

Universidad de Cantabria



Departamento de Ciencias y Técnicas
del Agua y del Medio Ambiente

TESIS DOCTORAL

Dinámica ecológica de una especie de almeja nativa y una introducida: Implicaciones para la conservación y la gestión del marisqueo

Ph.D. THESIS

Ecological dynamics of a native and a nonindigenous clam species: Implications for conservation and shellfishery management



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Andrés García Gómez

Santander, 2013

UNIVERSIDAD DE CANTABRIA



**E.T.S. INGENIEROS DE CAMINOS, CANALES Y PUERTOS
DPTO. DE CIENCIAS Y TÉCNICAS DEL AGUA Y DEL MEDIO AMBIENTE**

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Autor: Gorka Bidegain Cancer

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Andrés García Gómez

Santander, Enero de 2013

A María Luisa

A María

A Ama y Aita

A Patri

La libertad es uno de los más preciosos dones que a los hombres dieron los cielos; con ella no pueden igualarse los tesoros que encierran la tierra y el mar: por la libertad, así como por la honra, se puede y debe aventurar la vida.

Miguel de Cervantes Saavedra. *Don Quijote de la Mancha*.

Y se dio cuenta de que nadie jamás está solo en el mar.

Ernest Hemingway. *El viejo y el mar*.

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RESUMEN

De acuerdo con la normativa de estudios de doctorado de la Universidad de Cantabria en relación a los requerimientos exigidos para aquellas tesis redactadas en un idioma diferente al español, aprobada por Junta de Gobierno de 12 de marzo de 1999 y actualizada a 17 de diciembre de 2012, a continuación se presenta un resumen “suficientemente extenso” del documento original redactado en inglés.

1. Introducción

1.1. Exposición de motivos

Los recursos de interés marisquero están sometidos a una gran presión debido a su creciente demanda y valor comercial. Una disminución de las capturas continuada unida a una gestión inadecuada del recurso puede dar lugar, en ocasiones, al colapso del mismo (Castilla y Defeo, 2001). Por ello, es esencial que el modelo de gestión se base en un conocimiento sólido del estado de explotación de las poblaciones y de su dinámica ecológica. Este conocimiento es todavía más necesario cuando el modelo trata de gestionar el marisqueo de una especie introducida, como es la almeja japonesa (*Ruditapes philippinarum*) en el hábitat donde se distribuye una especie del mismo género, como es la almeja fina (*Ruditapes decussatus*), teniendo en cuenta el riesgo potencial de expansión de la primera y desplazamiento de la última a áreas muy restringidas, como ha ocurrido en otros estuarios de Europa (Auby, 1993; Marín et al., 2003).

Por ello, se ha prestado una considerable atención en evaluar las poblaciones de estas dos especies altamente explotadas y cultivadas en diferentes partes del mundo (FAO, 2012). Estas evaluaciones incluyen estimaciones de la densidad y de la biomasa con el fin de conocer el funcionamiento del modelo de gestión aplicado (Hancock y Urquhart, 1965; Defeo, 1996; Castilla et al., 1998). Las estimaciones se realizan en relación con el área de

gestión o de pesca y teniendo en cuenta la variabilidad asociada a la misma. Con el fin de evaluar el estado de las poblaciones de almejas y estudiar los patrones de distribución de las mismas, se han desarrollado diferentes metodologías de muestreo, que difieren en la sistemática del mismo, el tamaño de las muestras o de la malla de cribado para la obtención de juveniles (Byers, 2005; Lee, 1996; Borja y Bald, 2000; Bald y Borja, 2001:2005; Caill-Milly et al., 2006; Morsan, 2007), y de estimación de los stocks (Palacios et al 1994:2000; Rueda y Urban, 1998; Orensanz et al., 2003). Sin embargo, la falta de una metodología de muestreo estandarizado junto con el alto consumo de tiempo y de recursos ligados a este tipo de métodos, sobre todo cuando son aplicados a estuarios de gran extensión, hacen que sea necesaria la exploración de un procedimiento de evaluación que combine un diseño apropiado de muestreo con la experiencia de los mariscadores.

En particular, en el caso de la Bahía de Santander, como en los demás estuarios de la región de Cantabria, a pesar de que el marisqueo de almejas tiene una larga tradición, la evaluación del estado de sus poblaciones nunca se había llevado a cabo anteriormente. Por lo tanto, era necesario realizar un primer estudio para caracterizar ambas poblaciones y analizar su estado de explotación, para poder, así, evaluar el funcionamiento de las medidas de gestión aplicadas y, a la vez, servir de apoyo a un nuevo modelo de gestión basado en el conocimiento científico.

Sin embargo, la evaluación del estado de las poblaciones es ineficaz sin un conocimiento de su dinámica ecológica y su aplicación en la toma de decisiones mediante la implementación de un modelo de gestión racional del recurso. Así, aunque el conocimiento sobre aspectos de la dinámica poblacional de la almeja fina y la almeja japonesa (Pérez-Camacho, 1979; Olafsson et al., 1994; Laruelle, 1999; Urrutia et al., 1999; Chícharo y Chícharo, 2000; Solidoro et al., 2000; Pérez-Camacho et al., 2002:2003; Melia et al., 2004; Phillips, 2006; Flye-Sainte-Marie et al., 2007a,b; Humphreys et al., 2007; Dang, 2009) o de la interacción de las mismas con el hábitat que colonizan (Arnold et al., 2000; Chícharo y Chícharo, 2001a; Peña et al., 2005; Vincenzi et al., 2006) ha aumentado de forma considerable en la última década, la búsqueda de respuestas a preguntas importantes

como las siguientes sigue siendo una tarea difícil: ¿Cuál es el potencial de expansión de la almeja japonesa y qué factores determinan este potencial? ¿Puede la expansión de la almeja japonesa introducida en Europa causar una interacción competitiva que desplace a la almeja fina?

La expansión y el predominio de la almeja japonesa registrado en otros estuarios europeos hacen que sea también necesario abordar el estudio de su distribución potencial y de los factores ambientales que la regulan. Además, a pesar de que la almeja japonesa está considerada como una amenaza para la almeja fina, su impacto directo en cuanto a la interacción competitiva, no se ha estudiado todavía. Por otra parte, los aspectos que determinan la conectividad y la persistencia de las poblaciones, tales como la dispersión de larvas o el reclutamiento, son escasamente conocidos y los modelos biofísicos desarrollados para ello (Borsa and Millet, 1990; Herbert et al., 2012) necesitan ser mejorados, en particular, en cuanto a la predicción del reclutamiento (Metaxas y Saunders, 2009). Estos aspectos deben ser investigados desde el punto de vista de la sostenibilidad del marisqueo de ambas especies y, en particular, desde la óptica de la conservación de la almeja fina.

Por otra parte, es necesario señalar, que la gestión del marisqueo en estuarios donde coexisten estas dos especies se basa, generalmente, en medidas comunes para ambas especies, independientemente de las diferencias biológicas existentes o del fomento específico del cultivo de una única. Por ejemplo, la talla mínima de captura, una de las medidas más ampliamente utilizadas en las estrategias de gestión pesquera (Berthou et al., 2005, Gilbert et al., 2006), ha sido la misma (40 mm) para ambas especies en la Unión Europea hasta 2007. Aunque, posteriormente, se redujo a 35 mm para la almeja japonesa (Dang, 2010), esta situación sostenida durante mucho tiempo podría haber tenido consecuencias negativas y ser una de las razones de la sobreexplotación y el desplazamiento de la almeja fina (Juanes et al., 2012). En muchos estuarios, como los de toda la costa norte de la Península Ibérica y de otros países de Europa, las normativas regionales aplicables todavía se basan en la misma talla de captura para ambas

especies (por ejemplo, Orden DES/25/2011; Cannas, 2010). Por lo tanto, todavía hay una necesidad de investigar la talla idónea de captura de estas especies, con el fin de obtener, a largo plazo, un rendimiento máximo del marisqueo y apoyar las estrategias de conservación de la almeja fina.

1.2. Objetivos

Aspectos relevantes de la dinámica ecológica tales como la expansión de almeja japonesa, la interacción competitiva, la dispersión larvaria y reclutamiento o la estimación de la talla mínima de captura requerían ser estudiados. A la vez, se hacía necesario explorar una metodología idónea para la evaluación continua de estas poblaciones en estuarios de gran extensión.

En este contexto, el objetivo de esta tesis es avanzar en el conocimiento de la dinámica ecológica de la almeja fina nativa de Europa, *Ruditapes decussatus*, y la almeja japonesa introducida, *Ruditapes philippinarum*, con el fin de obtener información y desarrollar herramientas aplicables en las estrategias de conservación y de gestión del marisqueo. Dicho objetivo general, se puede desglosar a través de los siguientes objetivos específicos:

- 1) Analizar la distribución espacial, la estructura de población y estimar los stocks de la almeja fina *R. decussatus* y la almeja japonesa *R. philippinarum* utilizando un método de muestreo basado en técnicas de marisqueo artesanales, con el fin de evaluar el funcionamiento del modelo de gestión en la Bahía de Santander.
- 2) Estimar el hábitat potencial de ambas especies y explorar su papel en la regulación de la expansión de la población de la almeja japonesa.
- 3) Examinar el impacto de la interacción competitiva entre ambas especies y de la predación en un escenario de expansión de la almeja japonesa.

4) Desarrollar un modelo de dispersión larvaria integrando un submodelo de reclutamiento basado en el hábitat potencial, con el fin de determinar las zonas de puesta y reclutamiento más importantes y evaluar la conexión entre las mismas.

5) Evaluar la idoneidad de un modelo no paramétrico para el estudio de la relación talla-peso de ambas especies y la estimación de la talla mínima de captura.

La estructura de la tesis se organiza de la siguiente manera: en el capítulo I se exponen los motivos por los cuales se ha realizado el presente trabajo de investigación, se describen las especies estudiadas y el estado de conocimiento sobre los diferentes temas tratados en la tesis. Al final de este primer capítulo, se presentan los objetivos específicos diseñados para responder a las cuestiones planteadas. En el Capítulo II, se describen las características del ámbito de estudio. En los siguientes cinco capítulos (III, IV, V, VI, VII) se presentan los estudios desarrollados para la consecución de los objetivos específicos de la tesis. Cada uno de estos cinco capítulos está compuesto por un resumen, una breve introducción que incluye los objetivos específicos de cada estudio y los apartados de metodología, resultados y discusión, constituyendo una versión editada de los apartados correspondientes de los artículos ya publicados o en fase de revisión científica en revistas indexadas dentro del SCI. Por último, las conclusiones generales, las propuestas de gestión del marisqueo y las futuras líneas de investigación se describen en el capítulo VIII.

2. Ámbito de estudio

La Bahía de Santander, área natural de recreación y de importancia económica situada en la costa norte de España (Golfo de Vizcaya), y sus aguas adyacentes son el ámbito de estudio principal de la tesis. En ella se han llevado a cabo la toma de muestras, la experimentación y se han desarrollado y/o aplicado los modelos predictivos con el fin de conseguir los objetivos planteados en la tesis. La Bahía se sitúa entre los 43°28' y 43°24'N

de latitud y 3°49'9" y 3°48'8"W de longitud (Figura 1). El área del estuario es de 22.7 km² y los páramos intermareales representan un 67 % del total de la Bahía, concentrándose principalmente en la margen derecha de la misma. Una barra de arena unida a la desembocadura del río Miera bordea la bahía por el noreste mientras que en la margen oeste se sitúa la zona portuaria (Figuras 2 y 5). En la zona intermareal, principalmente, se distribuyen la almeja japonesa, *Ruditapes philippinarum*, introducida en la bahía a finales de los años 80 y la almeja fina, *Ruditapes decussatus*. El marisqueo de estas poblaciones se lleva a cabo mediante técnicas tradicionales. El sustrato varía desde arenoso, en las zonas más oceánicas del norte como la boca del Cubas o la playa de Pedreña, a fangoso en las zonas más internas o estuáricas de Astilero, Boo, Solía-Tijero o Cubas. Las zonas submareales no son muy profundas, con máximos de 10–12 m a lo largo del canal de navegación (Figura 2). Las condiciones hidrodinámicas están controladas mediante un régimen mareal semidiurno y un rango mareal medio de 3 m e interactúan con inputs variables de descargas provenientes, principalmente, del río Miera a través de la zona de Cubas y, en menor medida, provenientes de Boo, Tijero y Solía (Puentes et al., 2002).

Las Marismas de Santoña también son parte del ámbito de estudio de esta tesis en el Capítulo VII. Este estuario está situado al este de la Bahía de Santander, y tiene una extensión también importante de 18.7 km² (Figura 1 y 3). El área intermareal comprende el 57 % del total de las marismas y en sus páramos se lleva a cabo, igualmente, el marisqueo de las especies de estudio.

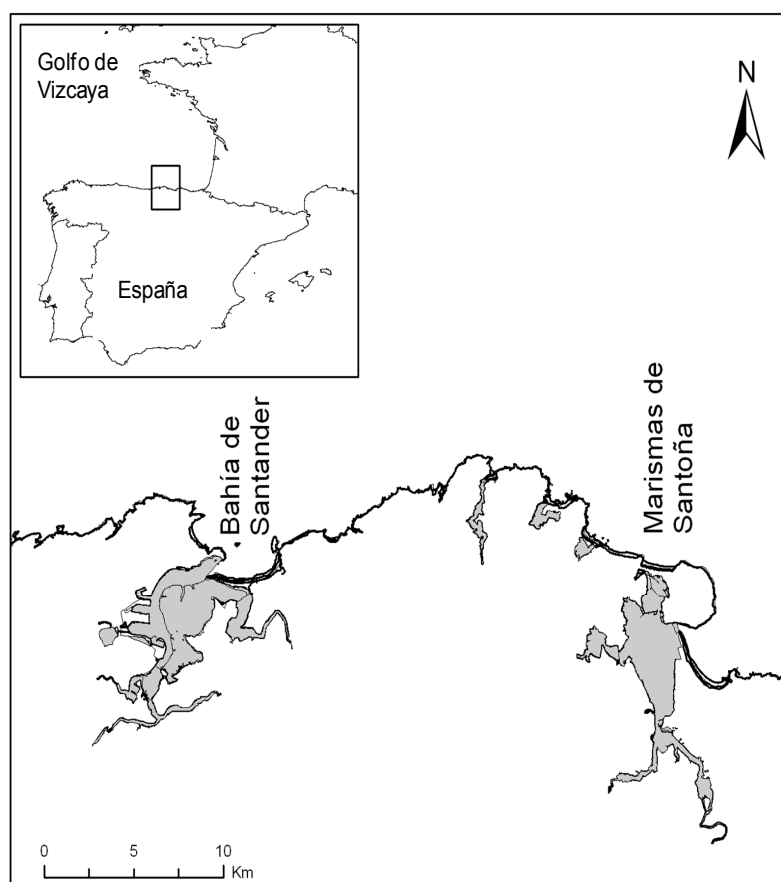


Figura 1 – Área de estudio: Bahía de Santander (principal ámbito de estudio) y Marismas de Santoña (área de estudio junto con la Bahía de Santander en el Capítulo VII), situadas en la costa norte de España (Golfo de Vizcaya).

El sustrato también varía desde arenoso en la zona de la boca del estuario y Montehano, a fangoso en la zona de Boo, y las rías de Rada y Limpias. Las zonas submareales son muy poco profundas, con máximos de 12 m, muy localizados en la boca del estuario (Figura 3). Las condiciones hidrodinámicas interactúan con las descargas variables provenientes del río Asón. Se puede ampliar información respecto al ámbito de estudio en Puente et al. (2002), Irabien et al. (2008), Galván et al. (2010), Juanes et al. (2012) o López et al. (2013).

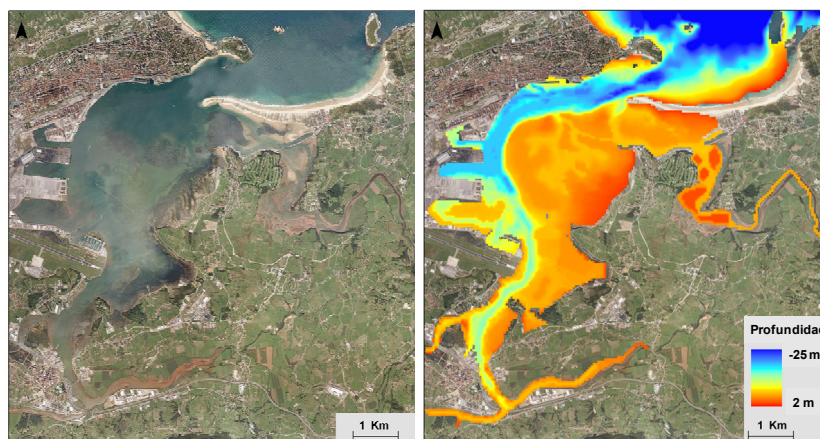


Figura 2 – Ortofoto (izquierda) y batimetría (m) (derecha) de la Bahía de Santander.

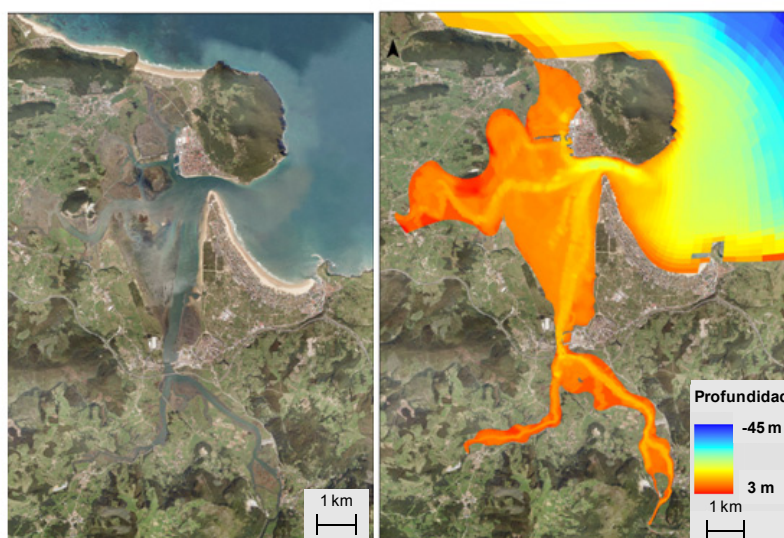


Figura 3 – Ortofoto (izquierda) y batimetría (m) (derecha) de las Marismas de Santoña.

