UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS

METABOLISMO Y FLUJOS DE NITRÓGENO DEL PASTO MARINO *Phyllospadix scouleri* Y SU VARIACIÓN BAJO CONDICIONES DE CALENTAMIENTO ASOCIADAS A OLAS DE CALOR MARINAS

TESIS

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

DOCTOR EN MEDIO AMBIENTE Y DESARROLLO

PRESENTA

PAULA BONET MELIÀ

Ensenada, Baja California, México. Enero de 2024

RESUMEN

Las praderas de *Phyllospadix* spp. (*P. scouleri* y *P. torreyi*) conforman complejos ecosistemas costeros de elevada productividad en las costas del Pacífico Norteamericano. Al igual que otras especies de pastos marinos, *Phyllospadix* spp. se encuentran bajo la amenaza de distintos impactos tanto de origen natural como antropogénico. En el contexto del cambio climático, se ha observado que las olas de calor marinas (OCMs) representan anomalías térmicas extremas que pueden impactar negativamente en el metabolismo, la productividad y la distribución de los pastos marinos. Sin embargo, el conocimiento acerca de sus efectos es aún limitado en algunos aspectos relacionados con la fisiología de estos, los cuales presentan una relación directa con importantes servicios ecosistémicos. En particular, se desconoce casi por completo cómo la exposición a elevadas temperaturas puede afectar al metabolismo del nitrógeno, y por tanto, al potencial de biofiltración de estas plantas. Esta tesis tuvo como objetivo principal evaluar los flujos y el metabolismo del nitrógeno (N) de *P. scouleri,* y su variación ante condiciones de calentamiento asociado a OCMs, utilizando distintos enfoques experimentales. Estos enfoques incluyeron: i) la comparación entre los efectos de una OCM con OCMs recurrentes (Capítulo 1), y ii) la comparación entre OCMs de diferente intensidad (severa y extrema) (Capítulo 2). Las respuestas del calentamiento sobre los flujos y metabolismo del N fueron examinadas a través de distintos descriptores, como la cinética de incorporación de amonio, nitrato y urea, la actividad enzimática asimilatoria, la capacidad de biofiltración, el contenido de N y los flujos de nitrógeno disuelto total. Es importante destacar que este estudio constituyó una de las primeras aproximaciones a la exploración de los efectos del calentamiento en dichos aspectos para *P. scouleri*. De forma general, y en base a los resultados de los dos experimentos, se pudo concluir que i) *P. scouleri* podría desempeñar un papel importante como biofiltro para aguas residuales que contienen amonio, nitrato y urea, ii) que la exposición a una única OCM y a OCMs consecutivas podría afectar a la capacidad de biofiltración de *P.scouleri,* iii) que la urea puede ser una fuente alternativa de N durante la exposición a OCMs, iv) que la incorporación y asimilación de amonio, nitrato y urea en *P. scouleri* puede variar según la intensidad de la OCM (severa o extrema) y la fase experimental (i.e. durante la exposición al calentamiento, o posterior al mismo), y v) que la incorporación y asimilación de amonio, nitrato y urea no exhibe un comportamiento lineal con el aumento de la temperatura.

ABSTRACT

Seagrass meadows of *Phyllospadix* spp. (*P. scouleri* and *P. torreyi*) are highly productive complex coastal ecosystems along the Pacific coast of North American. Like other seagrass species, *Phyllospadix* spp. are under threat from a variety of natural and anthropogenic impacts. In the context of climate change, marine heat waves (MHWs) have been observed as extreme thermal anomalies that can negatively impact seagrass metabolism, productivity, and distribution. However, knowledge of their effects is still limited in some aspects related to their physiology, which are directly related to important ecosystem services. In particular, it is almost completely unknown how exposure to elevated temperatures can affect nitrogen metabolism, and therefore, the biofiltration potential of these plants. The main objective of this thesis was to evaluate nitrogen (N) fluxes and metabolism of *P. scouleri*, and their variation under warming conditions associated with MHWs, using different experimental approaches. These approaches included: i) comparison between the effects of one MHW with recurrent MHWs (Chapter 1), and ii) comparison between MHWs of different intensity (severe and extreme) (Chapter 2). The responses of warming on N fluxes and metabolism were examined using different descriptors, such as ammonium, nitrate and urea uptake kinetics, assimilatory enzyme activity, biofiltration capacity, N content and total dissolved nitrogen fluxes. It is important to note that this study was one of the first approaches to investigate the effects of warming on these aspects for *P. scouleri*. Overall, based on the results of the two experiments, it could be concluded that i) *P. scouleri* could play an important role as a biofilter for wastewaters containing ammonium, nitrate and urea, ii) exposure to a single and consecutive MHWs could affect the biofiltration capacity of *P. scouleri*, iii) urea could be an alternative source of N during exposure to MHWs, iv) ammonium, nitrate and urea uptake and assimilation in *P. scouleri* can vary depending on the intensity of the MHW

(severe or extreme) and the experimental phase (i.e. during, or after, exposure to warming), and v) that ammonium, nitrate and urea uptake and assimilation do not show a linear behavior with increasing temperature.

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS
DOCTORADO EN MEDIO AMBIENTE Y DESARROLLO

METABOLISMO Y FLUJOS DE NITRÓGENO DEL PASTO MARINO *Phyllospadix scouleri* Y SU VARIACIÓN BAJO CONDICIONES DE CALENTAMIENTO ASOCIADAS A OLAS DE CALOR MARINAS

TESIS

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

DOCTORA EN MEDIO AMBIENTE Y DESARROLLO

PRESENTA

PAULA BONET MELIÀ

Aprobada por:

José Miguel Sandoval Gil

Dr. Jose M. Sandoval Gil
DIRECTOR

Dr. Guillermo A. Samperio Ramos
DIRECTOR

Ana Alexandre

Dra. Ana Alexandre
SINODAL

Dra. M.ª del Carmen Ávila López
SINODAL

Dr. Víctor F. Camacho-Ibar
SINODAL

AGRADECIMIENTOS

Quiero dar las gracias al Programa de Doctorado Medio Ambiente y Desarrollo, al IIO y a la UABC por brindarme la oportunidad de realizar mi tesis doctoral y contribuir a mi formación.

Al Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT) por el apoyo económico y permitir que pudiera dedicarme únicamente a la tesis.

A mis directores, Jose y Guille, por la oportunidad de realizar el doctorado y por todo el aprendizaje durante estos años.

A los miembros de mi comité de tesis, Ana, Víctor y Mª Carmen por la ayuda y tiempo dedicado.

A todos los miembros de CiNEMA por todas las reuniones, aunque a veces parecieran interminable.

A todos los miembros de botánica marina por su ayuda en los experimentos y en el laboratorio.

A todos los compañeros del doctorado por hacer más amenos los días de trabajo.

A mis amigos, por todos los momentos compartidos y por hacerme sentir como en casa, especialmente a ti, Gon.

A mi familia por su apoyo y entender que debía estar lejos de casa, en especial a mi madre, gràcis.

A quienes me ayudaron en mi momento más difícil, sin vosotros esta tesis no habría sido posible.

Finalmente, quiero darle las gracias a México, por recibirme con los brazos abiertos, por enseñarme tanto, por darme buenos amigos, pareja, viajes inolvidables y como no, por sus magníficas praderas de pastos marinos.

ÍNDICE

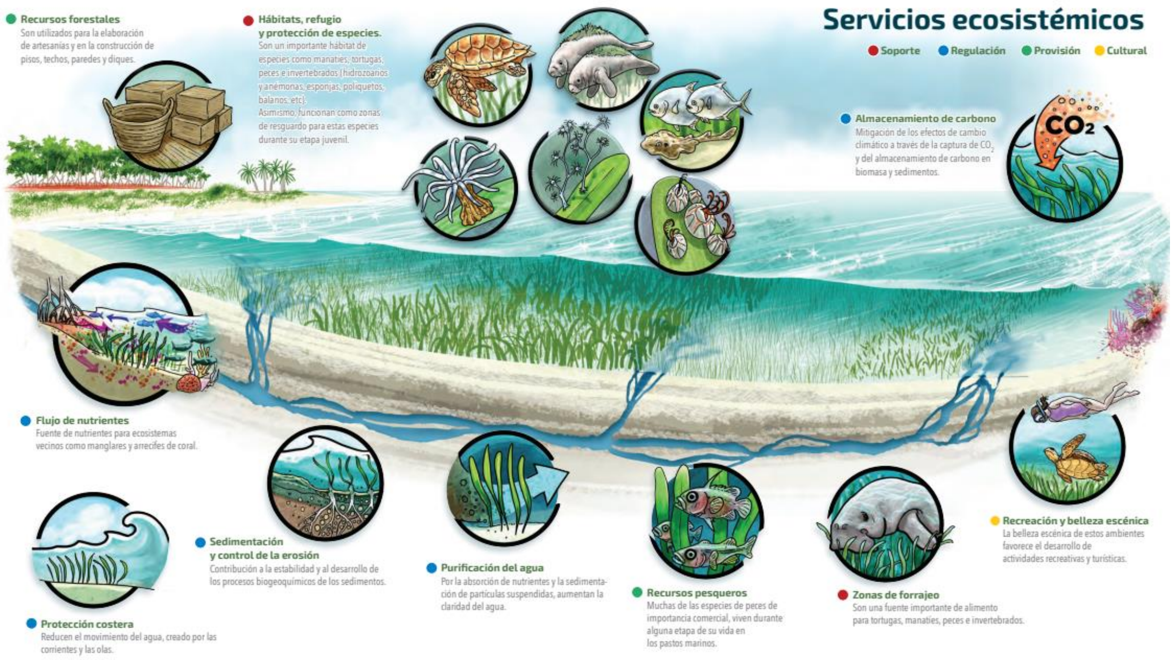
INTRODUCCIÓN

1. Pastos marinos: importancia y amenazas

Los pastos marinos son angiospermas que habitan en entornos marinos y tienen la capacidad de formar extensas praderas sumergidas (Gallagher et al., 2022). Por lo general, se desarrollan en sustratos arenosos o lodosos en aguas poco profundas (< 50 m; Esteban et al., 2018). No obstante, algunos géneros, como *Phyllospadix* spp., pueden crecer en sustratos rocosos desde áreas intermareales hasta aguas poco profundas (Ruiz-Montoya et al., 2021). Las angiospermas marinas se distribuyen prácticamente en todas las biorregiones del mundo (a excepción de la Antártida), y hasta la fecha se han identificado 12 géneros de pastos marinos, con alrededor de 65 especies diferentes (Charpy-Rouband y Sournia, 1990; den Hartog y Kuo, 2006).

Los pastos marinos representan uno de los ecosistemas sumergidos más productivos y ecológicamente relevantes en las zonas costeras (Duarte y Cebrián 1996; Duarte y Chiscano 1999). Además, desempeñan un papel fundamental como ingenieros del ecosistema y ofrecen una variedad de servicios ecosistémicos importantes. Por ejemplo, proporcionan hábitats y refugio a numerosas especies, sus rizomas y raíces estabilizan los sedimentos, protegen las costas contra la erosión, actúan como sumideros de carbono y contribuyen a mantener la calidad del agua (Fig. 1; Cuellar-Martínez et al., 2019; de los Santos et al., 2020).

En la actualidad, las praderas de pastos marinos se encuentran en una situación de amenaza debido a una combinación de impactos de origen natural y actividades humanas (Waycott et al., 2009; Dunic et al., 2021). Entre ellos se incluyen impactos derivados de la actividad pesquera, la degradación progresiva de la calidad del agua (manifestada por el aumento en la turbidez, los niveles de nutrientes y la acumulación de sedimentos), o el incremento en la temperatura del agua asociado al cambio climático (Smale et al., 2019; Hastings et al., 2020; Dunic et al., 2021). De forma general, estos impactos (y su combinación con otros estresores) han resultado en una disminución significativa de la extensión global de las praderas de pastos marinos, con estimaciones que sugieren pérdidas de hasta un 20% de su cobertura a nivel mundial (Orth et al., 2006; Waycott et al., 2009). Esta regresión plantea preocupaciones sobre la pérdida de la productividad y de los servicios ecosistémicos que proporcionan estos ecosistemas. Por eso, resulta esencial llevar a cabo estudios detallados de la respuesta del metabolismo del nitrógeno, elemento fundamental en la productividad y funcionalidad ecológica de los pastos marinas (McGlathery, 2008). Estos estudios son cruciales para la implementación de medidas efectivas de conservación y gestión destinadas a preservar estos ecosistemas y los servicios ecosostémicos que nos ofrecen.



