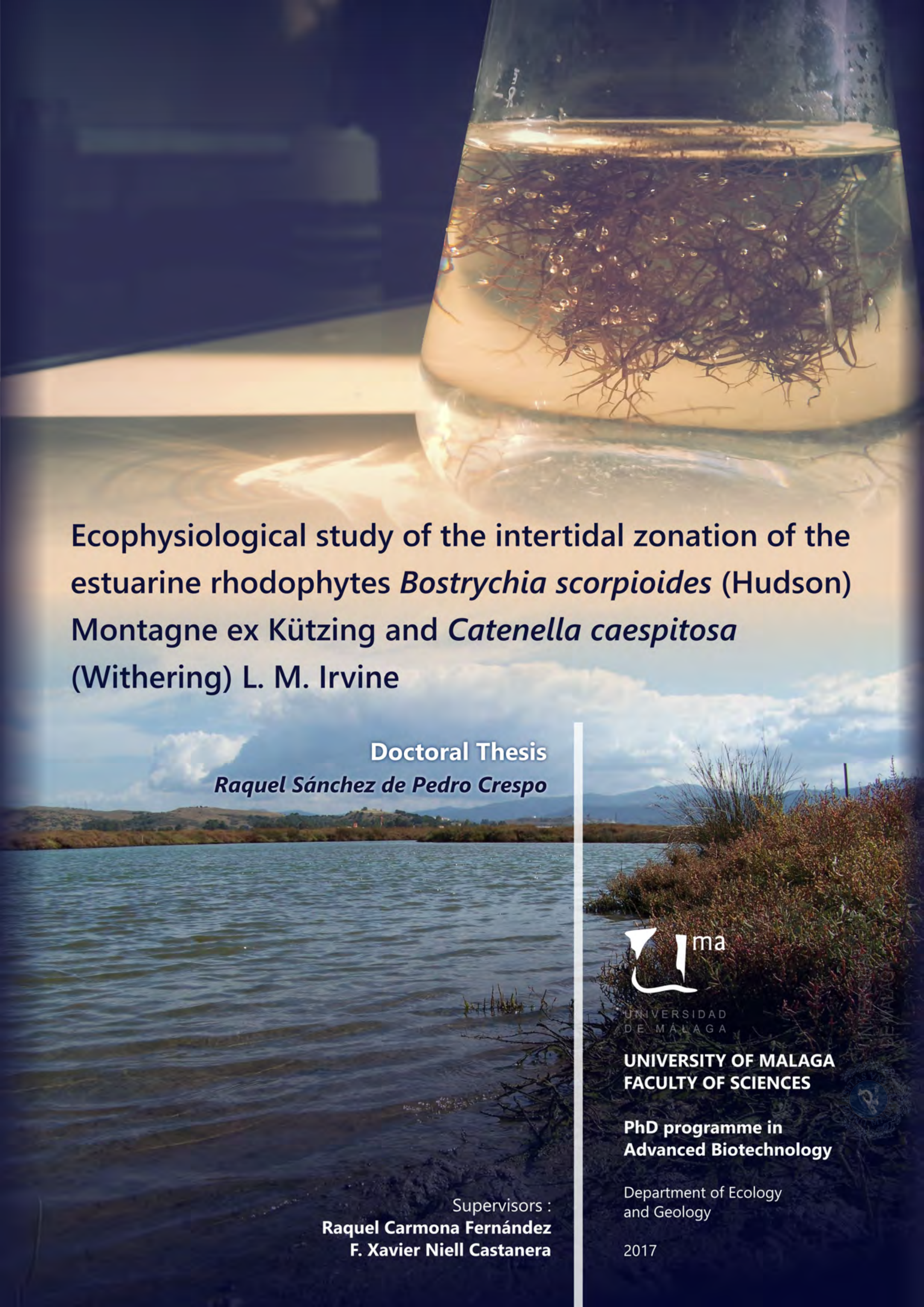

Ecophysiological study of the intertidal zonation of the estuarine rhodophytes *Bostrychia scorpioides* (Hudson) Montagne ex Kützing and *Catenella caespitosa* (Withering) L. M. Irvine

Doctoral Thesis

Raquel Sánchez de Pedro Crespo

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Memoria presentada para optar al
grado de Doctor en Ciencias Ambientales
por Raquel Sánchez de Pedro Crespo

Dirigida por por F. Xavier Niell Castanera y Raquel Carmona Fernández

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CERTIFICAN:

Que la presente memoria titulada "Ecophysiological study of the intertidal zonation of the estuarine rhodophytes *Bostrychia scorpioides* (Hudson) Montagne ex Kützing and *Catenella caespitosa* (Withering) L. M. Irvine" presentada por la Licenciada en Ciencias Ambientales, Raquel Sánchez de Pedro Crespo, ha sido realizada bajo nuestra dirección y el trabajo presentado y las publicaciones que lo avalan no han sido utilizados en tesis anteriores. Y considerando que representa el trabajo de Tesis Doctoral, autorizamos su exposición y defensa para optar al Grado de Doctor en Ciencias Ambientales.

Y para que así conste, a los efectos oportunos, firma el presente en Málaga a 21 de Diciembre de 2016.

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"The only thing that makes life possible is permanent, intolerable uncertainty; not knowing what comes next."

— Ursula K. Le Guin, *The Left Hand of Darkness*

Resumen

La presente tesis doctoral tiene como objetivo estudiar la ecofisiología de las macroalgas estuáricas *Bostrychia scorpioides* (Hudson) Montagne ex Kützing (Rhodophyta, Ceramiales) y *Catenella caespitosa* (Withering) L. M. Irvine (Rhodophyta, Gigartinales) y analizar el papel que desempeñan diferentes variables ambientales en su distribución y crecimiento en el intermareal del estuario del río Palmones.

Bostrychia scorpioides y *C. caespitosa* crecen sobre los talos basales leñosos de los macrófitos halófitos *Sarcocornia perennis* (Mill.) A. J. Scott y *Atriplex* (*Halimione*) portulacoides (l.) Aellen. Estas macroalgas se encuentran segregadas espacialmente a pequeña escala en el intermareal del estuario, a lo largo de un gradiente de luz, temperatura, emersión, y disponibilidad de nutrientes, según la diferente posición que ocupan en relación con la altura de marea. *Bostrychia scorpioides* se distribuye hacia los horizontes más altos del intermareal, mientras que *C. caespitosa* ocupa aquellos más bajos y próximos a la superficie del sedimento, coexistiendo ambas en una franja intermedia. Desde el punto de vista ecofisiológico, estas especies destacan por su adaptación a bajas irradiancias, debido al efecto atenuante del dosel de vegetación sobre el que crecen, y su alta tolerancia a la desecación, la cual determina sus límites superiores de distribución junto a la capacidad de recuperación fisiológica tras la reinmersión, tal como ocurre en la mayoría de macroalgas intermareales.

La tesis contribuye al debate científico sobre los factores ambientales que modelan la zonación de macroalgas en ecosistemas intermareales. Cabe destacar que la mayor parte de estudios de la zonación de macroalgas se han llevado a cabo en intermareales rocosos de ecosistemas costeros, siendo menor la información en estuarios. El interés de este estudio radica en las peculiaridades de la estructura y funcionamiento del ecosistema, como son los gradientes físico-químicos horizontales propios de los sistemas de interfase entre el medio marino y el ripario, así como el diferente tipo de sustrato y vegetación.

El presente estudio aborda diversos aspectos de las rodofíceas intermareales de zonas de estuario, a través de una aproximación ecofisiológica "integradora" y a "multiescala". La aproximación integradora busca unir las ventajas de experimentos de campo y laboratorio, mientras que la de multiescala busca combinar las respuestas fisiológicas y bioquímicas de cada especie a las variables ambientales con aquellas obtenidas a nivel de comunidad. La tesis se plantea tanto desde el punto de vista espacial como temporal, considerando especímenes localizados en diferentes microzonas del intermareal que presentan condiciones ambientales diferentes, que pueden dar lugar a respuestas metabólicas específicas y a lo largo de las estaciones.

En el **capítulo 2** se describen las especies y el sistema de estudio. La zonación de las rodofíceas de este estudio ocupa una franja de 30 cm en el intermareal estuárico, donde *C. caespitosa* ocupa la zona inferior-intermedia del intermareal (1.05-1.20 m), mientras que la intermedia-superior está dominada por *B. scorpioides* (1.15-1.30 m). El tiempo de inmersión, irradiancia y temperatura varían entre los distintos niveles intermareales. A lo largo de un ciclo semidiurno de marea *B. scorpioides* y *C. caespitosa* están 2.5 y 5 h en inmersión, destacando que la mayor parte del tiempo están emergidas. Los valores de irradiancia en los límites superiores e inferiores de distribución fueron de 122-208 y 32-51 µmol fotones m^{-2} s^{-1} para *B. scorpioides*, y de 30-72 y 1-11 µmol fotones m^{-2} s^{-1} para *C. caespitosa*, respectivamente. El coeficiente de atenuación de la luz a través del dosel de vegetación fue de 8.0 ± 0.4 m^{-1} en zonas más iluminadas y 11.0 ± 0.4 m^{-1} en las más sombrías, según la densidad del dosel de vegetación. La irradiancia media en la zona de coexistencia fue de 40-45 µmol fotones m^{-2} s^{-1}. La atenuación de la temperatura a través del dosel se determinó en invierno y verano, con valores medios para la zona intermareal intermedia de 13.7 y 23.4ºC, respectivamente, y mayores diferencias entre la temperatura del agua y el aire en verano (4.4ºC) que en invierno (3.2ºC). Se identificaron las concentraciones de nutrientes más frecuentes en el estuario a lo largo del periodo de estudio. El amonio es la forma de nitrógeno más abundante, con concentraciones que oscilan entre los 0 y 60 µM, con máximas concentraciones en invierno superiores a 80 µM. El nitrato está presente en un rango menor de concentraciones respecto al amonio (0 to 15 µM). Las concentraciones de fosfato normalmente varían entre 0 y 2 µM, aunque se encontraron máximas concentraciones en torno a 4 µM en invierno.

El **capítulo 3** tuvo como objetivo evaluar la capacidad de fotoaclimatación y los requerimientos energéticos para la fotosíntesis y el crecimiento de *B. scorpioides* y *C. caespitosa*, bajo condiciones controladas en laboratorio. Para ello se obtuvieron curvas fotosíntesis-luz y crecimiento-luz en especímenes de *B. scorpioides* y *C. caespitosa* previamente aclimatados a las condiciones estándar de laboratorio (15ºC, 45 µmol fotones m^{-2} s^{-1}, salinidad 36, pH 8.2). Se emplearon cinco irradiancias según los valores de campo (10, 20, 40, 70 y 230 µmol fotones m^{-2} s^{-1}). En los experimentos de fotosíntesis las algas se aclimataron durante tres días a las diferentes irradiancias. Las medidas de fotosíntesis obtenidas en todo el estudio se obtuvieron mediante el método de evolución y consumo de O_2 (electrodo tipo Clark). En el experimento de crecimiento las algas se cultivaron a las diferentes irradiancias durante dos semanas, con una renovación del medio de cultivo cada 3-4 días, y se determinó el contenido final de pigmentos (clorofila a, carotenoides totales y ficobiliproteínas). *Bostrychia scorpioides* y *C. caespitosa* mostraron diferencias en la mayoría de sus parámetros fotosintéticos y en sus curvas de crecimiento-luz. *Bostrychia scorpioides* presentó mayor capacidad fotosintética máxima y puntos de compensación para el crecimiento y la fotosíntesis más altos que *C. caespitosa*, lo que indica la menor capacidad de *B. scorpioides* para crecer a bajas irradiancias. *Catenella caespitosa* destacó por sus bajas tasas de respiración y bajos requerimientos lumínicos para crecer, que denotan una gran capacidad de aclimatación a bajas irradiancias. Los puntos de compensación para el crecimiento coincidieron con los valores de irradiancia encontrados en los límites inferiores de distribución de cada especie, explicándose así la ausencia de *B. scorpioides* en la zona inferior del intermareal (E_c de 23.8 µmol fotones m^{-2} s^{-1}), y la dominancia de *C. caespitosa* en dicho horizonte (10.2 µmol fotones m^{-2} s^{-1}). Sin embargo, dado que ambas especies poseen la misma capacidad de crecer a irradiancias saturantes para la fotosíntesis (230 µmol fotones m^{-2} s^{-1}), que se dan en la zona superior del intermareal, se concluyó que el crecimiento de *C. caespitosa* no estaría limitado por la luz en su hábitat natural.

El objetivo del **capítulo 4** fue determinar la capacidad de incorporación y requerimientos internos de nutrientes de *B. scorpioides* y *C. caespitosa*. Las algas se cultivaron en el laboratorio en ausencia de nutrientes durante 6 días, con el fin de determinar la máxima capacidad de incorporación de nutrientes tras un periodo de hambruna. Se obtuvieron cinéticas de incorporación para cada nutriente, durante 3 h, a 15ºC y a irradiancia saturante, con concentraciones iniciales de 5, 10, 20 y 40 µM para el amonio y el nitrato y 2, 5, 10 y 20 µM para el fosfato. Además, se estudió el efecto de la adición simultánea de amonio y nitrato (equimolar), con el fin de detectar posibles efectos inhibitorios en sus cinéticas. A partir del contenido interno de nutrientes y los tiempos medios de inmersión en el intermareal estuárico se estimaron las tasas de renovación o "turnover" del N y P. En un segundo grupo de experimentos se evaluó el efecto de la concentración de nutrientes en el crecimiento y la fotosíntesis, cultivando las algas durante 10 días a 15ºC y 45 µmol fotones m^{-2} s^{-1}. El medio de cultivo se enriqueció en amonio (0-80 µM), nitrato (0-10 µM) y fosfato (0-5 µM), obteniéndose 3 tratamientos (bajo, intermedio, alto) con N:P constante de 18, además de un cultivo control sin nutrientes.

Los resultados de este capítulo sugieren que los cambios en la disponibilidad de nutrientes (estacionales o mareales) pueden modificar la incorporación de nutrientes, respuesta fotosintética y composición interna de ambas especies. Ambas especies presentaron tasas de incorporación de amonio más altas que para el nitrato, y las interacciones entre las fuentes de N no fueron evidentes. Sin embargo, *B. scorpioides* posee mayor capacidad y afinidad en la incorporación de amonio y presentó mecanismos de rápida incorporación ("surge uptake"), mientras que *C. caespitosa* tiene una mayor habilidad para incorporar fosfato, mostrando cinéticas de saturación para el amonio y el nitrato. Además, *B. scorpioides* en base a su menor tasa de renovación de nutrientes internos respecto a *C. caespitosa*, posee una mayor independencia del aporte externo de nutrientes, lo que permite su crecimiento en la zona alta del intermareal donde los tiempos de inmersión son muy reducidos. Este hecho indicó que las tasas de renovación de los nutrientes internos pueden jugar un papel fundamental en la determinación de los límites de distribución de *B. scorpioides* y *C. caespitosa*. Respecto al crecimiento, la concentración de nutrientes no afectó a las tasas de ninguna de las especies, y se obtuvo una relación inversa entre las tasas de fotosíntesis neta y la concentración de nutrientes. En este sentido se sugirió que la falta de efecto de la concentración de nutrientes sobre el crecimiento puede reflejar su capacidad de soportar periodos intermitentes de disponibilidad de nutrientes en el estuario.

El **capítulo 5** se centró en analizar el efecto individual e interactivo de las variables de emersión sobre las respuestas fisiológicas (tolerancia y recuperación). En primer lugar, se obtuvieron las tasas de desecación o pérdida hídrica a dos temperaturas (15, 25 ºC) y bajo dos condiciones iniciales de hidratación ("blotted" 100% contenido hídrico, "wet" 250% contenido hídrico). En un segundo grupo de experimentos se estimaron las tasas de crecimiento tras 10 días de cultivo bajo distintas combinaciones de temperatura (T, 15, 25 ºC), irradiancia (I, 30, 230 µmol fotones m^{-2} s^{-1},), emersión (E, 0, 3 y 8 h d^{-1}, y "wet") y salinidad (S, 10, 20, 36, 45), siendo estas: T × I, T × E, S × E. Al final de los tratamientos se realizaron medidas fotosínteticas y análisis de la composición interna para evaluar el estado fisiológico de las algas. En el tercer grupo de experimentos se evaluó el efecto de la desecación en las respuestas fotosintéticas de *B. scorpioides* y *C. caespitosa*, y su capacidad de recuperación tras la emersión a corto (0-3 h) y a largo plazo (1-6 días de emersión continua). Además de las medidas fotosínteticas por evolución de oxígeno, se emplearon técnicas de medida de la fluorescencia del fotosistema II (PSII) por pulsos de amplitud modulada (PAM) del que se obtuvo el rendimiento cuántico óptimo y efectivo y curvas de transporte electrónico relativo (ETR).

Bostrychia scorpioides y *C. caespitosa* poseen diferente tolerancia a las variables de emersión, lo cual se ha observado de forma más clara en la diferente susceptibilidad de sus tasas de crecimiento a la desecación y la temperatura y su capacidad de recuperar la fotosíntesis tras la emersión continuada a largo plazo. A corto plazo no se detectaron diferencias interespecíficas en el grado de tolerancia, resistencia y recuperación tras periodos de emersión, lo que señala que las respuestas a la emersión a corto plazo no siempre coinciden con la capacidad para tolerar largo periodos de emersión y, por tanto, no pueden relacionarse con la posición que cada especie ocupa en el intermareal. Ambas especies fueron capaces de recuperar su actividad fotosintética tras periodos de emersión diarios y tras 3 días de emersión continua, y mantuvieron tasas positivas fotosíntesis hasta una pérdida hídrica del 50-60%. Estas respuestas indican que los tiempos de emersión diarios a los que están expuestas no suponen condiciones estresantes para ellas en su hábitat natural. Sin embargo, la exposición diaria a niveles severos de desecación y temperatura tuvieron una influencia mucho más drástica sobre el crecimiento de *C. caespitosa* que el de *B. scorpioides*. Además, tras 6 días de emersión continua la capacidad de recuperación fotosintética de *C. caespitosa* (recuperación del 8% del control en inmersión) se redujo en mayor medida que en *B. scorpioides* (recuperación del 21% del control).

La combinación de temperatura e irradiancia (T × I), bajo condiciones de inmersión, puso en evidencia la mayor sensibilidad del crecimiento de *C. caespitosa*, que fue mayor a 15ºC. El crecimiento de *C. caespitosa*, se redujo drásticamente con la emersión, mientras que el de *B. scorpioides* mostró una sensibilidad mucho menor frente a periodos diarios de emersión crecientes. La combinación de emersión y temperatura tuvo un efecto sinérgico negativo sobre el crecimiento de *B. scorpioides* y *C. caespitosa*, ya que a 25ºC la emersión tuvo un efecto más negativo sobre sus tasas de cercimiento, especialmente en *C. caespitosa*. La combinación de salinidad y emersión tuvo un efecto antagonista sobre el crecimiento de estas especies. La salinidad fue relevante bajo inmersión continua, donde se obtuvo un crecimiento máximo entre 10 y 20 para ambas especies. Sin embargo, a periodos de emersión creciente el efecto de la salinidad perdió importancia. Por otro lado, ambas especies pueden crecer en emersión siempre que no exista pérdida hídrica por desecación, aunque solo *B. scorpioides* mantiene la misma tasa de crecimiento en emersión húmeda (manteniendo el grado de hidratación) que en inmersión continua.