En ambos estuarios el marisqueo está fuertemente arraigado a las comunidades vecinas y se realiza de manera artesanal. En el caso de las almejas la pesca se realiza mediante rastrillado o con cuchillo en la zona intermareal (Figura 4). Las capturas de almeja fina y almeja japonesa constituyen el 80 % del total de los recursos marisqueros. En la Bahía de Santander, aunque las capturas son variables, en los últimos años se han declarado 10 ton anuales de almeja fina y 30 ton de almeja japonesa, mientras que en las marismas de

Santoña se han declarado 10 ton anuales de la primera y 20 ton de la segunda (Datos de la Consejería de Ganadería, Pesca y Desarrollo Rural del Gobierno de Cantabria). En el caso de la Bahía de Santander, existe una zona intermareal destinada al cultivo de almeja japonesa, mediante la fórmula de concesión, de la cual, actualmente, se explota eficazmente alrededor de 1 Ha.

La regulación del marisqueo de la almeja en la región de Cantabria se basa en ordenanzas anuales que limitan la talla mínima de captura y las zonas de captura y establecen vedas estacionales (ejemplo, GAN/35/2005). Hasta la fecha, estas medidas de gestión se aplican sin tener en cuenta las diferencias biológicas que existen entre la almeja fina y la almeja japonesa, siendo ésta última más resistente a condiciones desfavorables y de un crecimiento más rápido (Spencer et al., 1991; Solidoro et al., 2000; Usero et al., 1997).



Figura 4 – La técnica artesanal de marisqueo de almejas basada en el rastrillado del sedimento (izquierda) y en la recolección a mano de las almejas que aparecen en la superficie (círculos rojos) (derecha).

La única información científica disponible en relación con las especies de estudio en la Bahía de Santander es sobre sus relaciones biométricas (Arnal and Fernández-Pato, 1977:1978) y los datos de captura. Por lo tanto, la falta de datos, tanto del estado de explotación de las poblaciones como de aspectos de su dinámica ecológica, no ha permitido desarrollar un modelo de gestión del marisqueo basado en el conocimiento científico. En este contexto, en los años 2005 y 2010 el Gobierno de Cantabria mediante la

Consejería de Ganadería, Pesca y Desarrollo Rural y en colaboración con el IH Cantabria, realizó sendas evaluaciones del estado de las poblaciones de los recursos marisqueros del litoral de Cantabria y diferentes estudios científicos destinados a ampliar el conocimiento de la dinámica ecológica de estas especies. Parte de los estudios realizados en dichos estudios constituyen el eje principal de la presente tesis.

3. Capítulo III: Caracterización de las poblaciones de la almeja fina nativa *Ruditapes decussatus* y la almeja introducida *Ruditapes philippinarum* en la Bahía de Santander.

Este capítulo ha dado lugar a la publicación de un artículo de investigación en la revista indexada en el SCI Ocean and Coastal Management, vol. 69, pp. 316-326, por Juanes, J.A., Bidegain, G., Echavarri-Erasun, B., Puente, A., García, A., García, A., Bárcena, J.F., Álvarez, C., García-Castillo, G., en 2012 con el título "Differential distribution pattern of native Ruditapes decussatus and introduced Ruditapes philippinarum clam populations in the Bay of Santander (Gulf of Biscay). Considerations for fisheries management".

Dentro de este trabajo, se llevó a cabo la primera caracterización de las poblaciones de ambas especies en la Bahía de Santander en el año 2005. Para ello, se realizó un estudio de campo en las distintas zonas de marisqueo, donde se tomaron muestras a lo largo de transectos siguiendo técnicas tradicionales basadas en el rastrillado a mano (Figura 5). Se analizaron las distribuciones de tallas, densidades y los stocks de las poblaciones. Además, se aplicó un modelo hidrodinámico con el fin de comprender el transporte de larvas y los patrones de reclutamiento asociados a las corrientes de marea y el flujo de agua.

Los resultados ayudaron a evaluar el funcionamiento de las medidas de gestión establecidas en la Bahía de Santander, mostrando la existencia de una actividad marisquera ilegal sobre individuos por debajo de la talla mínima legal y en zonas vedadas.

Se observó un patrón de distribución de las especies con zonas donde ambas especies coexisten y zonas donde una de ellas predomina claramente sobre la otra, lo cual se refleja también en los stocks estimados en las diferentes zonas de pesca evaluadas (Figura 6).

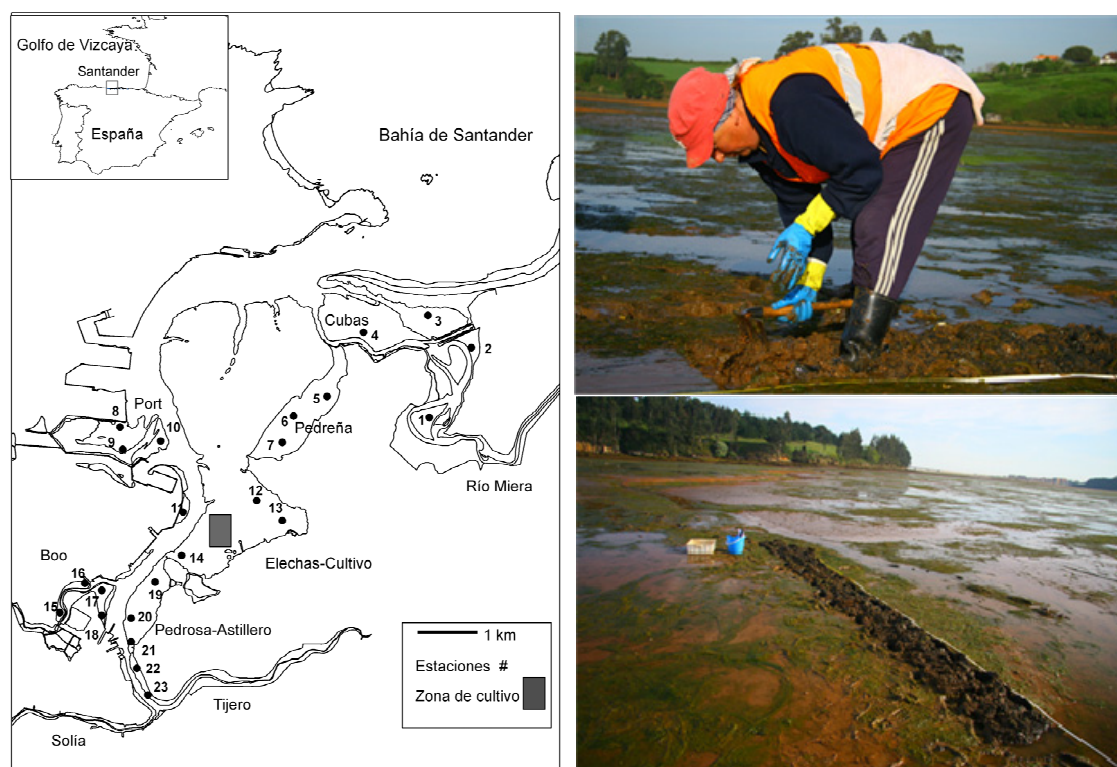


Figura 5 – Las estaciones de muestreo localizadas en las zonas de marisqueo de Cubas (1,2,3,4), Puerto (8, 9, 10), Pedreña (5, 6, 7), Elechas o zona de Cultivo (12, 13, 14), Boo (15, 16, 17, 18) y Pedrosa-Astillero (19, 20, 21, 22, 23) (izquierda) y una mariscadora extrayendo almejas mediante la metodología aplicada de muestreo, basada en la técnica artesanal de rastrillado en un transecto de 10 m x 1m en la zona de Cubas (Bahía de Santander) (derecha).

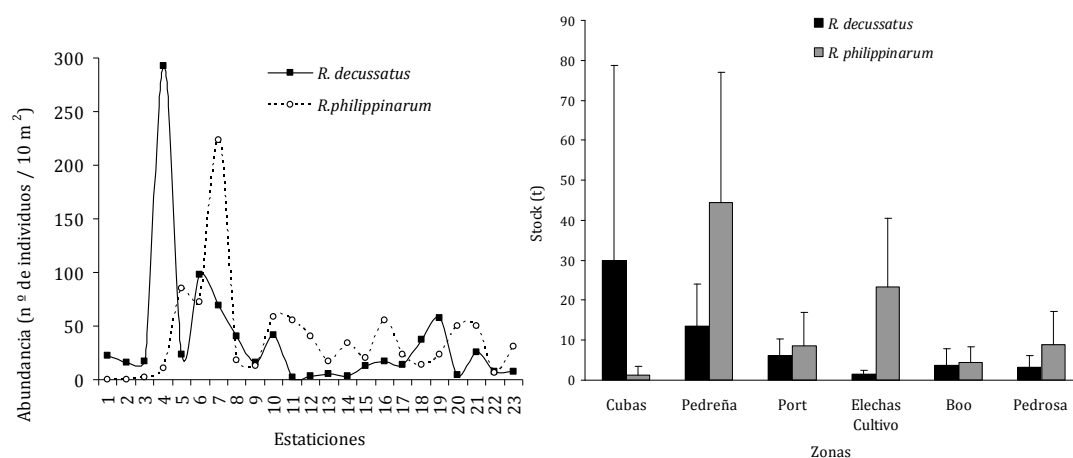


Figure 6 – Abundancia (izquierda) y stocks (derecha) de *R. decussatus* y *R. philippinarum* en cada una de las estaciones y en las zonas de pesca respectivamente.

Además, se observó que existía un reclutamiento limitado de *R. philippinarum* en las partes interiores de la ría de Cubas, en la zona noreste, y de *R. decussatus* en las zonas más internas del sur de la Bahía, asociado al flujo principal que llega al sur (Figura 7).

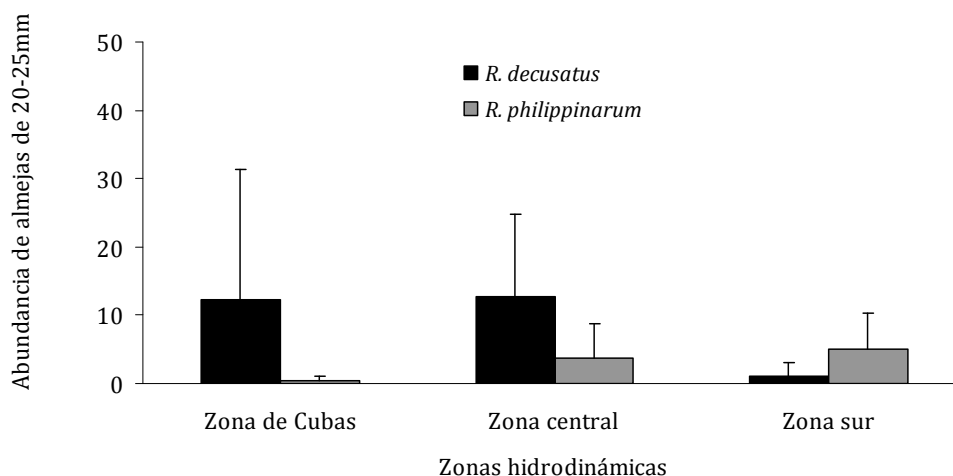


Figura 7 – Abundancia de individuos de talla 20-25 mm (talla de reclutamiento al arte) de *Ruditapes decussatus* and *Ruditapes philippinarum* en las zonas hidrodinámicas definidas: Zona de Cubas, asociada al flujo del canal de la Ría, Zona central que incluye las zonas de Pedreña y Puerto de Raos, asociada a un eddy, y la Zona sur asociada al flujo del canal de navegación principal que llega al sur (contiene la zonas de Cultivo-Elechas, Boo y Pedrosa-Astillero).

En resumen, el método de muestreo desarrollado proporcionó datos idóneos de abundancia y densidad de almejas adultas y de talla comercial, mostrando su viabilidad para la estimación de stocks comerciales y para conocer el status de las poblaciones de almejas en estuarios extensos. A la vez, el éxito de la vinculación de los mariscadores en esta metodología de muestreo puede ser un punto de partida para una futura gestión del recurso en base a la colaboración entre la administración y el colectivo de mariscadores.

4. Capítulo IV: Estimación de la expansión potencial de *R. philippinarum* mediante la aproximación de “nicho ecológico”.

Este capítulo ha dado lugar al artículo de investigación “Ecological niche modelling approach to predict the potential expansion of a nonindigenous clam” de Bidegain, G., Bárcena, J.F., García, A., Juanes, J.A., enviado a revisión a la revista Ecological Modelling indexada en el SCI.

Los patrones de distribución descritos de manera preliminar en la caracterización de las poblaciones realizada en el capítulo III requerían un estudio de mayor alcance dirigido a conocer el potencial de expansión de la almeja japonesa, teniendo en cuenta que en varios estuarios de Europa esta especie se ha convertido en dominante relegando a la almeja fina a ocupar zonas muy localizadas. Para ello, se estudió el hábitat potencial de ambas especies en la Bahía de Santander y se determinaron las áreas potenciales donde se registraba el predominio de una especie sobre la otra.

El modelo utilizado para ello fue el Análisis Factorial del Nicho Ecológico (ENFA) que se basa en datos de presencia. El área con un hábitat potencial alto o donde se observó un predominio de una especie sobre la otra especie fue considerablemente más extensa para la almeja fina que para la japonesa (Figuras 8 y 9). Este resultado, muestra el importante

papel que puede jugar el hábitat en la regulación de la expansión de la especie introducida en la Bahía.

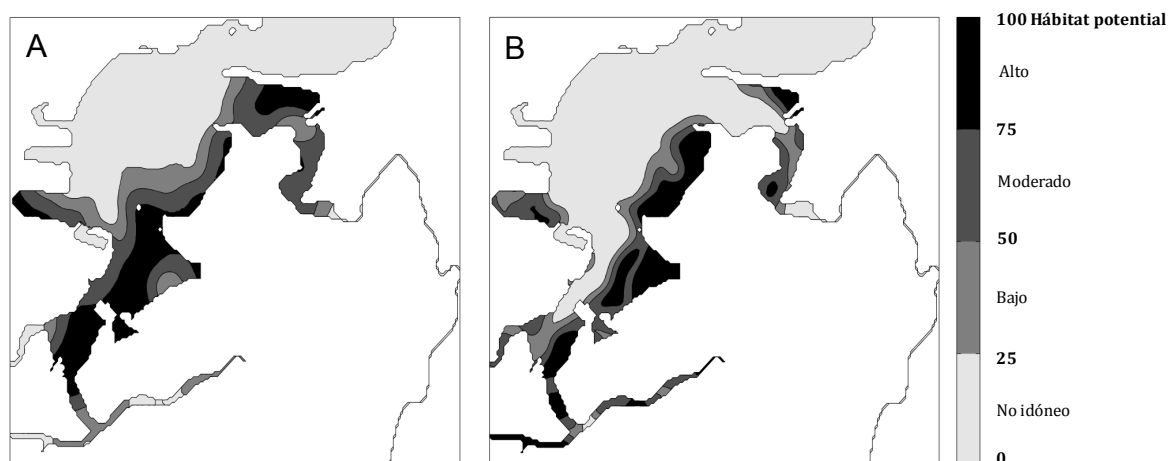


Figura 8 – Hábitat potencial de *R. decussatus* (A) y *R. philippinarum* (B) en la Bahía de Santander, clasificado en 4 clases del índice de hábitat potencial HSI (habitat suitability index): No idóneo (HSI <25), Bajo ($25 \leq \text{HSI} < 50$), moderado ($50 \leq \text{HSI} < 75$) y alto (HSI >75).

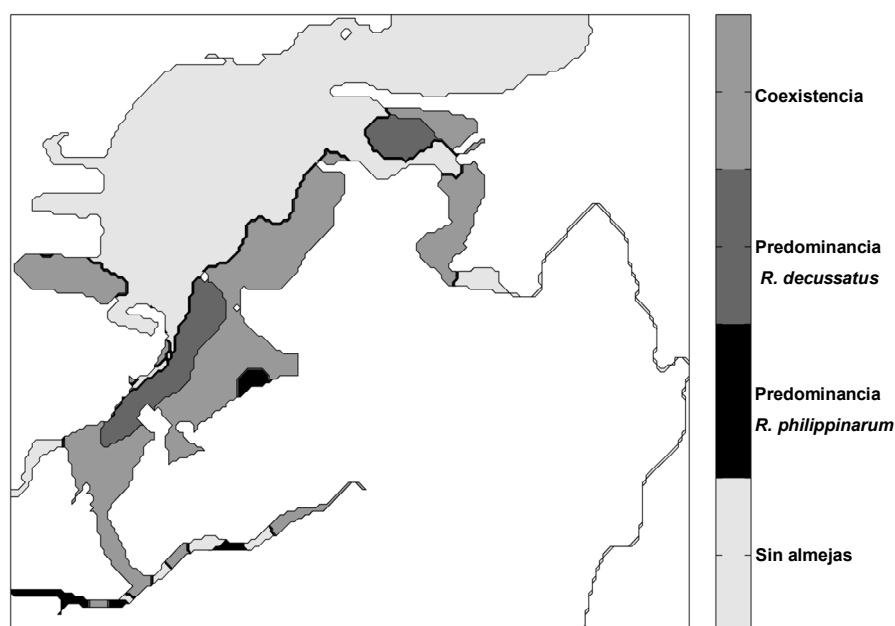


Figura 9 – Zonas potenciales de coexistencia de las dos especies y de predominancia de una especie sobre la otra.

Las variables ambientales que más determinaron la presencia de ambas especies fueron la batimetría, la velocidad de la corriente y la salinidad, aunque su importancia y la asociación (positiva o negativa) con el hábitat potencial fueron diferentes para cada una de ellas (ver Tabla 4.3, Chapter IV). El modelo ENFA parece ser una técnica idónea para predecir la distribución de estas especies y explorar el potencial de expansión de la especie introducida. Este resultado tiene importantes implicaciones tanto para la conservación como para la gestión del marisqueo o de la acuicultura en cuanto a la zonación de áreas de restauración o de cultivo.