Recursos forestales Son utilizados para la elaboración de artesanías y en la construcción de pisos, techos, paredes y diques.

Hábitats, refugio y protección de especies. Son un importante hábitat de especies como manatíes, tortugas, peces e invertebrados (hidrozoarios y anémonas, esponjas, poliquetos, balanos, etc).
Asimismo, funcionan como zonas de resguardo para estas especies durante su etapa juvenil.

Almacenamiento de carbono Mitigación de los efectos de cambio climático a través de la captura de CO_2 y del almacenamiento de carbono en biomasa y sedimentos.

Flujo de nutrientes Fuente de nutrientes para ecosistemas vecinos como manglares y arrecifes de coral.

Sedimentación y control de la erosión Contribución a la estabilidad y al desarrollo de los procesos biogeoquímicos de los sedimentos.

Purificación del agua Por la absorción de nutrientes y la sedimentación de partículas suspendidas, aumentan la claridad del agua.

Recursos pesqueros Muchas de las especies de peces de importancia comercial, viven durante alguna etapa de su vida en los pastos marinos.

Zonas de forrajeo Son una fuente importante de alimento para tortugas, manatíes, peces e invertebrados.

Recreación y belleza escénica La belleza escénica de estos ambientes favorece el desarrollo de actividades recreativas y turísticas.

Protección costera Reducen el movimiento del agua, creado por las corrientes y las olas.

Figura 1. Representación gráfica de los servicios ecosistémicos clave proporcionados por los pastos marinos. Modificado de Teutli et al. (2020).

2. Aspectos generales del metabolismo del nitrógeno en los pastos marinos

La disponibilidad de nutrientes, particularmente el nitrógeno (N), desempeña un papel fundamental en la productividad y funcionalidad ecológica de las praderas de pastos marinos (McGlathery, 2008). El N se presenta en una mezcla compleja de compuestos disueltos con diferentes concentraciones, algunos de ellos disponibles para su utilización por parte de los pastos marinos (Vonk et al., 2008). Estos nutrientes pueden provenir de fuentes externas, como aguas residuales, deposiciones atmosféricas o fertilizantes, y también de fuentes internas a través del reciclaje interno en la estructura clonal de la pradera (Romero et al., 2006; McGlathery, 2008).

Los pastos marinos tienen la capacidad de adquirir nitrógeno al incorporar compuestos orgánicos e inorgánicos disueltos a través de sus hojas y raíces (Touchette y Burkholder, 2000).

La incorporación de N suele responder a una cinética de Michaelis-Menten,

$$V= (V_{max}\ \text{x}\ S)/(K_m + S)$$

donde V y V_{max} representan la velocidad y tasa máxima de incorporación de N, respectivamente, K_m es la constante de saturación media, y S es la concentración del nutriente. La afinidad de incorporación ($\alpha = V_{max}/K_m$) refleja la eficiencia de absorción de nutrientes a concentraciones sub-saturantes (Touchette y Burkholder, 2000). Sin embargo, algunos estudios han demostrado respuestas lineales en la incorporación de nitrógeno inorgánico para especies como *Zostera marina*, *Thalassia hemprichii* y *Amphibolis antarctica* (Roth y Pregnall, 1988; Paling y McComb, 1994; Stapel et al., 1996). Las razones detrás de estas dos tipos de cinéticas aún no se comprenden completamente, pero podrían reflejar respuestas de aclimatación frente a la disponibilidad de N (McGlathery, 2008).

La fracción inorgánica disuelta de nitrógeno (NID) en los entornos costeros está mayormente conformada por nitrato y amonio, considerados como las principales fuentes de N para los pastos marinos (Lee et al., 2007). Generalmente, los pastos marinos incorporan NID de manera más eficiente a través de sus hojas que de sus raíces

(Alexandre et al., 2015), aunque ambos compartimentos vegetativos pueden contribuir de manera importante al sustento de las necesidades de necesidades de N en la planta (Sandoval-Gil et al., 2015). El nitrato se incorpora mediante un sistema de transporte activo de alta afinidad y dependiente de sodio (Rubio et al., 2018). El amonio se incorpora a través de un sistema de baja afinidad basado en el movimiento pasivo de NH_4^+ a través de canales de membrana, y un sistema de alta afinidad con transporte activo (McGlathery, 2008). Se han documentado mayores valores de V_{max} y α para el amonio, atribuidos a los menores costos metabólicos necesarios para su asimilación (Touchette y Burkholder, 2000) y a las características de los sistemas de transporte en la membrana plasmática (Rubio et al., 2018).

El nitrógeno orgánico disuelto (NOD) principalmente en forma de urea y aminoácidos, puede suponer una fuente importante de N complementaria para los pastos marinos en condiciones de baja disponibilidad de NID (La Nafie et al., 2014; Alexandre et al., 2015; Viana et al., 2019). Aunque se ha prestado menos atención a su estudio, la incorporación de NOD parece estar asociada a un sistema de transporte activo de alta afinidad y dependiente de sodio, similar al nitrato (Azcón-Bieto y Talón, 2013). Los pastos marinos pueden incorporar DON a tasas comparables a las de nitrato (Vonk et al., 2008; Van Engeland et al., 2011; Alexandre et al., 2015). No obstante, sus tasas de consumo son mucho menores que las correspondientes al NH_4^+ debido al elevado consumo de energía necesario para la absorción de DON (McGlathery, 2008).

Tras su incorporación, el N puede ser asimilado gracias a la actividad de distintas enzimas para luego ser almacenado y/o movilizado por translocación entre los compartimentos vegetativos (Viana et al., 2019). El nitrato se asimila a través de una reducción a nitrito por la enzima nitrato reductasa (NR) y después, el nitrito es reducido a amonio gracias a la enzima nitrito reductasa (NiR). El amonio se asimila a través del ciclo glutamina sintetasa (GS)/glutamato sintetasa (GOGAT) para la síntesis de aminoácidos (Touchette y Burkholder, 2000; Azcón-Bieto y Talón, 2013). Estos procesos se sustentan en la producción de energía metabólica y esqueletos de carbono provenientes de los procesos fotosintéticos (Azcón-Bieto y Talón, 2013).

De forma general, el uso eficiente del nitrógeno en los pastos marinos dependerá de i) la disponibilidad de los compuestos nitrogenados, ii) las capacidades de incorporar y asimilar N, iv) la capacidad de almacenar y reciclar N inorgánico y orgánico, y v) de la pérdida de compuestos de N por flujos de liberación/exudación.

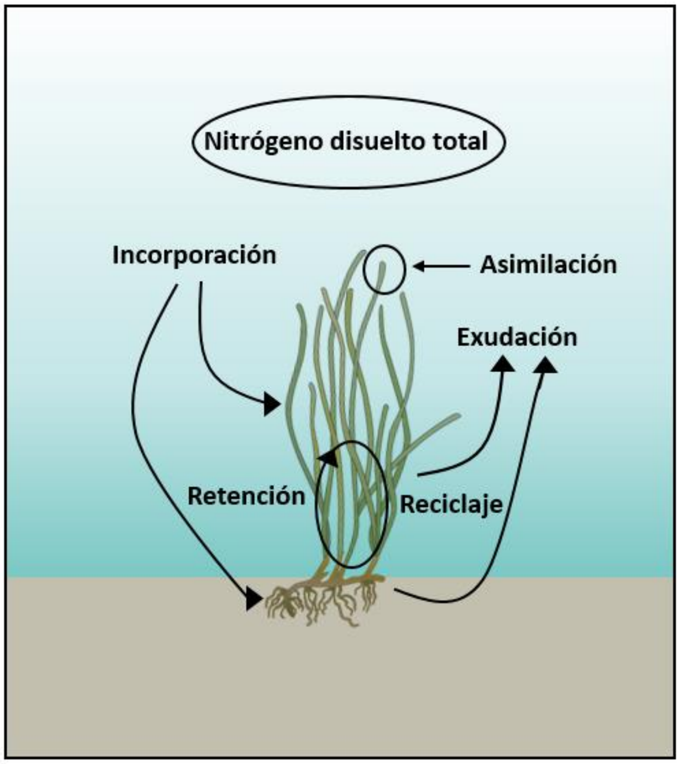


Figura 2. Representación esquemática de los principales flujos de N en un pasto marino.

3. Efectos del calentamiento en el metabolismo del N de los pastos marinos

En la actualidad, hay una creciente preocupación por comprender y anticipar los impactos del cambio climático en los ecosistemas costeros conformados por pastos marinos (Jordà et al., 2012; Koch et al., 2013; Nguyen et al., 2021a). En este contexto se ha observado que el aumento de la temperatura del agua de mar puede tener consecuencias en la productividad, tasas metabólicas, estructura de la comunidad y distribución de los pastos marinos (Egea el al., 2018; Nguyen et al., 2021b; Egea et al., 2023; Smith et al., 2023). En comparación con el calentamiento paulatino de los océanos, el calentamiento anómalo y abrupto asociado a las olas de calor marinas (OCMs) puede provocar alteraciones más severas en las comunidades biológicas (Smith et al., 2023). En pastos marinos, estas alteraciones se han demostrado a sus distintos niveles biológicos, desde el metabólico al de comunidad (Koch et al., 2013; Thomson et al., 2015; Arias-Ortiz et al., 2018; Smale et al., 2019; Smith et al., 2023; Egea et al., 2023). Una OCM se define como un aumento temporal (mínimo 5 días) y anómalo en la temperatura superficial del mar, con temperaturas superiores al percentil 90 según un período de referencia histórico de 30 años (McGregor et al., 2015; Hobday et al., 2016), aunque existen diversos tipos de OCMs (moderadas, fuertes, severas y extremas) clasificadas por su intensidad y duración (Hobday et al., 2018). Los modelos climáticos proyectan un aumento en la intensidad y la frecuencia de las OCMs (Oliver et al., 2018; Marín et al., 2021), por lo que es crítico realizar estudios que predigan su impacto. Existe una cantidad considerable de información sobre de los efectos del estrés térmico en varios aspectos ecofisiológicos de los pastos marinos (p.e., fotosíntesis; Marín-Guirao et al., 2019; Serrano et al., 2019; Vivanco-Bercovich et al., 2022). Sin embargo, las respuestas específicas relacionadas con el metabolismo del nitrógeno (N) son mucho más escasas (Ontoria et al., 2019; Alexandre et al., 2020; Vivanco-Bercovich et al., 2023). Algunos estudios han documentado que las OCMs pueden afectar negativamente las capacidades de incorporar y retener N en *Phyllospadix scouleri* y *Cymodocea nodosa* (Vivanco-Bercovich et al., 2023; Ontoria et al., 2019), mientras que otros trabajos han encontrado el efecto contrario (Alexandre et al., 2020). Muchos de los estudios realizados hasta la fecha se han centrado en evaluar el efecto de un mismo tipo de OCM, basado en un único incremento de temperatura mantenido durante un periodo determinado (Costa et al., 2021; Deguette et al., 2022; Egea et al., 2023). Las características de las OCMs pueden ser diversas, y otros trabajos (aunque escasos) se ha preocupado por integrarlas en sus diseños experimentales (DuBois et al., 2020; Nguyen et al., 2020; Saha et al., 2020; Pazzaglia et al., 2022; Vivanco-Bercovich, 2023). Se necesita seguir avanzando en el conocimiento de las respuestas de pastos marinos en distintos escenarios posibles de calentamiento, como OCMs de diferente intensidad y recurrentes, así como incluir periodos experimentales de recuperación de la temperatura a niveles promedio. Sin duda, esto es indispensable para un mejor examen de la tolerancia y resiliencia de pastos marinos frente a este tipo de estresor térmico.

