



TESIS DOCTORAL

Distribución geográfica de macroalgas boreoatlánticas en su límite sur:
mecanismos, patrones de cambio e impactos ecológicos

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CERTIFICA

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral “Distribución geográfica de macroalgas boreoatlánticas en su límite sur: mecanismos, patrones de cambio e impactos ecológicos”, son aptos para ser presentados por Linney Duarte Mora ante el tribunal que en su día se consigne, para aspirar al Grado de Doctor en Conservación de Recursos Naturales por la Universidad Rey Juan Carlos de Madrid.

VºBº Director Tesis

Dra. Rosa M. Viejo G.

*A mis padres, por todo y por tanto,
y a la memoria de mi tío Miguel Mora*

Diseño de portada: Kevin Ng

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Entender los factores que determinan la distribución de las especies y los cambios que se producen en las fronteras de distribución son temas claves en ecología y biología evolutiva. En los últimos años los efectos antropogénicos sobre los sistemas naturales y el cambio climático global han intensificado considerablemente el interés sobre los procesos que se producen en los límites de distribución. De especial importancia son las poblaciones en los límites sur, que tienen un gran valor ecológico, evolutivo y de conservación, y sin embargo han recibido menos atención que poblaciones en latitudes norte. Estas poblaciones pueden extinguirse o persistir bajo escenarios de cambio climático. Las poblaciones marginales pueden localizarse en refugios climáticos, presentar adaptaciones o respuestas fenotípicas a las condiciones ambientales. Las comunidades intermareales de costas rocosas constituyen excelentes sistemas para el estudio de los factores determinantes de la distribución de especies y los efectos del cambio climático. Las algas pardas de gran porte, formadoras de dosel, dominan muchos de estos sistemas intermareales y constituyen especies claves, ingenieros autogénicos que crean hábitats para otras especies, siendo por tanto cruciales en el mantenimiento de la diversidad costera. Estudios recientes indican que la interacción entre factores biológicos y abióticos puede originar cambios en la distribución futura de especies de algas. En la Península Ibérica encontramos el límite sur de distribución de muchas macroalgas de gran porte y afinidades boreoatlánticas, algunas de las cuales experimentan cambios en su distribución geográfica. Entre estas especies, se encuentran dos *Fucales* intermareales, con diferencias en su ciclo de vida (ej. longevidad y capacidad de dispersión), las macroalgas *Fucus serratus* L. e *Himanthalia elongata* (L.) S.F. Gray, que han sufrido cambios en su distribución en el N de España a lo largo del pasado siglo. En esta tesis, reportamos el cambio en la distribución geográfica en el último siglo de especies formadoras de dosel como *Fucus serratus* y *Himanthalia elongata*, que no tienen equivalentes funcionales en los sistemas marinos. *Himanthalia* prácticamente ha desaparecido de la cornisa Cantábrica, mientras que las poblaciones de *F. serratus*, se mantienen en el oeste de la Península Ibérica, a pesar de observarse también drástica pérdida en su abundancia. Por otra parte, la distribución parcheada de poblaciones de *F. serratus* en las Rías Bajas, parece estar ligada a distintos factores tales como: la abundancia de amonio, las bajas temperaturas del agua en el verano, alta variabilidad en la salinidad y la presencia de *Himanthalia elongata*. En general, poblaciones de *Fucus serratus* en las localidades estudiadas muestran señales de adaptación local. Identificándose también que *F. serratus* ocupa lugares mas benignos en las Rías Bajas que en Lugo. En este límite sur de distribución geográfica (Península Ibérica) de *F. serratus*, también detectamos cambios en la estructura de las comunidades bentónicas, con alteraciones en las posiciones tróficas de algunos consumidores, los cuales en algunos casos, ocupan posiciones tróficas más bajas, específicamente en localidades donde *F. serratus* ha prácticamente desaparecido.

INTRODUCCIÓN GENERAL



"A journey of a thousand miles must begin with a single step."
Lao Tzu

La estructura y dinámica de los límites de distribución geográfica de las especies y los mecanismos que las determinan son uno de los temas de mayor interés en ecología y biología evolutiva (MacArthur 1972, Gaston 2003). Las fronteras de distribución se relacionan frecuentemente con límites fisiológicos, interacciones entre especies, la presencia de barreras en la dispersión o una combinación de estos factores (Gaston 2003, Parmesan y col. 2005, Sagarin y col. 2006). La estructura espacial de los límites de distribución de especies es compleja y variable, ya que se pueden observar fronteras abruptas, donde en un espacio reducido se produce la transición desde la presencia continua de poblaciones a la ausencia total de la especie, pero también pueden aparecer poblaciones marginales aisladas y dispersas que presentan una elevada persistencia temporal o que son, por el contrario, esporádicas (Gaston 2009). Las fronteras pueden permanecer estáticas durante largos periodos de tiempo, o desplazarse, contrayéndose o expandiéndose, como consecuencia de la evolución paulatina del nicho de la especie o debido a cambios en las condiciones ambientales (Gaston 2009, Sexton y col. 2009). En los últimos años, ante los efectos antropogénicos a escalas locales sobre los sistemas naturales y el cambio climático global, se ha reforzado el interés sobre la estructura, dinámica y mecanismos que determinan los patrones de distribución geográfica de las especies, así como las consecuencias que dan lugar a esos desplazamientos de fronteras en la estructura y función de las comunidades y la diversidad biológica (Channell y Lomolino 2000, Thomas y col. 2004, Parmesan y col. 2005).

De hecho, existen ya numerosas evidencias de desplazamientos en la distribución geográfica de organismos ligadas al cambio climático reciente (ej. Walther y col. 2002, Parmesan y Yohe 2003, Lenoir y col. 2008). No obstante, son relativamente más frecuentes los estudios realizados sobre los procesos de colonización que se producen en el límite de distribución frío, más cercano a los polos (o límite superior, en distribuciones altitudinales), en general en expansión, que los procesos que ocurren en el límite cálido, más próximo al ecuador (o bajo en

altitud), “the rear edge” (Hampe y Petit 2005, Jump y col. 2009). Asimismo, los límites fríos y cálidos se han relacionado tradicionalmente con mecanismos diferentes, lo que puede determinar respuestas divergentes al cambio climático. Dado que la riqueza de especies en muchos grupos se incrementa en zonas tropicales y que las condiciones climáticas parecen relativamente benignas en dichas latitudes, se ha sugerido que las interacciones biológicas son más importantes hacia el ecuador, y por ello, los límites sur se han relacionado con factores biológicos más que con una tolerancia limitada de las especies a condiciones físicas (MacArthur 1972). Sin embargo, los resultados de una revisión reciente, realizada a partir de 125 estudios, indican que los límites cálidos se relacionan más frecuentemente con factores abióticos que con interacciones biológicas (Cahill y col. 2014). Por otro lado, otra revisión de Sunday y colaboradores (2012) sugiere que las causas determinantes de límites de distribución cálidos (sur en el hemisferio norte) son diferentes en el medio marino y terrestre. Al menos en el caso de organismos ectotermos, los límites sur se presentan asociados a rangos de tolerancia térmicos en especies marinas, mientras que en organismos terrestres otros factores, como las interacciones biológicas podrían jugar un papel determinante. Los resultados de revisiones recientes son, en cierta medida, contradictorios, y en la actualidad se desconocen las causas próximas que determinan los límites de distribución de muchas especies, en concreto el límite cálido de distribución. Las interacciones biológicas pueden además verse modificadas por las condiciones ambientales, lo que añade complejidad a las respuestas de las especies al cambio climático (Davis y col. 1998). Así, se ha observado que ante condiciones ambientales estresantes en el límite sur de distribución diversas especies son más susceptibles a patógenos y a depredadores (Tomanek y Somero 1999, Mieszkowska y col. 2006).

Las poblaciones situadas en el límite de distribución cálido o sur tienen un gran valor ecológico, evolutivo y de conservación (Hampe y Petit 2005), al presentar con frecuencia una elevada diversidad y/o diferenciación genética respecto a poblaciones centrales (ej. Petit y col. 2003, Hewitt 2004, Neiva y col.

2012, Nicastro y col. 2013). Este patrón se ha observado en especies del hemisferio norte que durante los procesos glaciales ocurridos durante el Cuaternario se han refugiado en áreas a bajas latitudes (Provan 2013, Hampe y Jump 2011 y referencias incluidas). Las poblaciones marginales pueden encontrarse en micro climas favorables para su desarrollo (Lennon y col. 2002, Gaston 2009, Oliver y col. 2009), y presentar adaptaciones locales (Matías y col. 2014) o respuestas fenotípicas a estos ambientes marginales (Jueterbock y col. 2013 y referencias incluidas).

Ante el cambio climático y los factores antropogénicos que actúan a escalas más locales las especies pueden adaptarse, desplazarse o extinguirse (Thuiller y col. 2008, Pearson y col. 2009). El desplazamiento de límites de distribución, de hecho, supone procesos de extinción y colonización locales. La extinción de especies no es un proceso aleatorio, ya que algunas son más susceptibles que otras a los factores antrópogenicos y ambientales (Bracken y col. 2008). Si las especies que desaparecen a escala local son aquellas que juegan un papel clave en los ecosistemas, su desaparición alterará el funcionamiento general de los mismos.

Los ecosistemas marinos y en concreto los sistemas costeros se encuentran entre los más valiosos a nivel global desde el punto de vista ecológico y socioeconómico (Barbier y col. 2011) y en la actualidad se encuentran amenazados por el cambio climático e impactos antropogénicos que actúan a escala más local (Hughes 2005 y referencias en este respecto, Harley y col. 2006). La temperatura superficial del océano se ha incrementado un promedio de 0.11°C por década en el período de 1971-2010, y se ha producido una reducción del pH del mismo (Orr y col. 2005, Harley y col. 2012, IPCC 2014). La pérdida en la cobertura de hielo (Dyurgerov y Meier 1997) ha contribuido a los cambios en el nivel del mar, indicándose que entre los años 1900-2010 el nivel promedio del mar se incrementó en 1.7 mm yr^{-1} , mientras que entre 1993-2010 el incremento fue de 3.3 mm yr^{-1} (IPCC 2014). Se prevén además cambios globales en el oleaje, afloramiento y salinidad de los océanos debido a distintos factores como variaciones en los vientos

en las superficies de los océanos, cambios en las precipitaciones, y la pérdida de glaciares (Bakun 1990, Bopp y col. 2001, Grabemann y Weisse 2008, Hoegh-Guldberg y Bruno 2010, IPCC 2014 y referencias en este respecto). Estudios recientes reflejan impactos ya detectados en la distribución y fenología de diversos organismos marinos relacionados con el calentamiento global o la acidificación de los océanos (Beaugrand y col. 2008, Doney y col. 2009, Hoegh-Guldberg y Bruno 2010, Harley y col. 2012, Koch y col. 2013).

En sistemas costeros las macroalgas de gran porte, formadoras de dosel (la gran mayoría algas pardas, Phaeophyceae) constituyen ingenieros autogénicos o especies fundadoras, que modifican el ambiente, creando estructura para otras especies además de ser fuentes de materia y energía para organismos situados en niveles superiores de la red trófica (Jones y col. 1997, Stephenson y col. 1986). En diversas costas del mundo se ha observado la retracción de la distribución o la reducción de abundancia en poblaciones marginales de macroalgas, que se han relacionado con el cambio climático reciente y otros factores antropogénicos (Vogt y Schramm 1991, Sagarin y col. 1999, Alcock 2003, Schiel y col. 2004, Lima y col. 2007, Ugarte y col. 2009, Wernberg y col. 2011). Por ejemplo en Australia aumentos de la temperatura del agua y altos niveles de CO₂ han dado lugar a la extinción local de Laminariales y al incremento de algas de menor porte (Connell y Russell 2010, Russell y col. 2009). En costas europeas también se han detectado este tipo de cambios, desde comunidades dominadas por algas de gran porte a sistemas con abundancia de especies cespitosas, cambios relacionados con contaminación urbana o cambios climáticos recientes (Airoldi y col. 2008 y referencias incluidas). En Japón poblaciones de macroalgas han prácticamente desaparecido, siendo remplazadas por barreras de corales, lo que también se relaciona con el incremento en la temperatura y el aumento en la abundancia de peces tropicales (Nakamura y col. 2013 y referencias en este respecto).

Sin embargo, son muy poco los estudios que reflejan como la pérdida de algas formadoras de dosel y la simplificación del hábitat afecta a la redes tróficas (Airoldi y col. 2008, Byrnes y col. 2011, Coll y col. 2011). Los cambios en redes

tróficas pueden analizarse mediante el uso de abundancias naturales de isótopos estables de carbono y nitrógeno (Peterson y Fry 1987, Forero y Hobson 2003). El valor de $\delta^{15}\text{N}$ de cada especie indica su posición trófica, mientras que el $\delta^{13}\text{C}$ sugiere la posible dieta (DeNiro y Epstein 1978, Peterson y Fry 1987, Rau y col. 1983). Los análisis de isótopos estables por tanto permiten evaluar el impacto de los cambios recientes en la distribución de especies de macroalgas, en concreto de las extinciones de poblaciones marginales en el límite sur, sobre la estructura de las redes tróficas costeras (ej. Rau y col. 1983, Forero y Hobson 2003, Laurand y Riera 2006).

Los organismos intermareales de costas rocosas, como macroalgas e invertebrados sésiles, son idóneos para estudios de patrones y mecanismos de distribución, y se consideran indicadores tempranos de los impactos del cambio climático en medios costeros, ya que están sujetos a condiciones ambientales como la temperatura cercanas a sus límites de tolerancia fisiológicos (Helmuth y col. 2006 y referencias en este respecto). Además, la distribución de estos organismos costeros puede considerarse unidimensional, y son especies fáciles de manipular, por ejemplo de trasplantar de una costa a otra (Raffaelli y Hawkins 1996, Correa y col. 2006).

Las costas atlánticas y cantábricas de la Península Ibérica constituyen un escenario ideal para el estudio de patrones de distribución, mecanismos determinantes y cambios en especies de macroalgas intermareales de gran porte. En esta zona, que ha constituido un refugio durante la última glaciación para diversas especies de macroalgas de afinidad boreoatlántica, se localiza el límite de distribución sur para estas especies (Gómez y Lunt 2006, Bennet y Provan 2008, Provan 2013) y las poblaciones marginales aquí presentes albergan *pools* genéticos únicos (Provan 2013). Además existen numerosos datos históricos sobre la distribución de varias especies de algas desde principios del siglo XIX en esta zona, desde el Golfo de Vizcaya hasta las costas Atlánticas Gallegas (ej. Sauvageau 1897, Fischer-Piette 1963, Anadón y Niell 1980, Anadón 1983, Gorostiaga y col. 2004). Estudios anteriores indican que en los límites sur de

distribución varias Fucales se localizan en áreas más protegidas al oleaje, a diferencia de las zonas centrales de distribución, describiéndose también mayor abundancia de herbívoros que en el norte de Europa (Southward y col. 1995). En las costas de la Península Ibérica se están produciendo cambios en la abundancia local, en algunos casos con extinciones y modificaciones en la distribución de varias de estas algas pardas de gran porte (Lima y col. 2007, Fernández 2011, Viejo y col. 2011, Díez y col. 2012, Martínez y col. 2012, Nicastro y col. 2013). Investigaciones apuntan al mismo tiempo al incremento en la temperatura superficial del océano en la Península en los últimos 50 años (Lima y col. 2007, deCastro y col. 2009).

Entre las especies que tienen en la Península Ibérica su límite sur de distribución se encuentran las algas Fucáceas *Fucus serratus* L. y *Himanthalia elongata* (L.) S.F Gray (Lüning 1990). Ambas especies presentan dos límites de distribución en la Península Ibérica: uno situado en el noreste de la costa cantábrica y otro en costas Atlánticas, en el norte de Portugal (Lüning 1990). La distribución de *F. serratus* es bastante diferente en la cornisa cantábrica que en la zona atlántica: en la primera aparece muy continua en la costa oeste (Arrontes 1993, 2002), mientras que en las costas atlánticas solamente aparecen poblaciones muy dispersas dentro de grandes ensenadas o rías (Pazó y Niell 1977). La capacidad de dispersión de *F. serratus* es más limitada que la de *H. elongata* (Arrontes 2002, Coyer y col. 2003), lo que sugiere diferencias en estas especies en su respuesta a cambios ambientales (Alcock 2003, Hiscock y col. 2004). En el último siglo se han detectado cambios en la distribución de ambas especies en el N de España (Anadón and Niell 1981, Arrontes 1993, 2002, Alcock 2003), que parecen estar ligados a factores ambientales (Martínez y col. 2012), además condiciones físicas negativas han mostrado afectar la reproducción de *F. serratus* (Viejo y col. 2011).

OBJETIVOS

El objetivo general de esta tesis doctoral fue la determinación de la estructura y dinámica de los límites de distribución sur (Península Ibérica) de las macroalgas *Himanthalia elongata* (L.) S.F. Gray y en especial de *Fucus serratus* L., investigando los cambios recientes en dichos patrones, los mecanismos determinantes de los mismos y las consecuencias de estos cambios en la estructura de las comunidades intermareales y la red trófica costera. Para conseguir este objetivo, se desarrollaron los siguientes objetivos específicos:

1. Examinar los cambios históricos y recientes en la distribución y abundancia de *Himanthalia elongata* y de *Fucus serratus* en la Península Ibérica, y relacionar estos cambios con las variaciones ambientales (temperatura superficial de las aguas) y las características del ciclo de vida de las especies (longevidad y capacidad de dispersión).
2. Determinar las causas de la distribución parcheada y persistente de *Fucus serratus* dentro de las rías en la costa Atlántica del noroeste de España.
3. Comparar el crecimiento de individuos y la tasa de reclutamiento de poblaciones de *F. serratus* dentro de Rías con las de poblaciones remanentes en la costa N (costa de Lugo), relacionando las variaciones detectadas con cambios en el ambiente físico y biótico y con la posible presencia de diferenciación adaptativa entre poblaciones atlánticas y cantábricas.
4. Determinar las consecuencias de la extinción local de poblaciones de *Fucus serratus* en la estructura y función de las comunidades intermareales, comparando la composición y abundancia de algas y epifauna asociada, y la estructura de la red trófica en localidades donde la macroalga *F. serratus* todavía es abundante con localidades marginales donde la abundancia de la alga se ha reducido drásticamente en los últimos años.

METODOLOGÍA GENERAL

Los materiales y métodos de esta tesis están descritos de forma detallada en cada uno de los capítulos. No obstante, a continuación se explica brevemente la metodología empleada.

1. Áreas de estudio

En general el área de estudio se desarrolló en las costas rocosas del océano Atlántico y el mar Cantábrico o Golfo de Vizcaya de la geografía española (Fig. 1a).

En el capítulo I se visitaron 217 lugares a lo largo de la Península Ibérica. El capítulo II fue realizado en distintos lugares de la costa Atlántica, específicamente en la Ría de Muros y Noya, y en la Ría de Arosa (Fig. 1b), donde históricamente la macroalga *F. serratus* tiene una distribución bastante parcheada. El capítulo III también se realizó en las costas de las Rías de Muros y Arosa, y en las costas Cantábricas en Lugo (Fig. 1c), donde *F. serratus* mantiene poblaciones abundantes. El capítulo IV presenta datos obtenidos en las costas Cantábricas (Fig. 1d), en específico en localidades de Asturias (Novellana y Cadavedo), donde recientemente se ha identificado que *F. serratus* presenta una drástica reducción en su abundancia; las otras dos localidades están en Lugo, Galicia (San Pedro y Area Longa) donde la macroalga tiene una mayor permanencia temporal.

2. Distribución geográfica de *Fucus serratus* L. y *Himanthalia elongata* (L.) S.F. Gray

Para el capítulo I se realizó una revisión bibliográfica y recopilación de datos de herbarios sobre la distribución geográfica de las algas formadoras de dosel: *Fucus serratus* y *Himanthalia elongata*, desde finales del siglo XIX hasta nuestros días. Además, se realizaron rastreos detallados en el campo para determinar la presencia o ausencia de las dos especies en localidades del noroeste

Peninsular. Uno de ellos, fue llevado a cabo en los años 2004-2006 por el equipo de investigación (ver Martínez y col. 2012), y el segundo fue realizado en 2008-09 y 2011 (rastreos personales).

3. Experimentos de trasplantes de *Fucus serratus*

En el capítulo II se realizaron trasplantes con individuos juveniles y embriones de *Fucus serratus* en la Ría de Muros y Noya, situada en la Provincia de A Coruña, y la Ría de Arosa que se encuentra entre las Provincias de A Coruña y Pontevedra. Los trasplantes con juveniles se realizaron durante dos períodos del año, entre localidades donde la especie *Fucus serratus* mantiene poblaciones abundantes (localidades origen), y localidades interiores y exteriores respecto a las de origen, donde la especie nunca ha sido descrita, pero otras especies como *Ascophyllum nodosum* y *Fucus vesiculosus* si son abundantes. También en el capítulo III se realizaron trasplantes con juveniles de *F. serratus* entre las localidades origen de las Rías y localidades de Lugo, donde poblaciones de *F. serratus* se han mantenido a través del tiempo. Para los experimentos de trasplantes se utilizaron alrededor de 140 individuos.

En los experimentos de trasplantes con embriones de *F. serratus* se emplearon discos artificiales hechos con una mezcla de epoxy. Los discos fueron colocados en localidades de la Ría de Muros y Noya, y la Ría de Arosa bajo el dosel de *F. serratus*. Posteriormente se trasplantaron parte de estos discos a localidades internas y externas respecto a la localidad de origen, dentro de cada una de las Rías (capítulo II). Asimismo discos artificiales colocados y retirados periódicamente se utilizaron para realizar estimas de reclutamiento natural en las distintas localidades de las Rías y Lugo, a lo largo del año, cuyos resultados se presentan en el capítulo III.

4. Experimentos en el laboratorio

Debido a los resultados obtenidos en el experimento de trasplantes de juveniles de *F. serratus* entre localidades de Lugo y de las Rías, se decidió posteriormente realizar dos experimentos en el laboratorio con acuarios: uno de preferencia alimentaria de macro-herbívoros y otro de crecimiento de frondes de *F. serratus*, cuyos detalles aparecen en el capítulo III. El experimento de preferencia alimentaria se realizó con tres especies de macro-herbívoros: los isópodos *Dynamene bidentata* Philippi y *Idotea balthica* Pallas, y el gasterópodo *Gibbula pennanti* Philippi, e individuos de *Fucus serratus* procedentes de localidades de las Rías (Ofreixo y Isla de Arosa), y localidades de Lugo (San Pedro y Peizas, ver Fig. 1c). El otro experimento de crecimiento de frondes de *F. serratus* se realizó con algas procedentes de estas localidades y fueron sujetos a las mismas condiciones ambientales (luz, fotoperiodo, nutrientes y temperatura del agua).

5. Medida de variables físicas

En el capítulo I se presentó la temperatura media superficial del agua de Agosto desde 1870 al 2009 (colaboración con el equipo del Dr. Gómez-Gesteira, Laboratorio de Física Ambiental de la Universidad de Vigo). Para el capítulo II y III se obtuvieron datos de temperatura del agua y de salinidad. Se colocaron durante dos años registradores de temperatura en distintas localidades dentro de las dos Rías (la Ría de Muros y Noya, y la Ría de Arosa), y en localidades de la costa de Lugo, para la obtención de datos diarios de temperatura del agua. Además, se obtuvieron datos de salinidad de localidades cercanas a los lugares de estudio (información recopilada por el Instituto Tecnológico para el control de Medio Marino de Galicia, Xunta de Galicia, Vilagarcía de Arousa, Provincia de Pontevedra, España). Asimismo, se tomaron muestras de nutrientes inorgánicos en el agua de mar, en localidades de las Rías Bajas y de Lugo, en 3 fechas distintas y cuyos resultados se presentan en los capítulos II y III.

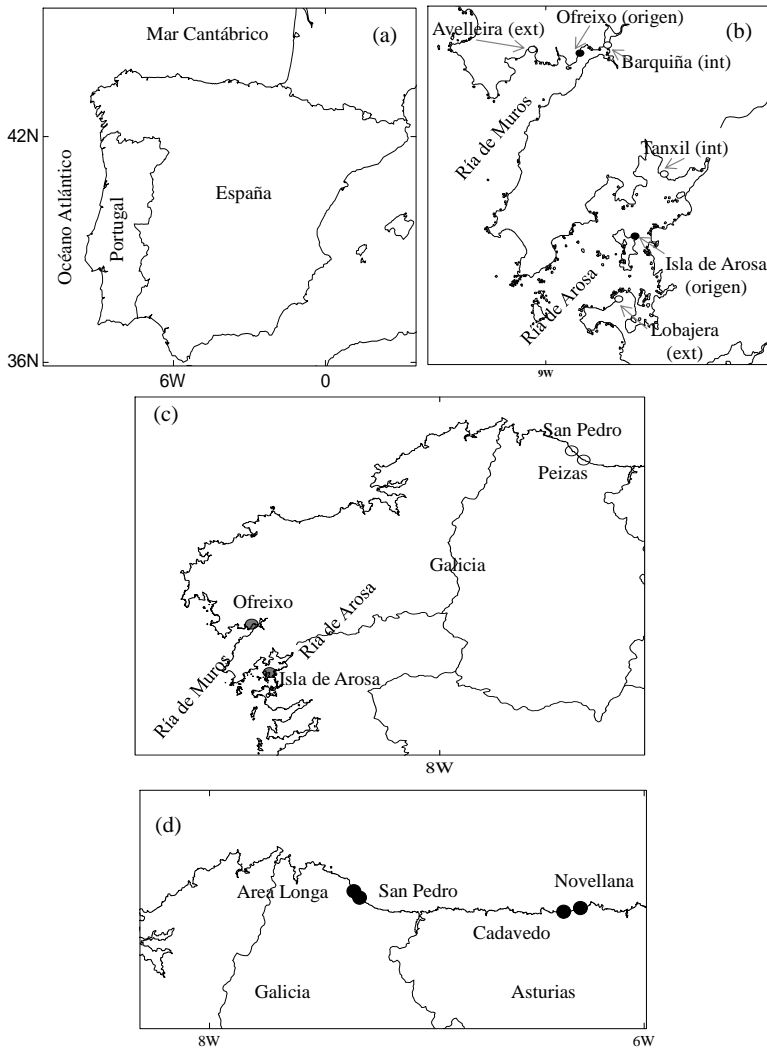


Figura 1. Localidades de estudio en costas rocosas del Océano Atlántico y Mar Cantábrico.

6. Análisis para el estudio de redes tróficas

Para el capítulo IV se tomaron muestras bentónicas, de las algas más abundantes e invertebrados consumidores de algas para los análisis de isótopos estables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$). Las muestras fueron recolectadas en zonas con reducida abundancia de *F. serratus* (localidades en Asturias), y en localidades con mayor permanencia temporal de la macroalga formadora de dosel (localidades en Lugo,

Galicia). Además se hizo el análisis de las posibles dietas de algunos de los herbívoros con el modelo mixto Bayesiano SIAR (Parnell y col. 2010).

7. Modelo de hábitat

En el capítulo II se realizó un modelo de distribución de hábitat de *Fucus serratus* en las dos Rías Bajas estudiadas (la Ría de Muros y Noya, y la Ría de Arosa). Las variables físico y químicas seleccionadas fueron la temperatura media de verano (2 años de estudio), nitrato + nitrito, amonio, fosfato, salinidad y la desviación estándar de la salinidad. Además, se seleccionó la presencia de *F. vesiculosus*, *H. elongata* y el tipo de sustrato como variables explicativas de la presencia de *F. serratus* en estas áreas.

8. Análisis estadísticos

Algunos de los datos de los capítulos II, III, y IV se analizaron utilizando análisis de varianza (ANOVA). En los capítulos II y III los datos de cobertura de algas y macro-invertebrados se analizaron utilizando PERMANOVA (Anderson et al. 2005). Además, SIMPER análisis fueron utilizados para identificar los macro-invertebrados que contribuye a las diferencias entre áreas o localidades (Clarke 1993). Diferencias entre Rías o localidades en el reclutamiento natural de embriones *F. serratus* fueron analizados usando Binomial Negativo (Generalized Lineal Models). Por último en el capítulo IV los valores de los sitios de $\delta^{13}\text{C}$ y $\delta^{15}\text{N}$ de algas e invertebrados fueron analizados usando modelos de regresión tipo II (Major axis method, Legendre & Legendre 1998).

Para alcanzar los objetivos de esta tesis doctoral, la misma se ha estructurado en cuatro capítulos que han sido escritos en inglés. A continuación se presenta un breve resumen del contenido de los mismos.

Capítulo I. *Recent and historical range shifts of two canopy-forming seaweeds in North Spain and the link with trends in sea surface temperature.* Se examinó la distribución reciente e histórica de las algas formadoras de dosel *Fucus serratus* L. y *Himanthalia elongata* (L.) S.F. Gray. En este trabajo se realizó una revisión bibliográfica y de herbarios, así como también se ejecutaron rastreos de la distribución de éstas macroalgas en la Península Ibérica. Cambios recientes e históricos en la distribución de las macroalgas se relacionaron con cambios en la temperatura superficial del agua en los últimos 100 años y las características del ciclo de vida de las especies (longevidad y capacidad de dispersión). Los resultados de este capítulo han sido publicados en *Acta Oecologica* (Duarte y col. 2013. 51: 1-10).

Capítulo II. *Drivers of the scattered distribution of the foundational alga Fucus serratus L. inside estuarine refuges at its southern range limit.* En este estudio se realizaron experimentos de trasplantes con juveniles de *Fucus serratus*, los cuales se llevaron a cabo durante dos períodos del año entre localidades donde la especie *F. serratus* mantiene poblaciones abundantes en las Ría de Muros y Noya y la Ría de Arosa (localidades origen) y localidades interiores y exteriores respecto a la de origen dentro de las Rías donde la especie nunca ha sido descrita, pero otras Fucales como *Ascophyllum nodosum* y *Fucus vesiculosus* son abundantes. Además, se realizaron trasplantes con embriones de *F. serratus*. Los discos fueron colocados en localidades de las Rías bajo el dosel de *F. serratus* y posteriormente se trasplantaron parte de estos discos a localidades internas y externas respecto a la localidad de origen, dentro de la misma Ría.

Finalmente, se ejecutaron modelos de hábitats, con el fin de poder explicar los factores involucrados en la distribución parcheada de *F. serratus* en las Rías Bajas.

Capítulo III. *Phenotypic differentiation among peripheral populations of the canopy-forming alga Fucus serratus at its retreating southern edge.* En este capítulo examinamos poblaciones marginales de *Fucus serratus* en las costas Atlánticas (noroeste de Galicia) y Cantábricas (Lugo, Galicia) donde la especie mantiene poblaciones. Comparándose la viabilidad de individuos de estas poblaciones marginales, que posiblemente presentan adaptaciones locales. También, se ejecutaron experimentos en el laboratorio con acuarios: un experimento de preferencia alimentaria con tres especies de macro-herbívoros (los isópodos *Dynamene bidentata* y *Idotea baltica*, y el gasterópodo *Gibbula pennanti*) y individuos de *Fucus serratus* procedentes de distintas poblaciones. El otro experimento de crecimiento de frondes de *F. serratus* procedentes de distintas localidades que fueron sujetos a las mismas condiciones ambientales (luz, fotoperiodo, nutrientes y temperatura).

Capítulo IV. *Effects of the alga Fucus serratus decline on benthic assemblages and trophic linkages at its southern range edge.* En este último capítulo se estudiaron los efectos de la drástica retracción de la macroalga *Fucus serratus* en las comunidades bentónicas y en las cadenas tróficas en su límite sur de distribución geográfica. Para esto se realizaron comparaciones entre localidades situadas en la misma latitud y con antecedentes de poblaciones abundantes de *F. serratus*. Toma de muestras de las comunidades bentónicas para análisis de isótopos estables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) en zonas con reducida abundancia de *F. serratus* (localidades marginales de distribución) y en localidades con mayor permanencia temporal de la macro alga formadora de dosel. Los resultados de este capítulo han sido publicados en Marine Ecology Progress Series (Duarte y col. 2015. 527: 87-103. doi: 10.3354/meps11248)

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**Recent and historical range shifts of two canopy-forming seaweeds in
North Spain and the link with trends in sea surface temperature**



F. serratus. Oleiros
August 2004, (R. Viejo)



H. elongata. Vilela beach.
July 2009, (L. Duarte)

Study published:

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ABSTRACT

Geographical range shifts of two canopy-forming seaweeds, *Himanthalia elongata* (L.) S.F. Gray and *Fucus serratus* L. were investigated at their southern range boundary in Northern Spain from the end of nineteenth century to 2009. Given the good dispersal abilities of *H. elongata* and its short life-span, we hypothesize that this species will track environmental changes at a faster rate than the perennial and short-distance disperser *F. serratus*. Our results show a continuous and drastic westward retraction of *H. elongata*, which has nowadays virtually vanished in Northern Spain, whereas *F. serratus* is still found in the westernmost area. Despite this, the first species is still relatively abundant in the Iberian Peninsula, whereas the presence of the latter is scattered and reduced. Overall, range shifts fit with the warming trend in sea surface temperature (SST), whereas it is unlikely that increases in grazing pressure or coastal pollution have driven the observed changes, particularly the rapid trend in recent years. Differences in species traits are linked to range dynamics. The higher persistence of *F. serratus* at eastern shores may thus be related to its longer life span and its greater thermal tolerance. The presence of sporadic populations of *H. elongata* outside the zone of continuous distribution can be attributed to long-distance dispersal events during cold pulses. Relict populations in isolated and estuarine locations were left behind in the contracting range margins, particularly for *F. serratus*. In Northern Spain, the westward retreat of large canopy-forming algae seems to be a general phenomenon, involving other species such as kelps. Therefore, an evident reorganization of coastal assemblages is expected, though the temporal extent of changes and the consequences for ecosystem services need to be evaluated.

Key-words: Biogeography, Canopy-forming algae, *Fucus serratus*, *Himanthalia elongata*, Range boundary, Sea surface temperature

1. Introduction

A comprehensive understanding of future consequences of climate change in species distributional patterns requires determining the nature of the range boundaries and their causes (Davis et al. 1998 a,b, Thomas & Lennon 1999, Gaston 2003, Parmesan et al. 2005). Defining the position of a species range limit is not straightforward, as the spatial structure of this limit is commonly complex, with zones where the presence of the species is continuous, sparse but persistent or sporadic (Gaston 2003 and references therein). In some cases, these limits are quite constant within long time, whereas other range boundaries shift frequently, retreating and expanding over time (Gaston 2009).

The range shifts could reflect spatial responses of the species to changes in environmental conditions. Indeed, the recent shifts in species geographic ranges documented in both marine and terrestrial systems have been commonly linked to global warming (Walther et al. 2002, Gaston 2003, 2009, Parmesan et al. 2005, Sagarin et al. 2006, Thomas et al. 2006, Lima et al. 2007, Beaugrand et al. 2008). Nevertheless, lags in the species reaction to environmental changes may occur as a consequence of dispersal limitations or life history traits, for instance the individual longevity (e.g. García et al. 1999, Sexton et al. 2009 and references therein). Disequilibria of the range boundaries with contemporary climatic conditions may thus differ between species with contrasting dispersal abilities or life history characteristics (e.g. Araújo & Pearson 2005, Hampe 2011).

An ideal system for the study of species' distribution mobility is the coastal realm and particularly the intertidal area of rocky shores. The simplicity of the geographic ranges of coastal species, which can be considered uni-dimensional, facilitates these studies. Moreover, species found in the intertidal zone are early indicators of the impacts of climate change, as they are frequently living on the limit of their physiological tolerance (Helmuth et al. 2006). Canopy-forming algae are important components of intertidal rocky shores. As these species act as autogenic engineers (*sensu* Jones et al. 1997) variations in their distribution may trigger changes in the whole system.

An increasing number of studies have documented shifts in macroalgae distributions, and these variations have been commonly attributed to rising sea surface temperatures (e.g. Southward et al. 1995, Sagarin et al. 1999, Schiel et al. 2004, Mieszkowska et al. 2005, Lima et al. 2007). In most of these studies only two observations at different times were compared. However, in Atlantic shores of the Iberian Peninsula, there is a rich historical record of intertidal species distributions from late nineteenth century to the present (e.g. Sauvageau 1897, Miranda 1931; Fischer-Piette 1955a, 1963, Ardré 1970, 1971, Anadón et al. 1979, 1983, Anadón & Niell 1981, Margalet & Navarro 1992, Margalet et al. 1993, Arrontes 1993, Morán, 1999, Alcock 2003).

Cold-temperate macroalgae have two southern distribution boundaries in this region, they are abundant at the Northwest corner and disappear both towards the inner part of the Bay of Biscay in Northern Spain and towards southern Portugal (Sauvageau, 1897, Fischer-Piette 1955a, Lüning 1990, Dinter 2001). Two of these species are the canopy-forming algae *Fucus serratus* L. and *Himanthalia elongata* (L.) S.F. Gray. Historical records suggest changes in their distribution in Northern Spain over the last century linked to changes in sea temperature (Fischer-Piette 1955a, Anadón & Niell 1981, Arrontes 1993, 2002, Gómez-Gesteira et al. 2008, deCastro et al. 2009). Nevertheless, only Alcock (2003) presented both biological and environmental information in his unpublished PhD dissertation. Furthermore, recent habitat distribution models related the current geographic range of these two species to a combination of climatic variables (Martínez et al. 2012). These two algae present different dispersal abilities and individual longevity. Thus, *F. serratus* has limited dispersal capacities and a life-span of up to 5 years (Rees 1932, Arrontes 1993, 2002, Coyer et al, 2003), while *H. elongata* is able to disperse over longer distances and plants are usually biennial (Sauvageau 1918, Moss 1969).