5. Capítulo V: Estimación de la dispersión larvaria y el reclutamiento mediante un modelo lagrangiano basado en el hábitat potencial

Este capítulo ha dado lugar al artículo de investigación “Predicting clam larval dispersal and recruitment using habitat suitability-based particle tracking model” de Bidegain, G., Bárcena, J.F., García, A., Juanes, J.A., enviado a revisión a la revista Journal of Marine Systems indexada en el SCI.

En este trabajo se integraron los resultados de hábitat potencial obtenidos en el capítulo anterior en un modelo de dispersión de larvas, con el fin de obtener predicciones del asentamiento y reclutamiento de ambas especies. Para ello, se desarrolló un modelo lagrangiano, denominado LARVAHS, que contempla el comportamiento de las larvas y la hidrodinámica del ámbito de estudio. Se llevó a cabo un estudio específico en la Bahía de Santander, intentando completar de este modo, los resultados y consideraciones preliminares obtenidos sobre el reclutamiento tras la aplicación del modelo

hidrodinámico en el estudio de caracterización de las poblaciones. El modelo fue alimentado con datos registrados de viento, caudales del río Miera y mareas observadas para obtener predicciones durante la época de puesta de las almejas. La liberación teórica de huevos se realizó desde siete zonas de puesta establecidas y se analizó la dispersión de las larvas sometidas a advección, difusión turbulenta vertical y comportamiento larvario.

El modelo incorporó un submodelo de reclutamiento para simular el comportamiento de asentamiento y la mortalidad post asentamiento, basado en el hábitat potencial obtenido previamente en el capítulo anterior mediante el análisis factorial del nicho ecológico (ENFA). Se simularon seis escenarios desde cada zona de puesta, mediante la combinación de tres periodos (primavera, verano y otoño) y la fase de marea en el momento de la puesta (marea viva y muerta).

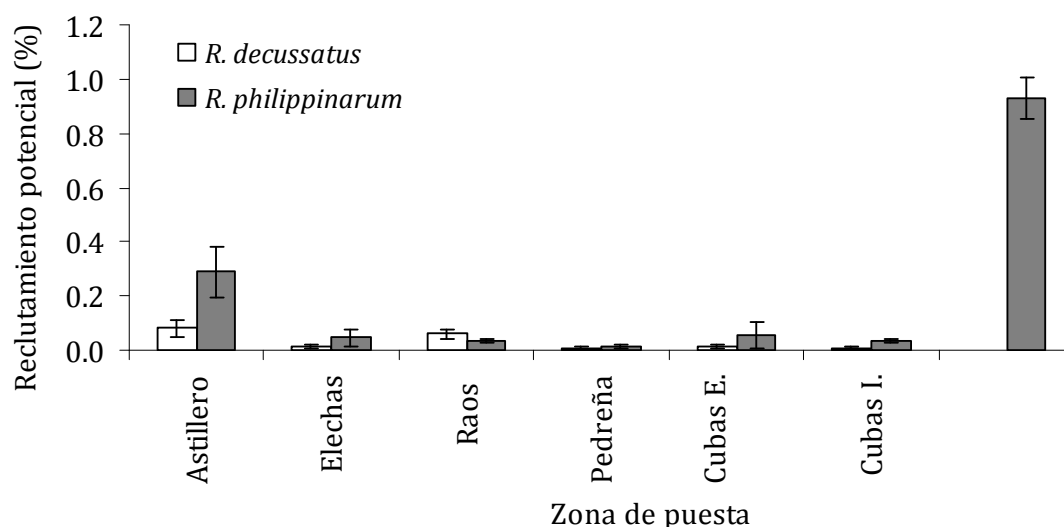


Figura 10 – Porcentaje de larvas de *R. decussatus* and *R. philippinarum* potencialmente reclutadas (eje y) provenientes de cada una de las zonas de puesta (eje x). Las barras de error representan el error estándar de la media de los diferentes escenarios estacionales (primavera, verano, otoño) y de marea (viva, muerta).

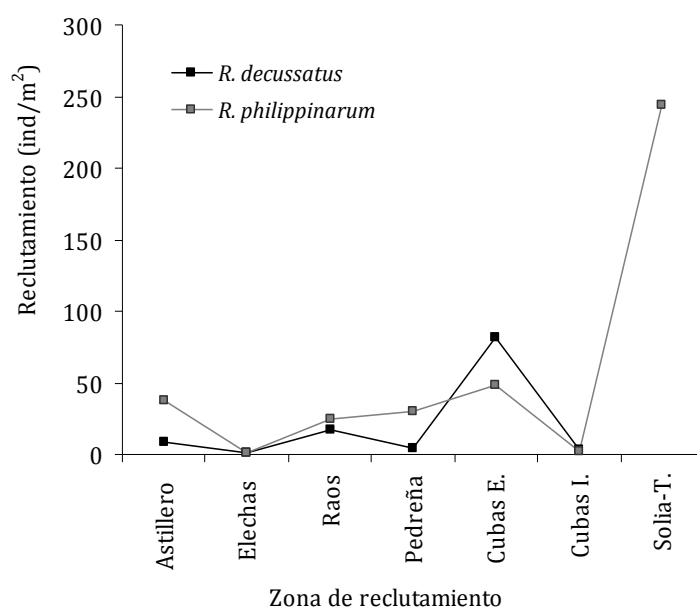


Figura 11 – Densidad de reclutamiento potencial en las zonas con un hábitat potencial alto ($HSI > 75$), calculada como la suma de larvas reclutadas en las diferentes estaciones y escenarios de marea provenientes de las diferentes zonas de puesta dividida por el área dada con ese hábitat potencial en cada zona de reclutamiento.

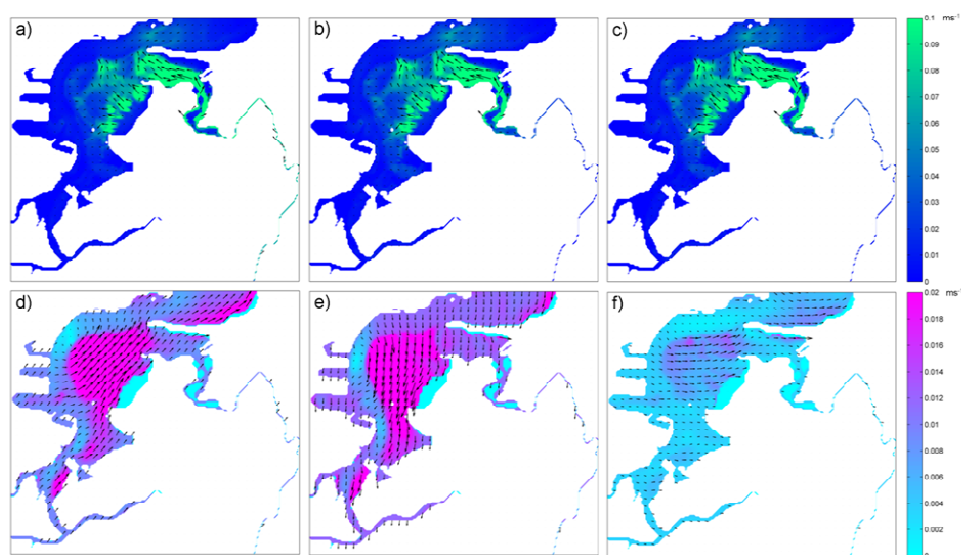


Figura 12 – Corrientes de marea (ms^{-1}) (a, b, c) y de viento (d, e, f) para los escenarios de primavera, verano y otoño.

El modelo LARVAHS puso de relieve el papel del comportamiento específico de la larva (duración del estado planctónico), estación (los vientos predominantes) (Figura 12) y la ubicación de la zona de desove en el éxito del reclutamiento, junto con el efecto de la marea (ver Tabla 5.3, Chapter VII). Además, el modelo ha demostrado ser una herramienta valiosa para determinar las principales zonas de puesta (Figura 10) y reclutamiento (Figura 11) y explorar la conectividad entre las mismas (Figura 13).

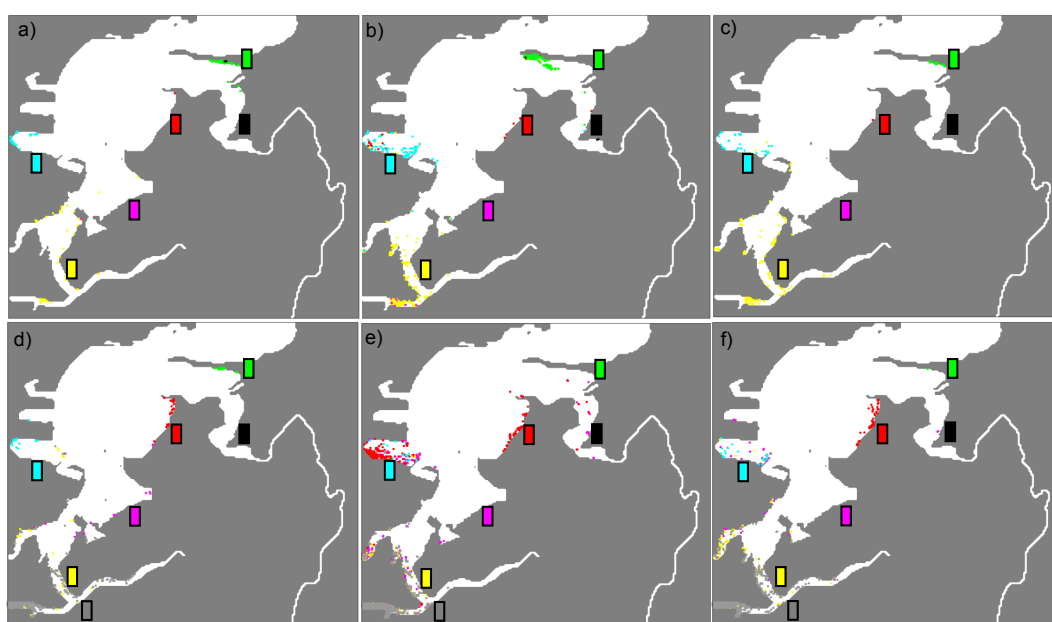


Figura 13 – Reclutamiento estimado para *R. decussatus* en los escenarios de primavera (a), verano (b) y otoño (c) y para *R. philippinarum* en los mismos escenarios (d, e, f) respectivamente. Los rectángulos representan las zonas de puesta (ver detalle en Figura 5.2) y los puntos de colores las larvas reclutadas $\times 10^5$ provenientes de cada zona dependiendo del color: Astillero (amarillo), Elechas (rosa), Raos o Puerto (azul), Pedreña (rojo), Cubas Exterior (verde), and Cubas Interior (negro).

En consecuencia, el modelo parece tener un potencial importante como herramienta para la toma de decisiones en la gestión del marisqueo con respecto a la expansión de la almeja japonesa, a la hora de determinar la localización de las zonas idóneas de cultivo o de restauración con el fin de conseguir un mayor rendimiento de la producción y la sostenibilidad de la almeja nativa.

6. Capítulo VI: Estudio de la influencia de la expansión de la almeja introducida *R. philippinarum* sobre el desplazamiento de la especie nativa *R. decussatus*

Este capítulo ha dado lugar a la publicación de un artículo de investigación en la revista indexada en el SCI Journal of Experimental Marine Biology and Ecology, vol. 4451, pp. 44-52 por Bidegain, G. y Juanes, J.A. en 2013 con el título "Does expansion of the introduced Manila clam Ruditapes philippinarum cause competitive displacement of the European native clam Ruditapes decussatus?"

Además del hábitat potencial y la dispersión larvaria, la competición interespecífica o la predación son factores que podrían tener también una considerable importancia en la regulación de ambas poblaciones y, por lo tanto, en la expansión o la predominancia de almeja japonesa sobre la almeja fina en diferentes estuarios de Europa. En este estudio, se analizó la interacción competitiva entre la almeja fina y la almeja japonesa, con el fin de determinar si esta interacción podría afectar directamente a su crecimiento y mortalidad. A la vez, se examinó el efecto de la exposición a la predación. Para ello, se llevó a cabo un experimento en un páramo intermareal (Elechas) situado en la margen este de la Bahía de Santander (ver sección 2. Ámbito de Estudio). Mediante un diseño experimental de bloques al azar, se manipuló la densidad relativa de estas especies en recintos de 0.25 m² con mallas laterales de 8 mm y cubiertos de ella en los casos de tratamientos no expuestos a la predación (Figura 14).

Los resultados obtenidos muestran que el aumento de la densidad de almeja japonesa, simulando un escenario de expansión, no afecta al crecimiento o la mortalidad de la almeja nativa y que la depredación juega un papel esencial en la regulación de ambas poblaciones de especies, limitando así, considerablemente el aumento de su densidad, particularmente en el caso de la almeja japonesa.

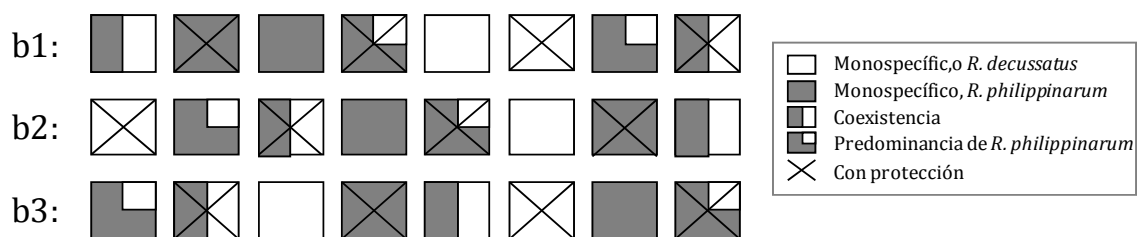


Figura 14 – Esquema del diseño experimental de bloques (b1, b2, b3) al azar.

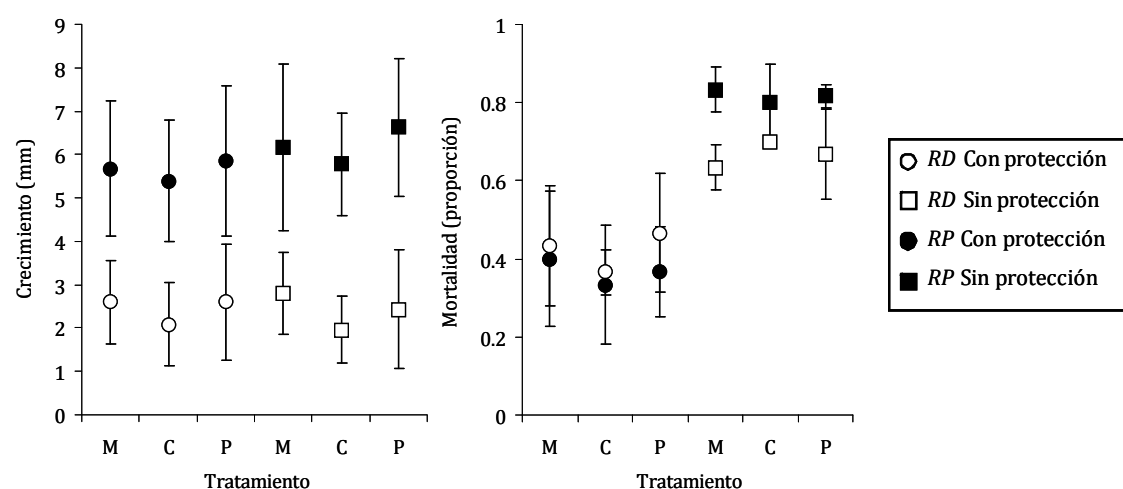


Figura 15 – Crecimiento (izda.) y mortalidad (dcha.) de *Ruditapes decussatus* (RD) y *Ruditapes philippinarum* (RP) (media \pm SD) durante el experimento (1 año) para 3 tratamientos de densidad relativa en recintos con protección y sin protección de 0.25 m²: Monoespecífico (M), (2) coexistencia (C), and (3) predominancia de *R. philippinarum* (P).

En general, los resultados indican que la predación tiene un efecto mayor en comparación con la interacción competitiva para ambas poblaciones (Figura 15). Además, al extrapolar las densidades experimentales a otros estuarios de Europa, parece que la interacción competitiva entre estas especies podría estar jugando un papel en la disminución drástica de la almeja nativa, pero solamente en los casos en que las densidades de almeja japonesa son extremadamente altas.

7. Capítulo VII: Estimación de la talla mínima de captura de *R. decussatus* y *R. philippinarum* mediante un modelo no paramétrico

Este capítulo ha dado lugar a la publicación de un artículo de investigación en la revista indexada en el SCI Ocean and Coastal Management, vol. 71, pp. 52-63 por Bidegain, G., Sestelo, M., Roca-Pardiñas, J., Juanes, J.A. en 2013 con el título "Estimating a new suitable catch size for two clam species: Implications for shellfishery management".

El marisqueo juega un papel relevante en la regulación de estas poblaciones de almeja por lo que la aplicación de unas medidas de gestión inadecuadas podría desembocar en una sobreexplotación de las mismas. En este sentido, en el caso de las especies de estudio, una de las medidas más comunes en la gestión de los recursos marinos vivos, la talla mínima de captura, requería ser investigada teniendo en cuenta que, a pesar de sus diferencias biológicas, en muchos estuarios de Europa donde coexisten, sigue siendo la misma para ambas especies. Por ello, en el último estudio de esta tesis se analizaron los patrones de ganancia de peso por unidad de talla y se estimaron las tallas mínimas de captura para cada especie. Para ello, se tomaron muestras de ambas especies en diferentes zonas de la Bahía de Santander y Marismas de Santoña. La relación talla-peso se calculó utilizando dos modelos, el modelo alométrico clásico y un modelo de regresión no paramétrica basado en suavizadores lineales de Kernel (Modelo KNP). El modelo considerado es el siguiente:

$$PS = m_0 + \begin{cases} m_1(LM) + \varepsilon_1 & \text{if } F = 1 \\ m_2(LM) + \varepsilon_2 & \text{if } F = 2 \end{cases}$$

donde ε_1 y ε_2 son los errores para el nivel del factor, m_0 representa el efecto global de la longitud (LM , longitud máxima) en la variable respuesta PS (peso seco); y m_1 y m_2 los efectos específicos de la longitud asociados con los niveles 1 (*R. decussatus*) y 2 (*R. philippinarum*) del factor F (especie).