4. *Phyllospadix* spp. en Baja California y olas de calor marinas

En las costas del Pacífico Norteamericano, se encuentran las extensas praderas de *Phyllospadix scouleri* y *Phyllospadix torreyi*, comúnmente llamadas en inglés como *surfgrasses.* Estos pastos marinos dominan desde la franja intermareal hasta los ~7 m de profundidad, conformando praderas con elevada productividad vegetativa (> 8000 g PS m^{-2} por año; Ramírez-García et al., 1998; García-Pantoja et al., 2020). Los extremos de su distribución se localizan desde Columbia Británica (Canadá) hasta Isla Margarita en la península de Baja California Sur (México; Den Hartog 1970; Phillips 1979) y son los únicos pastos marinos insulares presentes en la Reserva de la Biósfera Islas del Pacífico de Baja California. Estas especies se diferencian de la mayoría de los pastos marinos porque presentan particularidades anatómicas, morfológicas (p.ej., rizomas engrosados y con la superficie ondulada) y fisiológicas (Ruiz-Montoya et al., 2021) que les confieren una elevada capacidad para colonizar sustratos rocosos y soportar la exposición a un hidrodinamismo y oleaje extremo (Cooper y McRoy 1988; Barnabas 1994; Kuo y Stewart 1995). Además, en comparación con otros pastos marinos, sus hojas muestran tasas de incorporación de N notablemente altas, presumiblemente para compensar su baja absorción por las raíces (Terrados y Williams, 1997). Ya que *Phyllospadix* spp. presenta una elevada biomasa (2800 g PS m^{-2}) perenne, y su distribución en el intermareal coincide con zonas urbanizadas, se ha propuesto que podrían funcionar como biofiltros para vertidos de aguas residuales (García-Pantoja et al., 2020), aunque no existe evidencia empírica de dicha afirmación. A pesar de conformar comunidades de alto valor ecológico y económico (Ramírez-García et al., 1998), se sabe muy poco acerca de aspectos ecofisiológicos y los descriptores relacionados con el metabolismo de N de estas especies; esto se acentúa aún más al comparar con la información disponible con la especie *Zostera marina*, también formadora de comunidades clímax en bahías y esteros de esta región (Cabello-Pasini et al., 2003; Den Hartog y Kuo 2006; Sandoval-Gil et al., 2016).

Figura 3. Las praderas mixtas de *Phyllospadix torreyi* y *Phyllospadix scouleri*. (A) En la zona intermareal de la costa de Ensenada y (B) en la zona submareal de la Isla de Todos Santos, Baja California.

En los últimos años, la costa de Baja California (México) ha experimentado una sucesión de olas de calor marinas (OCMs; Bond et al., 2015; Sen Gupta et al., 2020, Wei et al., 2021). La más significativa, conocida como "the Blob", ocurrió entre 2013 y 2015, y resultó en anomalías térmicas de hasta +5 °C a más de 50 m de profundidad (Bond et al., 2015; Zaba y Rudnick, 2016; Sen Gupta et al., 2020; Wei et al., 2021). En esta región, ya se ha demostrado que las anomalías térmicas asociadas a las OCMs y ENSO pueden impactar a la productividad y estructura de las comunidades de macrófitos marinos, como a las praderas de *Z. marina* (Echavarría et al., 2006) y a los bosques de algas pardas Laminariales (Arafeh-Dalmau et al., 2020). Algunos estudios indican que el crecimiento y la supervivencia de *Phyllopadix* spp. pueden verse comprometidos bajo condiciones de calentamiento (Drysdale y Barbour, 1975). Sin embargo la información acerca de los potenciales efectos de OCMs en la ecofisiología de estas especies es aún muy escasa (Vivanco-Bercovich et al., 2022; Vivanco-Bercovich et al., 2023).

5. Objetivos

Objetivo general: evaluar los flujos y el metabolismo del nitrógeno en *Phyllospadix scouleri,* y su variación ante distintos escenarios de calentamiento asociados al impacto de olas de calor marinas.

Para logran este objetivo, se llevaron a cabo dos experimentos de mesocosmos, correspondientes a dos objetivos específicos.

Objetivos específicos

i) Evaluar la capacidad de *P. scouleri* para actuar como biofiltro de vertidos de aguas residuales, así como analizar su variación ante la exposición a una única OCM o dos OCMs consecutivas.

ii) Evaluar los efectos de la OCMs de diferente intensidad sobre el metabolismo y flujos de N (incorporación, asimilación y exudación) de *P.scouleri*

La tesis está estructurada en dos capítulos, en formato de artículo científico. En el **Capítulo I** se comparan las respuestas del metabolismo del N en *Phyllospadix scouleri* frente a la exposición de una única OCM o dos OMCs consecutivas. En el **Capítulo II**, se comparan los efectos de OCMs de diferente intensidad (severa y extrema) y el periodo post-OCMs en el metabolismo y flujos de N en *P.scouleri*.

Tabla 1. Características generales y descriptores biológicos correspondientes a los dos experimentos realizados.

	Experimento 1 (Capítulo 1)	**Experimento 2 (Capítulo 2)**
Tema general	OCM única y OCMs consecutivas	OCMs de diferente intensidad (severa y extrema)
Especie utilizada	*P. scouleri*	*P. scouleri*
Pradera donadora	Isla Todos Santos	Isla Todos Santos
Fecha	Agosto 2020	Octubre 2021
Mesocosmos	Indoor	Outdoor
Duración total del experimento	39 días	27 días
Temperatura promedio de la OCM	24°C	22-25°C
Temperatura máxima de la OCM	24°C	25-28°C
Temperatura del control	18°C	17-20°C
OCMs consecutivas	✓	
Periodos post OCM		✓
Descriptores		
Cinéticas de incorporación de nitrógeno	✓	✓
Enzimas asimilatorias	✓	✓
Capacidad de biofiltración	✓	
Contenido de nitrógeno total	✓	✓
Flujo de nitrógeno disuelto total		✓

6. Referencias

Alexandre, A., Hill, P. W., Jones, D. L., & Santos, R., 2015. Dissolved organic nitrogen: a relevant, complementary source of nitrogen for the seagrass zostera marina. Limnology and Oceanography, 60(5), 1477-1483. https://doi.org/10.1002/lno.10084

Barnabas, A., 1994. Anatomical, histochemical and ultrastructural features of the seagrass *Phyllospadix scouleri*. Aquatic Botany, 49, 167-182. https://doi.org/10.1016/0304-3770(94)90036-1.

Cabello-Pasini, A., Muñiz-Salazar, R.,H Ward,Annual. D., 2003. Variations of biomass and photosynthesis in Zostera marina at its southern end of distribution in the North Pacific. Aquatic Botany, 76(1), 31-47. https://doi.org/10.1016/S0304-3770(03)00012-3.

Alexandre, A., Quintã, R., Hill, P. W., Jones, D. L., & Santos, R., 2020. Ocean warming increases the nitrogen demand and the uptake of organic nitrogen of the globally distributed seagrass *Zostera marina*. Functional Ecology, 34(7), 1325-1335.

Alexandre, A.C.S., Da Silva, J.S., Bouma, T.J., Santos, R., 2011. Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass *Zostera noltii*. J. Exp. Mar. Biol. Ecol. 401 (1–2), 7–12. https://doi.org/10.1016/j.jembe.2011.03.008.

Arafeh-Dalmau, N., Montaño-Moctezuma, G., Martínez, J.A., Beas-Luna, R., Schoeman, D.S., Torres-Moye, G., 2019. Extreme marine heatwaves alter kelp forest community near its equatorward distribution limit. Front. Mar. Sci. 6, 499. https://doi.org/10.3389/fmars.2019.00499

Arias-Ortiz, A., Serrano, O., Masqué, P., Lavery, P. S., Mueller, U., Kendrick, G. A., Duarte, C. M., 2018. A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. Nature Climate Change, 8, 338–344. https://doi.org/10.1038/s41558-018-0096-y

Azcón-Bieto, J., Talón, M., 2008. Fundamentos de fisiología vegetal. McGraw-Hill Interamericana de España, S.L.

Charpy-Robaud, C., and Sournia, A., 1990. The comparative estimation of phytoplanktonic, microphytobenthic, and macrophytobenthic primary production in the oceans. Mar. Microb. Food Webs 4, 31–57.

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. https://doi.org/10.3390/oceans2030025

Cuéllar-Martínez, T., Sanchez-Cabeza, J., Pérez-Bernal, L. H., & Sandoval-Gil, J. M., 2019. Relevance of carbon burial and storage in two contrasting blue carbon ecosystems of a North-East Pacific coastal lagoon. Science of The Total Environment, *675*, 581-593. https://doi.org/10.1016/j.scitotenv.2019.03.388

de los Santos, C.B., Scott, A., Arias-Ortiz, A., Jones, B., Kennedy, H., Mazarrasa, I., McKenzie, L., Nordlund, L.M., de la Torre-Castro, M. de la T., Unsworth, R.K.F., Ambo-Rappe, R., 2020. Seagrass ecosystem services: assessment and scale of benefits. Blue Value Seagrasses Environ. People 19–21.

Deguette, A., Barrote, I., Silva, J., 2022. Physiological and morphological effects of a marine heatwave on the seagrass *Cymodocea nodosa*. Sci. Rep. 12, 7950. https://doi.org/10.1038/s41598-022-12102-x

Duarte C. M, Cebrián J., 1996. The fate of marine autotrophic production. Limnology and Oceanography. 41(8), 1758–1766. https://doi.org/10.4319/lo.1996.41.8.1758

Duarte CM, Chiscano CL., 1999. Seagrass biomass and production: a reassessment. Aquatic Botany 65: 159–174

DuBois, K., Williams, S.L., Stachowicz, J.J., 2020. Previous exposure mediates the response of eelgrass to future warming via clonal transgenerational plasticity. Ecology 101. https://doi.org/10.1002/ecy.3169

Echavarría-Heras, H., Solana-Arellano, E., Franco-Vizcaíno, E., 2006. The role of increased sea surface temperature on eelgrass leaf dynamics: onset of El Niño as a proxy for global climate change in San Quintín Bay, Baja California. Bull. South.

Calif. Acad. Sci. 105 (3), 113–127. https://doi.org/10.3160/0038-3872(2006)105 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. https://doi.org/10.1111/gcb.15684

Sandoval-Gil, J., Alexandre, A., Santos, R., Camacho-Ibar, V.F., 2016. Nitrogen uptake and internal recycling in *Zostera marina* exposed to oyster farming: eelgrass potential as a natural biofilter. Estuar. Coasts 39, 1694–1708. https://doi.org/10.1007/s12237-016-0102-4.

Terrados, J., Williams, S.L., 1997. Leaf versus root nitrogen uptake by the surfgrass *Phyllospadix torreyi*. Mar. Ecol. Prog. Ser. 149, 267–277. https://doi.org/10.3354/meps149267.

Egea, L., Jiménez-Ramos, R., Romera-Castillo, C., Casal-Porras, I., Bonet-Melià, P., Yamuza-Magdaleno, A., Cerezo-Sepúlveda, L., Pérez-Lloréns, J. L., & Brun, F. G., 2023. Effect of marine heat waves on carbon metabolism, optical characterization, and bioavailability of dissolved organic carbon in coastal vegetated communities. Limnology and Oceanography, 68(2), 467-482. https://doi.org/10.1002/lno.12286

Egea, L., Jiménez–Ramos, R., Vergara, J. J., Hernández, I., & Brun, F. G., 2018. Interactive effect of temperature, acidification and ammonium enrichment on the seagrass *Cymodocea nodosa*. Marine Pollution Bulletin, *134*, 14-26. https://doi.org/10.1016/j.marpolbul.2018.02.029

Esteban, N., Unsworth, R.K.F., Gourlay, J.B.Q., Hays, G.C., 2018. The discovery of deep-water seagrass meadows in a pristine Indian Ocean wilderness revealed by tracking green turtles. Mar. Pollut. Bull., Securing a future for seagrass 134, 99–105. https://doi.org/10.1016/j.marpolbul.2018.03.018

Gallagher, A.J., Brownscombe, J.W., Alsudairy, N.A., Casagrande, A.B., Fu, C., Harding, L., Harris, S.D., Hammerschlag, N., Howe, W., Huertas, A.D., Kattan, S., Kough, A.S., Musgrove, A., Payne, N.L., Phillips, A., Shea, B.D., Shipley, O.N., Sumaila, U.R., Hossain, M.S., Duarte, C.M., 2022. Tiger sharks support the characterization of the world's largest seagrass ecosystem. Nat. Commun. 13, 6328. https://doi.org/10.1038/s41467-022-33926-1

García-Pantoja, J.A., Ruiz-Montoya, L., Sandoval-Gil, J.M., Vivanco-Bercovich, M.V., Ferreira-Arrieta, A., Zertuche-González, J.A., Guzmán-Calderón, J.M., Norzagaray-López Orión; Samperio-Ramos, G., Montaño-Moctezuma, G., Hernández-Ayón, M., 2020. Fijación neta de carbono por pastos marinos (*Phyllospadix* spp.) en una isla del Pacífico Mexicano, in: Estado actual del conocimiento del ciclo del carbono y sus interacciones en México: Síntesis a 2020, Síntesis Nacionales. Programa Mexicano del Carbono en colaboración con la Universidad Autónoma Metropolitana-Xochimilco, Texcoco, Estado de México, México, p. 602.

