cambridge University Press*. **1**, 99–145. (doi:10.1017/CBO9780511525551)
2. Orth R J, Carruthers T J B, Dennison W C, Duarte C M, Fourqurean J W, Heck Jr K L, Hughes A R, Kendrick G A, Kenworthy W J, Olyarnik S, *et al.* 2006 A Global Crisis for Seagrass Ecosystems. *BioScience*. **56**, 987–996. (doi: 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2)
3. Spalding M, Taylor M, Ravilious C, Short F, Green E. 2003 The distribution and status of seagrasses. *World Atlas Seagrasses*. 5–26.
4. Short F, Carruthers T, Dennison W, Waycott M. 2007 Global seagrass distribution and diversity: A bioregional model. *J Exp Mar Biol Ecol*. **350**, 3–20. (doi:10.1016/j.jembe.2007.06.012)
5. Lee K S, Park S R, Kim Y K. 2007 Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *J Exp Mar Biol Ecol*. **350**, 144–175. (doi:10.1016/j.jembe.2007.06.016)
6. Cullen-Unsworth L, Unsworth R. 2013 Seagrass Meadows, Ecosystem Services, and Sustainability. *Environ Sci Policy Sustain Dev*. **55**, 14–28. (doi:10.1080/00139157.2013.785864)
7. Bruno J F, Bertness M D. 2001 Habitat modification and facilitation in benthic marine communities. In Bertness MD, Gaines SD, Hay ME (eds) *Marine Community Ecology*, pp. 201–218. Sunderland, MA: Sinauer Associates.
8. Duffy J E. 2006 Biodiversity and the functioning of seagrass ecosystems. *Mar Ecol Prog Ser*. **311**, 233–250. (doi:10.3354/meps311233)
9. Ruíz J M, Boudouresque C F, Enríquez S. 2009 Mediterranean seagrasses. *Bot Mar*. **52**, 369–381. (doi:10.1515/BOT.2009.058)
10. Unsworth R K F, Cullen-Unsworth L C, Jones B L H, Lilley R J. 2022 The planetary role of seagrass conservation. *Science*. **377**, 609–613. (doi:10.1126/science.abq6923)
11. Said N E, McMahon K, Lavery P S. 2021 Accounting for the influence of temperature and location when predicting seagrass (*Halophila ovalis*) photosynthetic performance. *Estuar Coast Shelf Sci*. **257**, 107414. (doi:10.1016/j.ecss.2021.107414)
12. Marbà N, Duarte C M. 2010 Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Glob Change Biol*. **16**, 2366–2375. (doi:10.1111/j.1365-2486.2009.02130.x)

13. Waycott M, Duarte C M, Carruthers T J B, Orth R J, Dennison W C, Olyarnik S, Calladine A, Fourqurean J W, Heack Jr K L, Hughes A R, *et al.* 2009 Accelerating lossof seagrasses across the globe threatens coastal ecosystems. *Proc Natl Acad Sci USA*. **106**, 12377–12381. (doi:10.1073/pnas.0905620106)

14. Calvin K, Dasgupta D, Krinner G, Mukherji A, Thorne P W, Trisos C, Brovkin V, Engström K, Fishman T, Jones C, Le Quéré C. 2023 IPCC: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland. Available at: https://www.ipcc.ch/report/ar6/syr/.

15. Nguyen H M, Ralph P J, Marín-Guirao L, Pernice M, Procaccini G. 2021 Seagrasses in an era of ocean warming: a review. *Biol Rev*. **96**, 2009–2030. (doi:10.1111/brv.12736)

16. Chefaoui R M, Duarte C M, Serrão E A. 2018 Dramatic loss of seagrass habitat under projected climate change in the Mediterranean Sea. *Glob Change Bio*l. **24**, 4919–4928. (doi:10.1111/gcb.14401)

17. Beca-Carretero P, Rotini A, Mejia A, Migliore L, Vizzini S, Winters G. 2020 *Halophila stipulacea* descriptors in the native area (Red Sea): A baseline for future comparisons with native and non-native populations. *Mar Environ Res*. **153**, 104828. (doi:10.1016/j.marenvres.2019.104828)

18. Boudouresque C F, Bernard G, Pergent G, Shili A, Verlaque M. 2009 Regression of Mediterranean seagrasses caused by natural processes and anthropogenic disturbances and stress: a critical review. *Bot Mar*. **52**, 395–418. (doi:10.1515/BOT.2009.057)

19. Wesselmann M, Chefaoui R M, Marbà N, Serrao E A, Duarte C M. 2021 Warming Threatens to Propel the Expansion of the Exotic Seagrass *Halophila stipulacea*. *Front Mar Sci*. **8,** 759676**.** (doi:10.3389/fmars.2021.759676)

20. Marbà N, Jordà G, Bennett S, Duarte C M. 2022 Seagrass Thermal Limits and Vulnerability to Future Warming. *Front Mar Sci*. **9**, 860826**. (**doi:10.3389/fmars.2022.860826)

21. Jorda G, Marbà N, Bennett S, Santana-Garcon J, Agusti S, Duarte C M. 2020 Ocean warming compresses the three-dimensional habitat of marine life. *Nat Ecol Evol*. **4**, 109–114. (doi:10.1038/s41559-019-1058-0)

22. Dattolo E, Marín-Guirao L, Ruiz J M, Procaccini G. 2016 Long-term acclimation to reciprocal light conditions suggests depth-related selection in the marine foundation species *Posidonia oceanica*. *Ecol Evol*. **7**, 1148–64. (doi:10.1002/ece3.2731)

23. Riera R, Becerro M A, Stuart-Smith R D, Delgado J D, Edgar G J. 2014 Out of sight, out of mind: Threats to the marine biodiversity of the Canary Islands (NE Atlantic Ocean). *Mar Pollut Bull*. **86**, 9–18. (doi:10.1016/j.marpolbul.2014.07.014)

24. Fabbri F, Espino F, Herrera R, Moro L, Haroun R, Riera R, González-Henriquez N, Bergasa O, Monterroso O, Ruiz de la Rosa M, *et al.* 2015 Trends of the seagrass *Cymodocea nodosa* (Magnoliophyta) in the Canary Islands: Population changes in the last two decades. *Sci Mar*. **79**, 7-13. (doi:10.3989/scimar.04165.19B)

25. Reyes J, Sansón M, Afonso-Carrillo J. 1995 Distribution and reproductive phenology of the seagrass *Cymodocea nodosa* (Ucria) Ascherson in the Canary Islands. *Aquat Bot*. **50**, 171–180. (doi:10.1016/0304-3770(95)00451-5)

26. Pavón-Salas N, Herrera R, Hernández-Guerra A, Haroun R. 2000 Distributional Pattern of Seagrasses in the Canary Islands (Central-East Atlantic Ocean). *J Coast Res*. **16**, 329–335.

27. Marín-Guirao L, Ruiz J M, Dattolo E, Garcia-Munoz R, Procaccini G. 2016 Physiological and molecular evidence of differential short-term heat tolerance in Mediterranean seagrasses. *Sci Rep*. **6**, 28615. (doi:10.1038/srep28615)

28. Rodriguez C, Ballesteros E. 2013 Guía de las macroalgas y fanerógamas marinas del Mediterráneo Occidental. Barcelona, Spain: Omega.

29. Silva J, Barrote I, Costa M M, Albano S, Santos R. 2013 Physiological Responses of *Zostera marina* and *Cymodocea nodosa* to Light-Limitation Stress. *PLoS ONE*. **8**, e81058. (doi:10.1371/journal.pone.0081058)

30. Olsen Y S, Sánchez-Camacho M, Marbà N, Duarte C M. 2012 Mediterranean Seagrass Growth and Demography Responses to Experimental Warming. *Estuaries Coasts*. **35**, 1205–1213. (doi:10.1007/s12237-012-9521-z)

31. Massa S I, Arnaud-Haond S, Pearson G A, Serrão E A. 2009 Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal). *Hydrobiologia*. **619**,195–201. (doi:10.1007/s10750-008-9609-4)

32. Moore K A, Short F T, Larkum A, Orth R, Duarte C M. 2006 Zostera: Biology, Ecology, and Management. *Springer*. 361–386. (doi:10.1007/1-4020-2983-7_16)

33. Cabaço S, Machás R, Santos R. 2009 Individual and population plasticity of the seagrass *Zostera noltii* along a vertical intertidal gradient. *Estuar Coast Shelf Sci*. **82**, 301–308. (doi:10.1016/j.ecss.2009.01.020)

34. Sokolova I M, Frederich M, Bagwe R, Lannig G, Sukhotin A A. 2012 Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrate. *Mar Environ Res*. **79**, 1–15. (doi:10.1016/j.marenvres.2012.04.003)

35. Govers L L, Suykerbuyk W, Hoppenreijs J H T, Giesen K, Bouma T J, Van Katwijk M M. 2015 Rhizome starch as indicator for temperate seagrass winter survival. *Ecol Indic*. **49**, 53–60. (doi:10.1016/j.ecolind.2014.10.002)

36. Marín-Guirao L, Bernardeau-Esteller J, García-Muñoz R, Ramos A, Ontoria Y, Romero J, Pérez M, Ruiz JM, Procaccini G. 2018 Carbon economy of Mediterranean seagrasses in response to thermal stress. *Mar Pollut Bull*. **135**, 617–629. (doi:10.1016/j.marpolbul.2018.07.050)

37. Ralph P J, Durako M J, Enríquez S, Collier C J, Doblin M A. 2007 Impact of light limitation on seagrasses. *J Exp Mar Biol Ecol*. **350**, 176–193. (doi:10.1016/j.jembe.2007.06.017)

38. Ruiz J M, Romero J. 2001 Effects of *in situ* experimental shading on the Mediterranean seagrass *Posidonia oceanica*. *Mar Ecol Prog Ser*. **215**, 107–120. (doi:10.3354/meps215107)

39. Costa M M, Silva J, Barrote I, Santos R. 2021 Heatwave Effects on the Photosynthesis and Antioxidant Activity of the Seagrass *Cymodocea nodosa* under Contrasting Light Regimes. *Oceans*. **2**, 448–460. (doi: 10.3390/ oceans2030025)

40. Hansen A B, Pedersen A S, Kühl M, Brodersen K E. 2022 Temperature Effects on Leaf and Epiphyte Photosynthesis, Bicarbonate Use and Diel O2 Budgets of the Seagrass *Zostera marina* L. *Front Mar Sci*. **9**, 822485. (doi:10.3389/fmars.2022.822485)

41. Eggert A. 2012 Seaweed responses to temperature. In Seaweed Biology: Novel Insights into Ecophysiology, Ecology and Utilization (eds C. Wiencke, K. Bischof), pp. 47–66. Berlin, Heidelberg: *Springer*. (doi:10.1007/978-3-642-28451-9_3)

42. Collier C J, Uthicke S, Waycott M. 2011 Thermal tolerance of two seagrass species at contrasting light levels: Implications for future distribution in the Great Barrier Reef. *Limnol Oceanogr*. **56**, 2200–2210. (doi:10.4319/lo.2011.56.6.2200)

43. York P H, Gruber R K, Hill R, Ralph P J, Booth D J, Macreadie P I. 2013 Physiological and Morphological Responses of the Temperate Seagrass *Zostera muelleri* to Multiple Stressors: Investigating the Interactive Effects of Light and Temperature. *PLoS ONE*. **8**, e76377. (doi:10.1371/journal.pone.0076377)

44. Burkholz C, Duarte CM , Garcias-Bonet N. 2019 Thermal dependence of seagrass ecosystem metabolism in the Red Sea. *Mar Ecol Prog Ser*. **614**, 79–90. (doi:10.3354/meps12912)

45. Duarte C M. 2011 The role of marine biota in the functioning of the biosphere. *Proc. R. Soc. B*. **278**, 1429-1437.

46. Gacia E, Marbà N, Cebrián J, Vaquer-Sunyer R, Garcias-Bonet N, Duarte C M. 2012 Thresholds of irradiance for seagrass *Posidonia oceanica* meadow metabolism. *Mar Ecol Prog Ser*. **466**, 69–79. (doi:10.3354/meps09928)

47. Savva I, Bennett S, Roca G, Jordà G, Marbà N. 2018 Thermal tolerance of Mediterranean marine macrophytes: Vulnerability to global warming. *Ecol Evol*. **8**, 12032–43. (doi:10.1002/ece3.4663)

48. Wesselmann M, Anton A, Duarte C M, Hendriks I E, Agustí S, Savva I, Apostolaki E T, Marbà N. 2020 Tropical seagrass *Halophila stipulacea* shifts thermal tolerance during Mediterranean invasion. *Proc R Soc B Biol Sci*. **287**, 20193001. (doi:10.1098/rspb.2019.3001)

49. Beca-Carretero P, Olesen B, Marbà N, Krause-Jensen D. 2018 Response to experimental warming in northern eelgrass populations: comparison across a range of temperature adaptations. *Mar Ecol Prog Ser*. **589**, 59–72. (doi:10.3354/meps12439)

50. Aoki L R, McGlathery K J, Wiberg P L, Al-Haj A. 2020 Depth Affects Seagrass Restoration Success and Resilience to Marine Heat Wave Disturbance. *Estuaries Coasts*. **43**, 316–28. (doi:10.1007/s12237-019-00685-0)

51. Minguito-Frutos M, Boada J, Pagès J F, Marco-Méndez C, Arthur R, Adams M P, Alcoverro T. 2023 Species-specific acclimatization capacity of key traits explains global vertical distribution of seagrass species. *Glob Ecol Biogeogr*. **32**, 976–86. (doi:10.1111/geb.13673)

52. Collier C J, Lavery P S, Masini R J, Ralph P J. 2007 Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. *Mar Ecol Prog Ser*. **337**, 103-115. (doi:10.3354/meps337103)

53. Mcmahon K, Collier C, Lavery P S. 2013 Identifying robust bioindicators of light stress in seagrasses: A review. *Ecol Indic*. **30**, 7–15. (doi:10.1016/j.ecolind.2013.01.030)

54. Kong E, Ow Y X, Lai S, Yaakub S M, Todd P. 2019 Effects of shading on seagrass morphology and thermal optimal of productivity. *Mar Freshw Res*. **71**, 913-921. (doi:10.1071/MF19173)

55. Collier C J, Lavery P S, Ralph P J, Masini R J. 2009 Shade-induced response and recovery of the seagrass *Posidonia sinuosa*. *J Exp Mar Biol Ecol*. **370**, 89–103. (doi:10.1016/j.jembe.2008.12.003)

56. Minguito-Frutos M, Adams M P, Alcoverro T, Vilas M P, Alonso D, Mayol E, Bernardeu-Esteller J, Marín-Guirao L, Ruiz JM, Boada, J. 2023 Quantifying the role of photoacclimation and self-facilitation for seagrass resilience to light deprivation. *Front. Plant Sci.* **14**, 1186538. (doi:10.3389/fpls.2023.1186538)