The general aims of this study are to compile and describe in detail the sequence of historical and recent changes in the distribution and abundance of these two canopy-forming seaweeds across the North Coast of Spain, and relate these changes to patterns in sea surface temperature (SST) from 1870 to 2009. The historical information is complemented with data from two field surveys carried out in 2004-2006 and 2008-2009. Potential expansions and retractions will be related to the correspondent temporal and spatial SST trends. Given the good dispersal capacity of *Himanthalia elongata* and its short life span, we hypothesize that this species would rapidly follow environmental changes, while *Fucus serratus* would not respond as quickly as *H. elongata* by expanding its distribution range especially during favorable periods.

2. Materials and Methods

2.1. Biogeographical survey

We reviewed the large-scale studies made on the distribution of *Himanthalia elongata* and *Fucus serratus* in NW Spain (Sauvageau 1897, Fischer-Piette 1955a, 1955b, 1963, Margalet & Navarro 1992, Margalet et al. 1993, Alcock 2003) as well as several publications and reports that examined the distribution of these species along shorter stretches of coast (Bornet 1892, Lázaro é Ibiza 1889, Sauvageau, 1918, Miranda 1931, Fischer-Piette & Seoane, 1962, Fischer-Piette & Ginsburg-Ardre, 1963, Niell 1977, Pazó & Niell 1977, Anadón et al. 1979, Anadón & Niell 1981, Anadón 1983, Arrontes 1993, Morán 1999, Gorostiaga et al. 2004).

We also carried a detailed field-survey along the Northwestern coast of Spain in 2004-2006, from San Antonio beach in Llanes (Asturias), west of Cape Peñas (43°27'N, 4°59'W), to Baiona in Galicia (42°7'N, 8°50'W) (Fig. 1). The presence/absence of *Himanthalia elongata* and *Fucus serratus* was recorded in 217 locations, which were visited during spring tides and referenced on the ground with a hand-held GPS Garmin 60 (Garmin Corporation, Olathe, Kansas, USA). In order to assure the reliability of the absences in each location, we inspected the intertidal fringe (of variable width) corresponding to a linear distance of 600-1000 m at each location (see Martínez et al, 2012, for further details). A second survey was done in 2008-2009 covering the North Coast of Spain until Gandario beach near La Coruña, Galicia (43°20'N, 8°13'W) (Fig. 1). The visited locations were those with presence of the target species in the previous surveys of 2004-2006. In 2011 four locations were added to this last survey, further East, in Cantabria province.

In order to estimate the abundance of *Fucus serratus* we carried out a quantitative sampling in 2008-2009 at six locations where the species was present: three of them were in the present range boundary of the species in West Asturias, and three of them in North Galicia, where it has a long temporal persistence (hereafter marginal and central areas; see Fig. 1). The locations were Cadavedo (43°33'N, 6°22'W), Novellana (43°34'N, 6°17'W) and Oleiros (43°34'N, 6°13'W) in the marginal area and Morás (43°43'N, 7°27'W), San Pedro (43°37'N, 7°19'W) and Area Longa (43°36'N, 7°17'W) in the central area. At each location, three sites about 20-50 m apart were randomly selected. Sampling was done during three dates: May and July 2008 and February 2009. At each date, four plots of 0.25 m² (also randomly selected) were sampled per site during low spring tides. Nevertheless, due to logistical constraints only two sites and three plots per site were sampled in central locations in May 2008. Primary and secondary cover (cover of algae directly on the substratum, i.e. holdfasts and short turf-forming individuals of ca 5 cm maximum length, and overstory canopies respectively) were estimated as point intercepts using a grid with 81 regularly spaced points.

2.2. Sea Surface Temperature (SST)

The distribution of cold-water species in Northern Spain has been linked to the spatial trends in August sea temperatures (Lüning 1990, Arrontes 1993). Therefore, a sea surface temperature (SST) database was used to investigate the temporal and spatial variability of August SST in Northern Spain.

SST was obtained from the UK Meteorological office, Hadley Center HadISST1.1-Global sea-Ice coverage and SST (<http://badc.nerc.ac.uk/data/hadisst>) (Rayner et al. 2003). Data are available from 1870 to nowadays, with monthly periodicity on a $1^\circ \times 1^\circ$ grid with global coverage. In the present study, 8 data points were considered along the northern coast of Spain (at 44° N) covering from 1 to 9° W (see Fig. 1).

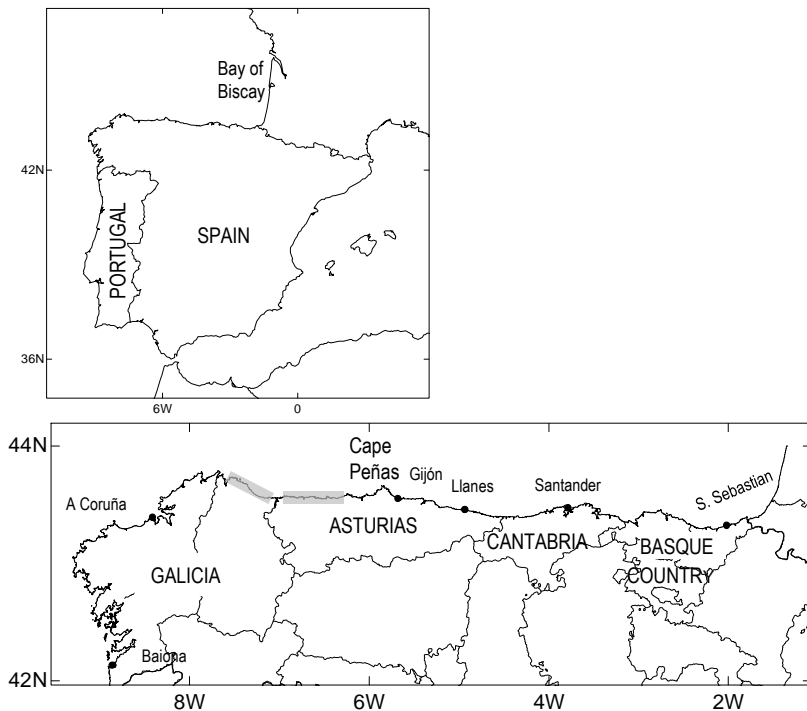


Figure 1. Study area in Northern Spain. The names of regions and some locations are shown for reference. Black squares indicate the points where the reconstructed SST data were considered. Central (C, north Galicia) and marginal (M, west Asturias) zones of *Fucus serratus* distribution, where quantitative sampling was done, are marked with grey bands (see text for details).

2.3. *Maps and Data Analysis*

The Universal Transverse Mercator (UTM) coordinate locations of the historical records were determined at a spatial resolution of 1 x 1 km with the help of the description of the sites in the original papers, consulting historic maps (e.g. San Vicente de la Barquera port in 1870, www.puertosedecantabria.es) and 1:50.000 topographic maps (Spanish Military Cartography) of the distinct areas. These data and the locations from our surveys were plotted using the software DMap 6.0.

To evaluate temporal trends in SST, mean values were calculated by averaging the data corresponding to the 8 points along the coast of Northern Spain. Linear regressions of August SST anomalies (C°) versus time (year) were then calculated for each period with species range shifts. Regressions were calculated using the public domain package R (R Development Core Team 2008). Moreover, August SST recorded at the position of the distributional boundary in the first historical data set (i.e. those corresponding to the end of the XIX century) was taken as a rough thermal limit for each species. Mean August SST of the 4 years prior to the biological data records was used, taking into account the species life-span (ranging from 2 to 5 years) and the potential lag in their responses to SST. The spatial location of this August isotherm was then tracked through time and compared with the position of the range limit for each species in subsequent periods.

3. **Results**

3.1. *Geographic Distribution and Species Abundance*

3.1.1. *Himanthalia elongata*

Current and historic surveys indicate that the distribution of *Himanthalia elongata* has experienced a severe retraction in Northern Spain, where it now has its most restricted distribution range since the late 1800s.

At the end of XIX century, the species was abundant and reproductive towards the East until at least Santander ($43^{\circ}28'N$, $3^{\circ}48'W$) in the Cantabria province (Lázaro é Ibiza 1889, Sauvageau 1897; see Fig. 2A). At this time, the species was also present further East, in the inner part of the Bay of Biscay, though feasibly constituting sporadic populations. Hence, *Himanthalia elongata* was collected in 1827 by Bory de Saint Vicent in San Sebastian, the Basque coast (Herbarium of the Natural History Museum of Paris, ref. PC0599273; on line access, <http://coldb.mnhn.fr/>). The two compiled specimens have the entire holdfasts. Moreover, few reproductive individuals were described in Guéthary, France ($43^{\circ}25'N$, $1^{\circ}36'W$) from 1904 to 1911 (Sauvageau 1918, Fig 2A). These specimens however vanished in subsequent years.

In 1949 *Himanthalia elongata* was absent at any visited location in Cantabria and the Basque country (Fischer-Piette 1955b). It was observed around Gijón east of Cape Peñas (Miranda 1931) and in 1955, Fischer-Piette (1963) also found the macroalgae at three locations in this area, the most eastern one being Lastres ($43^{\circ}31'N$, $5^{\circ}16'W$). Therefore, using a conservative estimate, there was a shift in *H. elongata* distribution from Santander in Cantabria to Lastres in Asturias, i.e. about 116 km towards the west (a retraction in species distribution), from the end of the nineteenth century to 1955 (see Table 1). Afterwards, the presence of a sporadic population was described in Ubiarco, Cantabria ($43^{\circ}24'N$, $4^{\circ}06'W$) from 1958 to 1961 (Fischer-Piette & Ginsburg-Ardre 1963; Fig 2B). Individuals were mostly non-reproductive and colonized shaded areas.

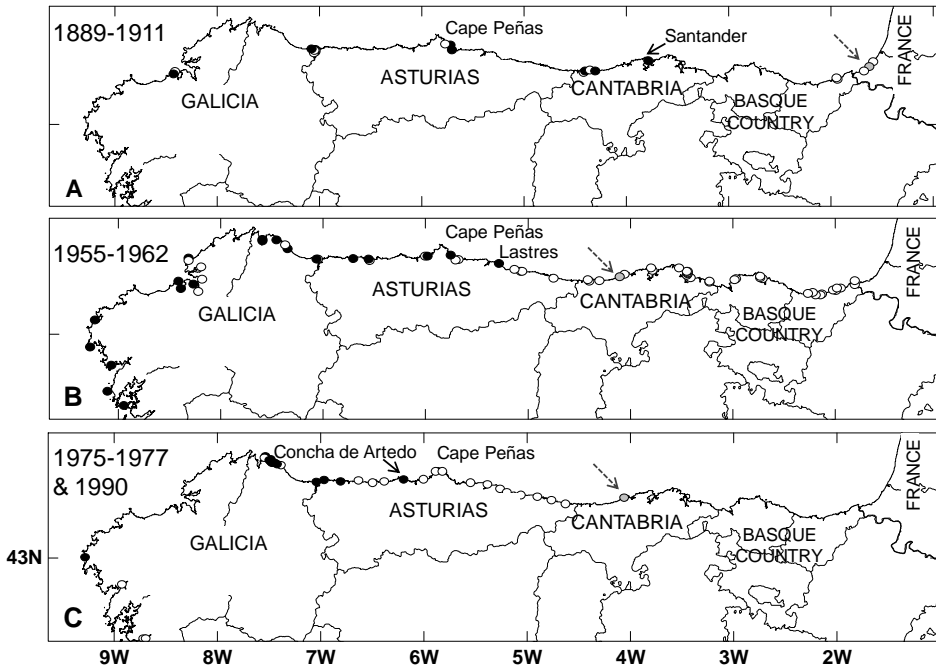


Figure 2. Distribution of *Himanthalia elongata*. Locations visited are indicated by black (presence), white (absence) and grey (sporadic populations) circles and also pointed by grey arrows. (A) Surveys of 1889-1911, (B) 1955-1962, (C) 1975-77 and 1990. Names of the range boundary locations are shown. See text for bibliographic references.

In subsequent years, *Himanthalia elongata* disappeared from the eastern coast of Asturias and in 1975-1977 the range boundary was apparently located in Concha de Arredo ($43^{\circ}34'N$, $6^{\circ}10'W$), west of Cape Peñas (Anadón & Niell 1981; Anadón, 1983; Fig. 2C). Therefore, from 1955 to the late 1970's there was a new retraction of the species distribution (Table 1). From the late 1970's to 2000 the range boundary of the species apparently remained at Concha de Arredo, W Asturias (Morán 1999, Alcock 2003). *H. elongata* was not recorded from this site towards the East (Gorostiaga et al. 2004), except at one isolated location, in Suances, Cantabria ($43^{\circ}25'N$, $4^{\circ}02'W$) in 1990 (Margalet & Navarro 1992; Fig. 2C).

Very different results are revealed in our surveys of 2004-2006 and 2008-2009, which indicated that *Himanthalia elongata* disappeared from Concha de Artedo and towards the West in a stretch of coast > 100 km within a very short period (Fig. 3A). In 2008-2009 the species just remained in two locations in Northern Spain within the coastal section from Concha de Artedo to Cariño port in Galicia (43°44'N, 7°51'W): Tapia port (43°34'N, 6°56'W) and in Vilela (43°45'N, 7°41'W). By June 2011 reproductive individuals and several recruits of *H. elongata* were still found in Vilela but the small population in Tapia port vanished. From Cariño port towards the West, the species still remains nowadays abundant and reproductive, being recorded at several locations in western shores of Galicia (Fig. 3A). Our results hence indicate a retraction of the species distribution from Concha de Artedo to at least Vilela i.e. about 130 km towards the West in the short period from 2004-2006 to 2008-2009. This means a retraction of more than 330 km since the end of XIX century in Northern Spain (see Table 1 for a summary).

3.1.2. *Fucus serratus*

Similarly to *Himanthalia elongata*, at the end of the XIX century the distribution range of *Fucus serratus* stretched out at least until Santander in Cantabria (Lázaro é Ibiza, 1889; see Fig. 4A). Moreover, we cannot disregard the presence of *F. serratus*, at least as transient populations, in the inner part of the Bay of Biscay, as stated by the specimen collected in San Sebastian by René du Parquet in 1856 (Herbarium of the Natural History Museum of London, ref. BM001062001).

In 1949 the limit of distribution of the species was apparently at San Vicente de la Barquera, in Cantabria (Fig. 4B), although it was only observed in the sheltered area whereas at the end of the nineteenth century, the species had been recorded in both sheltered and more wave-exposed areas at this site (Sauvageau, 1897). The boundary in open shores moved towards the West, to Gijón (43°33'N, 5°41'W) in Asturias. Populations west of Ribadeo became frequent and very reproductive, though the maximum size of individuals was only 15 cm at that time (Fischer-Piette, 1955b).

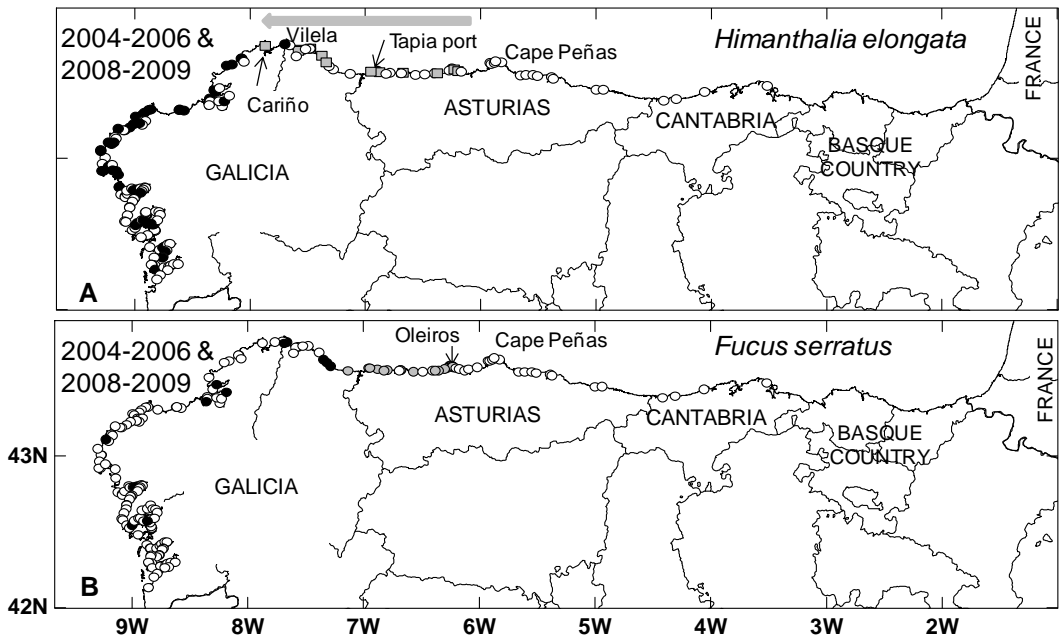


Figure 3. Distribution of (A) *Himanthalia elongata* and (B) *Fucus serratus* from 2004-2006 to 2008-2009. The black circles in the maps indicate the presence of the species, and the white ones the absence. In (A) grey squares represent local extinctions from 2004-2006 to 2008-2009 and in (B) grey circles represent marginal locations with very low *Fucus serratus* cover and presence of sparse individuals by 2008-2009. The grey arrow shows the retraction towards the west observed at this time. See text for bibliographic references.

In 1955 the most eastern population of the species *Fucus serratus* in open shores of North Spain was located in Lastres (43°31'N, 5°16'W), east of Cape Peñas, though the estuarine population in San Vicente de la Barquera remained (Fischer-Piette, 1963). The presence of the species in Lastres cannot be considered an expansion of *F. serratus*, as this site was not surveyed in 1949 (see above, Fischer-Piette, 1963). The period of 1949 to 1955 was highlighted as environmentally favorable for the species, as the size of individuals increased to 36-65 cm (Fischer-Piette, 1955b, 1963)

Table 1. Summary of changes on the geographic distribution of *Himanthalia elongata* and *Fucus serratus* in North Spain from 1889 to 2009. In the case of *Fucus serratus* trends are shown for wave-exposed shores.

<i>Himanthalia elongata</i>			<i>Fucus serratus</i> (wave-exposed shores)			References
Period	Dist. changes	Distrib. trend (estimated distance)	Period	Dist. changes	Distrib. trend (estimated distance)	
1889-1911 to 1955	From Cantabria (Santander) to E Asturias (Lastres)	Contraction (≈116 km west)	1894-1896 to 1955	From Cantabria (Santander) to E Asturias (Lastres)	Contraction (≈116 km west)	Lázaro é Ibiza 1889, Sauvageau 1897, Fischer-Piette 1955b, 1963
1955 to 1975-1977	From E Asturias (Lastres) to W Asturias (Concha de Arredo)	Contraction (≈84 km west)	1955 to 1975-77	From E Asturias (Lastres) to W Asturias (Barayo)	Contraction (≈107 km west)	Fischer-Piette 1963, Anadón & Niell 1981, Anadón 1983
Late 1980's to 2000	Absence in E Asturias, presence in W Asturias (Concha de Arredo)	No apparent changes in distribution	1977 to 1985-1989	From Barayo to Novellana-Oleiros	Expansion (≈26 km east)	Arrontes 1993, Navarro & Margalet 1993, Morán 1999, Arrontes 2002, Alcock 2003
2004-2006 to 2008-2009	From E Asturias (Concha de Arredo) to Galicia (Vilela)		2004-2006 and 2008-2009	No changes in distribution, drastic changes in abundance	From Oleiros to Cegoñas (73 km west)	Our field surveys
1889 to 2008-09	From Cantabria (Santander) to Galicia (Vilela)	Contraction (≈ 330 km west)	1894-96 to 2008-09	From Santander to Oleiros	Contraction (≈197 km west)	

Fucus serratus retracted its range distribution to Barayo ($43^{\circ}34'N$, $6^{\circ}36'W$), 76 km west of Gijón and 173 km from the very sheltered location San Vicente de la Barquera, between the years 1955 and 1975-1977 (Anadón and Niell, 1981; Fig. 4C; Table 1). At some point between 1985 and 1989, however, the species range expanded to Novellana ($43^{\circ}34'N$, $6^{\circ}17'W$), 26 km towards the East (Arrontes pers. comm.; Arrontes, 1993; Fig. 4C). A very small population was also described in Luanco ($43^{\circ}37'N$, $5^{\circ}46'W$) located east of Novellana and near Gijón. In 1990, the species was experimentally introduced in Oleiros ($43^{\circ}34'N$, $6^{\circ}13'W$), a location near Novellana. From 1990 to 2000 the number of plants increased exponentially in Oleiros and the species was abundant in almost any rocky platform westwards from this site (Morán, 1999; Arrontes, 2002; authors pers. obs.). Therefore, the period ranging approximately from 1985 to 2000 was favorable for the species, which expanded its distribution in North Spain, though in a reduced stretch of coast (Table 1).

From 2004-2006 to 2008-2009 we did not find variation in the distribution range of *Fucus serratus*, as the boundary limit was still located around Oleiros, West of Cape Peñas (Fig. 3B). However, a sharp decline in the abundance in relation to data from 2000 was found in the populations of W Asturias, from Oleiros to Cegoñas ($43^{\circ}33'N$, $7^{\circ}7'W$) near the border between Galicia and Asturias (Fig. 3B; see Table 1 for a summary of the trends). Percent cover of *F. serratus* in 2008-2009 was much lower in W Asturias (marginal area) than in N Galicia (central area), with average values $< 45\%$ in the marginal area ($< 8\%$ in most of the locations and sampling dates) and $> 90\%$ in N Galicia (Table 2). Very dispersed and small plants remained in all the 12 locations visited in W Asturias, with two exceptions, one site at Cadavedo and Tapia port, where abundant populations still remained until 2009. Nonetheless, in Tapia port the individual size and abundance of *F. serratus* population seemed to decline by 2011 (authors pers. obs.). Nowadays, the drastic decline in the marginal area of Northern Spain (W Asturias) virtually reduces the presence of this species in the Iberian Peninsula to

the scattered but persistent populations inside big estuaries (rías) in Galicia and a few semi-exposed locations in N Galicia (Fig. 3B).

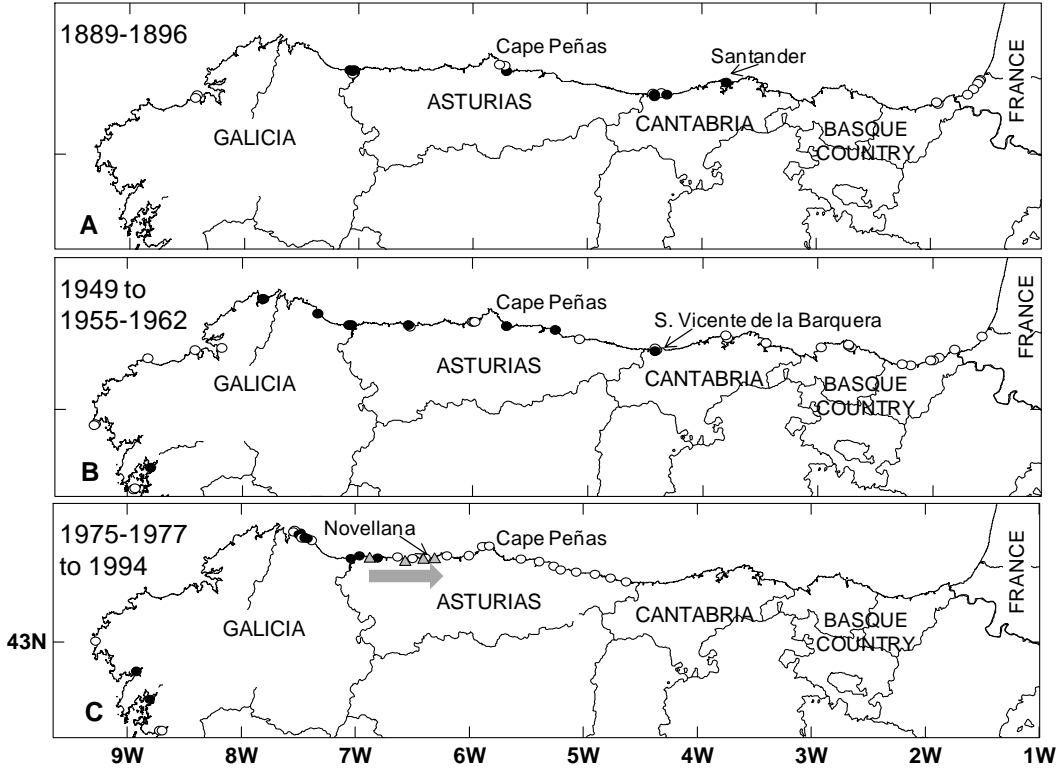


Figure 4. Distribution of *Fucus serratus*. The black circles in the maps indicate the presence of the species, and the white ones the absence. (A) Surveys of 1889-1896, (B) 1949 to 1955-1962, (C) 1975-1977 to 1994. The grey triangles show new locations where *F. serratus* was recorded between 1990 and 1994. The grey arrow shows the short expansion towards the east observed at this time. See text for bibliographic references.

Table 2. Percent cover of *Fucus serratus* (primary and secondary cover pooled) in marginal locations (W Asturias) and central (N Galicia) of its distributional range in N Spain. Sites within locations were pooled, n = 48 except in May 2008 for central locations where n = 6. Cd= Cadavedo, No=Novellana, SP=San Pedro, Mo= Morás, Al= Area Longa.

Date	Marginal			Central		
	Cd	No	Ol	SP	Mo	Al
May 2008	18.9 (± 7.2)	0.8 (± 0.5)	3.5 (± 0.5)	103.9 (± 1.9)	109.3 (± 4.5)	101.0 (± 2.1)
Jul. 2008	41.6 (± 11.4)	0.1 (± 0.1)	4.2 (± 1.0)	101.7 (± 1.1)	109.3 (± 3.2)	103.5 (± 1.0)
Feb. 2009	7.8 (± 9.3)	0.2 (± 0.5)	3.3 (± 2.5)	91.3 (± 8.2)	101.0 (± 10.4)	98.1 (± 4.2)

3.2. August Sea Surface Temperature (SST) on the North coast of Spain

From 1870 to 1889, August sea surface temperature on the North Coast of Spain experienced a significant decrease of $0.039\text{ }^{\circ}\text{C year}^{-1}$ (expressed by the slope of regression of August annual anomalies versus year), ($r^2 = 0.269$, $p < 0.05$, $n = 20$; Fig. 5A). This cool period corresponds to the time before the first published records, which showed the widest species distributions in the studied area (see above). Temperatures were spatially heterogeneous, with lower values at lower longitudes, i.e. towards the West (Fig. 5B). From 1890 to 1911, when the presence of a few reproductive individuals of *Himanthalia elongata* were described in Guéthary-France (Sauvageau 1897, 1918) no significant trend was observed in August anomalies, except a highly temporal variability (Fig. 5A).

During the period of 1912 to 1949, SST increased by $0.022\text{ }^{\circ}\text{C year}^{-1}$ ($r^2 = 0.165$, $p < 0.05$, $n = 38$), corresponding with the contraction of both *Fucus serratus* and *Himanthalia elongata* distribution. After the very cool August SST of 1912 (with the lowest anomaly over the whole period, about $-1.85\text{ }^{\circ}\text{C}$) came warmer years, such as 1937 and 1947 (Fig. 5B). August SST showed a trend to decrease during the period of 1949 to 1955 (Fig. 5A), which is described as a favourable time for *F. serratus*. This trend however was not statistically significant (sample size was low, $n = 6$). From 1956 to 1977 no significant trend was observed in SST,

even when a contraction in both species distributions was detected during this period. The year 1975 was the warmest of the period (August anomaly 1.07 °C).

From 1978 to 1990, when *Himanthalia elongata* was present sporadically in Cantabria and there was a brief expansion of *Fucus serratus* in W Asturias (see above), we however observed that August SST anomalies increased by 0.146 °C year⁻¹, with a good fit between time and temperature trends ($r^2 = 0.538$, $p < 0.01$, $n = 13$). The whole period of 1978 to 2009 shows an evident warming trend, with August SST increasing by 0.053 °C year ($r^2 = 0.359$, $p < 0.001$, $n = 32$). Since 1987 all the anomaly values were positive and 1999, 2006 and particularly 2003 were the warmest years (anomalies > 1.5-2 °C; Fig. 5A). Warming occurred all over the coast of North Spain, but temperature increases was clearly greater towards the East, i.e. lower longitudes (Fig. 5B).

August SST in Cantabria, where the range margin of both species was located by the end of XIX century, was 19.9 °C (mean value of the previous 4 years). From late 1880's to 1990's the spatial position of the distribution boundary for both species coincide with the location of 19.9 °C isotherm (4-previous-years mean value), which also moved to the West (Fig. 6). After 1990 SST increased remarkably, particularly towards the East (see above) and by 2000 the August SST average value of 19.9 °C was located at the most western area in North Spain. In 2008-2009, *Himanthalia elongata* range boundary moved further west, tracking spatial changes in sea temperatures, whereas in the case of *Fucus serratus* there is a spatial decoupling between its present distribution and the temperature trend (Fig.

6

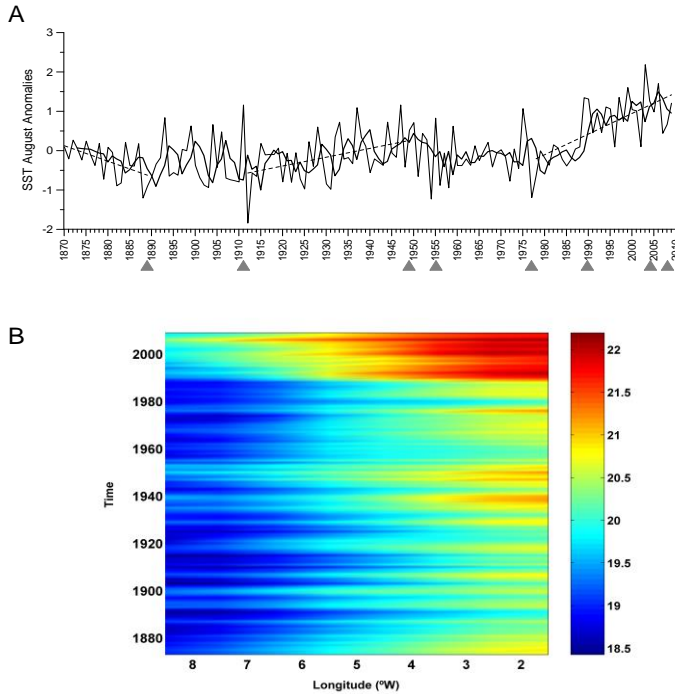


Figure 5. (A) Temporal trend for August SST anomalies at the North Coast of Spain from 1870 to 2009 (average values for the whole coastline). Raw data and a running average of previous 4 years are shown (thin and thick lines respectively). Significant trends of raw data are also indicated (discontinuous line) (B) August SST (running average of previous 4 years) along the coastline of N Spain from 1870 to 2009. Triangles indicate the points in time of the biological surveys.

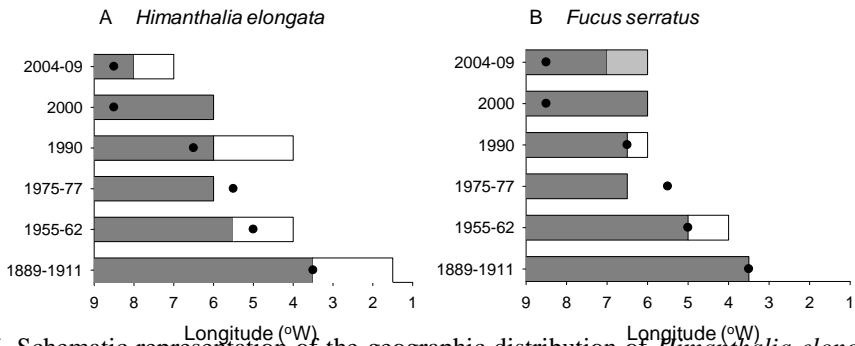


Figure 6. Schematic representation of the geographic distribution of *Himanthalia elongata* (A) and *Fucus serratus* (B) in the north coast of Spain (longitude 9° to 1° W) from 1889 to 2009. Bars indicate the area where the presence of species was continuous (dark grey) or sparse and sporadic (white). In (B) the light grey bar indicates the recent decline in species abundance at the range boundary. Black dots show the location of 19.9 °C August SST isotherm (value at the species range border in 1889), the westward displacement of this value through time, and the degree of spatial coupling with species boundaries.

4. Discussion

Historic data and our surveys show that the geographic range of the two canopy-forming algae *Himanthalia elongata* and *Fucus serratus* have experienced an evident retraction in Northern Spain from the end of the XIX century to our days. Overall, these retreats agree with the SST warming trend, the rise in temperature being more intense towards the inner part of the Bay of Biscay and especially in recent decades (this study, Gómez-Gesteira et al. 2008, deCastro et al. 2009 and references therein). The shrink has been more drastic for *H. elongata* than *F. serratus*, as the first species nearly disappeared from Northern Spain, whereas *F. serratus* is nowadays still found in the westernmost area, though with drastic declines in abundance.

Our results agree with other studies that have reported shifts in species distributions and particularly coastal seaweeds, commonly linked to SST warming (e.g Southward et al. 1995, Sagarin et al. 1999, Schiel et al. 2004, Mieszkowska et al. 2005, Lima et al. 2007, Ugarte et al. 2009). Furthermore, the westward retreat of large canopy-forming algae in North Spain appears to be a general trend, also including kelp species such as *Saccorhiza polyschides*, *Laminaria ochroleuca* and *L. hyperborea* (Fernández & Anadón 2008, Fernández 2011).

The higher range shift observed for *Himanthalia elongata* than *Fucus serratus* may reflect a higher resistance of the latter to higher temperatures. Thus, despite the historic range boundaries for both species roughly match similar August SST values (around 19.9 °C; see results); recent laboratory experiments show growth tolerance of *F. serratus* to higher sea temperature than *H. elongata* (around 24 °C for adult plants of *F. serratus*, 18 °C for reproductive individuals of *H. elongata*; Martínez et al, unpublished data). Furthermore, an upper survival limit of about 25 °C was also indicated for *F. serratus* (Lüning 1984).

In addition to trends in SST, other climatic and non-climatic variables may play a role in determining range boundaries and the observed differences between species range shifts. *Fucus serratus* is found higher on the shore than *Himanthalia elongata* (Anadón 1983, Little & Kitching 1996) and thus it is probably more

influenced by climatic factors related to emersion times (see comments in Viejo et al. 2011). High cloud cover was also linked to its presence in Northern Galicia (Alcock 2003). Accordingly, both air temperature and cloudiness are significant in explaining the current presence of *F. serratus* in NW Iberian Peninsula (Martínez et al. 2012). The influence of competition interacting with physical conditions cannot be disregarded, as under unfavorable environmental conditions the species may be outcompeted by other seaweeds (see Arrontes 1993). Further studies including higher resolution data of climatic factors, e.g. SST and air temperatures, and distributions of potential interacting species such as *Fucus vesiculosus*, may refine our understanding of the observed shifts.

On the other hand, it is unlikely that the observed retraction of the target species in Northern Spain, particularly the rapid trend in recent years, was influenced by increases in grazing pressure or anthropogenic factors. Certainly, grazers are more abundant in southern versus northern Europe (Southward et al. 1995) and sea warming may favor grazing gastropods such as limpets (Schiel et al. 2004). Nevertheless, the distribution of both *Fucus serratus* and *Himanthalia elongata* shifted towards the West in Northern Spain, precisely where the limpets *Patella intermedia* Murray in Knappand and *P. vulgata* L attain larger sizes (Alcock 2003).

Anthropogenic stressors, in particular coastal pollution have been the cause of conspicuous declines and even local extinctions of canopy-forming algae in different regions around the world (Airoldi 2008 and references therein). However, in Northern Spain, pollution has considerably decreased in the last years following the European Water Framework Directive (WFD 2000/60/CE), with the exception of some embayment and commercial ports interspersed along the northern Spanish Coast (Casado-Martínez 2006, García-Barcina et al. 2006 Juanes et al. 2008, Puente et al. 2008, Borja et al. 2009).

Species distributions may indeed respond to extreme conditions, instead to average longer-term trends (Easterling et al. 2000). In our study the retreats during the late century could be a response to the progressive warming trend, already

detected by several other authors (deCastro et al. 2009, Goikoetxea et al. 2009), but also to the heat waves of 1999, 2003 and 2006 (see results; Cerrano et al. 2000, Garrabou et al. 2009, García-Herrera et al. 2010).