En el **capítulo 6** se analizó la variabilidad intra- e interespecífica en la ecofisiología de *B. scorpioides* y *C. caespitosa* en relación al gradiente de marea y las posible implicaciones a nivel de interacciones bióticas. Para ello, se recolectaron especímenes de los límites superiores (U) e inferiores (L) de sus respectivos rangos de distribución intermareal, y se obtuvieron curvas de fotosíntesis-luz, tasas de incorporación de nutrientes y la composición interna (contenido en pigmentos, composición elemental y contenido en osmolitos). Los resultados revelaron que existe variabilidad fenotípica en las respuestas fisiológicas y composición bioquímica de *B. scorpioides* y *C. caespitosa*. Estas diferencias fueron mayores en los límites del área de su coexistencia, debido a sus diferentes mecanismos de aclimatación a la luz y a la disponibilidad de nutrientes. *Bostrychia scorpioides* presentó una mayor variabilidad en las respuestas fotosintéticas y contenido en pigmentos, que coincide con el rango más amplio de irradiancias al que está expuesta (30-230 µmol fotones m^{-2} s^{-1}) en comparación con el de *C. caespitosa* (0-70 µmol fotones m^{-2} s^{-1}). Los talos de *B. scorpioides* situados en sus límites inferiores de distribución presentaron mayor capacidad y eficiencia fotosintética que los de la zona superior. Estos cambios estuvieron ligados a una aclimatación pigmentaria, ya que no se detectaron al expresar los datos respecto al contenido en clorofila a. Por otra parte, se encontraron diferencias intraespecíficas en las tasas de incorporación de amonio y fosfato en *C. caespitosa*. Aunque en inmersión *C. caespitosa* posee tasas mayores de incorporación, en zonas intermareales intermedias su capacidad disminuye, dando ventaja competitiva a *B. scorpioides* que mantiene tasas superiores. Aunque la disponibilidad de nutrientes disminuye hacia las partes superiores del intermareal, no se encontraron diferencias interespecíficas en el contenido total interno de N. Sin embargo, la incorporación de amonio disminuye con la altura de marea en *C. caespitosa*, mientras que no se encontraron diferencias en *B. scorpioides* en la incorporación de nitrato.

Bostrychia scorpioides de la parte superior de su distribución presentó tiempos mayores de renovación interna de N, lo que respalda la hipótesis de que especímenes localizados en posiciones intermareal superiores, incluso a nivel intraespecífico, poseen una mayor capacidad de retener el N, y por lo tanto dependen en menor medida del aporte externo de nutrientes. En cambio, *C. caespitosa* no presentó dicha capacidad de ajuste de su tasa de renovación o “turnover”, por lo que esta especie necesita una inmersión más frecuente. No obstante, los valores de C:N y contenido total de N no muestran que esta especie esté limitada por N en el campo. Si bien la capacidad de ambas especies para incorporar P se redujo hacia los límites superiores de su zonación, tal reducción solo repercutió en el contenido interno de P de *C. caespitosa*. Los tiempos de renovación del P fueron mayores en talos localizados a niveles superiores del intermareal (20 vs 2 d), lo que sugiere que durante emersiones prolongadas (hasta 10-20 d) estas especies podrían estar limitadas por P, lo que se demuestra según los valores de N:P obtenidos superiores a 40. Por último, se encontraron mayores concentraciones de los osmolitos D-dulcitol y D-sorbitol en especímenes de la zona superior de distribución de *B. scorpioides*, lo cual podría contribuir a su capacidad de soportar periodos de emersión más prolongados en zonas más altas del intermareal estuárico.

Los **capítulos 7 y 8** abordan la dinámica estacional de la biomasa, crecimiento, fotosíntesis y composición interna de las especies objeto de estudio, a través de muestreos mensuales en el estuario. En el capítulo 7, además se analizó la distribución espacial de la biomasa en relación a su zonación y a lugares con diferente densidad de dosel de vegetación (iluminados vs. sombríos). Entre noviembre 2013 y noviembre 2014 se obtuvieron datos mensuales de diferentes variables ambientales y se determinó la biomasa de cada especie, con el fin de relacionar los cambios temporales en la biomasa y el crecimiento con éstas. *Bostrychia scorpioides* presentó una mayor dominancia en áreas más iluminadas, distribuyéndose su biomasa de forma más homogénea a lo largo del intermareal. Por el contrario, *C. caespitosa* fue más abundante donde las condiciones ambientales fueron más benignas (en zonas sombrías y en los niveles inferior e intermedio del intermareal). En este sentido, el aumento de densidad en el dosel de vegetación aminora el estrés físico, facilitando así el crecimiento de *C. caespitosa*, dada su menor tolerancia a la emersión (capítulo 5), y limita el de *B. scorpioides*, debido a sus mayores requerimientos lumínicos para crecer (capítulo 3). En consecuencia, las variaciones en la densidad del dosel no alteraron la biomasa algal total.

La biomasa de ambas especies presentó un claro patrón estacional, con un aumento de fotosíntesis, crecimiento y contenido interno de nutrientes de invierno a primavera. Ambas especies presentaron una rápida capacidad de integrar los cambios ambientales en el estuario, ya que no se observó retardo alguno en las correlaciones entre el crecimiento y las variables ambientales. La aplicación de técnicas estadísticas multivariantes permitió identificar el tiempo de inmersión mensual y la disponibilidad de nutrientes como las variables con mayor influencia en su crecimiento. No obstante, el crecimiento de *B. scorpioides* se vio afectado en mayor medida por la concentración de fósforo soluble en el agua del estuario, mientras que el de *C. caespitosa* mostró una mayor relación con el nitrógeno inorgánico soluble, aunque solo hasta concentraciones de 40 µM. Durante la segunda mitad del estudio se observó una mayor abundancia de biomasa algal, coincidiendo con un periodo de menores perturbaciones físicas y biológicas, en el sentido de la ausenciade lluvias torrenciales y proliferación de ulváceas.

En el **capítulo 8**, se analizaron las respuestas de fotoaclimatación y los cambios en la composición interna (pigmentos, composición elemental y osmolitos) de *B. scorpioides* y *C. caespitosa* obtenidos a lo largo de 1 año (febrero 2014 - febrero 2015). Las rodofíceas intermareales de este estudio presentaron diferentes respuestas en cuanto a su fotofisiología, composición interna y aclimatación osmótica. De ello se destaca la diferente respuesta estacional de cada especie: *Bostrychia scorpioides* presentó una plasticidad fotofisiológica mayor que *C. caespitosa* frente a las variaciones ambientales estacionales, siendo esta última menos sensible a estos cambios sin presentar grandes variaciones. Estas respuestas fueron acordes a las diferencias fotofisiológicas obtenidas en experimentos de laboratorio (capítulo 3). Las diferencias interespecíficas en el contenido interno no fueron tan evidentes como para las respuestas fotosintéticas. El contenido en pigmentos y en composición elemental aumentó de verano a invierno, en consonancia con el ciclo anual de los nutrientes en el estuario. Además, se ha detectado una relación positiva entre las concentraciones de nitrógeno inorgánico disuelto (DIN) y el N total para cada mes. Sin embargo, las variaciones en el contenido de P total son mínimas y no se relacionaron de forma significativa con el fósforo soluble total del medio. Los valores de los índices C:N y N:P de muestras de campo indicaron que las especies no están limitadas por N pero sí por P en el estuario. Por otro lado, los osmolitos de cada especie presentaron patrones estacionales muy diversos, que ponen en evidencia la influencia de múltiples variables ambientales sobre su síntesis. Dado que las medidas se realizaron en talos recolectados del horizonte intermedio de su zonación intermareal, las diferentes respuestas obtenidas no pueden atribuirse a la exposición diferencial a los factores abióticos (luz, emersión, nutrientes, etc.), sino a la propia plasticidad fenotípica de cada especie frente a condiciones ambientales fluctuantes.

En la discusión general (**capítulo 9**) se abordan de forma transversal los objetivos de la tesis, con el fin de (1) integrar y contrastar las respuestas ecofisiológicas obtenidas en experimentos de campo y laboratorio, (2) identificar y destacar las características ecofisiológicas de cada especie y (3) discutir las implicaciones ecológicas para su zonación y las posibles relaciones interespecíficas.

En primer lugar, las respuestas de crecimiento porporcionaron una medida de respuesta a las variables ambientales integradora y de mayor significancia ecológica. En base a los experimentos de laboratorio, el tiempo de emersión diario fue la variable con mayor influencia en el crecimiento de *B. scorpioides* y *C. caespitosa*, pero particularmente para *C. caespitosa*, ya que reduce su crecimiento de forma drástica (capítulo 5). Esta afirmación está en consonancia con la mayor influencia del tiempo mensual de inmersión/emersión sobre el crecimiento estacional, y con la disminución de la biomasa total hacia los límites superiores de la zonación intermareal (capítulo 7). La irradiancia y la emersión tuvieron la misma importancia relativa para el crecimiento de *B. scorpioides*, indicando que las reducciones de irradiancia o el aumento de emersión afectan su crecimiento en la misma medida. Evidencia de esto se encontró en la ausencia de diferencias entre la biomasa de *B. scorpioides* entre los límites superior e inferior de su horizonte (capítulo 7). Por otro lado, la irradiancia tuvo una menor importancia que la emersión para el crecimiento de *C. caespitosa*, lo cual se debe a su mejor adaptación fotofisiológica a bajos niveles lumínicos, tal como se ha demostrado en experimentos de laboratorio (capítulo 3) y campo (capítulos 6, 7, 8). La temperatura tuvo mayor influencia en el crecimiento de *C. caespitosa* que en el de *B. scorpioides*, debido a la mayor sensibilidad de *C. caespitosa* a esta variable y su efecto combinado con la emersión (capítulo 5), lo cual se ha observado en el campo en la menor abundancia de *C. caespitosa* en microambientes más expuestos al estrés de emersión (zonas iluminadas, capítulo 7).

La concentración de nutrientes no tuvo un efecto significativo sobre las tasas de crecimiento obtenidas en cultivo (capítulo 4). Este resultado aparentemente contradice aquel obtenido en el estudio estacional, donde se observó que la concentración de fósforo soluble reactivo y nitrógeno inorgánico disuelto contribuyen en gran medida al crecimiento de *B. scorpioides* y *C. caespitosa*, respectivamente. ¿Cómo puede explicarse esta diferencia? En base al análisis de las tasas de crecimiento obtenidas en laboratorio, la tasa de renovación del medio de cultivo tuvo un efecto significativo en el crecimiento de estas especies. En los experimentos de esta tesis el medio de cultivo se renovó diariamente, excepto en los del capítulo 3, que se cambiaba cada 3-4 días y donde las tasas de crecimiento fueron menores. En este punto ha de considerarse además que *B. scorpioides* y *C. caespitosa* están en inmersión dos veces al día a lo largo de ciclos mareales regulares, periodos durante los cuales reciben un aporte continuo de nutrientes, en su mayoría procedentes de la redisolución del amonio y el fosfato desde el sedimento vía la acción mareal. Por lo tanto, aunque en este estudio se han empleado concentraciones de nutrientes similares a las del agua del estuario, la renovación diaria del medio de cultivo podría no ser suficiente para inducir cambios significativos en su crecimiento. De hecho, en la mayoría de experimentos de laboratorio se observó dilución de nutrientes internos y moléculas de acumulación de N (ej. ficobiliproteinas y clorofila a) por crecimiento, lo que sugiere que el crecimiento obtenido se basa en los nutrientes internos y no en el aporte externo.

Las comparaciones entre las tasas de crecimiento obtenidas en campo y laboratorio evidenciaron diferentes aspectos. Por un lado, las pérdidas de biomasa de *B. scorpioides* fueron menores en laboratorio que en el campo, lo que sugiere que *B. scorpioides* está probablemente expuesta a condiciones ambientales más severas que las que se han empleado en los experimentos de laboratorio (más de 8 h de emersión diaria y temperaturas superiores a 25 ºC). Por otro lado, se encontraron similares pérdidas de biomasa en campo y laboratorio para *C. caespitosa*, lo que sugiere que las condiciones ambientales a las que *C. caespitosa* está expuesta en su hábitat natural no son más extremas que las empleadas en los cultivos de crecimiento. Si bien las altas tasas obtenidas en el laboratorio evidencian que *C. caespitosa* tiene un potencial de crecimiento mucho mayor, las condiciones a las que éstas se obtuvieron no se dan en su hábitat natural, pues nunca están en inmersión continua y el incremento de irradiancia va ligado a una emersión más severa.

Bostrychia scorpioides posee una mayor variabilidad fenotípica a escala espacial (zonación) y estacional. Esta especie presentó una mejor adaptación a alta irradiancia, a través de cambios en sus respuestas fotosíntesis irradiancia y ajustes en el contenido pigmentario, además de maximizar sus tasas de fotosíntesis a altas irradiancias (capítulos 3, 6), y presentó una mayor plasticidad fotofisiológica a nivel estacional. Por otro lado, esta especie redujo las tasas de renovación de nutrientes internos en las posiciones de marea donde el aporte de nutrientes es menor (capítulos 4, 6). Además, mantuvo tasas de crecimiento positivas frente a periodos severos de emersión diaria recuperando su actividad fotosintética tras 6 días de emersión continua. Este conjunto de respuestas fisiológicas permite a esta especie afrontar condiciones ambientales más fluctuantes, lo que explica su clara dominancia en lugares más expuestos a la luz, su presencia en los niveles más altos del intermareal y su distribución homogénea de biomasa entre los distintos niveles mareales (capítulo 7).

Catenella caespitosa mostró una gran capacidad fotofisiológica adaptada a bajas irradiancias, con bajísimos requerimientos lumínicos para su crecimiento, la minimización de las tasas de respiración y un alto contenido de carbono específico por superficie de talo. Respecto a *B. scorpioides* presentó una menor variabilidad fisiológica a lo largo de su zonación, así como a escala estacional (capítulos 6, 7). Esta respuesta fotofisiológica, junto a su baja capacidad para reducir la demanda interna de N en posiciones más altas en el intermareal y su mayor sensibilidad a las variables de emersión, explican su dominancia en las zonas intermareales más bajas y en lugares sombríos, donde el estrés está aminorado por el dosel de vegetación (capítulo 7).

Los resultados de esta tesis demuestran que la zonación intermareal de *B. scorpioides* y *C. caespitosa* está principalmente controlada por los factores físicos. Sin embargo, no se puede descartar el papel adicional de las interacciones bióticas entre las especies en base a los resultados obtenidos. En primer lugar, debe considerarse que el intermareal estuárico está restringido al intermareal superior, donde los factores físicos ganan relevancia. Si hubiese una interacción competitiva entre las especies, ocurriría donde existiese disponibilidad de un recurso común potencialmente limitante para el crecimiento, lo que constituye el prerrequisito del principio universal de exclusión competitiva. La colonización del sustrato representa el recurso más evidente por el cual se puede producir competencia entre macroalgas, pero en este caso no varía a lo largo del intermareal. Por otro lado, la disponibilidad de luz y nutrientes disminuye en la zona del intermareal inferior y superior, respectivamente. En este sentido, la competencia directa por esos recursos es más probable que ocurra a niveles de intermareal medio, donde todos los recursos están medianamente disponibles. En la zona intermedia ninguna de las especies estuvo limitada por nutrientes, ya que se produce la inmersión diaria y además sus respuestas fotofisiológicas fueron similares (capítulo 3). Sin embargo, los resultados del capítulo 6 mostraron que sus diferencias interespecíficas aumentan hacia los límites de la zona intermareal media. Según la hipótesis inicial en relación al uso de recursos, una especie tendrá ventaja competitiva cuando tenga mayor habilidad de adquirir todos los recursos. Hacia el límite inferior de la distribución de *B. scorpioides*, *C. caespitosa* podría crecer sobre *B. scorpioides* y es posible que *B. scorpioides* perdiera biomasa por la menor disponibilidad de luz. Sin embargo, dado que la luz per se limita el crecimiento de esta especie, es difícil discernir a partir de los resutlados si la competencia con *C. caespitosa* tendría un papel adicional en la determinación del límite inferior de zonación de *B. scorpioides*. Por el contrario, en el límite superior de distribución de *C. caespitosa*, *B. scorpioides* es mejor competidor por la luz, presenta menores “turnover” de nutrientes y posee una mayor tolerancia a la emersión. Siguiendo un razonamiento similar al previo, *B. scorpioides* crecería sobre *C. caespitosa* a esas condiciones, como se demostró en los experimentos de emersión (capítulo 5), y en su mayor dominancia en zonas expuestas a mayor estrés abiótico (capítulo 7). En consecuencia, si *B. scorpioides* creciese sobre *C. caespitosa*, su biomasa recuciría el estrés por emersión hasta cierto punto, lo que facilitaría el crecimiento de *C. caespitosa* y, al mismo tiempo, *C. caespitosa* podría crecer bajo los talos de *B. scorpioides* dada su mejor adaptación a baja irradiancia.

La coexistencia entre especie aparentemente similares puede ocurrir a través de distintos caminos, bajo la principal influencia de factores físicos, biológicos o combinaciones de estos y además variar entre ecosistemas intermareales debido a diferencias locales en las condiciones ambientales. El solapamiento de los horizontes de *B. scorpioides* y *C. caespitosa* en zonas intermedias (en mayor grado en zonas sombrías) pone en evidencia que las similares respuestas en condiciones intermedias favorecen su coexistencia en lugar de su competencia, estando en consonancia con la hipótesis de perturbación intermedia, que sugiere la existencia de refugios que escapan de la exclusión competitiva. Aunque ambas especies requieren los mismos recursos para crecer, poseen diferentes requerimientos lumínicos, tasas de renovación y presentan diferentes habilidades de incorporación de nutrientes. Bajo la perspectiva de la hipótesis de partición del nicho, *B. scorpioides* y *C. caespitosa* podrían considerarse competidores que se han segregado espacialmente como resultado de una especialización en la adquisición de recursos, particionando así sus nichos. Tomando en consideración ambas hipótesis, *B. scorpioides* y *C. caespitosa* colonizarían la zona intermedia que es óptima para su crecimiento, y se segregarían espacialmente de forma progresiva, explicando de ese modo la zonación observada.

Los resultados manifiestan una alta variabilidad espacial y temporal en las respuestas fisiológicas, abundancia y distribución, que está en consonancia con el alto nivel de fluctuación y variabilidad del sistema intermareal estuárico. Pese a la gran heterogeneidad asociada a las fluctuaciones del ecosistema estuárico, *B. scorpioides* y *C.*

caespitosa presentaron un claro patrón estacional en cuanto a crecimiento, producción de biomasa y nutrientes internos, que aumentan de inverno a finales de primavera. Además, ambas especies poseen una rápida respuesta fisiológica a los cambios en el estuario, lo que junto a la alta plasticidad fenotípica de *B. scorpioides* y la adaptabilida o resistencia de *C. caespitosa* pueden conferir una alta resiliencia a esta asociación de algas frente a perturbaciones ambientales.

En la última década el estuario del río Palmones ha experimentado un proceso de progresiva eutroficación y salinización, a lo que se suma el marcado efecto del cambio climático en el sur de España, con el ligado aumento de temperatura, la disminución de las precipitaciones estivales y la mayor frecuencia de lluvias torrenciales. A estos cambios ambientales se suma la pérdida del 50% de superficie de las marismas en Andalucía en los últimos 50 años. Bajo esta perspectiva, el conocimiento ecofisiológico adquirido a través de esta tesis puede servir como base conceptual para el desarrollo de modelos de simulación ecológicos, con el fin de predecir las respuestas de esta comunidad de macroalgas intermareales estuáricas a diferentes escenarios futuros, en relación a dichos cambios ambientales.