57. Duarte C M. 1991 Seagrass depth limits. *Aquat Bot*. **40**, 363–377. (doi:10.1016/0304-3770(91)90081-F)

58. Burkholder J M, Tomasko D A, Touchette B W. 2007 Seagrasses and eutrophication. *J Exp Mar Biol Ecol*. **350**, 46–72. (doi:10.1016/j.jembe.2007.06.024)

59. Dattolo E, Ruocco M, Brunet C, Lorenti M, Lauritano C, D'Esposito D, De Luca P, Sanges R, Mazzuca S, Procaccini G. 2014 Response of the seagrass *Posidonia oceanica* to different light environments: Insights from a combined molecular and photo-physiological study. *Mar Environ Res.* **101**,225–236. (doi:10.1016/j.marenvres.2014.07.010)

60. 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. (doi:10.3389/fpls.2017.01142)

Chapter 2:

Light dependence of seagrass upper thermal thresholds

Pilar De Pablo Tobajas[1], Andrea Anton[2], Núria Marbà[2*]

[1] Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[2] Global Change Research Group, IMEDEA (CSIC-UIB), Institut Mediterrani d'Estudis Avançats, Miquel Marquès 21, 07190, Esporles, Illes Balears, Spain

*Corresponding author:

Global Change Research Group, IMEDEA (CSIC-UIB), Institut Mediterrani d'Estudis Avançats, Miquel Marquès 21, 07190, Esporles, Illes Balears, Spain. Tel: +34 971611720 Email: nmarba@imedea.uib-csic.es

Keywords: seagrass – depth – marine heat waves – growth – survival – metabolism

Abstract:

In warming scenarios, many primary producers tend to migrate vertically to cooler habitats. However, light-limited conditions in these deeper ecosystems can impact on the thermal tolerance of species. This study focuses on three key seagrass species in the Mediterranean Sea (*Cymodocea nodosa*, *Posidonia oceanica* and *Zostera noltei*). Three mesocosm experiments were conducted, exposing the plants to various temperature treatments (ranging from 16ºC to 40ºC, depending on the species) for 10 to 20 days. Light intensity was used as a proxy of depth, applying two light treatments, which were archived though shading: High Light (191 μmol m^{-2} s^{-1}) and Low Light (55 μmol m^{-2} s^{-1}),simulating environmental conditions near their upper and lower depth limits. We measured growth and survival rates to calculate thermal thresholds (optimum, upper critical and lethal temperatures), and deactivation energy from a thermal performance curve. Additionally, metabolic rates (Net Primary Production, Gross Primary Productionand Respiration) were measured for *Z. noltei*. We tested whether the plants at different light intensities, and consequently at varying depths, have similar upper thermal thresholds and optimal temperatures, as well as exhibit similar growth and metabolic responses throughout a thermal gradient. Upper thermal thresholds and deactivation energies were unaffected by light intensity; however, significant differences were observed in growth rates and metabolic responses, particularly in the transition toheterotrophic systems, with lower performance under low light conditions. Among the three species, *C. nodosa* demonstrated a higher capacity to thrive under warmer conditions and could survive in deeper environments. Moreover, unexpectedly high upperthermal limits were observed for *P. oceanica* when compared to previous studies, suggesting a possible thermal adaptation of clones to recent rising temperatures. Our findings indicate a habitat contraction of seagrass species is expected under future climatechange scenarios, in which deep meadows may also be vulnerable to warming conditions.

Introduction

Seagrass meadows are among the most productive and diverse marine habitats in the world (1), contributing to many ecosystem services (2), such as coastal protection, nurseries areas, water filtration, nutrient cycles, carbon sinks, etc (3,4). Despite their crucial function on the ecosystem, seagrass species are highly threatened. Current estimates indicate a global decline of 19.1% (5602 km^2) in the area of studied seagrass meadows since 1880 (5). The principal reasons are anthropogenic activities, as well as global warming, and associated marine heat waves (6).

On a global scale, there is a projection that sea surface temperature will rise by 2^oC by the year 2100 (7), as well as an increase of frequency, intensity and duration of marine heat waves (8). This ocean warming can have catastrophic impacts on seagrass meadows. For instance, significant die-off events of *P. oceanica* in the Mediterranean Sea during the 2003 and 2006 heat waves, resulted in a mortality of the 13% of shoot population (9). Likewise, there was loss of 36% of seagrass meadow in Shark Bay, Australia during the 2010/2011 heat wave (10). This excess of heat can result in shifts on species distribution towards more suitable habitats (11).

This migration typically results in a poleward shift of their biogeographical ranges (12), as well as the tropicalization phenomenon in temperate regions, caused by the change of species diversity by colonization of more thermally tolerant organisms (13,14). For example, an increase in the abundance and number of tropical fish species was observed in the northern Gulf of Mexico between 1970 and 2007 (15) Additionally, species can change their vertical distribution by migrating to deeper and cooler environments, which are within their thermal boundaries (11). For instance, demersal fish in the North Sea are currently performing a vertical displacement of 3-6 m per decade (16). However, with these migration other critical environmental factors vary with depth, such as the availability of light.

The light intensity, which is a crucial factor that support the metabolism of primary producers (17), decreases exponentially with depth due to absorption and scattering processes (18). It is important to consider that the seagrasses have relatively high light requirements in order to support the metabolic rates of their non-photosynthetic tissues (19), restricting their depth boundaries (11). Numerous studies have indicated that prolongated light reduction can lead to metabolic imbalance, resulting in a carbon store depletion, growth decreased, and, in some cases, higher mortality (20,21), as well as a shift from an autotrophic to a heterotrophic system in warming scenarios (22). Indeed, light plays a crucial role in driving the physiological performance of various species, but also in determining their lower vertical limits (23). However, there is a limited number of studies that examinate the interaction between both factors. Some of these researches use some temperature treatments at varying light intensities to measure morphological and physiological responses, such as growth, survival, biomass, metabolism and photosynthetic efficiency, of some seagrass species (24,25).

Research that considers the effects of these interactions on the thermal performance of species is even less common. Some ecological models have predicted the seagrass distribution based on their thermal thresholds at saturated light conditions (26, 27). Under these conditions, models project that there would be a reduction in the seagrass suitable depth range, with the exclusion of the shallowest and deepest limits of the entire habitable depth range of the meadow under warming scenarios (11,17). However, it could be possible that seagrass thermal limits vary depending on the light availability. To understand the nature of the interactions between the vertical distribution of the species and thermal stress, a theoretical-practical experiment can be conducted to calculate their thermal performance curves (TPCs).

TPCs are characterised by a lower lethal thermal limit, an exponential increase until reaching a maximum biological activity at the optimum temperature, followed by an abrupt decline towards the upper lethal thermal limit (14). For instance, this can result in a bell-shaped temperature-response pattern in terms of growth rate (28). Nevertheless, these thermal boundaries can vary among populations due to genetic adaptations to local conditions, phenotypic plasticity in response to environmental fluctuations or short-term physiological regulations (29, 30). From the analysis of the TPCs, it is possible to obtain the optimum, lower and upper critical temperatures, as well as activation and deactivation energy (Eh) of the rising and falling phase of the curve, respectively. This parametrization facilitates comparisons between species and populations (14,31,32). In addition, these variables contribute to understanding the distinct responses exhibited by individual species under different light conditions.

In this study, we use the TPCs to evaluate the thermal performance under light treatments of three seagrass species (*Cymodocea nodosa*, *Posidonia oceanica* and *Zostera noltei*), in terms of growth, survival and metabolic rates, as well as thermal thresholds, particularly the optimal, upper critical (75th and 90th percentiles of the performance curve) and lethal temperature. The light intensities, used as a proxy for depth, were selected as light treatments: High Light (191 μmol m^{-2} s^{-1}) and Low Light (55 μmol m^{-2} s^{-1}), mimicking the environmental conditions close to their upper and lower depth limit. Through these analyses, we aim to better understand the interaction between light availability and temperature, as well as their potential synergistic effects on seagrass productivity and future vertical distribution under warming scenarios. Our specific questions are: 1) Do the thermal optimum and upper thermal thresholds of these three species vary depending on light availability? 2) Does Eh, which reflect sensitivity to warming temperatures exceeding the thermal optimum, changes under varying light conditions? 3) How does the seagrass metabolic rates fluctuate in relation to light intensity?

Material and methods

a. Study area

The study was conducted using three different species, *C. nodosa*, collected at Tenerife (Canary Islands, Spain) at 8 m depth during September 2023; *P. oceanica*, collected at Mallorca (Balearic Islands, Spain) at 2 m depth during February 2024; and *Z. noltei*, collected at Cabrera (Balearic Islands, Spain) at 2 m depth during June 2024 (Figure 2.1). The light availability during harvesting was measured *in situ* using a mini-PAR sensor.

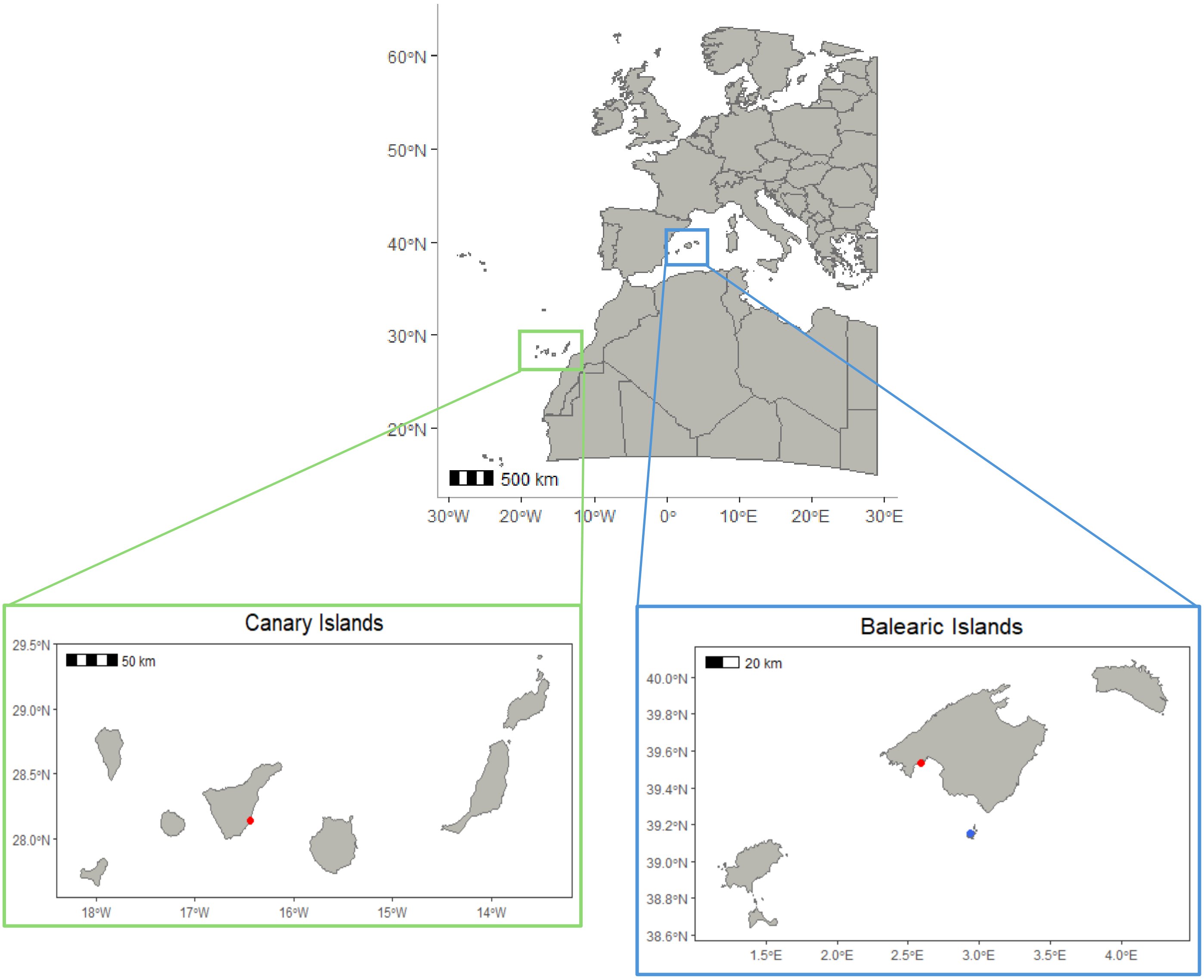

Figure 2.1. The study area where the three species of seagrasses were collected: the Canary Islands (left), where *C. nodosa* was sampled and the Balearic Islands (right), from which *P. oceanica* (red dot) and *Z. noltei* (blue dot) were harvested.

b. Experimental design

All collected plants were transported to the laboratory within cool boxes and air supply for *P. oceanica* and *Z. noltei*, whereas *C. nodosa* plants were carefully wrapped in seawater-soaked towels, placed in an isothermal box, and transported by plane.

Upon arrival, the plants were acclimated to *in situ* environmental conditions in a temperature-controlled chamber for a duration of 3 to 6 days with air supply. Salinity levels were maintained around 36.5 g L^{-1} for Mediterranean species, and 36 g L^{-1} for Atlantic species. The temperature was regulated to 24ºC for *C. nodosa*, 16ºC for *P. oceanica* and 22ºC for *Z. noltei*. Additionally, the plants were exposed to a photoperiod of 12 h light : 12 h dark.

Following the acclimatization period, the plants were divided into vertical (ortotropic) and apical (plagiotropic) shoots. In the *C. nodosa* experiment, vertical shoots were used; for *P. oceanica*, both shoot types were utilized (excluding the rhizomes and roots); and for *Z. noltei*, only apical shoots were selected (fragments of three internodes of horizontal rhizomes and three shoots were used due to its smaller size, however physiological responses were measured only on the apical shoot). Single shoots were transported to individual experimental units. Each one consisted of a double-layered transparent plastic bag, filled with 1.5 L of filtered seawater for *C. nodosa* and *Z. noltei* individuals, whereas *P. oceanica* required 1.8 L due to its larger size. Per temperature treatment, 14 experimental bags, 7 for each light treatment, were distributed randomly within an experimental bath container of 150 L (Figure 2.2). Additionally, two aquariums were maintained with extra shoots, one at control temperature and other set to increase the temperature as the other treatments. Extra plants were stored in case of death individuals, the presence of fungi before starting the treatment, or the need to extend the experimental thermal range with additional temperature treatments.

Figure 2.2. Overview of the experimental setup (left) and the distribution of each experimental bags, divided into light treatments, during the experiment (right).