Hartog, C. den, Kuo, J., 2006. Taxonomy and biogeography of seagrasses, in: Seagrasses: biology, ecology and conservation. Springer Netherlands, Dordrecht, pp. 1–23. https://doi.org/10.1007/978-1-4020-2983-7_1

Hastings R, Cummins V, Holloway P., 2020. Assessing the Impact of Physical and Anthropogenic Environmental Factors in Determining the Habitat Suitability of Seagrass Ecosystems. Sustainability, 12(20):8302. https://doi.org/10.3390/su12208302

Hobday, A. J., Oliver, E. C. J., Gupta, A. S., Benthuysen, J. A., Burrows, M. T., Donat, M. G., Holbrook, N. J., Moore, P. J., Thomsen, M. S., Wernberg, T., & Smale, D., 2018. Categorizing and naming marine heatwaves. Oceanography, *31*(2). https://doi.org/10.5670/oceanog.2018.205

Hobday, A.J., Alexander, L.V., Perkins, S.E., Smale, D.A., Straub, S.C., Oliver, E.C.J., Benthuysen, J.A., Burrows, M.T., Donat, M.G., Feng, M., Holbrook, N.J., Moore, P.J., Scannell, H.A., Sen Gupta, A., Wernberg, T., 2016. A hierarchical approach to defining marine heatwaves. Prog. Oceanogr. 141, 227–238. https://doi.org/10.1016/j.pocean.2015.12.014

Jordà, G., Marbà, N., & Duarte, C. M., 2012. Mediterranean seagrass vulnerable to regional climate warming. Nature Climate Change, *2*(11), 821-824. https://doi.org/10.1038/nclimate1533

Koch, M. S., Bowes, G., Ross, C., & Zhang, X., 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Global Change Biology, *19*(1), 103-132. https://doi.org/10.1111/j.1365-2486.2012.02791.x

La Nafie, Y. A., Van Engeland, T., Van Katwijk, M., & Bouma, T. J., 2014. Uptake of nitrogen from compound pools by the seagrass *Zostera noltii*. Journal of Experimental Marine Biology and Ecology, *460*, 47-52. https://doi.org/10.1016/j.jembe.2014.06.007

Lee, K., Park, S. R., & Kim, Y. K., 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. Journal of Experimental Marine Biology and Ecology, *350*(1-2), 144-175. https://doi.org/10.1016/j.jembe.2007.06.016

Marín, M., Feng, M., Phillips, H.E., Bindoff, N.L., 2021. A global, multiproduct analysis of coastal marine heatwaves: distribution, characteristics, and long-term trends. J. Geophys. Res. Oceans 126, e2020JC016708. https://doi.org/10.1029/2020JC016708

Marín-Guirao, L., Entrambasaguas, L., Ruiz, J. M., & Procaccini, G., 2019. Heat-stress induced flowering can be a potential adaptive response to ocean warming for the iconic seagrass *Posidonia oceanica*. Molecular Ecology, 28(10), 2486-2501. https://doi.org/10.1111/mec.15089.

McGlathery, K. J., 2008. Seagrass habitats (pp. 1037-1071). Nitrogen in the marine environment. https://doi.org/10.1016/b978-0-12-372522-6.00023-2

McGregor, G.R., Bessemoulin, P., Ebi, KE., Menne, B., 2015. Heat waves and Health: Guidance on Warning–System Development G.R. World Meteorological Organization and World Health Organization. WMO–No. 1142. pp 96.

Nguyen, H.M., Bulleri, F., Marín-Guirao, L., Pernice, M., Procaccini, G., 2021b. Photo-physiology and morphology reveal divergent warming responses in northern and southern hemisphere seagrasses. Mar. Biol. 168, 129. https://doi.org/10.1007/s00227-021-03940-w

Nguyen, H.M., Kim, M., Ralph, P.J., Marín-Guirao, L., Pernice, M., Procaccini, G., 2020. Stress memory in seagrasses: first insight into the effects of thermal priming and the role of epigenetic modifications. Front. Plant Sci. 11, 494. https://doi.org/10.3389/fpls.2020.00494

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

Oliver, E.C.J., Burrows, M.T., Donat, M.G., Sen Gupta, A., Alexander, L.V., Perkins-Kirkpatrick, S.E., Benthuysen, J.A., Hobday, A.J., Holbrook, N.J., Moore, P.J., Thomsen, M.S., Wernberg, T., Smale, D.A., 2018. Projected Marine Heatwaves in the 21st Century and the Potential for Ecological Impact. Front. Mar. Sci. 6.

Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J. M. G., Romero, J., & Pérez, M., 2019. Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass. Marine Environmental Research, *145*, 27-38. https://doi.org/10.1016/j.marenvres.2019.02.002

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A Global Crisis for Seagrass Ecosystems. BioScience 56, 987. https://doi.org/10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2

Paling, E. I., and McComb, A. J., 1994. Nitrogen and phosphorus uptake in seedlings of the seagrass *Amphibolis antarctica* in Western Australia. Hydrobiol. 294, 1–4. https://doi.org/10.1007/BF00017618

Pazzaglia, J., Badalamenti, F., Bernardeau-Esteller, J., Ruiz, J.M., Giacalone, V.M., Procaccini, G., Marín-Guirao, L., 2022. Thermo-priming increases heat-stress tolerance in seedlings of the Mediterranean seagrass *P. oceanica*. Mar. Pollut. Bull. 174, 113164. https://doi.org/10.1016/j.marpolbul.2021.113164

Romero, J., Lee, K., Pérez, M., Mateo, M. Á., & Alcoverro, T., 2006. Nutrient dynamics in seagrass ecosystems. En Springer eBooks (pp. 227-254). https://doi.org/10.1007/1-4020-2983-7_9

Roth, N., & Pregnall, A. M., 1988. Nitrate reductase activity in *Zostera marina*. Marine Biology, *99*(4), 457-463. https://doi.org/10.1007/bf00392552

Rubio, L., García-Pérez, D., García-Sánchez, M. J., & Fernández, J. A., 2018. NA^+-Dependent High-Affinity nitrate, phosphate and amino acids transport in leaf cells of the seagrass *Posidonia oceanica* (L.) delile. International Journal of Molecular Sciences, *19*(6), 1570. https://doi.org/10.3390/ijms19061570

Ruiz-Montoya, L., Sandoval-Gil, J.M., Belando-Torrentes, M.D., Vivanco-Bercovich, M., Cabello-Pasini, A., Rangel-Mendoza, L.K., Maldonado-Gutiérrez, A., Ferrerira-Arrieta, A., Guzmán-Calderón, J.M., 2021. Ecophysiological responses and self-protective canopy effects of surfgrass (*Phyllospadix torreyi*) in the intertidal. Mar. Environ. Res. 172, 105501. https://doi.org/10.1016/j.marenvres.2021.105501

Saha, M., Barboza, F.R., Somerfield, P.J., Al-Janabi, B., Beck, M., Brakel, J., Ito, M., Pansch, C., Nascimento-Schulze, J.C., Jakobsson Thor, S., Weinberger, F., Sawall, Y., 2020. Response of foundation macrophytes to near-natural simulated marine heatwaves. Glob. Change Biol. 26, 417–430. https://doi.org/10.1111/gcb.14801

Sandoval-Gil, J. M., Camacho-Ibar, V. F., Del Carmen Ávila-López, M., Hernández-López, J., Zertuche-González, J. A., & Cabello-Pasini, A., 2015. Dissolved inorganic nitrogen uptake kinetics and $\boldsymbol{\delta}^{15}$N of *Zostera marina* l. (eelgrass) in a coastal lagoon with oyster aquaculture and upwelling influence. Journal of Experimental Marine Biology and Ecology, *472*, 1-13. https://doi.org/10.1016/j.jembe.2015.06.018

Serrano, Ó., Arias-Ortiz, A., Duarte, C. M., Kendrick, G. A., & Lavery, P., 2021. Impact of marine heatwaves on seagrass ecosystems. En Ecological studies (pp. 345-364). https://doi.org/10.1007/978-3-030-71330-0_13

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., Burrows, M. T., Alexander, L. V., Benthuysen, J. A., Donat, M. G., Feng, M., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Sen Gupta, A., Payne, B. L., & Moore, P. J., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nature Climate Change, 9(4), 306-312. https://doi.org/10.1038/s41558-019-0412-1.

Smith, K.E., Burrows, M.T., Hobday, A.J., King, N.G., Moore, P.J., Sen Gupta, A., Thomsen, M.S., Wernberg, T., Smale, D.A., 2023. Biological Impacts of Marine Heatwaves. Annu. Rev. Mar. Sci. 15, null. https://doi.org/10.1146/annurev-marine-032122-121437

Stapel, J., Tl, A., Bhm, V. D., Groot, D. M. T., Phw, V. D. H., & Ma, H., 1996. Nutrient uptake by leaves and roots of the seagrass *Thalassia hemprichii* in the Spermonde archipelago, Indonesia. Marine Ecology Progress Series, *134*, 195-206. https://doi.org/10.3354/meps134195

Teutli, C., Mendoza-González, G., Chiappa-Carrara, X., Gallardo, A., 2020. Guía de Servicios Ecosistémicos de las praderas de pastos Marinos de la Península de Yucatán.

Thomson, J. A., Burkholder, D. A., Heithaus, M. R., Fourqurean, J. W., Fraser, M. W., Statton, J., & Kendrick, G. A., 2015. Extreme temperatures, foundation species, and abrupt ecosystem change: An example from an iconic seagrass ecosystem. Global Change Biology, 21(4), 1463-1474. https://doi.org/10.1111/gcb.12694.

Touchette, B. W., & Burkholder, J. M., 2000. Review of nitrogen and phosphorus metabolism in seagrasses. Journal of Experimental Marine Biology and Ecology, *250*(1-2), 133-167. https://doi.org/10.1016/s0022-0981(00)00195-7

Van Engeland, T., Bouma, T. J., Morris, E. P., Brun, F. G., Peralta, G., Lara, M. A. C., Hendriks, I. E., Soetaert, K., & Middelburg, J. J., 2011. Potential uptake of dissolved organic matter by seagrasses and macroalgae. Marine Ecology Progress Series, *427*, 71-81. https://doi.org/10.3354/meps09054

Viana, I. G., Saavedra-Hortúa, D. A., Mtolera, M. S. P., & Teichberg, M., 2019. Different strategies of nitrogen acquisition in two tropical seagrasses under nitrogen enrichment. New Phytologist, *223*(3), 1217-1229. https://doi.org/10.1111/nph.15885

Vivanco-Bercovich, M., Belando-Torrentes, M. D., Figueroa-Burgos, M. F., Ferreira-Arrieta, A., Macías-Carranza, V., García-Pantoja, J. A., Cabello-Pasini, A., Samperio-Ramos, G., Cruz-López, R. & Sandoval-Gil, J. M., 2022. Combined effects of marine heatwaves and reduced light on the physiology and growth of the surfgrass *Phyllospadix torreyi* from Baja California, Mexico. Aquatic Botany, 178, 103488. https://doi.org/10.1016/j.aquabot.2021.103488

Vivanco-Bercovich, M., Sandoval-Gil, J. M., Bonet-Meliá, P., Cabello-Pasini, A., Muñíz-Salazar, R., Montoya, L. R., Schubert, N., Marín-Guirao, L., Procaccini, G., & Ferreira-Arrieta, A., 2023. Marine heatwaves recurrence aggravates thermal stress in the surfgrass *Phyllospadix scouleri*. Marine Pollution Bulletin, *199*, 115943. https://doi.org/10.1016/j.marpolbul.2023.115943

Vonk, J. A., Middelburg, J. J., Stapel, J., & Bouma, T. J., 2008. Dissolved organic nitrogen uptake by seagrasses. Limnology and Oceanography, *53*(2), 542-548. https://doi.org/10.4319/lo.2008.53.2.0542

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. 106, 12377–12381. https://doi.org/10.1073/pnas.0905620106

Zaba, K. D., & Rudnick, D. L., 2016. The 2014–2015 warming anomaly in the Southern California current system observed by underwater gliders. Geophysical Research Letters, *43*(3), 1241-1248. https://doi.org/10.1002/2015gl067550

Cooper, L.W., McRoy, C.P., 1988. Anatomical adaptations to rocky substrates and surf exposure by the seagrass genus *Phyllospadix*. Aquat. Bot. 32, 365–381. https://doi.org/10.1016/0304-3770(88)90108-8

Den Hartog, C., 1970. The sea-grasses of the world. N.-Holl. Amst.