Abbreviations

α	Photosynthetic efficiency, coefficient of statistical significance
AIC_c	Corrected Akaike's Information Criterion
ANOVA	Analysis of variance
ASW	Artifical seawater
B	Biomass
chl *a*	Chlorophyll a
C:N	Carbon to nitrogen ratio
C:P	Carbon to phosphorus ratio
r	Coefficient of correlation
R	Coefficient of multiple correlation
DIN	Dissolved inorganic nitrogen
DW	Dry weight
E	Irradiance
E_0	Incident irradiance
E_c	Light compensation point
E_k	Light saturating point
E_z	Irradiance at tidal elevation z
ETR_m	Maximum electron transport rate
F_v/F_m	Maximum quantum yield
FW	Fresh weight
K_m	Half saturation constant for nutrient
K_s	Half saturation constant for light
LAT	Lowest astronomical tide
LMWC	Low molecular weight carbohydrates
MLR	Multiple linear regression
N:P	Nitrogen to phosphorous ratio
NPR	Net photosyntetic rate
NSW	Natural seawater
P-E	Photosynthesis-irradiance
PBP	Phycobiliproteins
PCA	Principal component analysis
P_m	Maximum photosynthetic rate
P_g	Gross photosynthetic rate
P_{sat}	Saturating photosynthesis
P_{subsat}	Subsaturating photosynthesis
PSII	Photosystem II
RGR	Relative growth rate
R-PC	R-phycocyanin
R-PE	R-phycoerytrin
RWC	Relative water content
SA:B	Surface area to biomass ratio
SA:V	Surface area to volume ratio
TSP	Total soluble phosphorus
V_{max}	Maximum uptake rate
X_0	Substrate compensation point

Contents

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Chapter 1

Introduction

Introduction 1

1.1 Spatial and temporal variability in intertidal ecosystems

Within aquatic ecosystems, tidal environments have offered a good and attractive model to test many central hypotheses in ecology in relation to spatial and temporal distribution of bethic organisms and their diversity (Connell, 1961; Menge and Sutherland, 1976; Connell, 1972; Paine, 1974; Niell, 1976b). Tidal environments are highly fluctuating ecosystems subject to daily emersion and submersion conditions, where organisms are exposed to variations in the environmental conditions and the availability of essential resources for their growth and survival (Niell, 1979). This set of ecological conditions defines an unique habitat where the physiology of organisms is well adapted coping with the regular regime of fluctuations (Davison and Pearson, 1996; Paine et al., 1998). Numerous and valuable ecological studies have been carried out in these ecosystems, from tropical (Menge and Lubchenco, 1981; Alongi, 1987; Dawes et al., 1991) to polar latitudes (Dayton et al., 1974; Lüning and Asmus, 1991), thriving to understand the mechanisms that explain the distribution of benthic organisms from the subtidal to the uppermost intertidal zone (Stephenson and Stephenson, 1949; Dayton and Hessler, 1972; Paine, 1974; Dring and Brown, 1982). After nearly one century of research, intertidal ecology is a continually developing field of interest for marine scientists (Underwood, 2000; Benson, 2002; Tomanek and Helmuth, 2002).

Macroalgae are important primary producers in coastal and estuarine ecosystems (Zedler, 1980; Valiela et al., 1997; Duarte and Cebrián, 1996). Macroalgal communities funnel energy into marine food webs (Duggins and Dethier, 1985; Menge, 1995), export biomass (Niell, 1976b; Duarte and Cebrián, 1996; Nielsen et al., 2004), offer refugee to other organisms (Raffaelli and Hawkins, 1996; Bracken et al., 2007) and act as a sink and source of nutrients (McGlathery et al., 1997). In addition, they are an important economic resource for humans, since they have been used in aquaculture as food supply, or as sources of high value chemicals for industry among many other applications (Vadas, 1979; Chopin et al., 2001; McHugh, 2003).

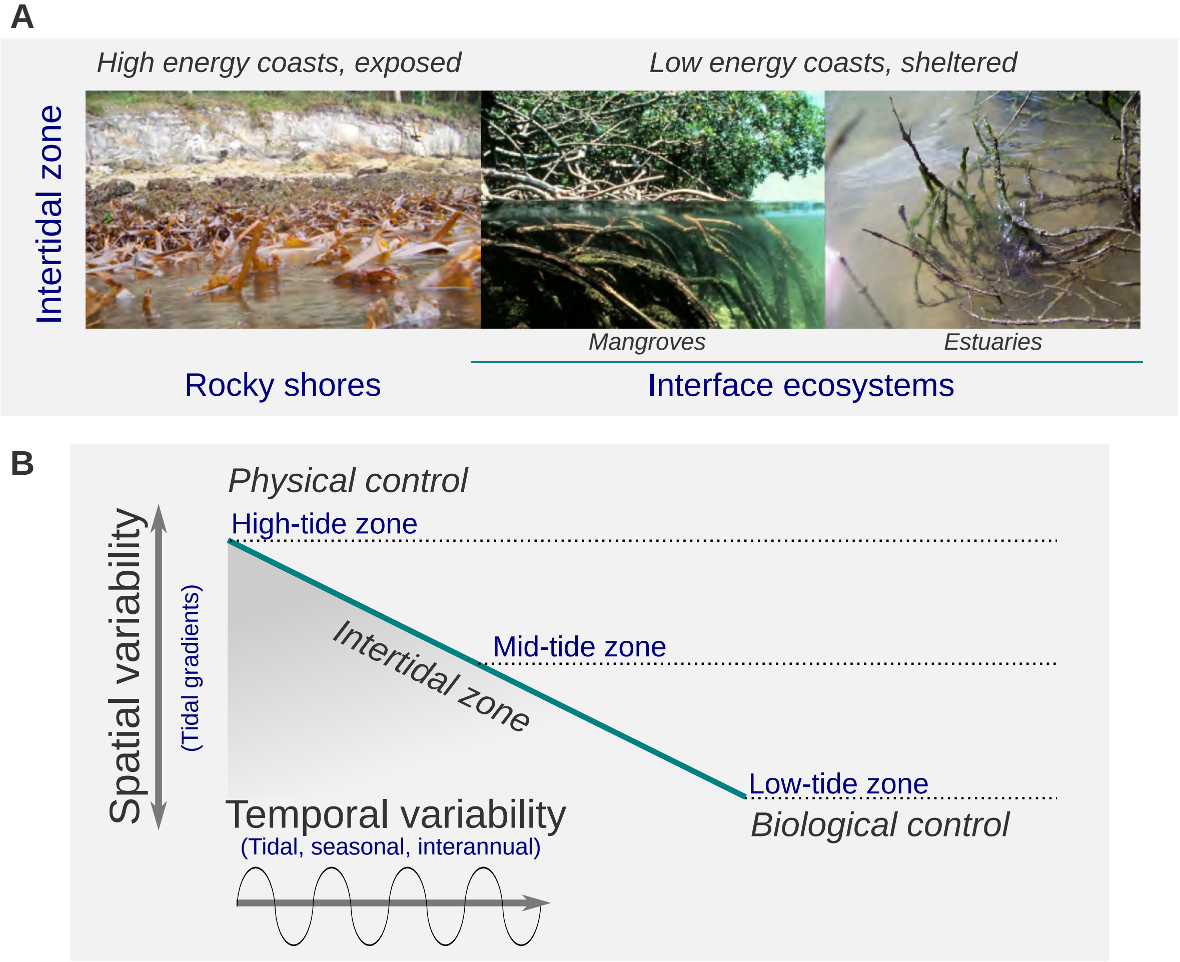

Figure 1.1. A) Examples of intertidal ecosystems (from left to right): intertidal zone in the Galician coast (northern Spain); above and below water view at the edge of a mangal (Source: Public domain, US NOAA); estuarine intertidal zone in the Palmones River Estuary (southern Spain). B) Spatial and temporal scales of variability in intertidal ecosystems.

Many of the classical studies on intertidal environments have dealt with the distribution patterns of macroalgae in vertically discrete horizontal zones, which is defined as "macroalgal zonation" (Baker, 1909; Stephenson and Stephenson, 1949; Mann and Steinke, 1988; Davison and Pearson, 1996), which is mainly apparent on marine rocky shores but also in interface ecosystems (Brinkhuis, 1977; Phillips et al., 1996; Melville and Pulkownik, 2007) (Fig. 1.1). It is widely accepted that the interplay between abiotic and biotic factors control the spatial and temporal distribution of intertidal communities (Sanders, 1968; Connell, 1972; Niell, 1977). As a general rule, physical factors (i.e. emersion, temperature, salinity, nutrients) ultimately restrict the upper limits of intertidal organisms (Colman, 1933; Lewis, 1964; Schonbeck and Norton, 1978; Dring and Brown, 1982; Davison and Pearson, 1996; Phillips et al., 1996; Melville and Pulkownik, 2007), whereas biological interactions (competition, facilitation and herbivory pressure) and light play a major role towards the lower intertidal zone (Lewis, 1964; Connell, 1972; Schonbeck and Norton, 1980; Hurd and Dring, 1990; Chapman and Johnson, 1990; Lüning, 1990) (Fig. 1.1). All these variables act and fluctuate at daily, seasonal and interannual scales, altering patterns of macroalgal primary production (Niell, 1977; Lapointe, 1987; Valiela et al., 1997; Schramm, 1999).

Physiological acclimation and adaptation to environmental gradients allow macroalgae to modify their resource acquisition, leading to inter- and intraspecific phenotypic and genotypic differentiation (Wattier and Maggs, 2001; Bischof et al., 2006; Hays, 2007; Zardi et al., 2011; Krueger-Hadfield et al., 2013). On the other hand, genotypic differentiation has been recently identified among conspecifics located at different tidal elevations or from different intertidal microhabitats, where tidal action and emersion-related variables act as the main selective pressures (Hays, 2007; Zardi et al., 2011; Krueger-Hadfield et al., 2013; Clark et al., 2013; Mota et al., 2015; Muangmai et al., 2016).

1.1.1 Abiotic variables

Spatial gradients in resource availability and regulating factors (temperature, salinity, pH, water motion) impact on the metabolic processes of seaweeds, determining their composition, distribution and abundance (Baker and Bohling, 1916; Zaneveld, 1937; Lewis, 1964; Lüning, 1990; Hurd et al., 2014). Intertidal macroalgae are diurnally confronted with physical limitations and fluctuations in the availability of key resources through tidal cycles (Thomas et al., 1987; Hurd and Dring, 1991; Skene, 2004; Kim et al., 2008). This challenging environment creates a "trade-off" where the energetic costs of emersion tolerance should be balanced by the potential benefits in a way that survival is ensured (Johnson et al., 1998; Hunt and Denny, 2008). Nevertheless, intertidal macroalgae possess different mechanisms that compensate the lack of nutrient supply and thalli dehydration during prolonged emersion periods (Hurd and Dring, 1990; Surif and Raven, 1990; Kim et al., 2008).

Light is an underlying environmental variable responsible for primary production of coastal ecosystems (Kirk, 2011) and partially affects zonation of macroalgae (Johnson and Skutch, 1928; Lüning, 1981; Markager and Sand-Jensen, 1992). Physiological changes in pigment content and photosynthetic activity enable their growth under different light environments (Bjorkman and Holmgren, 1963; Coutinho and Yoneshigue, 1988; Beach and Smith, 1996a,b; Fairhead and Cheshire, 2004). Such photoacclimation processes gain additional importance when light becomes limiting for macroalgal growth as a result of changes in water depth and turbidity (Lüning, 1981; Vergara et al., 1997; Aumack et al., 2007; Roberts et al., 2014), canopy density (Beanland and Woelkerling, 1983; Altamirano et al., 2004; Vis et al., 2006) or self-shading (Gerard, 1984;

Beach and Smith, 1996a; Vergara et al., 1998; Copertino et al., 2009). Photosynthetic and growth kinetic parameters have been used to assess the capacity of macroalgae to acclimate to changing environmental light (Gantt, 1990; Falkowski and LaRoche, 1991; Delebecq et al., 2013), and their values usually follow the distribution of macroalgae at different tidal levels, which occur at scales from microscale (Williams and Carpenter, 1990; Beach and Smith, 1996a) to hundreds of metres (Lüning, 1981; Chapman and Johnson, 1990). Algae exposed to higher light regimes usually exhibit a higher maximum photosynthesis (P_m) and light saturation point (E_k), than those living in shaded habitats with lower photosynthetic efficiency (α) and E_c values (Falkowski and Raven, 2007). On the other hand, morphological adaptations such as a thin thallus, high surface area to volume (SA:V) quotient or low thallus specific carbon (TSC) are advantageous for growing in dim light (Markager and Sand-Jensen, 1992, 1996), in addition to their effects on other physiological processes such as nutrient uptake (Rosenberg and Ramus, 1984; Taylor et al., 1998).

Previous studies have associated the differences in nutrient uptake of co-occurring species to their differential tidal zonation (Thomas et al., 1987; Phillips et al., 1996; Kim et al., 2008). Since nutrients are intermittently available for upper intertidal species, their uptake abilities can be different from lower intertidal ones (Hurd and Dring, 1990). A relationship between higher N uptake rates and the upper position in the intertidal zone has been reported for several intertidal seaweeds (Phillips and Hurd, 2003, 2004; Kim et al., 2008; Bracken et al., 2011). These differential patterns in uptake can also occur between high and low shore specimens of the same species, as a result of phenotypic variations to environmental conditions (Phillips and Hurd, 2004; Bracken et al., 2011; Benes and Bracken, 2016). Similarly, adjustments in their internal nutrient content and turnover rates can occur in response to different nutrient availability, due to variable nutrient concentrations or differential inputs, exclusively under submersion (Thomas et al., 1987; Phillips and Hurd, 2003; Kim et al., 2008; Young et al., 2009), which can induce spatial and seasonal differences in the biochemical composition (Niell, 1976a; Brinkhuis, 1977; Lapointe et al., 1981; Levitt and Bolton, 1991; Hernández et al., 1993; Pérez-Lloréns et al., 2004). Seaweeds from the upper shore sometimes display high resilience to nutrient limitation via a high nutrient storage capacity (Thomas et al., 1987; Beach and Smith, 1996a), which helps them sustaining growth during long periods of low availability.

The interplay between light and nutrient availability along the tidal gradient and across seasons have a major role in their spatial and temporal patterns of macroalgal productivity. Photophysiological acclimation and nutrient acquisition are interrelated processes, as light modifies the nutrient requirements for growth (Lapointe, 1987; Henley et al., 1991). For instance, at a physiological level, N assimilation promotes the C flow via respiratory pathways in autotrophs (Turpin et al., 1988), nitrate reductase requires light active photosynthesis, whilst pigments such as phycobiliproteins can serve as nitrogen storage compounds in the case of red seaweeds (Fujita, 1985; Vergara et al., 1993; Beach and Smith, 1996a; Sampath-Wiley et al., 2008). Moreover, photoperiod and annual nutrient cycles control the endogenous circannual rhythms of growth in some species (Lüning, 1993; Raven and Geider, 2003). Macroalgae that have evolved towards this mechanism (e.g. kelps) have successfully developed in ecosystems with markedly annual nutrient cycles, as for example polar regions (Rosenberg and Ramus, 1982; Schaffelke and Lüning, 1994; Gordillo et al., 2006).

Emersion tolerance of seaweeds has been regarded as one of the main structuring factors in intertidal communities (Doty, 1946; Johnson et al., 1974; Dring and Brown, 1982; Davison and Pearson, 1996). As a general trend, species inhabiting the upper intertidal parts sometimes also present higher resistance mechanisms to desiccation, temperature, hyperosmotic conditions and excess irradiance than those occupying lower tidal levels (Strömgren, 1977; Karsten and Kirst, 1989a; Davison and Pearson, 1996; Li and Brawley, 2004; Bischof et al., 2006). However, abiotic conditions may restrict the settlement, growth and reproduction of seaweeds towards the upper intertidal zone (Lewis, 1964; Matta and Chapman, 1995; Davison and Pearson, 1996; Wright et al., 2004; Hunt and Denny, 2008). In upper intertidal macroalgae photosynthesis increases after slight water loss (Stocker and Holdheide, 1938; Brinkhuis et al., 1976; Gao et al., 1999), but it declines at further dehydration due to the interruption of the electron transport between photosystems (Bewley, 1979; Ji and Tanaka, 2002; Gao et al., 2011). Severe dehydration can also alter the biochemical composition (Hurd and Dring, 1991; Sampath-Wiley et al., 2008; Kim et al., 2013), disrupts cell integrity (Holzinger and Karsten, 2013) and modify the activity of many proteins involved in key physiological functions (Murthy et al., 1986; Flores-Molina et al., 2014; Parages et al., 2014; López-Cristoffanini et al., 2015).

Intertidal macroalgae present diverse adaptative features and physiological mechanisms of emersion tolerance that allow them to cope with daily and seasonal fluctuations (Brown, 1987). Some species possess functional forms and growth patterns that minimise water losses, allowing them to withstand emersion conditions (Schonbeck and Norton, 1979; Littler and Littler, 1980; Collado-Vides et al., 1997; Beach and Smith, 1997; Hunt and Denny, 2008). Additionally, many intertidal seaweeds recover or enhance physiological activity following submersion (Thomas and Turpin, 1980; Hurd and Dring, 1991; Skene, 2004; Ji et al., 2005; Kim et al., 2008), although it depends on the extent of the previous emersion period (Wiltens et al., 1978; Dring and Brown, 1982). In addition, recent studies have evidenced active physiological and biochemical responses that help maintaining cell integrity and reduce emersion-induced oxidative stress (Aguilera et al., 2002; Karsten, 2012; Holzinger and Karsten, 2013; Parages et al., 2014).

Interactive effects among emersion-related variables can lead to varying responses in intertidal seaweeds along the tidal environment (Schonbeck and Norton, 1979; Fernández et al., 2015), affecting each physiological process differently and thus can generate differential seasonal patterns in productivity (Lapointe et al., 1981; Brown, 1987; Levitt and Bolton, 1991). Combinations of environmental variables can have additive, antagonistic or synergistic effects on ecosystems than when they act independently (Matta and Chapman, 1995; Crain et al., 2008; Fernández et al., 2015). Likewise, daily and seasonal changes in tidal regime (i.e. timing of the tides) (Williams and Dethier, 2005; Hurd et al., 2014) and meteorological conditions (Dethier and Williams, 2009; Lamote et al., 2012) can improve or enhance the effect of abiotic variables, and thereby modify their physiological response in their natural habitats (Raffaelli and Hawkins, 1996; Cox and Smith, 2015).

1.1.2 Biotic variables

Biotic interactions among intertidal benthic organisms play also a prominent role as structuring factors of zonation at intermediate and lower intertidal zones (Connell, 1961; Lewis, 1964; Paine, 1974; Lubchenco, 1978; Niell and Varela, 1984). Predation and competition are the most studied types of interaction, probably derived from their more direct and visible effects (Dayton, 1971; Menge and Sutherland, 1976). Intra- and interspecific competition have been described for several marine benthic organisms (Connell, 1961; Paine, 1974; Jackson, 1977; Buss and Jackson, 1979; McCook et al., 2001), including intertidal macroalgae (Lubchenco, 1980; Chapman and Johnson, 1990; Karez, 2003b). Among seaweeds indirect competition for resource availability (light, substrate, nutrients) is the most dynamic and complex process of interaction (Miller, 1967; Dudgeon et al., 1999; Underwood, 2000). Davison and Pearson (1996) hypothesised that the intertidal distribution limits are controlled by competitive exclusion (Hardin, 1960), although the stress caused by nutrient limitation or desiccation mediates these interspecific interactions. Nevertheless, competitive interactions can also lead to character convergence rather than exclusion, specifically in the case of plants that compete for substitutable resources (e.g. different nitrogen sources) (Grant, 1972; Goldberg and Barton, 1992; Tilman, 1994; Fox and Vasseur, 2008).