Within each bath experimental container, two water pumps and two aeration tubes were used to facilitate the air exchange between the atmospheric-water layer. Additionally, an IKS-AQUASTAR system was connected to a temperature sensor and a heater located inside the bath container. This system allows for precise regulation of water temperature in each experimental unit. The bags were suspended with their surface fully open to facilitate gas exchange and were illuminated with a photoperiod of 12 h light : 12 hdark using fluorescent aquarium lamps with normal and ultraviolet radiation. Salinity was monitored daily using a conductimeter, and distilled water was added to compensate for evaporation, maintain salinity levels of 36-38 g L^{-1} for Atlantic species, and 36.5-37.5 g L^{-1} for Mediterranean species (see Supplementary Materials, Table 3.1). Seawater within experimental bags was renewed twice a week to sustain water quality.

At the end of the acclimation period, bath container temperatures were risen at 1^{o}C day^{-1} until reaching the target temperature. The experimental temperatures used for each species were: 24 (control), 27, 30, 32, 34, 36, 38 and 40^{o}C for *C. nodosa*; 16 (control), 19, 22, 24, 26, 28, 30, 32, 34 and 36^{o}C for *P. oceanica*; and 22 (control), 24, 26 28, 30, 32, 34,36 and 38^{o}C for *Z. noltei* (see Supplementary Materials, Figure 3.1). The seagrass responseat control temperature was repeated three times (four in the case of *P. oceanica* experiment): at the beginning (Control 1), in the middle (Control 2) and at the end of the experimental period (Control 3). We did so to test if the duration of the experiment affected the seagrass growth responses. In addition, two light treatments were used: High Light conditions (191 µmol m^{-2} s^{-1}), which represent the light availability near the upper limit of the meadow, whereas Low Light conditions (55 µmol m^{-2} s^{-1}) mimic the light intensity close to the lower limit, using three layers of mesh. Light conditions were measured using a mini-PAR sensor.

The plants were maintained at the target temperature for 10 days for fast-growing species (*C. nodosa* and *Z. noltei*), and 20 days for slow-growing species (*P. oceanica)*, to facilitate the observation of measurable growth at the end of the experimental period (Figure 2.3)

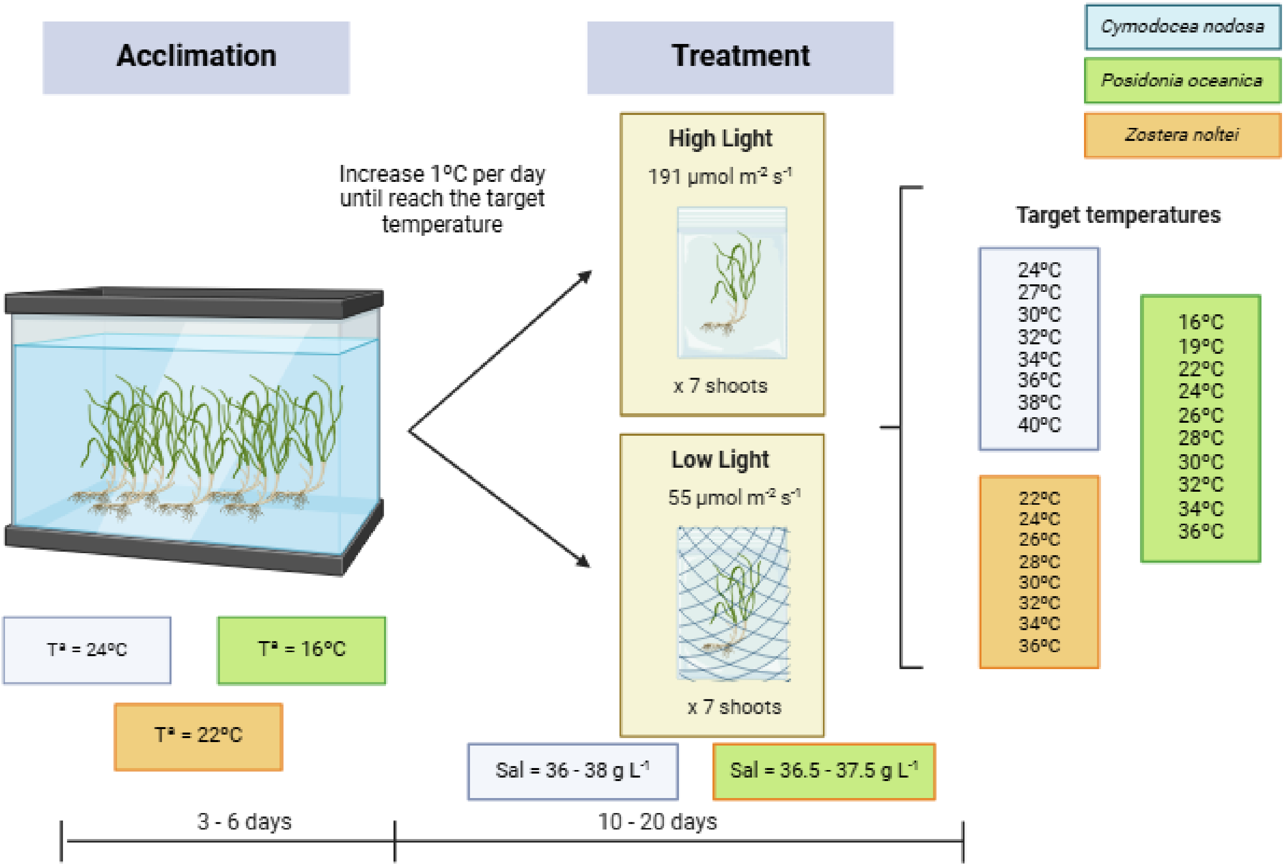

Figure 2.3. Distribution of experimental time periods, which includes the acclimation, followed by the light treatment period, with their respective temperatures and salinity levels for each phase.

In the *C. nodosa* experiment, a problem occurred in the sensor system controlling the temperature, resulting in a decrease in the temperature to 19ºC on the experimental units, impacting the 40ºC and control 3 treatments, leading to an early conclusion of the experiment. According to Savva *et al.* (32), the minimum temperature for *C. nodosa* is 12.9ºC. Therefore, the observed mortality can be attributed to the warming period rather than the decrease in temperature.

c. Growth rate

The growth rate of the species was assessed using a leaf marking technique (33). At the beginning of the temperature treatment, each shoot was marked with two parallel punctures near the apical meristem using a hypodermic needle in the case of *C. nodosa* and *P. oceanica*, whereas, for *Z. noltei*, two perpendicular punctures were made due to its smaller leaf size. Additionally, total wet weight of the shoots was measured.

At the end of the treatment, the leaf elongation, defined as the distance the punctures migrated towards the leaf tip, was measured, as well as the presence of new leaves (i.e. young leaves without punctures), the leaf width and the rhizome length (vertical and horizontally). Then, leaves were cut at the marked points and the tissues were separated into new and old tissues, and roots, rhizomes and petioles were also separated. All fresh tissues were weighed together per light treatment, with roots also were weighed individually. Subsequently, the samples were placed in an oven for 24 h and weighed again dry.

The leaf growth rate was calculated using the following equations:

$$LGR_1 = \frac{NLL}{t}$$

Where LGR_1 represents the leaf growth rate, in distance units (cm d^{-1} shoot $^{-1}$), NLL means the length of new leaf tissues (cm shoot $^{-1}$), and t the duration of the treatment (d).

$$LSW = \frac{DLW}{TL}$$

Where LSW represents the leaf specific weight (gDW cm^{-1}), DLW means dry leaf weight, including new and old leaf tissues (gDW), and TL the sum of all total leaf lengths per light treatment, including new and old tissues (cm shoot $^{-1}$).

Leaf growth rate, in mass units, (LGR_2, gDW d^{-1} shoot $^{-1}$) was estimated as,

$$LGR_2 = LGR_1 \text{ x LSW}$$

Where LGR_1 represents the leaf growth rate (cm d^{-1} shoot $^{-1}$) and LSW the leaf specific weight (gDW cm^{-1}).

d. Thermal performance curve

The thermal performance of each species was characterized by fitting the growth rate responses to a Gaussian function (34):

$$Y = ae^{[-0.5\left(\frac{T-b}{c}\right)^2]}$$

Where Y represents the measured growth rate response, T is the temperature (^{o}C), a (Amplitude) is the height of the centre of the distribution in Y units, b (Mean) is the temperature value at the centre of the distribution, and c (SD) is the width of the temperature distribution. From the fitted function, the T_{opt} is identified as b, whereas the $T_{max75\%}$ was calculated as the temperature at which the optimal growth rate is reduced by half (75th percentile of the performance curve), and $T_{max90\%}$ as the temperature at which the optimal growth rate is reduced one tenth (90th percentile of the curve), accompanied by their respective confidence intervals (CI) $_{95\%}$.

e. Deactivation energy

The thermokinetics of the biological activities are defined by the Van't Hoff-Arrhenius relation when integrated into the metabolic theory of ecology. The warming sensitivity of each species was evaluated through the Eh, which represents the falling phase of measured response within the thermal niche beyond the T_{opt}. This sensitivity was estimated as the slope of a linear regression model between the ecophysiological trait and temperature treatment, including the biological activity at T_{opt}. The Eh was calculated using the metabolic theory of ecology equation:

$$R = R_0 e^{-Eda/kT}$$

Where R_0 represents the state-depended scaling coefficient of the organism, Eh is the deactivation energy, k is Boltzmann's constant (8.62 x 10^{-5} eV K^{-1}), T is temperature (Kelvin), and R is the biological trait, specifically the growth rate response. The corresponding IC $_{95\%}$ were also calculated.

f. Survival rate

An individual was considered dead when there was a loss of turgor in the plant, the meristem exhibited as a soft and darkened appearance, and leaves were easily detached from the petiole. The survival rate was estimated using a logistic growth equation. From the fitted function, the upper lethal limit (L_{T50}) was determined as the temperature at which the 50% individuals' mortality occurs, using the following equation:

$$L_{T50} = \frac{a}{b}$$

Where y represents the L_{T50} (ºC), a is the constant coefficient, and b is the coefficient of the predictor variable in the fitted function.

g. Metabolic rate

At the end of the treatment, the plants of *Z. noltei* in the third experiment were incubated inside 200mL chambers, with two replicates per incubator, resulting in a total of three incubators and six replicates per light treatment. The incubators were positioned inside a controlled temperature container using the IKS-AQUASTAR system. They were placed, alternately between light treatments (with and without mesh) around a motor, which facilitate the continuous movement of water inside the incubator. Dissolved O_2 (DO) concentration and temperature were measured at the beginning and at the end of the incubation period (collecting three values of each replicate), using a ProSOLO DO meter. The incubation period was defined as 3 h of light and 3 h of dark conditions at different temperatures. Dead plants were excluded from the analysis.

The metabolic rates were measured as the changes in DO concentration within the incubators to estimate Net Primary Production (NPP), Gross Primary Production (GPP) and Respiration (R). NPP was calculated based on the fluctuation in DO concentrations during the light conditions, whereas R was estimated as DO changes under dark conditions. GPP was calculated as the sum of NPP and the absolute value of R, as indicated by the following equation. The values were expressed in mg h^{-1} $shoot^{-1}$.

$$GPP = NPP + |R|$$

h. Statistical analysis

A Wilcoxon signed-rank test was used to investigate the potential impact of fungi presence on shoot growth by comparing the growth rate of infected and non-infected shoots. A comparison of thermal limits (T_{opt}, $T_{max75\%}$, $T_{max90\%}$) with their respective $CI_{95\%}$ between shoot types was performed for *P. oceanica* experiment (significant differences if there is not an $CI_{95\%}$ overlapping).

To determine significant differences between control groups of each experiment, a one-way ANOVA or Kruskal-Wallis test was employed, depending on the data distribution and the presence of outliers. In the case of differences, a Tukey or Dunn test was performed respectively. To compare TPCs between light treatments, we examined the thermal thresholds (T_{opt}, $T_{max75\%}$, $T_{max90\%}$) and Eh, comparing their $CI_{95\%}$, as well as the upper thermal limit (L_{T50}). Additionally, to evaluate differences between growth rates, metabolic rates under light treatments, we analysed the overlapping between $CI_{95\%}$ and performed a t- test to investigate the impact of the light intensity of the variability of the data (Response ~ Temperature + Light vs Response ~ Temperature). The significance level (α) was adjusted to 0.05 for all statistical analyses, which were conducted using R software. All graphics were created in R-studio, using the ggplot2 package.

Results

a. Experimental design

For growth rate responses, no significant differences were found between the control groups in either *C. nodosa* experiment (one-way ANOVA, F value = 2.719; df = 2, 16; $p > 0.05$ under HL; Kruskal-Wallis test, $Chi^2 = 0.69293$; df = 2; $p > 0.05$ under LL conditions) or in *P. oceanica* experiment (Welch F-test one-way ANOVA, F value = 0.787; df = 3, 23; $p > 0.05$ under HL; Kruskal-Wallis test, $Chi^2 = 6.481$; df = 3; $p > 0.05$ under LL conditions). However, for *Z. noltei*, significant differences were identified between the Control 3 and the others (Kruskal-Wallis test, $Chi^2 = 12.856$; df = 2; $p < 0.05$; Dunn post-hoc test, $p < 0.05$ under HL; Kruskal-Wallis test, $Chi^2 = 11.867$; df = 2;$p < 0.05$; Dunn post-hoc test, $p < 0.05$ under LL conditions) (see Supplementary Materials, Figure 3.2). These differences were attributed to the increase of temperature beyond the optimal one for 6 h at the end of the experiment, affecting only the Control 3 remaining in the study. Consequently, the data showed that the Control 3 was unable to recover from the heat-shock.

For metabolic rates, significant differences in NPP were observed between Control 1 and the others under both HL and LL conditions (one-way ANOVA test, F value = 20.2; df = 2, 6; $p < 0.05$; Tukey test, $p < 0.05$ in HL; one-way ANOVA, F value = 43.39; df = 2, 6; $p < 0.05$; Tukey test, $p < 0.05$ in LL). For Respiration rates, significant differences were observed between the Control 1 and the others under LL conditions (Kruskal-Wallis test, $Chi^2 = 5.6$; df = 2; $p > 0.05$ in HL; and one-way ANOVA test, F value = 19.19; df = 2, 6; $p < 0.05$; Tukey test, $p < 0.05$ in LL). Regarding GPP, significant differences were found between the Control 1 and the others under HL conditions, whereas under LL conditions, significant differences were observed between the Control 1 and Control 2 plants (one-way ANOVA test, F value = 42.12; df = 2, 6; $p < 0.05$; Tukey test, $p < 0.05$ in HL; and Kruskal-Wallis test, $Chi^2 = 6.4889$; df =2; $p < 0.05$; Dunn test, $p < 0.05$ in LL) (see Supplementary Materials, Figure 3.3). These differences between controls during the experimental period can be attributed to an accidental increase of temperature beyond the optimal along 6 h, in which the Control 3 was affected, as well as an increase in salinity (until 60 g L^{-1} in some replicates), which impacted the Control 2 and 3 plants during a period of 48 h.