In addition to different ranges of tolerance to the physical environment, the variation between the target species in their range shifts is probably related to divergences in their life history. Thus, the longer life-span of *Fucus serratus* up to 5 years versus the biennial cycle of *Himanthalia elongata* (Rees 1932, Moss 1969) could cause a slower retraction of the first species in response to climatic changes. It may also explain its discrete expansion around 1990's within a warming period (see results). During unfavorably warm years the species may grow slowly and/or reduce its reproduction, but still persist. It may then take advantage of short cold periods, yet maintaining the expansion during subsequent warm years. Stronger upwelling episodes of cold water occurred during summer months in 1988, 1989 and 1990 in NW Spain (Alvarez et al. 2010). Moreover, the number of days of upwelling per year from April to September was high in 1986, 1987 and 1989 (Fig. 10d in Llope et al. 2006). Expansion of the kelp *Saccorhiza polyschides* towards the inner part of the Biscay Bay in the early 1980's was likewise associated with cooler temperatures during these years in the area, within the rising SST trend observed since the seventies (Gorostiaga 1987, Borja and Gorostiaga 1990; Fernández et al. 1988, Gómez-Gesteira et al. 2008).

Differences in dispersal abilities of the target species are also involved in the dynamics of their range limits (see Arrontes, 2005 and references therein). Transient populations reported for *Himanthalia elongata* (see results) may be the consequence of long-distance dispersal events during short pulses of cool, environmentally favorable years. The species exhibits high dispersal abilities by means of detached and floating receptacles (Sauvageau 1918). The dispersal capacity of *Fucus serratus* is much more limited (Arrontes 2002, Coyer et al. 2003) and during favorable periods it restricts the spatial extend of the spread, the species being able to survive and reproduce beyond the range edge (Arrontes 1993, 2005).

The occurrence of *Fucus serratus* at isolated locations outside its "normal" range commonly reflects the permanence of populations left behind the retreating margin during warming periods (our results; Fischer-Piette 1955b, Alcock 2003). These "relict" populations are located in estuarine, wave-sheltered sites, for instance in San Vicente de la Barquera or Tapia Port. Even *Himanthalia elongata*, which colonizes more wave-exposed environments (Lüning 1990, Little & Kitching, 1996, Lagos & Cremades 2004), found a temporary refuge in Tapia port during its recent retreat.

The restriction to very sheltered places at the southern range margin has been documented for several fucoid species and intertidal invertebrates (Sauvageau 1897, Ballantines 1961, Southward et al. 1995, Ladah et al. 2003). These sites may provide an auspicious environment for the species within inhospitable regional conditions (Vadas et al. 1992, Hampe & Jump 2011). The muddy film, which often covers estuarine rocky shores, may offer protection against desiccation and photoinhibitory light stress (Ladah et al. 2003). Moreover, estuarine places may constitute a refuge from wave action and increasing grazing pressure under warming conditions (Schiel et al. 2004, Jonsson et al. 2006) or decreasing nutrient supply under decreases in upwelling intensity in the region (see contrasting results on upwelling intensity trends in Northern Spain in Llope et al. 2006 and Alvarez et al. 2010). More extreme windstorm events are expected under some climate change scenarios in several regions of Western Europe, including Spain (Leckebusch & Ulbrich 2004). Thus, other factors may interact with SST trends and determine the presence of relict populations in very sheltered locations.

Even when retreat in Northern Spain range has been more drastic for *Himanthalia elongata* than *Fucus serratus*, the first species is still common and abundant along the Western Atlantic shores of Spain, while the presence of *F. serratus* is there reduced to a few sparse locations inside big embayments or rías (this study; Pazó & Niell 1977; Lagos & Cremades 2004, Martínez et al. 2012). Therefore, the distribution of *F. serratus* in NW Iberian Peninsula is more

constrained and scattered than the range of *H. elongata*, despite the latter virtually vanished in Northern Spain.

Summarizing, a drastic retraction of the seaweeds *Fucus serratus* and *Himanthalia elongata* occurred in Northern Spain in parallel to SST trends. The observed differences between species in the speed of their range shifts and the nature of the distributional boundary (e.g presence of isolated populations outside the range margin) may be linked to differences in their life-history, thermal tolerance and dispersal abilities. The range retreat of large, canopy-forming algae appears a general phenomenon linked to warming in this coastal area, affecting other species such as kelps (Fernández 2011). Canopy-forming species are indeed key constituents of coastal systems determining the structure and functioning of assemblages through trophic and non-trophic interactions (Schiel 2006). Their loss, which has been observed in different coasts around the world, though related to different causes, represents relevant changes in the ecosystem structure and function (Airoldi et al. 2008). A profound reorganization of coastal assemblages is expected in Northern Spain, but the temporal extent of the variations and their consequences for coastal services needs to be evaluated.

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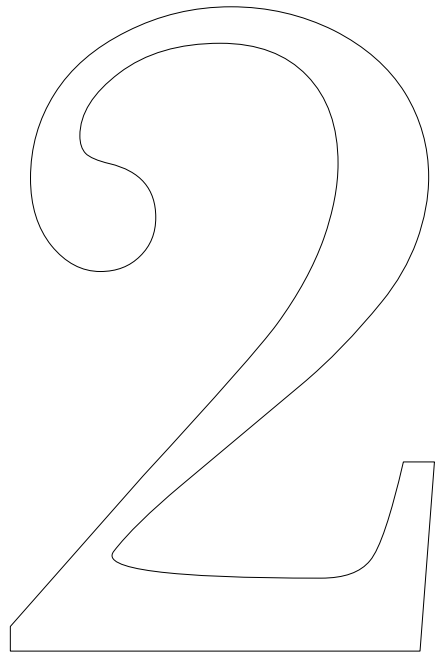
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**Drivers of the scattered distribution of the foundational alga *Fucus serratus* L.
inside estuarine refuges at its southern range limit**



Ofreixo, Ría de Muros. June 2013
(L. Duarte)



Isla de Arosa, Ría de Arosa. June 2013
(L. Duarte)

Linney Duarte, Rosa Viejo. Manuscript under preparation.

ABSTRACT

Diverse empirical research have tried to identify the factors causing geographic range limits of species, essential in forecasting species' responses in a scenario of climate change. In exposed and semi-exposed shorelines of North Europe, large brown seaweeds such as fucoids are abundant, while further south in Europe these species are restricted to sheltered and estuarine areas. The southern geographic distributional limit of the cold-temperate brown macroalgae *Fucus serratus* L. is situated in the NW of the Iberian Peninsula, where it has a very peculiar distribution in the Atlantic coasts, inside coastal inlets or Rías. In these Rías, *F. serratus* is absent from proximate locations within the same Rías, while other fucoids are very common. We obtained habitat models at small spatial scales (Rías) for the intertidal species, and conducted a series of experiments with juveniles and germlings of *F. serratus* in two of the Rías in Galicia, NW Spain: the Ría de Muros y Noya and the Ría de Arosa. For the transplants experiments, in each Ría we selected a location of origin, where *F. serratus* is present, and two other locations where the species is absent, one external and the other internal when compared to the location of origin. Then, *F. serratus*' juveniles and germlings transplants were done between the locations of origin and the internal and external locations. In general, our results showed that after two months the average number of germlings of *F. serratus* was significant higher in origin than in exterior and interior locations. Furthermore, transplanted juveniles of *F. serratus* showed a tendency of lower elongation and weight than juveniles in origin locations. Finally, our habitat models indicated that the scattered presence of *F. serratus* in the two Rías could be related to the presence of *Himanthalia elongata*, high ammonium concentrations, lower summer sea temperatures and high temporal salinity variability. Undoubtedly, the future of these marginal populations of *F. serratus* in these refuge areas in the Rías Bajas will depend on the capacity of the species to cope with the imminent climate change.

Key-words: Geographic distribution, Iberian Peninsula, *Fucus serratus*, coastal inlets, Rías Bajas, Biotic factors, Abiotic factors, Habitat distribution models

1. Introduction

The geographic distribution of terrestrial and marine species in nature and the factors that determine these limits is a topic of great interest to ecologists and biogeographers (Wallace 1878, Baker 1963, Schall & Pianka 1978). Contemporary ecological research has proposed that different processes operating at distinct spatial and temporal scales determine the biogeographic pattern of species, such as biotic and abiotic factors, which act in isolation or in combination (Gaston 2003, Case et al. 2005, Parmesan et al. 2005, Sagarin et al. 2006). The range limit of species has been conservatively seen as a transition area between a suitable and a less hospitable habitat, shifting from large to smaller populations (Guo et al. 2005, Kawecki et al. 2008). Nonetheless, recently this view has been challenged as the biogeographical pattern of a species is not a systematic one and it has been acknowledged that it is a more complex mosaic (Gaston 2009). Indeed, species' range limits can be very variable, where spatial changes may happen suddenly in order to maintain viable populations, or the disappearance of suitable habitats is more gradual and scattered (Gaston 2003). Moreover, in some cases, these limits are quite persistent along wide periods, whereas other range boundaries shift frequently, retracting and expanding over time (Gaston 2009, Sexton et al. 2009). Also, the marginal boundary of a species may be subjected by environmental stress, species interactions and dispersal limitations (Case et al. 2005). Undoubtedly, understanding species boundaries and the factors linked to them have nowadays received a renovated interest in order to forecast species extinction and loss of biodiversity given global climate change, anthropogenic factors and the establishment, and spread of invasive species (Channell & Lomolino 2000, Sagarin & Gaines 2002, Thomas et al. 2004, Parmesan et al. 2005).

At the periphery of the geographic distribution, species can be restricted to very specific habitats, similar to those occupied elsewhere, or even a different set of habitats (Gaston 2009, Oliver et al. 2009). If range boundaries are determined by climatic factors, then one might expect species to persist in the most favorable sites

available at the periphery, providing the highest possible fitness. Then, at the warm southern limit of the distribution of species, these might be restricted to habitats having especially cool microclimates, increasing the chances of species completing their life cycle. Actually, recent empirical work indicate that species occur in a subset of those habitats that occupied in core zones, especially favorable sites in marginal areas (Lennon et al. 2002, Oliver et al. 2009). On the other hand, where biotic interactions are important at the range edges, species would be expected to persist in relatively unfavorable sites, if these habitats provide refuges from their competitors or natural enemies.

The distribution of rocky shore species has been widely investigated in relation to biotic factors, such as grazing, competition and dispersal capacities (Thomas 1994, Burrows 2009, Mangialajo et al. 2012). And abiotic factors, like wind, temperature and ice exposure (Kiirikki 1996, Reichert 2008, Martinez et al. 2012), as well as the interaction between these factors (Underwood & Jernakoff 1981). Undoubtedly, intertidal species are in the boundary of land and sea, been consequently exposed to drastic environmental changes and frequently on the limit of their physiological tolerance (Helmuth et al. 2006). In these ecosystems macroalgae usually are the dominant species, forming canopies and acting as autogenic engineers changing the environment and creating the structure for other species (Jones et al. 1997).

In North Europe shorelines, large canopies of brown macroalgae are abundant in exposed and semi-exposed areas, while further south in Europe the geographic distribution of these seaweeds is restricted to sheltered and estuarine areas “refuges” (Ballantine 1961, Lewis 1964, Lüning 1990, Southward et al. 1995). This pattern seems to be related to environmental conditions of the area, becoming progressively more stressful for fucoid algae towards the south (Lüning 1990). It is also noteworthy that rocky shores of south Europe are associated with a higher presence of grazers than N Europe, which has been related to warmer

temperatures in the region (Ballantine 1961, Hawkins & Hartnoll 1985, Hawkins et al. 1992; Southward et al. 1995).

In this study, the species selected was the cold-temperate macroalgae *Fucus serratus* L, which southern range limit, is located in the North West of the Iberian Peninsula. In the Atlantic coasts of Spain this habitat-forming macroalgae has a very peculiar distribution, scattered inside coastal inlets or Rías, which has been persistent through time (Ardre 1957, Niell 1977, Pazó & Niell 1977). Remarkably, the species is absent from other proximate locations within the same Rías where other fucoids such as *F. vesiculosus* dominated. As far as we know that are not specific studies that investigate the biotic and abiotic factors that feasibly cause this particular distribution of *F. serratus* in the Rías Bajas.

The investigation was performed in two of the Rías Bajas in Galicia, NW Spain: the Ría de Muros y Noya and the Ría de Arosa. These Rías are mixed estuaries (Varela et al. 2005, Martinez-Urtaza et al. 2008), located in the Eastern North Atlantic Upwelling system (Wooster et al. 1976, Varela et al. 2005), characterized by wind driven coastal upwelling in spring-summer, which brings colder-nutrient rich water (Fraga 1981, Alvarez et al. 2008, 2012, Carballo et al. 2009). Consequently, these areas are well known by the higher yields of the fishery and aquaculture industry (Blanton et al. 1987, Carballo et al. 2009).

To determine the biotic and abiotic factors that feasibly may explain the particular scattered distribution of the macroalgae *Fucus serratus* in the Ría de Muros y Noya, and the Ría de Arosa, we conducted transplants with germlings and juveniles of this macroalgae. These experiments were done in locations of the Rías where *F. serratus* is dominant and abundant “origin locations” and exterior and interior locations respecting the origin locations, where *F. serratus* is absent. Furthermore, in order to explain the distribution of *F. serratus* in both Rías, we developed habitat distribution models for the species at small spatial scale.

3. Materials and methods

2.1 Study area

Sampling surveys and experiments were conducted at two of the so-called “Rías Bajas”, which are four large SW-NE coastal inlets in the Atlantic shores of Galicia: the Ría de Muros y Noya (hereafter Ría de Muros), and the Ría de Arosa (Fig. 1). The Ría de Muros is the northern most of the Rías. It covers a surface area of 90 km² with a mean volume of 2060 hm³ and a length of 13 km. The freshwater inflow to this Ría is provided by the rivers Tambre and Tines, the largest being provided by the first one (Carballo et al. 2009). Ría de Arosa is the most extensive of the four Rías Bajas, with a surface area of 230 km², a mean volume of 4800 hm³ and a length of 33.1 km. Two main rivers flow into this inlet: the Ulla and Umia. Almost all the exchange with the shelf takes place through the southern mouth, as the northern mouth is very shallow (10 m) (Rosón et al. 1995).

*2.1.1. Occurrence of *F. serratus* and other foundational brown algae inside the Rías*

The presence/absence of *Fucus serratus* along the northwestern coasts of Spain had been previously recorded by a detailed field survey carried out during 2004-2006 (see Martínez et al. 2012). Specifically, in the two selected Rías the inspection was done from May to September 2005 and covered a total of 52 rocky locations (26 in each ría). The target species is conspicuous and therefore easy to detect, but in order to further confirm the reliability of the absences a linear distance of about 600-1000 m was tracked at each location (see Martínez et al. 2012 for additional information). The occurrence of other Fucacean species was also noted, in particular *Fucus vesiculosus* L, *F. ceranoides* L, *Pelvetia canaliculata* (L.) Decne & Tour, *Ascophyllum nodosum* (L.) Le Jolis and *Himanthalia elongata* (L.) S.F. Gray. We repeated the survey in the same locations

during low spring tides of June and October 2011. Furthermore, to determine the temporal persistence of *F. serratus* populations inside the Rías, we reviewed the publications on the historical distribution of the species in this area (Ardre 1957, Donze 1968, Pazó and Niell 1977).

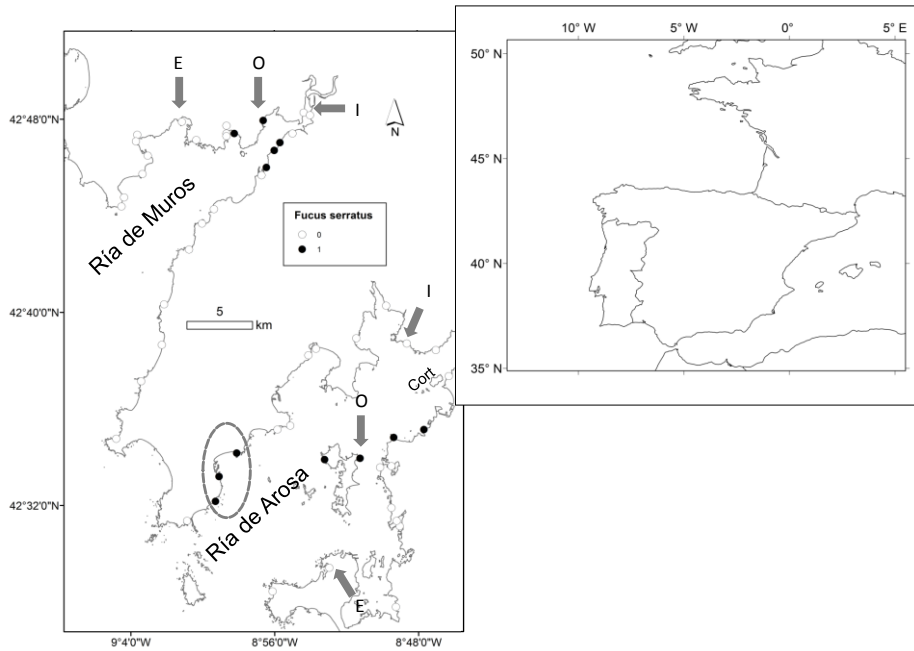


Figure 1. Study area and surveyed locations with presence and absence of *Fucus serratus* (sampling of 2011). The exterior (E), origin (O) and interior (I) locations where the transplant experiments were carried out are indicated with grey arrows in each Ría. Ría de Muros: Pta. Avelleira (E), Ofreixo (O), Punta Barquiña (I). Ría de Arosa: Playa de Tanxil (I), Isla de Arosa (O), Lobajera-Chan (E). Cort: Isla de Cortegada. Encircled with a grey dashed line in Ría de Arosa are locations where the abundance of *F. serratus* declined from 2005 to 2011-13.

2.1.2. Study sites for transplant experiments

For the transplant experiments with both germlings and juveniles, three locations with rocky substratum were selected per Ría, two of them in exterior and

interior positions of the Rías in relation to an ‘origin’ location, where the macroalgae *Fucus serratus* is at present still abundant and temporally persistent (see results of the field surveys and bibliographic review). The criterion for selecting outer and inner locations, apart from the lack of *F. serratus*, was the presence of the abundant cover of other fucoids.

The selected locations were: Isla de Arosa (42°33'N, 8°51'W), Lobajera-Chan (42°49'N, 8°88'W) and Tanxil (42°64'N, 8°81'W) as origin (O), exterior (E) and interior (I) locations respectively in the Ría de Arosa (Fig. 1). Then, Ofreixo (42°47'N, 8°56'W), Punta Avelleira (42°79'N, 9°02'W) and Punta Barquiña (42°80'N, 8°90'W) as origin, exterior and interior locations, respectively, in the Ría de Muros (Fig. 1).

2.2 *Settlement and transplants of F. serratus' germlings*

We used artificial discs with a rough surface (4 cm in diameter and 0.5 cm thick) for the settlement of *Fucus serratus* germlings in the field. These discs were made of an epoxy resin (Fetadit 55/63; Fetasa, Madrid, Spain) using silicone molds, following a procedure similar to the one used by Johnson (1994). Before using the discs, these were rinsed in tap water and then immersed into seawater (previously filtered with Whatman GF/C filters) for 48 hours. When reutilized, they were brushed and immersed in a 10% solution of bleach (sodium hypochlorite) to eliminate germlings and other organic rests, and subsequently rinsed in tap and filtered seawater.

Discs were drilled to create a central hole and attached with stainless steel screws to polycarbonate plates (14 x 14 cm and 0.3 cm thick), which were likewise fasten onto the rocky platform. A total of 12 plates with four discs each (i.e. 48 discs) were placed in the ‘origin’ locations from both Rías (i.e. Ofreixo and Isla de Arosa) during low spring tides of September 2012. Plates were located at mid-shore level (about 1.4 m above the Lowest Astronomical Tide) underneath *F. serratus* canopy, and separated from each other at a minimum distance of 1 to 3 m.

The transplant experiment was set up during spring tides of November 2012. Then, all the 48 discs were collected at each origin locations during low spring tides and the number of germlings of *F. serratus* counted in a sample of 23 discs per location. Counts were made at 25x magnification with a stereo microscope on the complete artificial surface within hours after collection. Discs were then placed into plastic containers with sea water and proper aeration, and returned back into the field the same night or the following day at low tide. Twelve discs randomly chosen were transplanted to exterior locations and 12 to interior locations, while the remaining 22-23 (two and one discs in Muros and Arosa respectively were discarded for the experiment, due to very low recruitment) were returned into the origin locations. In inner and outer locations, plates with discs were fixed under furoid canopies at a similar shore level than in the origin locations. The transplant experiment took place during 2 months, until January 2013. All discs were then collected at each location and embryos were counted again with a stereo microscope.

At this time of the year, *F. serratus* and *Ascophyllum nodosum* presented reproductive structures (receptacles) in the studied area. As new settlement onto the discs might confound the results of the transplant experiment, two types of controls were installed: a) in origin locations, receptacles of *F. serratus* fronds covering half of discs were removed, in areas of about 50 x 50 cm around plates, whereas the canopies over the other half were left intact; b) 6-7 new discs were added at the start of the experiment in origin locations, underneath un-manipulated *F. serratus* canopies, and in recipient locations with presence of reproductive *A. nodosum*, to record new settlers. Due to logistic constrains, however, new discs were only placed in the Ría de Muros. The first control, i.e. removal of *F. serratus* receptacles at the start of the experiment, resulted ineffective for avoiding new recruitment over the experimental period (two months), as the final number of embryos did not differ between discs under canopies with and without receptacles (ANOVA for

Treatment effect: $F_{1,10} = 0.35$, $p = 0.569$; $F_{1,9} = 0.03$, $p = 0.863$ for Ría de Arosa and Muros respectively). Only the discs underneath un-manipulated canopies were subsequently used in the experiment. New settlers were however recorded on the control discs in origin locations (but not in recipient location with presence of *A. nodosum*), and this settlement was then considered when analyzing the results of the experiment (see above).

2.3 Juvenile transplants

Transplant of juveniles (i.e. non-reproductive individuals of <15 cm length) were done at two different periods during the growing season of *Fucus serratus* (Arrontes 1993), hereafter named first and second trials. The first trial ran from February to May 2012 in the Ría de Arosa, and from a shorter period (due to logistic limitations), from March to May 2012 in the Ría de Muros. The second trial was done simultaneously in both Rías from the beginning of June to the end of July 2012. We used juvenile specimens and short periods of time for these transplant experiments (2 - 3 months) in order to prevent the development of receptacles and then the accidental introduction of the species in locations where historically has been absent.

In the origin locations of both Rías, we selected 72 juvenile individuals. Juveniles grew attached onto shells of *Mytilus galloprovincialis* and barnacles *Balanus* sp, in addition to the rocky substratum. We removed 54 out of these 72 selected individuals, attached to a fragment of rock or mussel shells/barnacles. This was done with the help of a hammer and a chisel, taken care of not damaging the fronds. The rest of 18 juveniles were left un-manipulated and used as controls. Detached individuals were transplanted in origin (hereafter named 'auto-transplants') and external and internal locations (N= 18) at mid-shore level, about 1.4 m above LAT. Three groups of 6 plants were placed in sites of about 1 m², which were randomly selected within each location and separated by 3-5 m. Controls were located around these sites. Auto-transplants were subject to similar

transportation times and conditions than the rest of transplanted individuals. Each specimen was marked with a numbered plastic label. The piece of rock or the mussel shell/group of barnacles with a juvenile attached was fixed onto the rocky substratum with cement. Loss of several transplanted individuals occurred due to attachment failure.

Length (from the base of the holdfast to the tip of the longest branch) and maximum circumference of controls, auto-transplants and transplanted algae were measured in the field (with an accuracy of 0.1 cm) at the start and end of each experimental trial. Those algae with a circumference < 2 cm could not be measured accurately and then were approximate to 2 cm. Volume was calculated with the length (l) and circumference (c) of each algae ($v = lc^2$), as described by Åberg (1990). At the end of both trials algae were collected and taken to the laboratory where they were dried at 60 °C for 48 hours and weighted.

2.4 *Cover of furoid algae and density of grazers*

A quantitative sampling was carried out to estimate the abundance of *Fucus serratus* and other coexisting Fucacean algae, such as, *F. vesiculosus* L, *Ascophyllum nodosum* (L.) Le Jolis and *Himanthalia elongata* (L) S.F Gray, and of macrograzers in the origin, exterior and interior locations within each ría. Sampling of algae was done in May 2012 while grazer density was estimated in two dates, May and July 2012. At each location we randomly selected three sites, which were about 10 to 20 m apart. At each site, four plots (of 0.25 m² for algae and 0.09 m² for grazers) were randomly sampled during low spring tides. Cover of fucoids was estimated by the point-intercept method using a 50 x 50 cm PVC frame, with a grid made of double thread and 81 regularly spaced intersections. Both primary and secondary covers (holdfast of adult plants and juveniles < 5 cm length, and overstory canopies respectively) were estimated, and values transformed to percentages. Total cover may then sum $> 100\%$. A cover of 1% was assigned to fucoids present in the plots but not recorded. Density of macrograzers (gastropods

and chitons) was also estimated *in situ* with the help of 30 x 30 cm PVC frames subdivided in 25 squares.

2.5 Environmental variables: sea water temperature, salinity and dissolved inorganic nutrients

Temperature (°C) was measured with Tidbit loggers (Onset Corporation, MA, USA) placed at mid-shore level at 23 out of those 52 locations where macroalgae occurrence was recorded (10 and 13 in the Ría de Muros and the Ría de Arosa respectively, see Appendix 1). Data loggers were fastened onto the rocky substratum with stainless steel screws in July (Ría de Muros) and December 2011 (Ría de Arosa) and programmed to record temperature every 30 minutes until September 2013. Data were downloaded periodically and two readings selected per day, corresponding to the high-tide moments.

Mechanical failure or loss of data loggers due to human interference generated gaps of variable duration in the data set. Therefore, temperature time series had to be interpolated for some loggers to subsequently estimate average temperatures. For the study period, high-tide temperature data completeness averaged 89 and 93% in Ría de Arosa y Muros respectively (range 43-100%). Missing records were interpolated using linear regressions of temperature data from the location in question plotted against temperature data from a different location. The criteria for selecting locations for interpolating missing data were: (1) they had no gaps in the period to be completed, (2) they showed the greatest Pearson product-moment correlation r value with the site showing missing data over the two-year period (r values ranged 0.97-0.99), of sites that fulfilled the other criteria, and (3) they showed a linear correlation (by visual inspection of scatterplots). Interpolated temperature data were then used to calculate average summer temperatures (July-September), winter temperatures (Jan-March) and total average values over the period December 2011 to September 2013.

Surface (0 to 5 m) salinity (‰) and dissolved inorganic nutrients concentrations (nitrate, nitrite, ammonium and orthophosphate, μM) were obtained at approximately weekly intervals in 18 oceanographic stations inside Rías (8 and 10 stations in Ría de Muros and Arosa respectively, see Appendix 1) over a two-year period (January 2012 to December 2013) from the Instituto Tecnológico para o Control do Medio Mariño de Galicia (Inctemar, Xunta de Galicia, Vilagarcía de Arousa, Spain). Average values for salinity and inorganic nutrients were then calculated over this 2-year period.

2.6 *Statistical analyses*

We used a Generalized Linear Model (GLM) with Negative Binomial distribution and a log link function to analyze the differences between Rías and Locations (origin, exterior and interior), considered fixed and orthogonal factors, in the final counts of the *F. serratus*’ germlings. In order to avoid confounding effects of new settlement during the course of the experiment, taking place in origin locations, the average number of recruits on control discs was subtracted from each count in origin locations. The negative binomial instead of the Poisson distribution was used in the GLM due to over dispersion of data (over dispersion parameter = 15.17; Zuur et al. 2009).

Juvenile transplants data were analyzed by one-way analyses of variance (ANOVAs), including *a priori* planned comparisons to test for transplant artefacts after significant effects of Treatment (Underwood 1997). Initial length and volume, and elongation (final- initial length) and final dry weights were the dependent variables analyzed. Results from different rías were analyzed separately, given the unbalanced design, due to the loss of transplanted specimens (see above), and the fact that in the first trial transplants were conducted at different times in Ría de Muros and Ría de Arosa.

ANOVAs were also used to analyze the cover of *F. serratus*, total cover of fucoids and density of grazers in the origin, inner and outer locations. The factors

analyzed were Ría (two levels, fixed), Location (origin, interior and exterior, fixed), Site (random and nested within Location) and in the case of grazers, Date (two levels, fixed). Differences in the assemblage of macrograzers were also evaluated using permutational multivariate analyses of variance (PERMANOVA; Anderson 2001, Anderson et al. 2008) on a Bray-Curtis similarity matrix calculated on fourth-root transformed data. The analysis was done using 9999 random permutations. When a significant pseudo-F was found, *a posteriori* comparisons were also performed. Furthermore, we used SIMPER analyses (Clarke 1993) to detect the species that contributed the most to these differences.

2.7. *Spatial analysis*

In order to obtain environmental information (salinity, temperature and inorganic nutrient concentration) in those locations where biological data (occurrence of *Fucus serratus* and other fucoids) were recorded, the information was processed with the Geographical Information System (GIS) software ArcGIS version 10.1 and the extension Spatial Analyst by ESRI (Redlands, CA). Data were analyzed by the inverse-distance weighted-interpolation algorithm with the following settings: power, 2 (Euclidean distance); 12 points; and variable search radius. Interpolation was done independently for each ría. Maps of the study area were obtained in format Shapefile (vector data) as BCN200 (Base Cartografica Numerica scale, 1:200,000) from the National Geographic Institute of Spain.

2.8. *Habitat distribution model for *F. serratus* inside rías*

To analyze the occurrence of *F. serratus* inside the Rías de Muros and Arosa we applied generalized linear models (GLMs) with a binomial distribution and a clog-log link function, as there were considerable more absences than presences (Zuur et al. 2009). The average temperature in summer (July-September, two-year study period), the nitrite + nitrate, ammonium and phosphate concentrations (total average over the study period), salinity and standard deviation

of salinity (i.e. estimation of temporal variability in freshwater inflow) were pre-selected as explanatory variables, in addition to the occurrence of *F. vesiculosus* and *H. elongata* (abundant furoid species coexisting with *F. serratus* inside rías), and the type of substratum. In particular, the presence of soft sediment covering the rocky substratum was determined through personal inspections during the field survey, and a dummy variable was created, with the zero value assigned to those locations with presence of a continuous muddy cover.

Pair-wise Spearman correlations between environmental variables were examined prior to final selection of explanatory variables. Correlations between nitrate + nitrite, ammonium concentrations, and salinity were significant and > 0.7 in absolute value (commonly applied threshold for collineality, see Dormann et al. 2013). Only one of these three variables was then included in alternative models. We preferred a linear over a nonlinear response to avoid overfitting taking into account the reduced number of data (52 locations), and also for consistency with the spatial scale analyzed, where environmental gradients are potentially short. Nevertheless, we fitted all possible combinations of linear and quadratic terms (subject in the last case to the condition that the corresponding linear term was included in the model), with no interactions, and used the Akaike Information Criterion adjusted for small sample size (AICc; Burnham & Anderson 2002) to rank models. Model selection was performed using ‘MuMIn’ package (Barton 2012; R Development Core Team 2012).

Additionally, hierarchical partitioning (HP) was used to assess the independent and joint effects of each explanatory variable included in the models. Binomial regression and log-likelihood as the goodness-of-fit measure were used, and statistical significance of the independent contributions was tested by a randomization routine (1,000 permutations) based on Z scores (Mac Nally 2002). HP was conducted using the ‘hier.part’ package (Walsh & Mac Nally 2004). HP should be applied with caution when > 9 explanatory variables are used, as the ranking of importance of the predictors may change depending on the variable

orders (Olea et al. 2010). However, we have 9 variables and after checking the potential effect of changing the order of variables, no effect was found.

Model accuracy was tested by checking the correct and incorrect classification of predicted values. Relative true presences or sensitivity of the model (RTP), relative true absences or specificity (RTA), and the kappa coefficient (k) were calculated. The prevalence of *F. serratus* was used as the threshold probability to calculate these performance metrics (Landis & Koch 1977, Fielding & Bell 1997).

3. RESULTS

3.1 Past and present geographic distribution of *Fucus serratus* in the study Rías Bajas

Historic surveys described the distribution of *Fucus serratus* in the rocky intertidal coasts of the Rías Bajas as scattered, constituted by isolated populations inside these large embayments (Pazó and Niell 1977). In particular, isolated and abundant populations were described in Ofreixo in the Ría de Muros, Isla de Cortegada in the Ría de Arosa, and Meira in the Ría de Vigo since 1950's-1960's (Ardre 1957, Donze 1968, Pazó & Niell 1977).

Our surveys in 2004-05 inside the Rías de Arosa and Muros recorded the presence of populations of *F. serratus*, apart from the origin sites for the transplant experiments (i.e. Ofreixo and Isla de Arosa in the Rías de Muros and Arosa respectively), in the following locations (see Fig. 1): Agrocobo (42°44'N, 8°58'W), Boa (42°46'N, 8°55'W) and Ormanda Beach (42°46'N, 8°56'W) in Ría de Muros; Castañeiras Beach (42°32'N, 8°59'W) Riveira (42°33'N, 8°59'W), Río Azor (42°57'N, 8°96'W) in the north coast of the Ría de Arosa; and Vilaxoan (42°35'N, 8°47'W), Sinas Beach (42°34'N, 8°49'W), and the Isla de Arosa (exterior site; 42°34'N, 8°53'W), in the south coast of Ría de Arosa. We did not survey Isla de Cortegada in the Ría de Arosa. In Ría de Pontevedra no populations were found in surveyed locations, and only one in a location of the Ría de Vigo (see Martinez et

al. 2012). In the new survey of 2011 (and subsequent visits in 2013), we found an evident decrease in the abundance and individual sizes in the populations of *F. serratus* from the three locations on the north coast of the Ría de Arosa (see Fig. 1), in particular in Río Azor, where just one testimonial individual was found in 2013. These three populations were nevertheless considered in the distribution model for the species occurrence (see below).

3.2 *Transplants of germlings and juveniles of Fucus serratus*

Just before transplantation (November 2012), the number of settled embryos per disk was 178 ± 19 (mean \pm SE, $N = 46$), without differences between Rías (ANOVA for Ría effect, $F_{1,44} = 0.44$, $P = 0.509$). Two months after transplantation, in January 2013, the average number of germlings remaining on discs was significantly higher in origin than in exterior and interior locations (Fig. 2, Table 1, non-significant interaction Location \times Treatment, significant effect of origin). Also, there was a non-significant trend for a higher number of germlings in the origin location of the Ría de Muros (Ofreixo) than in the Ría de Arosa (Isla de Arosa, Fig. 2). It is worth noticing that for the analysis, the average value in control discs was subtracted from each count of the origin locations, to avoid confounding effects due to new recruitment (see ‘Material and methods’). The same results were obtained when the maximum value (33 germlings) instead of the mean value of control discs was discounted (analysis not shown).

In the transplant experiments with juveniles, the initial size (length and volume) of individuals did not differ among Treatments (auto-transplant, control, exterior and interior), either in the first or second trials (ANOVA for Treatment effect: $F_{3,136} = 4.60$, $P = 0.408$, $F_{3,136} = 0.30$, $P = 0.823$, $F_{3,136} = 1.85$, $P = 0.140$, $F_{3,136} = 1.02$, $P = 0.384$ for initial length and volume of the first and second trial respectively). In the first trial, however, significant differences were found between Rías, with higher initial sizes in Ría de Arosa than in Muros (ANOVA for Ría effect: $F_{1,136} = 4.60$, $P = 0.030$ for length and $F_{1,136} = 5.23$, $P = 0.024$ for volume).

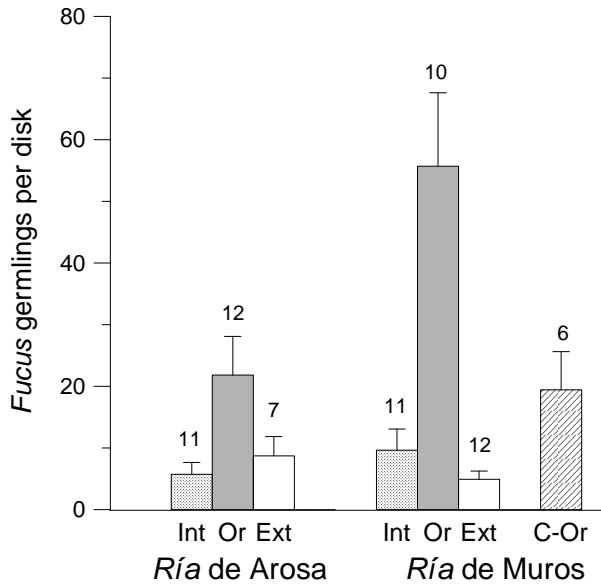


Figure 2. Final counting of *Fucus serratus* germlings (January 2013) at the distinct locations (Interior -Int, Origin-Or and Exterior-Ext) of the Rías de Arosa and Muros. The number of germlings in control discs in the origin location of Ría de Muros is also shown. Data are means \pm SE, N as indicated.