A fundamental question in competition theory is how distinct co-habitant species should be to make possible their coexistence in the same apparent space, which has been addressed in rocky shore communities (Connell, 1972; Lubchenco, 1980; Worm and Karez, 2002). Patterns of coexistence can be reached through different mechanisms and several equilibrium states can occur (Lewontin, 1969; Sutherland, 1974; Brauer et al., 2012). Niche-partitioning can allow the coexistence of similar species that compete for a potentially limiting resource, based on species-specific differences in resource acquisition (Schoener, 1974; Dudgeon et al., 1999; Muangmai et al., 2016). Evidence of this was found in the coexistence of two similar intertidal macroalgae (*Chondrus crispus* Stackhouse and *Mastocarpus stellatus* (Stackhouse) Guiry), although the decreased production of one of the species was also mediated by herbivory and exposure (Dudgeon et al., 1999). On the other hand, facilitative and mutualistic interactions among organisms may also contribute to coexistence patterns, and their study has gained relevance in the last two decades (Bertness and Leonard, 1997; Stachowicz, 2001; Bracken et al., 2007; Kéfi et al., 2016).

There is an increasing interest in how intraspecific variability can modify the outcome of ecological interactions, and a theoretical framework has been developed (Bolnick et al., 2011). Edwards and Connell (2012) suggested that the ability of benthic organisms optimising the resource acquisition along the tidal gradient may modify the strength and outcome of their interspecific interactions, gaining relevance at intermediate and low tidal levels (Lewis, 1964; Olson and Lubchenco, 1990). Recent insights in general competition theory have included light as a resource in mechanistic models of interspecific competition (Ryabov and Blasius, 2011; Brauer et al., 2012), based on the early approaches of Tilman (1982) for nutrient competition between two species. These models may offer new scenarios for the study of resource competition in intertidal environments, where light availability increases with tidal elevation, and nutrient load not only depends on nutrient levels and ratios, but also on the time available for their uptake (Johnson et al., 1974; Kim et al., 2008).

1.2 The intertidal rhodophytes from the Palmones River Estuary

The Palmones River Estuary (Algeciras Bay, southern Spain) is a shallow, temperate and partially mixed estuary located at the end of a small catchment Mediterranean basin. Due to its proximity to the Atlantic Ocean its tidal regime is Atlantic-influenced, and the water fluxes between the estuary and the bay are driven by the local meteorological conditions (Avilés and Niell, 2005; Sánchez-Garrido et al., 2014). The high seasonal and inter-annual variability in the environmental variables modify the estuarine dynamics and thus the conditions for optimal performance of their inhabiting communities (Moreno and Niell, 2004). Within the intertidal zone these changes are more pronounced, due to daily emersion and submersion tidal cycles and the fluxes of matter and energy that take place among the saltmarsh, sediment and estuarine water (Fig. 1.2).

For almost 30 years this ecosystem has been extensively studied, with major interest in nutrient dynamic and its relation with eutrophication (Avilés and Niell, 2005), tides (Clavero et al., 1997, 1999) and flood events (Avilés and Niell, 2005), sediment characterisation (Rubio et al., 2003), scales of variability (Moreno and Niell, 2004), organic matter recycling (Arrojo et al., 2009), ecophysiology of saltmarsh vegetation (Palomo et al., 2004; Palomo and Niell, 2009), the role of microbial mats (Carrasco et al., 2008) and the impact of anthropogenic activities (Jiménez et al., 1987; Clavero et al., 1997).

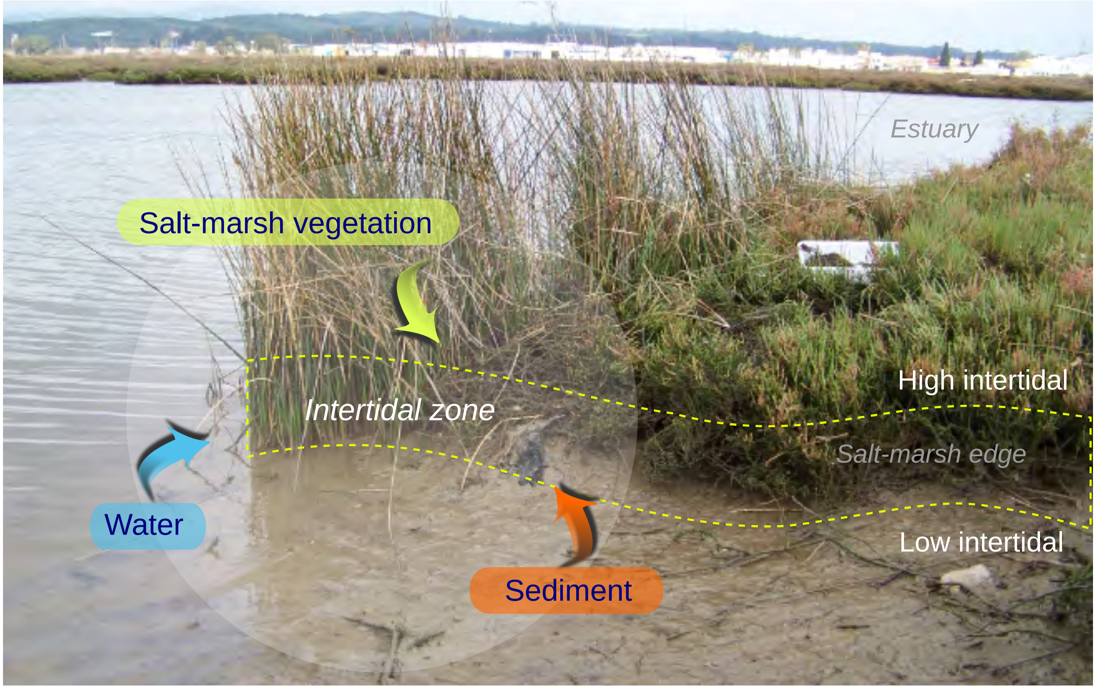

FIGURE 1.2. Intertidal zone of the Palmones River Estuary and the different compartments of the ecosystem.

The intertidal rhodophytes *Bostrychia scorpioides* (Hudson) Montagne ex Kützing (Rhodophyta, Ceramiales) and *Catenella caespitosa* (Withering) L. M. Irvine (Rhodophyta, Gigartinales) represent a sub-system of the estuary that has been recently studied in detail from an ecophysiological perspective (Mercado and Niell, 1999, 2000; Ruiz-Nieto, 2014). In the Palmones Estuary these epiphytic species grow entangled and attached to the lower parts of the lignified stems of halophylic macrophytes *Sarcocornia perennis* (Mill.) A. J. Scott and *Atriplex (Halimione) portulacoides* (l.) Aellen (Palomo et al., 2004). Preliminary field observations in the Palmones Estuary revealed that *B. scorpioides* grows towards the upper intertidal zone and *C. caespitosa* occupies the lower zone, whereas both species coexist and overlap at intermediate tidal levels (Fig. 1.3).

This algal association has been reported in many other temperate saltmarshes as Bostrychetum (Chapman, 1938; Post, 1967; Peña-Salamanca, 2008), and similar distributional patterns for *Bostrychia* and *Catenella* species have been reported in other locations (Mann and Steinke, 1988; Coppejans and Gallin, 1989; Davey and Woelkerling, 1985; Aikanathan and Sasekumar, 1994; Pedroche et al., 1995; Batelli, 2004; Melville et al., 2005). From an ecophysiological perspective, the most common traits of these rhodophytes are their low light and osmotic tolerance (Chapman, 1938; Yarish and Edwards, 1982a; Davey and Woelkerling, 1985; Mann and Steinke, 1988; Karsten and Kirst, 1989a; Peña-Salamanca et al., 1999). These species are frequently exposed to low environmental light levels, mainly due to the shading effect of the canopy vegetation under which they grow, increased water depth during submersion at high tide (Karsten et al., 1994; King and Puttock, 1994; Peña-Salamanca et al., 1999) and water turbidity (Dawes et al., 1978; Mathieson and Dawes, 1986). In fact, differences in acclimation abilities to such dim light conditions seem to play a fundamental role controlling the lower distributional limits of macroalgae in these ecosystems (Melville and Pulkownik, 2007). On the other hand, as for most of intertidal seaweeds, their upper distributional limits have been related to their differential tolerance to emersion factors (Dawes et al., 1978; Davey and Woelkerling, 1985; Mercado and Niell, 2000) and their capacity for

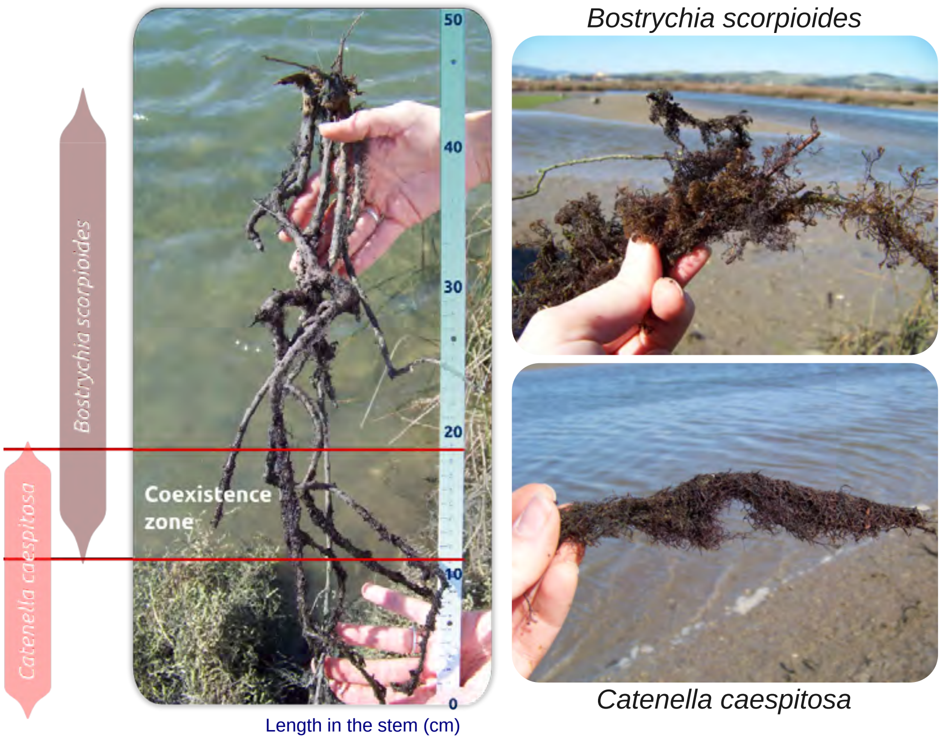

FIGURE 1.3. Intertidal zonation limits of *B. scorpioides* and *C. caespitosa* on a stem of the halophyte *S. perennis* from the Palmones Estuary (left). Stems covered by *B. scorpioides* (upper right) and *C. caespitosa* (lower right).

recovering nutrient uptake or photosynthesis upon resubmersion (Datta and Datta, 1999; Peña-Salamanca et al., 1999).

The intertidal zonation of *B. scorpioides* and *C. caespitosa* represents the main interest of this study, in relation to the structure and functioning of the estuarine system and the effect of tidal gradients on their physiology. As many desiccation tolerant seaweeds, the species in this study were unique, if not rare, and thus their ecophysiology is of scientific interest. In fact, Karsten and Kirst (1989a) denominated *B. scorpioides* as a "terrestrial" red alga, given its ability to tolerate incomplete turgor pressure regulation, which seems to be decisive for its uppermost intertidal distribution.

Previous investigations on the physiology of *B. scorpioides* and *C. caespitosa* in Palmones Estuary have addressed specific questions in relation to their C acquisition mechanisms and use (Mercado and Niell, 1999, 2000; Ruiz-Nieto, 2014; Ruiz-Nieto et al., 2014). At their intertidal position and frequent emersion periods both species have extensive access to atmospheric CO_2, which is the main source of inorganic carbon in both species (Ruiz-Nieto et al., 2014). In addition, Mercado and Niell (2000) reported higher photosynthetic rates of *B. scorpioides* under emersed than submersed conditions, which has been also suggested for *C. caespitosa* by Ruiz-Nieto (2014). Furthermore, the regulatory effect of salinity and ionic changes on the C acquisition of *B. scorpioides* and *C. caespitosa* have been investigated by Ruiz-Nieto (2014), who found that physiological responses within the natural range of salinity were not distinct. This fact suggest that the lack of physiological responses to certain variables, especially those related with emersion, can be an advantage coping with the fluctuating estuarine environment.

1.3 Background and objectives

In relation to the observed zonation, the scales of spatial and temporal environmental variability and previous ecophysiological knowledge on intertidal and estuarine macroalgae, several aspects are addressed in this thesis.

Acclimation to the light gradient under the canopy of saltmarsh vegetation in the Palmones Estuary has not been quantified in terms of physiological responses for *B. scorpioides* and *C. caespitosa*, when light should be of high relevance to their zonation, as noted for similar species (Beanland and Woelkerling, 1983; Phillips et al., 1996; Melville et al., 2005; Melville and Pulkownik, 2007). Nutrient acquisition abilities can vary with external nutrient concentration, internal nutritional status and available time for uptake along the small-scale intertidal, and also influence their spatial distribution (Datta and Datta, 1999; Phillips and Hurd, 2003). If *B. scorpioides* and *C. caespitosa* have almost similar photosynthetic, nutrient uptake and growth kinetic parameters (interspecific) and each of them shows parallel responses to irradiance and nutrient levels (intraspecific), they would be equally capable of surviving at the same tidal location in the intertidal zone. If they are not found to be so, it could be hypothesized that biological processes or physical factors other than their photophysiology and nutrient uptake capability are preventing them from coexisting.

Although species of these genera are emersion-tolerant, studies on their physiological recovery after emersion periods are rather limited (Peña-Salamanca et al., 1999). In this regard, it can be hypothesised that if both species are equally able to recover from emersion on a daily basis, zonation would depend upon the energetic trade-off between growth and protection when they are exposed to longer periods of emersion. As well, as interactions between tidal submergence and meteorological conditions (e.g. irradiance, temperature, relative humidity) can alter the severity of emersion conditions, responses to combinations of these environmental variables may shed light on their specific ranges of emersion tolerance.

Growth and distributional patterns of *B. scorpioides* and *C. caespitosa* may also vary in relation to microclimatic conditions and temporal fluctuations, as occur in most intertidal ecosystems (Raffaelli and Hawkins, 1996). According to the apparent distribution pattern between the two species, the tidal gradients along their low-amplitude intertidal zonation should be steep enough to allow the occurrence different microhabitats. On the other hand, varying microhabitats may also result from increasing densities in the canopy vegetation of *S. perennis*, which can ameliorate the exposure of the two rhodophytes to emersion stress (reduced light, temperature and dehydration). In this sense, and considering the intermediate disturbance hypothesis (Tilman, 1982), maximum productivity of these species might occur at (1) intermediate tidal levels and (2) in sheltered or shaded zones where disturbances are buffered, as some studies on similar epiphytic rhodophytes have evidenced (Beanland and Woelkerling, 1983). In addition, if both species respond similarly to these microhabitats, their relative abundances and physiological responses would be comparable at a given tidal or microclimatic condition, whereas if the contrary is observed, it would suggest that differential ecophysiological traits possibly contribute to their spatial and temporal distribution.

Seasonal variations account for the greatest changes in salinity, temperature and nutrient concentrations in Palmones Estuary (Clavero et al., 1997, 1999; Izquierdo, 2001), which are variables that can exert a major control on growth, photosynthesis and biochemical composition of estuarine rhodophytes (Brinkhuis, 1977; Dawes et al., 1978; Yarish and Edwards, 1982a; Mann and Steinke, 1988; Karsten and West, 1993). Macroalgal biomass and distribution in interface ecosystems is usually highly variable

(Beanland and Woelkerling, 1983; Davey and Woelkerling, 1985; Yokoya et al., 1999), which can be attributed to the regular environmental fluctuations.

Very few studies have examined physiological traits and ecological aspects of cohabiting species at both spatial and temporal scales in interface ecosystems, probably due to the low number of species respect to marine rocky shores (Wilkinson, 1980). It should be also considered that macroalgal zonation in saltmarshes is restricted to high shore levels, where biotic interactions loose relevance (Raffaelli and Hawkins, 1996) or are mediated by abiotic factors (Davison and Pearson, 1996). In the context of competition theory (Hardin, 1960; Tilman, 1982; Brauer et al., 2012), *B. scorpioides* and *C. caespitosa* would be more likely to compete for resources if their growth was controlled by the same environmental factors in the same extent. However, if each species is physiologically constrained by a different resource (i.e. light, nutrients) or regulating factor (i.e. desiccation, temperature, salinity), then they might exclude each other where (spatial) and when (temporal) each species uses more efficiently the most limiting resource, and their coexistence would be possible as long as none of them dominates the overall resource supply.

This thesis aims at determining **how different environmental variables affect primary production and spatial distribution of *B. scorpioides* and *C. caespitosa* along the spatial gradient (zonation) and temporal scales (tidal, seasonal, inter-annual) in the Palmones Estuary**.

The **specific objectives** of the present work are the following:

1. To evaluate the photoacclimation capacity and the energetic requirements for growth and photosynthesis (Chapter 3).
2. To determine the nutrient uptake capacity and internal nutrient requirements under simulated natural conditions (Chapter 4).
3. To study the physiological response (tolerance and recovery) to single and multiple emersion variables (temperature, salinity and desiccation) (Chapter 5).
4. To analyse the intra- and inter-specific variability in the ecophysiology of the two species in relation to the tidal gradient and their possible implications in their biotic interactions (Chapter 6).
5. To examine the seasonal dynamics of biomass and photophysiology of the two species, as function of environmental factors and microclimatic conditions (canopy cover and tidal level) (Chapters 7 and 8).

1.4 Thesis outline

This study aimed to contribute to gain a deeper knowledge on diverse ecophysiological aspects of intertidal estuarine rhodophytes, by following an "integrative" and "multi-scale" ecological approach. The "integrative" approach strives to unite ecophysiological results from field and laboratory experiments. *In situ* experiments provide direct field information but usually high variable data, due to the action of multiple environmental factors in nature even at small-scale (Coleman, 2002; Moreno and Niell, 2004). Experiments under controlled laboratory conditions allow using more complex and diverse techniques to assess the overall physiology, but can take assumptions that are far from the field conditions (Hurd et al., 2014). Nevertheless, if the latter are performed at actual ecological conditions, these results can be interpreted in their natural context within the limitations of experimental design (Davison and Pearson, 1996; Hurd et al., 2014). The "multi-scale" approach seeks to combine single biochemical and physiological responses of *B. scorpioides* and *C. caespitosa* to environmental variables with the multivariate ecological responses of the whole macroalgal assemblage.

The ecophysiological responses of *B. scorpioides* and *C. caespitosa* to environmental variables was explored at daily and seasonal scales, and at different microhabitats (e.g. shaded/sun-exposed sites or upper/lower tidal levels), thus aiming to obtain complementary insights on their physiology. In this sense, responses to spatial and temporal heterogeneity at different scales were transversal to this study, due to both the environmental characteristics intrinsic of intertidal environments and estuarine habitats.

Along the chapters of this dissertation, *in situ* and *ex situ* approximations are intermingled, going back and forth from the field to the laboratory to contrast the initial hypotheses and get a deeper understanding on their physiological mechanisms. Each chapter addresses and discusses specific research questions with, in some cases, common objectives from different approaches. First, the species and the system of study are described in detail in Chapter 2. Following next, Chapters 3, 4 and 5 subsequently deal with the single and combined responses to primary ecological factors (light, nutrients, temperature, salinity and emersion). A Chapter on the intraspecific variations under field conditions and its possible implications on their interspecific interactions follows (Chap. 6). The seasonal changes in biomass, photosynthesis and biochemical composition are assessed in long-term *in situ* studies in Chapters 7 and 8, respectively. Finally, the main findings are interrelated and contrasted in the general discussion (Chap. 9), with an especial emphasis on the ecological implications for their spatio-temporal distribution.