Drysdale, F.R., Barbour, M.G., 1975. Response of the marine angiosperm *Phyllospadix torreyi* to certain environmental variables: A preliminary study. Aquat. Bot. 1, 97–106. https://doi.org/10.1016/0304-3770(75)90015-7

Sen Gupta, A., Thomsen, M., Benthuysen, J.A., Hobday, A.J., Oliver, E., Alexander, L.V., Burrows, M.T., Donat, M.G., Feng, M., Holbrook, N.J., Perkins-Kirkpatrick, S., Moore, P.J., Rodrigues, R.R., Scannell, H.A., Taschetto, A.S., Ummenhofer, C.C., Wernberg, T., Smale, D.A., 2020. Drivers and impacts of the most extreme marine heatwave events. Sci. Rep. 10, 19359. https://doi.org/10.1038/s41598-020-75445-3

Bond, N.A., Cronin, M.F., Freeland, H., Mantua, N., 2015. Causes and impacts of the 2014 warm anomaly in the NE Pacific. Geophys. Res. Lett. 42, 3414–3420. https://doi.org/10.1002/2015GL063306

Wei, X., Li, K., Kilpatrick, T., Wang, M., Xie, S., 2021. Large-scale conditions for the record-setting southern california marine heatwave of August 2018. Geophys. Res. Lett. 48. https://doi.org/10.1029/2020GL091803

Phillips, R.C., 1979. Ecological notes on *Phyllospadix* (potamogetonaceae) in the Northeast Pacific. Aquat. Bot. 6, 159–170. https://doi.org/10.1016/0304-3770(79)90059-7

Kuo, J., Stewart, J.G., 1995. Leaf anatomy and ultrastructure of the North American marine angiosperm *Phyllospadix* (Zosteraceae). Can. J. Bot. 73, 827–842. https://doi.org/10.1139/b95-091

CAPÍTULO 1 – Marine heatwaves can limit the role of surfgrasses as biofilters for wastewaters

Published in Marine Pollution Bulletin
Available at doi.org/10.1016/j.marpolbul.2023.115651

ABSTRACT

Marine heatwaves (MHWs) can have detrimental effects on seagrasses, but knowledge about the impacts on their ecosystem services remains scarce. This work evaluated *Phyllospadix scouleri* (surgrass) as a biofilter for wastewater discharges, and how warming associated with MHW may affect this ecological function. The nitrogen uptake kinetics and assimilation abilities for ammonium, nitrate, and urea were examined under two different warming scenarios (single and repeated events) simulated in a mesocosm. N-uptake kinetics were related to urban sewage discharges close to surfgrass meadows. Our results revealed that surfgrasses can serve as effective biofilters because of their high nitrogen uptake rates and above-average canopy biomass. Nonetheless, exposure to both experimental warmings resulted in a significant decline in their ability to incorporate and assimilate nitrogen. Consequently, MHWs may reduce the capacity of surfgrasses to function as nitrogen sinks and green filters for sewage waters, jeopardizing their role as Blue Nitrogen systems.

Keywords: *Phyllospadix scouleri,* nitrogen uptake, nitrogen assimilation, climate change, wastewater.

1. Introduction

Seagrasses of the genus *Phyllospadix*, commonly called surfgrasses *(Phyllospadix torreyi* and *Phyllospadix scouleri)*, form vast meadows that dominate the rocky intertidal landscapes along the North American Pacific coasts, from British Columbia in Canada to Baja California, Mexico (Ramírez-García et al., 2002). As documented for other seagrasses (de los Santos et al., 2020), the extensive distribution and high productivity of sufgrasses have the potential to support various socioecological benefits. However, these benefits remain largely unexplored.

Seagrasses can act as effective biofilters, removing nutrients from the water column and limiting eutrophication (Sandoval-Gil et al., 2016; Bernardeau-Esteller et al., 2023). The biofiltering potential of surfgrasses is facilitated by their presence in the intertidal and shallow subtidal bottoms which usually overlaps with wastewater discharges in urbanized areas. Further, their very long leaves and high shoot biomass (up to ~115 cm and ~2800 g DW m- 2, Ramírez-García et al., 1998) provide a substantial amount of plant surface, which can effectively remove and trap nutrients (García-Pantoja, 2023). As compared to other seagrasses, their leaves can take up nitrogen at higher external concentrations, likely compensating for their low N uptake by roots (Terrados and Williams, 1997).

P. torreyi and *P. scouleri* meadows can help to maintain water quality in urban areas that are typically impacted by sewage discharges (Tanahara et al., 2021).

Like other seagrass species, surfgrasses are threatened by anthropogenic climate forcings such as global warming and marine heatwaves (Vivanco-Bercovich et al., 2021). Warming associated with marine heatwaves (MHWs) can negatively affect seagrasses by altering their metabolism, productivity, community structure, and distribution (Nguyen et al., 2021; Smith et al., 2023). MHWs are projected to increase in frequency and intensity (IPCC, 2022), and their impacts in seagrass ecosystems can be devastating (Arias-Ortiz et al., 2018). Particularly in Baja California (Mexico), MHWs and other warming anomalies (e.g., El Niño-Southern Oscillation) have had adverse effects on the productivity and structure of seagrass meadows (e.g., *Zostera marina*; Echavarría-Heras et al., 2006) and other marine forests (Arafeh- Dalmau et al., 2020). However, the effects of seawater warming on sufgrasses have received little attention (Vivanco-Bercovich et al., 2021). As a result, we still lack a full understanding of how this phenomenon affects their socio-ecological services, including their role as biofilters.

The value of seagrasses as biofilters is dependent on their high N uptake/assimilation rates, the retention capacity of N in the plant’s clonal structure, and the complex interaction between biotic/abiotic factors that condition N-fluxes in the population (McGlathery, 2008). These characteristics also dictate the efficacy of seagrasses as naturebased solutions to mitigate eutrophication and as Blue Nitrogen systems, a term recently introduced by

Hughes et al. (2022). Although there is substantial research on the effects of thermal stress on various of their physiological attributes (e.g., photosynthesis and respiration; Serrano et al., 2021a, 2021b; Deguette et al., 2022), the effect on N-metabolism is practically unexplored (Kaldy, 2014; Ontoria et al., 2019; Alexandre et al., 2020).

The purpose of this study was to assess the capacity of *P. scouleri* to act as biofilters for wastewater discharges by measuring the uptake rates of ammonium, nitrate, and urea. The activity of key enzymes involved in nitrogen assimilation, glutamine synthetase and nitrate reductase, were also quantified. Additionally, the study analyzed the effects of warming on these descriptors of nitrogen metabolism. The experiment included the simulation of single and repeated MHWs in a mesocosm.

2. Material and methods

Plant collection and experimental design

Phyllospadix scouleri plants were collected in August 2020 at Todos Santos Bay (31◦, 48′25.66″ N, 116◦ 47′46.41″ W). Whole plants (including orthotropic and plagiotropic shoots, rhizomes, and roots) were collected to preserve their clonal structure. The plants were transported to the laboratory within 2 h, in large coolers filled with seawater. Subsequently, they were placed in twelve independent tanks (~200 shoots per tank, 60 L) filled with filtered seawater (1 µm, UV). The tanks were continuously aerated, while water circulation and temperature were regulated by submersible pumps, chillers, and heaters. LED lamps supplied an irradiance level of approximately 150 µmol photon m^{-2} s^{-1} to the leaves, and a photoperiod of 10 L: 14D was adjusted to obtain a light dose of about 3.6 mol photon m^{-2} day^{-1}. Light and temperature were adjusted based on average values recorded at the collection site by submersible sensors Onset-HOBO MX2202, which were previously calibrated against a 2π quantum radiometer (LI190R; LI-COR, Bioscience). *P. scouleri* plants were kept at a constant temperature (18 °C) and irradiance for five days. After this short acclimation period, four independent tanks were assigned to each of the three experimental treatments. In the control treatment (C), the temperature was maintained at a constant at 18 °C. The MHW treatment exposed the plants to a single heatwave, while the MHWx2 treatment exposed them to two consecutive heatwaves.

The temperature and duration of the experimental MHWs (24 °C for 7 days), the time interval between heatwaves in MHWx2 (5 days), and the rates of temperature increase/decrease at the onset/conclusion of the heatwaves (±2 °C per day), were determined using recorded data from the donor meadow (Fig. S1 in Suppl. Mat.; data sourced from Marine Heatwave Tracker, Schlegel, 2020). The experimental MHWs fell under category III (severe) according to the classification proposed by Hobday et al. (2018). The salinity was kept constant (33–33.5 ‰) by adding de-ionized water, while pH was maintained within similar conditions of the sampling site (7.9–8.1). Salinity and pH levels were monitored using a submersible multi-parameter probe (YSI Pro Plus, USA). The water quality was maintained by partially replacing seawater every three days. The analysis of various biological descriptors (outlined below) was conducted at the conclusion of the heatwave in treatment MHW, and at the end of the second heatwave in the treatment MHWx2.

Nitrogen uptake kinetics, N-content, and N-assimilatory enzymes

To measure the kinetics of N uptake, the shoots were incubated for 30 min under four different concentrations of isotopically labeled ammonium, nitrate, and urea (5, 20, 40, and 100 µM; $^{15}NH_4Cl$, $K^{15}NO_3$,^{15}N-urea, al. % = 99; Cambridge Isotope Laboratories) diluted with artificial seawater. These wide ranges of N concentrations were selected to determine the plant's N-uptake rates at N-concentrations similar to those measured in surfgrass meadows close to wastewater discharges (see Tanahara et al., 2021 for a complete description of the concentrations and analytical procedures). The incubations were conducted in separate transparent plastic chambers (6 L), with one chamber dedicated to each nutrient and concentration ($n = 3$). Each incubation was made with three shoots randomly collected from each tank and treatment (C, MHW, and MHWx2), keeping a weight: volume ratio of ~0.3 g FW L^{-1}. To preserve the vegetative integrity, a small portion (~1 cm) of the rhizome was conserved in each shoot. Incubations were carried out in large laboratory incubators (VWR). The temperature and irradiance were kept at the same levels as those of the respective treatments. Nitrogen uptake rates were calculated following the equations in Sandoval-Gil et al. (2016). Briefly, uptake rates were plotted against substrate concentration (µM), and the uptake kinetic parameters were derived using the Michaelis–Menten model [i.e., $V = (V_{max} \times S) / (K_m + S)$], where V is the uptake rate (μmol N g^{-1} DW h^{-1}), V_{max} is maximum uptake rate (μmol N g^{-1} DW h^{-1}), S is substrate concentration (μM), and K_m represents the half-saturation constant (μM). The affinity constant (α) was calculated as V_{max}/K_m. Total N content was analyzed in four shoots not exposed to isotopically labeled N, that were randomly collected from each tank and treatment. Dried and ground leaf tissues were analyzed for ^{15}N and N-content at the University of California Davis-Stable Isotope Facility, using an elemental analyzer interfaced with a continuous flow isotope ratio mass spectrometer. The potential uptake rates at N-concentrations of the wastewater discharges (V_{amb}) near surfgrass meadows were calculated from the Michaelis–Menten equations obtained above and nutrient data from Tanahara et al. (2021).

Nitrogen content (% N) was determined in leaf tissues of three shoots per tank and treatment, following Sandoval-Gil et al. (2016).

The enzymes responsible for N-assimilation were analyzed in the leaf tissues of three shoots per tank and treatment. The nitrate reductase (NR) activity was measured *in vivo*, following the method described by Alexandre et al. (2004). Glutamine synthetase activity (GS) was measured *in vitro*, according to the method described by Sagi et al. (2002).

Statistical analysis

One-way analysis of variance (ANOVA) and pairwise multiple comparisons (*post-hoc* Tukey test) were used to test for significant differences ($p < 0.05$) in V_{max}, K_m, and %N among treatments (C, MHW, MHWx2). The Shapiro-Wilk test and Levene test, respectively, verified the assumptions of normality and homoscedasticity. When these assumptions were not met, even after data transformation, a non-parametric analysis were used (Kruskal-Wallis test followed by Mann-Whitney *U* test). This was the case for Vmax and Km from nitrate uptake rates, GS, and NR. All statistical analyses were performed using the SIGMAPLOT 11 statistical package (Systat Software Inc, USA).