During the experiment of *C. nodosa* and *P. oceanica*, the presence of a fungi was observed in some shoots, primarily located near the area of the cut rhizome and at the base of the leaves. Specially, the fungi were observed in treatments at higher temperatures, ranging from 24ºC to 40ºC. The results showed significant differences in growth rates between infected and non-infected shoots of *C. nodosa*, and, thus, the infected individuals were excluded from the study (see Supplementary Materials, Figure 3.4 and Table 3.2). However, in the case of *P. oceanica*, there were no significant differences in growth rates, allowing for the inclusion of infected shoots in the analysis (see Supplementary Materials, Figure 3.4).

For the *P. oceanica* experiment, a total of three vertical and four apical shoots per light treatment were used. The study of Marbà and Duarte *et al.* (35) observed that apical shoots tend to grow at a faster rate than vertical shoots. However, no significant differences under light treatments were observed between thermal thresholds (see Supplementary Materials, Figure 3.5). Consequently, both shoot types were included in the study.

b. Growth rate

Temperature had a significant effect on the growth rate of the species, exhibiting a clear bell-shaped curve across the range of temperatures assessed. There were no significant differences on optimal temperature (T_{opt}) neither upper critical temperatures ($T_{max75\%}$ and $T_{max90\%}$) between light treatments across the species (Table 2.1, Figure 2.4), although average $T_{max90\%}$ for *P. oceanica* growth was 2.88ºC higher at HL than LL conditions. However, T_{opt}, $T_{max75\%}$ and $T_{max90\%}$ varied across the species studied, *P. oceanica* exhibiting optimal growth rates at 7.18ºC under HL - 5.27ºC under LL, and 3.18ºC under HL – 1.8ºC under LL conditions lower than *C. nodosa* and *Z. noltei*, respectively. Regarding the temperature at which the growth rate was reduced by half, $T_{max75\%}$ for *P. oceanica* was 3.26ºC under HL – 3.63ºC under LL conditions lower than *C. nodosa*, although similar values were observed in comparison to *Z. noltei*. For the temperature at which the growth rate was reduced by one tenth, $T_{max90\%}$ for *P. oceanica* was similar under HL – 2.29ºC lower under LL conditions than *C. nodosa*, and 5.39ºC under HL – 2.58ºC under LL conditions higher than *Z. noltei*.

Moreover, no statistically significant differences were observed on growth rates across the thermal range tested among light treatments for *C. nodosa* (T-test, F value = 0.8369; df = 98, 97; $p > 0.05$, with $CI_{95\%}$ overlapping) (Figure 2.4). However, significant differences were observed in the growth rates of *P. oceanica* and *Z. noltei* between light intensities (T-test, F value = 22.204; df = 130, 129; $p < 0.05$ for *P. oceanica*; and T-test, F value = 16.054; df = 124, 123; $p < 0.05$ for *Z. noltei*; with $CI_{95\%}$ non-overlapping), indicating 1.5-fold approximately higher growth rates under HL conditions (Figure 2.4).

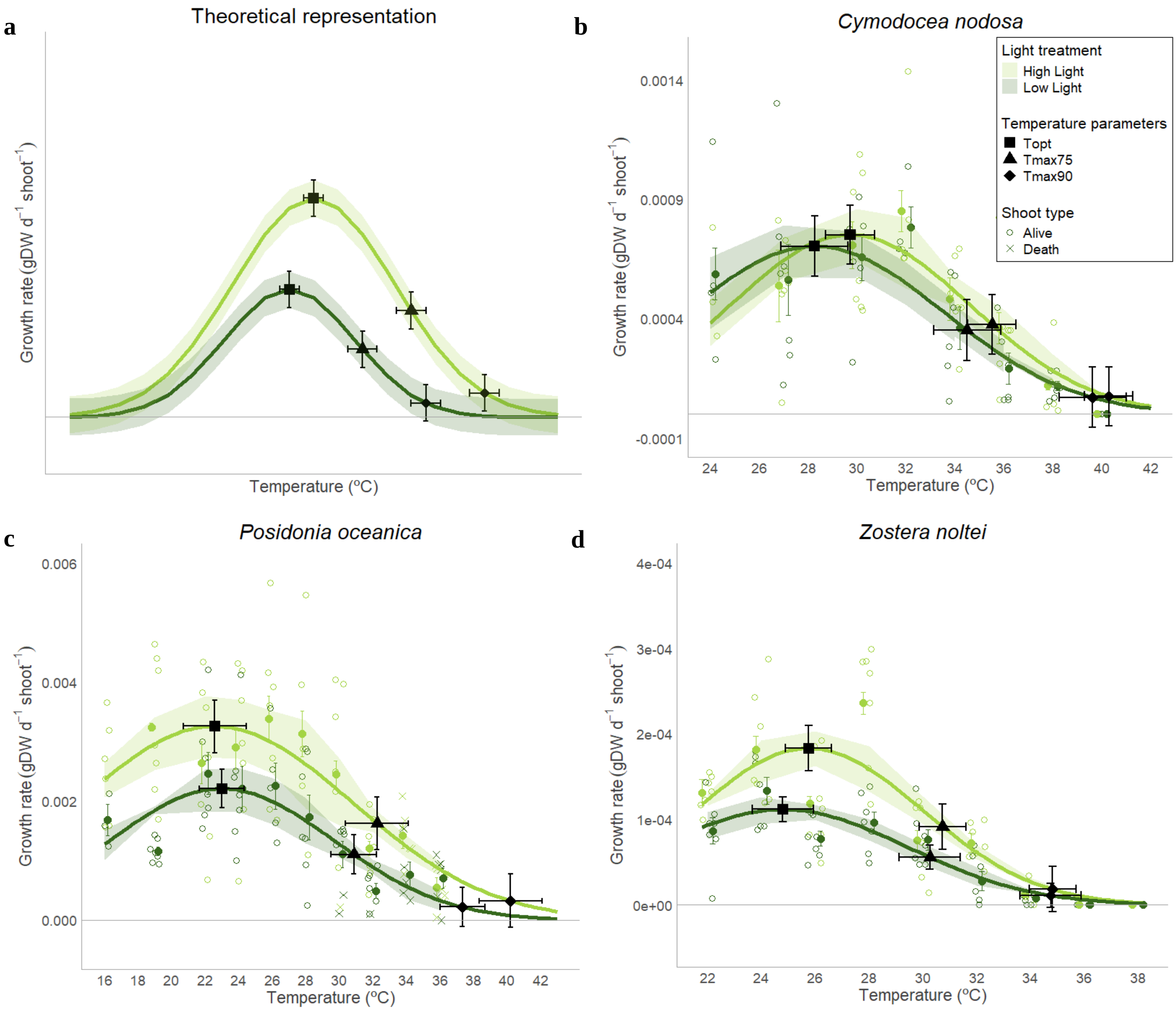

Figure 2.4. Theoretical thermal responses of seagrass growth rate (a); TPC of *C. nodosa* (b); *P. oceanica* (c) and *Z. noltei* growth rates (d). The light treatments (HL vs LL conditions) and their respective shared area, which represents the upper and lower $CI_{95\%}$, are also shown. Thermal parameters (T_{opt}, $T_{max75\%}$ and $T_{max90\%}$) obtained are shown on the graft. Consider that the growth data was fitted using a Gaussian function. The fill dots indicate the mean of growth rate at each temperature treatment, accompanied by standard error bars. The emptied dots represent the growth rate of alive shoots, whereas crosses indicate the growth rate of dead shoots at the end of the thermal treatment.

The Eh ranged from -2.123 eV to -0.960 eV across the species, however no significant differences were observed on the falling phase of the thermal performance between light treatments in all species (Table 2.1).

Table 2.1. Temperature related parameters (T_{opt}, $T_{max75\%}$, $T_{max90\%}$) ± $CI_{95\%}$ obtained from the Gaussian model, and the Eh ± $CI_{95\%}$ of the decline phase of TPC of the studied species are shown under light treatments (HL vs LL conditions).

Temperature Parameters	***Cymodocea nodosa***		***Posidonia oceanica***		***Zostera noltei***	
	High Light	**Low Light**	**High Light**	**Low Light**	**High Light**	**Low Light**
T_{opt} (ºC)	29.72 ± 0.99	28.27 ± 1.38	22.58 ± 1.87	23.00 ± 1.34	25.76 ± 0.86	24.80 ± 1.14
$T_{max75\%}$ (ºC)	35.52 ± 0.99	34.50 ± 1.38	32.26 ± 1.87	30.87 ±1.34	30.74 ± 0.86	30.27 ±1.14
$T_{max90\%}$ (ºC)	40.29 ± 0.99	39.63 ± 1.38	40.22 ± 1.87	37.34 ± 1.34	34.83 ± 0.86	34.76 ± 1.14
Eh (eV)	- 2.123 ± 0.826 (R2 = 48.79, p < 0.05, N = 31)	- 1.920 ± 0.666 (R2 = 55.49, p < 0.05, N = 30)	- 1.110 ± 0.417 (R2 = 38.97, p < 0.05, N = 47)	- 0.960 ± 1.657 (R2 = 29.01, p < 0.05, N = 44)	- 2.030 ± 0.706 (R2 = 50.92, p < 0.05, N = 35)	- 1.875 ± 0.631 (R2 = 55.08, p < 0.05, N = 32)
L_{T50} (ºC)	40.00	39.97	32.70 ± 2.95	32.15 ± 1.71	35.00	35.89 ± 1.77

c. Survival rate

No significant differences in the upper lethal temperature (L_{T50}) between light treatments were observed for the species studied (Table 2.1, Figure 2.5).

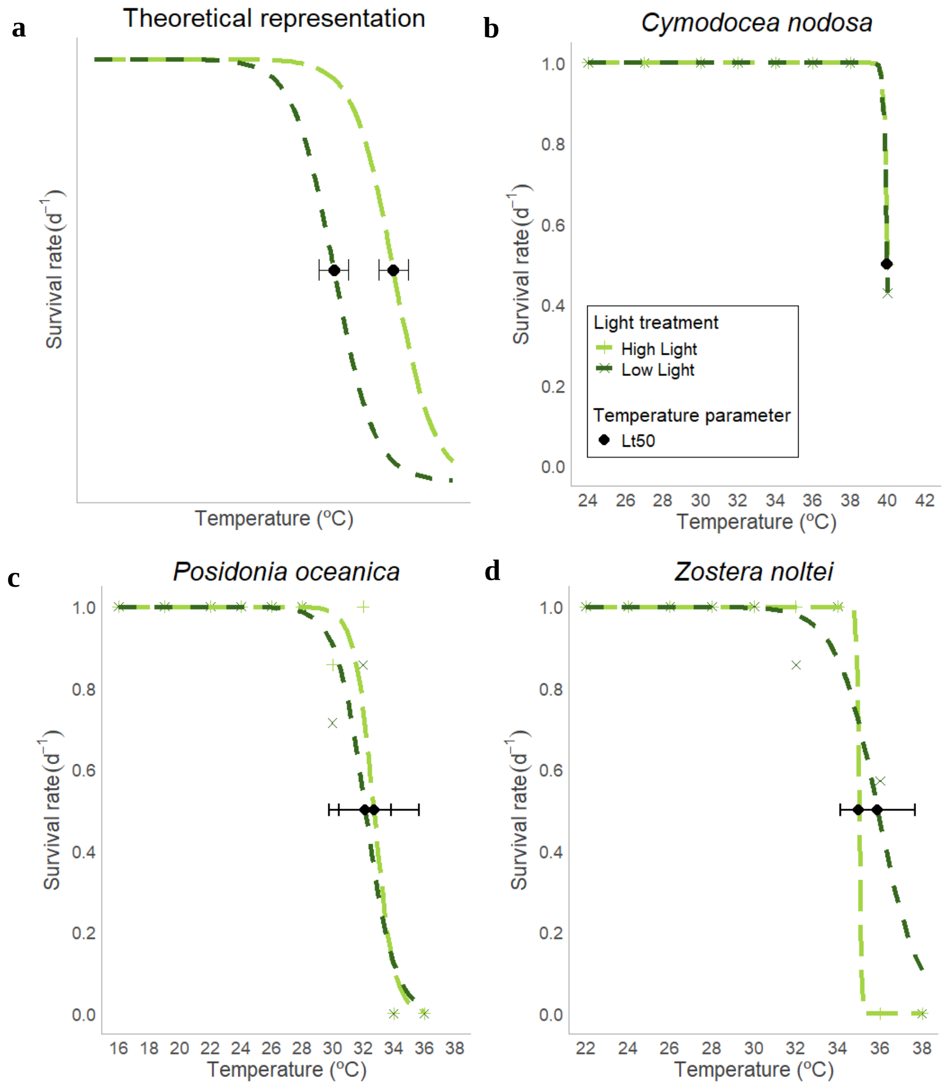

Figure 2.5. Theoretical survival curve within a thermal niche (a); *C. nodosa* (b); *P. oceanica* (c) and *Z. noltei* survival rates (d). Light treatments (HL vs LL conditions) and the upper thermal thresholds (L_{T50}), with their respective $CI_{95\%}$, are indicated in the graph. Consider that the survival data was fitted using a logistic growth model.

d. Metabolism

In terms of NPP, no significant differences were observed on T_{opt} between HL (23.81 ± 0.84°C) and LL conditions (23.19 ± 1.54°C). Furthermore, the thermal threshold at which primary production shifts from autotrophy to heterotrophy was found to be above 28.67 ± 0.84 °C under HL conditions. This threshold is notably higher than the 25.94 ± 1.54 °C observed under LL conditions.

Significant differences were found in NPP, GPP and Respiration rates between light treatments (t-test F value = 36.115; df = 34, 33; $p < 0.05$ in NPP; t-test F value = 7.3486; df = 34, 33; $p < 0.05$ in Respiration; t-test F value = 26.651; df = 34, 33; $p < 0.05$ in GPP, with non-overlapping $CI_{95\%}$) (Figure 2.6).