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Chapter

2

Description of the system of study

Description of the system of study

2

2.1 Study species

The two intertidal rhodophytes used in the this study were *Bostrychia scorpioides* (Hudson) Montagne and *Catenella caespitosa* (Withering) L. M. Irvine (Fig. 2.1), which are found in mangroves and estuaries from warm and temperate latitudes, although they are also present on marine rocky shores. They can colonise a broad variety of substrates such as the roots of the mangrove-community in tropical estuaries (Post, 1963; Beanland and Woelkerling, 1983; Davey and Woelkerling, 1985), the lower stems of saltmarsh vascular plants (Dawes et al., 1978), the soft-bottom muddy surface, or sheltered rocks and artificial substrates (Munda, 1974; Van Reine, 1983; Batelli, 2004). In addition, these genera posses a great capacity to grow under a wide range of environmental conditions (Almodóvar and Biebl, 1962; Davey and Woelkerling, 1985; Phillips et al., 1996; Peña-Salamanca et al., 1999; Melville and Pulkownik, 2007; Karsten, 2012).

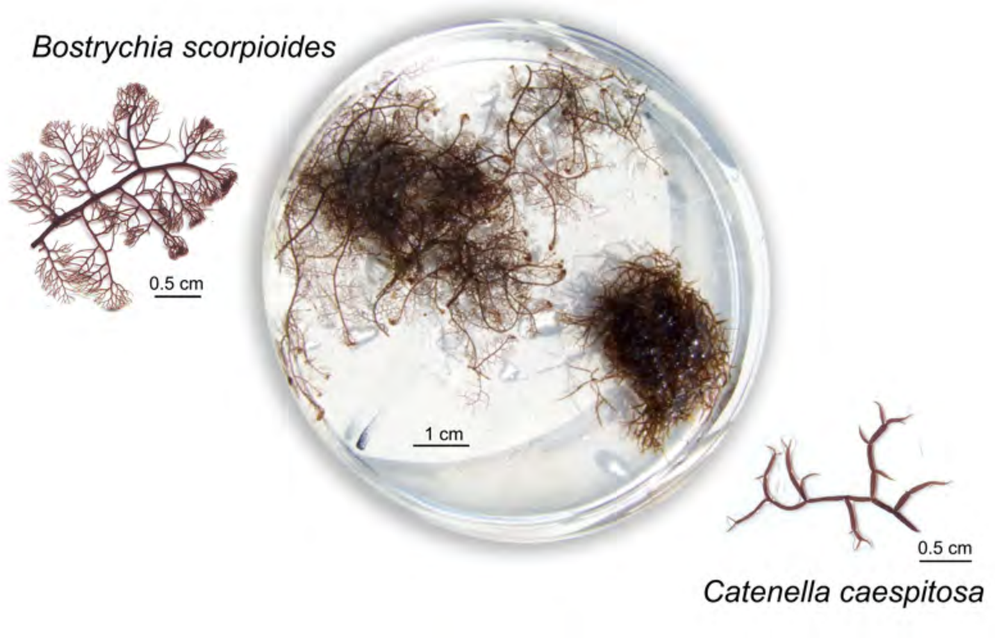


FIGURE 2.1. Specimens of *B. scorpioides* and *C. caespitosa* on a petri dish and detailed features of their thalli.

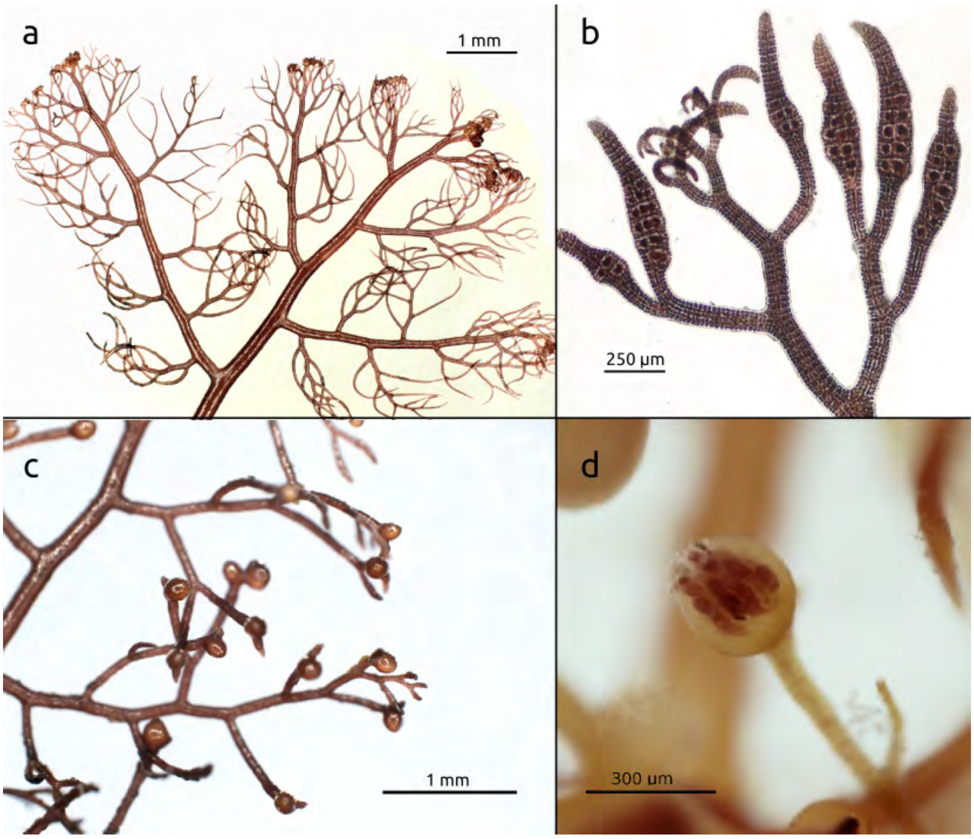


FIGURE 2.2. Thalli of *B. scorpioides* from the Palmones Estuary. a) Vegetative thalli. b) Mature tetrasporophyte. c) Female gametophyte with mature cystocarps. d) Detail of cystocarp with carpospores.

Bostrychia scorpioides and *C. caespitosa* occur in western Europe and usually form the association Bostrychietum (Post, 1967; Nienhuis, 1970). In the Iberian Peninsula, this association has been reported in the intertidal zone of the north coasts (Niell, 1978; Sánchez et al., 1996; Gorostiaga et al., 2004; Martínez Gil et al., 2007), the Tagus river estuary (Henriques, 2009), and saltmarshes in southwest of Spain (Mercado and Niell, 2000; Hernández et al., 2010; Ruiz-Nieto, 2014).

2.1.1 *Bostrychia scorpioides* (Hudson) Montagne ex Kützing

Bostrychia scorpioides is a rhodophyte of the order Ceramiales and family Rhodomelaceae. This species is characterised by filiform, flexuous and heavily branched fronds of 2-10 cm long, from dark purple to reddish brown, with circinated apices, forming entangled tufts. Main filaments are cylindrical, slightly flattened dorsoventrally, with pseudodichotomic primary laterals alternately arranged and disposed on the same plane. Branches of first degree are fully covered with short, slender, alternate laterals (branchlets) (Fig. 2.2 a). Apices bear several series of smaller branchlets, generally subulate and thornlike (Fig. 2.2 a). Specimens influenced by freshwater sources are usually less ramified and more slender, presenting morphological differences in comparison with marine specimens (Van Reine and Sluiman, 1980). Its internal structure consists of central siphonous cells 4-5 times as long as broad, clothed by 5-7 pericentral cells and cortex. *Bostrychia scorpioides* is a perennial species with both sexual and asexual propagation. It is a dioecious species with a life-history of the *Polysiphonia* type (isomorphous, triphasic) (Van Reine and Sluiman, 1980). In some other species of its genus from locations exposed to lower temperatures only the basal fraction of the thallus is perennial, and the whole thalli are regenerated (Yarish and Edwards, 1982b). Mature tetrasporophytes posses tetrahedral tetrasporangia, arranged in a double row that forms terminal stichidial branchlets with tetrahedral tetraspores (Fig. 2.2 b). Gametophytes present subglobose, urnshaped, subapical cystocarps (Fig. 2.2 c,d). In the Palmones estuary the different reproductive stages have been identified throughout this study, except for male gametophytes, which are often difficult to differentiate from young tetrasporophytes (Newton, 1931; Van Reine and Sluiman, 1980).

This subcosmopolitan species is commonly found near high-water mark in sheltered coasts of temperate regions, in the northern hemisphere between the 3 °C isotherm of January and by the 25 °C isotherm of July (air temperatures) (Sluiman, 1978). *Bostrychia scorpioides* is mainly distributed in the west European coast, South Africa, South America, South of Australia and New Zealand (Newton, 1931).

2.1.2 *Catenella caespitosa* (Withering) L.M. Irvine

Catenella caespitosa is a rhodophyte in the order Gigartinales and family Caulacanthaceae. Some synonims are *Catenella repens* (Lightfoot) Batters and *Catenella opuntia* (Goodenough & Woodward) Greville. Fronds are filiform, more or less flattened or subcylindric, from blackish-purple to brown, of 2 cm long. Filaments are irregularly branched, conforming a prostrate system of creeping stolons with haptera and bearing several erect shoots. Thalli are strongly constricted into flattened segments of less than 1 cm at irregular intervals (Fig. 2.3 a). Its internal structure consists of central, elongated and freely arranged medular cells, covered by a group of smaller and compacted cortical cells perpendicular to the thallus surface. This species presents both sexual reproduction and vegetative propagation by fragmentation (Van Reine, 1983). It has a isomorphous triphasic life-cycle as *B. scorpioides*. Tetrasporophytes present tetrasporangia between outer cortical cells, where they are embedded (Fig. 2.3 b). Monoecious gametophytes develop spermophores and female laterals that form cystocarps when fertilised (Fig. 2.3 c). Both spermatangia and cystocarps are prominent on the thallus (Fig. 2.3 c,d) (Newton, 1931).

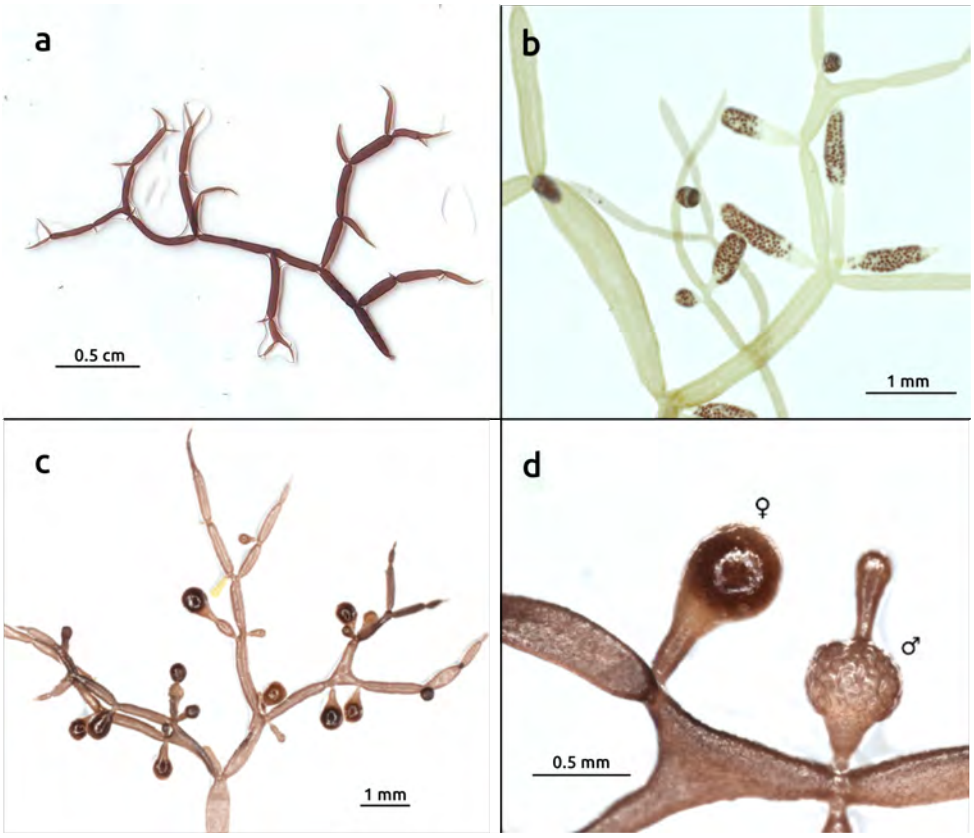


FIGURE 2.3. Thalli of *C. caespitosa* from the Palmones Estuary. a) Vegetative thalli. b) Mature tetrasporophyte. c) Gametophyte (n) with mature cystocarps. d) Detail of a cystocarp and a spermatangium.

Catenella caespitosa inhabits sheltered and shaded zones near the high tide level in estuaries (Eston et al., 1992; Cunha and Costa, 2002) and rocky shores (Munda, 1993; Araújo et al., 2005), but it can also be found unattached on the sediment surface or in small crevices under dim light conditions (Batelli, 2004; Silva et al., 2010). This subcosmopolitan species is widely distributed in temperate regions (Dixon and Irvine, 1977; Dawes and Mathieson, 2008), but over a broader geographical area than *B. scorpioides* (Van Reine, 1983; Batelli, 2004). Its morphology varies from filiform to compact forms, which is associated to different degrees of exposure (Van Reine, 1983). Estuarine specimens usually present both growth patterns, and they have been observed over the present study.

2.1.3 Morphological characteristics

Morphological characteristics of the two species of the study were determined to obtain conversion factors among thallus weight, area and volume. The relationship between fresh weight and dry weight (FW:DW) was obtained from independent replicates by linear regression analyses ($n = 30$, $r^2 = 0.98$), in specimens acclimated to the laboratory

TABLE 2.1. Morphological characteristics of *B. scorpioides* and *C. caespitosa* from Palmones estuary. Values are mean ± SE (n = 40).

Morphological characteristic	*B. scorpioides*	*C. caespitosa*
Percentage DW (%)	28.75 ± 0.4	15.25 ± 1.8
SA:V (cm^{-1})	6.0 ± 2.8	10.6 ± 1.6
SA:B ($cm^2\ g^{-1}$FW)	82 ± 5	105 ± 4
SA:B ($cm^2\ g^{-1}$DW)	285 ± 17	673 ± 27

(see acclimation conditions in section 2.2.4). FW was obtained after blotting thalli with towel paper until reaching constant weight. DW was obtained by drying them in a oven at 60°C for 24 h. Percentage DW was higher in *B. scorpioides* than in *C. caespitosa* (Table 2.1). Surface area:volume (SA:V) quotient and the surface area per unit dry biomass (SA:B) were determined on 40 specimens of each species by the procedure described by Rosenberg and Ramus (1984). Flat images were obtained from the thalli by scanning them in a CanoScan LiDE 25 (Canon USA, Melville, New York, USA) and thallus area was quantified using ImageJ (Image Processing and Analysis in Java, National Institute of Health, Bethesda, Maryland, USA). Volume of the thalli was determined by means of volume displacement techniques. Lower values of SA:V and SA:B were obtained for *B. scorpioides* than *C. caespitosa* (Table 2.1).

2.2 Study site

2.2.1 The Palmones River Estuary

The Palmones River Estuary (36° 10’13.4076"N, -5° 26’27.5742"W) is located in Algeciras Bay, Cádiz (Southern Spain), where both Mediterranean and Atlantic waters meet (Fig. 2.4). The particularity of the Palmones Estuary in comparison with other wetland areas in the south Iberian Peninsula is its Atlantic influence, and being subjected to a high inter-annual, seasonal and daily environmental variability (Moreno and Niell, 2004; Avilés and Niell, 2005). It has a total extension of 5.6 km in length, measuring an average 75 m in width and 1.5-2 m in depth at extreme low tides (lowest astronomical tide, LAT) (Clavero et al., 1997).

This partially mixed shallow estuary has weak tides with an amplitude between 0.6-2 m (Clavero et al., 1997; Izquierdo, 2001), with a highest astronomical tide of 2.06 m above LAT, and at a daily basis extensive areas of mud emerge at low tide. Water renewal time changes seasonally from 0.25 to 1.47 days (Niell et al., 2005; Clavero et al., 1997). Salinity in the estuarine water varies over a tide cycle between 29 and 35 (Clavero

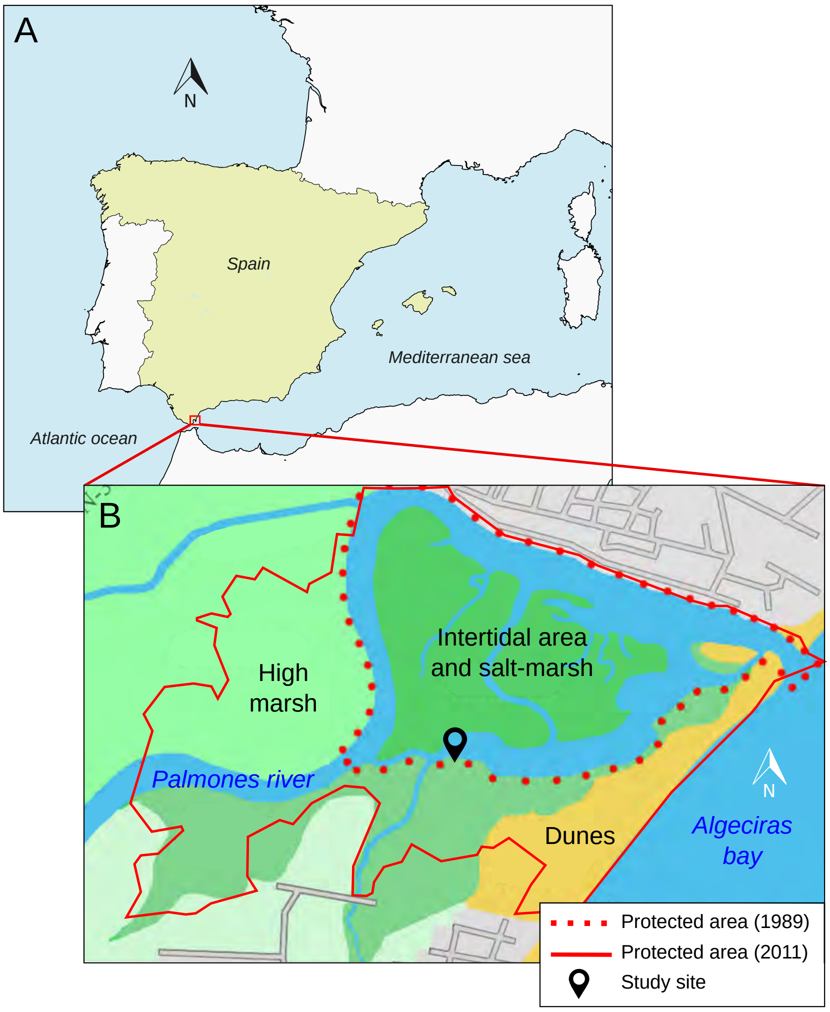


FIGURE 2.4. A) Location of the Palmones River Estuary. B) Environmentally protected area in the estuary and saltmarshes and location of the study site. Image modified from https://commons.wikimedia.org/w/index.php?curid=6001286 under creative commons license.

et al., 1997). Nevertheless, values between 15 and 37 have been observed in more recent field surveys and a progressive salinisation has been noted (Ruiz-Nieto, 2014). Mean water temperature in winter is 14ºC and 23ºC during summer, when rainfall is almost absent and air temperature rises above 30ºC. This estuary is classified as eutrophic, where ammonium is currently the major form of dissolved inorganic nitrogen (DIN) (Niell et al., 2005), although its concentration is highly variable (Vergara et al., 1997; Clavero et al., 2000), as in other estuaries (Kennison et al., 2011). On the contrary, nitrate and phosphate concentrations remain more stable over the year and at lower levels (Niell et al., 2005).