3. Results

The uptake rates of ammonium, nitrate and urea fitted well ($R^2 > 0.98$, $p < 0.05$) to the Michaelis–Menten model (Fig. 1A–C). Values of V_{max} for ammonium and nitrate significantly decreased by ~70–76 % (F = 59.13, $p < 0.001$; F = 25.78, $p = 0.001$) in plants exposed to single and double marine heatwaves (MHW, MHWx2) relative to control plants (Fig. 1D, E). The half-saturation constant (K_m) for these nutrients followed a similar trend since warmed plants showed 2–4-fold lower values than control plants (Fig. 1G, H; F = 21.97, $p = 0.002$; F = 5.19, $p = 0.048$). V_{max} and K_m for urea did not vary significantly among treatments, even though lower values were detected in the MHW treatment (Fig. 2F, I). The affinity (α) for ammonium was ~170 % higher in MHWx2 than in control plants (Fig. 1J). In contrast, control plants exhibited the highest α for nitrate (2 to 3-fold higher than MHW and MHWx2; Fig. 1K). Values of α for urea increased by ~265 % in MHW plants compared to C and MHWx2 (Fig. 1L).

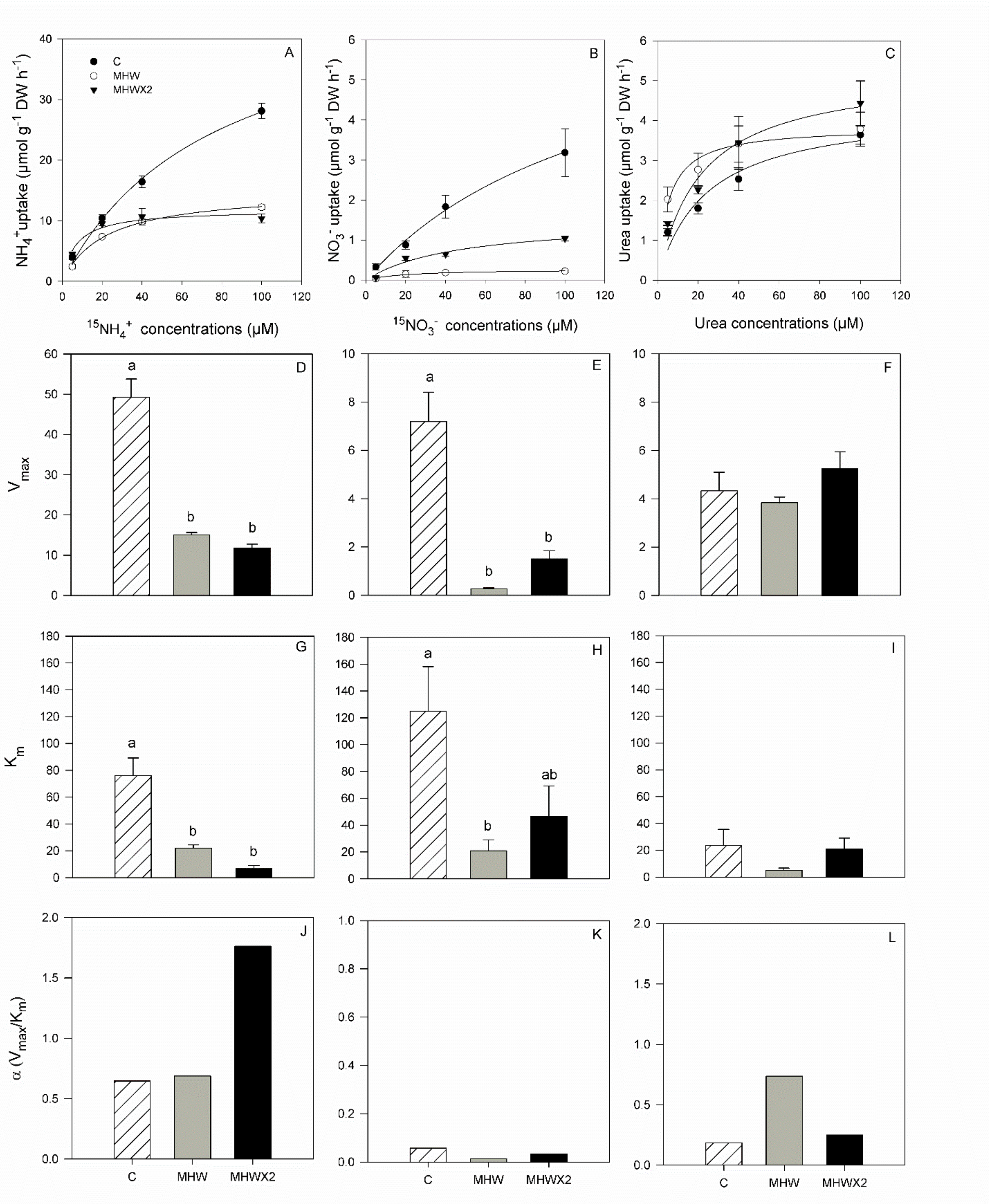


Figure 1. Kinetics of the uptake rates (μmol g^{-1} DW h^{-1}) for (A) ammonium, (B) nitrate and (C) urea measured in *P. scouleri* under the different experimental treatments, C

(control, 18 °C), MHW (exposure to a warming of 24 ◦C), and MHWx2 (exposure to two consecutive warmings). The descriptors derived from N-uptake kinetics are also showed: Maximum uptake rates (V_{max}; D–F), half-saturation constant (K_m; G–I), and uptake affinity (α; J–L). Statistical differences among treatments are indicated by different letters. Values are mean ± standard error ($n = 3$).

The activity of the enzymes nitrate reductase (NR) and glutamine synthetase (GS) was significantly decreased in plants exposed to repeated warming (MHWx2) compared to the control (H = 14.69, $p < 0.001$; H = 16.34, $p < 0.001$). This reduction was more pronounced for NR (- 85 %) than for GS (- 38 %) (Fig. 2).

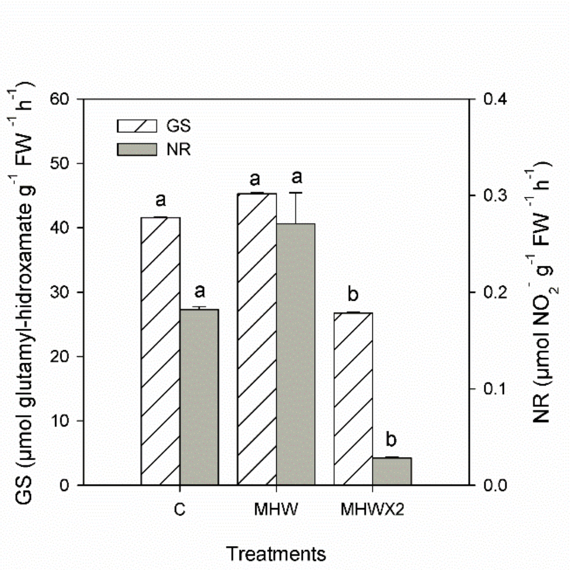


Figure 2. Activity of the enzymes glutamine synthetase (GS) and nitrate reductase (NR) measured in *P. scouleri* under the different experimental treatments, C (control, 18 °C), MHW (exposure to a warming of 24 °C), and MHWx2 (exposure to two consecutive warmings). Statistical differences among treatments are indicated by different letters. Values are mean ± standard error (n = 3).

Ammonium, nitrate, and urea concentrations in the areas receiving the wastewater discharges in Todos Santos Bay ranged from 0.01 to 170, 0 to 45.41, and 0.019 to 0.26 µM, respectively (Fig. 3; data from Tanahara et al., 2021). The ammonium uptake rates at ambient concentrations (V_{amb}) did not saturate in control plants, even at maximum concentrations of ~170 µM (Fig. 3A). This means that the uptake of ammonium by *P. scouleri* at the range of ammonium concentrations in wastewaters is below saturation levels, showing the high capacity of this species to take up ammonium from wastewater-impacted environments. On the other hand, the ammonium uptake rates of plants exposed to MHW and MHWx2, saturated at concentrations above 50 µM, showing a significant reduction in the uptake capacity of *P. scouleri*. The uptake rates of nitrate did not saturate within the range of concentrations in sewage waters in any of the warming treatments. Still, these decreased by ~82 % for MHW and ~48 % for MHWx2 relative to control plants (Fig. 3B). Mean values of Vamb for nitrate were higher in control plants (0.05 ± 0.008 µmol N g^{-1} DW $^{h-1}$) than in MHW (0.009 ± 0.001) and MHWx2 (0.026 ± 0.003). The maximum concentration of urea in the bay wastewaters (0.26 µM) was well below the Km calculated from the uptake kinetics of *P. scouleri* in both treatments (5–25 µM; Fig. 3C). Mean values of Vamb for urea were higher in plants exposed to MHW (0.05 ± 0.0004) than to MHWx2 or control (0.01 ± 0.001 µmol N g^{-1} DW $^{h-1}$).

Leaf %N of *P. scouleri* was not significantly different among treatments (F = 0.38, p = 0.69), and values ranged from 2.41 to 2.54 % DW.

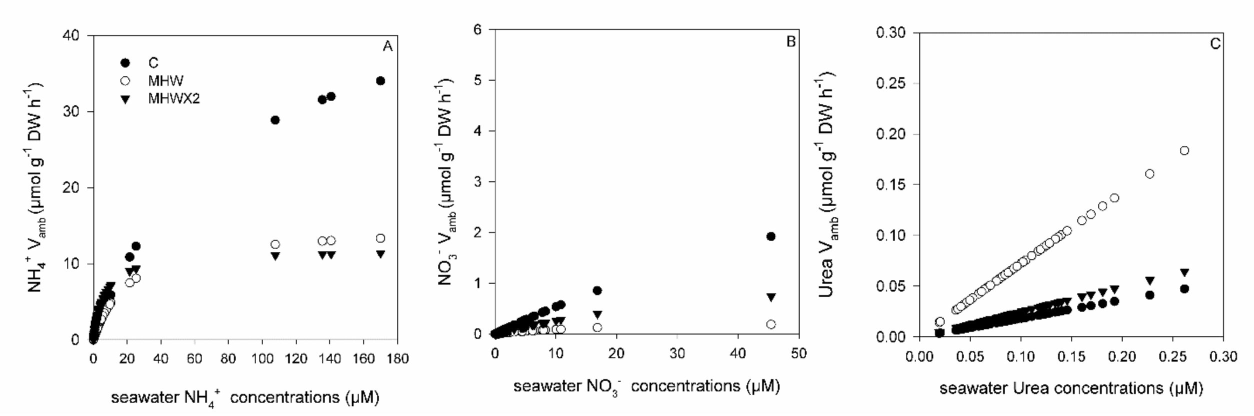


Figure 3. Potential N-uptake rates of *P. scouleri* at ambient concentrations (V$_{amb}$) of ammonium (A), nitrate (B) and urea (C) from wastewater discharges, under the different experimental treatments: C (control, 18 °C), MHW (exposure to a warming of 24 °C), and MHWx2 (exposure to two consecutive warmings).

4. Discussion

The N-uptake capacities of *Phyllospadix scouleri* measured in our study were generally higher than those reported for other seagrass species. The maximum uptake rates and half-saturation constant for ammonium and nitrate (V_{max} ~49 and ~7 μmol N g- 1 DW h-1; K_m ~76 and ~124 μM) in this species greatly surpass maximum values found in other temperate seagrasses, like *Zostera noltii*, *Halophila stipulacea*, *Cymodocea nodosa*, *Zostera marina*, or *Amphibolis antarctica* (refer to Table S1; Touchette and Burkholder, 2000; Alexandre et al., 2011; Alexandre et al., 2014; Alexandre and Santos, 2020). The ammonium affinity constant (α = 0.64) is also comparable to the highest values measured for other seagrasses (Table S1; Touchette and Burkholder, 2000; Alexandre et al., 2011; Li et al., 2020). Although studies on urea uptake kinetics are scarce, our data indicate that uptake rates for this compound can be higher than those measured in other seagrasses (e.g., *Thalassia hemprichii*, *Halodule uninervis*, and *Cymodocea rotundata*; Vonk et al., 2008). Moreover, the uptake rates for all nitrogen sources showed no saturation within the wide range of concentrations observed in wastewater discharges in Todos Santos Bay, and therefore, *P. scouleri* could efficiently remove nutrients available in the surrounding environment. The high V_{max} for ammonium is noteworthy as it aids in the successful removal of this nutrient directly from seawater that is heavily influenced by wastewaters, even when concentrations are as high as 170 μM. This has been also documented in *Z. marina*, supporting its biofiltering role for ammonium excreted by oysters in a coastal lagoon (Sandoval-Gil et al., 2016). The N-uptake kinetic of *P. scouleri*, along with its high and long-lasting (perennial) aboveground biomass productivity (Ramírez-García et al., 1998; García-Pantoja, 2023), establish it as an effective biofilter for urban wastewaters. The capacity for removing ammonium may be greater than that of nitrate and urea, due to a 10-fold higher V_{max} and α for the former (refer to control values in Fig. 2). These differences have been linked to the greater metabolic costs of assimilating nitrate and urea (Touchette and Burkholder, 2000), as well as the features of their plasma membrane transportation mechanisms (Rubio et al., 2007; Rubio et al., 2018). Although the uptake of urea saturated at lower concentrations than those of nitrate (i.e., urea K_m < nitrate-K_m), the similar values of α indicate that both could be incorporated similarly at sub-saturating concentrations. This also reinforces the notion of urea as a complementary nitrogen source, as shown in other seagrasses (Vonk et al., 2008; La Nafie et al., 2014).