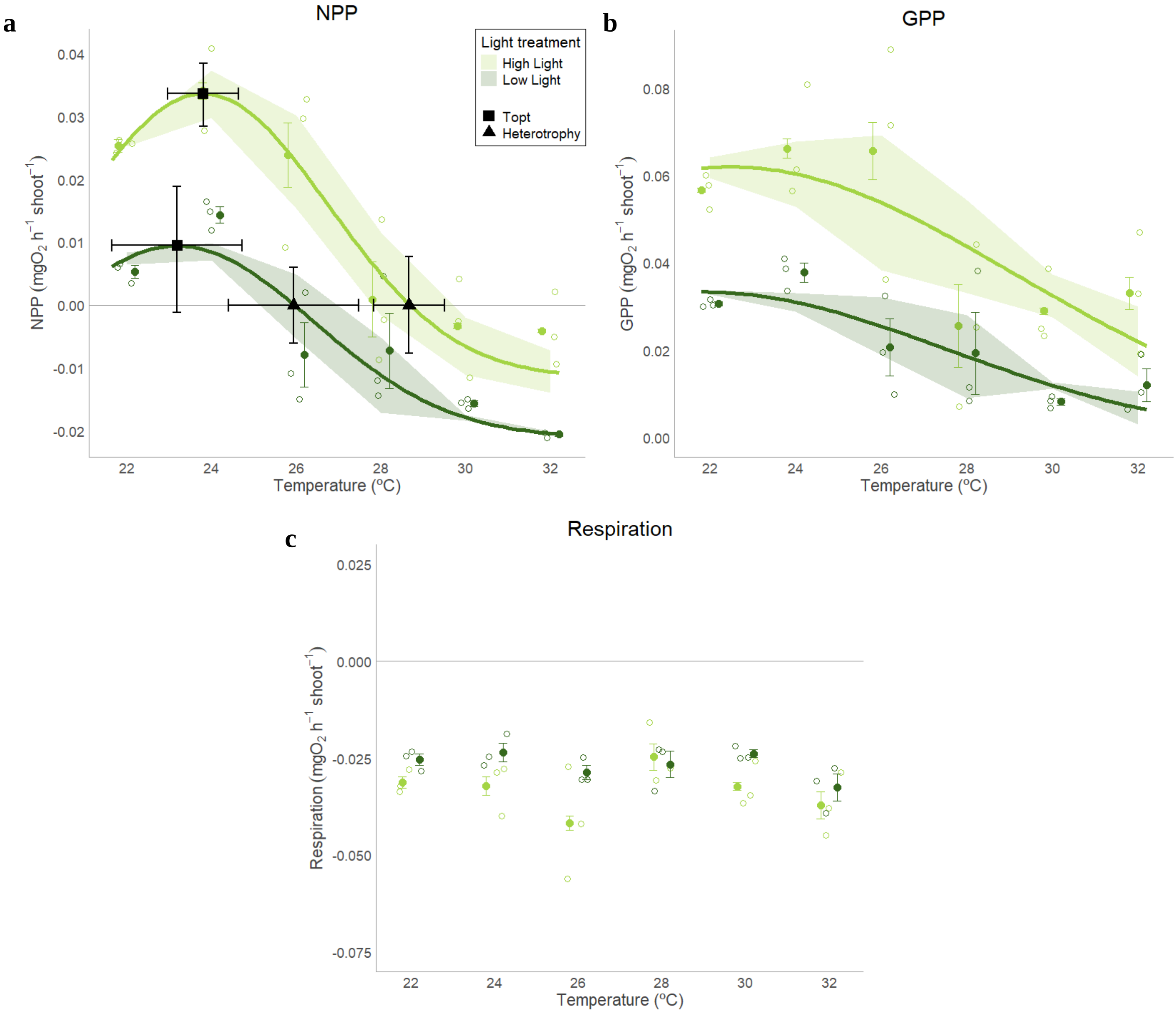

Figure 2.6. Thermal responses of NPP (a), GPP (b), and Respiration rates (c) of Z. *noltei*. Consider than the NPP and GPP data was fitted using a Gaussian model. Light treatments (HL vs LL conditions) are accompanied with their respective shared area, which represents the upper and lower $CI_{95\%}$. The fill dots indicate the mean values for each temperature treatment, paired with their respective standard error bars.

Discussion

Our results demonstrate a clear effect of temperature on seagrass survival, as well as growth and metabolism rates (NPP and GPP), characterized by a bell-shaped curve for the three species studied. However, the response to light availability varied between species. For both *P. oceanica* and *Z. noltei*, significant differences in their performance curve were found between light intensities, with higher growth rates observed under HL conditions. These differences are evident in the optimal growth rates, which is 3.26 ± 0.45 mgDW d^{-1} $shoot^{-1}$ under HL compared to 2.2 ± 0.32 mgDW d^{-1} $shoot^{-1}$ under LL conditions for *P. oceanica*, and 0.18 ± 0.03 mgDW d^{-1} $shoot^{-1}$ under HL compared to 0.11 ± 0.01 mgDW d^{-1} $shoot^{-1}$ under LL conditions for *Z. noltei*. Similar patterns have been observed in previous research, (e.g. 24,36–38), which reported a decline in growth under both light-limited and elevated temperature conditions. The results suggest that the light intensities used on the experiment limited the growth of both species, indicating that light availability is a critical factor that affect seagrass productivity. Although this productivity is significantly different at optimal conditions, these differences in growth decrease as the temperature exceeds the optimum, until overlapping at the upper thermal end in both curves. Moreover, the optimum temperature remained similar in the three species (including *P. oceanica* and *Z. noltei*), indicating no horizontal displacement on the performance curve due to light availability. These values are comparable to other studies, (e.g. 36,39), which also found no differences in optimum temperatures under shaded conditions in seagrass species.

In contrast to the results observed for *P. oceanica* and *Z. noltei*, no significant differences were found in the performance curve for *C. nodosa*. The optimal growth rates were 0.75 ± 0.12 mgDW d^{-1} $shoot^{-1}$ under HL compared to 0.70 ± 0.13 mgDW d^{-1} $shoot^{-1}$ under LL conditions, showing a minimal decrease in low light treatment. This unexpected result may be explained by the lower minimum light requirement (MLR) of *C. nodosa* compared to the other species, whose productivity is more dependent on light availability. Studies have reported a MLR of 4.4% Surface Irradiance (SI) (40) and 7.3% SI (41) for *C. nodosa* compared to 10 – 16% SI (42) and 11.5% SI (40) for *P. oceanica*, and 11% SI (43) and 17.6% SI (44) for *Z. marina*, a species closely related to *Z. noltei*. These lower MLR values may be linked to the ability of *C. nodosa* to exhibit photo-acclimatory responses under reduced light conditions. Such responses include enhanced photosynthetic efficiency and a reduction in respiratory demands, providing *C. nodosa* with a competitive advantage in low light environments (45,46), allowing it to maintain similar growth rates across varying light intensities.

In addition to comparing the growth responses between light treatments, warming sensitivity was assessed using three approximations: upper critical temperatures ($T_{max75\%}$ and $T_{max90\%}$), as well as Eh, estimated from the TPC, which represent the theoretical thermal sensitivity at physiological and organismal levels, and a lethal thermal limit (L_{T50}), defined as the temperature at which the 50% individuals' mortality, reflecting population level response. These parameters have been widely applied in seagrasses studies (e.g. 14, 47), as well as in other marine species, such as macroalgae, corals, echinoderms, molluscs, crustaceans and fish (e.g. 31,32, 48). As observed in the TPCs, there is a maximum divergence between growth curves under optimal conditions, but these differences decrease as temperatures rises. These overlapping of growth curves at higher temperatures reflect similar upper critical temperatures ($T_{max75\%}$ and $T_{max90\%}$) between light treatments, as well as similar Eh on the falling phase of the TPC. These thermal parameters are supported with L_{T50} observations. This absent of differences in thermal thresholds between light intensities can be attributed to irreparable damages of the photosynthetic apparatus at high temperature (49,50), so the plant cannot use the available light for photosynthesis (24,25). This suggests that light intensity becomes less relevant for plant productivity under lethal thermal stress.

These optimal and upper thermal thresholds under saturated light conditions were also compared to other studies employing the same methodology (29, 32) (Table 2.2). To achieve a more accurate comparison, additional upper critical temperatures were calculated: $T_{max80\%}$ (the 80^{th} percentile of the TPC) and $T_{max95\%}$ (the 95^{th} percentile of the TPC). Small variations on T_{opt} values were observed for both species (*C. nodosa* and *P. oceanica*). However significant differences were found in $T_{max95\%}$ for *C. nodosa,* which was 4.27°C higher in our study compared to Bennet *et al.* (29), and in both $T_{max80\%}$, which was 3.52°C higher in our study compared to Savva *et al.* (32), and $T_{max95\%}$, which was 12.7°C higher in our study compared to Bennet *et al.* (29), for *P. oceanica*. According to *C. nodosa*, the discrepancy between the upper thermal limits of our study and previous research could be attributed to the fact that the plants in this study were collected from the Canary Islands, instead of the Balearic Islands, as in the other studies. Consequently, these seagrass meadows are adapted to different environmental conditions (51), potentially leading to genetic differentiation between Mediterranean and Atlantic populations, which facilitate the existence of local ecotypes (52). These specific adaptations may enable them to tolerate different thermal thresholds. Conversely, the donor population of *P. oceanica* shoots was the same for our and Savva *et al.* (32) study. Hence, the observed differences in *P. oceanica* upper thermal limits among experiments could be attributed to phenotypic plasticity or thermal adaptation of the clones, which may help to mitigate the effects of increasing temperatures. Since year 2000, several marine heat waves have been recorder in the western Mediterranean have leaded summer seawater temperature often to exceed the T_{opt} for *P. oceanica* (32), significantly affecting the meadow survival (9). Furthermore, projections predict that this species could be functionally extinct in the Western Mediterranean by 2050 (11, 53). Consequently, these findings provide important insights into the thermal tolerance of the species and its potential future distribution in the Mediterranean Sea under climate change scenarios.

Table 2.2. Comparison of thermal thresholds (T_{opt}, $T_{max80\%}$ and $T_{max95\%}$) of *C. nodosa*, *P. oceanica* and *Z. noltei* between our results and the studies of Bennett *et al.*, (29) and Savva *et al.*, (32).

Temperature Parameters		Our study	Bennett *et al.*, (2022)	Savva *et al.*, (2018)
Cymodocea nodosa	T_{opt}	29.72 ± 0.99	28.00 ± 0.40	29.50 ± 1.30
	$T_{max80\%}$	38.55 ± 0.99	-	36.90 ± 2.90
	$T_{max95\%}$	**41.77 ± 0.99**	**37.50**	-
Posidonia oceanica	T_{opt}	22.58 ± 1.87	20.00 ± 0.70	25.80 ± 1.30
	$T_{max80\%}$	**37.32 ± 1.87**	-	**33.80 ± 0.50**
	$T_{max95\%}$	**42.70 ± 1.87**	**30.00**	-
Zostera noltei	T_{opt}	25.76 ± 0.86	-	-
	$T_{max80\%}$	33.34 ± 0.86	-	-
	$T_{max95\%}$	36.10 ± 0.86	-	-

Furthermore, metabolic measurements of *Z. noltei* were additionally conducted to further investigate the impact of light availability on additional physiological parameters of seagrass productivity. For NPP, the performance curves exhibit similar shape as growth curves, with significantly higher net production under HL conditions. These differences are evident in the optimal net production rates: 45.2E-02 ± 7.70E-03 mgO_2 h^{-1} $shoots^{-1}$ under HL compared to 3.06E-02 ± 6.00E-03 mgO_2 h^{-1} $shoots^{-1}$ under LL conditions (1.46 mgO_2 h^{-1} $shoots^{-1}$ higher under HL conditions). Moreover, similar optimum temperatures were observed between light treatments. However, the temperature at which the meadow system shifts from autotrophic (NPP > 0) to heterotrophic (NPP < 0) was 2.73°C lower under LL conditions. This value is critical for the plant production because, at this point, the photosynthetically fixed carbon cannot supply the respiratory demands, causing the system to act as an O_2 sinks (22). Interestingly, this metabolic shift does not seem to significantly affect the growth rate curves suggesting that plant metabolic responses to warming are evident sooner than those of growth, acting as an early warming indicator, and/or that shoot carbon supply after the metabolic shift might be provided by carbon reserves stored in seagrass tissues which might delay the occurrence of changes in growth. Additionally, the temperature at which NPP = 0 can serve as a proxy for the thermal threshold of the organism's viability (31). Our findings suggest that under limited light conditions, the negative impact of warming on seagrass production is intensified, potentially presenting a significant challenge for primary producers growing close to the deepest limit of their depth distribution range in the context of climate change. In the case of GPP, a clear temperature dependency was observed, though the optimal temperature was not reached. This could be due to the experimental thermal range exceeding the optimal temperature or to an inadequate fitting of the curve. A negative effect of increasing temperatures on GPP rates was observed, contrary to other studies (e.g. 54,55), where GPP increased with temperature. However, this thermal trend was not observed in respiration rates, which remained stable despite rising temperatures. These unexpected results coincided with the study of Collier *et al.* (24), who reported that leaf respiration was unaffected by temperature. This contradicts other studies (e.g. 28, 54–57), which observed increased respiration with temperature. The absence of a thermal effect on respiration in this experiment may be attributed to the limited amount of below-ground biomass used, including roots and rhizomes. The above:below ground biomass ratios for this study ranged from 72.01 ± 18.46 to 349.22 ± 110.08 (see Supplementary Materials, Table 3.3), in contrast to the study conducted by Plus *et al.* (55), which reported lower ratios between 0.6 ± 0.04 to 1.9 ± 0.25. This below ground biomass has significantly higher respiratory demands, particularly under warming temperatures (58), leading to a positive temperature response in respiration.

The results obtained indicate that the optimal and upper thermal thresholds for shoot growth and survival of the three species studied were not affected by light availability, and therefore, they would not be affected the depth distribution range. However, the growth rate and net primary production were affected by light availability, and thus, by depth. This suggests that deep meadows could decrease their growth and become heterotrophic systems at lower temperatures. Therefore, deeper meadows may exhibit a reduced capacity to recover from unfavourable conditions, such as herbivory (59,60), storm events (61, 62), boat anchoring (63,64), seasonal fluctuations (65) and marine heat waves (9). Consequently, our results indicate that the habitat contraction projected for this century under warming scenarios by Jorda *et al.* (11) could be more accurate because shallow populations will be exposed to temperatures exceeding their thermal thresholds, while deep populations, under light-limited conditions, might struggle to maintain the viability of their meadows (11,17).

It is important to note that shallow and deep meadows can develop different adaptations due to the pronounced summer stratification of water column, as well as different light environments (66). Previous research (e.g. 67), observed differences inthe heat tolerance of *P. oceanica* meadows at different depths, with shallow plants demonstrating a better response to cope with warming temperatures compared to plants from deeper areas. Moreover, there is recent evidence that seagrass meadows can acclimatize to low light conditions (68, 69). Since in our study we only collected shallow plants, using light intensity as a proxy for depth, the thermal thresholds observed in this experiment could differ to experimental ones. Therefore, field studies, in the form of translocations or common-garden experiments, are essential to validate the optimum and upper thermal thresholds obtained in the mesocosm experiments presented here and provide more accurate predictions of the future vertical distribution of the seagrass under climate change scenarios.