Table 1. a) Likelihood ratio test (LRT) for GLMs with Binomial Negative distribution and a log link function testing differences between Rías (Ri: Arosa, Muros) and locations (Lo: origin, exterior and interior) in the final counts of *F. serratus*’ germlings. The full model (M1) was simplified by removing the interaction term Ri x Lo; b) Parameter estimates (fitted values) and standard errors (SE) of the simplified model (M2). The fitted value for the interior location of Ría de Arosa was taken as reference level and the other estimates are differences from this reference level. Dispersion parameter of M2 is 1.40.

a) Model	Theta parameter	df	2loglik	Δ df	LRT	p
M1, Full (Ri, Lo, Ri x Lo)	0.5705	59	-417.84			
M2 (-Ri x Lo)	0.6296	57	-412.67	2	5.174	0.075
b) Parameters	Estimate	ES	z-value	p		
Intercept	1.6201	0.3872	4.184	<0.001		
Ri: Muros	0.4932	0.3485	1.415	0.157		
Lo: Exterior	0.1390	0.4338	0.321	0.749		
Lo: Origin	1.1543	0.4314	2.675	0.007		

Our results showed no significant effects of transplant treatments on the elongation or final dry weights of individuals (Table 2). Nevertheless, there was a non-significant trend for specimens transplanted into exterior locations presenting lower elongation values and final weights than juveniles remaining in the origin locations (auto-transplants, see Fig. 3). Additionally, in the first trial in the Ría de Arosa we detected a significant effect of the manipulation, with controls presenting higher elongation values than auto-transplants (Fig. 3, Table 2).

Table 2. One-way analysis of variance (ANOVA) for the effect of treatments (auto-transplants, control, exterior and interior) on the elongation (final length – initial length) and final dry weight of juveniles of *Fucus serratus*. Test a priori were shown after significant effect of Treatment. Unbalanced data (see Fig. 3), except for second Trial of Ría de Muros, were n = 9. C = Control, AT = Auto-transplant; I =Interior.

1 st Trial					Ría de Muros			
Elongation								
Source	df	MS	F	P	df	MS	F	p
Treatment					3	3.363	0.694	0.561
C vs AT	1	19.627	4.870	0.039				
AT vs I	1	0.486	0.079	0.786				
Residual	22	3.790			40	4.845		
Dry weight								
Treatment	2	5.445	1.863	0.179	3	0.546	1.380	0.263
Residual	22	2.922			40	0.396		
2 nd Trial					Ría de Muros			
Elongation								
Treatment	3	2.512	1.946	0.142	2	7.341	2.392	0.113
Residual	32	1.291			24	3.069		
Dry weight								
Treatment	3	0.261	0.718	0.548	2	0.591	1.602	0.222
Residual	32	0.363			24	0.369		

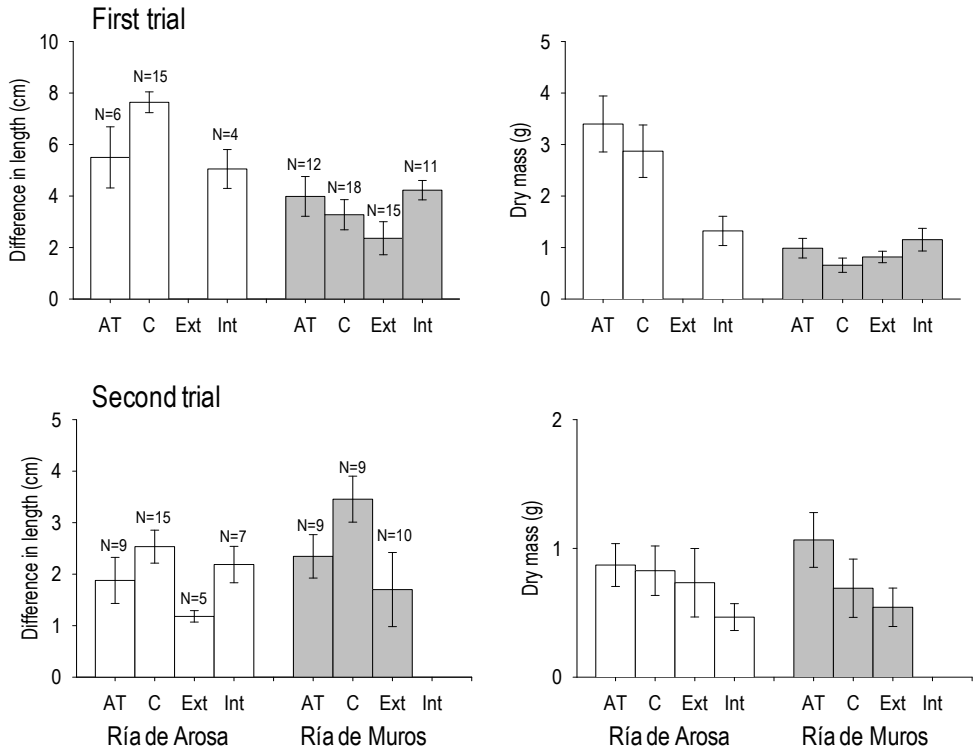


Figure 3. Elongation (final-initial length, cm) and final dry weight (g) of *Fucus serratus* juvenile transplants in the Rías de Arosa and Muros. Treatment abbreviations: AT = auto-transplants, C = controls, Ext = exterior, Int = interior. Data are means \pm SE, N as indicated.

3.3 Survey of algae and herbivores in the distinct locations

As expected, in origin locations there was a high cover of *Fucus serratus*, which form belts in the mid-intertidal area of these rocky shores with *F. vesiculosus* (Fig. 4a). *Himanthalia elongata* was also present. In exterior locations of both Rías *F. vesiculosus* prevailed, while in the interior ones *F. vesiculosus* and *A. nodosum* co-dominated (Fig. 4a). The total cover of fucoids tends to be higher in the Ría de Muros than Arosa (Table 3, marginally significant effect of Ría). There

was also a high variability in cover at small spatial scales, which also differed between Rías (significant Ría x Site interaction, Table 3).

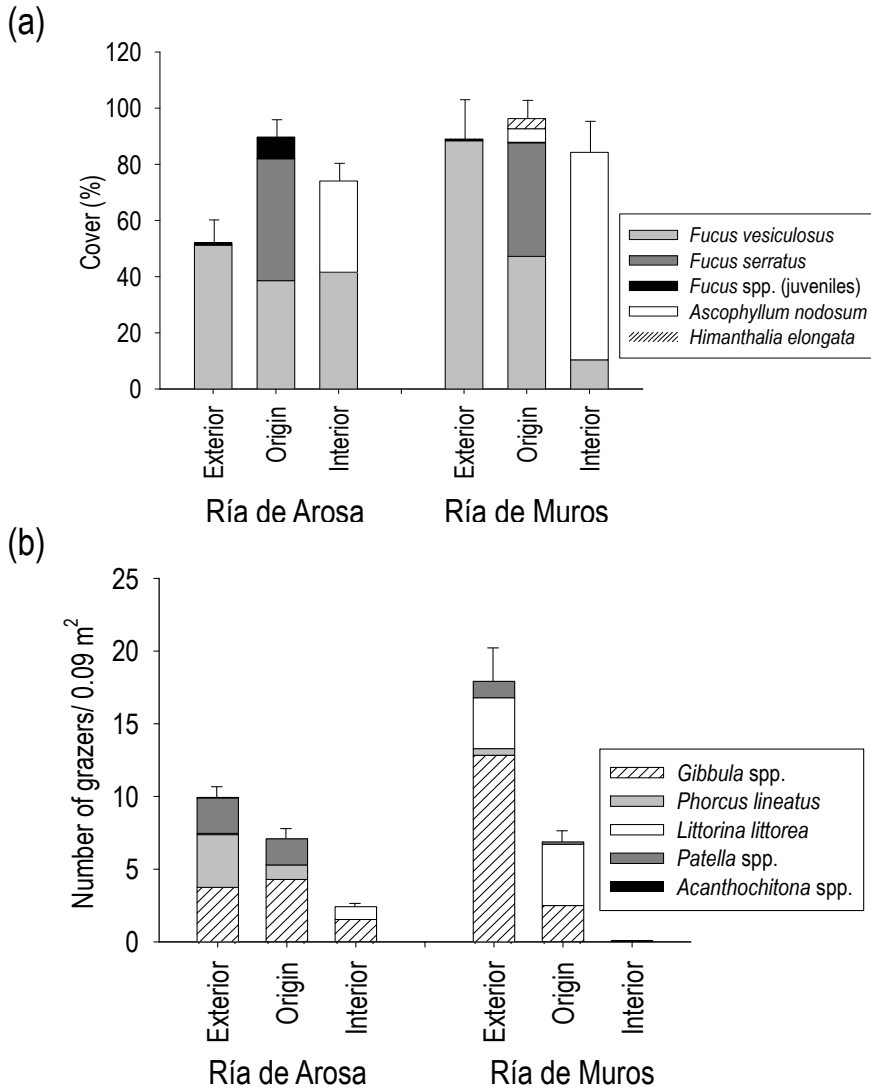


Figure 4. a) Mean percent cover of fucoids in origin, exterior and interior locations of Rías of Arosa and Muros in NW Spain. Locations surveyed in May 2012; b) Number of macrograzers in exterior, origin and interior locations of the study area. Data from different dates (May and July 2012) were pooled. Data are means \pm SE, N = 12 in a), N = 24 in b).

Table 3. ANOVA for differences between Rías (Muros, Arosa), among Locations (interior, origin, exterior) and Sites on the total cover of Fucacean species. Homogeneous variances.

Source	df	MS	F	P
Rías	1	5165.964	6.113	0.048
Location, Lo	2	2789.971	1.810	0.243
Ría x Lo	2	1412.471	1.671	0.265
Site (Lo)	6	1541.114	7.470	<0.001
Ria x Site (Lo)	6	845.124	4.096	0.002
Residual	53	206.308		

Results also indicated high small-scale variability in the total density of grazers and species composition (significant effects of Site, independently or in combination with other factors, Table 4). There were as well differences between locations (interior, exterior and origin) dependent on the Ría, but not on the sampling Date (significant interaction Ría x Location in ANOVA and PERMANOVA, Table 4). Thus, in both Rías there was a gradient, from higher to lower density of molluscan grazers, from the external to the interior location, but differences in the assemblage appeared more evident in the Ría de Muros than in the Ría de Arosa (Table 4, a *posteriori* comparisons in PERMANOVA). The species that mostly contributed to differences between the exterior and the interior locations were *Gibbula* spp. and *Littorina littorea* in the Ría de Muros, and *Phorcus lineatus* and *Patella* spp. in the Ría de Arosa, all of them more abundant in the exterior location (SIMPER analyses; see Fig. 4b). Actually, in the interior location of Ría de Muros, grazers were nearly absent (Fig. 4b).

Table 4. ANOVA (a) and PERMANOVA (b) for testing the effects of Date, Rías Location and Sites on the total density of macro-grazers, and the species composition and abundance of the grazing assemblage, respectively. *A posteriori* comparisons after significant interaction Ría x Location in PERMANOVA are also shown. In ANOVA, variances were homogenous at $p = 0.01$. In PERMANOVA the Monte-Carlo asymptotic p-value was used for Location and for the *a posteriori* comparisons of Locations within Rías. E = Exterior location, I = interior, O = Origin.

a) ANOVA					b) PERMANOVA		
Source	df	MS	F	P	MS	F	P
Date, Da	1	10.562	0.080	0.787	1083.331	0.361	0.699
Ría	1	122.840	2.516	0.164	36172.409	72.424	<0.001
Location,	2	1918.132	53.302	<0.001	53396.239	66.081	<0.001
Site (Lo)	6	35.986	1.679	0.133	808.045	0.799	0.683
Da x Ría	1	21.007	0.160	0.703	2005.237	1.288	0.303
Da x Lo	2	3.271	0.025	0.976	2674.144	0.890	0.486
Da x Site	6	131.972	6.157	<0.001	3004.410	2.971	0.001
Ría x Lo	2	363.549	7.445	0.024	33446.702	66.967	<0.001
Ría x	6	48.833	2.278	0.041	499.453	0.494	0.943
Da x Ría	2	35.715	0.273	0.770	1931.466	1.240	0.338
Da x Ría x	6	131.042	6.114	<0.001	1557.290	1.540	0.091
Residual	108	21.433			1011.238		
<i>A posteriori</i> comparisons Ría x Location							
				Ría de Arosa		Ría de Muros	
				t	P	t	P
E vs I				5.759	<0.001	11.960	<0.001
E vs O				2.693	0.039	3.433	0.007
I vs O				5.477	0.002	10.453	<0.001

3.4. *Habitat distribution model of F. serratus inside the Rías de Muros and Arosa*

Two models were the “best”, according to AICc criteria: one which linked *F. serratus* occurrence to that of *Himanthalia elongata* and to high ammonium concentrations (total average over a two-year period, hereafter model 1), and a second one (model 2) relating the presence of the target species again to the occurrence of *H. elongata*, but also to low average values of sea temperature in summer and high temporal salinity variability (Table 5a, Fig. 6). It is worth mentioning that the external locations selected for transplant experiments presented lower nutrient concentrations and salinity variability, and higher average seawater temperature in summer than origin and inner locations (Fig. 6).

Model 1 was however better than Model 2 according to the following criteria: i) it was the most parsimonious (less number of explanatory variables), ii) it includes the two explanatory variables with the greatest independent contribution to species occurrence and statistically significant in HP analysis (Fig. 5), and iii) it was the most accurate, according to the corrected predictions and kappa coefficients; in particular model 1 predicted higher proportion of absences than model 2 (Table 5b). As above indicated (see methods), ammonium concentrations were correlated to values of nitrite + nitrate and salinity, and then the occurrence of the species could also be linked to these factors.

Table 5. a) Summary of the best models according to the AICc criteria for *F. serratus* occurrence inside Rías. Him = occurrence of *Himanthalia elongata*; Ammonium = Ammonium average concentration over the study-period; SeaT-Summer: average sea surface temperature in summer; SalinitySD: Standard Deviation of Salinity; b) Performance metrics of the models. Relative true presences (sensitivity, RTP), relative true absences or specificity (RTA) and kappa coefficients are shown. The prevalence was 0.21.

	a)					b)			
	Intercept	Him	Ammonium	SeaT	Salinity	AICc	RTP	RTA	k
				Sum	SD				
Model 1	-9.231	+	4.623			46.5	0.73	0.78	0.42
Model 2	25.570	+		-2.014	2.918	47	0.73	0.68	^a 0.31
									^b

^a good and ^b poor, according to Landis and Kock 1977

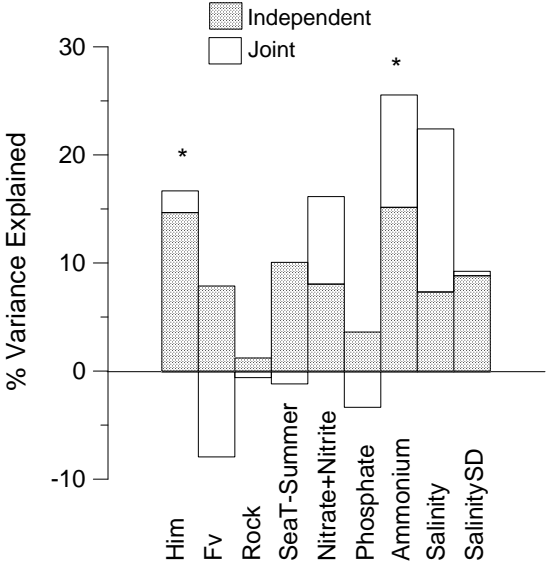


Fig. 5. The independent and joint contributions (given as the percentage of the total explained variance) of the predictor variables for the occupancy of *F. serratus*, as estimated from hierarchical partitioning. Asterisks indicate the variables which independent contributions to explaining variation were significant (randomization tests, $p < 0.05$). Him: *Himanthalia elongata* occurrence; Fv: *Fucus vesiculosus* occurrence; Rock: type of substratum; SeaT-Summer: Sea Surface Temperature in summer (July-September); Nitrate+Nitrite, Phosphate and Ammonium: Mean values of inorganic nutrient concentrations during the study period; Salinity: average salinity; SalinitySD: standard deviation of Salinity over the study period.

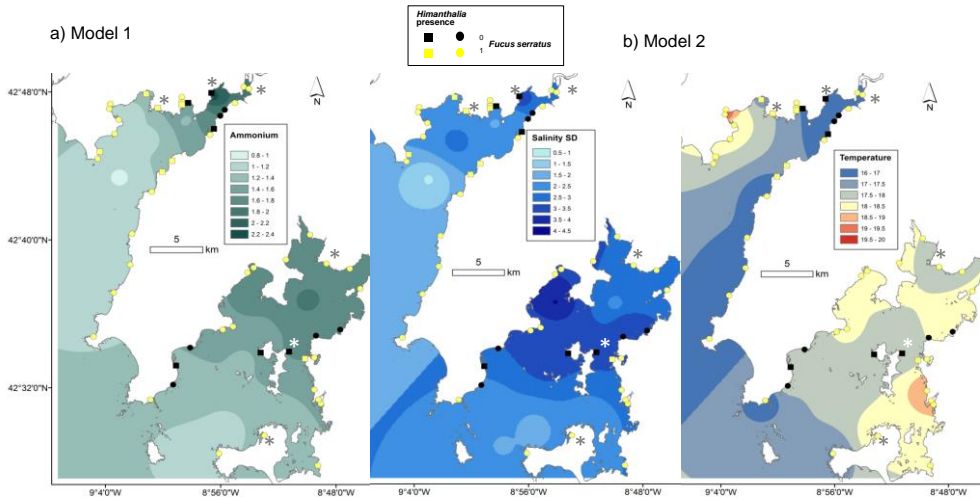


Figure 6. Maps showing the distribution of *F. serratus* in the studied Rías (absences in yellow symbols and presences in black symbols), and the values of the explanatory variables included in Models 1 and 2 (see Table 5): occurrence of *Himanthalia elongata* (yellow and black squares representing the presence of the species), mean ammonium concentrations (μM), the Standard Deviation of Salinity (Salinity SD), and the mean seawater temperature in summer ($^{\circ}\text{C}$). Asterisks indicated the selected locations for the transplant experiments in both rías.

4. DISCUSSION

The canopy-forming algae *Fucus serratus* is abundant and coexist with other macroalgae species like, *F. vesiculosus*, *Ascophyllum nodosum* and *Himanthalia elongata* in origin locations at both study Rías. Overall, our experiment with germlings of *F. serratus* indicates that there was a higher number of germlings in origin than exterior and interior locations, without significant differences between origin locations (Ofreixo and Isla de Arosa). Also, there is a tendency of lower length and less weight in transplanted juveniles, especially those juveniles left in exterior locations. Then, in general these results show that individuals of *F. serratus* survived better in origin locations, which populations have a historical permanence. Furthermore, this study suggest that the scattered geographic distribution of *F. serratus* in the Rías de Arosa and Muros may be linked to the presence of the macroalga *H. elongata*, high ammonium

concentrations, lower summer sea temperatures and high temporal salinity variability.

The geographic distribution of macroalgae in the intertidal realm may be the outcome of the interplay of a number of physical and non-environmental factors (Lüning 1990, Little & Kitching, 1996, Lobban & Harrison 1997). Competition with other canopy-forming species could explain the absence of *F. serratus* in exterior and interior locations. For instance, *F. vesiculosus* is described to have more tolerance than *F. serratus* to higher temperatures and wave action (Pazo & Niell 1977). One could also argue that in exterior locations, where we reported a higher density of macro-herbivores (see “Results”, Fig. 4b), grazing could inhibit the development of *F. serratus*, as some intertidal macro-herbivores have been reported to eat algae in their different stages (Lubchenco et al. 1982, Fredriksen 2003, Norderhaug et al. 2003, Schaal et al. 2010). Even, our epoxy discs had abrupt borders, which could have limited the access of grazers to the germlings. Additionally, we did not notice signs of grazing in the transplant of juveniles, especially in exterior locations. Nonetheless, it is known that grazers are more abundant in southern vs northern Europe (Southward et al. 1995) and species like the gastropods *Littorina* spp, *Gibbula* spp. and *Patella vulgata* have been described to feed on diatoms, embryos of algae and adult algae (Fischer-Piette 1948, Castenholz 1961, Williams 1990, Crothers 2001). Other studies have as well reported that the periwinkle *Littorina littorea* graze on small germlings of *Fucus* (Lubchenco 1983), while other species like *L. obtusata* and *L. mariae* feed on *A. nodosum* and epiphytes of *F. serratus* (Williams 1990). Also, *Patella vulgata* forages on *A. nodosum*, *Laminaria digitata*, *F. vesiculosus* and *F. serratus* among other macroalgae (Jones 1948, Lorenzen 2007).

On the other hand, the lower number of germlings of *F. serratus* in interior locations cannot be linked to grazing, as especially in the interior location of the Ría de Muros, macro-herbivores are practically absent (see “Results”, Fig. 4b). The

transition from lower to high muddy rocky sections in origin to interior locations was visually detected in our surveys, which further explains the overall lower numbers of grazers in interior locations, as in habitats with heavy sedimentation grazers feeding habits and movement are very restricted (Airoldi & Hawkins 2007, Schiel et al. 2006). The effects of sediments on sessile species, like macroalgae, certainly depend on the species, growth stage of the algae and the sediment characteristics (Santos 1993, Chapman & Fletcher 2002). And one could argue that the lack of *F. serratus* in these interior locations of the Rías may be feasibly related to the higher cover of a muddy film. Nevertheless, sediments could positively influence algae by offering protection against photoinhibitory light stress and desiccation (Ladah et al. 2003). In the case of *F. serratus*' embryos have been reported to be negatively affected by grazing and no by sedimentation (Araujo et al. 2012). Furthermore, a laboratory study described that the effects of sediments on *F. serratus* propagules depend on the sediment type (Chapman & Fletcher 2002). Other revisions have shown detrimental effects of sediments in the abundance, growth and reproduction of algae as well as in the settlement and survival of germlings (Devinny & Volse 1978, Santos 1993, Umar et al. 1998, Airoldi & Cinelli 1997). For instance, in the Baltic Sea the abundance and distribution of the habitat-forming algae *Fucus vesiculosus*, especially in its juvenile stage are described to be affected by sediment deposition (Eriksson & Johansson 2003).

Other factors apart from grazing and sediment deposition could also explain the scattered distribution of *F. serratus* in our study locations. In the case of *F. serratus* and at a regional scale (Iberian Peninsula) the geographic distribution of the macroalgae has been explained by cloudiness, air and sea surface temperatures (see Martínez et al. 2012). Also, remarkably empirical work shows that the geographic distribution of the macroalgae *F. serratus* and *H. elongata* at larger spatial scales is very different (Iberian Peninsula), as

Himanthalia is found in more exposed coasts than *F. serratus* (see Martínez et al. 2012, Duarte et al. 2013). Contrary, in the interior locations of the Rías, *H. elongata* and *F. serratus* populations occupied the same habitats (see “Results” and Fig. 6).

Empirical work has also shown the importance of wave action in the distribution of seaweeds along the intertidal coast (Dayton 1975, Blanchette 1997, Taylor & Schiel 2003, Jonsson et al. 2006). Surely, *F. serratus* is found in semi-exposed and protected areas of the coast (Ballantine 1961; Cremades et al. 2004). Then, in our models we did not include the predictable variable wave exposure as the Rías are more protected areas, even the south mouth of the Ría de Muros is a wave exposed area (see Martínez et al. 2012), but *F. vesiculosus* was not present and only red algae were observed (Viejo pers. obs.). Exterior locations in these study behave as semi-exposed areas with a dominant presence of the furoid *F. vesiculosus* (Cremades et al. 2004), while interior locations are sheltered areas with a higher presence of the macroalgae *Ascophyllum nodosum*, which is abundant and common in these exposure habitats (Moyse & Smith 1963, Cremades et al. 2004, Oliveira et al. 2006, Olsen et al. 2010).

Our habitat model describe that the presence of *F. serratus* was favored by lower summer sea temperatures, which is in accordance with a laboratory study that describes that high temperatures affect the growth of germlings of *F. serratus* (Arrontes 1993). Certainly, sea surface temperatures in origin locations of both Rías (see “Results”) are in the limited or a slightly higher that the maximal optimal sea temperature (18°C) indicated for *Fucus serratus* (Pazó and Niell, 1977). And below the survival limit of 25°C described for the species by Lüning (1984), and the 24°C limited temperature reported for the growth of the seaweed in laboratory experiments (Martínez et al. unpublished data). Furthermore, our model also suggest the presence of *Fucus serratus* in the Rías Bajas locations exposed to high concentrations of inorganic nutrients, specifically ammonium, which may be provided by the intense upwelling events describe in

spring-summer in these areas (Fraga 1981, Álvarez et al. 2008, 2012). Moreover, upwelling events are not negligible in these areas in autumn and winter (Álvarez et al. 2012). The nutrients provide by these upwelling events are feasibly more frequent in the south mouth of the Rías, while in the north mouths the higher concentration of nutrients may be supplied by rivers (Álvarez et al. 2005). Surely, there is high influence of continental runoff from distinct rivers in the northern mouth of the Rías (Álvarez et al. 2005, Martínez-Urtaza et al. 2008, Carballo et al. 2009, Iglesias et al. 2009) that contribute with inorganic nutrients and the variability in surface salinity. Furthermore, in the northern mouth of the Ría de Arosa lower salinity values are observed at the surface than at the bottom influenced by the rainfall pattern in winter (Álvarez et al. 2005). Also, higher input of nutrients at a small spatial scale due to human influence has been detected in the Rías Bajas (Villares & Carballeira 2006).

The combined effect of lower density and abundant light in transplants of juveniles may be an advantage for the growth of algae like *Fucus serratus* (Reed 1990; Creed et al. 1997). Then, it is reasonable to infer that in both trials the lack of a canopy could have seriously affected the auto-transplant and transplant of juvenile algae and mostly during emersion periods. Desiccation has been reported to limited photosynthesis and promotes tissue damage in algae and especially the growth and survival of germlings of algae (Schonbeck & Norton 1980, Bell 1993, Chapman 1995). Nonetheless, germlings of *F. serratus* were under the canopy of *F. serratus*, then desiccation cannot explain the lower number detected in the exterior and interior locations of the Rías de Muros and Arosa.

In our study, *F. serratus* in the Rías may be adapted to lower salinity values and or high variability in salinity. Overall, empirical work has shown that salinity alone does not seem to determine algae geographic distribution, as many algae species can be euryhaline species (Bird et al. 1979, Druehl 1981, Rusell 1987). Certainly, *F. serratus* has been reported in estuarine areas (Burrows 1964, Pazo & Niell 1977), and its occurrence in the Baltic Sea confirms that the species is

able to adapt to lower levels of salinity (Malm et al. 2001), while laboratory work indicates maximal growth of *F. serratus* at 20 to 30 ‰ (Bird et al. 1979).

The particular scattered geographic distribution of *Fucus serratus* in the two Rías Bajas feasibly depend on grazing pressure, the higher levels in some specific areas of ammonium, lower summer temperatures, high variability in salinity and the occurrence of *Himanthalia elongata*. Nonetheless, recent observations indicate drastic range shifts in the geographic distribution of *F. serratus* and changes in the abundance of its populations in the Iberian Peninsula, which seems to be related to increases in sea temperature (Viejo et al. 2011, Duarte et al. 2013). In this study we detected in the northern rocky coasts of the Ría de Arosa the drastic reductions in populations of the species in three locations (see “Results”). All these findings indicate that the future of populations *F. serratus* in these refuge locations in the Ría de Muros and the Ría de Arosa may be at risk, given that it has been projected a growth in the number of grazers due to the increase of temperatures (Schiel et al. 2004). Moreover, in the Iberian Peninsula decreasing trends in upwelling events have reported, related also to the rise in sea temperatures (Álvarez-Salgado et al. 2008; Álvarez et al. 2008, Pérez et al. 2010).

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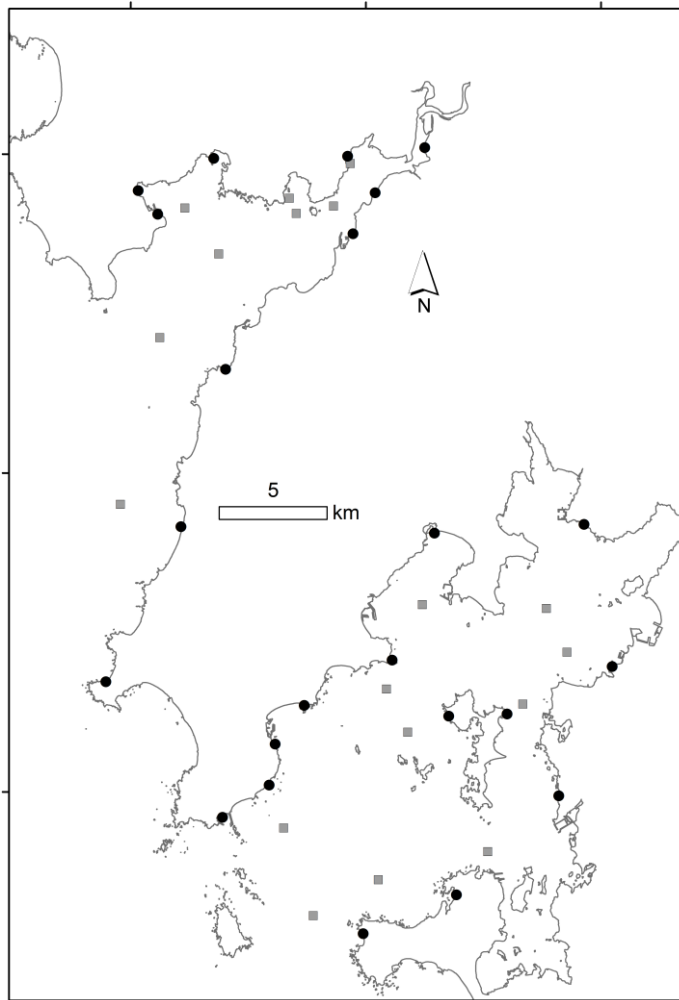
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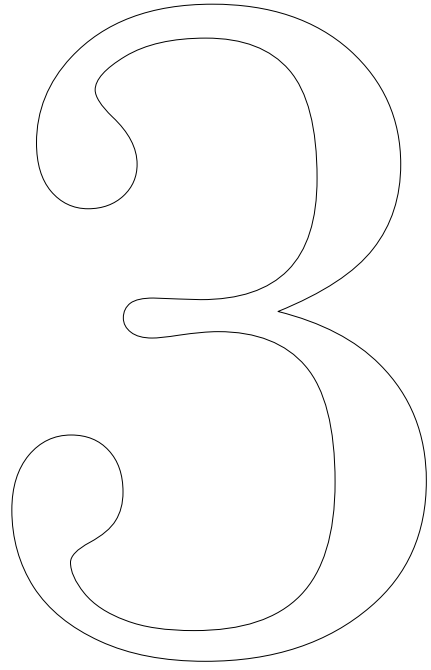
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SUPPLEMENTARY MATERIAL

Appendix 1. The 23 coastal locations (black circles) where sea temperature was obtained in situ with Data loggers, and the 18 oceanographic stations (grey quadrats) where salinity and inorganic nutrient data were obtained from the Instituto Tecnolóxico para o Control do Medio Mariño de Galicia (Inctemar, Xunta de Galicia).





Phenotypic differentiation among peripheral populations of the canopy-forming alga *Fucus serratus* at its retreating southern edge



San Pedro, Lugo. February, 2013 (L. Duarte)



Peizas, Lugo. June 2013 (L. Duarte)

Linney Duarte, Rosa Viejo. Manuscript under preparation



ABSTRACT

Peripheral or marginal populations are smaller and more fragmented than core populations, and frequently occur under highly variable and more pronounced environmental conditions. Yet, some marginal populations are able to adapt and persist in these habitats. In the Iberian Peninsula, peripheral populations have unique genetic pools and this geographical area is also a glacial refuge and the southern edge of many macroalgae species, such as the canopy-forming macroalgae *Fucus serratus* L. We compared the environmental conditions and the viability of peripheral populations of *F. serratus* at its southern edge in North West Spain. We have examined the differences among 4 locations: 2 locations are in the Atlantic coasts inside large embayments or Rías, and the other 2 are situated in the Cantabrian coasts in Lugo. At this rear edge, marginal populations of *F. serratus* inhabit under very different environmental conditions, and these are feasibly more benign in the Rías than in Lugo. Overall, the macroalgae showed signs of home-site advantage, as thalli transplanted in their own habitat showed higher elongation than foreign thalli. Natural recruitment of the macroalgae was spatial and temporal more persistent in the peripheral populations of the Rías, while in Lugo the recruitment of germlings was more variable. Populations of *F. serratus* especially in the Lugo locations are feasibly more susceptible to environmental variations, and population growth and persistence could be threatened in a recent future. Further studies should focus on comparisons among peripheral populations, especially, in geographical areas where the shift of canopy-forming algae is happening and at a very fast rate

Key-words: Rear edge, Peripheral populations, Intertidal rocky coasts, Macroalgae, Canopy-forming algae, *Fucus serratus*

1. Introduction

Marginal or peripheral populations are described as smaller in size, highly fragmented and or completely isolated, when compared with central or core populations (Brown 1984, Leppig & White 2006, Kawecki 2008). Peripheral populations are also often exposed to extreme situations and or large oscillations in the environment (Cassel-Lundhagen et al. 2009, Sexton 2014), and are under the influence of strong natural selection (Kawecki 2008). The persistence of these populations will clearly depend on the stability between mortality and recruitment rates (Guo et al. 2005). Populations at the rear edge can take advantage of good environmental years to reproduce and growth and they can also be found in micro environments, where environmental conditions are ideal for their development (Lennon et al. 2002, Kawecki 2008, Oliver et al. 2009), finding climate relict populations that have withstood shifts in their geographic distribution and extinction processes (Hampe & Petit 2005, Hampe & Jump 2011, Woolbright et al. 2014). Even, empirical work has described that marginal populations have less genetic variation than core populations of the same species (Lesica & Allendorf 1995, Schwartz et al. 2003), peripheral populations usually hold high inter-population genetic differentiation (Petit et al. 2003). Despite, the recognition of the great ecological, evolutionary and conservation value of peripheral populations (Channel & Lomolino 2000, Hampe & Petit 2005), these clearly have received very little attention compared with core populations.

Adaptation to marginal habitats involve complex processes such as, dispersion capacity, competition for local resources, the habitat quality and gene flow (see review in Kawecki 2008). Species with a relatively poor dispersal capacity could be expected to adapt to the local environment, due to the fact that they are most likely to experience changes in their allele frequencies (Guo et al. 2005, Patricelli et al. 2013), while gene flow from core populations into marginal ones, may limit adaptation at the rear edge (see review in Bridle & Vines 2007, Kawecki 2008). Then, locally adapted populations are those that possess higher

fitness than foreign genotypes (Kawecki & Ebert 2004, Kawecki 2008). Overall, adaptive differentiation has been detected over regional scales (Galloway & Fenster 2000, Santamaría et al. 2003), but they are as well some examples of local adaptation at small spatial scales (i.e. Knight & Miller 2004, see review in Sanford & Kelly 2011).

In this context, rocky intertidal coasts possess striking vertical and horizontal zonation, creating a variety of habitats within a very short spatial scale (Raffaelli & Hawkins 1996, Menge & Branch 2001). Nonetheless, local adaptation in rocky coasts ecosystems has been understudied (Bergström & Kautsky 2006, Hays 2007, see review in Sanford & Kelly 2011). Furthermore, in the marine realm, macroalgae are ideal species to study adaptive differentiation, as they are sessile species, with generally limited dispersal capacities (Norton 1992), and are well suited for manipulative field experiments (i.e. McGlathery 1992, Hays 2007, Koehl et al. 2008).

The Iberian Peninsula has been a refuge during the last glaciation for many macroalgae, been as well the southern range edge for many seaweeds (Lüning 1990, Coyer et al. 2003, Hoarau et al. 2007, Provan & Maggs 2011). Then, overall marginal or peripheral populations in the Iberian Peninsula have unique genetic pools (Provan 2013). Recent observations have also detected the shift of canopy-forming macroalgae in this geographical area, which overall has been associated to changes in the physical conditions of this area, such as increases in sea surface temperature (Lima et al. 2007, Fernández & Anadón 2008, Fernández 2011, Viejo et al. 2011, Díez et al. 2012, Duarte et al. 2013, Nicastro et al. 2013).

The rocky intertidal coasts of North West Spain in the Iberian Peninsula represent the southern range edge and a glacial refuge for the native perennial brown macroalga *Fucus serratus* L. (Lüning 1990, Coyer et al. 2003, Hoarau et al. 2007). Despite this, the genetic diversity of the seaweed has been reduced, feasibly due to the processes of colonizations and extinctions (Coyer et al. 2003). This furoid, with limited dispersal capacities (Arrontes 2002, Coyer et al. 2003), has two range limits in the NW Iberian Peninsula, one in northern Spain and the other

one in northern Portugal (Fischer-Piette 1957, Arrontes 1993). The species is present in the NW corner of the Iberian Peninsula, but it is absent towards the inner part of the Bay of Biscay (Lüning 1990, Arrontes 1993). Historical records and recent surveys indicated that this macroalga has a very particular distribution in the Atlantic coast, appearing as very disperse populations inside large embayments, the Rías Bajas (Pazó & Niell 1977, Martínez et al. 2012), while recently the geographic distribution of *F. serratus* along the Cantabrian coast has been shifting (Arrontes 2002, Duarte et al. 2013).