Our results indicate that exposure of *P. scouleri* to both single and repeated MHWs resulted in a significant decline in Vmax (from ~50 to ~10 µmol N g^{-1} DW h^{-1}) and Km (from ~80 to ~15 µM) for ammonium. Meanwhile, exposure to MHWx2 increased the affinity for ammonium by nearly 3-fold. This indicates that repeated MHWs have the potential to enhance the uptake of nutrients at lower (sub-saturating) concentrations, while significantly decreasing the capacity of *P. scouleri* to act as a biofilter at saturating ammonium concentration. As shown in Fig. 3A, values of Vmax dropped from ~30 to 10 µmol N g^{-1} DW h- 1 at external concentrations above 100 µM. Repeated heatwaves strongly reduce Vmax, Km, and α, leading to a decrease in *P. scouleri's* nitrate removal capacity. Warming also induced a decline in the activity of N-assimilatory enzymes, GS and NR. This implies that MHWs can compromise not only N-incorporation but also its assimilation, retention and utilization as internal N organic compounds. The activities of GS and NR activities only decreased in the MHWx2 treatment, which may indicate the physiological deterioration of *P. scouleri* following repeated warming events. Similar experimental conditions for this species have previously shown evidence of metabolic decline in key biological traits such as photosynthesis (Vivanco-Bercovich, 2023). However, our findings contradict the previously observed increase in nitrogen uptake/assimilation observed for other seagrasses when exposed to warming (e.g., *Z. noltii* and *Z. marina*; Alexandre et al., 2004; Kaldy, 2014; Alexandre et al., 2020). These discrepancies may potentially be explained by differences in methodological approaches (e.g., warming intensity and duration) and species-specific responses to warming, but further evidence is needed to fully understand the underlying factors. In our study, the heatwaves did not affect the maximum uptake capacity of *P. scouleri* for incorporating urea, although its uptake affinity increased after a single heatwave. This indicates that the transmembrane transports for DIN and DON displayed different thermo-sensitivity (Glass, 2003), potentially supporting the removal of urea from urban sewage discharges.

Based on measurements of *P. scouleri* canopy biomass at the collection site and other surfgrass beds (~2800 g DW m^{-2}; Ramírez-García et al., 2002; García-Pantoja, 2023), and assuming that maximum ammonium, nitrate and urea concentrations (i.e., 170, 45.41

and 0.26 μM, respectively) are present at mean sewage flow rates of 50–500 L/s, a meadow covering area of approximately 84 m^{-2} could completely remove all N inputs. This meadow area represents a small fraction of the surfgrass's total area along its bathymetric distribution (Ramírez-García et al., 1998). Considering that MHWs can dramatically reduce the nutrient biofiltering capacities of *P. scouleri*, larger areas of surfgrass meadows will be required to eliminate all nitrogen in wastewater effluents. Severe alterations in plant growth and meadow structure resulting from MHWs, along with adverse synergistic effects resulting from light limitation associated with nutrient loading, could further reduce the biofiltration capacity of the ecosystem (Vivanco-Bercovich et al., 2021).

Using the nitrogen stock biomass from Todos Santos Bay (2.54 % N, 2800 g DW m^{-2}; García-Pantoja, 2023), we estimate that *P. scouleri* meadows have the ability to retain up to 70.74 ± 11.52 g N m^{-2}. This represents a high N retention capacity compared to other species, e.g., *Enhalus acoroides* (11 g N m^{-2}; Ashikin et al., 2019), *Phyllospadix iwatensis* (34 g N m^{-2}; Hasegawa et al., 2005), and *Z. marina* (50 g N m^{-2}; Park et al., 2013). The nitrogen retention capacity of *P. scouleri* is thought to derive from its high leaf biomass. However, the exudation of organic nitrogen compounds, translocation mechanisms, and leaf longevity also have a significant impact on the plant's internal nitrogen storage capacity (Hasegawa et al., 2005). While the retention of nitrogen by surfgrass meadows appears to be less influenced by warming, compared to their uptake kinetics and assimilatory activities, the accumulation of nitrogen may be threatened indirectly by the effects of marine heatwaves (MHWs). Thus, the presence of MHWs may not only compromise the ability to remove DIN from the water column, but also hinder the potential to assimilate and store these nitrogen compounds in plant compartments.

5. Conclusions

In conclusion, our study demonstrates the potential significance of surfgrasses as nitrogen sinks and natural biofilters in urbanized shores with sewage or wastewater discharges, adding relevance to the Blue Nitrogen ecosystem service. However, we also discovered that MHWs have negative implications on the N-metabolism of surfgrasses, resulting in a reduction in their ability to remove nutrients from the water column and assimilate them. This ultimately limits their role as natural biofilters. However, our knowledge of the functionality of seagrasses as green filters and Blue Nitrogen systems is still developing, requiring further research efforts to investigate critical knowledge gaps. Examples of such gaps include the recovery capacities of surfgrasses after MHWs, specific responses of ecotypes, the interaction with their epiphytic microbiome, and with other environmental stressors associated with wastewater discharges (e.g., reduction in water transparency). The preservation of surfgrass ecosystems can be deemed a nature-based approach to mitigate coastal eutrophication.

6. References

Alexandre, A.C.S., Santos, R., 2020. Nutrition of the seagrass *Cymodocea nodosa*: pulses of ammonium but not of phosphate are crucial to sustain the species growth. Mar. Environ. Res. 158, 104954 https://doi.org/10.1016/j.marenvres.2020.104954.

Alexandre, A.C.S., Da Silva, J.S., Santos, R., 2004. The maximum nitrate reductase activity of the seagrass *Zostera noltii* (Hornem.) varies along its vertical distribution. J. Exp. Mar. Biol. Ecol. 307 (1), 127–135. https://doi.org/10.1016/j.jembe.2004.02.002.

Alexandre, A.C.S., Da Silva, J.S., Bouma, T.J., Santos, R., 2011. Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass *Zostera noltii*. J. Exp. Mar. Biol. Ecol. 401 (1–2), 7–12. https://doi.org/10.1016/j.jembe.2011.03.008.

Alexandre, A.C.S., Georgiou, D., Santos, R., 2014. Inorganic nitrogen acquisition by the tropical seagrass *Halophila stipulacea*. Mar. Ecol. 35 (3), 387–394. https://doi.org/10.1111/maec.12128.

Alexandre, A.C.S., Quinta, R., Hill, P.W., Jones, D.R., Santos, R., 2020. Ocean warming increases the nitrogen demand and the uptake of organic nitrogen of the globally distributed seagrass *Zostera marina*. Funct. Ecol. 34 (7), 1325–1335. https://doi.org/10.1111/1365-2435.13576.

Arafeh-Dalmau, N., Schoeman, D.S., Montaño-Moctezuma, G., Micheli, F., RogersBennett, L., Olguín-Jacobson, C., Possingham, H.P., 2020. Marine heat waves threaten kelp forests. Science 367 (6478), 635. https://doi.org/10.1126/science.aba5244.

Arias-Ortiz, A., Serrano, O., Masqué, P., Lavery, P.S., Mueller, U., Kendrick, G.A., Rozaimi, M., Esteban, A., Fourqurean, J.W., Marbà, N., Mateo, M., Murray, K., Rule, M.E., Duarte, C.M., 2018. A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. Nat. Clim. Chang. 8 (4), 338–344. https://doi.org/10.1038/s41558-018-0096-y.

Ashikin, C.N., Rozaimi, M., Arina, N., Fairoz, M., Hidayah, N., 2019. Nitrogen dynamics within an estuarine seagrass meadow under heavy anthropogenic influence. Mar. Pollut. Bull. 150, 110628 https://doi.org/10.1016/j.marpolbul.2019.110628.

Bernardeau-Esteller, J., Sandoval-Gil, J.M., Belando, M.D., Ramos-Segura, A., García-Muñoz R., Marín-Guirao, L., Ruiz, J.M., 2023. The role of *Cymodocea nodosa* and *Caulerpa prolifera* meadows as nitrogen sinks in temperate coastal lagoons. Diversity 15 (2), 172. https://doi.org/10.3390/d15020172.

Deguette, A., Barrote, I., Silva, J., 2022. Physiological and morphological effects of a marine heatwave on the seagrass *Cymodocea nodosa*. Sci. Rep. 12 (1) https://doi.org/10.1038/s41598-022-12102-x.

Echavarría-Heras, H., Solana-Arellano, E., Franco-Vizcaíno, E., 2006. The role of increased sea surface temperature on eelgrass leaf dynamics: onset of El Niño as a proxy for global climate change in San Quintín Bay, Baja California. Bull. South. Calif. Acad. Sci. 105 (3), 113–127. https://doi.org/10.3160/0038-3872 (2006)105.

García-Pantoja, J.A., 2023. Evaluación de las praderas de *Phyllospadix torreyi* y *Phyllospadix scouleri* como sistemas fijadores de carbono azul en una isla del Pacífico Mexicano. Degree Thesis. Universidad Autónoma de Baja California, México.

Glass, A.D.M., 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. Crit. Rev. Plant Sci. 22 (5), 453–470. https://doi.org/10.1080/07352680390243512.

Hasegawa, N., Iizumi, H., Mukai, H., 2005. Nitrogen dynamics of the surfgrass *Phyllospadix iwatensis*. Mar. Ecol. Prog. Ser. 293, 59–68. https://doi.org/10.3354/meps293059.

Hobday, A.J., Oliver, E.C.J., Gupta, A.S., Benthuysen, J.A., Burrows, M.T., Donat, M.G., Holbrook, N.J., Moore, P.J., Thomsen, M.S., Wernberg, T., Smale, D.A., 2018. Categorizing and naming marine heatwaves. Oceanography 31 (2). https://doi.org/10.5670/oceanog.2018.205.

Hughes, A.D., Charalambides, G., Franco, S.F., Robinson, G., Tett, P., 2022. Blue nitrogen: a nature-based solution in the blue economy as a tool to manage terrestrial nutrient neutrality. Sustainability 14 (16), 10182. https://doi.org/10.3390/su141610182.

IPCC, 2022. In: Pörtner, H.-O., Roberts, D.C., Tignor, M., Poloczanska, E.S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., Rama, B. (Eds.), Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. https://doi.org/10.1017/9781009325844.

Kaldy, J.E., 2014. Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. J. Exp. Mar. Biol. Ecol. 453, 108–115. https://doi.org/10.1016/j.jembe.2013.12.020.

La Nafie, Y., Van Engeland, T., Van Katwijk, M., Bouma, T.J., 2014. Uptake of nitrogen from compound pools by the seagrass *Zostera noltii*. J. Exp. Mar. Biol. Ecol. 460, 47–52. https://doi.org/10.1016/j.jembe.2014.06.007.

Li, S., Zhang, Y., Li, Z., Zhang, P., 2020. Uptake kinetics of nitrogen and phosphorus by *Zostera marina*. Chin. J. Plant Ecol. 44 (7), 772–781. https://doi.org/10.17521/cjpe.2019.0335.

de los Santos, C.B., Scott, A., Arias-Ortiz, A., Jones, B., Kennedy, H., Mazarrasa, I., McKenzie, L., Nordlund, L.M., de la Torre-Castro, M. de la T., Unsworth, R.K.F., Ambo-Rappe, R., 2020. Seagrass ecosystem services: assessment and scale of benefits. Blue Value Seagrasses Environ. People 19–21.

McGlathery, K.J., 2008. Seagrass habitats. In: Capone, D.G., Bronk, D.A., Mulholland, M. R., Carpenter, E.J. (Eds.), Nitrogen in the Marine Environment. Elsevier, pp. 1037–1060.

Nguyen, H.T., Ralph, P.J., Marín-Guirao, L., Pernice, M., Procaccini, G., 2021. Seagrasses in an era of ocean warming: a review. Biol. Rev. 96 (5), 2009–2030. https://doi.org/10.1111/brv.12736.

Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J.M., Romero, J., Pérez, M., 2019. Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass. Mar. Environ. Res. 145, 27–38. https://doi.org/10.1016/j.marenvres.2019.02.002.