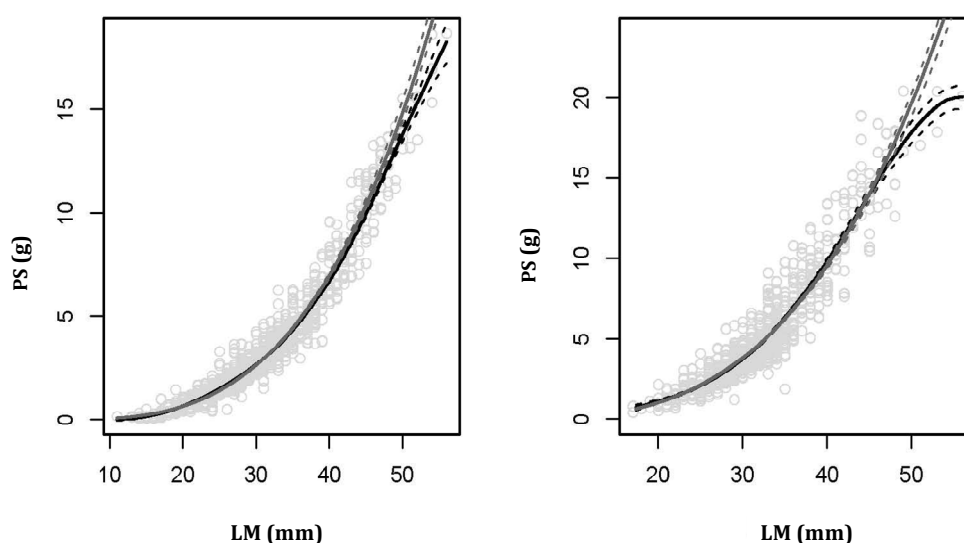


Figura 16 - Relaciones talla-peso (LM, longitud máxima; PS, peso seco) para *Ruditapes decussatus* (izda.) y *Ruditapes philippinarum* (dcha.). Las líneas sólidas grises representan el modelo alométrico y las sólidas negras al modelo KNP. Los intervalos de confianza basados en bootstraps (del 95%) se presentan con líneas discontinuas.

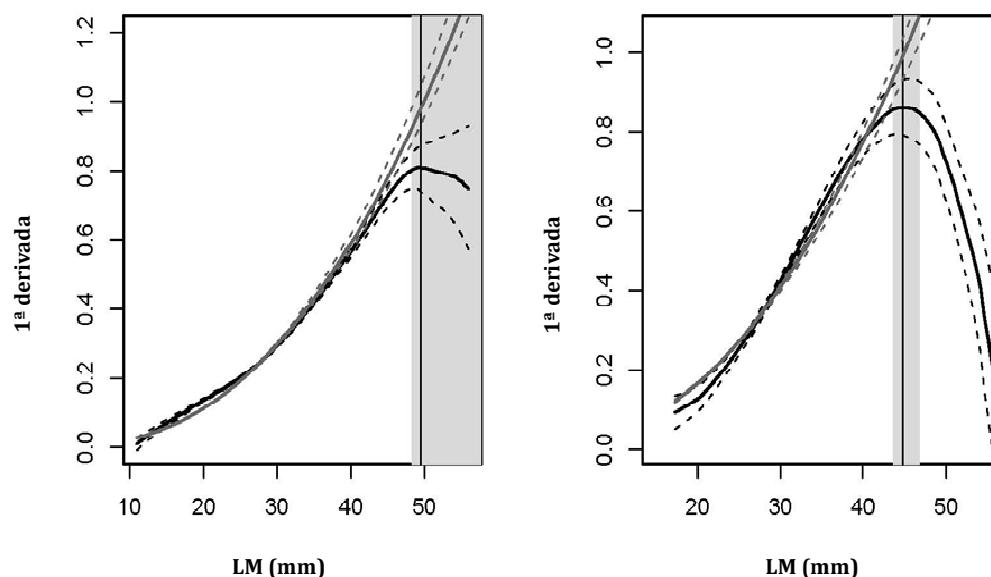


Figura 17 - Primera derivada de las regresiones iniciales para *Ruditapes decussatus* (izda.) and *Ruditapes philippinarum* (dcha.). Las líneas sólidas grises representan la 1ª derivada del modelo alométrico y las sólidas negras del modelo KNP. Los intervalos de confianza basados en bootstraps (del 95%) se presentan con líneas discontinuas.

En general, los resultados obtenidos mostraron que el modelo no paramétrico presentaba un mejor ajuste de los datos para ambas especies (Figura 16). La primera derivada de la regresión no paramétrica se utilizó para estimar la talla mínima de captura para cada especie, correspondiéndose con el tamaño en el que la primera derivada alcanza su máximo. Las tallas mínimas de captura estimadas fueron significativamente diferentes entre especies (Figura 17), siendo mayores que las actualmente establecidas en la UE. Por otra parte, se observó un efecto de los estuarios y de zonas dentro de cada estuario en los patrones de ganancia de peso por unidad de talla (ver Tabla 7.1, Chapter VII). Este estudio confirmó al modelo no paramétrico como una alternativa idónea para analizar la relación talla-peso y estimar la talla mínima de captura de las especies estudiadas, pudiendo ser el punto de partida para considerar su revisión con el fin de obtener un aumento de la biomasa disponible y efectos positivos en la conservación de la almeja fina.

8. Capítulo VIII: Conclusiones generales y líneas futuras de investigación

Los resultados de esta tesis aportan información y herramientas para apoyar la toma de decisiones en los modelos de gestión del marisqueo, tanto en general, para los estuarios donde estas especies habitan y son explotadas, como en particular, para la Bahía de Santander. En este apartado se resumen las principales conclusiones de la tesis, sus implicaciones en la gestión del marisqueo y las futuras líneas de trabajo a desarrollar a partir de este trabajo.

8.1. Conclusiones generales

- La metodología de muestreo utilizada, basada en técnicas artesanales de marisqueo y llevada a cabo por los propios mariscadores, permite obtener datos apropiados de abundancia, densidad y distribución de tallas de almejas adultas y de talla comercial,

demostrando su capacidad para ser utilizada en la estimación de stocks y el estado de explotación del recurso.

- La captura de individuos por debajo de la talla mínima legal en la Bahía de Santander podría desembocar en una reducción de ambas poblaciones, particularmente de la almeja fina, por tener un crecimiento más lento, afectando así al actual patrón de coexistencia.
- El análisis factorial del nicho ecológico (ENFA) ha demostrado ser una herramienta idónea para predecir la distribución potencial de las especies objetivo y explorar el potencial de expansión de la almeja introducida *R. philippinarum*.
- El hábitat potencial juega un papel importante como factor limitante de la expansión de *R. philippinarum* en la Bahía de Santander. La superficie con un hábitat potencial alto, o donde potencialmente predomina una especie sobre la otra, es considerablemente más extensa para *R. decussatus*.
- El modelo lagrangiano LARVAHS desarrollado en esta tesis se puede considerar como razonablemente idóneo para predecir la dispersión larvaria y el reclutamiento de las especies bentónicas considerando el comportamiento larvario, la variabilidad hidrodinámica y el hábitat potencial. El modelo ha demostrado tener una alta capacidad para (i) analizar el efecto de la localización de las zonas de puesta y las condiciones hidrodinámicas en el reclutamiento final y (ii) estimar las zonas de puesta y reclutamiento más importantes, junto con la conexión entre las mismas.
- La mayor duración de la fase larvaria de *R. decussatus* parece ser la principal razón para explicar su mayor dispersión y menor tasa de retención y reclutamiento en la bahía.

- La localización de las zonas de puesta, las corrientes inducidas por el viento y la fase de la marea en el momento de la puesta tienen un efecto significativo en el reclutamiento.
- En cuanto a la interacción de ambas especies, un escenario de expansión de *R. philippinarum* no se puede definir como una amenaza para la almeja fina *R. decussatus* en términos de competencia interespecífica en la Bahía de Santander.
- La predación juega un papel muy importante en la regulación de las poblaciones de ambas especies, pero particularmente en el caso de *R. philippinarum*.
- El modelo no paramétrico basado en suavizadores de kernel (KNP) ha demostrado tener capacidad para analizar la relación talla-peso y estimar la talla mínima de captura de especies con un patrón de ganancia de peso diferenciado a lo largo de su desarrollo.
- La talla mínima de captura estimada es distinta entre especies y, en ambos casos, mayor que la establecida actualmente tanto en la UE como a nivel regional.

8.2. Implicaciones para la gestión del marisqueo

En este apartado se plantean varias medidas de gestión o aplicaciones de las metodologías y herramientas desarrolladas.

- El muestreo basado en la técnica de pesca de los mariscadores de rastrillado del sedimento podría ser un método adecuado para evaluar el estado de las poblaciones de almeja y el funcionamiento de las medidas de gestión en los estuarios donde se lleva a cabo un marisqueo artesanal. Además, es aconsejable involucrar a los mariscadores en el muestreo y la evaluación como punto de partida de una gestión conjunta de los

recursos con el fin de lograr una progresiva acumulación de conocimientos para mejorar la ordenación pesquera.

- El análisis factorial del nicho ecológico (ENFA) para la estimación del hábitat potencial es una herramienta adecuada aplicable en la localización de zonas óptimas de restauración de *R. decussatus* o de cultivo de ambas especies. De la misma manera, la toma de decisiones en relación con las zonas óptimas de veda puede ser apoyada con esta herramienta.
- El modelo LARVAHS de dispersión larvaria basado en el hábitat potencial también ha demostrado ser una herramienta con un importante potencial para apoyar las estrategias de restauración y gestión de la acuicultura. Así, las zonas de puesta con un hábitat potencial alto, desde donde (después de la dispersión larvaria) se obtengan unas tasas importantes de reclutamiento final, deben ser tenidas en cuenta en estas estrategias dirigidas a la conservación y mejora de los rendimientos de la pesca de *R. decussatus*. Una mejor comprensión de la depredación también ayudará en la localización de sitios de restauración o santuarios.
- En vista de los resultados obtenidos respecto a la interacción competitiva, la estrategias de conservación de *R. decussatus* podrían estar más centradas en otros aspectos diferentes al del potencial impacto directo de *R. philippinarum*, siempre que se controle el aumento de la densidad de la misma.
- Así, para controlar la expansión y la densidad de esta especie introducida, a diferencia de *R. decussatus*, una zona de hábitat potencial alto pero con un éxito menor en el reclutamiento final podría ser una mejor opción a la hora de ubicar las zonas de cultivo para esta especie.
- La talla mínima de captura estimada para estas especies debería de ser un punto de partida para considerar su revisión teniendo en cuenta sus potenciales efectos

positivos sobre el rendimiento de la pesca de ambas especies y la conservación de la especie nativa.

8.3. Futuras líneas de investigación

Esta tesis ha revelado la existencia de posibles mejoras en los procedimientos de estimación o predicción descritos para alcanzar los objetivos, así como la posibilidad de explorar nuevos aspectos en el complejo campo de la dinámica ecológica de estas especies. Estas mejoras y las líneas a seguir han sido analizadas en detalle en las secciones de discusión y conclusiones de cada capítulo. En este apartado del resumen, se mencionan los aspectos más relevantes de la tesis que requieren ser priorizados en futuras líneas de investigación.

- En cuanto a la metodología de muestreo utilizada para la evaluación del estado de las poblaciones de almeja en los estuarios de gran extensión, sobre todo en aquellos con densidades bajas de almeja, se requiere un esfuerzo en cuanto a la estimación de la longitud o área mínima del transecto. Por otra parte, es necesario investigar sobre un tipo de muestreo que complete la evaluación de los individuos recién reclutados y juveniles (<15 mm), sin un consumo excesivo de tiempo ni recursos, con el fin de abarcar toda las clases de tallas de la población. Además, la aplicación de esta metodología en otros estuarios y otras especies de almeja es esencial para poder caminar hacia una metodología de muestreo estandarizada.
- En cuanto a las predicciones de la distribución potencial de ambas especies, la integración de un mayor número de variables ambientales con una mejor resolución espacial, junto con un mayor esfuerzo en la adquisición de datos de presencia permitirá un mayor refinamiento de los modelos ENFA. Por otra parte, la integración de la variable depredación en el modelo, o su posterior consideración en el análisis del

reclutamiento final, deben ser estudiados teniendo en cuenta su importante papel en la regulación de las poblaciones de almeja.

- El modelo LARVAHS puede servir como marco para orientar las investigaciones sobre la predicción de la dinámica larvaria y, en particular, sobre el asentamiento y reclutamiento, teniendo en cuenta la integración en el mismo de un submodelo de reclutamiento basado en el hábitat potencial. Sin embargo, es deseable dirigir futuras investigaciones hacia la obtención de una mayor resolución espacial y el entendimiento de procesos biológicos importantes, tales como el comportamiento larvario, la mortalidad, el crecimiento. Otra línea de investigación, se podría enfocar hacia el desarrollo de un modelo enteramente 3D con una mayor capacidad de cálculo y gestión de memoria. La calibración y validación del modelo a través de un esfuerzo encaminado a la obtención de muestras de individuos recién reclutados, incluso larvas en la columna de agua, es, de la misma manera, altamente recomendable.
- Una línea dirigida hacia el estudio de la interacción competitiva entre las especies estudiadas es también necesaria. Por un lado, a pesar de las densidades utilizadas en el experimento eran idóneas para simular un escenario de expansión en la Bahía de Santander, es recomendable utilizar densidades más altas con tal de intentar alcanzar el nivel crítico para desencadenar la competencia. Por otra parte, es aconsejable la experimentación a corto plazo para estudiar los potenciales efectos de la interacción en los movimientos de la almeja o en la migración, cubriendo otros estuarios y zonas diferentes dentro de estuarios con el fin de tener una mejor comprensión de la interacción. Por último, la experimentación sobre individuos recién reclutados o juveniles ayudará a determinar la importancia de la competencia en esta fase de la vida más crítica.
- En cuanto a la estimación de la talla de captura mínima idónea es esencial un esfuerzo a largo plazo que abarque el muestreo de almejas en diferentes estaciones del año con el fin de evaluar la influencia del desarrollo gonadal en la ganancia de peso y explorar el efecto de la falta de individuos de tallas grandes.

SUMMARY

The objective of the thesis is to advance in the understanding of ecological dynamics of the European native grooved carpet shell clam *Ruditapes decussatus* and the introduced Manila clam *Ruditapes philippinarum*, in order to support specific conservation strategies and shellfisheries management of these species in large estuaries and, specifically, in the Bay of Santander (N Spain, Gulf of Biscay), the main study site. Ecologically relevant aspects requiring investigation, such as the expansion of the Manila clam, competitive interaction, larval dispersal and recruitment or suitable specific catch size together with the methodologies applied for the assessment of these clam populations in large estuaries have been studied in this thesis.

First, a characterization of both species populations was performed in the Bay of Santander. For this purpose a field survey was carried out in the different shell-fishing areas where samples were taken on transects, following traditional exploitation techniques. Size frequency distributions, densities and stocks were evaluated and a hydrodynamic model was applied in order to understand larval transport and recruitment patterns associated to the tidal currents and water flow.

The results allowed evaluating the performance of the management measures in Bay of Santander, showing (i) that illegal fishing activity is performed in closed areas and on individuals under the minimum legal size, (ii) areas where both species coexist and areas where one of them dominates and (iii) a limited recruitment in the inner parts of Cubas tidal fresh for *R. philippinarum* and in the southern zones for *R. decussatus*. In summary, the sampling method developed provides adequate data of abundance and density of adult and commercial clams, showing its feasibility to estimate standing stocks and to know the exploitation situation of different zones in a large estuary. In addition, the sampling method involving shell-fishermen seems to be a good starting point for a future co-management strategy.

Although distribution patterns of these clam populations were described in this first characterization study, a more detailed study was required to deal with the expansion potential of the introduced Manila clam, since in several estuaries of Europe it has become a widespread and predominating species supplanting the native carpet shell clam by relegating it to occupy restricted areas. Thus, secondly, the habitat suitability for both species and potential predomination areas were determined. For this purpose, the Environmental Niche Factor Analysis (ENFA) modeling approach was used.

The highly suitable and potential predomination areas were considerably wider for the native species than for the introduced species, showing the important role of habitat suitability as a limiting factor for the expansion of the Manila clam. The environmental variables that most determined the presence of both clams in this study were bathymetry, current velocity and salinity, although their importance and association (positive or negative) with high predicted habitat suitability was different between species. The model showed that ENFA provides a promising technique to achieve distribution predictions of clams and explore the potential of expansion of the introduced or invasive species, supporting conservation, shellfishery and aquaculture strategies regarding placement of restoration sites or farming areas.

The next study in this thesis was to integrate habitat suitability results in a larval evolution model in order to predict settlement and recruitment patterns. A particle-tracking model, named LARVAHS, was developed coupled to a hydrodynamic model to investigate potential larval dispersal and recruitment patterns influenced by larval behavior and hydrodynamics. A specific study was carried out in the Bay of Santander (N Spain) to better understand the preliminary results obtained from the previous application of the hydrodynamic model in the characterization of the population. The model was fed with observed winds, freshwater flows and astronomical tides to obtain predictions during clam spawning period. Dispersion of larvae from 7 spawning zones was tracked and

subjected to three-dimensional advection, vertical turbulent diffusion and imposed vertical migration behavior. An habitat suitability-based (ENFA, Environmental Niche Factor Analysis) settlement-recruitment submodel was incorporated into the model to simulate settlement behavior and post-settlement mortality. Three simulation periods (spring, summer and autumn) and 2 initial releases (spring / neap tide) were combined in 6 different modeling scenarios. The LARVAHS model highlighted the role of season (i.e. predominating winds) and the spawning zone location on recruitment success together with the effect of the tidal phase at spawning. The model has proven to be a valuable tool for determining major spawning and nursery grounds and to explore connectivity between them, having important implications for shellfishery management regarding the expansion of Manila clam and aquaculture or restoration activities.