As other seaweeds, the canopy-forming algae *F. serratus* is an ecosystem engineer that provides refuge and it can be a source of energy for intertidal consumers (Watson & Norton 1987, Jones et al. 1997, Christie et al. 2009). Then, undoubtedly is of great interest to investigate the patterns and the potential local adaptation of these marginal populations (Jueterbock et al. 2013), in order to provide some indications about how this canopy-forming macroalgae may react to the imminent climate change. In the present work, we explore the environmental differences and the viability of peripheral populations of *F. serratus* in 2 distinct marginal areas in NW Spain. In particular, we examine two locations in the Rías Bajas, in the Atlantic coast, where populations of *F. serratus* are still dominant. The other 2 locations are in the Cantabrian coast in Lugo, where recently the geographic distribution of the species has shifted considerably. Comparing the viability of individuals of *F. serratus* among these peripheral populations, we examine the possible adaptation of these populations to their particular habitats. As other studies have reported, we expect that individuals of *F. serratus* transplanted back to their origin location will present higher fitness than foreign ones, which will be consistent with local adaptation (Bergström & Kautsky 2006, Hays 2007).

2. Materials and methods

2.1. Study area

The surveys and experiments were conducted at 4 locations from 2 distinct areas in North West Spain: the so-called Rías Bajas, large embayments in the Atlantic coasts, and Lugo province, in the Cantabrian shores (Fig. 1). The selected locations were: Isla de Arosa ($42^{\circ}33'N$, $8^{\circ}52'W$) and Ofreixo ($42^{\circ}79'N$, $8^{\circ}94'W$) inside the Rías (the Ría de Arosa and Ría de Muros, respectively), and San Pedro ($43^{\circ}37'N$, $7^{\circ}19'W$) and Peizas ($43^{\circ}35'N$, $7^{\circ}16'W$) in Lugo. Locations from Lugo and Rías are approximately 240 km apart (Fig. 1).

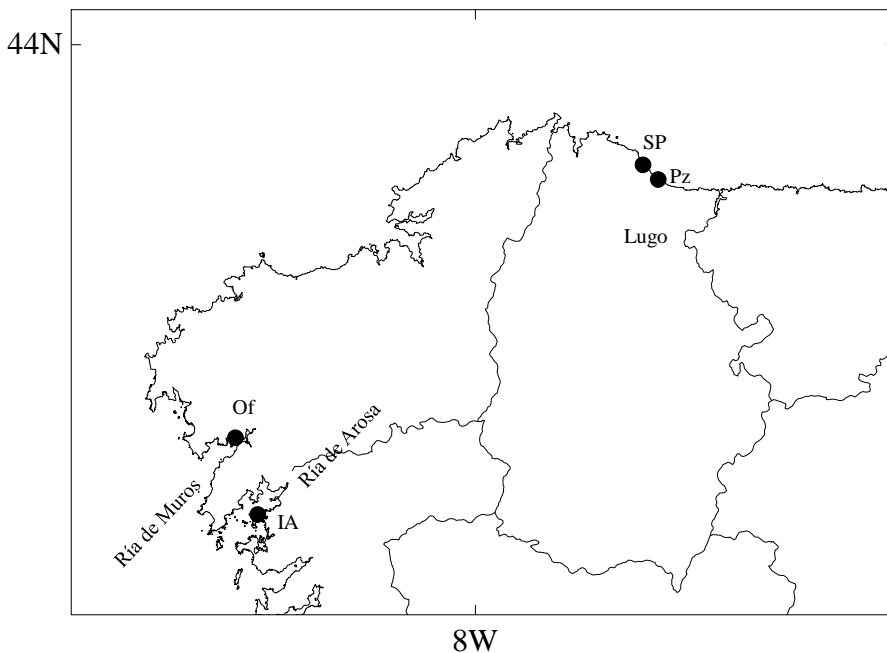


Figure 1. Study locations in NW Spain: Ofreixo (Of) and Isla de Arosa (IA) in the Ría de Muros and Ría de Arosa, respectively. San Pedro (SP) and Peizas (Pz) in Lugo.

2.2. Sampling of fucoids and macro-grazers

We estimated the abundance of *Fucus serratus* and other fucoid seaweeds, such as *Fucus vesiculosus* L, *Ascophyllum nodosum* (L.) Le Jolis, and *Himanthalia elongata* (L) S.F Gray, and the abundance of macro-grazers in the study locations in the middle of the zone dominated by *F. serratus*, at roughly 1.4 m above the Lowest Astronomical Tide. Sampling was done once for algal cover (May 2012), and at two dates for grazers (May and June 2012) during low spring tides. In each location we took three sites randomly, and four random plots per site (of 0.25 m² for algae and 0.09 m² for grazers). In order to determine the primary and secondary cover of fucoids, we employed the point-intercept method using a 50 x 50 cm PVC frame (a grid made of double thread with 81 regularly spaced intersections). Cover of fucoids was transformed to percentages. A cover of 1% was given to species present into the plots but not recorded. A 30 x 30 cm PVC frame segmented in 25 squares was used to estimate the density of macro-grazers (gastropods and chitons), which were identified *in situ*.

2.3. Recruitment of germlings

The natural recruitment of *Fucus serratus* was estimated in all study locations at mid-shore level (about 1.4 m above the Lowest Astronomical Tide). We used artificial substrata for the settlement of the germlings in the field (Johnson 1994). Artificial discs with a rough surface (4 cm in diameter and 5 mm thick) were made using an epoxy resin (Fetadit 55/63; Fetasa, Madrid, Spain). Before taking the discs into the field they were rinsed in tap water and left immersed for 48 hours in sea water (previously filtered with Whatman GF/C filters). When reutilized, discs were carefully brushed and submerged in a 10% solution of bleach (sodium hypochlorite) in order to eliminate any organic remains. These discs were then immersed in tap and sea water, as previously explained. The discs were attached with stainless steel screws to polycarbonate plates (14 x 14 cm, 3 mm thick) that were then fastened to the rocky platforms in the four study locations. Plates were placed at a minimum distance of 1-3 m from each other under the

canopy of *F. serratus*. Discs were left in the field from September 2012 to February 2013 (N = 24), and a new trial was placed from May to October 2013 (N = 12). In both time intervals the discs were periodically removed and the germlings counted with the help of a stereo microscope. Counting was done 1, 2, 4 and 5 months after the start of the essay at each location and period, except for the Lugo locations (Peizas and San Pedro), where the last count was not done in the first period (September-February). A variable number of discs, ranging from 4 to 24 were counted each time. Germlings of *F. serratus* were counted within hours after collection of the discs from the field, and after that the discs were placed in sea water with proper aeration and returned back into the field the same night or the following day.

2.4. *Reciprocal transplants of Fucus serratus' juveniles*

Transplant experiments were conducted at the study locations in two different periods, corresponding with the growing season of *F. serratus* (Arrontes 1993). First, from February to May 2012 and then from June to July 2012 (hereafter named first and second trial). The experiment was done in the middle of the rocky coast zones dominated by *F. serratus* during low spring tides, at similar tidal heights than the natural recruitment essay. We transplanted individuals between pairs of locations from different areas, i.e. Lugo and Rías. Precisely, transplants were done between Isla de Arosa and San Pedro, and Ofreixo and Peizas. Then, each location served both as source and recipient for transplanted thalli. The first trial was done from February to May 2012 between Isla de Arosa and San Pedro, and for a shorter period (due to logistic constraints) from March to May 2012 between the locations of Ofreixo and Peizas. Transplants between the same pairs of locations were done simultaneously in the second trial. Only non-reproductive, juvenile stages of *F. serratus* (<15 cm in length) were used, and the experiment lasted for very short periods of time (2-3 months) in order to prevent gene flow between distant populations.

Juveniles developed attached to the shells of the bivalve *Mytilus galloprovincialis* Lamarck, barnacles *Balanus* sp, or to the rocky substratum. We removed 36 out of 54 selected juveniles in each location with a fragment of rock or the mussel shells/barnacles, which they used as substrata. This was done with the support of a hammer and a chisel and taken care of not damaging the fronds. In each location, we transplanted back detached individuals from the same location (hereafter named 'auto-transplants', N = 18) and thalli that came from another origin (hereafter named 'transplants', N = 18). Both transplants and auto-transplants were numbered with a plastic label and cemented on to the rocky substratum. Un-manipulated juveniles were also labeled and left as controls (N = 18). Three groups of 18 algae (6 transplants, 6 auto-transplants and 6 controls) were placed in sites of about 1 m², which were randomly selected within each location and separated by 3-5 m. Juveniles used as auto-transplants were exposed to similar transportation times and conditions than the rest of transplanted individuals, in order to assure that these were suitable controls for the transplantation process. At the beginning (mid-February/end of March in the first trial, start of June in the second trial) and at the end of each trial (start of May or end of July) we measured the length (from the base of the holdfast to the tip of the longest branch) and the maximum circumference of thalli from the three treatments with an accuracy of 0.1 cm. Circumferences < 2 cm were approximate to 2 cm. The volume of each alga was estimated as: $v = lc^2$ (where l = length, c = circumference; Åberg 1990). At the end of the experiment, juveniles from each trial were gathered and cleaned of epiphytes and then dried at 60°C for 48 hours. Furthermore, a group of dried algae of the second trial (treatments: auto-transplants and transplants, N = 3-7) were sent to the University of Vigo (Centro de Apoyo Tecnológico) in order to determine the total carbon and nitrogen content using a CNH elemental analyzer.

2.5. *Environmental parameters*

Seawater temperature (°C) was measured at each study location with Tidbit loggers (Onset Corporation, MA, USA), fixed on to the rocky substratum with stainless steel screws at the same tidal height than the field experiments. One data logger was placed per location in July 2011 (in both locations of Lugo and Ofreixo), and in December 2011 in the Isla de Arosa. Tidbits recorded temperature every 30 minutes until September 2013. Every four to six months the data were downloaded and two readings per day were taken, matching the high-tide times.

Surface salinity data (0-5 m) was provided by the Instituto Tecnológico para o Control do Medio Mariño de Galicia (Intecmar, Xunta de Galicia, Vilagarcía de Arousa, Spain) at approximately monthly intervals in 2 stations close to the Rías locations from the period of January 2012 to December 2013: Vilanova station (42°34'N, 9°25'W) and Freixo (42°47'N, 8°56'W), close respectively to Isla de Arosa, and Ofreixo.

Dissolved inorganic nutrients (nitrate + nitrite and orthophosphate concentrations) were measured at the study locations in February, April and June 2013. Three water samples were taken per location and date. Samples were placed in plastic tubes (seawater previously filtered with polycarbonate filters of 0.2 µm pore size). Immediately after samples were gathered the tubes were covered with aluminum foil and frozen within one hour until they were analyzed in September 2013 using a Skalar auto-analyzer (Skalar, SAN ++) at the laboratory of the University Rey Juan Carlos in Madrid, Spain.

2.6. *Growth of *F. serratus*' thalli under the same environmental conditions: a common garden experiment*

Fronds of *Fucus serratus* of similar sizes, about 7 cm length (N = 20 per location) were collected from the study sites during the first spring tides of September 2013. Algae were transported from the study locations wet and with ice to the laboratory. Thalli were placed in 4 PVC aquaria (~800 mL, 35 x 25 x 15 cm; 5 fronds from each location, a total of 20 per aquarium) with sea water previously

filtered with Whatman GF/C filters and kept at a mean temperature of 18.0 °C. Temperature was controlled in aquaria with seawater chillers Titan 150 (AB Aqua Medic GmbH, Bissendorf, Germany). Tanks received light from above by cool white florescent lights and irradiance levels were $110 \mu\text{molm}^{-2}\text{s}^{-1}$ under a 14:10 h light:dark photoperiod. The initial length and wet weight of thalli were obtained after blotting them dried with paper towels. Then, 8 days after the experiment began the water of the tanks was replaced with new filtered seawater where inorganic nutrients were added (4.25 mg of NaNO_3^-/L and 0.69 mg of $\text{NaH}_2\text{PO}_4^-/\text{L}$). The experiment ran for 15 days and at the end the 80 thalli were re-measured.

2.7. Feeding preference experiment

The results of the reciprocal transplant experiments and the differences in nitrogen content of thalli of *F. serratus* suggested the presence of differential grazing on thalli of the fucoid depending on the location of origin (see ‘Results’). Therefore, a preference experiment was set up to test this hypothesis. During the spring tides of May 2013 non-reproductive fronds of *F. serratus* were collected in the study locations and transported to the laboratory wet and with ice. They were left for one night in open PVC tanks with seawater and proper aeration. The feeding assay was initiated the following day, at $110 \mu\text{molm}^{-2}\text{s}^{-1}$ of irradiance at the air-water interface and a 13:11 h light:dark photoperiod. Three species of grazers which are common in the studied area were used: the isopods *Dynamene bidentata* Adams, and *Idotea balthica* Pallas, and the gastropod *Gibbula pennanti* Philippi. The grazers were only collected from fronds of Lugo locations. After the algae were blotted dried with paper towels and cleaned from all visible epibionts, each piece of frond used for the experiment was cut to approximate the same size and their wet weights were determined to the nearest 0.1 g. We used 26 PVC tanks (~200 ml volume; 16 x 13 x 8 cm) for this assay. Each tank was filled up with natural sea water filtered with Whatman GF/C filters and proper aeration, at a mean ($\pm\text{SE}$) temperature of $14.6 (\pm 0.6)^\circ\text{C}$ (data obtained from two Tidbit loggers placed for a whole day in two tanks, measures taken every 15 minutes; N = 202). The average

seawater temperature at this time of the year was 14.7 and 14.5 °C in locations from Rías and Lugo respectively (average data from May 2012-2013 in the two studied locations in each area). Four fronds, one from each location, were introduced into the tanks. For *D. bidentata* and *G. pennanti* we used 8 tanks each with two grazers. For *I. balthica* we had 6 tanks with two grazers each. Grazers were not starved prior to the experiment, given that starvation may alter feeding behavior (Cronin & Hay 1996 a, b). Four tanks with algal pieces were left without grazers in order to measure autogenic changes in the algae (Renaud et al. 1990). The feeding experiment ran for one week, and at the end, algae were again blotted dried and weighted.

2.8. Statistical analyses

Differences in the total cover of fucoids and of *Fucus serratus* were analyzed through nested analyses of variance (ANOVA), with Location as a fixed factor (four levels) and Site as a random factor, nested within Location (three levels). An ANOVA was also used to test for differences in density of grazers, adding Date (fixed, two levels), as an orthogonal factor in the previous design. The abundance and composition of macro-grazer assemblages were also compared among locations, dates and sites by Permutational Multivariate ANOVA (PERMANOVA, Anderson et al. 2005). The multivariate analysis was based on Bray-Curtis distance measures on fourth-root transformed data. The analyses were done using 9999 random permutations.

Differences between locations in the recruitment of germlings of *Fucus serratus* were analyzed using Negative Binomial Generalized Lineal Models (GLM) for count data (Zuur et al. 2009). The binomial negative instead of the Poisson distribution was used due to the over dispersion of data (dispersion parameter $\phi > 50$; Zuur et al. 2009). Analyses were done for the recruitment one and four months after the placement of discs in the field.

Results of the reciprocal transplant experiments were analyzed by two-way ANOVAs, with Location and Treatment as fixed and orthogonal factors, including

a priori planned comparisons to test for transplant artefacts (Underwood 1997). The dependent variables used were initial length and volume, elongation (final-initial length) and final dry weights. Since the first trial started at different times for Isla de Arosa and San Pedro than Ofreixo and Peizas, results were analyzed separately. The effect of Treatment (control, auto-transplant and transplant) either as main effect or in interaction with Location was split into two *a priori* comparisons: (1) Controls *versus* Auto-transplants, (C *vs.* AT), to test for transplant artefacts and (2) controls (C & AT) *versus* Transplants (T), to test for the effect of the reciprocal transplant. In case P-values were <0.25 in the first comparisons, the effect of transplants was analyzed by comparing AT *versus* T. In this case, the two planned comparisons were non-orthogonal.

Differences in the nitrogen content of thalli were tested with two-way ANOVA, with Location and Treatment (AT and T) as fixed and orthogonal factors. Spatial and temporal differences in inorganic nutrients were also tested with two-way ANOVAs with Location and Date as orthogonal and fixed factors.

Results of the common garden experiment were analyzed with an analysis of covariance, with Location as a fixed factor and the initial wet weight as co-variable. The interaction with the covariate was non-significant and the model was then simplified. The minimal model was found by pooling those Locations with non-significant differences in intercept and slope. This minimal model was compared to the simplified model (all Location levels included) by using analysis of variance.

For the feeding preference experiment, the amount of each thalli consumed by grazers was estimated as its loss in biomass (blotted wet mass) calculated as: $(H_o \times C_f/C_i) - H_f$, where H_o and H_f were pre-assay and post-assay wet weights of thalli given to the three grazers, while C_i and C_f corresponded to the average pre- and post- experiment wet weights of controls from the correspondent Location of origin, in order to correct by autogenic changes during the essay (Cronin & Hay 1996 a,b, Prince et al. 2004). Due to a methodological problem with weights of thalli from San Pedro (that showed a strong mass decrease in controls), we had to

exclude them from the statistical test. Data were then analyzed by a two-way ANOVA with the Species (fixed, three levels) and Location (fixed, three levels) as orthogonal factors. The consumption from different thalli within the same aquaria was not independent (Steinberg 1988), and therefore, we only used 1 randomly chosen thallus for each aquaria (3 replicates for *Dynamene* and *Gibbula*, and 2 for *Idotea*). ANOVAs were done with Statistica 9.0 (StatSoft Inc, Tulsa, OK, USA) and GLMs executed using the public domain package R 3.0.3 (R Development Core Team, 2014).

3. Results

3.1. Abundance of fucoids and macro-grazers

Differences among locations were detected in the total cover of fucoids (ANOVA for Location effect: $F_{3,8} = 8.10$, $P = 0.008$; Fig. 2a). These differences were clearly marked by the lower cover of fucoids in the Lugo location of Peizas (see Fig. 2a; SNK test: $P_z < SP \sim IA \sim Of$). Also, our results showed that the total cover of *F. serratus* was different among the study locations, with higher percent cover of the target species in San Pedro (ANOVA for Location effect: $F_{3,8} = 9.65$, $P = 0.005$; Fig. 2a). There were no differences in both total cover and cover of *F. serratus* at small spatial scale (among Sites; ANOVA of Site (L) effect: $F_{8,36} = 1.27$, $P = 0.286$ for total cover; $F_{8,36} = 1.05$, $P = 0.487$ for *F. serratus* cover). Cover of fucoids was more diverse in locations within Rías than in Lugo, with greater abundance of *Fucus vesiculosus* L, *Himanthalia elongata* (L.) S.F. Gray, and *Ascophyllum nodosum* (L.) Le Jolis in the first area (see Fig. 2a).

Macro-grazers were more abundant in locations from Rías than in those from Lugo (Table 1a; Figura 2a, SNK test). Differences among Locations were also detected in the species composition and abundance of the grazing assemblage (Table 1b; *a posteriori* comparisons). In the Isla de Arosa higher abundances of *Patella* spp. and *Gibbula* spp. were observed, in Ofreixo the most abundant species

was *Littorina littorea*, while in locations from Lugo, San Pedro and Peizas *Gibbula* spp. was the dominant group (Fig. 1b).

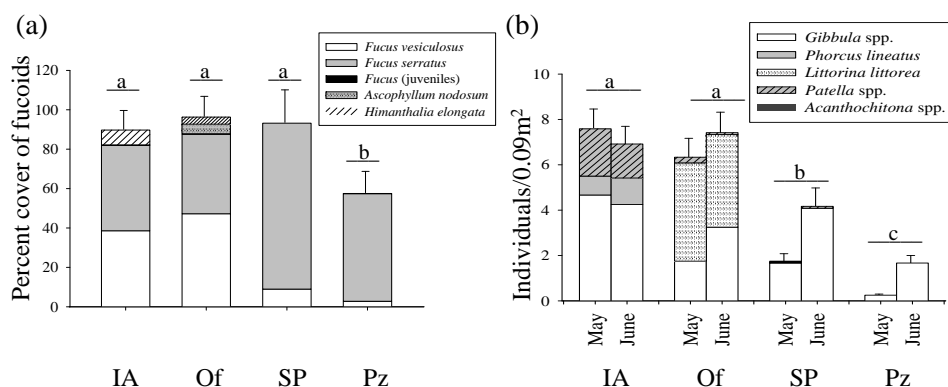


Figure 2. (a) Percent cover of fucoids in the study locations (abbreviations as in Fig. 1). Sampling was done in May 2012. (b) Total number of grazers in the same locations. Sampling was carried out in May and June 2012. (N = 12 in a & b).

3.2. Natural recruitment

The recruitment of *Fucus serratus* was more temporally and spatially variable in Lugo than in the Rías Bajas. The number of germlings that settled in San Pedro (one of Lugo locations) during early autumn (September to October 2012, one-month period) was much higher than the recruitment observed in the same location in spring, May to June 2013, and in both cases differed (being lower and higher, respectively) from the recruitment in Rías locations, Isla de Arosa and Ofreixo (Fig. 3a; Table 2a). After four months, the number of recruits remaining in San Pedro (period September 2012 to January 2013) did not differ from those in Isla de Arosa (Table 2b), i.e. in the location from Lugo, survival was lower than in the Rías (compare recruitment in San Pedro in Fig. 3a,b). No recruitment was detected at any of the studied periods in the other location from Lugo, Peizas (Fig. 3a,b). On the other hand, survival differed between locations within Rías, being higher in Ofreixo than in Isla de Arosa in a four-month period, regardless of the studied period (September to January vs. May to September, Fig 3b, non-

significant Location x Period interaction, Table 2c). Overall, in locations of Rías the recruitment of *F. serratus* was fairly consistent over the whole year of observations (October 2012 to October 2013; Fig. 3c), while in San Pedro the number of recruited embryos was much higher in October 2012 to February 2013 than in June to October 2013 (Fig. 3d). Moreover, during the whole year, the presence of embryos in Peizas was only detected in July 2013 (Fig. 3d) and October 2013 (just in 2 out of 12 discs in this last case).

Table 1. ANOVA (a) and PERMANOVA (b) testing the effects of Date, Location and Site (L) on the total density of grazers and on the abundance and composition of the assemblage of grazers respectively. In b) a *posteriori* comparisons for differences among Locations are also shown. *Monte Carlo asymptotic p-values were used in the pairwise comparison. Abbreviations as in Fig. 1.

a) ANOVA					b) PERMANOVA		
S	df	MS	F	P	MS	Pseudo-F	P
D	1	27.09	1.40	0.270	1231.9	1.70	0.202
L	3	224.98	11.20	0.003	11092	29.40	<0.001
S	8	20.08	1.71	0.112	377.4	0.99	0.473
D	3	9.90	0.51	0.685	396.3	0.55	0.792
D	8	19.29	1.64	0.129	724.6	1.90	0.024
R	72	11.77			380.5		
					<i>A posteriori</i> comparisons		
					among Locations		
					t	P	
					Of, IA	6.062	0.001
					SP,Pz	1.915	0.011
					Of,SP	3.986	<0.001
					Of,Pz	3.402	<0.001
					IA,SP	2.510	0.003
					IA, Pz	3.222	<0.001

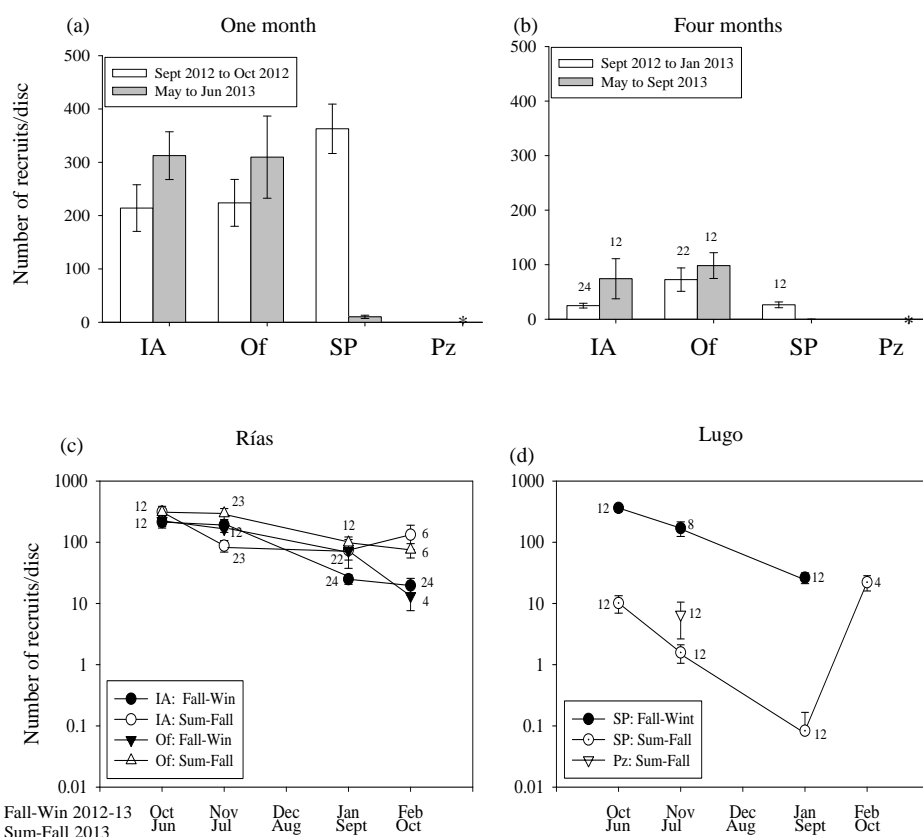


Figure 3. Natural recruitment of *Fucus serratus* at the study locations. (a- b) One and four months recruitment respectively (N = 12 in a). Asterisk indicated that no embryos were recorded in Peizas at any period. (c-d) Temporal variation in the natural recruitment of *F. serratus* from September 2012 to October 2013 in the locations from Rías and Lugo respectively. In Peizas the recruits were detected only at one month, July 2013, which also shown. Location abbreviations as in Fig. 1.

3.3. Reciprocal transplants of juveniles

The initial length of juveniles at both trials did not differ among locations or treatments (ANOVA for Location effect in first and second trials: $F_{3,204} = 1.43$, $P = 0.234$; $F_{3,204} = 1.07$, $P = 0.361$; for Treatment effect: $F_{2,204} = 0.78$, $P = 0.458$; $F_{2,204} = 2.07$, $P = 0.129$; non-significant interaction Location x Treatment, test not shown). The initial volume of the thalli used in the first trial was neither significantly different among locations or treatments (ANOVA for Location and Treatment effects respectively: $F_{3,204} = 0.89$, $P = 0.444$; $F_{2,204} = 0.85$, $P = 0.429$).

However, the initial volume of juveniles of the second trial did differ among locations, with plants of San Pedro being smaller than those from other locations (ANOVA for Location effect: $F_{3,204} = 3.26$, $P = 0.022$; SNK test: $SP < IA \sim Of \sim Pz$).

Table 2. Negative Binomial Generalized Lineal Model (GLM) testing the effects of Period (P) and Location (L) in the recruitment (germling counts) of *Fucus serratus* (a) Recruitment after one month in 2 different periods and 3 locations. The interaction $P \times L$ was significant and thus parameter estimates of the full model are shown. Isla de Arosa in May-June 2013 was taken as a reference level, and the rest of estimates are differences from the reference; (b) Four-month recruitment in 3 locations during 1 period. (c) Four-month recruitment, in 2 periods and 2 locations. The full model (M) was simplified by removing the interaction term $P \times L$. Likelihood ratio test, and parameters estimates for the final Model (M1) are shown. Isla de Arosa in May-June 2013 was also taken as reference level. Location abbreviations as in Fig. 1.

(a) Periods: Sept-Oct 2012 and May-Jun 2013 (one-month recruitment)				
Locations : IA, Of , SP				
Parameters	Estimate	ES	Z Value	P
IA, May-Jun 2013	5.76	0.21	27.44	<0.001
IA, Sept-Oct 2012	-0.39	0.30	-1.32	0.186
Of, May-Jun 2013	-0.03	0.30	-0.08	0.934
Of, Sept-Oct 2012	0.07	0.42	0.16	0.870
SP, May-Jun 2013	-3.44	0.31	-11.10	<0.001
SP, Sept-Oct 2012	3.97	0.43	9.24	<0.001
(b) Period: Sept 2012-Jan 2013 (four- months recruitment) Locations: IA, Of, SP				
Parameters	Estimate	ES	z Value	P
IA	3.22	0.24	13.33	<0.001
Of	1.07	0.35	3.08	0.002
SP	0.06	0.42	0.14	0.889
(c) Periods: Sept2012-Jan 2013 and May-Sept 2013 (four-months recruitment)				
Locations: IA, Of				
Model	θ parameter	df	2 Log Lik	P
(M), Full (P, L, $P \times L$)	0.72	66	-693.22	
(M1), Final (P, L)	0.71	67	-694.94	0.189
Parameters	Estimate	ES	z Value	P
IA, May-Sept 2013	4.08	0.28	14.44	<0.001
IA, Sept 2012-Jan 2013	-0.73	0.30	-2.41	0.016
Of, May-Sept 2013	0.80	0.29	2.80	0.005

In the first trial of reciprocal transplants between Isla de Arosa (Rías) and San Pedro (Lugo), we detected artifacts due to the manipulation (control individuals had a higher elongation than auto-transplanted algae, Table 3a, Fig. 4a). We also detected a significant treatment effect, with local thalli (auto-transplants, AT) outperforming ‘foreign’ thalli (transplants, T) both in elongation and final dry mass (Table 3a, *a priori* comparison AT vs. T). These differences were more evident for elongation in San Pedro and for final dry mass in Isla de Arosa (Figs. 4a,b). Nonetheless, the results should be interpreted carefully, as only three auto-transplants remained in San Pedro at the end of experiment and thus, data were clearly unbalanced. The first trial of reciprocal transplants between Ofreixo (Rías) and Peizas (Lugo) led to similar results. In this case, no artifacts due to transplantation were detected (*a priori* test C versus AT were non-significant, Table 3b). Significant treatment effects (i.e. outperformance of resident vs. foreign thalli) were identified in elongation and final dry mass (Table 3b, Figs. 4a,b). In the second trial, outcomes clearly depended on the recipient location (Table 3c, significant interaction L x Controls [C & AT] vs. T)). A home advantage, with local individuals (C and AT) outperforming foreign thalli (T) was detected in Isla de Arosa and Peizas (Figs. 4c,d; Table 3c). In this last location, transplants from Ofreixo (Rías) presented evident breakages (negative elongation) and very low final weights (Fig. 4d). In both trials, thalli transplanted from Ofreixo (Rías) to Peizas (Lugo) presented serious damage, with signs of grazing, and in some cases only the midrib of thalli remained (see ‘Figure S1 in Supplementary Material’). Results also showed that individuals from San Pedro grew less than those from other locations (Figs. 4c,d). Nevertheless, it is important to notice that these thalli presented smaller initial sizes (see above).

3.4. Nitrogen content of thalli

Fucus serratus juveniles from Ofreixo presented clearly higher nitrogen content than those from the other locations (ANOVA for Location effect: $F_{3,42} = 47.98$, $P < 0.001$; Fig. 5, SNK test). Accordingly, individuals growing at this Ría

location also exhibited a darker color than the rest of *F. serratus*. Remarkably, our results also shown a trend to higher nitrogen content in juveniles from the Rías (Isla de Arosa & Ofreixo) transplanted to Lugo (San Pedro & Peizas), in relation to the thalli remaining in Rías ($T > AT$), while the opposite trend ($AT < T$) was observed from the thalli of the Lugo locations (ANOVA, marginally significant interaction Location x Treatment effect: $F_{3,42} = 2.88$, $P = 0.047$; Fig. 5).

Table 3. ANOVA for the effects of Location and Treatments on the difference in length (cm) and final dry mass (g) in the reciprocal transplant experiments. a) unbalanced data, $N = 3-15$, b-c) balanced data, $N = 12$ and 7 respectively. C = controls; AT = auto-transplants (control of manipulation); T = transplants. Location abbreviations as in Fig. 1.

(a)First trial: IA-		Difference in length (cm)			Dry mass (g)			
Source	df	MS	F	P	MS	F	P	
Location (L)	1	0.001	<0.001	0.991	12.64	3.42	0.071	
Treatments:								
C vs. AT	1	37.57	5.10	0.029	5.29	1.43	0.238	
AT vs. T	1	116.99	15.89	<0.001	21.41	5.80	0.020	
Location x	2	13.10	1.78	0.180	6.77	1.83	0.172	
Residual	45	7.36			3.69			
(b)First trial: Of -		df	MS	F	P	MS	F	P
Location (L)	1	2.57	0.34	0.565	0.02	0.06	0.809	
Treatments:								
C vs. AT	1	7.52	0.98	0.325	0.03	0.11	0.739	
(C & AT) vs. T	1	37.62	4.91	0.030	1.654	5.70.9	0.019	
Location x	2	4.01	0.52	0.595	0.60	2.05	0.136	
Residual	66	7.67			0.29			
(c) Second trial: all		df	MS	F	P	MS	F	P
Location (L)	3	33.43	13.72	<0.001	1.90	8.86	<0.001	
Treatments:								
C vs. AT	1	2.17	0.89	0.349	0.11	0.49	0.485	
(C & AT) vs. T	1	69.67	28.60	<0.001	1.08	5.02	0.028	
Location x								
L x (C vs. AT)	3	2.16	0.89	0.453	0.13	0.60	0.617	
L x[(C & AT)	3	7.59	3.12	0.031	0.78	3.64	0.017	
Residual	72	2.44			0.21			

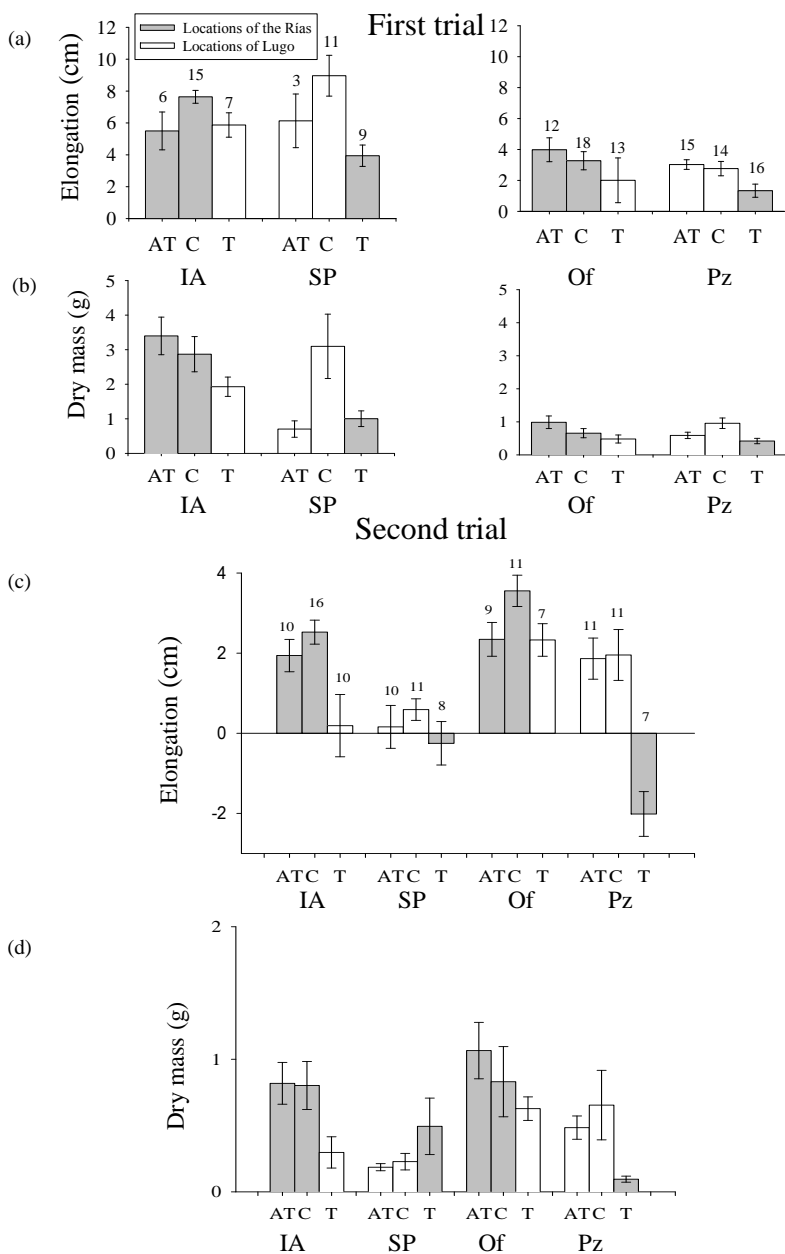


Figure 4. Reciprocal transplants of juveniles of *Fucus serratus* between Rías and Lugo locations (First and Second Trial). Elongation (final-initial length, cm) and final dry mass (g) are shown Treatment abbreviations: AT = auto-transplants, C = controls, T = transplant. Data are means \pm SE. Location abbreviations as in Fig. 1.