Our findings for the thermal performance of *C. nodosa* are particularly noteworthy. This species exhibits higher thermal thresholds than the other two species and exhibit a great ability to maintain growth under low light conditions. This suggests that *C. nodosa* may be a strong candidate to become one of the species that will occupy

Mediterranean Sea ecosystems under future warming scenarios. In contrast, other temperate species, such as *P. oceanica* and *Z. noltei*, are already suffering a progressive decline in Mediterranean habitats (e.g. 9,70-72), as well as rising temperatures exceeding their T_{opt} (32). The tolerance of *C. nodosa* to both higher temperatures and low light conditions suggest that it may benefit from the projected warming trends over the next century (73), potentially occupying empty niches of more vulnerable species, as well as expand its vertical distribution to deeper ecosystems.

Overall, our findings suggest that seagrass meadows under light-limited conditions, such as those near the deep distribution limit, may be vulnerable to warming temperatures, resulting in a contraction of the suitable depth range of the seagrass species. Due to limited literature, these results provide pioneering insights into the impact of climate change events on the vertical distribution of seagrass meadows. The outcomes of this studies could also be extrapolated to other primary producers, such as corals, for which some relevant studies have already been conducted (74–76), compared warming sensitivity between shallow and mesophotic communities, as well as to macroalgal communities. Understanding how abiotic factors like light availability and temperature interact, and how these interactions may create synergistic effects, is essential to improving predictions regarding the future vertical distribution of seagrass ecosystems under warming scenarios. These insights will help refine models and conservation strategies for marine ecosystems in the face of climate change.

Acknowledgements

The research of this master thesis has been funded by the projects OCEAN CITIZEN (Marine forest coastal restoration: an underwater gardening socio-ecological plan; European Union under the Horizon Europe program (grant agreement no. 101093910) and CYCLE (Complex DYnamics of CoastaL Ecosystems: Resilience to Climate Change; Spanish Research Agency, PID2021-123723OB-C21). The present research was carried out within the framework of the activities of the Spanish Government through the "Maria de Maeztu Centre of Excellence" accreditation to IMEDEA (CSIC-UIB) (CEX2021-001198).

References

1. Ruíz J M, Boudouresque C F, Enríquez S. 2009 Mediterranean seagrasses. *Bot Mar*. **52**, 369–381. (doi:10.1515/BOT.2009.058)

2. Cullen-Unsworth L, Unsworth R. 2013 Seagrass Meadows, Ecosystem Services, and Sustainability. *Environ Sci Policy Sustain Dev*. **55**, 14–28. (doi:10.1080/00139157.2013.785864)

3. Duffy J E. 2006 Biodiversity and the functioning of seagrass ecosystems. *Mar Ecol Prog Ser*. **311**, 233–250. (doi:10.3354/meps311233)

4. Unsworth R K F, Cullen-Unsworth L C, Jones B L H, Lilley R J. 2022 The planetary role of seagrass conservation. *Science*. **377**, 609–613. (doi:10.1126/science.abq6923)

5. Dunic J C, Brown C J, Connolly R M, Turschwell M P, Côté I M. 2021 Long-term declines and recovery of meadow area across the world's seagrass bioregions. *Glob Change Biol*. **27**, 4096–4109. (doi:10.1111/gcb.15684)

6. Orth R J, Carruthers T B, Dennison W C, Duarte C M, Fourqurean J W, Heck Jr K L, Hughes A R, Kendrick G A, Kenworthy W J, Olyarnik S *et al.* 2006 A Global Crisis for Seagrass Ecosystems. *BioScience*. **56**, 987–996. (doi:10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2)

7. Calvin K, Dasgupta D, Krinner G, Mukherji A, Thorne P W, Trisos C, Brovkin V, Engström K, Fishman T, Jones C, Le Quéré C. 2023 IPCC: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland. Available at: https://www.ipcc.ch/report/ar6/syr/.

8. Oliver E C J, Donat M G, Burrows M T, Moore P J, Smale D A, Alexander L V, Benthuysen J A, Feng M, Sen Gupta A, Hobday A J, *et al.* 2018 Longer and more frequent marine heatwaves over the past century. *Nat Commun*. **9**, 1324. (doi:10.1038/s41467-018-03732-9)

9. Marbà N, Duarte C M. 2010 Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Glob Change Biol*. **16**, 2366–2375. (doi:10.1111/j.1365-2486.2009.02130.x)

10. Arias-Ortiz A, Serrano O, Masqué P, Lavery P S, Mueller U, Kendrick G A, Rozaimi M, Esteban A, Fourqurean J W, Marbà N, *et al.* 2018 A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. *Nat Clim Change*. **8**, 338–344. (doi:10.1038/s41558-018-0096-y)

11. Jorda G, Marbà N, Bennett S, Santana-Garcon J, Agusti S, Duarte C M. 2020 Ocean warming compresses the three-dimensional habitat of marine life. *Nat Ecol Evol*. **4**, 109–114. (doi:10.1038/s41559-019-1058-0)

12. Poloczanska E S, Brown C J, Sydeman W J, Kiessling W, Schoeman D S, Moore P J, Brander K, Bruno J F, Buckley L B, Burrows M T, *et al.* 2013 Global imprint of climate change on marine life. *Nat Clim Change*. **3**, 919–925.(doi:10.1038/nclimate1958)

13. Sghaier Y R, Zakhama-Sraieb R, Benamer I, Charfi-Cheikhrouha F. 2011 Occurrence of the seagrass *Halophila stipulacea* (Hydrocharitaceae) in the southern Mediterranean Sea. *Bot Mar*. **54**, 575–582. (doi:10.1515/BOT.2011.061)

14. Wesselmann M, Anton A, Duarte C M, Hendriks I E, Agustí S, Savva I, Apostolaki E T, Marbà N. 2020 Tropical seagrass *Halophila stipulacea* shifts thermal tolerance during Mediterranean invasion. *Proc R Soc B Biol Sci*. **287**, 20193001. (doi:10.1098/rspb.2019.3001)

15. Fodrie F J, Heck K L, Powers S P, Graham W M, Robinson K L. 2010 Climate-related, decadal-scale assemblage changes of seagrass-associated fishes in the northern Gulf of Mexico. *Glob Change Biol*. **16**, 48–59. (doi:10.1111/j.1365-2486.2009.01889.x)

16. Dulvy N K, Rogers S I, Jennings S, Stelzenmüller V, Dye S R, Skjoldal H R. 2008 Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. *J Appl Ecol*. **45**, 1029–1039. (doi:10.1111/j.1365-2664.2008.01488.x)

17. Aoki L R, McGlathery K J, Wiberg P L, Al-Haj A. 2020 Depth Affects Seagrass Restoration Success and Resilience to Marine Heat Wave Disturbance. *Estuaries Coasts*. **43**, 316–328. (doi:10.1007/s12237-019-00685-0)

18. Lee K S, Park S R, Kim Y K. 2007 Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *J Exp Mar Biol Ecol*. **350**, 144–175. (doi:10.1016/j.jembe.2007.06.016)

19. Collier C J, Lavery P S, Ralph P J, Masini R J. 2008 Physiological characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. *Mar Ecol Prog Ser*. **353**, 65-79. (doi:10.3354/meps07171)

20. Collier C J, Waycott M, Ospina A G. 2012 Responses of four Indo-West Pacific seagrass species to shading. *Mar Pollut Bull*. **65**, 342–354. (doi:10.1016/j.marpolbul.2011.06.017)

21. Lee K S, Dunton K H. 1997 Effect of *in situ* light reduction on the maintenance, growth and partitioning of carbon resources in *Thalassia testudinum* banks ex König. *J Exp Mar Biol Ecol*. **210**, 53–73. (doi:10.1016/S0022-0981(96)02720-7)

22. Burkholz C, Duarte C M, Garcias-Bonet N. 2019 Thermal dependence of seagrass ecosystem metabolism in the Red Sea. *Mar Ecol Prog Ser*. **614**, 79–90. (doi:10.3354/meps12912)

23. Silva J, Santos R. 2003 Daily variation patterns in seagrass photosynthesis along a vertical gradient. *Mar Ecol Prog Ser*. **257**, 37–44. (doi:10.3354/meps257037)

24. Collier C J, Uthicke S, Waycott M. 2011 Thermal tolerance of two seagrass species at contrasting light levels: Implications for future distribution in the Great Barrier Reef. *Limnol Oceanogr*. **56**, 2200–2210. (doi:10.4319/lo.2011.56.6.2200)

25. York P H, Gruber R K, Hill R, Ralph P J, Booth D J, Macreadie P I. 2013 Physiological and Morphological Responses of the Temperate Seagrass *Zostera muelleri* to Multiple Stressors: Investigating the Interactive Effects of Light and Temperature. *PLoS ONE*. **8**, e76377. (doi:10.1371/journal.pone.0076377)

26. Adams M P, Collier C J, Uthicke S, Ow Y X, Langlois L, O'Brien K R. 2017 Model fit versus biological relevance: Evaluating photosynthesis-temperature models for three tropical seagrass species. *Sci Rep*. **7**, 39930. (doi:10.1038/srep39930)

27. Marbà N, Jordà G, Bennett S, Duarte C M. 2022 Seagrass Thermal Limits and Vulnerability to Future Warming. *Front Mar Sci*. **9**, 860826. (doi:10.3389/fmars.2022.860826)

28. Beca-Carretero P, Olesen B, Marbà N, Krause-Jensen D. 2018 Response to experimental warming in northern eelgrass populations: comparison across a range of temperature adaptations. *Mar Ecol Prog Ser*. **589**, 59–72. (doi:10.3354/meps12439)

29. Bennett S, Vaquer-Sunyer R, Jordá G, Forteza M, Roca G, Marbà N. 2022 Thermal Performance of Seaweeds and Seagrasses Across a Regional Climate Gradient. *Front Mar Sci*. **9**, 733315. (doi:10.3389/fmars.2022.733315)

30. Eggert A. 2012 Seaweed responses to temperature. In Seaweed Biology: Novel Insights into Ecophysiology, Ecology and Utilization (eds C. Wiencke, K. Bischof), pp. 47–66. Berlin, Heidelberg: Springer. (doi:10.1007/978-3-642-28451-9_3)

31. Anton A, Randle J L, Garcia F C, Rossbach S, Ellis J I, Weinzierl M, Duarte CM. 2020 Differential thermal tolerance between algae and corals may trigger the proliferation of algae in coral reefs. *Glob Change Biol*. **26**, 4316–4327. (doi:10.1111/gcb.15141)

32. Savva I, Bennett S, Roca G, Jordà G, Marbà N. 2018 Thermal tolerance of Mediterranean marine macrophytes: Vulnerability to global warming. *Ecol Evol*. **8**, 12032–12043. (doi:10.1002/ece3.4663)

33. Short F T, Duarte C M. 2001. Methods for the measurement of seagrass growth and production. *Global Seagrass Research Methods*, pp. 473. Amsterdam, Netherlands: Elsevier Science B.V. (doi:10.1016/B978-044450891-1/50009-8)

34. Angilletta Jr M J. 2006 Estimating and comparing thermal performance curves. *J Therm Biol*. **31**, 541–545. (doi:10.1016/j.jtherbio.2006.06.002)

35. Marbà N, Duarte C M. 1998 Rhizome elongation and seagrass clonal growth. *Mar Ecol Prog Ser*. **174**, 269–80. (doi:10.3354/meps174269)

36. Beca-Carretero P, Azcárate-García T, Julia-Miralles M, Stanschewski C S, Guihéneuf F, Stengel D B. 2021 Seasonal Acclimation Modulates the Impacts of Simulated Warming and Light Reduction on Temperate Seagrass Productivity and Biochemical Composition. *Front Mar Sci*. **8**, 731152. (doi:10.3389/fmars.2021.731152)

37. Dattolo E, Marín-Guirao L, Ruiz J M, Procaccini G. 2017 Long-term acclimation to reciprocal light conditions suggests depth-related selection in the marine foundation species *Posidonia oceanica*. *Ecol Evol*. **7**, 1148–1164. (doi:10.1002/ece3.2731)

38. Moreno-Marín F, Brun F G, Pedersen M F. 2018 Additive response to multiple environmental stressors in the seagrass *Zostera marina* L. *Limnol Oceanogr*. **63**, 1528–1544. (doi: 10.1002/lno.10789)

39. Kong E, Ow Y X, Lai S, Yaakub S M, Todd P. 2019 Effects of shading on seagrass morphology and thermal optimal of productivity. *Mar Freshw Res*. **71**, 913-921. (doi:10.1071/MF19173)

40. Olesen B, Enríquez S, Duarte C M, Sand-Jensen K. 2002 Depth-acclimation of photosynthesis, morphology and demography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. *Mar Ecol Prog Ser*. **236**, 89–97. (doi:10.3354/meps236089)

41. Dennison W C, Orth R J, Moore K A, Stevenson J C, Carter V, Kollar S, Bergstrom P W, Batiuk R A. 1993 Assessing Water Quality with Submersed Aquatic Vegetation. *BioScience*. **43**, 86–94. (doi:10.2307/1311969)

42. Ruiz J M, Romero J. 2001 Effects of in situ experimental shading on the Mediterranean seagrass *Posidonia oceanica*. *Mar Ecol Prog Ser*. **215**, 107–120. (doi:10.3354/meps215107)

43. Olesen B, Sand-Jensen K. 1993 Seasonal acclimatization of eelgrass *Zostera marina* growth to light. *Mar Ecol Prog Ser*. **94**, 91-99. (doi:10.3354/meps094091)

44. Bertelli C M, Unsworth R K F. 2018 Light Stress Responses by the Eelgrass, *Zostera marina* (L). *Front Environ Sci*. **6**, 39. (doi:10.3389/fenvs.2018.00039)

45. Marín-Guirao L, Bernardeau-Esteller J, Belando M D, García-Muñoz R, Ramos-Segura A, Alcoverro T, Minguito-Frutos M, Ruiz J M. 2022 Photo-acclimatory thresholds anticipate sudden shifts in seagrass ecosystem state under reduced light conditions. *Mar Environ Res*. **177**, 105636. (doi:10.1016/j.marenvres.2022.105636)

46. Silva J, Barrote I, Costa M M, Albano S, Santos R. 2013 Physiological Responses of *Zostera marina* and *Cymodocea nodosa* to Light-Limitation Stress. *PLoS ONE*. **8**, e81058. (doi:10.1371/journal.pone.0081058)

47. Anton A, Baldry K, Coker D J, Duarte C M. 2020 Drivers of the Low Metabolic Rates of Seagrass Meadows in the Red Sea. *Front Mar Sci*. **7**, 69. (doi:10.3389/fmars.2020.00069)

48. Giomi F, Barausse A, Duarte C M, Booth J, Agustí S, Saderne V, Anton A, Daffonchio D, Fusi M. 2019 Oxygen supersaturation protects coastal marine fauna from ocean warming. *Sci Adv*. **5**, eaax1814. (doi:10.1126/sciadv.aax1814)