Saltmarsh areas of Pamones river estuary are covered by shrubby halophytic vegetation, mainly composed by the *Sarcocornia perennis* (Miller) A. J. Scott and *Arthrocnemum macrostachyum* (Moric.) Moris, and also by *Atriplex portulacoides* (L.) Allen (ex *Halim-*

ione portulacoides) and *Juncus maritimus* Lam., the latter associated to freshwater inputs (Palomo et al., 2004; Luque et al., 2007). In the low estuary some areas are covered by several species of the macrophyte *Spartina*, intermingled with the saltmarsh vegetation, as well as patches of *Zostera noltii* Hornem. covering the mudflats (Peralta et al., 2000; Luque et al., 2007).

The area of study is listed under several figures of environmental protection (Table S1). In 2011 the protected area was extended from 58 to 113 ha (Consejería de Medio Ambiente, 2011), with the new limits shown in Figure 2.4.

2.2.2 Light, temperature and nutrient measurements

All irradiances measured in the field and laboratory experiments were made measured using a spherical sensor (US-SQS/L, Walz, Germany), connected to a radiometer (LI-COR®LI-250A, Li-Cor Biosciences, Lincoln, Nebraska, USA). Temperature measurements were taken by a conventional thermometer.

Estuarine water samples were directly taken from the surface water, at flood tide, during each field survey carried out along the study (2012-2015). Samples were kept in 100 mL plastic containers, transported in a cooler to the laboratory, filtered (Whatman GF/C filter, Whatman International Ltd., Maidstone, UK) and preserved at -20ºC for later analysis.

Nutrient concentration in water samples were analysed in an automated continuous flow analyser QuAAtro AQ2 AACE (Seal Analytical Ltd., Fareham, U.K.), using standard methods for ammonium (Slawyk and MacIsaac, 1972), nitrate (Shinn, 1941; Wood et al., 1967) and phosphate (Murphy and Riley, 1962).

2.2.3 Macroalgal intertidal zonation

In the Palmones Estuary *B. scorpioides* and *C. caespitosa* are spatially segregated within a narrow intertidal zonation with a vertical extent of 30 cm, but they overlap at intermediate tidal levels (Fig. 2.5). *Catenella caespitosa* occupies the lower to intermediate intertidal zone (1.05-1.20 m above LAT), whereas the intermediate to uppermost one is dominated by *B. scorpioides* (1.15-1.30 m above LAT) (Fig. 2.6).

Vertical distributional limits of *B. scorpioides* and *C. caespitosa* were verified using surveying techniques related to established tidal baselines in the middle estuary (study site in Figure 2.4). The water level was monitored every hour over a semidiurnal spring tidal cycle (n = 3), by fixing a benchmark to the sediment in the intertidal zone. These values were related to the tidal data measured from the nearest tide gauge (Algeciras

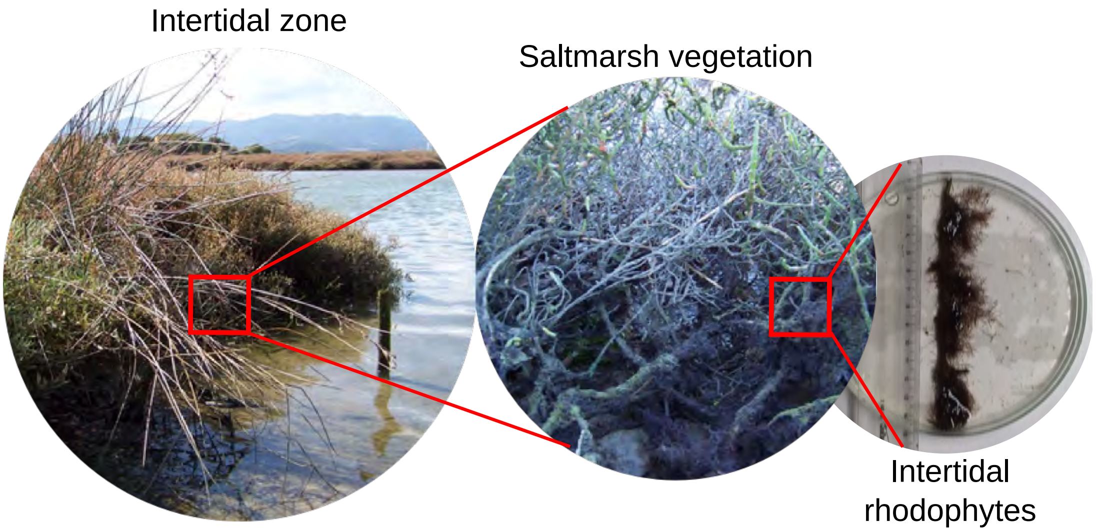


FIGURE 2.5. Location of the rhodophytes *B. scorpioides* and *C. caespitosa* in the intertidal zone of the Palmones Estuary and detail of an stem of *S. perennis* covered by them.

Bay, 36.18° N, 5.40° W), at 3 km from the sampling point, and available from public data bases (State Ports).

In spite of the narrow vertical extent of the macroalgal zonation, irradiance, air temperature and submersion hours per tidal cycle differ substantially among tidal levels (Fig. 2.6). Mean submersion times in a 12 h tidal cycle are 2.5 and 5 h for *B. scorpioides* and *C. caespitosa*, respectively, showing that the intertidal rhodophytes are mostly emersed throughout the tidal cycle.

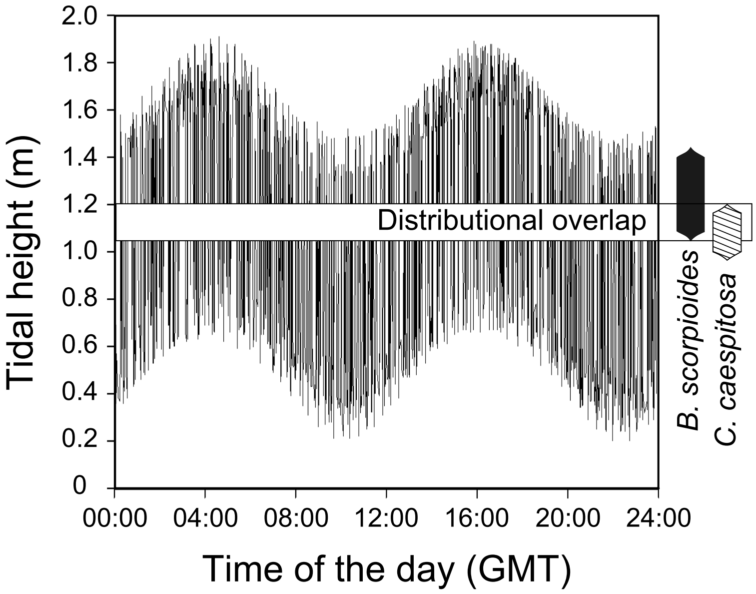


FIGURE 2.6. Tidal regime in the Palmones Estuary and intertidal distributional limits of *Bostrychia scorpioides* and *Catenella caespitosa*. Data extracted from tide heights in 2011 measured in Algeciras Port (36º 07.1′ N, 05º 26.6′ W) by the Spanish Institute of Oceanography (Ministerio de Defensa, Secretaria General Técnica, 2010).

2.2.3.1 Light and temperature gradients in the intertidal zone

In order to know the actual ranges of light and temperature for the macroalgae *in situ* measurements were taken along the intertidal zone. Light attenuation within the *S. perennis* canopy was determined on clear sunny days between late morning and early afternoon over the study period. Irradiance was measured beneath the vertical profile at several depths (n = 3), from the top of the plant canopy to the mudflat sediment below the macroalgae, and in two zones with different canopy density (sun-exposed and shaded zones), by using a spherical sensor conected to a radiometer (see section 2.2.4). *Bostrychia scorpioides* and *C. caespitosa* were distributed 0.25-0.60 m from the top of the canopy of *S. perennis*, which corresponded to 1-1.3 m above LAT as shown in Fig. 2.7. The decrease in radiation versus depth was computed based on the Lambert-Beer Extinction Law using the standard light attenuation equation:

$$E_z = E_0 \times exp^{(-k \times z)} \tag{2.1}$$

where E_z is the irradiance (µmol photons m^{-2} s^{-1}) at a certain depth from the top of the plant canopy z (m), E_0 is the incident irradiance at the top of the canopy (µmol photons m^{-2} s^{-1}), and k is the specific vertical attenuation coefficient of downward irradiance (m^{-1}).

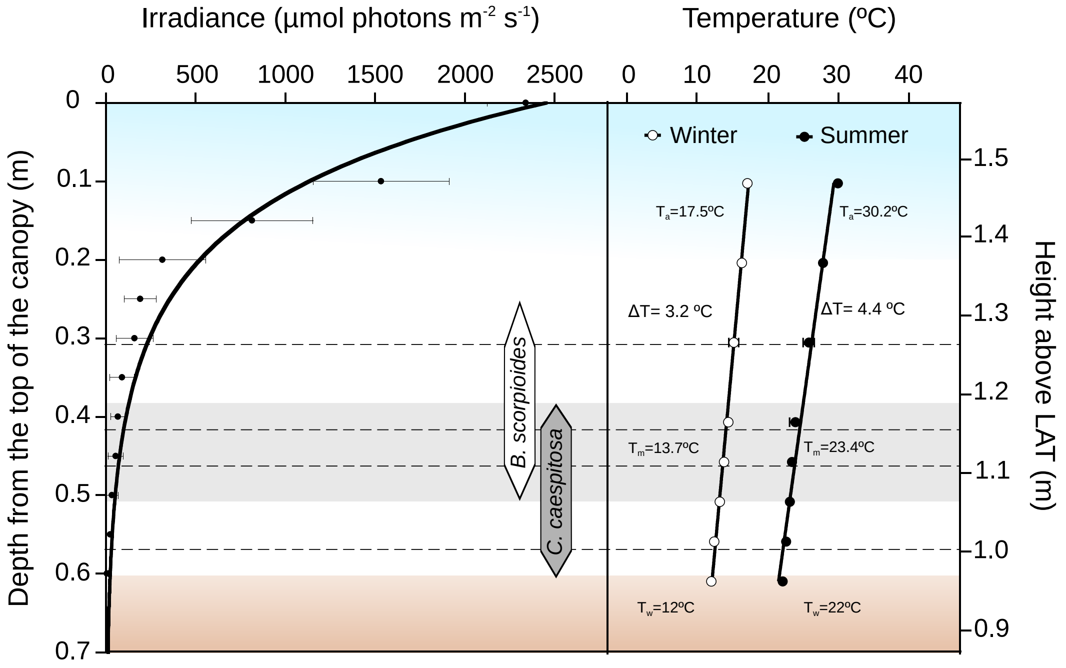


FIGURE 2.7. Light attenuation curve and temperature gradients (winter, summer) beneath the canopy of *Sarcocornia perennis* and vertical distribution of *B. scorpioides* and *C. caespitosa* on its basal stems in relation to their position in the estuarine intertidal, in metres above LAT. Air temperature (T_a). Water temperature (T_w). Temperature at the overlap zone (T_m). Difference between maximum and minimum air and water temperature (ΔT). Values are mean ± SD (n = 3).

Irradiance at the upper and lower limits of distribution was 122-208 and 32-51 µmol photons m^{-2} s^{-1} for *B. scorpioides* and 30-72 and 1-11 µmol photons m^{-2} s^{-1} for *C. caespitosa*, respectively (Fig. 2.7). The light attenuation coefficient (k) for the canopy was 8.0 ± 0.4 m^{-1} for sun-exposed zones and 11.0 ± 0.4 m^{-1} in shaded ones. Percentage of incident irradiance (% SI) at the upper zonation limits ranged between 1.5-7% for *B. scorpioides*, whereas *C. caespitosa* only received 0.3-2%. Mean irradiance at the distributional overlap zone was 40-45 µmol photons m^{-2} s^{-1}, which corresponded to 2% SI.

Temperature attenuation by the canopy of saltmarsh vegetation was determined in the intertidal zone of the study site (Fig. 2.4) in summer and winter 2012 (n = 3, Fig. 2.7). Differences between air and water temperature became greater during summer (4.4ºC) than in winter (3.2ºC), due to the high air temperature and the daily renewed estuarine water, which is cooled down by the tidal inputs (Fig. 2.7). Mean temperature at the coexistence zone was 13.7 and 23.4ºC in winter and summer, respectively (Fig. 2.7).

These marked light and temperature gradients can contribute to the zonation of the two rhodophytes, aspects that will be considered in chapters 3, 5, 7 and 8.

2.2.3.2 Environmental nutrient concentrations

Environmental nutrient concentrations in this study are defined as the most frequent concentrations of ammonium, nitrate, and phosphate. Water samples from the estuary were taken every month from March 2010 to May 2011 to determine nutrient concentration, following the sampling and analysis methods described in section 2.2.2.

Ammonium concentration fluctuated over the studied period and showed two modal values (Fig. 2.8): in 54% of samples, concentration ranged between 0 and 20 µM

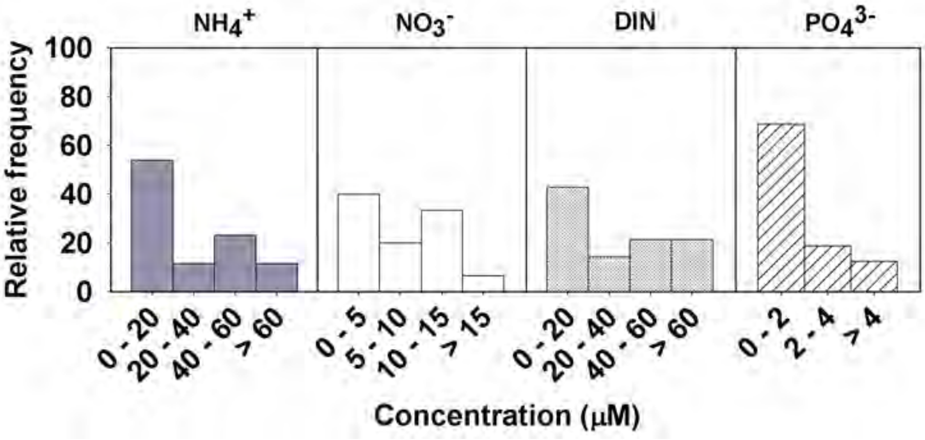


FIGURE 2.8. Environmental nutrient concentrations expressed as the observed frequency of each concentration range over the study period.

NH_4^+, and in 27%, between 40 and 60 µM NH_4^+. The highest ammonium concentrations were detected in early winter (85.3 µM NH_4^+). Nitrate concentration also presented two modal values (Fig. 2.8). In 40% of samples, this nutrient ranged from 0 to 5 µM NO_3^-, corresponding with samples from late spring, whereas in 33% of samples, it ranged from 10 to 15 µM NO_3^-, related to winter values. Total DIN concentrations in the estuary ranged from 0 to 20 µM in 50% samples and were greater than 60 µM in 25% samples (Fig. 2.8). Phosphate concentration varied between 0 and 2 µM in 69% of samples, and only in 12% of samples it slightly exceeded 4 µM PO_4^{3-}, with a maximum mean value of 4.26 ± 0.12 µM PO_4^{3-} observed in winter (Fig. 2.8).

According to the high variability of nutrient concentration in the Palmones Estuary and the different available time for their uptake along the intertidal zone, the nutrient uptake kinetics of *B. scorpioides* and *C. caespitosa* will be examined in this study in a range of concentrations beyond the mean levels found in the field, over the common submersion period at which these species are exposed to and in specimens collected at different tidal levels (chapters 4, 6).

2.2.4 Algal collection and culture maintenance

Algal samples used in the experiments were collected from the mid estuary, at a sampling poing 868 m from the river mouth indicated as "study site" in Figure 2.4. Thalli of *B. scorpioides* and *C. caespitosa* were carefully removed from the lignified stems of saltmarsh vegetation, at low tide, at identical tidal elevations relative to their distributional overlap zone (1.10-1.20 m), to prevent variations within each species related to tidal position, despite their narrow horizons (see subsection 2.2.3). Macroalgae were placed in plastic containers of 1L with a minimal amount of estuarine water and transported in a cooler to the laboratory. Vegetative thalli of both species were selected to reduce physiological variability (Harrison and Hurd, 2001), then rinsed in natural non-filtered seawater (NSW) and gently cleaned of sediment and scrubbed of epiphytes.

Algal cultures were kept under constantly submersed conditions, in 1.5 L perspex cylinders containing GF/C filtered natural seawater (NSW), with a pH of 8.2 (adjusted adding NaOH or HCl) and a salinity of 36. Adequate aeration (1 L min^{-1}) was provided to prevent the formation of boundary layer around thalli. Cultures were maintained in a walk-in cold room at 15 ± 1 °C, and irradiance was set at 45 µmol photons m^{-2} s^{-1}, provided by fluorescent lamps (cool daylight, F-18W/54-765 Sylvania) under 12:12 h light:dark (L:D) photoperiod. This irradiance and temperature corresponded to the zone of distributional overlap of these species in their natural habitat (Fig. 2.7), not being limiting for growth (see chapters 3 and 5).

For all laboratory experiments artificial seawater (ASW) was used instead of NSW, with the composition showed in table 2.2, adding the corresponding concentrations of NO_3^-, NH_4^+ and PO_4^{3-} for each experiment.

Table 2.2. Composition of the artificial seawater used in the laboratory experiments

Compound	Concentration
NaCl	0.5 M
KCl	0.01 M
$CaCl_2$	0.01 M
$MgCl_{2}6H_{2}O$	7 mM
$MgSO_{4}7\ H_{2}O$	0.03 M
$NaHCO_3$	2.5 mM

Chapter

3

Photosynthetic responses

Título: Understanding the intertidal zonationof two estuarine red macroalgae from *ex situ* photoacclimative responses

Autores: Sánchez de Pedro, R., Niell, F.X., Carmona, R.