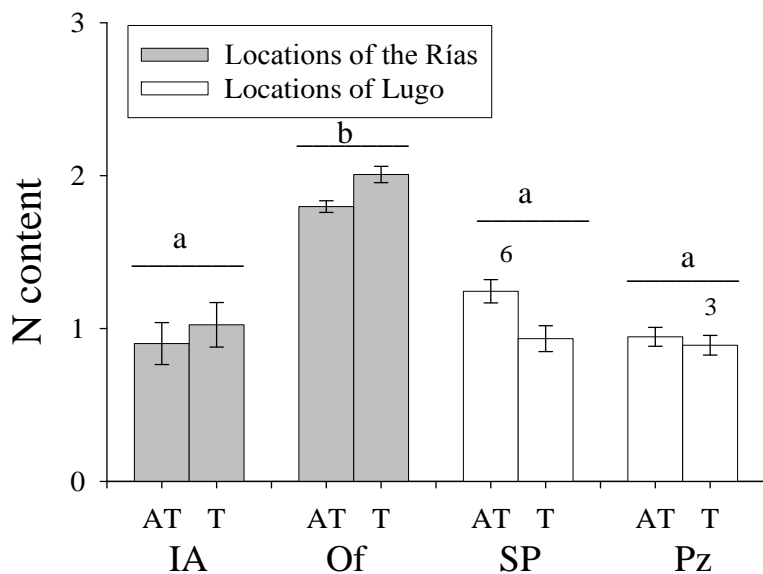


Figure 5. a) Nitrogen content (% dry mass) of *Fucus serratus* juveniles of the second trial (N = 7 except where indicated). AT = auto-transplants, T = transplants. The transplants (T) corresponded here to the origin location (instead the recipient location) of the juveniles (e.g. transplants of Isla de Arosa were juveniles from this location which were transplanted to San Pedro, Lugo). Mean sharing lower case letters do not differ significantly based on SNK tests. Location abbreviations as in Fig. 1.

3.5 Environmental parameters

Overall, the pattern of sea temperature observed in the study locations over two years revealed that water temperatures were cooler in the Rías than in the Lugo locations during summer, especially in Ofreixo (Mean summer SST \pm SE: 17.01 ± 0.35 , 18.59 ± 0.23 for Rías and Lugo locations respectively; Fig. 6a). The opposite trend was observed in winter, as mean temperatures were 12.97 °C for the Rías and 12.58 °C for the Lugo locations (Fig. 6a). Furthermore, readings depicted higher temperatures in Rías than in Lugo locations in the spring of 2012 (Fig. 6a). Salinity presented high seasonal variability in the stations within Rías, in particular in Arosa, with lower salinity in winter than in summer (Fig. 6b).

The location of Ofreixo presented the highest nitrite + nitrate concentrations in April and February (ANOVA, Date x Location interaction effect: $F_{6,24} = 29.21$, $P < 0.001$; SNK test, Of > IA ~Pz ~SP in February and April, Of~IA~Pz~SP in June; Fig. 6c). Regarding the orthophosphate values (PO_4^{3-}), differences did not depend on the date and were obviously marked by the higher values of both Rías locations, especially Ofreixo (Fig. 6d; ANOVA for Location effect: $F_{3,24} = 21.31$, $P < 0.001$).

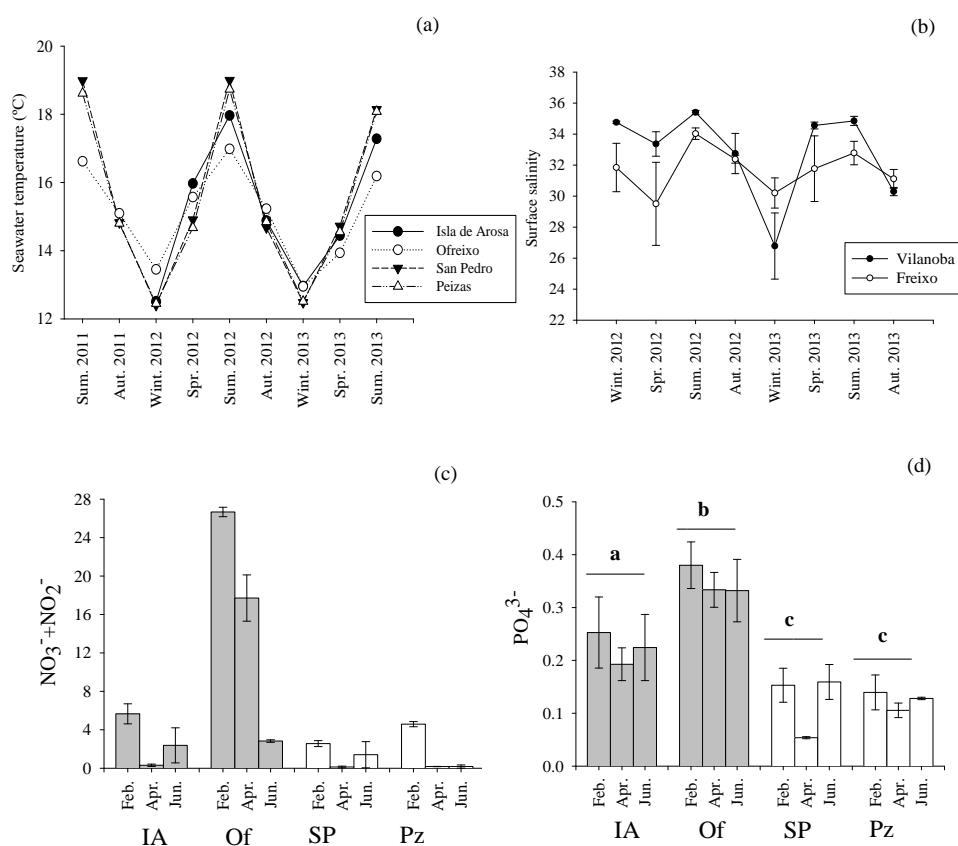


Figure 6.a) Seawater temperature (°C) in the study locations from July 2011 to September 2013. Seasonal average values are shown b) Surface salinity (‰) in stations closer to the locations from Rías, during the period January 2012 to December 2013. Seasonal average values are shown. c) $\text{NO}_3^- + \text{NO}_2^-$ and PO_4^{3-} concentrations (μM). Average values \pm SE are shown. Samples were taken in February, April and June 2013 (N = 9). Mean sharing lower case letters did not differ significantly based on SNK tests. Location abbreviations as in Fig. 1

3.6. Juveniles of *F. serratus* growing at the same physical conditions

Our results clearly indicated differences in the response of thalli from different locations, when exposed to the same environmental conditions at the laboratory. The individuals of Ofreixo (one of the locations from the Rías) presented the highest growth (the increase in wet weight was, as average, 0.132 g above that of thalli from the rest of locations, see Table 4; Figure S2 in Supplementary material).

3.7. Feeding preference experiment

The preference for thalli with different origin did not vary among the macro-grazers (ANOVA, Species effect: $F_{2,9} = 0.60$, $P = 0.571$; Species x Location interaction : $F_{4,9} = 0.61$, $P = 0.665$; Fig. 7). Clearly, the isopods *Dynamene bidentata* and *Idotea balthica*, and the gastropod *Gibbula pennanti* preferred to eat the pieces of *Fucus serratus* from Ofreixo than from Isla de Arosa and Peizas (Fig. 7; ANOVA, Location effect: $F_{2,9} = 7.02$, $P = 0.015$; SNK test: Of > IA ~ Pz). Our results also suggested that pieces of *F. serratus* from Isla de Arosa grew in the presence of grazers, mainly, of *I. balthica* and *G. pennanti* (Fig. 7; negative changes in wet weight indicated a higher final weight in presence of grazers, after correcting by autogenetic changes in controls, see ‘Material and Methods’).

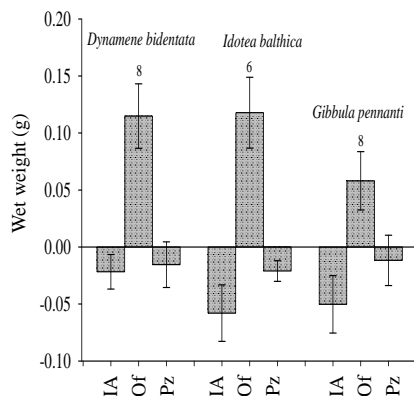


Figure 7. Change in wet weight (g) of *Fucus serratus* fronds consumed by three grazers in the feeding preference experiment. Location abbreviations as in Fig. 1.

4. Discussion

The distribution of *F. serratus* at its rear edge (Iberian Peninsula) and after its recent and drastic decline detected in Cantabrian coasts (Viejo et al. 2011, Duarte et al. 2013) is nowadays virtually limited to the scattered but persistent populations inside large embayments (*Rías*) in Galicia, and a few locations in N Portugal (Araújo et al. 2014), and in N Spain (Lugo populations). We found high environmental heterogeneity and differences in the performance of peripheral populations inside the *Rías* in the Atlantic coasts vs those in semi-exposed locations of Lugo. These two groups of populations had high temporal persistence, being present at least since 1940-1960's. Thus, the location of Peizas, near the village of Cangas de Foz, shelters an abundant population of *F. serratus* since at least 1949 (Fischer-Piette 1955), while isolated populations inside *Rías*, as that in Ofreixo, have been recorded since 1960's (Donze 1968, Pazó & Niell 1977). Our results also showed signs of home-site advantage in peripheral populations, with local individuals outperforming overall foreign thalli. This home-site advantage suggests the presence of local adaptation in the studied populations, though carry-over effects, i.e. environmental effects during the development, could also lead to these results.

The environmental conditions experienced by *Fucus serratus* populations inside the *Rías* in the Atlantic coasts clearly differed than those in Cantabrian coasts, N Spain (Lugo). In fact, seawater temperature in summer was lower in *Rías* than in Lugo locations, and the seasonal variability was also reduced in these large embayments, while the supply of inorganic nutrients was much higher inside the *Rías*, in particular in the Ofreixo location. All these physical differences between these two geographical areas could be linked to the fact that the *Rías Bajas* are part of the Eastern North Atlantic Upwelling System (Wooster et al. 1976), which is one of the four major upwelling regions in the world (Álvarez-Salgado & Gago 2001). Intense upwelling events are reported from April to September in the western area of Galicia, bringing cooler and nutrient-rich water to surface waters (Fraga 1981, Alvarez et al. 2008, 2012), while in the Northern Galicia *Rías*,

upwelling events are more sporadic (Ospina-Alvarez 2010). The influence of continental runoff from the rivers Ulla and Umia in Ría de Arosa, and Tambre in Ría de Muros could as well contribute to the greater input of inorganic nutrients and the high variability in surface salinity values in these areas (Alvarez et al. 2005, Iglesias et al. 2009, Martínez-Urtaza et al. 2008). Lower salinity values between N and W Spain occur at opposite times of the year, as in northern Spain these values corresponded to the summer months due, among other factors, to the influence of the French rivers, while in western Spain are observed in winter and are strongly related to the raining season and the rivers influences in these areas (Bode et al. 2012). Moreover, the locations in the Atlantic Rías are more sheltered to wave exposure than those in the Cantabrian rocky coasts (see Martínez et al. 2012). In contrast to seawater temperature, however, Atlantic Rías present higher air temperatures and reduced cloudiness than the western Cantabrian coasts (Alcock 2003, Martínez et al. 2012).

In response to the spatial heterogeneity in the physical conditions, *Fucus serratus* individuals from populations of these two areas, Rías and Lugo, may display phenotypic differentiation, through phenotypic plasticity or genetic mechanisms (local adaptation) or both. As genotypic frequency tend to be homogenized by gene flow among populations, local adaptation will be more common if the species have limited dispersion capacity than the spatial resolution of the environmental variability (Kawecki 2008, Hays et al. 2007 and references therein). The geographical distance among peripheral populations of *F. serratus* and the poor dispersal capacities of the species, with embryos recruiting very close to their parents (Arrontes 1993, 2002, Coyer et al. 2003), could enhance local adaptation. The results of the reciprocal transplant experiments indicate a home-site advantage, with higher performance (growth) of local *versus* foreign individuals of *F. serratus*, which may suggest the presence of local adaptation. However, carry-over effects, due to the past developmental history of juveniles in origin locations, may also explain the results. By using out-planting recruits, these environmental effects could have been removed. Actually, a reciprocal transplant

experiment with recruits was carried out, with spawned embryos on artificial surfaces in the field. Although maternal effects may still persist during several generations (Roach & Wulff 1987), and therefore they could remain in settled embryos in the field, in a related fucoid species their influence was apparently low (Hays et al. 2007). Nevertheless, the test with germlings failed, due to the presence of new settlement onto the artificial surfaces during the course of the experiment, which confounded the results. New assays with recruits settled in the laboratory under controlled conditions should be carried out in order to remove carry-over effects.

Home-site advantage was also feasibly the result of differential grazing pressure in foreign fronds, in particular in individuals from Ofreixo transplanted into Lugo. These fronds presented in some cases serious damage and negative elongation in the transplanted location (see results and Fig. S1 in the 'Supplementary Material'). They grew in its origin location under high concentrations of inorganic nutrients and presented the highest nitrogen contents. Observations in the field revealed that these algae from Ofreixo in fact exhibited a darker green color compared with the seaweeds from the other three locations (authors' pers. obs.). Seaweeds growing in excessive pulses of nutrients may have the capacity to store nutrients for future growth (Lapointe & Duke 1984, Fong et al. 1994) and the nutritional content of algae can affect the feeding behavior of grazers (Cronin & Hay 1996a, Erickson et al. 2006). The results of the feeding preference experiment support the presence in the field of a differential grazing of Ofreixo's fronds.

Moreover, in the feeding preference experiment growth of fronds was enhanced in presence of herbivores in comparison with control plants, in particular the fronds from Isla de Arosa. Grazers could help growth of algae by nutrient enrichment via their faeces (Duffy 1990, Bracken et al. 2007, Ling & Fong 2008). Previous studies have showed the higher N content in the tissue of the macroalgae *Acanthophora spicifera* (M. Vahl) Børgesen, due among other possible factors to the nutrient enrichment provided by a shrimp aquaculture (Ling & Fong 2008).

Natural recruitment of peripheral populations of *F. serratus* in Lugo was more spatially and temporally variable than in Rías, with low recruitment rates detected in Lugo during summer-autumn. These differences were observed despite the higher density of macro-invertebrates found inside Rías, where species such as chitons and gastropods can scrape food sources on hard substrata by using their radulas (Crothers 2001, Bhatnagar & Bansal 2009). Nonetheless, it is possible that the abrupt borders of the recruitment discs may have limited the access of grazers to the recruits of *F. serratus*. The low and variable recruitment in Lugo populations can be the result of episodic disturbances, for instance the practically absence of embryos in Peizas could in part be explained by the temporal accumulation of sand over the rocky platforms observed in this location, and particularly on the recruitment discs (authors' pers. obs.). Sand deposition may have diminished the availability of stable substratum for the settlement of the embryos in this location, reducing the availability of oxygen and nutrients for the development of the embryos (Daly & Mathieson 1977, Devlin & Volse 1978). Nevertheless, the marked differences in the number of recruits of the macroalgae between Rías and Lugo seemed to be greatly influenced by the lower number of *F. serratus* reproductive individuals in Lugo, as recruitment of fucoid seaweeds is highly determined by the abundance and fecundity of adults (Lamote & Johnson 2008). This is in accordance with observations of marginal populations in Portugal of the seaweed *F. guiryi*, which also showed high temporal variability in natural recruitment, given that reproductive fronds were mostly shed during several months (Zardi et al. 2015). Moreover, lower and more variable growth rate of marginal populations of *F. serratus* in the N of Portugal compared with central populations of the macroalgae in France have been detected, and linked to the smaller size of individuals of the species and the breakage of their fronds (Araújo et al. 2014). Even when this study reflects only one year of recruitment, and surely population's viability can be maintained with some good years of recruitment (Kawecki 2008), additional observations suggest that the current and future status of Lugo populations are feasibly threatened. Undoubtedly, changes in these

peripheral populations are happening, as two decades ago *F. serratus* was reproductive the whole year in the N of Spain (Arrontes 1993). Indeed, during our field observations, we visually noted that adult plants in Lugo presented a reduced number of receptacles and damaged fronds in 2013 (see Figs. S3a,b in the 'Supplementary Material'). In subsequent years, 2014 and 2015, plants of Lugo presented very damaged fronds, sometimes reduced to the midrib (see Figs. S3c,d in 'Supplementary Material').

It has been argued that rather than adapted to local conditions, the southern-edge populations of the Iberian Peninsula are actually maladapted and with limited potential response for the on-going climate change (Pearson et al. 2009). Even when the NW Iberian Peninsula is one of the former refugial areas from the Last Glacial Maximum for *F. serratus* (Hoarau et al. 2007), repeated extinctions and recolonizations have subsequently reduced its genetic diversity (Coyer et al. 2003). However, the cycles of extinction and colonization feasibly affected to populations towards the East in N Spain (Arrontes 1993, Duarte et al. 2013). It is unknown the adaptation potential of the Rías and Lugo peripheral populations and this will depend on their present genetic diversity, and the future physical conditions in the environments. As in this regard, there are evidences that indicate that populations of *F. serratus* from the Rías Bajas have a very different genetic pool than populations from the N of Spain (Ester Serrão's personal communication). There is a need for further studies to determine the inter and intra-population genetic diversity of *F. serratus* at this southern edge.

There is no doubt of the lack of empirical work concerning how gene flow among divergent populations will affect adaptive potential (Bridle & Vines 2007). This gene flow may be especially beneficial among edge populations, as it could enhance genetic variation and supply of favorable alleles adaptive at the range borders (Sexton et al. 2011). It would be interesting to evaluate if by creating gene flow among marginal populations of *F. serratus*, for instance through experimental crossing, it will increase the evolutionary capacity of these range limit populations to respond to climate change.

Under the present scenario of global climate change peripheral populations of the canopy-forming alga *Fucus serratus* seem to be experiencing drastic changes, especially in the Cantabrian coasts in Lugo, where presently its geographic distribution is retracting (Duarte et al. 2013). Certainly, the future of the peripheral populations of the Rías and Lugo will strongly depend on changes in sea temperatures and upwelling events. In this later aspect, recent investigations reveal the weakness of coastal upwelling in the Iberian Peninsula, corresponding with the rise in sea surface temperature (Álvarez-Salgado et al. 2008; Alvarez et al. 2008, Pérez et al. 2010). Specifically, in the coasts of Galicia and in Asturias, in NW Spain, a reduction in the intensity of the upwelling events has been described (Llope et al. 2006, Alvarez et al. 2008, 2010; Pérez et al, 2010, Bode et al. 2012).

The results of this study revealed that marginal populations of *F. serratus* in NW Spain inhabit very different environments. Furthermore, Lugo populations are feasibly more susceptible to environmental variations, and population growth and persistence could be threatened in a recent future. Further studies should focus on comparisons among peripheral populations, especially, in geographical areas where the shift of canopy-forming algae is happening and at a very fast rate.

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Figure S1. Juveniles of *Fucus serratus* from Ofreixo (Rías) transplanted into Peizas (Lugo), First trial. (a, b) The juvenile algae showed marks, as if they were grazed. In other cases only the midrib of the thalli remained (c, d).

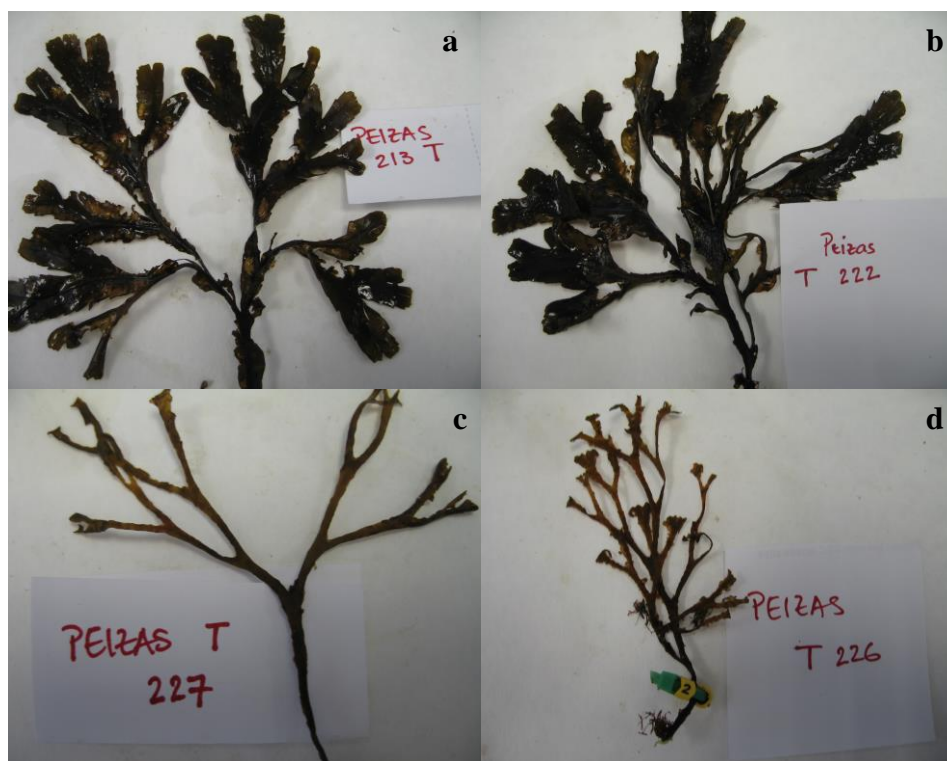


Figure S2. Final vs initial fresh weights of *F. serratus* fronds from different locations growing in the laboratory at the same environmental conditions. The fitted regression lines are also shown (see Table 4 for parameter estimates). The same results (i.e. growing differences between the fronds of Ofreixo and the rest of locations) were obtained when only thalli < 1g final weight were included (analysis not shown).

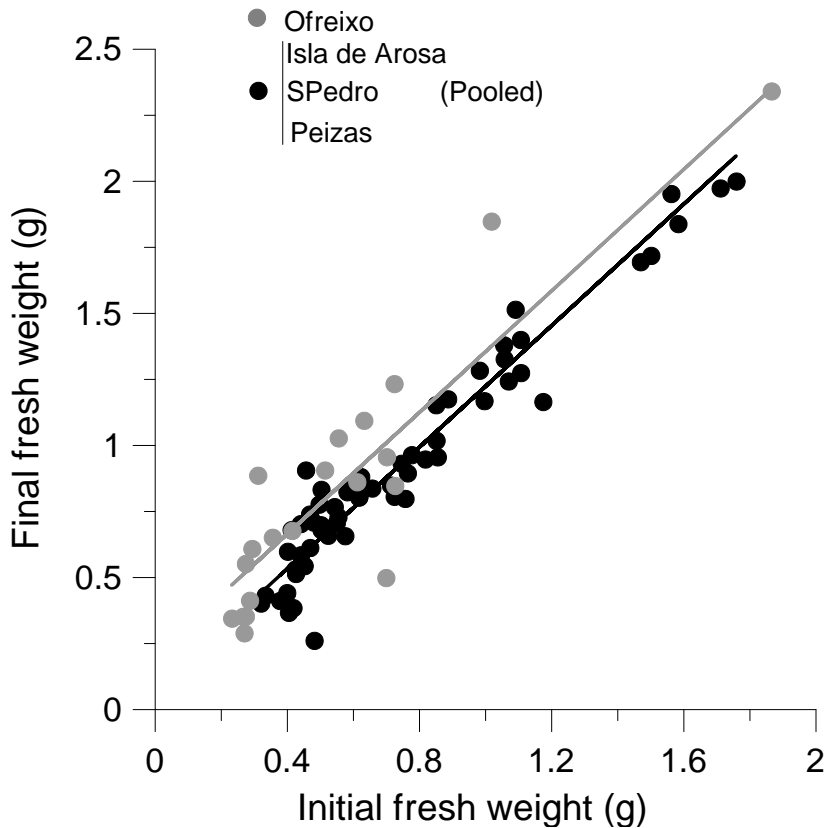
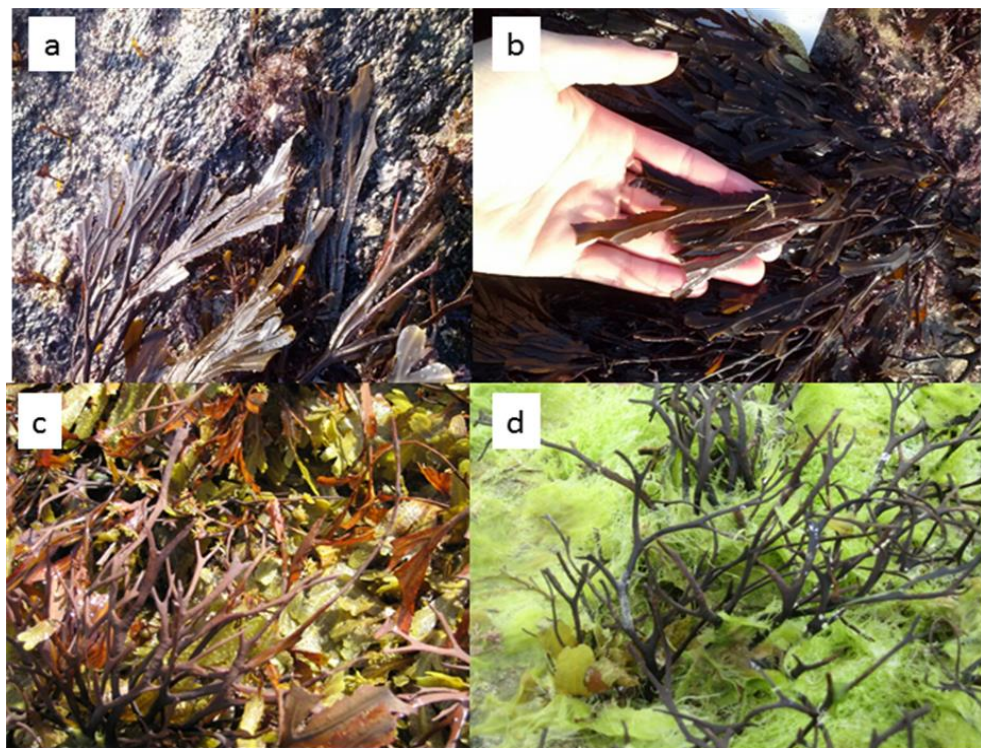
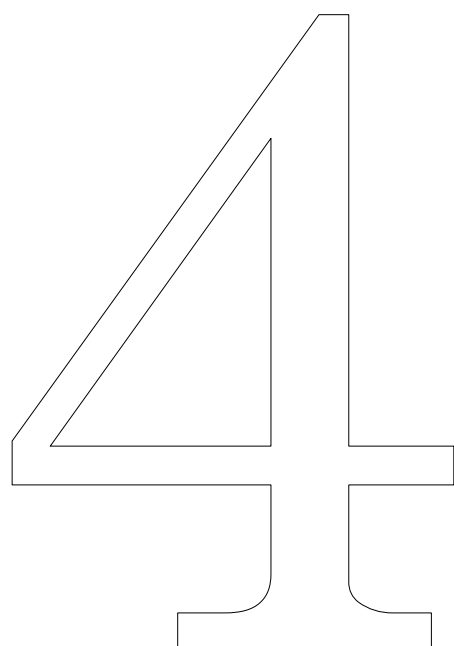


Figure S3. Fronds of *Fucus serratus* in San Pedro (Lugo location) a, b: adult plants, February 2013; c: adults and recruits, July 2014; d: adults, May 2015.





Effects of alga *Fucus serratus* decline on benthic assemblages and trophic linkages at its retreating southern range edge



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ABSTRACT

Canopy-forming seaweeds are important coastal ecosystem engineers that sustain diverse multi-trophic assemblages. Their losses, with the subsequent reduction in habitat complexity, have been documented across many parts of the world and are often attributed to climate change and other anthropogenic factors. The general aim of the present study was to understand the repercussions of the decline of the canopy-forming alga *Fucus serratus* L. at its retreating southern range edge in the diversity and food-web linkages of intertidal assemblages. Few studies have attempted to document changes in benthic food webs following canopy loss. We examined the differences among southern locations situated at different distances from the range margin: those at the very edge, where *F. serratus* experienced a dramatic decline during recent years (marginal locations), and those where *F. serratus* is still dominant (central locations). Comparisons were made among locations situated at the same latitude and sharing a recent history of *F. serratus* dominance. Trophic relationships were analyzed using natural abundances of carbon and nitrogen stable isotopes. We report clear changes in the structure of benthic assemblages and lower trophic positioning of some consumers, suggesting an overall shrinkage of the food web length at the contracting range edge of *F. serratus*, which will transfer to higher trophic levels. Under present and future climatic scenarios, shifts in the distribution of coastal ecosystem engineers could entail a reorganization of local natural assemblages and food webs. More attention should be given to measure how much these shifts can modify the whole coastal food webs and their functioning.

Key-words: Canopy-forming algae, *Fucus serratus*, Rear range edge margin, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, Stable isotope analysis, Food web

1. Introduction

There is a global concern about the impacts of species loss in ecosystem functioning, which has generated a large body of research in recent years (see Hooper et al. 2005 for a review). Species loss is not a random process in nature, as some species are more susceptible than others to a variety of factors, including anthropogenic pressures and physical stress

(Bracken et al. 2008). If the most sensitive species are those playing a key role in a natural system, their disappearance will alter the whole ecosystem functioning.

Canopy-forming seaweeds, such as fucoids and kelps, are important coastal ecosystem engineers that provide refuge for several species and sustain complex and diverse multi-trophic assemblages (Dayton 1985, Jones et al. 1997, Graham 2004, Schiel 2006). These seaweeds are an important part of the diet of basal macro-consumers at their adult stage or as algal sporelings, but also maintain a diverse community of algal epiphytes (Stephenson et al. 1986, Pavia et al. 1999, Van Alstyne et al. 1999). Their loss and the subsequent reduction in habitat complexity have been documented across many parts of the world and have often been attributed to climate change and other human-related stress factors (Airoldi et al. 2008, Byrnes et al. 2011, Wernberg et al. 2011, Strain et al. 2014). Hence, local extinctions of cool water canopy-forming algae have been documented at their contracting southern range margins, linked to recent ocean warming (e.g. Lima et al. 2007, Hawkins et al. 2009, Nicastro et al. 2013). A major challenge in ecology is not just to understand and predict changes in the distribution of key species under climate change, but also to identify the impacts of these changes on the associated assemblages and ecosystem functioning.

In temperate rocky shores, the loss of these canopy forming seaweeds frequently favors substrate colonization by small-sized turf-forming macro algae (Strain et al. 2014 and references therein). The shift from canopy to turf-dominated substrates may change faunal species composition (Christie et al. 1998, Wikström & Kautsky 2007) and reduce the resilience of the assemblages to large-scale

disturbances (Eriksson et al. 2006, 2007). Despite indirect evidence suggesting that canopy loss has consequences that likely propagate along food chains (Airoldi et al. 2008), few studies have attempted to document these shifts in food-web structure. The scarce empirical analyses performed to date have detected reductions in food-web complexity and length following canopy loss (Graham 2004, Byrnes et al. 2011).

A considerable number of canopy-forming, cold temperate macroalgae, which dominate intertidal and shallow subtidal rocky shores of northern Europe, reach their southern range limit in the Iberian Peninsula (Lüning 1990). Among these species is the brown seaweed *Fucus serratus* L., which has 2 range boundaries in the NW Iberian Peninsula: one in northern Portugal, and another in northern Spain (Fischer-Piette 1957, Arrontes 1993). Recent and drastic declines in marginal populations of *F. serratus* have been reported in northern Spain, which are related to a rise in sea surface temperature and other emersion-linked physical variables (Viejo et al. 2011, Martínez et al. 2012a,b, Duarte et al. 2013).

The general aim of this paper was to understand the changes in the diversity and food-web linkages of intertidal benthic assemblages at the southern range limit of *F. serratus* in northern Spain, which is experiencing habitat shifts from the dominance of *F. serratus* to turf-forming algae. In particular, we examined whether there were differences in the benthic communities and trophic structure among southern locations situated at different distances from the range margin: those at the very edge, where *F. serratus* has experienced a dramatic decline during recent years (marginal locations), and those where *F. serratus* is still dominant (central locations). Comparisons were made among locations situated at the same latitude and sharing a recent history of *F. serratus* dominance. Due to the diverse array of food sources that canopies supply to consumers, we hypothesized that the drastic decline of this furoid in marginal locations would affect the structure of the benthic food web, driving reductions in food-chain length and food web complexity.

Trophic relationships were analyzed using natural abundances of carbon and nitrogen stable isotopes (Peterson & Fry 1987, Forero & Hobson 2003). The $\delta^{15}\text{N}$ of animal tissue indicates trophic position, while the $\delta^{13}\text{C}$ ratio can suggest diet sources (DeNiro & Epstein 1978, Rau et al. 1983, Peterson & Fry 1987). This technique has been largely used in studies on trophic structure and feeding habits in marine research (e.g. Rau et al. 1983, Forero & Hobson 2003, Laurand & Riera 2006). Nonetheless, relatively few stable isotope studies have been done in rocky shores (Bustamante & Branch 1996, Bode et al. 2006, Hill & McQuaid 2008, Riera et al. 2009), and much less frequent are studies evaluating the impact of loss of habitat-formers on the structure of coastal food webs (e.g. Byrnes et al. 2011, Coll et al. 2011).

2. Materials and Methods

2.1. Study locations

The study was done in 4 locations on the northern coast of Spain distributed at different distances from the range margin of *Fucus serratus*. Two of the locations, Novellana (43° 34' N, 6° 17' W) and Cadavedo (43° 33' N, 6° 22' W) are situated in the marginal area, whereas San Pedro (43° 37' N, 7° 19' W) and Area Longa (43° 36' N, 7° 17' W) are about 100 km from the range boundary, hereafter called central area, but are in the same latitudinal position (Fig. 1). In each area, the 2 locations are exposed to moderate wave action and are about 10 km apart. In the central area, *F. serratus* is still abundant, whereas in the marginal area this species has experienced a drastic reduction in abundance during recent years (Arrontes 2002, Duarte et al. 2013)

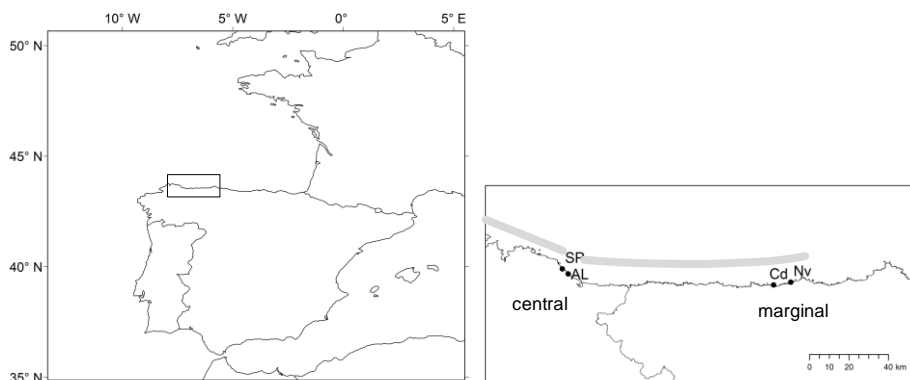


Figure 1. Locations of study areas: San Pedro (SP) and Area Longa (AL) (central area), and Novellana (Nv) and Cadavedo (Cd) (marginal area). Grey line indicates the distributional range of *F. serratus* in northern Spain at the time of the study.

2.2. Sample collection

Field surveys were carried out in October 2007 and July 2008 during low spring tides at each location. These 2 sampling seasons are representative of annual periods of low and high biomass of *F. serratus* in the study area (Arrontes 1993). Two sites per location separated by a distance of 50 to 100 m were randomly chosen at approximately 1.4 m above Lowest Astronomical Tide (maximum tidal range is around 4.3 m). At each site and date, primary and secondary cover of macroalgae and sessile animals was estimated by randomly sampling 50×50 cm plots ($n = 4$). This was done with the point method using a grid with 81 regularly spaced points. Destructive sampling was then done by scraping the surface of plots of 30×30 cm, which were randomly selected at each site (3 and 2 samples per site in October and July, respectively) for abundance estimations of vagile fauna and for isotopic analyses of fauna and algae. All samples were stored frozen at -20°C until sorted. They were then thawed and sieved at 1 mm, and the animals retained were identified to the smallest possible taxonomic level, counted, and weighed after drying them at 60°C for 48 h. Faunal species were identified with the help of distinct taxonomic keys (Chevreux & Fage 1925, Lincoln 1979, Llera et al. 1983, González & Méndez 1986). Abundant species (e.g. gastropods *Bittium reticulatum*

and *Rissoa* spp. >1000 individuals in some samples) were sub-sampled to estimate total biomass per replicate.

2.3. Isotopic analyses

The most abundant macroalgae, including *F. serratus*, and most abundant invertebrates known to consume benthic resources were analyzed for carbon and nitrogen isotopic composition. Reproductive tissue (receptacles) and vegetative parts of *F. serratus* were examined independently in July samples.

All samples (2–5 individuals per species and plot) were ground to a fine powder. The carbon and nitrogen isotopic composition of samples was determined using a Flash EA 1112 Elemental Analyzer coupled online via ConFlo III interface to a Delta V Mass Spectrometer (Elemental Analyzer Isotope Ratio Mass Spectrometer). Acetanilide was used as a standard between samples. The carbon and nitrogen isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviations from a standard material (urea and other elemental analyzer standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$.