49. Campbell S J, McKenzie L J, Kerville S P. 2006 Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. *J Exp Mar Biol Ecol*. **330**, 455–468. (doi:10.1016/j.jembe.2005.09.017)

50. Kim M, Qin L Z, Kim S H, Song H J, Kim Y K, Lee K S. 2020 Influence of Water Temperature Anomalies on the Growth of *Zostera marina* Plants Held Under High and Low Irradiance Levels. *Estuaries Coasts*. **43**, 463–476. (doi:10.1007/s12237-019-00578-2)

51. Dennison W C, Alberte R S. 1986 Photoadaptation and growth of *Zostera marina* L. (eelgrass) transplants along a depth gradient. *J Exp Mar Biol Ecol*. **98**, 265–282. (doi:10.1016/0022-0981(86)90217-0)

52. Tsioli S, Orfanidis S, Papathanasiou V, Katsaros C, Exadactylos A. 2018 Effects of salinity and temperature on the performance of *Cymodocea nodosa* and *Ruppia cirrhosa* : a medium-term laboratory study. *Bot Mar*. **62**, 97–108. (doi:10.1515/bot-2017-0125)

53. Chefaoui R M, Duarte C M, Serrão E A. 2018 Dramatic loss of seagrass habitat under projected climate change in the Mediterranean Sea. *Glob Change Biol*. **224**, 4919–4928. (doi:10.1111/gcb.14401)

54. Ouisse V, Migné A, Davoult D. 2010 Seasonal variations of community production, respiration and biomass of different primary producers in an intertidal *Zostera noltii* bed (Western English Channel, France). *Hydrobiologia*. **649**, 3–11. (doi:10.1007/s10750-010-0254-3)

55. Plus M, Deslous-Paoli J M, Auby I, Dagault F. 2001 Factors influencing primary production of seagrass beds (*Zostera noltii* Hornem.) in the Thau lagoon (French Mediterranean coast). *J Exp Mar Biol Ecol*. **259**, 63–84. (doi:10.1016/S0022-0981(01)00223-4)

56. Bulthuis D A. 1987 Effects of temperature on photosynthesis and growth of seagrasses. *Aquat Bot*. **27**, 27–40. (doi:10.1016/0304-3770(87)90084-2)

57. Gao Y, Jiang Z, Du M, Fang J, Jiang W, Fang J. 2019 Photosyntheticand metabolic responses of eelgrass *Zostera marina* L. to short-term high-temperature exposure. *J Oceanol Limnol*. **37**, 199-209. (doi:10.1007/s00343-019-7319-6)

58. Alcoverro T, Manzanera M, Romero J. 2001 Annual metabolic carbon balance of the seagrass *Posidonia oceanica*: The importance of carbohydrate reserves. *Mar Ecol Prog Ser*. **211**, 105–116. (doi:10.3354/meps211105)

59. Cebrián J, Duarte C M, Marbà N, Enríquez S, Gallegos M, Olesen B. 1996 Herbivory on *Posidonia oceanica*: magnitude and variability in the Spanish Mediterranean. *Mar Ecol Prog Ser*. **130**, 147–155. (doi:10.3354/meps130147)

60. Tomas F, Turon X, Romero J. 2005 Seasonal and small-scale spatial variability of herbivory pressure on the temperate seagrass *Posidonia oceanica*. *Mar Ecol Prog Ser*. **301**, 95–107. (doi:10.3354/meps301095)

61. Marco-Méndez C, Marbà N, Amores Á, Romero J, Minguito-Frutos M, García M, Pagès J F, Prado P, Boada J, Sánchez-Lizaso J L, *et al.* 2024 Evaluating the extent and impact of the extreme Storm Gloria on *Posidonia oceanica* seagrass meadows. *Sci Total Environ*. **908**, 168404. (doi:10.1016/j.scitotenv.2023.168404)

62. Oprandi A, Mucerino L, De Leo F, Bianchi C N, Morri C, Azzola A, Benelli F, Besio G, Ferrari M, Montefalcone M. 2020 Effects of a severe storm on seagrass meadows. *Sci Total Environ*. **748**, 141373. (doi:10.1016/j.scitotenv.2020.141373)

63. Francour P, Ganteaume A, Poulain M. 1999 Effects of boat anchoring in *Posidonia oceanica* seagrass beds in the Port-Cros National Park (north-western Mediterranean Sea). *Aquat Conserv Mar Freshw Ecosyst*. **9**, 391–400. (doi:10.1002/(SICI)1099-0755(199907/08)9:43.0.CO;2-8)

64. Pergent-Martini C, Monnier B, Lehmann L, Barralon E, Pergent G. 2022 Major regression of *Posidonia oceanica* meadows in relation with recreational boat anchoring: A case study from Sant'Amanza bay. *J Sea Res*. **188**, 102258. (doi:10.1016/j.seares.2022.102258)

65. Williams S L. 1988 Disturbance and recovery of a deep-water Caribbean seagrass bed. *Mar Ecol Prog Ser*. **42**, 63–71. (doi:10.3354/meps042063)

66. Dattolo E, Ruocco M, Brunet C, Lorenti M, Lauritano C, D'Esposito D, De Luca P, Sanges R, Mazzuca S, Procaccini G. 2014 Response of the seagrass *Posidonia oceanica* to different light environments: Insights from a combined molecular and photo-physiological study. *Mar Environ Res*. **101**, 225–236. (doi:10.1016/j.marenvres.2014.07.010)

67. Marín-Guirao L, Ruiz J M, Dattolo E, Garcia-Munoz R, Procaccini G. 2016 Physiological and molecular evidence of differential short-term heat tolerance in Mediterranean seagrasses. *Sci Rep*. **6**, 28615. (doi:10.1038/srep28615)

68. Mayol E, Boada J, Pérez M, Sanmartí N, Minguito-Frutos M, Arthur R, Alcoverro T, Alonso D, Romero J. 2022 Understanding the depth limit of the seagrass *Cymodocea nodosa* as a critical transition: Field and modeling evidence. *Mar Environ Res*. **182**, 105765. (doi:10.1016/j.marenvres.2022.105765)

69. Minguito-Frutos M, Adams M P, Alcoverro T, Vilas M P, Alonso D, Mayol E, Bernardeu-Esteller J, Marín-Guirao L, Ruiz JM, Boada, J. 2023 Quantifying the role of

photoacclimation and self-facilitation for seagrass resilience to light deprivation. Front. Plant Sci. 14, 1186538. (doi:10.3389/fpls.2023.1186538)

70. Marbà N, Díaz-Almela E, Duarte C M. 2014 Mediterranean seagrass (*Posidonia oceanica*) loss between 1842 and 2009. *Biol Conserv*. **176**, 183–190. (doi:10.1016/j.biocon.2014.05.024)

71. Martin P, Sébastien D, Gilles T, Isabelle A, De Montaudouin X, Emery É, Claire N, Christophe V. 2010 Long-term evolution (1988–2008) of *Zostera* spp. meadows in Arcachon Bay (Bay of Biscay). *Estuar Coast Shelf Sci*. **87**, 357–366. (doi:10.1016/j.ecss.2010.01.016)

72. Shili A, Trabelsi E B, Ben Maïz N. 2002 Benthic macrophyte communities in the Ghar El Melh lagoon (North Tunisia). *J Coast Conserv*. **8**, 135–140. (doi:10.1652/1400-0350(2002)008[0135:BMCITG]2.0.CO;2)

73. Boudouresque C F, Bernard G, Pergent G, Shili A, Verlaque M. 2009 Regression of Mediterranean seagrasses caused by natural processes and anthropogenic disturbances and stress: a critical review. *Bot Mar*. **52**, 395–418. (doi:10.1515/BOT.2009.057)

74. Gould K, Bruno J F, Ju R, Goodbody-Gringley G. 2021 Upper-mesophotic and shallow reef corals exhibit similar thermal tolerance, sensitivity and optima. *Coral Reefs*. **240**, 907–920. (doi:10.1007/s00338-021-02095-w)

75. Schramek T A, Colin P L, Merrifield M A, Terrill E J. 2018 Depth-Dependent Thermal Stress Around Corals in the Tropical Pacific Ocean. *Geophys Res Lett*. **45**, 9739–9747.(doi:10.1029/2018GL078782)

76. Tavakoli-Kolour P, Sinniger F, Morita M, Nakamura T, Harii S. 2023 Variability in thermal stress thresholds of corals across depths. *Front Mar Sci*. **10**, 1210662. (doi:10.3389/fmars.2023.1210662)

Chapter 3

Supplementary Materials

Table 3.1. Salinity levels of each thermal treatment on the *C. nodosa* (a), *P. oceanica* (b) and *Z. noltei* experiment (c), with their corresponding standard error.

a

Cymodocea nodosa	
Temperature (ºC)	Salinity (g L^{-1})
Control 1	36.50 ± 0.09
Control 2	36.48 ± 0.09
Control 3	36.13 ± 0.08
27	36.69 ± 0.07
30	37.45 ± 0.12
32	36.81 ± 0.07
34	37.26 ± 0.18
36	37.74 ± 0.20
38	37.16 ± 0.14
40	37.24 ± 0.11

b

Posidonia oceanica	
Temperature (ºC)	Salinity (g L^{-1})
Control 1	36.54 ± 0.07
Control 2	36.69 ± 0.06
Control 3	36.64 ± 0.04
Control 4	36.53 ± 0.02
19	36.76 ± 0.06
22	36.87 ± 0.06
24	36.79 ± 0.07
26	36.78 ± 0.03
28	37.00 ± 0.03
30	37.37 ± 0.06
32	37.40 ± 0.05
34	37.63 ± 0.06
36	37.47 ± 0.12

c

Zostera noltei	
Temperature (ºC)	Salinity (g L^{-1})
Control 1	36.77 ± 0.05
Control 2	36.43 ± 0.15
Control 3	36.72 ± 0.04
24	36.72 ± 0.05
26	36.77 ± 0.10
28	37.28 ± 0.15
30	38.61 ± 0.9
32	37.10 ± 0.08
34	38.15 ± 0.88
36	37.96 ± 0.24
38	38.15 ± 0.11

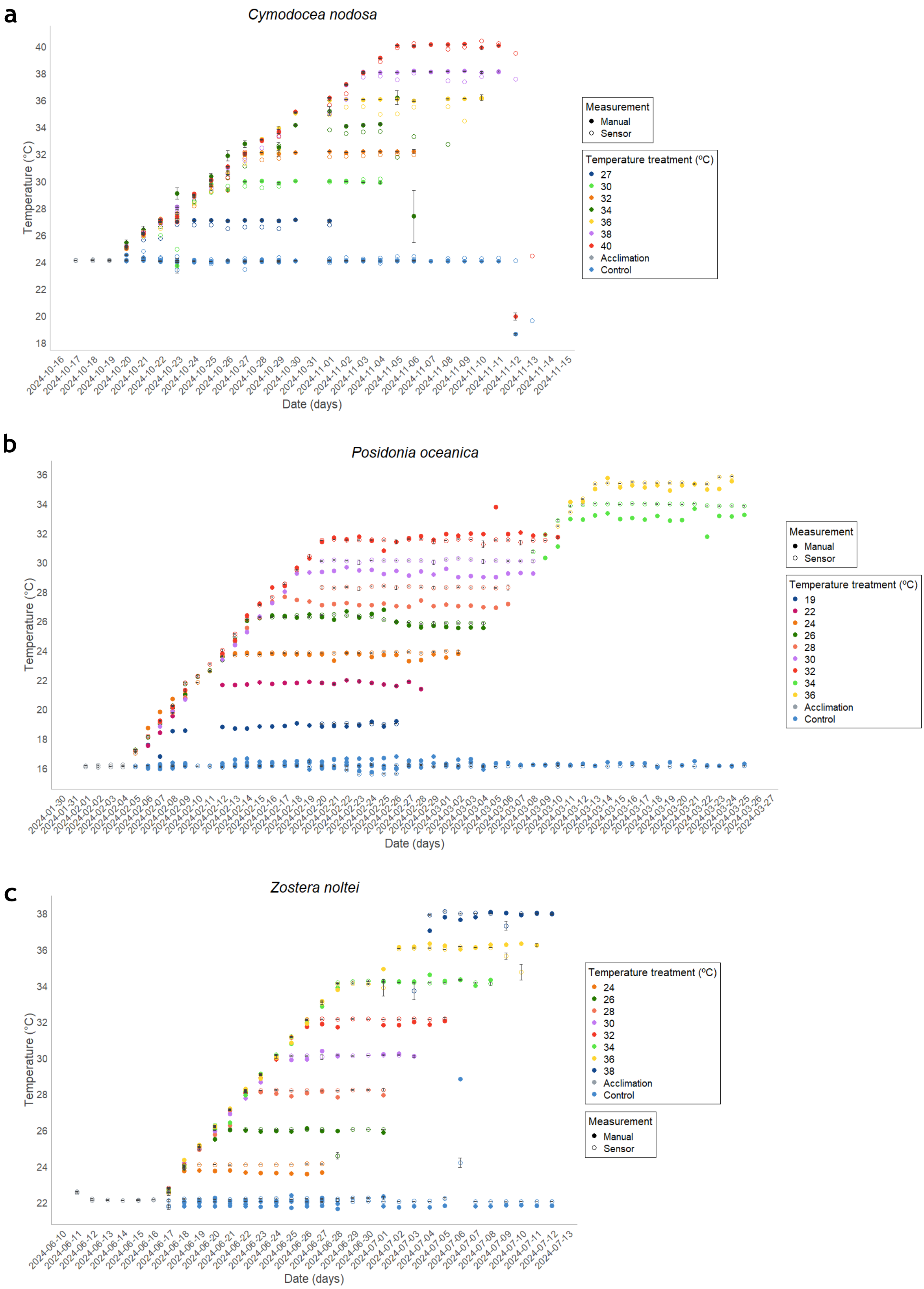

Figure 3.1. Temperature measurements of each thermal treatment throughout the *C. nodosa* (a), *P. oceanica* (b), and *Z. noltei* experiment (c), which include readings from each temperature sensor, accompanied by their respective standard error, as well as manual measurements conducted before the salinity correction.