Revista: European Journal of Phycology

Volumen: 49

Páginas: 538–549

Año de publicación: 2014

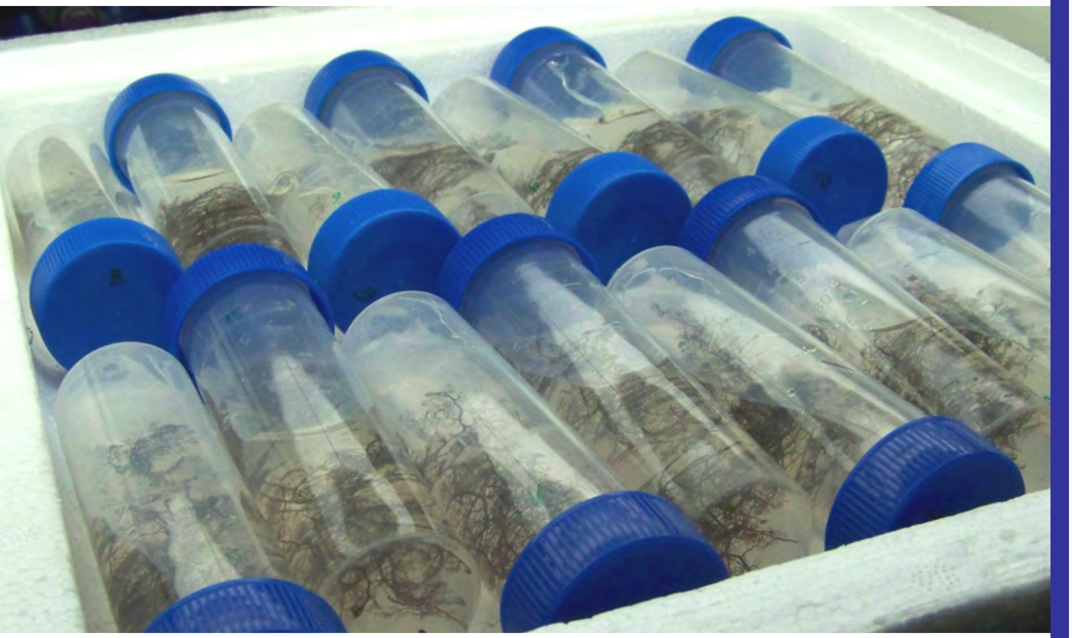
Resumen: This study investigated the influence of light on the distributional limits of *Bostrychia scorpioides* and *Catenella caespitosa* within a low amplitude intertidal zonation in the Palmones estuary, Spain. Changes in photosynthesis- and growth-irradiance curves and pigment content were examined ex situ at irradiances found in their natural habitat (10–230 µmol photons m^{-2} s^{-1}). The highest maximum photosynthesis rate (P_m) and photosynthetic efficiency (α) were found between 20–40 µmol photons m^{-2} s^{-1} in both species. *Bostrychia scorpioides*, the uppermost intertidal species, had a higher P_m, α, dark respiration rate and light compensation point (E_c) than *C. caespitosa* at all acclimation irradiances. Net photosynthetic rates measured at their respective acclimation irradiances showed photosynthetic responses of *B. scorpioides* to be maximized at high irradiances, while in *C. caespitosa* they did not change. Higher growth rates were obtained in *C. caespitosa* than in *B. scorpioides*, which may be related to its lower thallus specific carbon content. When irradiance decreased, the chlorophyll a content of *B. scorpioides* increased whereas in *C. caespitosa* R-phycoerythrin increased. E_c for growth of *B. scorpioides* coincided with the irradiance at its lower distributional limit in the estuary, below which this species showed losses in biomass. However, in *C. caespitosa* the sustained growth *ex situ* at saturating irradiances contrasts with its absence from the upper intertidal zone, where similar light regimes occur. We demonstrated that while light clearly restricts the growth of *B. scorpioides* to the uppermost intertidal zone, this environmental factor would not prevent *C. caespitosa* from growing at higher levels.

DOI: 10.1080/09670262.2014.978394

Chapter

4

Nutrient physiology



Título: Differential nutrient uptake by two segregated red algae in an estuarine intertidal zone

Autores: Sánchez de Pedro, R., Niell, F.X., Carmona, R.

Revista: Phycologia

Volumen: 52

Páginas: 461–471

Año de publicación: 2013

Resumen: *Bostrychia scorpioides* (Rhodophyta: Ceramiales) and *Catenella caespitosa* (Rhodophyta: Gigartinales) were segregated but slightly overlapping in the narrow intertidal of the Palmones River Estuary (Southern Spain). This study investigated whether there were interspecific differences in nutrient uptake capabilities and how meaningful these were to zonation. Uptake kinetics of ammonium, nitrate, and phosphate were measured over successive time intervals across a range of environmentally realistic concentrations. The interactions between ammonium and nitrate in mixedNsource uptake were also tested. Using internal N and P content, turnover rates were calculated for each species for periods of emersion and submersion. Nutrient uptake kinetics were saturable for almost all nutrients except for ammonium in *B. scorpioides*, which showed surge uptake responses through linear kinetics. V_{max} and affinity progressively decreased over 3 h for all nutrients in *C. caespitosa* and only for phosphate in *B. scorpioides*. At high dissolved inorganic nitrogen concentrations both species exhibited greater uptake rates for ammonium than nitrate; although, similar N uptake rates were obtained at the low ones. Ammonium contributed to the totalNuptake in *B. scorpioides* to a greater extent; whereas, in *C. caespitosa*, this only occurred at high N concentrations. In mixed N source uptake experiments, ammonium and nitrate uptake rates by *B. scorpioides* decreased when the other N source was equimolarly supplied, while in *C. caespitosa* these interactions were absent. *Catenella caespitosa* showed a V_{max} and affinity for phosphate fourfold higher than for *B. scorpioides*, with greater interspecific differences than those found for N uptake. *Catenella caespitosa* renewed internal nutrients faster and was more dependent on the external nutrient supply than *B. scorpioides*, which can withstand longer periods without nutrient supply due to its slower nutrient turnover rate. Different capabilities for nutrient acquisition in the two species suggested an adaptation to the nutrient availability at each intertidal position.

DOI: 10.2216/13–147.1

Chapter 5

Responses to environmental variables

(Capítulo con resultados pendiente de publicación)

Chapter

6

Intraspecific ecophysiological variability

Título: Intraspecific phenotypic variation in two estuarine rodophytes across their intertidal zonation

Autores: Sánchez de Pedro, R., Karsten, U., Niell, F.X., Carmona, R.

Revista: Marine Biology

Volumen: 163

Páginas: 221–549

Año de publicación: 2016

Resumen: This study investigated the influence of light on the distributional limits of *Bostrychia scorpioides* and *Catenella caespitosa* within a low amplitude intertidal zonation in the Palmones estuary, Spain. Changes in photosynthesis- and growth-irradiance curves and pigment content were examined ex situ at irradiances found in their natural habitat (10–230 μmol photons m^{-2} s^{-1}). The highest maximum photosynthesis rate (P_m) and photosynthetic efficiency (α) were found between 20–40 μmol photons m^{-2} s^{-1} in both species. *Bostrychia scorpioides*, the uppermost intertidal species, had a higher P_m, α, dark respiration rate and light compensation point (E_c) than *C. caespitosa* at all acclimation irradiances. Net photosynthetic rates measured at their respective acclimation irradiances showed photosynthetic responses of *B. scorpioides* to be maximized at high irradiances, while in *C. caespitosa* they did not change. Higher growth rates were obtained in *C. caespitosa* than in *B. scorpioides*, which may be related to its lower thallus specific carbon content. When irradiance decreased, the chlorophyll a content of *B. scorpioides* increased whereas in *C. caespitosa* R-phycoerythrin increased. E_c for growth of *B. scorpioides* coincided with the irradiance at its lower distributional limit in the estuary, below which this species showed losses in biomass. However, in *C. caespitosa* the sustained growth *ex situ* at saturating irradiances contrasts with its absence from the upper intertidal zone, where similar light regimes occur. We demonstrated that while light clearly restricts the growth of *B. scorpioides* to the uppermost intertidal zone, this environmental factor would not prevent *C. caespitosa* from growing at higher levels.

DOI: 10.1007/s00227-016-2997-5

Chapter

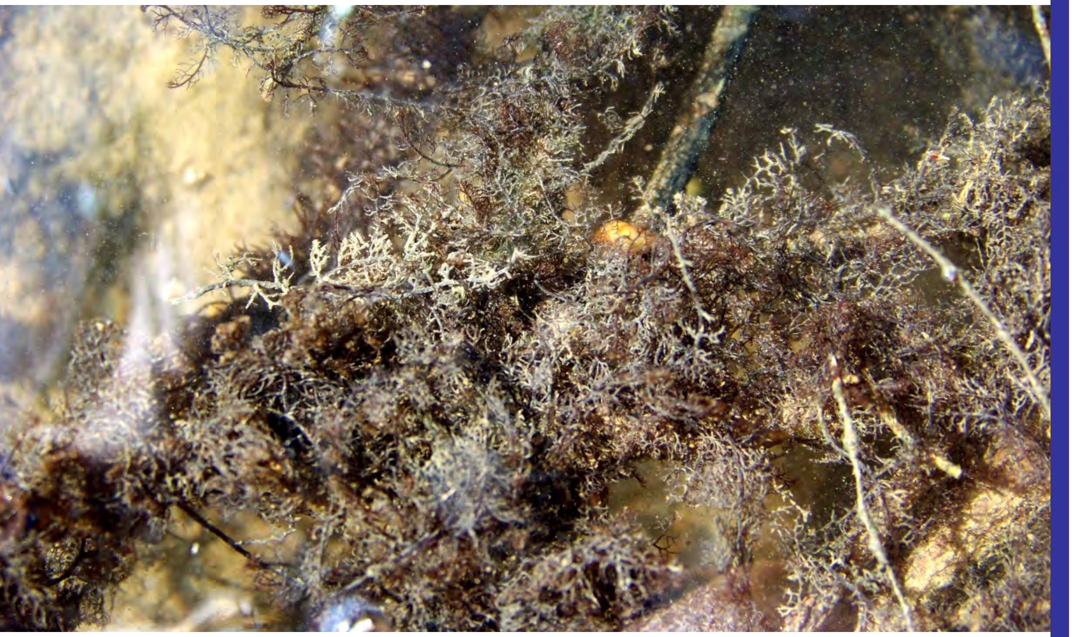
7

Spatial and seasonal variations in biomass and growth

(Capítulo con resultados pendiente de publicación)

Chapter
8

Seasonal variations in photosynthesis and biochemical composition



(Capítulo con resultados pendiente de publicación)

Chapter 9

General discussion and conclusions

General discussion and conclusions

9

> *"Heterogeneity and/or instability must be recognized as fundamental features of a natural situation"*
>
> — (Den Boer, 1968)

In this thesis different aspects of the ecophysiology of *B. scorpioides* and *C. caespitosa* have been assessed by several methods, in order to understand how different environmental variables affect their primary production, and therefore, their spatial and temporal distribution in the intertidal zone of the Palmones Estuary. The specific objectives and hypotheses initially stated have been addressed through laboratory and field experiments, and the underlying mechanisms of the distinct ecophysiological traits of each species have been discussed in detail in the previous chapters. This final synthesis aims to (1) integrate and contrast the responses obtained by the different approaches, (2) identify the most characteristic physiological traits of each species and (3) to discuss their ecological implications for their intertidal zonation and interspecific relationships.

Among the different physiological variables analysed in this study, growth rates provided the most significant results from an ecological perspective. Growth data from the field experiments were summarised by means of multivariate analysis, showing the major influence of tidal submersion and nutrient availability on their seasonal growth (Chapter 7). Following a similar approach, all the growth responses obtained from culture experiments in this and a previous study (Sánchez de Pedro, 2011) have been analysed by means of multiple linear regression, in order to ascertain the relative importance of each environmental variable (Table 9.1).

Daily emersion time was the variable with higher relative importance for the growth of *B. scorpioides* and *C. caespitosa*, but especially for *C. caespitosa*, explaining almost 50% of total variability (Table 9.1), as it drastically reduced its growth (Chapter 5). This statement strongly agrees with the high influence of tidal submersion/emersion in their seasonal growth and with the decline in total macroalgal biomass towards the upper intertidal limits (Chapter 7). Irradiance and emersion had the same relative importance for growth of *B. scorpioides* (Table 9.1), indicating that light reductions or increasing emersion can affect its growth similarly. Evidence of this was found in the similar biomass of this species found at low and upper parts of its horizon (Chapter 7). On the other hand, irradiance had much lower importance than emersion for the growth of *C. caespitosa* (16%), which is due to its better shaded-adapted photophysiology, as demonstrated by both culture (Chapter 3) and field experiments (Chapters 6, 7, 8). Moreover, temperature had a greater influence in the growth of *C. caespitosa* than *B. scorpioides*, which is due to its higher sensitivity to this variable and its additive effect with emersion as discussed in Chapter 5, and also evidenced by the lower biomass relative abundance of *C. caespitosa* at microenvironments exposed to higher temperature (sun-exposed zones, Chapter 7).

Salinity of the culture medium had no significant effects on the growth of *B. scorpioides* and *C. caespitosa* under laboratory cultures. The results of Chapter 5 showed that growth of these species was optimum at salinities between 10-20, but only under submerged conditions. This optimum salinity range was in agreement with the reported for many other estuarine rhodophytes, although most of them are euryhaline (i. e. 5-60) (Dawes et al., 1978; Jagtap and Untawale, 1981; Karsten and Kirst, 1989b; Rao and Rangaiah, 2008). The lack of sensitivity of physiological responses to changes in salinity is very common among estuarine algae and confers an ecological advantage in ecosystems that undergo broad osmotic fluctuations.

Nutrient concentration had no significant effect on the growth rates obtained in cultured experiments (see Chapter 4). This result contradicts those of seasonal experiments, that revealed that TSP and DIN had a major contribution to the growth of *B. scorpioides* and *C. caespitosa*, respectively. How can this difference be explained? According to the analysis of culture growth rates, the renewal rate of the culture medium had a significant effect on the growth of these species (Table 9.1). In the growth experiments

TABLE 9.1. Multiple linear regression analysis of the growth rates of *B. scorpioides* and *C. caespitosa* in relation to abiotic variables, obtained from different experiments (this study and Sánchez de Pedro (unpublished data)).

Species	*B. scorpioides*		*C. caespitosa*	
Intercept	3.75 ± 1.10		9.16 ± 1.27	
Variable	Predict. Coeff. ± SE	Relat. Import.	Predict. Coeff. ± SE	Relat. Import.
Emersion	-0.30 ± 0.05***	29%	-0.62 ± 0.05***	47%
Irradiance	0.01 ± 0.00***	28%	0.02 ± 0.00***	17%
Temperature	-0.08 ± 0.04*	3%	-0.26 ± 0.04***	12%
Renewal rate	-1.03 ± 0.25***	12%	-1.41 ± 0.28***	8%
R^2	0.74		0.74	
Model *P*	< 0.0001		< 0.0001	
n	50		50	

Regression coefficients (intercept and predictors) are displayed ± standard error (SE) and with associated significance (*** $P < 0.001$; ** $P < 0.01$;* $P < 0.05$), in addition to the relative importance of predictor variables, associated overall model R^2 and significance (Model *P*), and the number of observations (*n*). Salinity and nutrient concentration (DIN, TSP) were included in the analysis but did not have a significant influence.

of this thesis culture medium was always changed on a daily basis (except in Chapter 3), but when it was changed every 3-4 days it never exceeded 3.5 % d^{-1} (Sánchez de Pedro, 2011). It should be considered that these species are submerged twice a day in their natural habitat over regular tidal cycles, being exposed to a continuous supply of nutrients, which are redissolved from the eutrophic sediments by tidal action (Clavero et al., 1997; Avilés and Niell, 2005). Therefore, although concentrations used in the experiments simulated the most frequent ones in the estuary, a daily renewal time might not be enough for inducing significant changes in their growth rates. In this sense, in most of culture experiments a dilution effect by growth on internal nutrients and N-storage molecules (e.g. PBP, chl *a*) was observed, suggesting that these growth rates were mainly supported by internal nutrients rather than the external supply.

Growth rates obtained from seasonal changes in biomass ranged between -6.8 - 6 % d^{-1} in *B. scorpioides*. In culture experiments biomass losses were not as high as in the field (minimum RGR of -0.52 % d^{-1}, at 10 µmol photons m^{-2} s^{-1}, 15ºC, salinity 36), while surprisingly, maximum rates coincided (maximum RGR of 6 % d^{-1} at 230 µmol photons m^{-2} s^{-1}). This difference suggests that *B. scorpioides* is probably exposed to harsher environmental conditions in the field that those tested in the culture experiments (i. e. more than 8 h of daily emersion and temperatures higher than 25ºC). Likewise, field growth rates of *C. caespitosa* were between -4.9 - 3 % d^{-1}, while those obtained from culture experiments varied from -4.2 % d^{-1} (8 h daily emersion, subsaturating irradiance and 25ºC) to 7.6% (continuous submersion, saturating irradiance, 15ºC). Similar biomass losses in field and culture experiments suggest that the environmental conditions to which *C. caespitosa* is exposed in its natural habitat are ameliorated and not more extreme than those tested in the cultures. In addition, the 2.5-fold maximum growth rates obtained under laboratory conditions evidence that *C. caespitosa* has a much greater growth potential. However, that set of environmental conditions are unlikely to be found in the field, since they are never continuously submerged and because increases in irradiance occur concomitantly with higher levels of emersion.

Bostrychia scorpioides and *C. caespitosa* present many ecological similarities, being considered shade and desiccation tolerant in comparison with other algal groups or rhodophytes. In fact, emersion and irradiance were the variables with major influence in their growth and spatial distribution, followed by nutrient availability, being consistent with the conclusions drawn from similar studies in intertidal rhodophytes (Beanland and Woelkerling, 1983; Phillips et al., 1996; Datta and Datta, 1999; Phillips and Hurd, 2003; Melville et al., 2005). However, although this investigation confirms their common ecological traits, particularly at intermediate environmental conditions of irradiance, nutrient availability and emersion, the two rhodophytes clearly differ on their responses to these variables (i. e. *B. scorpioides* not being as much shade-tolerant as *C. caespitosa* and the latter not being as desiccation-tolerant as the former). Results from field and culture experiments have demonstrated that *B. scorpioides* and *C. caespitosa* posses different phenotypic plasticity in their physiological and biochemical responses.

Bostrychia scorpioides presented a higher physiological plasticity at both spatial (zonation) and seasonal scale. Across its intertidal distribution this species showed the ability to (1) modify its P-E responses by adjusting pigment content, also confirmed by its active photoacclimation and maximization of NPR at higher irradiances in the laboratory (Chapters 3, 6); (2) to reduce its internal N and P turnover at tidal levels where nutrient supply is reduced (Chapters 4, 6); and (3) to be able to sustain positive growth rates at severe daily emersion periods and recover photosynthesis after 6 days of continuous emersion. Its physiological plasticity was also evidenced seasonally by wide variations in its photosynthetic performance. This species exhibited features of shade-adapted autotrophs during winter (i.e. low R_d and E_c, high chl *a*), but light-adaptation ones in summer (i. e. high E_k, high R_d). This physiological performance allows *B. scorpioides* to cope with more fluctuating environmental conditions, which explains its dominance towards the upper intertidal zone and at sun-exposed sites (Fig. 9.1), as well as the homogeneous distribution of its biomass among tidal levels (Chapter 7). Furthermore, its high phenotypic plasticity might be due to a high genetic variability among individuals of the same population. Several studies carried out in Australian coastal ecosystems have identified haplotypes and cryptic species for this genus (Zuccarello et al., 1999; Zuccarello and West, 2003; Muangmai et al., 2016). The recent investigation of Muangmai et al. (2014) demonstrated that cryptic species of *B. intricata* (Bory) Montagne occupy different parts of the intertidal zone not-randomly, due to different physiological performances. The analysis of the genetic variability of the populations of *B. scorpioides* in specimens from the Palmones Estuary would be of great interest to explain its ecological plasticity.

In contrast to *B. scorpioides*, *C. caespitosa* showed little physiological variability within its distribution in the intertidal zone and at seasonal scale (Chapters 7, 8). However, the most remarkable trait of this species was its great shade-adapted physiology, based on its low light requirements for growth, minimisation of respiration rates and high thallus specific carbon, which are advantageous traits for growing at low light environments (Pedroche et al., 1995; Batelli, 2004). Its photophysiological performance together with its lower capacity to reduced its N turnover rate at upper tidal levels (Chapter 6) and its higher sensitivity to emersion related variables (Chapter 5) support its dominance at the lower intertidal zones and at shaded sites, where physical stress is ameliorated by the canopy vegetation (Fig. 9.1).