Apart from habitat suitability and larval dispersion, competitive interaction between these species and predation could also play an important role in the expansion and domination of Manila clam over the carpet shell clam. However, it is not clear if the non-indigenous clam is the direct responsible for the predominance patterns observed in several estuaries of Europe. Thus, the competitive interaction between these species was analyzed to determine whether this interaction could directly impact growth and mortality of the native clam populations. In addition, the effect of exposure to predators on both species was also examined. For this purpose, a field experiment was conducted on an intertidal area in the Bay of Santander. Relative density of these species was manipulated in a randomized block experimental design.

The results obtained show that the increased density of the Manila clam simulating species expansion scenario does not affect growth or mortality of the native clam and predation plays an essential role in regulating both species populations, limiting their density increase. Moreover, the Manila clam appears to be more negatively impacted by predation compared to the native clam. Overall, our results indicate that predation has a more significant effect on both populations when compared to competitive interaction. When

extrapolating our experimental densities to other estuaries of Europe, it seems that the competitive interaction between these species could be playing a role on the drastic decrease of the native clam only when extremely high densities occur.

Shellfishery also plays a relevant role in regulating clam populations. Moreover, management failures could lead to an extreme regulation or overexploitation. In this regard, the minimum size of capture required further investigation since it is the same for both species in many estuaries of Europe where they coexist, regardless of their biological differences. The last study of this thesis analyzed the weight gain patterns per unit of size and estimated the minimum suitable catch sizes. Data were obtained from individuals collected in the Bay of Santander and the neighbouring estuary of Marismas de Santoña. The length–weight relationship was estimated using the classic allometric model and a nonparametric regression model based on local linear kernel smoothers. Additionally, first derivatives were used to estimate a minimum capture size for this species, corresponding to the size at which the first derivative reached the maximum.

The nonparametric model resulted in a better fit of data for both species and the estimated minimum catch sizes were considerably different for each of them, being larger than those currently established in the EU. Moreover, an effect of estuaries and zones on individual weight gain patterns was found. This confirmed the nonparametric model as an alternative approach to analyze the length–weight relationship for the studied species and to estimate a minimum suitable catch size. The application of the specific catch sizes obtained in this study could be a starting point to consider the revision of minimum catch size of this species in order to obtain an increase in available commercial stocks of both species and positive effects on the conservation of the native species.

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Chapter I

Introduction and background to the research

Chapter I. Introduction and background to the research

1.1. Motivations for the research

Productive natural populations of shellfish resources are coming under pressure as rising demand and prices for these generally high-value species leads to their overexploitation. A decreasing trend in global catch rates and landings in the absence of proper management has often led to stock collapse (Castilla and Defeo, 2001). Moreover, shellfish populations suffer negative impacts from human activities other than fishing (e.g. organic and industrial discharges)(Caddy and Defeo, 2003). Thus, it is essential that a shellfishery management model should be based on a solid scientific knowledge of both the population exploitation status and ecological dynamics of the target species. This knowledge is even more relevant in cases of introduction of non-native species for aquaculture purposes, such as the Manila clam (*Ruditapes philippinarum*). The expansion of the introduced species can lead to the displacement of the native species to restricted areas, as it has already occurred in several bays and lagoons of Europe in case of the native carpet shell clam (*Ruditapes decussatus*)(Auby, 1993; Marin et al., 2003)

Therefore, considerable attention has been paid to the population assessments of these two species, since they are highly shell-fished and cultivated species in Europe and worldwide (FAO, 2012). Assessments of these clam species commonly include estimates of biomass and densities (e.g. Bald and Borja, 2001; Caill-Milly et al., 2006:2008; Cannas, 2011) which are essential to evaluate the performance of the shellfishery management model (e.g. Hancock and Urquhart, 1965; Defeo, 1996; Castilla et al., 1998). The estimation of abundance of clam species needs to be expressed as a total quantity by stock, shellfish bed or management area with some measure of the associated variance. In this regard, several studies on diverse topics of clams stock assessment methodologies have been

carried out (e.g. Palacios *et al.*, 1994:2000; Rueda and Urban, 1998; Orensanz *et al.*, 2003). Particularly, different sampling methods regarding sampling grid, size of samples or the mesh size to sieve the sediment (Byers, 2005; Lee, 1996; Borja and Bald, 2000; Bald and Borja, 2001:2005; Caill-Milly *et al.*, 2006; Morsan, 2007) have been applied to perform these estimations. However, the lack of a standardized sampling methodology and the expected high time and resource consumption of these sampling methods for large estuaries requires the exploration of assessment procedures that combine the appropriated technical design with the fishermen experience.

Despite the traditional shellfishery of clams has a long history in the Bay of Santander and other important estuaries of the region of Cantabria (northern coast of Spain, Gulf of Biscay) population assessment studies have never been conducted until 2005. Therefore, a study of the characterization of clam populations was required in order to evaluate the performance of management measures and to support a new scientific-based management model.

However, the characterization of a population or stock assessments alone, without a strong understanding of ecological dynamics oriented to management decision-making, is of course ineffective. Although understanding of population dynamics of the native clam *R. decussatus* and the nonindigenous clam *R. philippinarum* (e.g. Pérez-Camacho, 1979; Olafsson *et al.*, 1994; Laruelle, 1999; Urrutia *et al.*, 1999; Chícharo and Chícharo, 2000; Solidoro *et al.*, 2000; Pea and Pea, 2001; Pérez-Camacho *et al.*, 2002:2003; Melia *et al.*, 2004; Phillips, 2006, Flye-Sainte-Marie *et al.*, 2007a,b; Humphreys *et al.*, 2007; Dang, 2009,) or the interaction of clams with the habitat characteristics where they dwell (e.g. Arnold *et al.*, 2000; Chícharo and Chícharo, 2001a; Peña *et al.*, 2005; Vincenzi *et al.*, 2006) has consistently improved, finding answers to following important questions is still a difficult task. Does expansion of the introduced Manila clam cause competitive displacement of the European native clam? Which is the expansion potential of Manila clam and which environmental factors play an important role on it? Although the Manila

clam is considered as threat to the native grooved carpet shell clam, its impact has not been studied yet regarding competitive interaction. Moreover, this introduced species drastic expansion and predomination registered in other European estuaries is an issue that must be addressed through the examination of the factors regulating or enhancing the expansion. These issues need to be investigated from the point of view of fisheries sustainability and, in particular, from the field of conservation of the native species.

Moreover, determinant aspects for understanding the connectivity and persistence of these populations such as larval dispersal and recruitment are poorly understood and the biophysical models developed for this purpose (e.g. Borsa and Millet, 1990; Herbert et al., 2012) need to be importantly improved, particularly in the prediction of larval settlement and recruitment (Metaxas and Saunders, 2009). Advances in this direction are essential to (i) examine factors involving larval dispersal or retention in an estuary and (ii) to determine major spawning and nursery zones. This information may support these species fisheries management models.

In the estuaries where the study species coexist, several management models are based on measures that are usually common to both species regardless of biological aspects or if a species is more tracked to cultivation than other (Juanes et al., 2012). For instance, one of the most important and widely used measures in fishery management strategies, the minimum catch size (e.g. Berthou et al., 2005; Gilbert et al., 2006), was the same (40 mm) for both species to 2007 in the European Union and, although after that it was decreased to 35 mm for Manila clam (Dang, 2010), this situation could have had negative consequences and may be one of the reasons of the overexploitation and displacement of the native carpet shell clam, as pointed out by Juanes et al. (2012). Yet, regional regulations in many estuaries, such as those across all the north coast of Iberian Peninsula and in other countries of Europe, are still based on the same catch size for artisanal hand-raking fishing of both species (e.g. Orden DES/25/2011; Cannas, 2010). Therefore, there is

still a need to investigate the suitable specific catch size of these clams in greater detail, in order to obtain, in the long run, the maximum yield from the fishery of both species.

Within this context, it is essential to advance in the understanding of ecological dynamics of these two species in order to support specific conservation strategies and shellfisheries management.

1.2. Study species

1.2.1. Systematic position

The studied species are the European grooved carpet shell clam *Ruditapes decussatus* and the Manila clam *Ruditapes philippinarum*, congeneric bivalve molluscs that belong to family Veneridae (Rafinesque, 1815), also known as venus clams. Veneridae family or venerids are characterized as bivalves (Class Bivalvia, Linnaeus, 1758) with two muscles, the anterior and posterior adductors, which control the opening and closing of the shell valves. Numbering about 30.000 species, bivalves form the second most diverse class of molluscs after gastropods. Bivalves are exclusively aquatic, requiring fresh or sea water for production and respiration, and typically for feeding (Grzimek et al., 2004). Veneridae family is a very large family, with over 500 living species of bivalves, most of which are edible, and many of which are exploited as a food source. Many of the most commercially important venerid bivalves, as the present study ones, are commonly known simply as “clams” (Gosling, 2003). The systematic position of the studied species is presented in the Table 1.

Grooved carpet shell clam		Manila Clam
Phylum	Mollusca	
Class	Bivalvia (Linnaeus, 1758)	
Subclass	Heterodonta (Neumayr, 1884)	
Order	Veneroidea (Adams & Adams, 1856)	
Superfamily	Veneroidea (Rafinesque, 1815)	
Family	Veneridae (Rafinesque, 1815)	
Subfamily	Tapetinae (Adams & Adams 1857)	
Genus	Ruditapes (Chiamenti, 1900)	
Species	<i>Ruditapes decussatus</i> (Linnaeus, 1758)	<i>Ruditapes philippinarum</i> (Adams & Reeve 1850)

Table 1.1 - Systematic position of grooved carpet shell clam and Manila clam (Le Treut 1986; Laruelle, 1999).

1.2.2. Global distribution

Global distribution of both species is presented in Figure 1.1. The carpet shell clam is native to Europe, being found in the North Sea and the northeast Atlantic coasts, from Norway (61 ° N) and Azores to as far south as Senegal (12 ° N), and common in estuaries and lagoons of most of the Mediterranean basin (Paracha, 1982, Lubet, 1984; Breber, 1985; Poppe and Goto, 1991).

The Manila clam is a subtropical to low boreal species native of the Indo-Pacific coastal seas and its distribution area ranged from the Kuril islands (52°N, 150°E) in the Northern Pacific ocean to Pakistan (30°N, 65°E) in the Indian ocean. Because of its commercial value, Manila clam has been introduced, either intentionally or accidentally, to several

parts of the world becoming permanently established (Sladonja et al., 2011). It was introduced to Hawaii in 1929 (Yap, 1977) and ten years later to the North-West coast of the United States, as a hitchhiker species on shells of Pacific oyster individuals (Harbo 1997, Levings et al. 2002). Naturally established populations are now present from British Columbia to California (Harbo 1997; DFO, 1999).



Figure 1.1 – Geographical distribution of the European native grooved carpet shell clam *R. decussatus* (a, black line) and the Manila clam *R. philippinarum* (b, black line represents the original distribution; gray line represents the coasts where the species has been introduced and become naturally established) from Gosling (2003) and figures and reference therein.

Overfishing and irregular yields of the native grooved carpet shell led to imports of *R. philippinarum* into European waters (Sladonja et al., 2011). Between 1972 and 1975, the species was first introduced in France and at the end of the 70's also in Great Britain (Flassch and Leborgne, 1992; Jensen et al., 2004) followed by numerous transfers within European waters for aquaculture purposes, particularly to Portugal, Ireland, Spain, and Italy. Natural reproduction of the introduced individuals favored geographical expansion into the wild in these countries, where the Manila clam proved to be more resistant and faster growing than the endemic carpet-shell clam (Usero et al., 1997). Thus, natural populations are currently found on the South coast of Great Britain (Humphreys et al., 2007), all along the French and Spanish Atlantic coasts and on the Mediterranean coasts (mainly in the Adriatic Sea) (Laruelle, 1999; Cesari and Pellizzato, 1990). This species has

also been reported from different other places in the world, such as Norway (Mortensen and Strand, 2000), Morocco, Tunisia (Cesari and Pellizzato, 1990) and Israel (Ponurovsky and Yakolev, 1992).

1.2.3. Biology and ecology

Clams frequently occur in aggregations forming shell beds in marine, estuarine and freshwater habitats. They are infaunal and burrow into the protection offered by sand, mud or gravel using the foot (Grzimek et al., 2004). Since they are filter feeders, contact is maintained with the surface by way of siphons that extend from the posterior end of the animal (Gosling, 2003). Water flows in through the inhalant siphon, through the gills, where filtering of suspended food particles (i.e. phytoplankton, micorphytobenhos and detritus) takes place, and exits through the exhalant siphon (Jørgensen et al., 1986) (Figure 1.2). Some bivalves burrow only deep enough to cover the shell and have correspondingly short siphons, meanwhile others burrow very deeply and have long siphons (e.g. Lardies et al., 2001; Jaramillo et al., 2007).



Figure 1.2 - *R. decussatus* individual pumping water through the exhalation siphon. Photo credit: Brian Spencer, published on the MarLIN website.

Regarding the studied species, *R. decussatus* and *R. philippinarum* occurs in intertidal and very shallow subtidal sandy to muddy sediments in lagoons, bays or protected estuaries. They are very similar and difficult to distinguish externally. Most morphological differences are only identifiable by experienced observers. An initial examination of the shell shows *R. decussatus* species is distinguished from *R. philippinarum* by its much more pronounced decussate sculpture (criss-cross markings), the more angulated and less robust shell, the less pronounced striation. Both species show high colour variability, more pronounced in *R. philippinarum*. The maximum length for both species is around 75 mm (Gosling, 2003). The environmental conditions environmental may affect the shape and appearance of the shell (Cannas, 2010). However, internal examination reveals a clear difference between species: the longer and more separated siphons of *R. decussatus* comparing to the almost fully fused siphons of *R. philippinarum* (Figure 1.3). These differences in siphon length lead to higher burial depth of *R. decussatus* (~ 10-12 cm) than that of *R. philippinarum* (~ 2-4 cm) (Vilella, 1950; Lee, 1996; Gosling, 2003).

Sexual maturation phase can begin between 10-20 mm shell lengths in *R. decussatus* (Lucas, 1968; Urrutia et al., 1999) while in *R. philippinarum* it can start at a 5-10 mm (Ponuvorovsky and Yakolev, 1992 and references therein). However, the major contribution to reproduction is done by large individuals since fecundity increases with size for both species (Holland and Chew, 1974; Ponuvorovsky and Yakolev, 1992; Robert et al., 1993; Laruelle et al., 1994; Urrutia et al., 1999).

Ruditapes decussatus and *R. philippinarum* show inter-specific differences in breeding cycles. However, for both species the breeding period can extend from April to November and the number of spawning can be variable depending on the water temperature conditions and food supply (Gosling, 2003; Cannas, 2010). In each spawning season adult clams of both species released from 1 to 12 million gametes to the water (Yap, 1977; Chung et al., 2001; Park and Choi, 2004; Matías et al, 2009) which can be fertilized and metamorphosed to planktonic larvae with different stages.



Figure 1.3 - External and internal images of *Ruditapes decussatus* (a,b) and *Ruditapes philippinarum* (c,d).

Therefore, although these species are highly sedentary as adults, they can disperse importantly during the planktonic larval stage of around 15-21 days (e.g. Pérez-Camacho, 1980; Vela and Moreno, 2005; Young-Baek et al., 2005; Hinata and Furukawa, 2006). The supply of larvae is a determinant factor for the stability of benthic populations that depend upon the settlement and recruitment of the planktonic larvae to balance the adult mortality losses (e.g. Minchinton and Scheibling, 1991; Rodríguez et al., 1993; Olafsson et al., 1994). As other invertebrates, for these species survival and development of both planktonic life and benthic phases may largely depend on environmental conditions such

as sediment characteristics, hydrodynamics, food availability, salinity, water quality, competition or presence of predators (Vicenzi et al., 2006; Cannas, 2010).

R. philippinarum is a faster growing species and more resistant to unfavourable environmental conditions than *R. decussatus* (Breber, 1985:1991; Usero et al., 1997). Although growth can be highly variable since it depends on environmental conditions where clam dwells, in general, *R. philippinarum* can reach large sizes, for instance 35 mm, in 2-3 years whilst *R. decussatus* needs 4-5 years (Urrutia et al., 1999; Solidoro et al., 2000; Chessa et al., 2005; Humphreys et al., 2007; Dang, 2009).

1.3. Shellfisheries, population assessment and management

R. decussatus and *R. philippinarum* are highly exploited infaunal bivalves in Europe and worldwide, respectively. Cultivation and expansion of Manila clam has led this species to be the most important resource for commercial clam landings in Europe (Sladonja et al., 2011; FAO, 2012,). Harvest of *R. philippinarum* has experienced a worldwide significant increase in the last 20 years, currently representing one of the most productive aquaculture species in the world (3.6 million metric tons in 2010). China is by far the leading producer (97.4% of total annual production) while Italy had a smaller but yet conspicuous production of over 36000 tons and Spain around 1100 tons. Whilst, for *R. decussatus* total production in Europe was 3500 tons in 2010, being 1500 tons obtained from fishery and the remainders from aquaculture. Spain is the largest fishery producer of this native clam with 800 tons while Italy is the largest aquaculture producer with 1050 tons (FAO, 2012).

In this context, in the estuaries where both species coexist, shellfishery and aquaculture specific management models are essential. Regarding the frame of marine living resources

management, in 1982, the United Nations Convention on the Law of the Sea provided a new framework to improve management of marine resources. The new legal regime of the oceans gave coastal states rights and responsibilities for the management and utilization of fishery resources within their areas of national jurisdiction. Similarly, the development of a strategy to ensure sustainable use of living marine resources and their environment was one of the aspects of relations between man and the biosphere discussed at the United Nations Conference on Environment and Development (Rio de Janeiro, June 1992). Undoubtedly, the main potential mechanism for achieving sustainable development is improved management of these resources.