Park, S.M., Kim, Y., Kim, S.U., Lee, K., 2013. Nitrogen budget of the eelgrass, *Zostera marina* in a bay system on the south coast of Korea. Ocean Sci. J. https://doi.org/10.1007/s12601-013-0028-8.

Ramírez-García, P., Lot, A., Duarte, C.M., Terrados, J., Agawin, N.S.R., 1998. Bathymetric distribution, biomass and growth dynamics of intertidal *Phyllospadix scouleri* and *Phyllospadix torreyi* in Baja California (Mexico). Mar. Ecol. Prog. Ser. 173, 13–23. https://doi.org/10.3354/meps173013.

Ramírez-García, P., Terrados, J., Ramos, F., Lot, A., Ocaña, D., Duarte, C.M., 2002. Distribution and nutrient limitation of surfgrass, *Phyllospadix scouleri* and *Phyllospadix torreyi*, along the Pacific coast of Baja California (México). Aquat. Bot. 74 (2), 121–131. https://doi.org/10.1016/s0304-3770(02)00050-5.

Rubio, L., Linares-Rueda, A., García-Sánchez, M.J., Fernandez, ´ J.M.G., 2007. Ammonium uptake kinetics in root and leaf cells of *Zostera marina* L. J. Exp. Mar. Biol. Ecol. 352 (2), 271–279. https://doi.org/10.1016/j.jembe.2007.07.024.

Rubio, L., García-Pérez, D., García-Sánchez, M.J., Fernández, J.M.G., 2018. Na^+- dependent high-affinity nitrate, phosphate and amino acids transport in leaf cells of the seagrass *Posidonia oceanica* (L.) Delile. Int. J. Mol. Sci. 19 (6), 1570. https://doi.org/10.3390/ijms19061570.

Sagi, M., Scazzocchio, C., Fluhr, R., 2002. The absence of molybdenum cofactor sulfuration is the primary cause of the flacca phenotype in tomato plants. Plant J. 31 (3), 305–317. https://doi.org/10.1046/j.1365-313x.2002.01363.x.

Sandoval-Gil, J., Alexandre, A., Santos, R., Camacho-Ibar, V.F., 2016. Nitrogen uptake and internal recycling in *Zostera marina* exposed to oyster farming: eelgrass potential as a natural biofilter. Estuar. Coasts 39, 1694–1708. https://doi.org/10.1007/s12237-016-0102-4.

Schlegel, R.W., 2020. Marine heatwave tracker: when and where marine heatwaves are happening around the world. http://www.marineheatwaves.org/tracker.

Serrano, O., Arias-Ortiz, A., Duarte, C.M., Kendrick, G.A., Lavery, P.S., 2021a. Impact of Marine Heatwaves on Seagrass Ecosystems. Springer eBooks, pp. 345–364. https://doi.org/10.1007/978-3-030-71330-0_13.

Serrano, O., Arias-Ortiz, A., Duarte, C.M., Kendrick, G.A., Lavery, P.S., 2021b. Impact of marine heatwaves on seagrass ecosystems. In: Ecosystem Collapse and Climate Change. Springer International Publishing, pp. 345–364.

Smith, K.E., Burrows, M.T., Hobday, A.J., King, N.G., Moore, P.J., Sen Gupta, A., Thomsen, M.S., Wernberg, T., Smale, D.A., 2023. Biological impacts of marine heatwaves. Ann. Rev. Mar. Sci. 15 (1), 119–145. https://doi.org/10.1146/annurevmarine-032122-121437.

Tanahara, S., Canino-Herrera, S.R., Durazo, R., Félix-Bermúdez, A., Vivanco-Aranda, M., Morales-Estrada, E., Del Carmen Lugo-Ibarra, K., 2021. Spatial and temporal variations in water quality of Todos Santos Bay, northwestern Baja California, Mexico. Mar. Pollut. Bull. 173, 113148 https://doi.org/10.1016/j.marpolbul.2021.113148.

Terrados, J., Williams, S.L., 1997. Leaf versus root nitrogen uptake by the surfgrass *Phyllospadix torreyi*. Mar. Ecol. Prog. Ser. 149, 267–277. https://doi.org/10.3354/meps149267.

Touchette, B.W., Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism in seagrasses. J. Exp. Mar. Biol. Ecol. 250 (1–2), 133–167. https://doi.org/10.1016/s0022-0981(00)00195-7.

Vivanco-Bercovich, M., 2023. Respuestas de pastos marinos de Baja California (*Phyllospadix scouleri* y *Phyllospadix torreyi*) a estrés térmico asociado a olas de calor marinas. PhD Thesis. Universidad Autónoma de Baja California, México.

Vivanco-Bercovich, M., Belando-Torrentes, M.D., Figueroa-Burgos, M.F., FerreiraArrieta, A., Macías-Carranza, V., García-Pantoja, J.A., Cabello-Pasini, A., SamperioRamos, G., Cruz-López, R., Camacho-Ibar, V.F., Sandoval-Gil, J.M., 2021. Combined effects of marine heatwaves and reduced light on the physiology and growth of the surfgrass *Phyllospadix torreyi* from Baja California, Mexico. Aquat. Bot. 178, 103488 https://doi.org/10.1016/j.aquabot.2021.103488.

Vonk, J., Middelburg, J.J., Stapel, J., Bouma, T.J., 2008. Dissolved organic nitrogen uptake by seagrasses. Limnol. Oceanogr. 53 (2), 542–548. https://doi.org/10.4319/lo.2008.53.2.0542.

7. Supplementary material

Table S1. Leaf nitrogen uptake kinetic parameters of seagrass species. The parameters were estimated based on the Michaelis– Menten model. V_{max} = maximum uptake rate (µmol N g^{-1} dry weight h^{-1}); K_m = half-saturation constant (µM); and α = affinity coefficient (V_{max}/K_m). These data have been obtained from works after Touchette and Burkholder (2000) and Alexandre et al. (2011), who already presented summary tables for previous studies.

Species	**Nutrient**	**V_{max}**	**K_m**	**α**	**Source**
Halophila stipulacea	NH_4^+	9.79	57.95	0.17	Alexandre et al., 2014
Zostera marina	NH_4^+	40-102	50-210	0.20-0.95	Sandoval-Gil et al., 2015
Zostera marina	NH_4^+	28.91-82.08	49.92-150.62	0.55-0.65	Alexandre et al., 2020
Cymodocea nodosa	NH_4^+	14.90	44.38	0.34	Alexandre and Santos, 2020
Zostera marina	NH_4^+	51.8	68.1	0.76	Li et al., 2020
Phyllospadix scouleri	**NH_4^+**	**49.22**	**76.13**	**0.64**	**This study**
Zostera marina	NO_3^-	0.50-6.50	5-60	0.10-0.60	Sandoval- Gil et al., 2015
Posidonia oceanica	NO_3^-	6.30	8.70	1.38	Rubio et al., 2018
Zostera marina	NO_3^-	0.59-2.19	1.75-6.56	0.29-0.41	Alexandre et al., 2020
Cymodocea nodosa	NO_3^-	1.66	9.19	1.27	Alexandre and Santos, 2020
Zostera marina	NO_3^-	39.1	68.6	0.56	Li et al., 2020
Phyllospadix. scouleri	**NO_3^-**	**7.18**	**124.76**	**0.05**	**This study**
Phyllospadix scouleri	**Urea**	**4.32**	**23.61**	**0.18**	**This study**

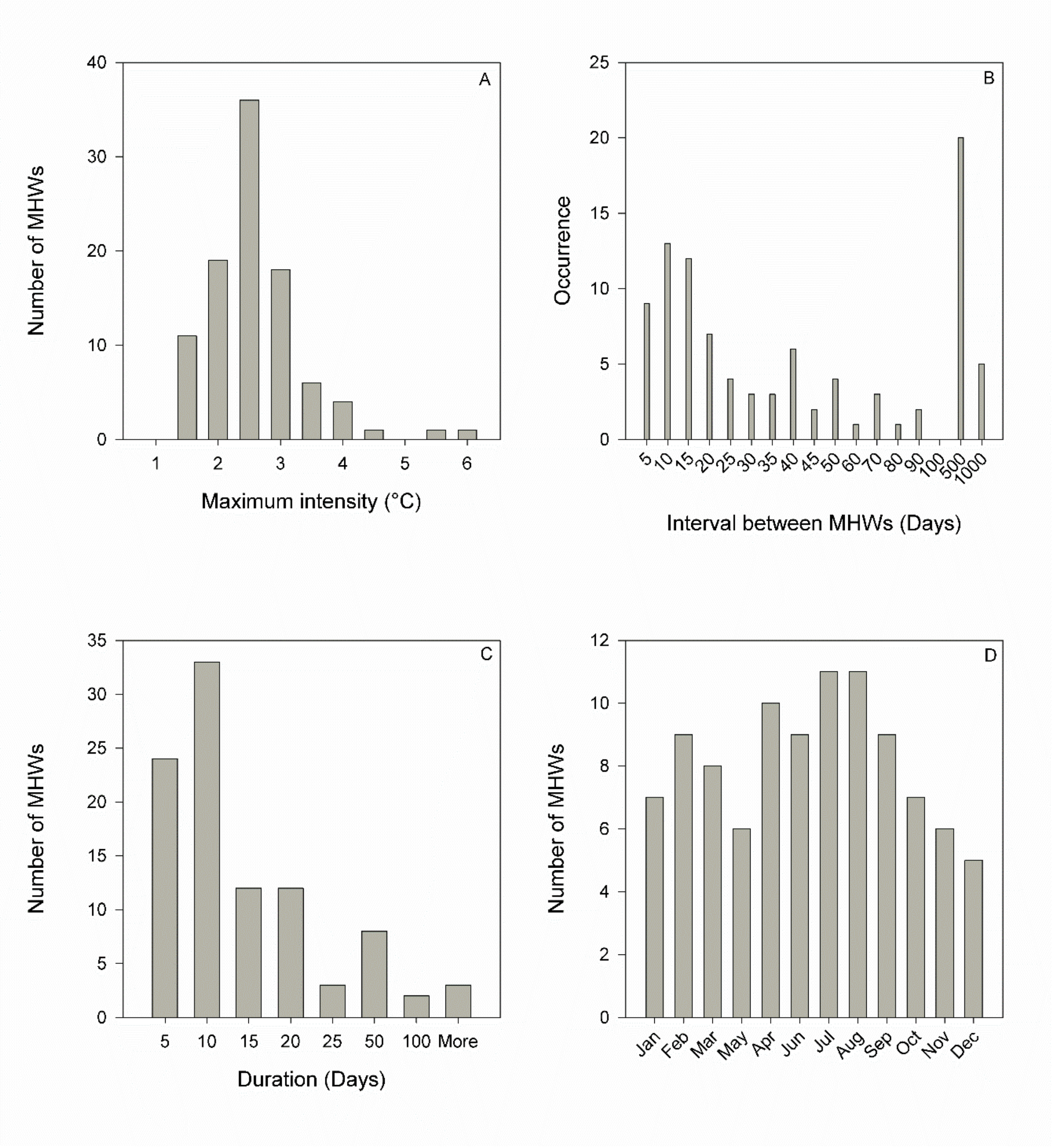


Figure S1. Characteristics of the MHWs that were registered in the beds of *Phyllospadix scouleri* (Todos Santos Island, B.C., Mexico) between the years 1982 – 2019. A – Monthly distribution of MHWs incidence; B – Maximum intensity of registered MHWs; C - Duration of the registered MHWs; D – Duration of interval periods between consecutive MHWs.

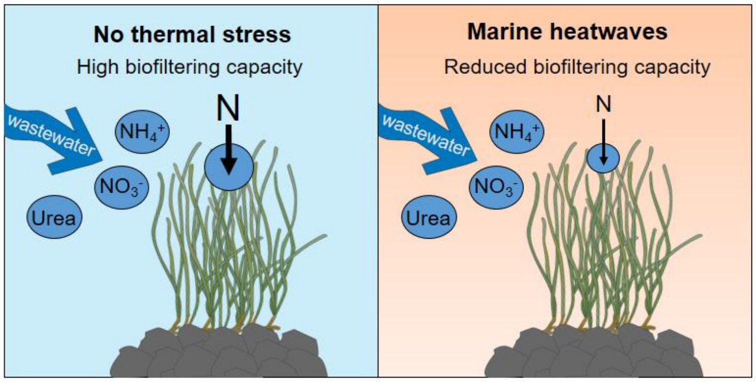


Figure S2. Graphical abstract.