Precision in the overall preparation and analysis was better than 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Animals >5 mm were analyzed for stable isotopes after removal of their guts, shells, and any skeletal parts, while for small individuals <5 mm (e.g. gastropods *Rissoa* spp, gammarid amphipods, and isopods) whole individuals were analyzed. Samples of small molluscs with shell and coralline algae were acidified to ensure removal of any carbonate residual. Acidification may introduce a bias in determining the natural abundance of ${}^{15}\text{N}$ (Mateo et al. 2008). Even when the cuticle composition of crustaceans such as isopods includes calcite (Neues et al. 2007), their isotopic signal was similar with and without the acidification treatment and hence these samples were not acidified (ANOVAs for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data for 5 species, $F_{1,8} = 0.02$, $p = 0.90$ and $F_{1,8} = 0.50$, $p = 0.49$, respectively).

2.4. Data analyses

Permutational multivariate analyses of variance (PERMANOVA, Anderson et al. 2008) were used to test for differences among locations, sites, and dates in macroalgae and macrofauna assemblage compositions. Location and Date were considered fixed and orthogonal factors, and Site was treated as a random factor nested within Location. Abundance of fauna and percentage cover of algae were fourth-root transformed prior to the computation of triangular dissimilarity matrices based on the Bray-Curtis measures (Anderson et al. 2008). A posteriori pairwise comparisons were performed after significant pseudo-F tests. Taxa contributing >50% to the cumulative percentage of average dissimilarity were detected using SIMPER analyses (Clarke 1993).

Those faunal species represented at each location and date, and with a sufficient number of replicates, were analyzed to detect spatial and temporal changes in isotopic signatures by using 2-way ANOVAs, where Location and Date were treated as fixed and orthogonal factors. Also, 2-way ANOVA was employed to test differences between *F. serratus* reproductive and vegetative parts at central locations (July data). Prior to the ANOVAs, Cochran's C-test was used to check the homogeneity of variances (Underwood 1997). When significant differences for Location or its interaction with Date were found, Student-Newman-Keuls (SNK) tests were performed as a posteriori tests.

The inclusion of Site within Location as a factor in the ANOVAs of isotopic signatures was not feasible due to insufficient within-site replication for the target species at each Location. In order to test the variability among sites within each location, we plotted the average of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each species found at one site vs. the values of the same species at the second site. Pearson's correlation coefficients (r) and the intercept and slope of Model II regressions (Major axis method, Legendre & Legendre 1998) were then calculated. The level of association and relations close to 1:1 (i.e. intercepts non-significantly different from 0 and slopes not differing from 1) would indicate that species had similar average isotopic signatures between sites within each location

PERMANOVAs and SIMPER analyses were performed using PRIMER v.6 (Anderson et al. 2008). ANOVAs were done using Statistica version 7.0. The Model II regressions were made with the R-package lmodel2 (version 1.7-0, R-Core Team 2012).

Dual-isotope plots were used to visually explore the relationships among benthic consumers and their potential food sources at each location and date. The relative trophic positions referred to selected isotopic baselines were estimated for the chitons *Achantochitona* spp. and the crab *Pirimela denticulata*. Since the chitons feed on macroalgae (e.g. Bode 1989), this group was considered the reference measure for the consumer. *P. denticulata*, on the other hand, is considered predator/scavenger/omnivore (WoRMS Editorial Board 2014), but detailed information on its diet is unknown. Other intertidal crabs described as predators use a diversity of food items in their diets, including peracarid crustaceans (isopods, amphipods; Williams 1981, Cannicci et al. 2002). As these small crustaceans are dominated by grazing species, which display close and low $\delta^{15}\text{N}$ values (see 'Results'), they were used as the isotopic baseline for *P. denticulata*. The Bayesian mixing model SIAR 4.1 (Parnell et al. 2010; R-core Team 2012) was used to estimate the contribution of the potential food sources to the diet of the crab *P. denticulata*. The crab feeds on some of the collected food items only if its isotopic signal lies inside the area bounded by a convex polygon, as defined by the values of potential food sources corrected by selected fractionation values (Parnell et al. 2010 and references therein). These polygons were superimposed on the dual - isotope plots to visually determine the importance of different food sources for the crab. For ^{13}C fractionation ($\Delta^{13}\text{C}$), we assumed a value of 0.3‰ (± 1.3 SD), as indicated by McCutchan et al. (2003) for consumers analyzed as a whole. For $\Delta^{15}\text{N}$, we selected the observed difference between the $\delta^{15}\text{N}$ of *P. denticulata* and the peracarid crustaceans in marginal locations, a mean value of 1.55‰ (± 0.38 SD). This value is similar to the average discrimination factor of 1.4‰ reported by Caut et al. (2009) for consumers raised on invertebrate diets; the value reported by Vanderklift & Ponsard (2003) for marine organisms

(1.48‰); and the range of 1–2‰ reported by Hill & McQuaid (2008) for organisms in rocky shores of South Africa.

4. Results

3.1. Species composition and abundance of algae and faunal assemblages

As expected, *Fucus serratus* cover was higher in the central locations of San Pedro and Area Longa than in the marginal locations of Cadavedo and Novellana, particularly if we consider secondary (canopy) cover (Fig. 2). Maximum average values of canopy cover were below 9% in marginal locations, while the minimum average value in central locations was >79% (see Fig. 2). Nevertheless, the primary cover of *F. serratus* greatly increased in July at one of the 2 marginal locations (Cadavedo; Fig. 2). Accordingly, the macroalgal assemblage showed differences between marginal and central locations in October, whereas in July Cadavedo did not differ from the central locations (PERMANOVA, Location \times Date, $F_{3,48} = 2.17$, $p = 0.049$; Cadavedo vs. central locations, $p > 0.20$ in July; for the complete analysis, see Table S1 in the Supplement material). Also, small- spatial scale variability (sites within locations) was more constant over time in marginal than in central locations (PERMANOVA, Date \times Site, $F_{4,48} = 3.69$, $p = 0.001$; differences between sites within marginal locations, $p < 0.05$ for both dates; see Table S1 for the complete analyses). Apart from *F. serratus*, the macroalgae contributing to differences between marginal and central locations were opportunistic species from the genus *Ulva* and *Ceramium*, and the turf-forming alga *Osmundea pinnatifida*, which were more abundant in the marginal locations, whereas in central locations we observed a higher cover of erect and crustose coralline algae (SIMPER for October data; see Table S2 in the Supplement material for the complete analysis). In July, these same species differentiated Novellana assemblages from those of Cadavedo and central locations, in addition to *Leathesia difformis* (more abundant in Novellana) and soft crustose species (more abundant in Cadavedo and central locations, SIMPER analysis, see Table S2).

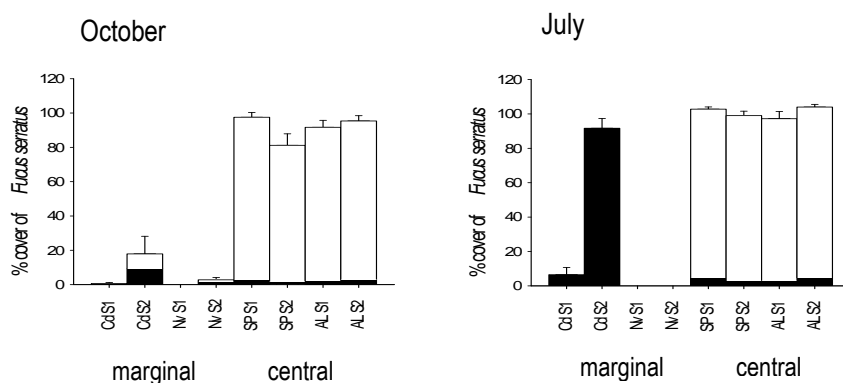


Figure 2. Total cover (primary + secondary) of *Fucus serratus* in the studied sites (S1 and S2), locations (abbreviations as in Fig. 1) and sampling dates (October 2007 and July 2008) (mean \pm SE, $n = 4$). Black and white bars indicate the primary and secondary cover respectively.

Macrofaunal assemblages showed differences among the locations situated in the central and marginal area of distribution of *Fucus serratus*. Overall, central locations were similar among each other, while marginal locations, Novellana and Cadavedo, differed from each other (Table 1, *a posteriori* comparisons). The marginal location of Novellana differed from the rest of locations (Table 1, *a posteriori* comparisons) and these differences were in species abundances rather than in composition. On the other hand, Cadavedo presented an intermediate situation between Novellana and central locations. Novellana was characterized by higher densities of the amphipods of the family Hyalidae, larval Diptera and crustaceans (Tanaidacea), whereas the gastropods *Rissoa* spp, *Barleeia unifasciata*, *B. reticulatum*, *Ocenebra erinaceus*, the chitons *Acanthochitona* spp. and the isopod *Dynamene bidentata* were more abundant in central locations and Cadavedo (Table 2). Dry weight data showed a similar pattern, as species like *O. erinaceus*, *Rissoa* spp, *Gibbula* spp, *Hinia* spp, *Tricolia pullus*, *Patella* spp. and *Acanthochitona* spp. differentiated central locations from the marginal location of Novellana (SIMPER analysis, see Table S3 in the Supplement material).

3.2. Variation in the isotopic signatures of macrofauna among locations

Four species were sufficiently represented across locations and dates to allow quantifying their changes in isotopic signatures (Table 3). The predator/scavenger crab *Pirimela denticulata* and the grazer chitons *Acanthochitona* spp. showed clear differences between marginal and central locations in $\delta^{15}\text{N}$ values, which were on average 2.7 ‰ and 2.1 ‰ respectively higher in central locations (Figure 3a; Table 4). The other 2 taxa analyzed, the gastropods *Bittium reticulatum* and *Rissoa* spp, presented $\delta^{15}\text{N}$ signatures 1.7 and 1.9 ‰, respectively, lower in the marginal location of Cadavedo (Fig. 3a; Table 4). Regarding the $\delta^{13}\text{C}$ values, no clear pattern was evident between marginal and central locations. The $\delta^{13}\text{C}$ values of *Acanthochitona* spp. and *Rissoa* spp. did not vary among locations, while those of *P. denticulata* and *B. reticulatum* were higher in the marginal location of Novellana than in the other locations (Fig. 3b, Table 4, SNK tests for *P. denticulata*: Novellana [Nv] > San Pedro [SP] ~ Cadavedo [Cd] > Area Longa [AL]; SNK test for *B. reticulatum*: Nv > SP ~ AL ~ Cd).

Table 1. Permutational ANOVA (PERMANOVA) for the effects of Date, Location and Site on faunal species composition and abundance (as densities and dry weights) at mid-shore level, $n = 2$. *A posteriori* comparisons for differences between locations are also shown. The Monte Carlo asymptotic p-value was used both for the Location term in PERMANOVA and the pairwise comparisons. Location abbreviations as in Fig. 1.

Source	df	Density			df	Dry weight		
		MS	F	p		MS	F	p
Date, D	1	3724.5	6.90	<0.001	1	3263.8	4.76	0.001
Location, L	3	3719.9	6.44	<0.001	3	3456.0	4.36	<0.001
Site (L)	4	577.8	1.22	0.193	4	793.6	1.74	0.010
D x L	3	1357.9	2.51	0.005	3	1225.7	1.79	0.059
D x S (L)	4	540.3	1.14	0.289	4	686.2	1.51	0.042
Residual	22 ^(a)	473.3			24	454.9		
<i>A posteriori</i> comparisons D x L					<i>A posteriori</i> comparisons L			
		Density				Dry weight		
		October		July				
		t	p	t	p	t	p	
Within areas	SP vs AL	1.60	0.090	1.32	0.194	0.94	0.545	
	Nv vs Cd	1.91	0.039	2.75	0.017	2.22	0.007	
Between areas	Nv vs SP	2.89	0.006	2.51	0.018	2.27	0.008	
	Nv vs AL	2.46	0.018	2.54	0.022	3.24	<0.001	
	Cd vs SP	1.78	0.047	1.88	0.048	1.35	0.152	
	Cd vs AL	1.63	0.071	1.79	0.077	1.88	0.017	

^(a) Two samples were not considered in the analyses to achieve homogeneous multivariate dispersion within groups

Table 2. Invertebrate species contributing to approximately the first 50% of the cumulative percentage of the average dissimilarities between Novellana (Nv) and the group of Cadavedo (Cd) and central locations (SIMPER analysis of densities). The order of contribution of each species and the cumulative percentage of contribution are indicated. Locations abbreviations as in Fig.1, n = 4-6 in Nv, 12-18 in the group SP, AL, Cd (October to July, respectively).

Species	Density (individuals/ 90 cm ²)							
	SP, AL, Cd Mean (SE)	October Nv Mean (SE)	Contribution Order	%	SP, AL, Cd Mean (SE)	July Nv Mean (SE)	Contribution Order	%
<i>Rissoa</i> spp.	728.9 (258.9)	28.0 (22.3)	1	7.1	910.0 (428.1)	5.0 (3.0)	1	8.9
Hyalidae	9.3 (3.3)	107.7 (28.4)	2	5.9	18.9 (8.5)	73.3 (25.0)	8	3.8
Tanaidacea	4.1 (1.8)	73.0 (25.8)	3	5.2				
Diptera (larvae)	4.0 (1.6)	33.3 (8.5)	4	4.5	0.8 (0.5)	7.8 (1.9)	7	3.9
<i>Barleeia unifasciata</i>	201.3 (60.4)	59.8 (25.9)	5	4.3	150.8 (56.3)	8.8 (1.7)	4	4.2
<i>Bittium reticulatum</i>	831.3 (223.4)	420.3 (98.8)	6	4.0	914.7 (212.2)	463.8 (161.3)	6	3.9
<i>Acanthochitona</i> spp.	35.7 (5.8)	3.7 (1.9)	7	3.7				
<i>Ocenebra erinaceus</i>	21.6 (5.5)	1.2 (0.5)	8	3.6	7.9 (1.7)	0.8 (0.5)	11	3.2
<i>Skeneopsis planorbis</i>	5.4 (3.5)	25.2 (22.6)	9	3.4	32.4 (15.1)	8.8 (5.1)	5	4.0
<i>Dynamene bidentata</i>	24.7 (6.0)	1.2 (0.5)	10	3.4	13.9 (3.2)	0.3 (0.3)	3	4.8
<i>Microdeutopus</i> spp.	5.8(3.6)	7.7 (3.8)	11	2.9	3.7 (1.1)	19.3 (6.3)	12	3.2
<i>Hinia</i> spp.	7.4 (2.4)	0.8 (0.4)	12	1.3	7.8 (2.3)	0.3 (0.3)	9	3.5
<i>Tricolia pullus</i>					20.5 (4.4)		2	5.6
<i>Idotea</i> spp.					5.2 (2.1)		10	3.4
Cumulative percentage of dissimilarity				47.4				43.6

Differences among locations were not influenced by the sampling date (Table 4, non-significant interaction Date x Location). Six other consumers were well represented in October, and their isotopic values were tested for differences among locations (Figure S1 in the Supplement material). Three out of the 6 species varied in their $\delta^{15}\text{N}$ signature among locations and only 1 showed clear-cut differences among the marginal and central locations. This species was the grazer isopod *Dynamene bidentata*, whose $\delta^{15}\text{N}$ signature was on average 1.7‰ lower at marginal locations. The predator polychaete *Perineireis cultrifera* showed values 2‰ lower at the marginal location of Cadavedo, while the grazer gastropod *Tricolia pullus* showed variability among locations with lower values in marginal locations. Significant differences also occurred for $\delta^{13}\text{C}$ values, but no clear trend between marginal vs. central locations was found (see Fig. S1).

The small-scale, between-sites variability of the ^{15}N isotopic signature of species was relatively low within marginal and central locations in October (the between-sites relationship did not differ from 1:1, high r values, Table 5), but it apparently increased at marginal locations in July (Fig. 4, Table 5, lower r values). In $\delta^{13}\text{C}$ signatures, a trend of central vs. marginal was not detected (Table 5).

Regarding the temporal changes, overall $\delta^{13}\text{C}$ values of the species were clearly higher in October than in July, particularly for *Rissoa* spp, whereas $\delta^{15}\text{N}$ values were higher in July than in October, although this trend was non-significant for *P. denticulata* (Fig. 3b, Table 4).

3.3. Variation in the isotopic signatures of food sources among locations

Isotopic signatures of the abundant macroalgae *Corallina* spp. and *Fucus serratus* did not show differences between central and marginal locations. Only the opportunistic algae *Ulva* spp. exhibited a trend of higher $\delta^{15}\text{N}$ values in central than marginal locations (mean \pm SE = 7.3 ± 0.3 ‰, 5.9 ± 0.1 ‰, $n = 4$ and 9 for central and marginal locations respectively, July data). *Corallina* spp. showed differences between dates, but not among locations (ANOVA for the effect of Date in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, $F_{1,3} = 63.80$, $p < 0.001$; $F_{1,3} = 13.96$ $p = 0.001$). Thus, similarly to the consumer's pattern, $\delta^{13}\text{C}$ values were higher in October than July (mean \pm SE =

11.6 ± 0.4 ‰ in October, -15.1 ± 1.2 in July, $n = 21$ and 16 respectively), while the opposite trend was observed for $\delta^{15}\text{N}$ (4.8 ± 0.2 ‰ in October, 7.0 ± 0.2 in July). The $\delta^{15}\text{N}$ signature of the canopy-forming *Fucus serratus* differed among locations, with lowest values in Area Longa (ANOVA for the effect of Location $F_{3,12} = 4.50$, $p = 0.025$, SNK tests, data from October, see Fig. 5).

The primary producers exhibited higher variability in carbon than in nitrogen isotopic composition, with *Corallina* spp. (Co) and reproductive parts of *Fucus serratus* (July data) presenting the highest $\delta^{13}\text{C}$ values, and the vegetative parts of *F. serratus* and *Ulva* spp. the lowest (Figs. 5 & 6).

The isotopic signatures of vegetative and reproductive parts (receptacles) of *F. serratus* were significantly different (ANOVA for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, $F_{1,8} = 33.41$, $p = 0.001$; $F_{1,8} = 13.77$, $p = 0.006$). Receptacles exhibited higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than vegetative parts (Fig. 6, *F. serratus* reproductive parts [Frep] vs. vegetative parts [Fs] in central locations; mean $\delta^{13}\text{C}$ signature \pm SE = -15.9 ± 0.5 ‰ for receptacles; -22.1 ± 0.7 ‰, for vegetative parts and mean $\delta^{15}\text{N}$ signature \pm SE = 7.4 ± 0.3 ‰ and 5.5 ± 0.3 ‰, $n = 4$ and 8 , respectively). Furthermore, this isotopic differentiation between reproductive and vegetative parts of *F. serratus* was greater than the variation detected among different algal species (Fig. 6, central locations).

Table 3. List of taxa used for isotopic analyses. Locations and dates where samples were taken are shown (O: October 2007, J: July 2008; location abbreviations as in Fig. 1). Trophic groups (TG) are indicated; D: detritivore, G (ma/mi): grazer (macroalgal/microalgal), P: predator, Sc: scavenger, Pp: primary producer, Abb: abbreviations. Nomenclature follows WoRMS Editorial Board (2014) and Guiry & Guiry (2014).

Species	Abb.	TG	Source	Locations				$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		n
				Nv	Cd	AL	SP	Mean	SE	Mean	SE	
Gastropoda												
<i>Rissoa</i> spp.	R	Gma/mi	Borja (1986)	O, J	O, J	O, J	O, J	-13.2	0.8	6.8	0.3	34
<i>Gibbula</i> spp. ^a	G	Gma/mi	Withers et al. (1975), Crothers (2001)	O	O, J	O, J	O, J	-16.7	0.2	8.8	0.1	46
<i>Barleeia unifasciata</i>	Bu	D,G	Borja (1986)	J	J	J	J	-17.6	0.3	8.8	0.2	15
<i>Bittium reticulatum</i>	Br	D	Borja (1986)	O, J	O, J	O, J	O, J	-16.3	0.4	7.0	0.2	38
<i>Nassarius</i> spp. ^b	Na	Sc	Tallmark (1980)		J	O, J	O, J	-16.6	0.4	9.8	0.3	19
<i>Tricolia pullus</i>	Tp	D	Fretter & Manly (1977)	O	O, J	O, J	O, J	-13.4	1.0	8.3	0.3	18
<i>Ocenebra erinaceus</i>	Oc	P	Barry (1981)			J	J	-17.2	0.1	10.0	0.2	8
Polyplacophora												
<i>Acanthochitona</i> spp.	Ac	Gma/mi	Purchon (1977), Bode (1989)	O, J	O, J	O, J	O, J	-16.1	0.5	9.0	0.2	38
Isopoda												
<i>Dynamene bidentata</i>	Db	Gma	Holdich (1976), Arrontes (1990)	O	O, J	O, J	O, J	-14.1	0.3	6.8	0.3	34
<i>Dynamene magnitorata</i>	Dm	Gma	Holdich (1976), Arrontes (1990)	O	O, J	O, J	O, J	-15.3	0.4	6.3	0.4	20
<i>Idotea</i> spp. ^c	I	Gma	Salemaa (1987)			J	J	-16.5	0.2	7.9	0.3	4
<i>Lekanesphaera</i> sp.	L	D	Mancinelli et al. (2005)		J			-15.5	0.6	5.2	0.1	4
Amphipoda												
Hyalidae ^d	H	Gma	Viejo & Arrontes (1992), Poore (1994)	O, J	O, J	O	O, J	-18.9	0.3	7.0	0.2	25
<i>Ampithoe rubricata</i>	Ar	Gma	Duffy & Hay 1991	O	J	O	O	-19.4	0.3	5.9	0.2	17

Table 3 continued

Species	Abb	TG	References	Locations				$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		n
				Nv	Cd	AL	SP	Mean	SE	Mean	SE	
<i>Ampithoe helleri</i>	Ah	D, G	Guerra-García et al. (2014)		J			-21.5	1.2	4.9	0.0	2
Tanaidacea	T	D, G	WoRMS Editorial Board (2014), Roca-Torecilla & Guerra-García	O, J	J			-15.8	0.3	6.7	0.2	14
Decapoda												
<i>Pilumnus hirtellus</i>	Ph	P	Schaal et al. 2010		O	O		-15.8	0.5	7.8	0.7	6
<i>Pirimela denticulata</i>	Pd	P, Sc	WoRMS Editorial Board (2014)	O, J	O, J	O, J	O, J	-14.0	0.3	9.1	0.4	28
Polychaeta												
<i>Perinereis cultrifera</i>	Pc	P	Schaal et al. (2010)	O	O	O	O	-18.8	0.3	8.6	0.3	14
Chlorophyta												
<i>Ulva rigida</i>	Ur	Pp		J	O, J	J		-20.4	0.8	5.9	0.3	10
<i>Ulva clathrata</i>	Uc	Pp		O	O		J	-18.7	0.3	6.1	0.4	6
Phaeophyta												
<i>Fucus serratus</i> (vegetative part)	Fs	Pp		O	O, J	O, J	O, J	-19.7	0.7	5.7	0.1	25
(reproductive)	Frep					J	J	-15.9	0.5	7.4	0.5	4
<i>Cladostephus</i>	Cl	Pp		J	J			-19.8	0.5	6.4	0.2	6
<i>Stypocaulon scoparium</i>	S	Pp		J	J			-19.4	0.6	5.9	0.3	6
Rhodophyta												
<i>Ceramium</i> spp.	Ce	Pp		O, J	O			-17.4	0.3	6.0	0.2	15
<i>Corallina</i> spp.	Co	Pp		O, J	O, J	O, J	O, J	-13.1	0.7	5.8	0.2	37

(^a) Mostly *Gibbula umbilicalis* and *G. pennanti*; (^b) *Nassarius incrassatus* and *N. reticulatus*; (^c) *Idotea baltica* and *I. granulosa*. (^d) *Apohyale prevostii* and *H. perieri*

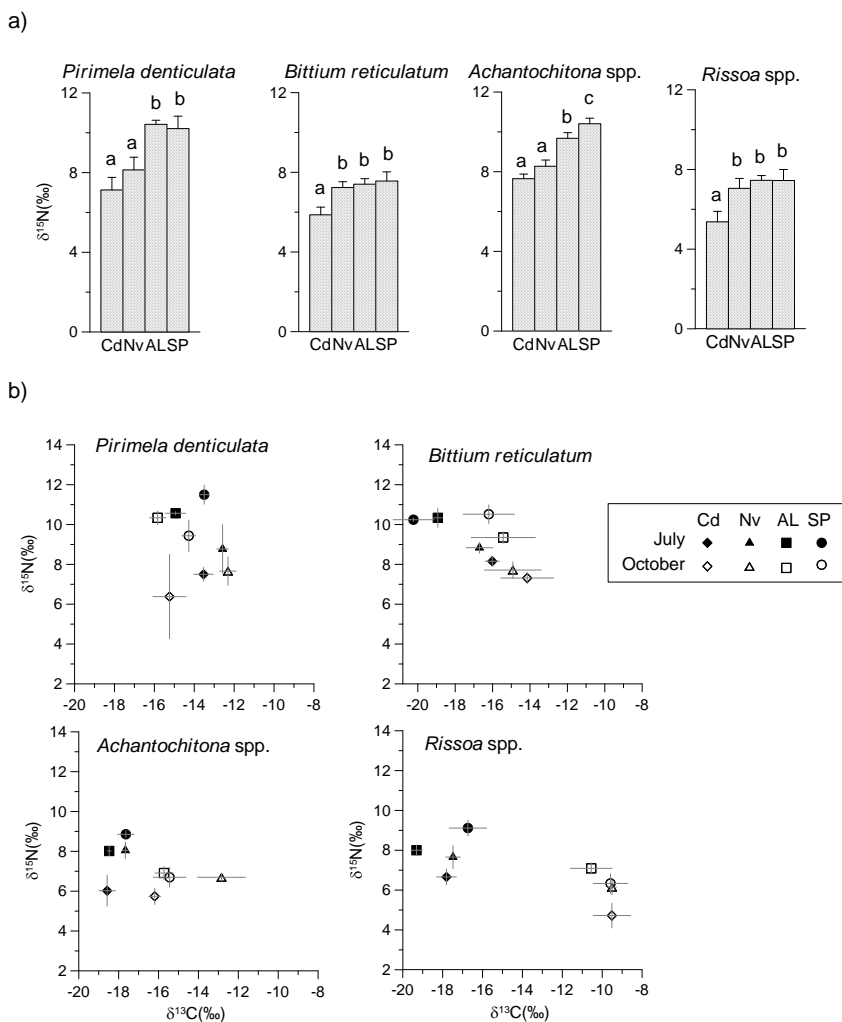


Figure 3. (a) $\delta^{15}\text{N}$ values (mean \pm SE, $n = 6-10$) for 4 consumers in marginal (Cd, Nv) and central (SP, AL) locations. Data from different sampling dates were pooled. Means sharing lower case letters did not differ significantly based on SNK tests. (b) $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ signatures (mean \pm SE, $n = 2-6$) for the same species. Location abbreviations as in Fig. 1.

3.4. Changes in trophic structure among locations

The dual-isotope plots revealed complex trophic linkages among the analyzed benthic organisms (Figs. 5 & 6). Primary consumers and macroalgae displayed similar $\delta^{13}\text{C}$ ranges (Figs. 5 & 6). Among the crustacean grazers, the $\delta^{13}\text{C}$ values of amphipods were close to the opportunistic and filamentous algae, but also to *F. serratus*, at least in October (Figs. 5 & 6). Isopods also had $\delta^{13}\text{C}$ signatures proximate to *F. serratus*, but mostly to reproductive parts (Frep in July, Fig. 6).

The gastropods *Rissoa* spp, which exhibited the highest $\delta^{13}\text{C}$ signatures among consumers in October, had values close to those of *Corallina* spp. at this date but not in July (Figs. 5 & 6). Among secondary consumers, the $\delta^{13}\text{C}$ signature of the polychaete *P. cultrifera* was close to the value of the amphipods and lower than the signal of the crab *P. denticulata* (see Fig. 5).

Primary consumers displayed high variability in their $\delta^{15}\text{N}$ signatures, with differences up to 4.5 ‰ (Figs. 5 & 6). Thus, the peracarid crustaceans (isopods, amphipods and tanaids) presented overall $\delta^{15}\text{N}$ signatures similar to or even lower than the values of their potential macroalgal food sources (mean $\delta^{15}\text{N}$ = 6.64‰, Figs. 5 & 6). The molluscs *Gibbula* spp, *Achantochitona* spp, *Tricolia pullus* and *Barleeia unifasciata*, exhibited $\delta^{15}\text{N}$ values clearly higher than primary producers and similar to or even higher than those of secondary consumers, predators and scavengers (overall mean $\delta^{15}\text{N}$ = 8.78 ‰, to Figs. 5 & 6). Finally, the $\delta^{15}\text{N}$ signatures of the small gastropods *Bittium reticulatum* and *Rissoa* spp. were similar to those of crustaceans in October, but closer to the rest of molluscs in July (Figs. 5 & 6).

Interestingly, the dual-isotope plots also revealed apparent changes in the $\delta^{15}\text{N}$ signature and trophic position of some consumers between marginal and central locations and a general trend for benthic food webs to become shorter in marginal locations. Thus, the nitrogen range, i.e. the difference between the mean $\delta^{15}\text{N}$ signatures of species with the highest and the lowest values (see Figs. 5 & 6), tended to be larger in central locations (5.30 ± 0.31 ‰ and 3.95 ± 0.50 ‰ in central and marginal locations respectively, mean \pm SE, $n = 4$; data from different dates were pooled). In particular, the grazer chiton *Achantochitona* spp. and the predator/scavenger crab *P. denticulata* exhibited higher $\delta^{15}\text{N}$ values in central locations (see above). Also, their relative $\delta^{15}\text{N}$ signatures, i.e. the difference in relation to a baseline measure (see 'Materials and methods'), was clearly higher (around double) in central locations. For the chitons, the $\delta^{15}\text{N}$ values were 3.97 ± 0.32 ‰ and 2.10 ± 0.05 ‰ in central and marginal locations, respectively (mean \pm SE, $n = 4$; ANOVA $F_{1,6} = 33.54$, $p = 0.001$) and 3.23 ± 0.26 ‰ and 1.55 ± 0.19 ‰

for *P. denticulata* ($F_{1,6} = 22.87$, $p = 0.003$). Rather than representing striking variations within species in the ^{15}N discrimination factors ($\Delta^{15}\text{N}$), these results feasibly reflect spatial changes in the diet and the trophic position of the consumers. Using the lowest value, 1.55‰, for average $\Delta^{15}\text{N}$ and 0.3‰ for $\Delta^{13}\text{C}$ as fractionation factors, the diet of the crab *P. denticulata* appeared to be composed of macroalgae (*Ceramium* spp. and *Corallina* spp.), isopods (*Dynamene* spp.) and the small gastropods *B. reticulatum* and *Rissoa* spp. in marginal locations, whereas the consumer apparently excluded the algae and incorporated larger gastropods (*Gibbula* spp. and *T. pullus*) and the chitons *Achantochitona* spp. in central locations (see the convex polygon in Fig. 5, October data). The potential food sources varied from October to July, but again *Gibbula* spp. were included in the diet of crabs in central but not in marginal locations (Fig 6). It was not possible to determine the range of proportions among these feasible food sources, as the variation among their isotopic signatures was limited and thus the Bayesian mixing models SIAR was subject to uncertainties (see Fig. S2 in the supplement material for results of SIAR with October data).

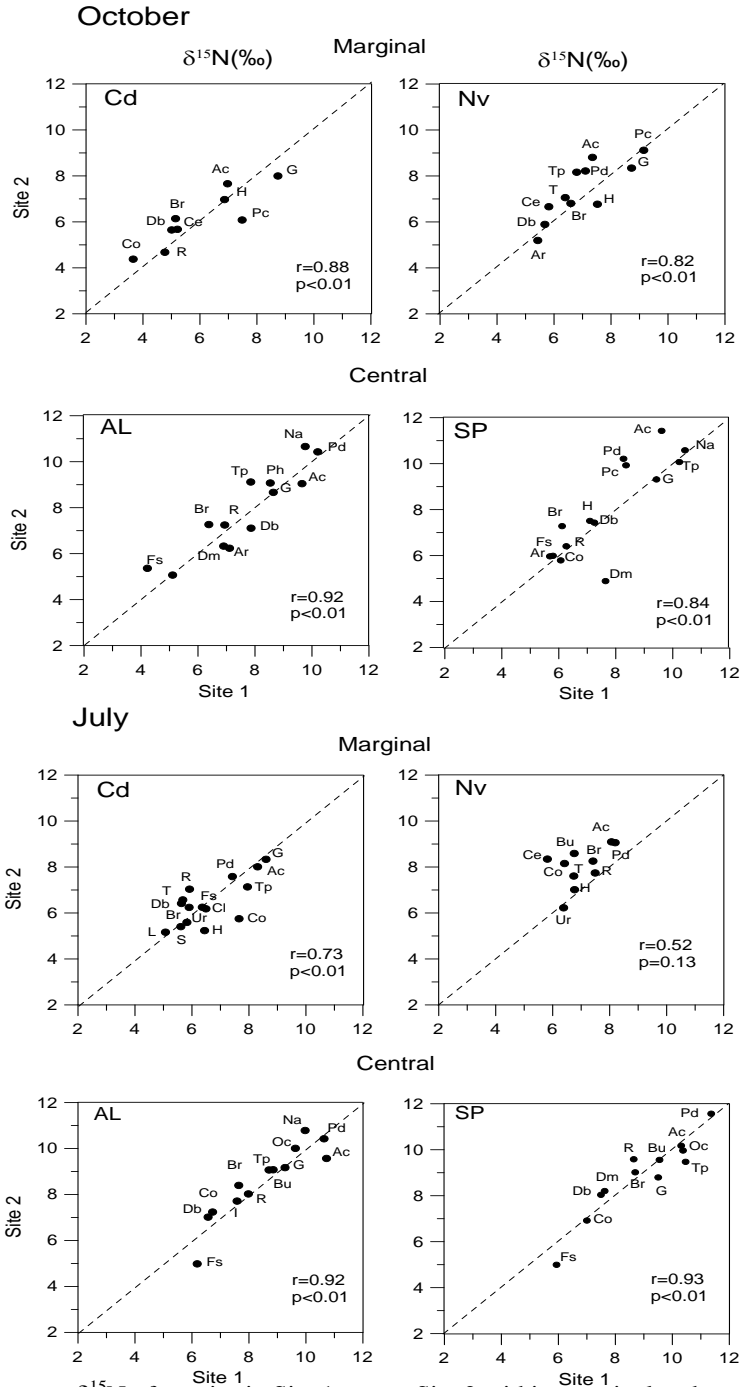


Figure 4. Average $\delta^{15}\text{N}$ of species in Site 1 versus Site 2 within marginal and central locations at both sampling dates. Abbreviations of species and locations are indicated in Table 3 and in Fig. 1 respectively. Dashed line shows 1:1 correlation. Pearson's correlation coefficients (r) and p values are also indicated.

Table 4. ANOVA for the effects of Date and Location in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of 4 consumers; unbalanced data, $n = 2-6$. D = date; L = location.