Since then, the development of strategies for the sustainable management of living marine resources and environmental protection of associated ecosystems, is one of the main commitments addressed by the competent authorities, both international, national and regional with an important legal regulation in this regard and their transposition to national legislation (60/2000/EC Water Framework Directive (EC, 2000), Directive 2008/56/EC of the Sea (EC, 2008), Biodiversity Strategy COM / 98/0042 (European Commission (EC), 1998), OSPAR 98/249/EC (EC, 1992a) Habitats Directive 92/43/EEC (EC, 1992b) COM/92/509 Convention on Biological Diversity (EC, 1992c), Act 3 / 2001 on Fisheries of the State Fisheries, Aquaculture Proposal at Sea (NOAA, 2006).

Currently, one of the questions which are being developed in this regard is the sustainable management of fisheries, involving "the regulation of marine living resources, based on the understanding of the structure and dynamics of ecosystems in the resource is part "(FAO, 1999). In this sense, the main objective is to ensure balanced and responsible exploitation of fisheries resources, promoting sustainable development, adopting the necessary measures to protect, conserve and restore these resources and their ecosystems. According to the FAO, species stock assessment may be described as the search for an exploitation level which gives the maximum yield in weight from the fishery (King, 1995; Sparre and Venema, 1997)

In the last two decades, efforts focused on population assessment studies have been intensified in order to establish the most appropriate shellfisheries management measures. Regarding the studied species, studies have been focused on population stock assessments, spatial distribution and size distribution analysis (Lee, 1996, Bald and Borja, 2001:2005; Cailly-Milly et al., 2006:2008; Sullivan, 2007; Morsan, 2007). For this purpose, different sampling methods regarding sampling grid, size of sample or mesh size to sieve the sediment have been performed (e.g. Byers, 2005; Lee, 1996; Borja and Bald, 2000; Caill-Milly et al., 2003; Morsan, 2007). In general, sampling methods are based on systematic sampling of small quadrats ($<0.50 \text{ m}^2$) where sediment is removed to a given depth and sieved using a mesh to collect all sizes individuals. However, the lack of a standardized sampling methodology and their expected high time and resource consumption for large estuaries requires the exploration of assessment procedures that combine the appropriated technical design with the fishermen experience.

In the Bay of Santander (N Spain), estuarine populations of these two species have been largely exploited by shellfishers and until now, the clam fishery management has been based on setting a minimal size of capture (total length of 40 mm) and seasonal closing areas by regional regulations regardless of the distribution patterns and biological differences between species. Moreover, the important role of fishermen in management (Brown, 2001; Scott, 2001) is not yet considered in this region, although some experiences in this direction have been very successful in the neighbour regions of Galicia, where the government regulations promote a co-management system between fishers' organisations ("cofradías") and the fisheries authority (Meltzoff, 1995; Molaes and Freire, 2003).

An efficient management should be based on a good knowledge of: (i) the biology of the target species; (ii) the available resource through stock evaluations; (iii) the fishing pressure and activity. Although knowledge of the biology of the studied species is wide (e.g. Pérez-Camacho, 1979; Pérez-Camacho et al., 2002:2003; Solidoro et al., 2000; Melia et al., 2004.; Flye-Sainte-Marie, et al., 2007a,b) and several stock evaluations have been done

in nearby estuaries in Spain and France (e.g. Borja, 1989:2000; Bald and Borja, 2001:2005; Caill-Milly et al., 2003:2006), the only available information in the Bay of Santander regarding this species is about their biometrical relationships of *R. decussatus* (Arnal and Fernández-Pato, 1977:1978) and clam annual captures from fishery statistics data. Hence, the lack of data has not allowed the implementation of scientific-based management measures and not even the possibility of evaluating the performance of the current management measures.

1.4. Distribution patterns and habitat suitability

In estuaries where a nonindigenous species dwell, the scientific-based management models require special attention regarding species distribution patterns studies, since natural or accidental invasions of marine nonindigenous species, together with deliberate introductions, can have significant impacts on the native communities (Carlton, 1996; Bachelet et al., 2004; Ruiz et al., 1999). However, management decisions for conservation of a native species or action against a certain introduced or invasive species are often blocked by a lack of basic and essential ecological information. In this regard, the study of the potential distribution of both the native and the nonindigenous species is essential in order to identify priority areas for conservation of species and habitats (e.g. Álvares and Brito, 2006; Brito et al., 2009; Tittensor et al., 2009; Valle et al., 2011) and to establish specific zone-based management models. This information is even more important when the studied species are exploitable and a sustainable fisheries management is desired (e.g. Galparsoro et al., 2009; Cilenti et al., 2011). Estimation of distribution areas of commercial species can be linked with the potential yield of the fishery (Vincenzi et al., 2006a,b) and, therefore, support zone-based management models can be used to establish different conservation or fishing strategy.

The Manila clam *Ruditapes philippinarum* is one of the most widely introduced species for aquaculture purposes as it was mentioned above. Currently, it is one of the most commercially exploited bivalve mollusks along the Pacific coast of the United States, the European Atlantic coast, the Adriatic and Aegean seas and the Indo-Pacific region (Jensen et al., 2004; Dang, 2010). In several disturbed estuaries or lagoons of Europe as Arcachon Bay (France) or the lagoon of Venice (Italy), this species has supplanted the European native carpet shell clam *Ruditapes decussatus* by occupying almost entirely their ecological niche and relegating it to occupy very restricted areas (Auby, 1993; Marin et al., 2003; Blanchet et al., 2004; Mistri, 2004; Caill-Milly et al., 2008). In the case of the Lagoon of Venice, it holds very high densities and has spread along the Adriatic coast at 30 km/year (Breber 2002). However, this expansion and extreme domination pattern of the introduced bivalve species has not yet been detected in other estuaries such as the Bay of Santander (Gulf of Biscay, N Spain) since the mean ratio *R. philippinarum*/*R. decussatus* presence is approximately 1:1. In fact one can observe zones where (1) both species coexist, (2) nonindigenous species predominates and (3) the native species predominates have been identified (Juanes et al., 2012). This species was first introduced in the Bay of Santander in the late 80s but until the late 90s the farming areas were testimonial. Nowadays, the operational farming area is not larger than 1 ha. However, being aware of the drastic decline of the native carpet shell clam in several estuaries of Europe, where the Manila clam has been introduced and has subsequently expanded, and prediction of the potential distribution of both species is essential. This spatial information could lead to support either the conservation measures for the native species or the appropriated fisheries management actions for a sustainable exploitation.

Predictive models are suitable tools to explore the environmental variables explaining the distribution of organisms (Guisan and Thuiller, 2005). In the last two decades the rapid development of Geographic Information Systems (GIS) along with powerful mathematical/statistical techniques and the growing availability of digital landscape data are allowing to model the potential distribution of species, being applicable to a wide

range of studies of biogeography, conservation biology, ecology, palaeoecology and wildlife or resource management (e.g., Mladenoff and Sickley, 1998; Guisan and Zimmermann, 2000; Schadt et al., 2002; Guisan and Thuiller, 2005; Araújo and Guisan, 2006; Olivier and Wotherspoon, 2006; Santos et al., 2006; Tole, 2006). In particular, these models have been used extensively in conservation plans for estimating the probability of occurrence of threatened species in terrestrial ecosystems (e.g. Araújo and Guisan, 2006; Hirzel et al., 2004). In recent years, the application of habitat suitability models in marine ecosystems has had a boost covering areas such as aquaculture (Aguilar-Manjarrez and Ross, 1995a,b; Longdill et al., 2008; Radiarta, 2008), fisheries management (Galparsoro et al., 2009), estuarine habitat management (Barnes et al., 2007, Valle et al., 2011) and conservation of a wide range of species such as cetaceans (Praca et al., 2009), migratory birds and turtles (Skov et al., 2008; Tian, 2008), polychaetes (Willems et al., 2008, Meißner et al., 2008), corals (Davies et al., 2008, Bryan and Metaxas, 2007; Dolan et al., 2008; Tittensor et al., 2009). In regard of bivalve species, the habitat suitability predictions has been mainly focused on commercial species such as oysters and clams in order to improve the management models or to restore habitats with aquaculture purposes (Soniat & Brody, 1988, Arnold et al., 2000; Vincenzi et al., 2006a,b). Moreover, the species distribution models have also been used to predict spatial patterns of biological invasions and to prioritize locations for early detection and control of outbreaks of the nonindigenous species in both terrestrial (e.g. Herborg et al., 2007; Meentemeyer et al., 2008; Strubbe and Matthysen, 2009) and marine environment (Inglis et al., 2006).

The analysis of the relationships between species and a set of environmental predictors that are likely to influence the suitability of the habitat for the focal species, is the basis of the predictive habitat models (Franklin, 1995; Guisan & Zimmermann, 2000). Most of the available methods are based on presence/absence data, yet often absence data are of limited use because certain areas within the study site may be suitable but not yet reached by the introduced species (Hirzel et al. 2002). Hence, an alternative approach is modelling presence-only data. This method characterizes the environmental conditions in the

“presence” locations by means of a factorial analysis and predicts the species distribution over the whole study area where the same environmental conditions are found (Hirzel et al., 2002; Braunisch et al., 2008). One of the examples of this approach is the Ecological Niche Factor Analysis (ENFA) developed by Hirzel et al. (2002). The ENFA is designed for the factors that explain most of the ecological distribution of the species. The procedure is similar to Principal Component Analysis (PCA), but in the case of ENFA factors have biological significance.

This technique assumes that a given species has a non-random distribution within an eco-geographical variable and that the individuals occupy the optimal range. By using only presence data, ENFA calculates habitat suitability based on the niche width of a species (Hutchinson, 1957) in relation to eco-geographical variables. These results in useful statistics related to the species niche and habitat suitability over large spatial areas (Hirzel and Arlettaz, 2003). ENFA is only able to fit linear dependencies of the species niche unless transformations or nonlinear combinations of variables are also incorporated (as layers). With respect to assessing the importance of each variable, ENFA results are more straightforward to interpret than other presence-only models based on heuristic and jackknifing estimates outputs (Tittensor et al., 2009). Thus, the ENFA has proven to be a valuable tool for predicting habitat suitability of marine benthic species (e.g. Leverette and Metaxas, 2005; Bryan and Metaxas, 2007; Willems et al., 2008; Galparsoro et al., 2009;; Tittensor et al., 2009; Valle et al., 2011) and for monitoring the potential spread of invasive or introduced species in terrestrial habitats (Hirzel, 2001; Hirzel et al., 2004; Casinello et al. 2006; Acevedo et al. 2007).

Therefore, the ENFA approach seems to be a suitable tool to determine the habitat suitability of this study species and its role on limiting on enhancing the expansion of the introduced Manila clam. Moreover, to conduct this analysis in the Bay of Santander (N Spain), where no dominant pattern has yet been detected, is essential and may importantly support management strategies.

1.5. Larvae dispersion and recruitment patterns

In intertidal and subtidal marine environments, many species such as clams are sessile or highly sedentary as adults, with dispersal occurring predominantly during a planktonic larval stage (Siegel et al., 2003). The supply of larvae, considered as the number of planktonic larvae available near suitable settlement sites (Minchinton and Scheibling, 1991; Gaines and Bertness, 1993; Jeffery and Underwood, 2000; Beaulieu et al., 2009), is a determinant of the final distribution and the stability of the benthic populations that depend upon the settlement and recruitment of the planktonic larvae to balance the adult mortality losses (e.g. Connell, 1985; Sinclair, 1989; Minchinton and Scheibling, 1991; Rodríguez et al., 1993; Olafsson et al., 1994). Therefore, knowledge of the patterns of larval dispersal between benthic habitat patches is critical to understanding the distribution, persistence and connectivity of marine populations together with their potential expansion (e.g. Botsford et al., 2001; Hastings and Botsford, 2006; Pineda et al., 2007). Thus, in the last decades, predicting the dispersion and supply of larvae has been one of the major goals of the study on population ecology (e.g. Rougharden et al., 1988; Siegel et al., 2003, Siegel et al., 2008) and specially on fisheries management and restoration activities (Kakino, 1996; Bostford et al., 1997; North et al., 2009; Savina et al., 2010; Kim et al., 2012). Population dynamic of exploited species can be more sensitive to recruitment dynamics since, besides to weather and oceanographic conditions, larval supply is linked to adult or spawning biomass which in turns depends on the fishery (Bakun, 1996; Hsieh et al. 2006).

The prediction of the larval supply needs to encompass (i) spawning stock abundance (Myers and Barrowman, 1996; Francis, 1997; Myers, 1997; Ye, 2000), (ii) larval dispersion, which in turn depends largely on the swimming behavior of larvae, the duration of the planktonic stage and the hydrodynamic conditions (e.g. Thorson 1950; Roegner, 2000; Pineda et al. 2007) and (iii) settlement, which refers to where and when they find a suitable habitat to metamorphose (Pineda et al. 2007; North et al., 2008). The

final recruitment success (i.e. the number of individuals reaching a juvenile nursery area) (North et al., 2009) is influenced by the previous settlement and the early post larval mortality (Hunt and Scheibling, 1997; Pineda et al. 2007).

Biophysical models integrating all or part of these factors are increasingly being used to predict larval transport and explore the role of different biological and physical factors on larval dispersal and settlement of marine benthic species (Metaxas and Saunders, 2009). Most of the larval evolution models developed draw on information from hydrodynamics (i.e. water flow) and simplified the larval behavior as a passive tracer of currents (e.g. Borsa and Millet, 1992; Chiswell and Booth, 1999; Incze and Naimie, 2000; Siegel et al., 2003; Aiken et al., 2007; Miyake et al., 2009;). They seem to be promising in that they can yield detailed connectivity matrices and also resolve dispersal trajectories, although they do not resolve an adequate level of detail in flow structures (Largier, 2003). In the last decade, important steps have been made for including larval behavior into these models such as age-dependent vertical migration or behavioral cues (Hinckley et al. 2001 Hinrichsen et al. 2005; Miller, 2007; North et al., 2008; Banas et al., 2009; Herbert et al, 2012). Estuaries, lagoons and bays have proven to be excellent systems to apply these numerical models to study how biological and physical processes influence larval supply. These systems provide important nursery grounds and adult habitats for benthic invertebrates with pelagic larval stages and their enclosed morphology, together with the predictable nature of tidal flows or salinity variations, makes them relatively easy to measure physical processes and larval trajectories (Thompson, 2011).

Therefore, taking into account the above mentioned aspects, larvae of exploited benthic invertebrate species in estuaries or bays should be, potentially, highly suitable to model and results can support decision making in fisheries management, aquaculture activities and conservation strategies. However, few studies have been conducted in order to predict benthic commercial invertebrates' larvae dispersion and settlement patterns within these systems. Commercial and widely distributed clams, oysters and abalones

have been the main objective species of biophysical models. *Ruditapes philippinarum* or Manila clam was modeled in Tokyo bay by Hinata and Tomisu (2005) and Hinata and Furukawa (2006), who assumed larvae to behave as a passive tracer of wind-induced currents. North et al. (2008) modeled the dispersion of two oyster species, *Crassostrea virginica* and *C. ariakensis*, in Chesapeake Bay using a hydrodynamic model, forced with observed winds and freshwater flow, together with a larval behavior and settlement submodels. The behavior submodel included swimming speed and behavioral cue components (salinity) that regulated the vertical velocity of particle movement. The purpose of the settlement sub-model was to determine if a pediveliger-stage particle encountered suitable habitat (i.e. presence of oyster reef), assuming that in the period of settlement chemical cues from living oysters promote larval settlement (Turner et al. 1994, Tamburri et al. 1996). Other studies have also shown that wind effects are important in larval distribution (Suzuki et al., 2002; Leis, 2006; Pineda et al., 2007; Ayata et al., 2009, 2010) and under some conditions wind-induced physical structure could be an important mechanism of retention of invertebrates larvae (Epifanio et al., 1989; Verdier-Bonnet et al., 1997).

Recently, Herbert et al. (2012) modeled the larval dispersal of the Manila clam *Ruditapes philippinarum* in the Poole Harbour (England) in order to explain its potential spread beyond the point of introduction in the UK. For this purpose, they modeled the larval dispersal adapting the larval behavior submodel of North et al. (2008) but not considering wind effects or any settlement submodel. The dispersion kernel of all positions of larvae over the last three days of life was used to define the major settlement zones.

Miyake et al. (2009) simulated larval dispersal processes for abalone (*Haliotis discus discus*, *H. gigantea*, and *H. madaka*) for evaluating harvest refugia and for identifying effective locations for reproductive sources and potential settlement sites. For this purpose, the authors used passive particles for the larval dispersion simulations and, in absence of a settlement submodel, a competent period for settlement was assumed (from

3 to 7 days after spawning). Stephens et al. (2006) modeled the dispersal and transport of larval New Zealand abalone (*Haliotis iris*) using also a coupled two-dimensional hydrodynamic and Lagrangian particle-trajectory models. Broekhuizen et al, (2011) simulated the dispersal of the oyster *Ostrea chilensis* Philippi in Tasman Bay (New Zealand) using a larval behavior model. The particle-tracking model for simulation of dispersal of different species of pelagic larvae, including benthic invertebrates (e.g. crab), was also applied by Roughan et al., (2011). They assumed larvae to disperse as passive particles and only considered coastal settlement (i.e. settlement occurred when a particle came within a third of a grid box of the coast) which by default accounted for habitat suitability. Recent study focused on a highly commercial fish species (cod, *Gadus morhua*) (Hinrichsen, et al., 2009) considered a settlement probability calculated with respect to habitat suitability regarding the minimum requirement of oxygen saturation for its recruitment success.

In summary, only few biophysical models include a habitat suitability approach in their settlement subroutines. However, they are simplified subroutines and commonly do not consider environmental variables to define the suitable habitat conditions for survival of species. The subsequent aim beyond determining larval dispersal and simplified settlement patterns therefore is to move towards including habitat suitability modeling to better understand recruitment success or post settlement mortality. A better understanding of these aspects will provide fundamental insights into the population dynamics and in fisheries management, since it is determinant to predict the future distribution patterns and stability of benthic populations.