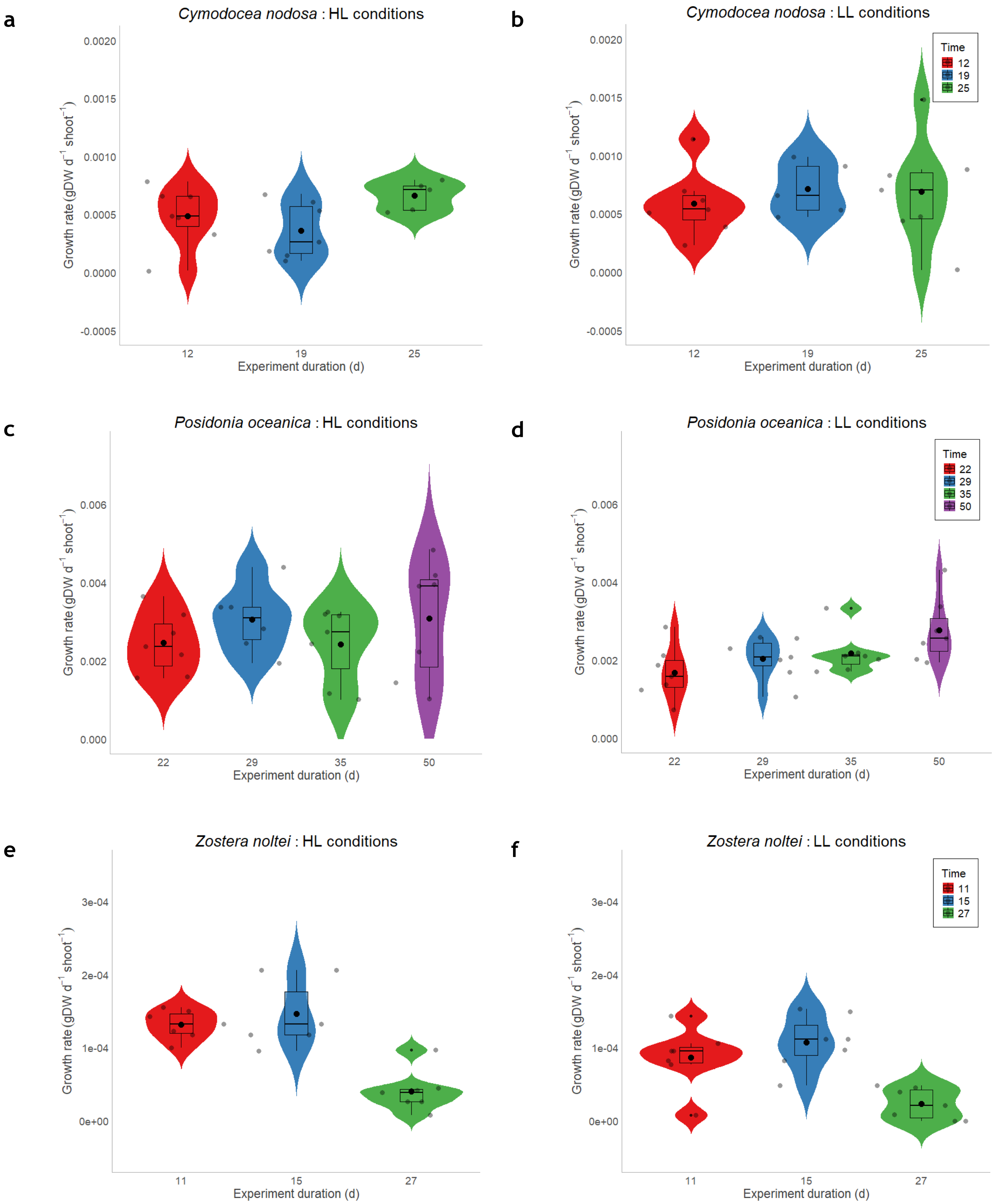

Figure 3.2. Violin plots of the distribution of growth rate data for *C. nodosa* controls under HL (a) and LL conditions (b), *P. oceanica* controls under HL (c) and LL conditions (d), and *Z. noltei* under HL (e) and LL conditions (f). A box plot and mean values for each group are also included.

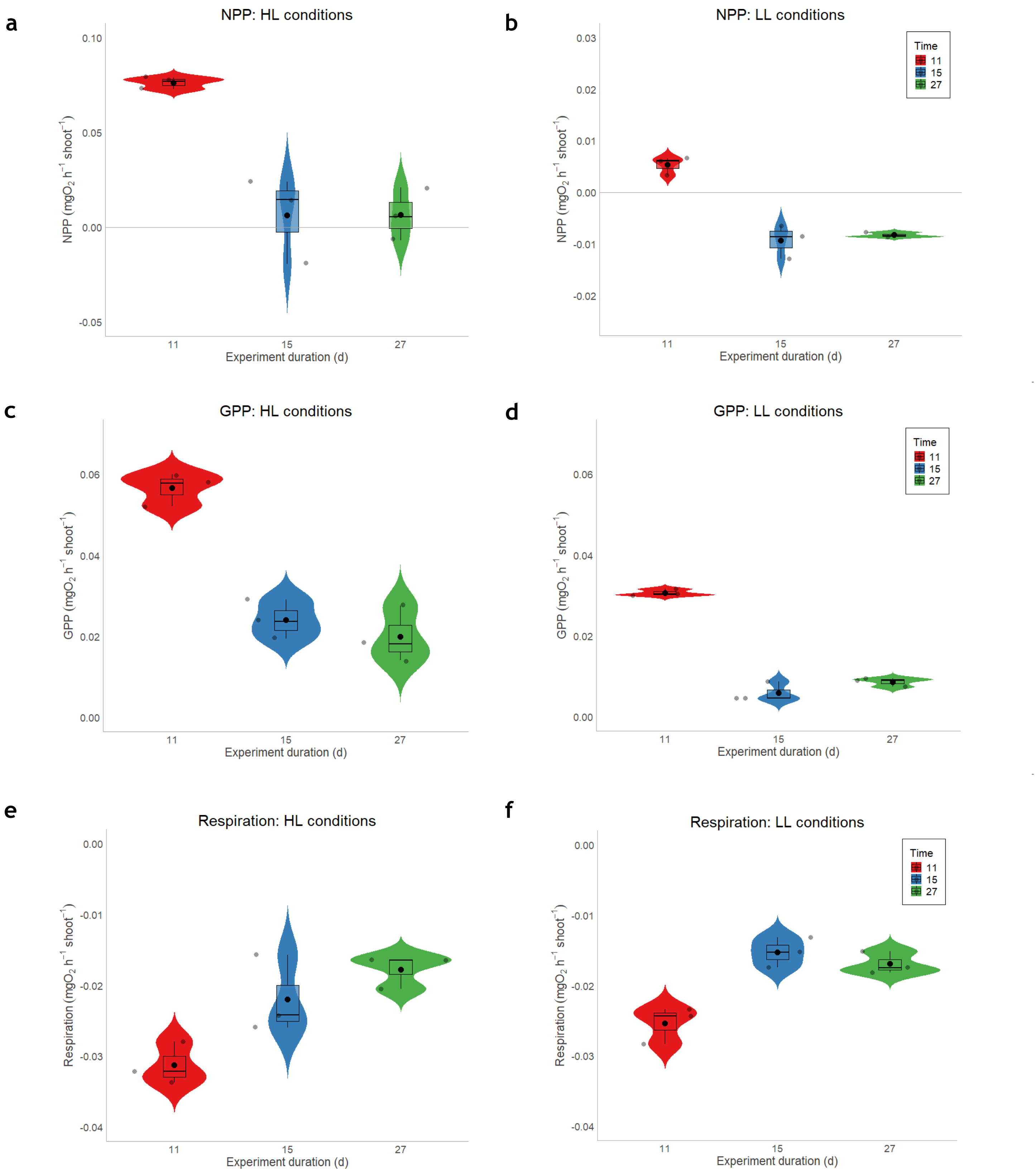

Figure 3.3. Violin plots for the NPP rates controls under HL (a) and LL conditions (b), GPP rates under HL (c) and LL conditions (d), and Respiration rates under HL (e) and LL conditions (f). A box plot and mean values for each group are also included.

Table 3.2. Number of replicates for the *C. nodosa* experiment after the exclusion of infected shoots, divided per temperature and light treatment.

Temperature (ºC)	Light treatment	
	High Light	**Low Light**
24	N = 7	N = 7
27	N = 7	N = 7
30	N = 7	N = 6
32	N = 5	N = 4
34	N = 7	N = 6
36	N = 6	N = 6
38	N = 5	N = 7
40	N = 6	N = 7

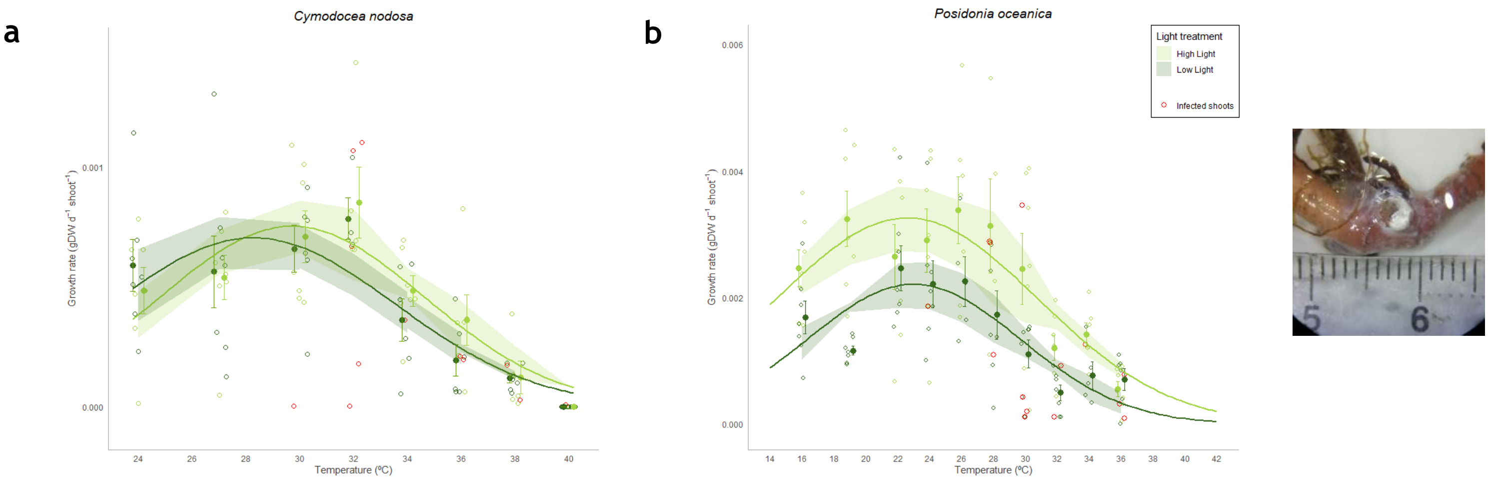

Figure 3.4. Thermal responses of *C. nodosa* (a), *P. oceanica* growth rates (b), both fitted using a Gaussian model, and photo of the fungi on the horizontal rhizome of *C. nodosa* (c). Light treatments (HL vs LL conditions) and their corresponding shares area, which represents the upper and lower $CI_{95\%}$, are included. The fill dots represent the mean of growth rate at each temperature treatment, accompanied by standard error bars. The red dots indicate the shoots that had been infected by the fungi.

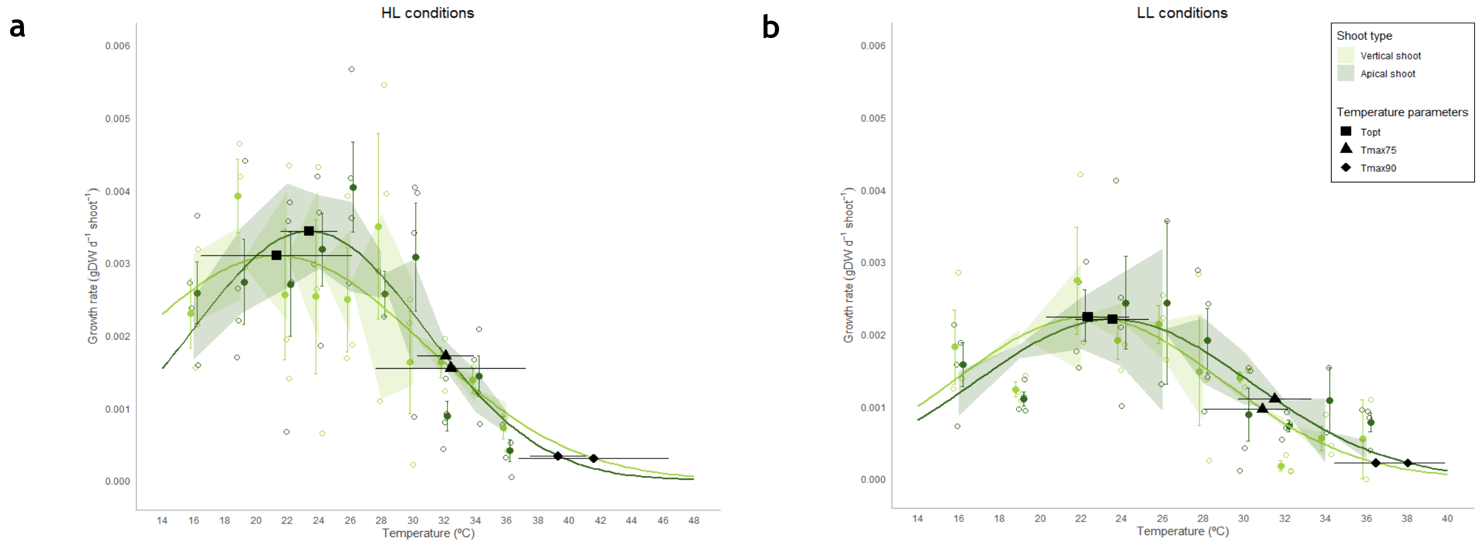

Figure 3.5. Thermal responses of growth rate for vertical and apical shoots of *P. oceanica* under HL (a) and LL conditions (b), fitted using a Gaussian model. The shoot type (vertical vs apical) and their corresponding shares area, which represents the upper and lower $CI_{95\%}$, are included. The thermal thresholds (T_{opt}, $T_{max75\%}$, $T_{max90\%}$), with their respective $CI_{95\%}$, are shown. The fill dots represent the mean of growth rate at each temperature treatment, alongside standard error bars.

Table 3.3. Above:below ground biomass ratios and their respective standard error at each temperature and light treatment of the *Z. noltei* experiment.

Temperature (ºc)	Light treatments	
	High Light	Low Light
Control 1	208.80 ± 39.90	165.91 ± 48.12
Control 2	125.67 ± 30.62	81.18 ± 19.84
Control 3	72.01 ± 18.46	76.21 ± 25.81
24	349.22 ± 110.08	174.42 ± 34.16
26	163.04 ± 47.21	172.32 ± 52.64
28	95.40 ± 24.36	149.72 ± 22.27
30	133.65 ± 13.94	191.18 ± 40.29
32	126.73 ± 25.90	152.38 ± 28.49
34	156.13± 23.89	212.28 ± 67.87
36	83.34 ± 17.28	245.23 ± 69.88
38	182.84 ± 84.51	133.40 ± 21.22