		$\delta^{15}\text{N}$			$\delta^{13}\text{C}$		
<i>Pirimela denticulata</i> ^(a)							
Source	df	MS	F	p	MS	F	p
D	1	8.524	4.315	0.050	4.038	5.841	0.025
L	3	19.219	9.728	<0.001	10.611	15.347	<0.001
D x L	3	1.054	0.534	0.664	1.017	1.470	0.251
Residual	21	1.976			0.691		
<i>Bittium reticulatum</i> ^(b)							
D	1	13.823	17.082	<0.001	85.097	34.716	<0.001
L	3	6.476	8.003	<0.001	8.348	3.406	0.030
D x L	3	1.380	1.705	0.187	3.490	1.424	0.255
Residual	30	0.809			2.451		
<i>Rissoa</i> spp.							
D	1	23.542	22.707	<0.001	478.019	129.508	<0.001
L	3	7.197	6.941	0.001	5.550	1.504	0.237
D x L	3	1.446	1.395	0.267	1.099	0.298	0.827
Residual	26	1.037			3.691		
<i>Achantochitona</i> spp.							
D	1	3.917	6.881	0.014	68.260	7.759	0.009
L	3	14.473	25.427	<0.001	18.318	2.082	0.124
D x L	3	0.942	1.654	0.199	2.915	0.331	0.803
Residual	29	0.569			8.797		

^(a)In $\delta^{15}\text{N}$ variances were homogeneous for a significance level of 0.01^(b)In $\delta^{13}\text{C}$ variances were heterogeneous, but the same results were obtained with PERMANOVA

Table 5. Pearson's correlation coefficients (r) and parameters (intercept and slope) of model II regressions (Major axis method) for the isotopic signatures of species between site 1 and 2 within each locality and sampling date. MA intcpt, MA slope= slopes and intercepts of the Major Axis regressions respectively (Correlation coefficients for $\delta^{15}\text{N}$ in Fig. 4). If the intercept differs from 0, and the slope from 1 (values included or not in the 95% confidence intervals) is indicated within brackets. (~0), (~1): parameters did not differ from these values; (<0) (<1) parameters are lower than these values; ns: non-significant

October								
Areas	Locations	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$				n
		r	p	MA intcpt	MA slope	MA intcpt	MA slope	
Marginal	Cadavedo	0.722	0.028	-3.88 (~0)	0.68 (~1)	1.71 (~0)	0.74 (~1)	9
	Novellana	0.589	0.056	ns	ns	-0.02 (~0)	1.07 (~1)	10
Central	Area Longa	0.823	0.001	-5.25 (~0)	0.69 (~1)	-0.01 (~0)	1.03 (~1)	13
	San Pedro	0.948	<0.001	1.76 (~0)	1.05 (~1)	-2.21 (~0)	1.32 (~1)	14
July								
Marginal	Cadavedo	0.859	<0.001	0.10 (~0)	1.04 (~1)	0.84 (~0)	0.85 (~1)	15
	Novellana	0.812	0.002	4.60 (~0)	1.21 (~1)	ns	ns	10
Central	Area Longa	0.971	<0.001	-0.26 (~0)	0.97 (~1)	-0.34 (~0)	1.05 (~1)	13
	San Pedro	0.895	<0.001	-7.32 (<0)	0.54 (<1)	-0.36 (~0)	1.03 (~1)	12

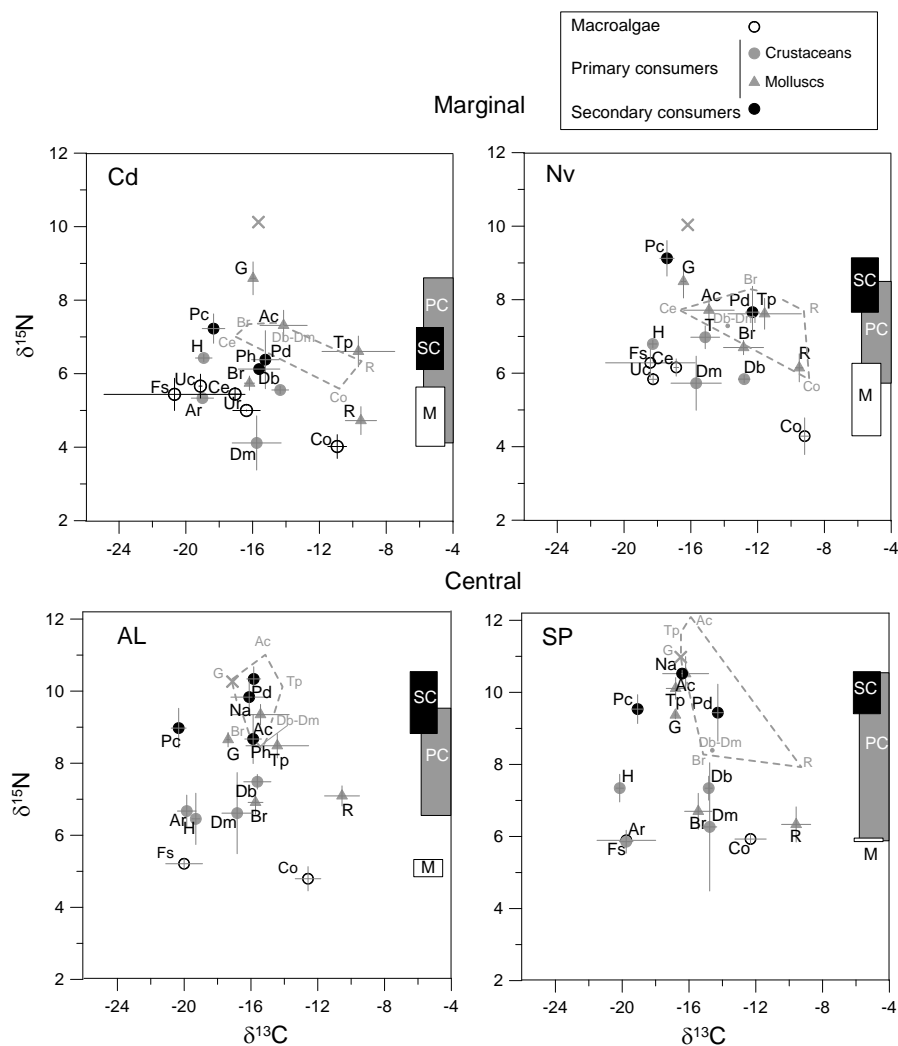


Figure 5. $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ signatures (mean \pm SE, $n = 2-6$) of organisms sampled in October in central and marginal locations. Vertical bars represent the $\delta^{15}\text{N}$ variation within the groups of secondary consumers (SC), primary consumers (PC), and macroalgae (M). The polygons represent the signatures of potential food sources corrected for fractionation (species indicated in gray font; *Dynamene bidentata* and *D. magnitorata* were pooled) for the crab *Pirimela denticulata*. Crosses indicate the positions of the corrected values of *Gibbula* spp. Location abbreviations as in Fig. 1. Abbreviations and diets of species are indicated in Table 3.

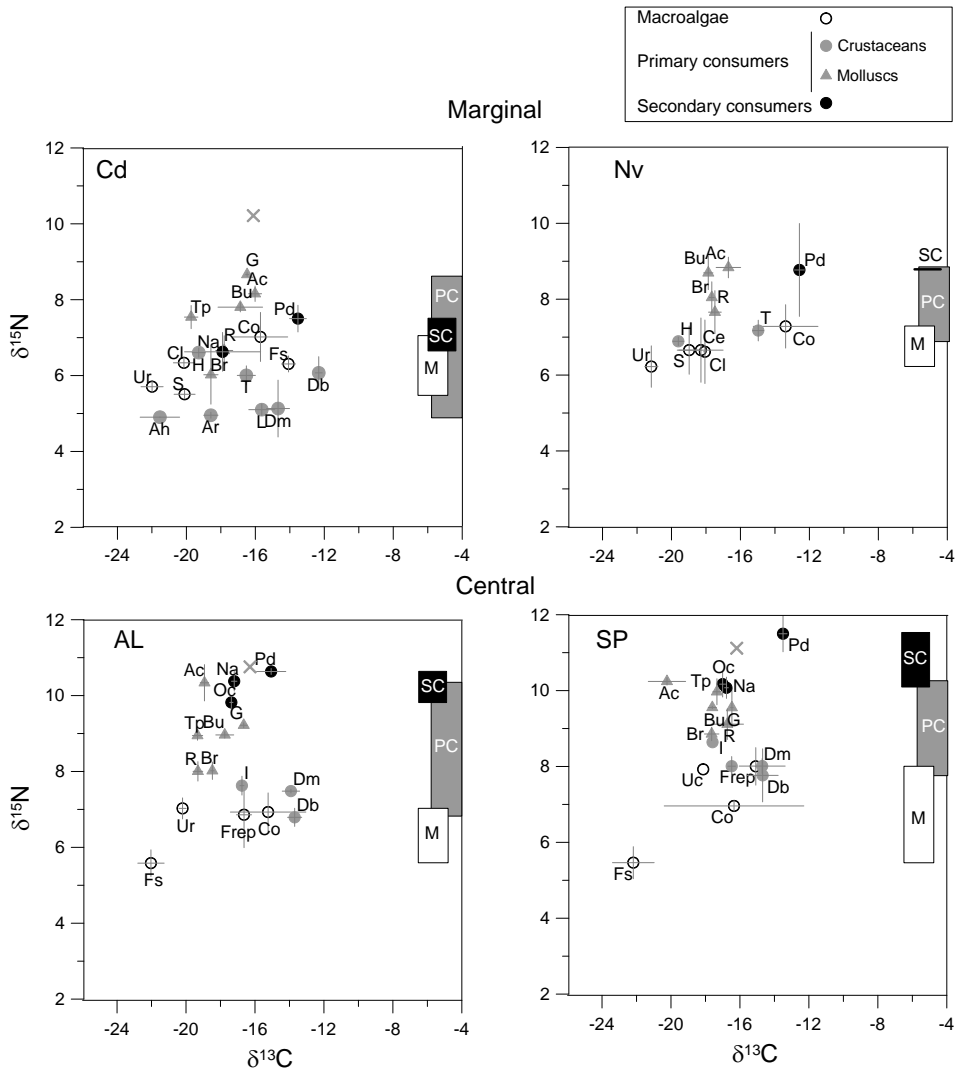


Figure 6. $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ signatures of organisms sampled in July in central and marginal locations (mean \pm SE, $n = 2-4$, except for *Ulva* in SP, where $n=1$). Vertical bars represent the $\delta^{15}\text{N}$ variation within the groups of secondary consumers (SC), primary consumers (PC), and macroalgae (M). Crosses indicate the position of corrected fractionation values of *Gibbula* spp. Location abbreviations as in Fig. 1. Abbreviations and diets of species are indicated in Table 3.

9. Discussion

The loss of the canopy of *F. serratus* implies the transition from a multilayered macroalgal habitat (i.e. a canopy of the furoid and species growing underneath) to a simpler habitat with a cover of smaller-sized algae. These habitat

transformations, with growth enhancement of ephemeral and turf-forming species, have been previously reported in different geographical areas, as a consequence of abiotic stress or anthropogenic factors (Airoldi et al. 2008, Strain et al. 2014 and references therein). In our study, while erect and crustose coralline algae and soft crusts proliferated under the canopy of *F. serratus*, in locations at the range edge, where the macroalgae were disappearing, opportunistic species (*Ulva* and *Ceramium* spp.) and the turf-forming *Osmundea pinnatifida* or the globular *Leathesia difformis* became more abundant. These might occur as a direct response to the canopy loss or, alternatively, both canopies and associated algae could be simultaneously reacting to environmental stressors. Canopies can control the development of the understory, limiting the growth of ephemeral, opportunistic algae and favoring other groups such as algal crusts or coralline species, feasibly by changing the abiotic conditions, i.e. reducing light, temperature or attracting grazers (Lilley & Schiel 2006, Eriksson et al. 2006, 2007).

Whereas the “vertical” complexity of macroalgal assemblages was reduced due to the canopy loss, the horizontal small-scale variability (between-site variation) was higher within marginal locations (see ‘Results’). This could reflect the patchy distribution of small-sized ephemerals and turf-forming species within boundary locations, but also the small-scale spatial pattern of *F. serratus* disappearance. Indeed, patches of *F. serratus* still remained at the end of this study in marginal locations (authors’ pers. obs.). The remnants were dwarf morphs, short specimens with a bushy appearance, also detected in other *Fucus* species (Viejo et al. 2011). The presence of remnants was more evident in Cadavedo than in Novellana, and this is why high values of primary cover were recorded at 1 site in this first location, coinciding with the seasonal peak of *F. serratus* biomass (Arrontes 1993). A recent survey in 2013 showed that the seaweed became locally extinct in Novellana and other marginal locations, i.e. the range boundary moved westwards along a stretch of coast of about 80 km (towards the “central” area), leaving behind a boundary of a few sparse and very small populations, such as Cadavedo (R. M. Viejo pers. obs.; see also Duarte et al. 2013).

Macrofauna tracked the shifts in macroalgal communities, since invertebrate assemblages of Novellana differed from the remaining ones. As in previous studies that evaluate the influence of canopy loss on the associated fauna (e.g. Lilley & Schiel 2006, Wikström & Kautsky 2007), we observed reductions in abundance rather than in species composition. There was a shift in the dominant species in Novellana, where amphipods of the family Hyalidae, Tanaidacea and larvae of Diptera were abundant, while gastropods, the chitons *Acanthochitona* spp, and the isopod *Dynamene bidentata* were less numerous. The marginal location of Cadavedo, with the remnant presence of *F. serratus*, represented an intermediate situation with respect to changes in faunal assemblages. The effects of *F. serratus* disappearance on macrofauna may occur because canopy offers shelter from predators, changes hydrodynamics and increases water retention at low tide, or because lost seaweeds constitutes an important food source (e.g. Arrontes 1999 and references therein, Bates & DeWreede 2007, Christie et al. 2009, Best et al. 2014). Canopies provided a diverse array of food sources for consumers, including the seaweed itself, which could be directly eaten by grazers or as detritus (Fredriksen 2003, Norderhaug et al. 2003, Schaal et al. 2010), but also the micro and macroalgal epiphytes, sessile animals and microorganisms growing on the thallus surface (Pavia et al. 1999, Lachnit et al. 2013).

The preferences for food and habitat vary among faunal species. The isopod *D. bidentata*, which was more abundant in central locations, has been commonly associated with canopy-forming algal hosts upon which the isopod feeds preferentially (Arrontes 1990; Viejo & Arrontes 1992; Morán & Arrontes 1994). On the other hand, the higher abundance of hyalid amphipods and dipteran larvae in the marginal location of Novellana might be related to their food preferences, as they feed on different resources, including filamentous and ephemeral species such as *Ulva* spp. (Henriques-Oliveira et al. 2003, Taylor & Steinberg 2005, Guerra-García et al. 2014). Our stable isotope analyses (the $\delta^{13}\text{C}$ signatures) indicated that the isopod *D. bidentata* could be feeding on *F. serratus*, in particular on reproductive parts, while amphipods may also consume species of

Ulva or *Ceramium*. Moreover, turf-forming and filamentous algae provide better shelter from predation and high adhesive surface for amphipods than erect flat fronds (Bushmann 1990; Hacker & Steneck 1990), such as those of *F. serratus*.

Benthic food webs, supported by macrophytes (macroalgae or sea grasses), are generally short in length when they are compared to other ecosystems, since most invertebrates are detritivores or herbivores and have high levels of omnivory (Fredriksen 2003, Hill & McQuaid 2008, Baeta et al. 2009, Schaal et al. 2010). Nonetheless, the presence of habitat-forming macrophytes by increasing habitat complexity may raise diversity within and among trophic levels, inducing omnivore species to feed at higher trophic levels, thereby enhancing food-web complexity (Coll et al. 2011, Rossi et al. 2015). Overall, low nitrogen ranges ($\sim 4 - 5 \text{ ‰}$) were detected in the studied rocky-shore systems, revealing short length of the food-webs. Moreover, the observed discrepancies between the discrete trophic groups in which the species were *a priori* classified, and their $\delta^{15}\text{N}$ signatures, indicated the presence of intra-guild variability in the diets and high levels of omnivory. In particular, grazing crustaceans (isopods, gammarid amphipods), presented $\delta^{15}\text{N}$ signatures similar or even lower than the values of their potential macroalgal food sources, while grazing gastropods such as *Gibbula* spp. and the chiton *Achantochitona* spp. exhibited $\delta^{15}\text{N}$ values $\sim 2 \text{ ‰}$ higher on average, proximate to the isotopic signal of secondary consumers. Differences in $\delta^{15}\text{N}$ signatures among primary consumers were up to 4.5 ‰ (see Figs 5 & 6). Low fractionation levels of small crustacean grazers (e.g. McCutchan et al. 2003, Michel 2011), and higher ^{15}N -enrichment of chitons and the topshell *Gibbula* spp. were reported in previous studies (Fredriksen 2003, Riera et al. 2009, Schaal et al. 2010). A high variability within the group of primary consumers might reflect differences between species in the trophic fractionation, due to metabolic and assimilation processes, as the form of N excretion (Vander Zanden & Rasmussen 2001, Vanderklift and Ponsard 2003). Both crustacean and marine molluscs are, however, primarily ammonotelic. Moreover, the observed range of $\delta^{15}\text{N}$ variation within the group of primary consumers is probably too large to be explained only

by these differences and rather reflects the variability in their feeding modes and diets. Chiton and gastropod species use their radulas to scrape a diversity of food sources on hard substrata, and in the case of the topshell, on the surface of the thalli of macroalgae (Bode 1989, Crothers 2001, Bhatnagar & Bansal 2009, Schaal et al. 2010). Their high $\delta^{15}\text{N}$ signatures, close to predators and scavengers ($>10\text{‰}$ for *Achantochitona* spp. at some locations, see Figs 5 & 6) indicated that they may also include animals in their diets. This is in accordance with the results of Camus et al. (2008), who found that the ingestion of animals was a frequent phenomenon in molluscan 'herbivores' of Chilean coasts, and consequently these species presented a high potential for omnivory. Even when molluscs may feed on remains of vagile animals and sessile fauna, in our study the tidal zone dominated by macroalgae presented low covers of sessile animals such as barnacles, mostly *Chthamalus* spp. (average percentage covers $< 0.7\text{‰}$), without apparent differences between central and marginal locations.

Remarkably, our stable isotope results also revealed food-webs disruptions at the range boundary of *F. serratus*, with shifts in the diet and lower trophic positioning of some consumers, and an overall trend for shrinkage of the food-web length at marginal locations. In particular, the $\delta^{15}\text{N}$ signatures of the chiton *Achantochitona* spp. and the crab *Pirimela denticulata* fell in marginal locations (both the absolute values but also the relative values referred to respective isotopic baselines, i.e. macroalgae and grazer peracarid crustaceans). The estimation of the trophic positioning of organisms is moderately sensitive to the selection of the trophic baseline (Post 2002). However, the spatial shift in the $\delta^{15}\text{N}$ signatures of the crab persisted when referred to macroalgae as a food source (average differences in central vs. marginal locations: 4.3 and 1.7 ‰, respectively), or even when referred to *Ulva* spp. (3.6 vs. 2.2 ‰), the only primary producer showing spatial trends. Furthermore, trophic positioning is very sensitive to the considered value of trophic fractionation of $\delta^{15}\text{N}$ (Post 2002), and this discrimination factor is variable, which also add difficulties to the reconstruction of diets in natural systems (Post 2002, Caut et al. 2009). Nevertheless, we interpret these consistent differences in the

average $\delta^{15}\text{N}$ signal of the 2 consumers between marginal and central locations as shifts in their diets and evident changes in their trophic positioning. We based this interpretation in the fact that differences were sustained with respect to trophic baselines, and they were similar or greater than the discrimination factors reported by different authors (see 'Materials and Methods'). Our results then suggest that consumers, such as the chiton *Achantochitona* spp. and the crab *Pirimela denticulata* increased the proportion of animals in their diets in central locations, i.e. in presence of canopies. In particular, the crab might consume macroalgae and small gastropods in marginal locations, while excluding the algae and incorporating larger gastropods (*Gibbula* spp.) and even the chitons *Achantochitona* spp. at central locations. These diet shifts are in accordance with the diverse diet of intertidal crabs, with species considered to be predators very often ingesting macroalgae (Williams 1981, Canicci et al. 2002). Moreover, the changes are in agreement with the observed shifts in faunal assemblages, as large molluscs, such as *Gibbula* spp. and *Acanthochitona* spp. were more abundant in the presence of *F. serratus*. We applied average fractionation values which were close to those reported in the literature by different authors in comparable systems (see 'Materials and methods'). Furthermore, the use of larger ^{15}N discrimination factors from the literature (e.g. 2.5 ± 2.5 ‰, Vander Zanden and Rasmussen 2001) would not allow for estimations of the diet of the crab in marginal locations, as the corrected values of the potential food items would lie well above the signal of the consumer. The use of fixed average fractionation values, in particular the measured $\Delta^{15}\text{N}$ of 1.55‰, requires cautious interpretation of the detailed, fine-grained variations in the crab diet. Yet, our results indicate that consumers feasibly fed more as secondary consumers in presence of canopies and increased the consumption of algae when the canopy-former was lost.

Greater between-site variability in the $\delta^{15}\text{N}$ signatures was also detected in July at marginal locations. This could be related to the mosaic of patches of ephemerals, turf-forming species and remnants of *F. serratus*, which might provoke diet shifts of consumers at these thin-grain scales in these locations (see

also Schaal et al. 2011). Alternatively, it could be the result of small-spatial shifts in the $\delta^{15}\text{N}$ isotopic signal of the basal sources (the macroalgae). The $\delta^{15}\text{N}$ signatures of some consumers did not follow the marginal vs. central pattern, which indicates that other factors, apart from canopy loss are feasibly affecting trophic linkages.

We also detected evident temporal patterns in stable nitrogen and carbon isotopes. Several consumers and the alga *Corallina* spp. were ^{15}N -enriched and ^{13}C -depleted in July with respect to October, both in marginal and central locations. Temporal shifts in the diets of consumers, as well as changes in the metabolic routes during the growth cycle of organisms and the use of different N sources by primary producers, may be involved in these variations. For instance, the proximity of the isotopic signal of *Rissoa* to the signatures of *Corallina* in October but not in July, suggested seasonal changes in the gastropod diet (Norderhaug et al. 2003 and references therein), with the inclusion of this alga (as previously reported by Borja 1986) only in October. On the other hand, temporal differences in isotopic ratios of macroalgae could be caused by changes in the biochemical processes during their growth cycle (Fredriksen 2003) and by the use of different N sources by primary producers through the year (Viana & Bode 2013). Also, the great isotopic differentiation between vegetative and reproductive parts of the thalli of *F. serratus* could be linked to within-thallus variation in biochemical processes (Brenchley et al. 1997). Intra-thallus variation in isotopic ratios has been previously reported by Fredriksen (2003) between new and old parts of the kelp *Laminaria hyperborea*.

Species loss at the current pace is often a consequence of recent climate change and other anthropogenic stressors (Harley et al. 2006, Strain et al 2014). Furthermore, when key species are also the most prone to become extinct, their loss would trigger major changes in the whole system, spreading further up in the food-web. This is the case of canopy-forming algae, which are among the first group lost in degraded coastal systems (Eriksson et al. 2006), and are as well very sensitive to climate change (Jueterbock et al. 2013). Indeed, recent studies have reported the contraction of the southern boundary for several fucoids and kelps in

the Iberian Peninsula as shifts linked to recent ocean warming (e.g. Fernández 2011, Duarte et al. 2013, Nicastro et al. 2013). These species of fucoids and kelps usually do not have functional equivalents in marine habitats (Schiel 2006). As more productive systems tend to support longer food chains and tangled food webs (Thompson et al. 2007 and references therein), canopy losses would lead to the decline of diversity and food-web complexity. Our results are in accordance with the scarce empirical analyses performed to date, detecting this sort of response following canopy loss (Graham 2004, Byrnes et al. 2011). The benthic invertebrate assemblage and their trophic linkages shifted following the disappearance of the canopy-forming algae *F. serratus* at its retreating range edge. More importantly, the loss of *F. serratus* may imply a decrease in food chain length and food-web complexity, which feasibly affect higher trophic levels, as fishes and seabirds feed on benthic fauna (Fredriksen 2003 and references therein). Under present and future climatic scenarios, the shifts in the distribution of ecosystem engineers could then entail a reorganization of local natural assemblages and coastal food-webs. More concern and attention should be given to measure how much these shifts can modify the whole coastal food-webs and their functioning.

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SUPPLEMENTARY MATERIAL

Table S1. PERMANOVA for the effects of Date, Location and Site on macroalgal assemblages, $n = 4$. *A posteriori* comparisons for differences between Locations per Date, and Location per Site are also shown. Monte Carlo asymptotic p-value was used both for the Location term in PERMANOVA and the pairwise comparisons. Nv = Novellana, Cd = Cadavedo, AL = Area Longa, SP = San Pedro.

	Density				
Source	df	MS	F		p
Date, D	1	3284.6	1.84		0.124
Location, L	3	11623.2	4.00		0.003
Site (L)	4	2919.3	6.03		0.001
D x L	3	3870.4	2.17		0.049
D x S (L)	4	1787.2	3.69		0.001
Residual	48	483.7			
<i>A posteriori</i> comparisons D x L					
		October		July	
		t	p	t	p
Within areas	SP vs AL	0.97	0.469	1.31	0.263
	Nv vs Cd	0.86	0.557	1.19	0.318
Between areas	Nv vs SP	3.30	0.041	2.34	0.082
	Nv vs AL	2.56	0.074	2.19	0.084
	Cd vs SP	2.84	0.048	1.58	0.204
	Cd vs AL	2.26	0.074	1.40	0.259
<i>A posteriori</i> comparisons D x S (L)					
		October		July	
		t	p	t	p
Sites within	SP	1.30	0.208	1.39	0.126
	AL	2.20	0.015	1.29	0.186
	Cd	2.07	0.023	3.01	0.004
	Nv	1.93	0.021	2.69	0.006

Table S2. Macroalgal species contributing to approximately the first 50% of cumulative percentage of the average dissimilarities between central and marginal locations in October, and between Novellana and the group of Cadavedo and central locations in July (SIMPER analyses, *Fucus serratus* excluded). The percentage cover, mean and SE, and the order and percentage of contribution of each species are indicated.

Species	October				July			
	Central	Marginal	Contribution		Cd, SP, AL	Nv	Contribution	
	SP, AL	Nv, Cd						
	Mean (SE) n = 16	Mean (SE) n = 16	Order	%	Mean (SE) n = 24	Mean (SE) n = 8	Order	%
<i>Ceramium</i> spp.		22.99 (3.86)	1	14.8	2.69 (0.98)	11.42 (1.96)	3	6.1
<i>Ulva clathrata</i>	0.20 (0.11)	15.35 (3.30)	2	11.4	0.27 (0.13)	4.91 (2.00)	5	5.5
<i>U. rigida</i>					1.81 (0.54)	12.01 (4.26)	2	6.5
<i>Phymatolithon</i> spp.	4.32 (0.92)		3	8.6	8.38 (3.95)	2.04 (0.79)	8	5.0
<i>Corallina</i> spp.	52.24 (4.51)	12.58 (2.37)	4	6.5	35.13 (5.94)	27.31 (5.20)	4	5.6
<i>Cladosthephus spongiosus</i>	5.91 (1.37)	3.15 (1.07)	5	5.7	3.54 (1.24)	6.30 (2.65)	6	5.5
<i>Osmundea pinnatifida</i>	0.64 (0.15)	3.37 (0.61)	6	5.3				
<i>Leathesia difformis</i>					0.38 (0.21)	4.92 (1.12)	1	6.9
Soft crustose species					6.86 (2.33)	2.47 (1.26)	7	5.4
Cumulative average % dissimilarity				52.3				46.4

Table S3. Invertebrate species contributing to approximately the first 50% of cumulative percentage of the average dissimilarities between Novellana and the central locations (SIMPER analysis of dry weight, data from different dates were pooled). The dry weight (mean and SE), and the order and percentage of contribution of each species are indicated. Locations abbreviations as in Fig. 1, n = 10 in Nv; 20 in the group SP and AL.

Species	Dry weight (g/ 90 cm ²)			
	SP, AL	Nv	Contribution	
	Mean (SE)	Mean (SE)	Order	%
<i>Ocenebra erinaceus</i>	2.14 (0.60)	0.07 (0.03)	1	9.4
<i>Rissoa</i> spp.	3.94 (1.48)	0.09 (0.08)	2	8.2
<i>Gibbula</i> spp.	4.43 (0.60)	0.70 (0.35)	3	7.6
<i>Nassarius</i> spp.	0.43 (0.06)	0.05 (0.02)	4	5.6
<i>Tricolia pullus</i>	0.18 (0.06)	0.01	5	4.5
<i>Patella</i> spp.	0.14 (0.05)	0.08 (0.04)	6	4.1
<i>Acanthochitona</i> spp.	0.56 (0.31)	0.15 (0.06)	7	4.0
<i>Dynamene bidentata</i>	0.06 (0.02)	0.01	8	3.8
<i>Barleeia unifasciata</i>	0.53 (0.01)	0.09 (0.04)	9	3.7
Cumulative percentage of dissimilarity				49.20

Figure S1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SE) for different consumers in the studied locations (October data). Locations abbreviations as in Fig. 1. The results of the ANOVA are shown. Means sharing lower case letters do not differ significantly based on SNK tests.

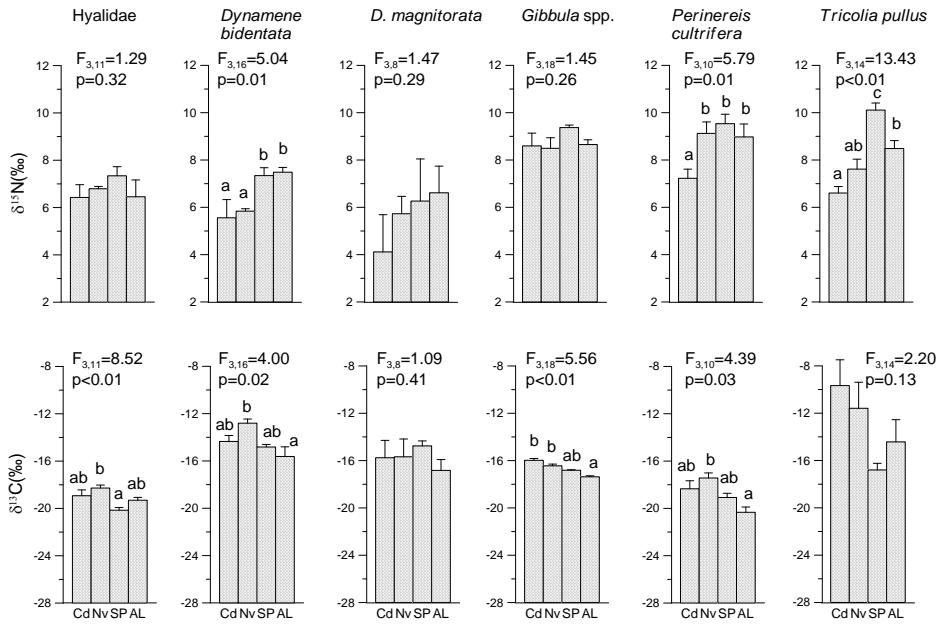
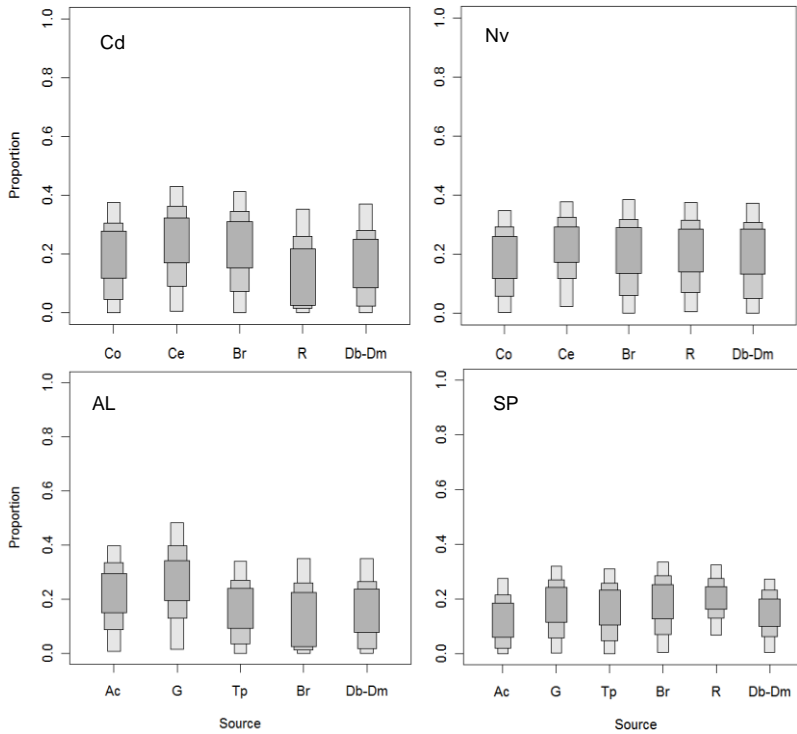


Figure S2. Contributions of potential food sources to the diet of the crab *Pirimela denticulata* as determined by the Bayesian mixing models SIAR. The 50, 75 and 95% credible intervals (dark to light boxes) are shown in marginal (Cadavedo, Cd; Novellana, Nv) and central (Area Longa, AL, San Pedro, SP) locations. Data from October. See Table 1 for species abbreviations. Db-Dm: data from those two species of isopods were pooled before running the analyses.



CONCLUSIONES GENERALES

1. Se ha reportado que desde finales del siglo XIX hasta nuestros días en la Península Ibérica, se ha observado una drástica retracción en el límite sur de distribución geográfica de las macroalgas *Fucus serratus* y *Himanthalia elongata*, y dicha retracción está ligada, entre otros factores, al aumento de la temperatura superficial del agua.
2. La distribución geográfica de *Himanthalia elongata* muestra una retracción más drástica que *Fucus serratus*. Identificándose que *Himanthalia* prácticamente ha desaparecido del Norte de España (costa Cantábrica), manteniendo poblaciones abundantes en las costas Atlánticas (noroeste de Galicia). Mientras que las poblaciones de *F. serratus*, se mantienen en el oeste de la Península Ibérica, a pesar de observarse también drástica pérdida en su abundancia y poblaciones aisladas.
3. La permanencia de *Fucus serratus* en el Norte de España está ligada a que esta especie tiene una esperanza de vida más larga que *H. elongata*, además de tener una mayor resistencia a las altas temperaturas.
4. La identificación de poblaciones esporádicas, fuera de la distribución continua de la macroalga *Himanthalia elongata* en los distintos periodos, con algunos pulsos de temperaturas más bajas, puede también deberse a la alta capacidad de dispersión de esta alga.
5. *Fucus serratus* tienen una distribución geográfica bastante parcheada en las costas rocosas de las Rías Bajas de Galicia en el noroeste de España, ocupando costas rocosas parcialmente expuestas o protegidas “refugios”. Identificándose asimismo que la especie coexiste con otras macroalgas como *F. vesiculosus*, *Ascophyllum nodosum* y *H. elongata*.

6. Esta particularidad en la distribución parcheada de estas poblaciones marginales de *F. serratus*, parece estar ligada a localidades ricas en nutrientes, específicamente amonio, con bajas temperaturas del agua en el verano, y con alta variabilidad en la salinidad. Además otras variables como la presencia de *Himanthalia elongata* y la herbivoría explican, la distribución geográfica de la especie en las Rías estudiadas.

7. Experimentos de trasplantes con juveniles y cigotos de *F. serratus* dentro de las Rías de Muros y la Ría de Arosa mostraron que el número de cigotos al final de los experimentos, fue significativamente mayor en las localidades origen. Además se observó una tendencia de menor tamaño en juveniles de la especie trasplantados en las localidades exteriores.

8. Se detectó una mayor diversidad en la cobertura de macroalgas en las localidades de las Rías estudiadas (Ofreixo y Isla de Arosa), que en las localidades de Lugo (San Pedro y Peizas). En las Rías observamos mayor abundancia de especies como *Fucus vesiculosus*, *Himanthalia elongata* y *Ascophyllum nodosum*. Asimismo, las Rías presentaron la característica de poseer una mayor abundancia de herbívoros que Lugo.

9. Por otra parte, las condiciones ambientales de poblaciones marginales de *F. serratus* son bastante diferentes en el noroeste de España. Identificándose que poblaciones de las Rías ocupan hábitats más benignos para la macroalga, que las poblaciones de *F. serratus* investigadas en las localidades de Lugo.

10. Además, en este límite sur de distribución geográfica (noroeste de España), la especie *Fucus serratus* muestra señales de adaptación local. En efecto, en los experimentos de trasplantes de individuos juveniles, aquellos trasplantados en sus propios hábitats, mostraron mayor elongación que individuos de las otras localidades.

11. Respecto al reclutamiento natural de *Fucus serratus* se observó que es más constante tanto temporal como espacial en las localidades de las Rías que en las localidades de Lugo. Observándose, que el mayor reclutamiento de *F. serratus* en San Pedro (Lugo) en un mes de observación, tuvo lugar en otoño, mientras que en las localidades de las Rías esto sucedió en primavera.

12. Poblaciones de *F. serratus* en Lugo, posiblemente son más susceptibles a los cambios ambientales que las poblaciones de las Rías Bajas. En efecto, durante el año de observaciones, las poblaciones de la macroalga en la localidad de Peizas (Lugo) prácticamente no reclutaron. Lo que parece estar estrechamente relacionado con el deterioro de los frondes de las algas durante estos años, y consecuentemente el poco número de receptáculos en las mismas.

13. En este límite sur de distribución geográfica de *F. serratus*, reportamos también cambios en la estructura de las comunidades bentónicas, con alteraciones en las posiciones tróficas de algunos consumidores, ocupando estos en algunos casos, posiciones tróficas más bajas, específicamente en localidades donde *F. serratus* ha prácticamente desaparecido.

14. En localidades donde *Fucus serratus* ha desaparecido vemos además el aumento de especies oportunistas como *Ulva* y *Ceramium* spp. Mientras que en localidades donde la especie se mantiene, observamos el mayor desarrollo de algas de menor porte como las algas coralinas.

15. Sin duda, la desaparición de *Fucus serratus* en su límite sur de distribución en la Península Ibérica, implica el acortamiento de las cadenas tróficas y cambios en la complejidad de las redes alimentarias.

16. La permanencia de la formadora de dosel *Fucus serratus*, en su límite sur de distribución geográfica en el noroeste de España, dependerá de la capacidad de reacción de la especie a los eminentes cambios climáticos.

17. Es necesario puntulizar la importancia de estudios e investigaciones anteriores, en relación a esta tesis, que sirven de base para proseguir y confirmar en el tiempo, que los cambios climáticos han sido factores que inciden en el crecimiento, desarrollo y permanencia de las especies investigadas.

Advierto al lector omitir esta sección, si la longitud de la misma sobrepasa sus límites personales de aceptación. Reconozco la humildad y pequeñez de este trabajo, ante la grandeza de innumerables y visionarios trabajos científicos, más la finalización de esta etapa ha sido el esfuerzo de innumerables personas y el sortear numerosas dificultades; y es por eso, que ni las palabras escritas de la mejor manera logran expresar mi inmensa gratitud a todas ellas. Además, de antemano pido disculpas a todas aquellas personas cuyos nombres no estén reflejados en estas líneas, los cuáles sin intención alguna haya dejado fuera. Y con toda mi alma muchas gracias por en algún momento de este camino ayudarme a seguir.

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Carianda: una de las cosas más bellas de la vida es sentarse y conversar como si no hubiera distancia y el tiempo no transcurriera. Siempre has creído en mí y has estado a mi lado con una fraternidad indescriptible. Amiga te quiero muchísimo.

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