1.6. Interaction between the study species

The spread of invasive species, identified as a major ecological threat in coastal marine communities (e.g. Cohen & Carlton 1998; Occhipinti-Amborgi, 2001; Perrings, 2002; Dawson et al., 2005; Claudet et al., 2010), can affect the native species through several mechanisms such as predation, parasitism, herbivory, vectoring of pathogens, modification of critical habitat, hybridization, and competition (Simberloff, 2000; Dudas et al., 2005; Occhipinti-Amborgi, 2007). The 1992 'Rio' Convention on Biological Diversity included the problems caused by introduced species as a priority item (Mooney and Neville, 2000). Moreover, Elliot (2003) considered that introduced marine organisms can be treated in the same way as chemical pollutants and incites the use of the term biological pollution. Therefore, the invasion research and managers usually consider that nonindigenous origin of a species is relevant to predict and value their negative impacts (Kueffer and Hadorn, 2008). Yet coastal aliens could also play a beneficial role in ecosystem functioning and can result in a net gain of species (Sax and Gaines, 2003) and in an increase of biomass production (Minchin and Rossental, 2002; FAO, 2009). As a consequence, the use of the origin for judging a species is arguable since it could have close affinity to "xenophobia" or "racism" (e.g. Simberloff, 2003; Warren, 2007). Nevertheless, a precautionary approach has been proposed for introduced or invasive species risk assessment, which assumes that an alien species is problematic until proven otherwise (Simberloff, 2005; Wittenberg and Cock, 2001).

In this context, pressure tends to increase on managers to minimize the impacts of nonindigenous species, and on scientists to provide control measures (Bax et al. 2001). Consequently, the study of interactions between the nonindigenous and native marine species has become a focus of conservation biology in order to correct identify potentially injurious nonindigenous species. Moreover, research that contributes to gain insight into the effects of the interaction between introduced commercial species and native communities is of a particular significance from a socioeconomic point of view since it can

affect positively or negatively the fisheries yield (e.g. Seijo et al., 1998; Reaser et al., 2007). In this regard, several species have been deliberately introduced for aquaculture purposes or in order to improve the fisheries yield: the oysters *Crassostrea gigas*, *C. ariakensis*, *C. rivularis*, and *Ostrea edulis* (Grizel and Héral, 1991; Langdon and Robinson, 1991; Mann, 1983), the hard clam *Mercenaria mercenaria* and the softshell clam *Mya arenaria* (Gollasch, 2006; Conde et al., 2010) and the Manila clam *Ruditapes philippinarum* (Breber, 1985). Moreover, the cultures of the Pacific cupped oyster *Crassostrea gigas* and Manila clam *Ruditapes philippinarum* were responsible for the introduction of the largest number (60) of nonnative invertebrates and algae, often attached to packaging material, fouling the shell or parasitizing bivalve tissues (Mineur et al., 2007; Savini et al., 2010).

Currently, *R. philippinarum* has become a widely distributed natural population and one of the most commercially exploited bivalve molluscs along the European coast (i.e. Atlantic coast, Adriatic and Aegean seas) contributing more than 90% to European yields of the two species (Gosling, 2003). As it was mentioned above, in several disturbed estuaries or lagoons of Europe this species has occupied almost entirely the ecological niche of the native clam *Ruditapes decussates* relegating it to occupy very restricted areas (Aubby, 1993; Marin, 2003; Mistri, 2004; Caill-Milly et al., 2008). Occhipinti-Ambrogi (2007) stated that the success of the nonindigenous species after establishment is governed by two different filters: a local dispersal filter, mainly connected to invasion pressure (i.e. introduced repeatedly) and a habitat suitability filter (i.e. environmental and community filter), which determine a species to reach stage of invasion where it is widespread and dominant. However, as it was mentioned above, this extreme dominance pattern of the introduced Manila clam has not yet been detected in other estuaries of Europe such as in Bahía de Santander (Juanes et al., 2012). Consequently, considering the differentiated stages of invasion of Manila clam in European estuaries and taking into account the absence of studies investigating the interaction with the native carpet shell clam or other biotic interactions, nowadays, the definition of this nonindigenous species as a direct threat for the European native clam is too daring.

Alien species can negatively affect native populations through interspecific competition, inter alia. Competition can be regulating the distribution patterns and the abundance of intertidal communities if one species indirectly inhibits the growth of another species inhabiting the same area (Simberloff 2002). Inter-specific competition is, by definition, a negative interaction between two or more species that utilize the same shared, limiting resource (Connell 1983). Resource competition, often measured through density-dependant reductions in growth or survival, has been observed in natural bivalve communities (Peterson 1985, Whitlatch et al. 1997). However, interspecific competition is relatively difficult to demonstrate unequivocally in invaded communities and while competition seems important and common in marine invasions that have examined it empirically, it must be taken into account that only half the studies published have been experimental (Byers, 2009).

Several authors have suggested that competition seems to be an unimportant force in the structure of soft-bottom marine communities in general, since soft-bottom fauna have a third dimension (i.e. depth) and hence, it may not be as effective a structuring force as on hard bottom shores (Dayton, 1984; Peterson, 1991 and Wilson, 1991). Regarding the non native species Manila clam, few experimental studies have been designed to test directly whether resources of any sort are limiting to other infaunal bivalve species or whether competition for limited resources is an important process. Byers (2005) investigated the interaction between *Ruditapes philippinarum* and the native bivalve *Prothoaca staminea* and did not found direct effects of Manila clam on *Protothaca*. Lee (1996) also studied the interaction between Manila clam and *Anomalocardia squamosa* and neither found intense interaction effects. Similarly, Lum (2011) found a lack of competition between Manila clam and varnish clam (*Nuttallia obscurata*).

However, the above mentioned assumption that an alien species is problematic until proven otherwise (Simberloff, 2005; Wittenberg and Cock, 2001) is a central tenant of conservation strategies. Therefore, it is also essential to study the interaction between

Manila clam and the European carpet shell clam in order to support appropriate management measures for the conservation of the native clam and sustainable exploitation of these valuable marine resources.

1.7. Length-weight relationship and minimum catch size

Regarding the sustainability of commercial clams, setting the minimum catch size of is one of the most important and widely used measures in shellfishery management strategies (e.g. Berthou et al., 2005; Gilbert et al., 2006). The estimation and respect of a specific suitable minimum catch size could have important positive effects on the conservation of the native species and on the yield of the clam fishery (e.g. Congleton et al. 2006; Getchis, 2007; Bald et al., 2009; Lum, 2011). Commonly, regional governments in each country sets its own regulations regarding size restrictions, closures, and harvest tools (e.g. Jenkins et al. 1997; Getchis 2007; Juanes et al., 2012). Bald et al. (2009) developed a dynamic model, capable of predicting clam population evolution in response to different management measures and suggested that best management actions were in order of efficiency: (1) the establishment of an appropriated minimum catch size, (2) the reduction in the capture season and (3) the increase of non fishing zones. Freire and García-Allut (2000) also considered the use of protected areas and minimum sizes as key regulation tools in European artisanal fisheries management models.

The studied clam species management models have also been commonly based on setting a minimal size of capture and closing areas through regional annual regulations (e.g. Orden DES/25/2011; Caill-Milly, 2006; Cannas, 2010). Regardless of the existing important biological differences between these species (i.e. Manila clam is more resistant and grew faster than the endemic carpet-shell clam (Usero et al., 1997)) the minimum catch size established across all the north coast of Iberian Peninsula is the same (40 mm) for both species. In other countries of Europe, the minimum catch size is also identical but

smaller (35 mm) for both species (Cannas, 2010). In addition, the minimum legal size established by the European Union for vessels shellfishery was 40 mm for both species to 2007 (Caill-Milly, 2006) and after that it was decreased to 35 mm for Manila clam (Dang, 2010). This situation could have had negative consequences and may be one of the reasons of the overexploitation and displacement of the native carpet shell clam as it was mentioned above (Juanes et al., 2012). Therefore, within this context, there is still a need to investigate the suitable specific catch size of the study species in greater detail, in order to obtain improved yields from the fishery and support the native clam conservation strategies.

In this regard, the estimation of adequate catch sizes for commercial marine invertebrates such as bivalves or crustaceans integrate several biological aspects such as individual size at sexual maturation, growth rate and length-weight relationship (Donaldson and Donaldson, 1992; XUNTA, 1992; Coutures and Chauvet, 2001; Camacho-Mondragon et al., 2012). The length-weight relationship has been studied in various marine species such as fishes (Froese, 2006; Nieto-Navarro et al. 2010) and crustaceans (Pinheiro and Fiscarelli, 2009) using different parametric models. For the grooved carpet shell clam and Manila clam, this relationship has usually been defined by the classic allometric model (Bald and Borja, 2001, 2004; Caill-Milly et al., 2003, 2006; Bradbury et al., 2005); where clam weight (DW, g) is related to shell length (ML, cm) by the equation $DW = a (ML)^b$, being a and b constants. In other bivalve species such as *Macoma baltica* or *Mercenaria mercenaria* this relationship has also been analyzed using the same model (Bachelet, 1980; Hoffman et al., 2006).

Sestelo and Roca-Pardiñas (2011) analyzed the potential of benefit of using the length-weight relationship to objectively estimate and a suitable catch size of the gooseneck barnacle *Pollicipes pollicipes*, in order to obtain, in the long run, the maximum yield from the fishery. These authors used a nonparametric model as an alternative to the classic allometric model to analyze the length-weight relationship. They record specific patterns

in the data at the end of the regression curve that could not be detected by the allometric model. This suggests that some valuable biological information is lost using the allometric model (Rabaoui et al., 2007). This may be due to the fact that the nonparametric models allow for a more flexible fit of the data than the parametric regression techniques because they do not specify in advance any function that links the covariates to the response. Moreover, they proposed the minimum catch size as the one at which the first derivative of the length-weight relationship regression curve reached the maximum, ensuring that over this point weight gain from one size to the next decreases. For this purpose, they used a nonparametric model considering the absence of a maximum in the first derivative of the allometric model. The success in the application of this nonparametric model to other marine commercial species (e.g. bivalves) could confirm this technique as an alternative method to study the length-weight relationship and estimate the specific minimum suitable catch size of species that display a differentiated weight gain pattern throughout its development.

1.8. Objectives of the thesis

The general objective of this thesis is to advance in the understanding of ecological dynamics of the European native grooved carpet shell clam *Ruditapes decussatus* and the worldwide introduced Manila clam *Ruditapes philippinarum* in order to support specific conservation strategies and shellfisheries management. Thus, the present thesis provides an opportunity to investigate ecologically relevant aspects requiring investigation, such as the expansion of the Manila clam, competitive interaction, larval dispersal and recruitment or suitable specific catch size together with the methodologies applied for the assessment of these clam populations in large estuaries have been studied in this thesis. The specific objectives of this thesis are focused on the following aspects:

- 1) To analyze the spatial distribution patterns, the population structure and the stocks of *R. decussatus* and *R. philippinarum*, using a shell-fishermen-based assessment method for large estuaries.
- 2) To predict habitat suitability for both species and explore its role regulating the expansion of Manila clam.
- 3) To examine the significance of competition among these species and predation in a scenario of expansion of the nonindigenous Manila clam.
- 4) To develop a larval evolution model integrating a habitat suitability-based recruitment submodel to determine the major spawning and nursery grounds and assess the potential connectivity between them.
- 5) To assess the suitability of a nonparametric model to study the length-weight relationship of both species and estimate a minimum suitable specific catch size.

1.9. Layout of thesis

The structure of the thesis is organized as follows:

In this Chapter I, the motivations for the research, the study species and the background to the research of the studied aspects are presented first. At the end of this chapter the specific objectives designed to answer the questions raised are outlined and the structure of the thesis is described. In Chapter II, a detailed description of the study site is presented.

The following five chapters (III, IV, V, VI, VII) address the objectives of the thesis. Each of the chapters includes an abstract, a brief introduction and methodology, results and discussion sections and has led to the publication of a research article in a SCI journal.

A brief summary of the investigations conducted in each study chapter is described as follows:

*Chapter III. Characterization of the native European clam *Ruditapes decussatus* and the nonindigenous Manila clam *Ruditapes philippinarum* clam populations in the Bay of Santander*

In this chapter the spatial distribution patterns, the population structure and the stocks of *R. decussatus* and *R. philippinarum* are analyzed in the Bay of Santander in the northern coast of Spain (see Chapter II), with particular attention in coexistence and the relationship between the hydrodynamic patterns and the current distribution of both species. A new sampling methodology is implemented to evaluate the performance of actual management measures.

Chapter IV: Ecological niche modeling approach to predict the potential expansion of a nonindigenous clam

In this chapter ENFA (Environmental Niche Factor Analysis) is used to locate areas that are most likely to provide suitable habitat for the native carpet shell clam *R. decussatus* and the worldwide introduced Manila clam *R. philippinarum* in the Bay of Santander. This study also examines the relative importance of several environmental variables in determining suitable habitat and determines areas where both species potentially coexist and areas where one species dominates in order to know the role of the habitat suitability regulating the expansion of Manila clam.

Chapter V: Expansion of a nonindigenous clam causes competitive impact or is regulated by predation?

A experimental study was conducted in order to examine the significance of competition among these two congeneric species and to explore the impact of the nonindigenous Manila clam on the native carpet shell clam. This study examines the effects of increasing density of *Ruditapes philippinarum* on mortality and growth of *Ruditapes decussates* and the effect of predation (fish, crabs, etc.) on both species in order their role of this biotic factor in the regulation of the expansion of the nonindigenous species.

Chapter VI: Predicting clam larval dispersal and recruitment using habitat suitability-based particle tracking model

A particle-tracking model is developed to study the larval transport, supply and recruitment of *Ruditapes decussatus* and *Ruditapes philippinarum*. The model includes (i) a larval behavior submodel and (ii) a settlement-recruitment submodel based on the habitat suitability resulted from the Environmental Niche Factor Analysis (ENFA) previously conducted. This study examines the effect of location of spawning zones and

hydrodynamic variables (i.e. tide and wind) on larval dispersion. It also determines the major spawning and nursery grounds and the potential connectivity between these grounds.

Chapter VII: Estimating a new suitable catch size for clams

The length-weight relationship of both studied species is estimated using two models: a classic allometric model and a nonparametric regression model based on local linear kernel smoothers. This study also investigates first derivatives of these regressions to estimate a new suitable specific capture size and, thus, obtain the maximum yield from the fishery.

Finally, general conclusions, conservation and management proposals and future research lines are described in Chapter VIII.

Chapter II

Study site

2.1. General environmental characteristics

The Bay of Santander, an important economic, recreational and natural area in the northern coast of Spain (Cantabria, Gulf of Biscay), was the main study site where sampling, experiments and modelling to achieve the objectives of the thesis were conducted or applied. The Bay is located between the 43°28' and 43°24'N of latitude and 3°49'9" and 3°48'8"W of longitude. The area of the estuary is 22.7 km² and the intertidal zone represents 67 % of the total area, concentrated mainly in the tidal flats of the right margin. The estuary is bordered by a sandy barrier linked to the mouth of the river Miera (Figure 2.1). It is important to note that the Santander Bay surface is a harbour area with the port located in the west margin of the Bay. The estuarine ecosystem was significantly stressed by urban and industrial continuous untreated discharges of more than 250,000 inhabitants until June 2001, when the new sewer system came into operation. The main discharge points of urban wastewater are located in the left side of the Bay, which receives, at present, overflows produced when the sewer system exceed its capacity (Echavarri-Erasun et al., 2010; López et al., 2013).

The morphology and functionality of the Bay are conditioned by fillings in the western margin of the estuary and periodical dredges to maintain the navigation channel operative. Galván et al. (2010) classified this estuary as morphologically complex and dominated by intertidal areas and tidal dynamics. The substratum of this area varies from sandy (northern open areas) to muddy (southern and inner areas). Subtidal zones are dominated by shallow waters (< 5m), with maximum depths of 10–12 m along the navigation channel (Juanes et al., 2012) (Figure 2.2). Hydrodynamic conditions are controlled by a semidiurnal tidal regime and 3 m mean tidal range, interacting with variable freshwater inputs coming mainly from the river Miera through the Cubas area in the northeastern area of the estuary, with a mean flow of 8 m³/s, and to a much lesser

large, from small southern streams through the Boo, Tijero and Solía tidal fresh areas (river inlets) (Figure 2.2) (Puente et al., 2002; Galván et al., 2010). Maximum current velocities of about 0.8 m/s are detected in the estuary mouth (Gobierno de Cantabria, 2008). Further details of this estuary and its sand flats are provided elsewhere (e.g. Puente et al., 2002; Galván et al., 2010; Juanes et al., 2012; López et al., 2013).

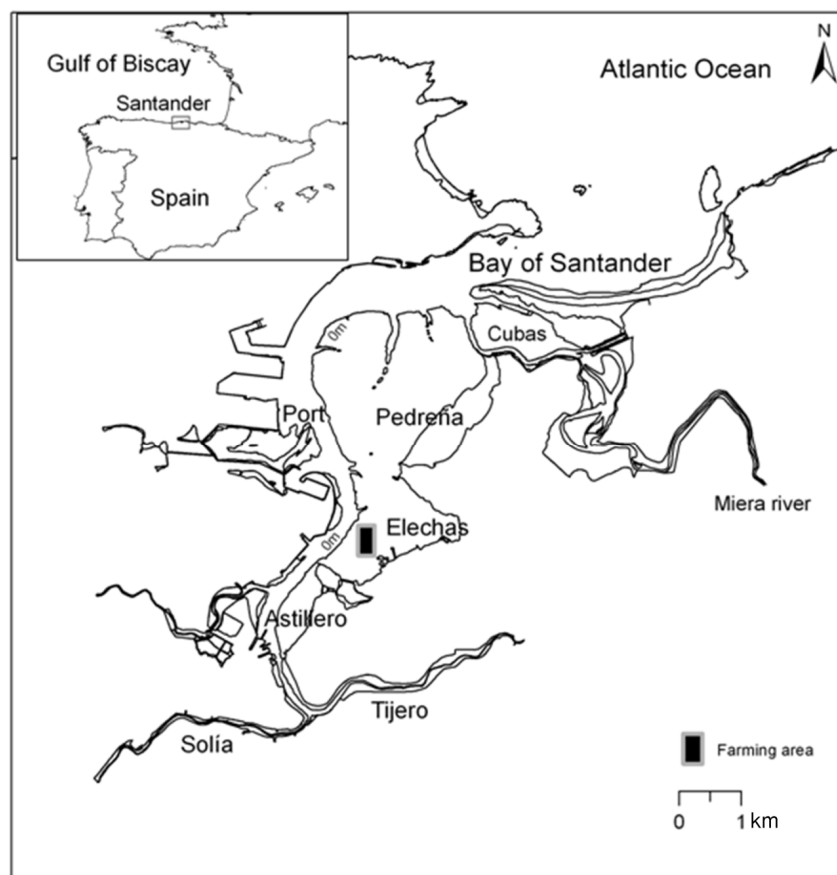


Figure 2.1 - Study area: Bay of Santander estuary, located in the northern coast of Spain (Gulf of Biscay). The main fishing tidal flat names and the Manila clam farming area are presented.