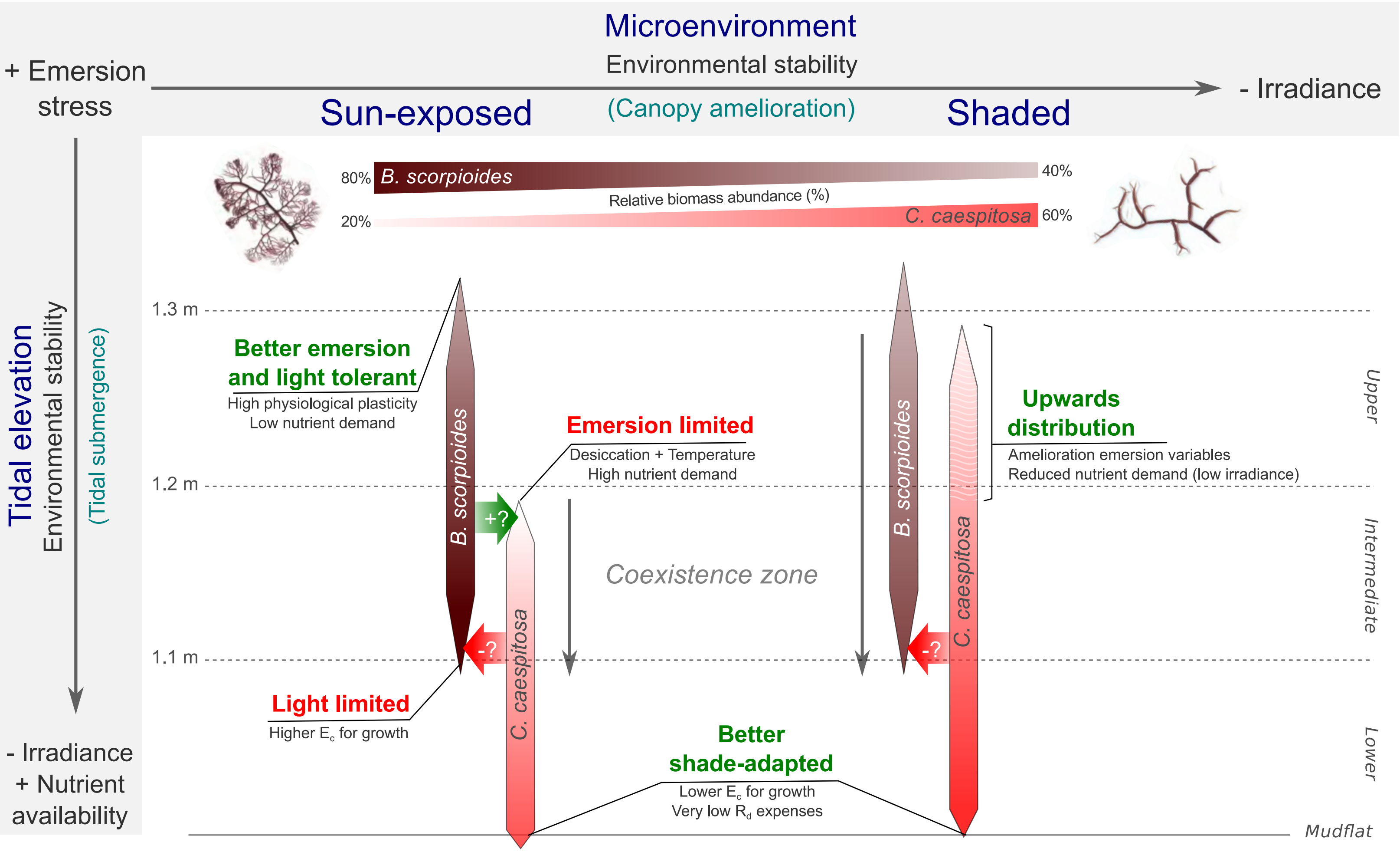


FIGURE 9.1. Schematic representation of the physically-controlled zonation of *B. scorpioides* and *C. caespitosa* in the intertidal zone of the Palmones Estuary. Horizonal axis represents environmental stability gradient from sun-exposed to shaded zones, associated to increasing density in the vegetation canopy. Vertical axis represents environmental stability along the tidal gradient, from lower to upper intertidal zones. Changes in biomass abundance of each species along the two axis is represented by different color intensities. Coloured arrows indicate possible competitive (red) and facilitative (green) interactions between the two species.

As reported in Chapter 7, the total macroalgal biomass did not vary among sites with different canopy densities but different dominance patterns were observed, both due to the species-specific ecophysiological performances discussed in the previous paragraph. Despite both species were always present and their distribution ranges overlap at sun-exposed and shaded zones (Chapter 7), it is possible that at more extreme microenvironments (exposed sites or below thicker canopies) they grow separately. This inference derived from the conceptual model of Figure 9.1 can be support by some direct field observations (pers. obs.).

The results of this thesis strongly support that the intertidal zonation of *B. scorpioides* and *C. caespitosa* is mainly controlled by physical factors. Nevertheless, it cannot be completely ruled out the additional role of biotic interactions based on the results of this study. First, it should be considered that the intertidal zone of this study is restricted to the eulittoral zone, where physical factors gain relevance (Raffaelli and Hawkins, 1996). If competitive interactions existed they would occur where potentially limiting common resources are available, which is considered the prerequisite of the "competitive exclusion principle" (Hardin, 1960). Colonisation substrate represent the most evident resource for which these species may compete (Davey and Woelkerling, 1985; Nedwell et al., 2002), but in this case it does not vary with tidal elevation. On the other hand, light and nutrient availability decreases at lower and upper intertidal zones, respectively. Therefore, direct competition for resources may probably occur at intermediate tidal levels, where these three resources are available. However, none of the species were nutrient limited within that zone, since nutrients are continuously supplied during submersion (high renewal time), and also both species have very similar photosynthetic and growth responses at that irradiance (45 µmol photons m^{-2} s^{-1})(Chapter 3). The results from Chapter 6 showed that their interspecific differences became greater towards the boundaries of the intermediate zone (Fig. 9.1). According to the initial hypothesis, one species will become a greater competitor when dominates the overall resource supply, considering that abiotic factors can mediate their resource acquisition abilities (Davison and Pearson, 1996). At the lower parts of the distribution of *B. scorpioides*, *C. caespitosa* might overgrow *B. scorpioides* and it is possible that *B. scorpioides* would loss biomass by overshadowing (red arrows in Figure 9.1). However, as light *per se* limits its growth, it is very difficult to ascertain from the results if competition with *C. caespitosa* may have a concomitant influence setting the lower zonation limit of *B. scorpioides*. At the boundary of the upper distribution of *C. caespitosa*, *B. scorpioides* is a better competitor for light, it reduces its turnover rates and also possesses a greater desiccation tolerance, thus dominating the overall resource supply (Fig. 9.1). Following a similar reasoning, *B. scorpioides* overgrows *C. caespitosa* at these conditions, as evidenced by the emersion-growth experiments (Chapter 5), and its much higher dominance in zones bearing higher abiotic stress (Chapter 7). In turn, if *B. scorpioides* overshadows *C. caespitosa*, its biomass might ameliorate abiotic stress to a certain extent, which would facilitate the growth of *C. caespitosa* and, at the same time, *C. caespitosa* could grow covered by thalli of *B. scorpioides* according to its better shade-adaptation (see green arrows in Figure 9.1). Hence, biotic interactions would only play a marginal role in the zonation of *B. scorpioides* and *C. caespitosa*.

Coexistence among apparently similar species can occur through different mechanisms, under the primary influence of physical, biological or both variables, and vary among intertidal ecosystems due to local environmental conditions (Lubchenco, 1980; Dudgeon et al., 1999; Karez, 2003a; Muangmai et al., 2016). The overlap of both species at intermediate tidal levels and its broader vertical extent in shaded zones (Fig. 9.1) evidence that similar physiological traits promote their coexistence rather than competition. This result is in agreement with the "intermediate disturbance hypothesis" (IDH, Connell, 1978; Tilman, 1982), which can lead to "spatial refuges" that escape from competitive exclusion (Worm and Karez, 2002). On the other hand, although both species require the same resources for growing, they have different light demands, turnover rates and uptake abilities (e.g. preferential use of NH_4^+ in *B. scorpioides* and greater P-uptake abilities in *C. caespitosa*). With regard to the "resource ratio hypothesis" (RRH, Tilman, 1977), *B. scorpioides* and *C. caespitosa* may be considered competitors that partially segregate as a result of a specialisation in resource acquisition, thus partitioning their niche. Taking these two hypotheses together, both species would colonise intermediate tidal areas that are optimal for their growth and then progressively segregate, thus supporting either their actual coexistence and spatial segregation.

The high environmental variability characteristic of the Palmones Estuary (Moreno and Niell, 2004) was also reflected in the physiological responses of the two rhodophytes of this study, particularly in *B. scorpioides*, due to its higher exposure to environmental heterogeneity at higher tidal positions. Seasonal and interannual changes accounted for the great variability in the abundance of *B. scorpioides* and *C. caespitosa* in their natural habitat, already pointed out for macroalgae from interface ecosystems (Beanland and Woelkerling, 1983; Davey and Woelkerling, 1985; Yokoya et al., 1999). Nevertheless, both species followed a clear and common annual pattern with increasing photosynthesis, growth and internal nutrient content from winter to spring. Despite the high fluctuating environment at which they are exposed to, these species possess a fast response to environmental changes in the estuary (less than one month), which together with the high physiological plasticity of *B. scorpioides* and resistance of *C. caespitosa* might confer a high resilience against environmental perturbations.

Future perspectives

Over the last decade the Palmones Estuary has undergone a progressive eutrophication (Niell et al., 2005) and salinisation (Ruiz-Nieto, 2014), in addition to the marked effect of climate change in the southern Spain, with increasing temperature, reducing rainfall and increasing flood events (Sousa et al., 2007). Moreover, 50% of saltmarsh surface in Andalusia has been lost in the past 50 years (Muñoz-Rojas et al., 2011). In this context, the ecophysiological knowledge gained by this thesis may serve as a firm basis for future modelling studies, in order to predict the responses of this macroalgal community to different future scenarios, related to the above environmental changes.

The two rhodophytes of this study showed a broad and similar tolerance to salinity, in accordance with many others estuarine rhodophytes (Dawes et al., 1978; Karsten and Kirst, 1989b; Ruiz-Nieto, 2014). Surprisingly, salinity and emersion had no additive effects on the growth of *B. scorpioides* and *C. caespitosa*, and the former variable only exerted a significant effect when they are fully submerged. In this sense, if salinity keeps on increasing in the estuary in the future, both species are likely to acclimate to these changes. Nevertheless, based on the assumption that under osmotic stress energy can be funelled into growth or osmotic protection, future studies should address the osmotic acclimation responses of these rhodophytes under combinations of emersion variables at biochemical (organic osmolytes, inorganic ions) and ultrastructural level.

With regards to the responses to emersion obtained in this thesis, it would be of high ecological interest to obtain photosynthetic measurements in air by CO_2 uptake. Such measurements may fill the gaps between the physiological responses of *B. scorpioides* and *C. caespitosa* to emersion obtained in this thesis and the well-described C uptake mechanisms identified for them in the study of Ruiz-Nieto et al. (2014). These authors highlighted that CO_2 is the only C source for these species, and in the case of *B. scorpioides* there is also evidence that its photosynthetic rates are higher under emersion (but hydrated conditions) than when it is is submerged (Mercado and Niell, 2000). In the case of *B. scorpioides*, the similar growth responses obtained either when submerged or continuously emerged but hydrated suggest that this species needs certain degree of emersion, as CO_2 availability is constrained when submerged, reinforcing the definition of this species as a "terrestrial" red algae by Karsten and Kirst (1989a). Lastly, the characterization of the underlying enzymatic and molecular mechanisms would be of high scientific interest to understand their physiological abilities coping with high environmental pressure without being actually stressed.

Conclusions

1. *Bostrychia scorpioides* possesses a high physiological variability at spatial (zonation) and seasonal scales. This species was better adapted to higher irradiances, presented a great photophysiological plasticity, was able to reduce its internal N and P turnover at tidal levels where nutrient supply is low and sustained positive growth rates at severe daily emersion periods. This performance allows this species to cope with more fluctuating environmental conditions, explaining its clear dominance at sun-exposed sites and towards the upper intertidal zone and its homogeneous biomass distribution among tidal levels.

2. *Catenella caespitosa* shows a clear shade-adapted photophysiology with remarkably low light requirements for growth, minimisation of respiration rates and high thallus specific carbon. This photophysiological performance together with its lower capacity to reduce its N turnover rate at increasing tidal positions and its higher sensitivity to emersion related variables support its dominance at the lower intertidal zones and shaded sites.

3. *Bostrychia scorpioides* had higher uptake rates and affinity for the most-abundant N source (NH_4^+) and presented surge uptake mechanisms, while *C. caespitosa* had a greater ability to take up PO_4^{3-} and saturation kinetics for NH_4^+ and NO_3^-.

4. Both species are able to fully recover photosynthetic activity on a daily basis (up to 8h) and after 3 days of continuous emersion. Emersion drastically reduces the growth of *C. caespitosa*, whereas that of *B. scorpioides* is much less sensitive to increasing daily emersion periods.

5. Considering field and laboratory studies responses to environmental variables, emersion, irradiance and nutrient availability are the most influential ones in the growth of *B. scorpioides* and *C. caespitosa*.

6. Increasing density of the canopy vegetation ameliorates physical stress, facilitating the growth of *C. caespitosa* due to its lower tolerance to emersion, and constrains the growth of *B. scorpioides*, due to its higher light requirements for growth. In consequence, changes in canopy density did not alter the total biomass of the macroalgal assemblage.

7. Biomass of the two rhodophytes followed a clear annual pattern, with increasing photosynthesis, growth and internal nutrient content from winter to spring. Both species were not N-limited during the study period, but P-limited.

8. The spatial distribution of *B. scorpioides* and *C. caespitosa* in the intertidal zone of the Palmones Estuary is strongly influenced by physical factors, having biotic interactions a marginal influence. Emersion variables and nutrient availability set the upper limits of both species , and prevents *C. caespitosa* from growing at the upper zone, while light limits the distribution of *B. scorpioides* to the intermediate and uppermost intertidal zones.

Conclusiones

1. *Bostrychia scorpioides* posee una mayor variabilidad fenotípica a escala espacial (zonación) y estacional. Esta especie presentó una elevada adaptación a alta irradiancia plasticidad fotofisiológica, capacidad de reducir las tasas de renovación de N y P internos en alturas de marea donde el aporte de nutrientes es menor y mantuvo tasas de crecimiento positivas en periodos severos de emersión diaria. Este conjunto de respuestas permite a esta especie afrontar condiciones ambientales más fluctuantes, lo que explica su clara dominancia en lugares expuestos a mayor irradiancia, su presencia en los niveles más altos del intermareal y la distribución homogénea de su biomasa entre los distintos niveles mareales.

2. *Catenella caespitosa* muestra una gran capacidad fotofisiológica adaptada a bajas irradiancias, con bajísimos requerimientos lumínicos para su crecimiento, la minimización de las tasas de respiración y un alto contenido de carbono específico por superficie de talo. Esta respuesta fisiológica, junto a su baja capacidad para reducir la demanda interna de N en posiciones más altas del intermareal y su mayor sensibilidad a las variables de emersión, explican su dominancia en las zonas intermareales más bajas y en lugares sombríos.

3. *Bostrychia scorpioides* posee mayor capacidad y afinidad en la incorporación de la fuente de N más abundante (NH_4^+) y presentó mecanismos de incorporación rápida (surge uptake), mientras que *C. caespitosa* tiene una mayor habilidad para incorporar PO_4^{3-} y presentó cinéticas de saturación para el NH_4^+ y el NO_3^-.

4. Ambas especies son capaces de recuperar su actividad fotosintética tras periodos de emersión diarios (hasta 8 h) y tras 3 días de emersión continua. El crecimiento de *C. caespitosa*, se reduce drásticamente con la emersión, mientras que el de *B. scorpioides* muestra una sensibilidad mucho menor frente a periodos diarios de emersión crecientes.

5. Considerando las respuestas a las variables ambientales obtenidas en experimentos de campo y laboratorio, la emersión, la irradiancia y la disponibilidad de nutrientes son aquellas más influyentes en el crecimiento de *B. scorpioides* y *C. caespitosa*.

6. El aumento de densidad en el dosel de vegetación aminora el estrés físico, lo cual facilita el crecimiento de *C. caespitosa*, dada su menor tolerancia a la emersión, y limita el de *B. scorpioides*, debido a sus mayores requerimientos lumínicos para

crecer. En consecuencia, las variaciones en la densidad del dosel no alteraron la biomasa algal total.

7. La biomasa de ambas especies presentó un claro patrón estacional, con un aumento de fotosíntesis, crecimiento y contenido interno de nutrientes de invierno a primavera. Ambas especies no estuvieron limitadas por N pero sí por P durante el periodo de estudio.

8. Los factores físicos influyen de forma directa en la distribución espacial de *B. scorpioides* and *C. caespitosa* en el intermareal del estuario del río Palmones, teniendo las interacciones bióticas un papel marginal. Las variables de emersión y la disponibilidad de nutrientes establecen los límites superiores de distribución de ambas especies, e impiden el crecimiento de *C. caespitosa* en la zona superior del intermareal, mientras que la luz es el principal factor que limita la distribución de *B. scorpioides* a la zona intermedia y superior de éste.

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List of Publications

This thesis was written as six inter-related chapters, of which three have been published (chapters 3, 4, 6) in peer reviewed journals. All content of each chapter is almost exactly as in the manuscripts, although they include some additional data.

Published articles

- **Sánchez de Pedro, R.**., Niell, F.X., Carmona, R., 2013. Differential nutrient uptake by two segregated red algae in an estuarine intertidal zone. *Phycologia* 52, 461–471. doi:10.2216/13–147.1 (Chapter 4)

- **Sánchez de Pedro, R.**, Niell, F.X., Carmona, R., 2014. Understanding the intertidal zonation of two estuarine red macroalgae from *ex situ* photoacclimative responses. *Eur. J. Phycol*, 49, 538–549. doi:10.1080/09670262.2014.978394 (Chapter 3)

- **Sánchez de Pedro, R.**, Karsten, U., Niell, F.X., Carmona, R., 2016. Intraspecific phenotypic variation in two estuarine rodophytes across their intertidal zonation. *Mar. Biol.*, 163: 221. doi: 10.1007/s00227-016-2997-5 (Chapter 6).

Conference contributions

- Carmona, R., Niell, F.X., Ruiz Nieto, M., **Sánchez de Pedro, R.**, 2012. A reassessment of Fucacean communities next to a cancelled waste in the Northwest of Spain. Effects of the improvement of the environmental protection technology. 10th IWA Symposium on Forest Industry Wastewaters and 8th International Conference on the Fate and Effects of Pulp and Paper Mill Effluents. Ciudad de Concepción (Chile). Collaborator.

- **Sánchez de Pedro, R.**, Niell, F.X., Carmona, R., 2014. Zonación intermareal a pequeña escala: Variabilidad intraespecífica en la fisiología de dos rodofíceas estuáricas, in: X Congreso de Ficología de América Latina Y El Caribe. Métepec, México. doi:10.13140/2.1.2331.8082. Poster.

- **Sánchez de Pedro, R.**, Niell, F.X., Carmona, R., 2015. Nutrient and light responses in two estuarine rhodophytes: implications for their zonation. *Sixth European Phycological Congress*. 50 - sup 1, pp. 118 - 119. Oral paper.
- **Sánchez de Pedro, R.**, Niell, F.X., Carmona, R., 2015. Zonation of two estuarine rhodophytes: Intraspecific variability at such a small-scale?. *Sixth European Phycological Congress*. 50 - sup 1, pp. 160. Poster.
- **Sánchez de Pedro, R.**, Niell, F.X., Carmona, R., 2015. Primary production dynamics of two rhodophytes in an Atlantized-Mediterranean estuary. Volumen de Comunicaciones presentadas en el *VIII Simposio sobre el Margen Ibérico Atlántico*, pp. 21 - 24. Depósito legal MA 1272-2015. Oral paper.

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In the intertidal zone of Palmones estuary (South Spain) the rhodophytes *Bostrychia scorpioides* and *Catenella caespitosa* are spatially segregated but grow together at intermediate tidal positions where their horizons overlap. **But how similar can two species be so they can coexist?** This dissertation aims to characterize the ecophysiological differences and similarities between the two species, and to identify the environmental variables with major influence in their zonation. Living together implies a shared use of common resources, and different competitive and facilitative interactions can take place under such a scenario.

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