The Bay of Santander houses important meadows of *Zostera marina* and *Nanozostera noltii*, the only two seagrass species present in the local sea environment. Furthermore, 8 habitats included in the Habitat Directive have been recognized in the estuary. Macroinvertebrates communities are among the most diverse and richest in the region. It

is remarkable Highlight the presence of the “Abra” community, which is the richest community identified in the “Cantabrian” estuaries and the high ecological value of some zones, specifically those located in the right side of the estuary and the areas directly influenced by the “Miera” river. Some of these zones are protected as an SCI according to the Habitat Directive. Moreover, it is important to note that that one of the largest and more productive areas of molluscs’ production in northern coast of Spain is located in this estuary.

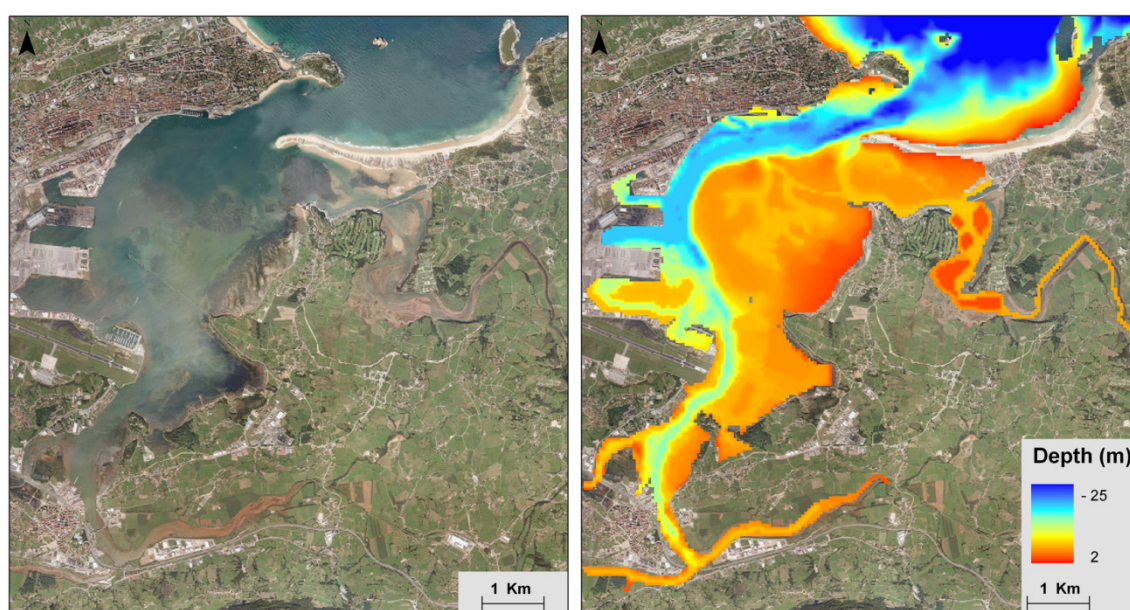


Figure 2.2 – Ortophotograph (left) and bathymetry (m) (right) of the study area (Bay of Santander).

Marismas de Santoña

In addition to the Bay of Santander, Marismas de Santoña wetlands were also considered as study site to achieve the objectives of Chapter VII. This estuary, located close to the Bay of Santander, is also an important estuary in northern Spain (Gulf of Biscay) with 18.7 km²

(Figure 2.3). Galvan et al. (2010) classified these transitional waters as morphologically complex and dominated by intertidal areas and tidal dynamics. The intertidal area represents 57 % of the total area of Marismas de Santoña, where shellfishing of *Ruditapes philippinarum* and *Ruditapes decussatus* bivalves is also conducted using traditional techniques (i.e. hand rakes, knives) like in the Bay of Santander.

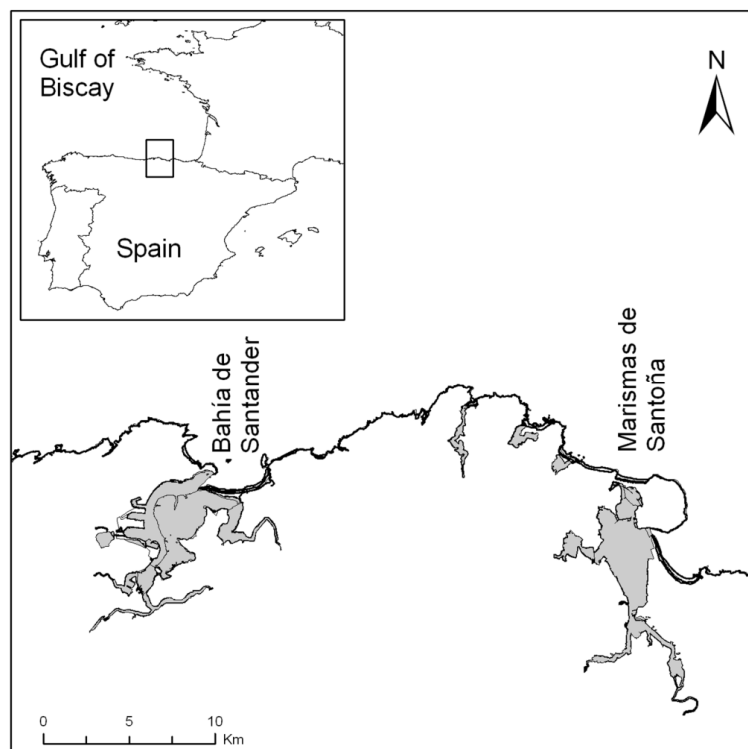


Figure 2.3 – Bay of Santander (Bahía de Santander) and Santoña estuary (Marismas de Santoña) located in the northern coast of Spain.

The substratum of this area varies from sandy (open and more oceanic areas) to muddy sediments (inner and more estuaric areas). Subtidal zones are dominated by shallow waters (< 5m) (Figure 2.4) and hydrodynamic conditions are controlled by a semidiurnal tidal regime and 3 m mean tidal range, interacting with variable freshwater inputs from Asón river. Further details of these estuary and sand flats are provided elsewhere (e.g. Irabien et al., 2008; Galván et al., 2010).

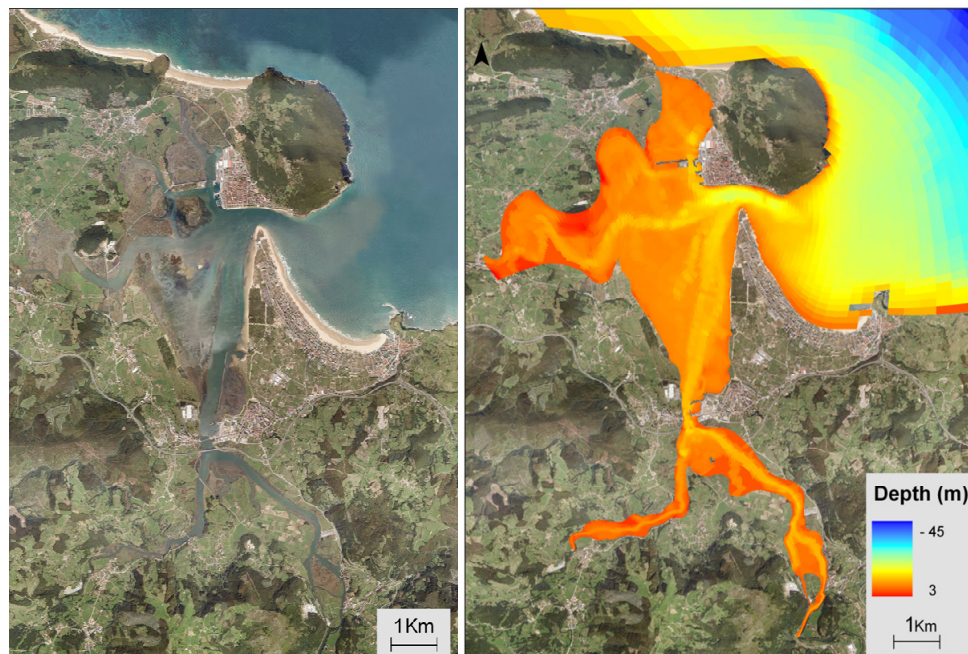


Figure 2.4 – Ortophotograph (left) and bathymetry (m) (right) of the study area (Marismas de Santoña).

2.2. Shellfishing and management

In the region of Cantabria, living largely in the coastal environment, the economic and ecological importance of shellfishing represents a feature to consider in the design and implementation of the management model of these resources. In addition, the shellfishing sector has a large social impact due to its cultural interest. It constitutes a historical and cultural heritage of the region (Gómez Navedo, 2002) since the type of extraction is performed by hands usually by women and is a traditional activity strongly rooted in the communities located in the vicinity of the estuaries and the coast. The characteristics of this activity and each resource exploited, together with the social and environmental characteristics of each geographical area, provide a note of uniqueness that must be considered in its management.

In the intertidal flats of the Bay of Santander and Marismas de Santoña, several species of infaunal bivalves are largely distributed. Regarding shellfishing species, the two most widely distributed commercial bivalves are the native carpet shell clam (*Ruditapes decussatus*) and the introduced Manila clam (*Ruditapes philippinarum*). Moreover, other important shellfishing species such as razor clams (*Solen marginatus* and *Ensis* spp.) and species used as bait for fishing such as infaunal crabs (*Upogebia* spp. and *Callinasa* spp.) or worms (*Diopatra neapolitana*, *Arenicola marina* and *Sipunculus nudus*) are also common.

The carpet shell clam and the Manila clam present highest capture rates (80% of total) and commercial interest (70% of total trade value of seafood removed with a maximum of 725000 euros in 2006). In the Bay of Santander, although the captures are variable, 40 t of clams has been declared in last years (10 t corresponds to carpet shell clam and 30 t to Manila clam). In Marismas de Santoña, in last years, around 30 t of clams has been captured (10 t of carpet shell clam and 20 t of Manila Clam) (data provided by the Fisheries Department of the Government of Cantabria).

The shellfishing of clams is usually done by women in the intertidal zones, using artisanal techniques such as looking for holes (i.e. marked by clam's siphons) and extracting clams using a knife or a hand rake (Figure 2.5, right and 2.6). Besides, some culture parks are located on the central south-eastern part of the Bay of Santander, where high densities of Manila Clam are farmed (Figures 2.1 and 2.5). The captures data provided by the Fisheries Department of the Government of Cantabria include extractions from the farming areas within the total capture data.



Figure 2.5 – Professional shellfishermen working at Manila clam culture zones (left) and a shellfisherwoman fishing clams by hand-raking the sediment (right) in the Bay of Santander (N Spain).



Figure 2.6 – The artisanal clam fishing technique based on hand raking of the sediment (left) and collecting the clams appearing to the surface (red circle) when the sediment is turned over by the rake (right).

To date, efforts in relation to the shellfishing management in the region of Cantabria have been aimed primarily at regulating the extraction by enacting various regulations regarding the establishment of shellfishing zones, minimum catch sizes, closed areas and seasons and licensing and authorization systems. This regulation is specified in an Order of the Cantabria published annually (eg. GAN/35/2005 Order of 17 June, Regulating the

closures, minimum size and collection of shellfish and other species of commercial interest for the 2005 season in the Region of Cantabria). Thus, regarding the study species, until now, the management of the fishery has been based on setting the same minimal size of capture for both species (total length of 40 mm) and seasonal closed areas by the mentioned regional regulation, regardless of the distribution patterns and biological differences between species. Moreover, regulatory controls on the quality of the shellfishing zones and resources are conducted according to the legislation.

The only scientific available information in the Bay of Santander regarding this species is about their biometrical relationships of *R. decussatus* (Arnal and Fernández-Pato, 1977:1978) and clam (nonspecific to 2007) annual captures from fishery statistics data. Hence, the lack of data has not allowed the implementation of scientific-based management measures and not even the possibility of making a specific assessment of exploitation status or interaction effects between clam species. Within this context, in 2005 and 2010, the regional Government conducted shellfish population assessments and several scientific studies through the Environmental Hydraulics Institute – IH Cantabria (University of Cantabria) (IH Cantabria, 2005:2011) in order to support the management of the shellfishery in the coast of Cantabria. This thesis has been based on data obtained during these two projects.



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Chapter III

Characterization of the native clam *Ruditapes decussatus* and the nonindigenous Manila clam *Ruditapes philippinarum* populations in the Bay of Santander

Chapter III. Characterization of the native clam *Ruditapes decussatus* and the nonindigenous Manila clam *Ruditapes philippinarum* populations in the Bay of Santander

*This study has led to the publication of a research article in the Journal Ocean and Coastal Management Journal, vol. 69, pp. 316-326, by Juanes, J.A., Bidegain, G., Echavarri-Erasun, B., Puente, A., García, A., García, A., Bárcena, J.F., Álvarez, C., García-Castillo, G., in 2012 with the title "Differential distribution pattern of native *Ruditapes decussatus* and introduced *Ruditapes philippinarum* clam populations in the Bay of Santander (Gulf of Biscay). Considerations for fisheries management".*

Abstract

The aim of the present study is to provide a first characterization of the grooved carpet shell clam *Ruditapes decussatus* (native) and the Manila clam *Ruditapes philippinarum* (nonindigenous) populations in the Bay of Santander in order to improve the management of these commercially exploited resources. For this purpose a field survey was carried out in different fishing areas where samples were taken on transects, following artisanal shellfisher exploitation techniques. Size frequency distributions, densities and stocks were evaluated for different fishing zones. In addition, a hydrodynamic model was applied in order to understand larval transport and recruitment patterns associated to the tidal currents and water flow. Within this context, the first evaluation of the clam populations in the Bay of Santander showed: (a) that fishing activity is performed on individuals under the minimum legal size (40 mm) and in closed areas, (b) a significant differences on density by zone (c) a distribution pattern with areas where both species coexist and areas where one of them dominates, (d) *R. decussatus* occurs at relatively low density in stations near the culture parks and (e) a limited recruitment in the inner parts of Cubas tidal fresh

for *R. philippinarum* and in the southern zones for *R. decussatus*. Based on this study, some managing guidelines are presented mainly focused on avoiding the overfishing of the native clam *R. decussatus*.

3.1. Introduction

Although, the knowledge of the biology of the studied species is wide (e.g. Pérez-Camacho, 1979; Pérez-Camacho et al., 2002:2003; Solidoro et al., 2000; Melia et al., 2004.; Flye-Sainte-Marie, et al., 2007a,b) and several stock evaluations have been done in nearby estuaries in Spain and France (e.g. Borja, 1989:2000; Bald and Borja, 2001:2005; Caill-Milly et al., 2003:2006), the only available information in the Bay of Santander regarding this species is about their biometrical relationships of *R. decussatus* (Arnal and Fernández-Pato, 1977:1978) and clam (nonspecific) annual captures from fishery statistics data. Hence, the lack of data has not allowed the implementation of scientific-based management measures and not even the possibility of making a specific assessment of overexploitation or interaction effects between clam species.

In this sense, several studies on diverse topics of stock assessment methodologies on molluscs (Palacios *et al.*, 1994:2000; Rueda and Urban, 1998; Orensanz *et al.*, 2003) and different sampling methodologies (sampling grid, size of quadrat sampled, mesh size) (Byers, 2005; Lee, 1996; Borja and Bald, 2000; Bald and Borja, 2001:2005; Caill-Milly et al., 2006; Morsan, 2007) have been carried out for clam population evaluations. However, the lack of a standardized sampling methodology and their expected high time and resource consumption for large areas requires the implementation of assessment procedures that combine the appropriated technical design with the fishermen experience.

Moreover, the importance of larval abundance and dispersion in determining the recruitment of benthic marine invertebrates, which presents a high spatial and temporal

variability (Borsa and Millet, 1992; McLachlan et al., 1996; Ripley and Caswell, 2006; Herbert et al., 2012) and the structure of their communities has been emphasized by several authors (Roughgarden et al., 1988; Pineda, 2000; Roegner, 2000), noting that larval transport within an estuary is largely dependent on hydrodynamic patterns. Therefore, the analysis of the influence of the flow of water and tidal currents on the distribution pattern of clam populations appears to be essential to understand other aspects of the dynamics of these species that will aid in decision making for resource management.

In this context, the main goal of the present study is to analyze the spatial distribution patterns, the population structure and the stocks of *R. decussatus* and *R. philippinarum*, with particular attention in coexistence and the relationship between the hydrodynamic patterns and the current distribution of both species. It is remarkable that larval dispersal and recruitment patterns are analyzed in detail using a biophysical model in Chapter VII. It is expected that results of this study may contribute to assess the feasibility of the new sampling methodology implemented to evaluate the performance of actual management measures and to allow for proposals of new management actions.

3.2. Material and methods

3.2.1. Sampling and laboratory procedures

Abundance, biomass, and population size structure for carpet-shell clam and Manila clam were analyzed in 23 stations (Figure 3.1), placed in areas where commercial operation of the resource is conducted in the Bay of Santander. These areas were selected by compiling information from shellfishermen, fishing inspectors and technical staff of the Main Directorate of Fishing.

Sampling was conducted during low tides (semidiurnal tide) in April 2005. At each station individuals were extracted by a professional shell-fisherman by means of the hand raking of the sediment (upper 15 cm) in a unique 10 m x 1m transect (Figure 3.2) This operation was similar to their fishing extraction technique. Taxonomic determination of each individual was carried out in the laboratory, followed by fresh weight (FW, g) and shell length (± 0.1 mm) measurements.

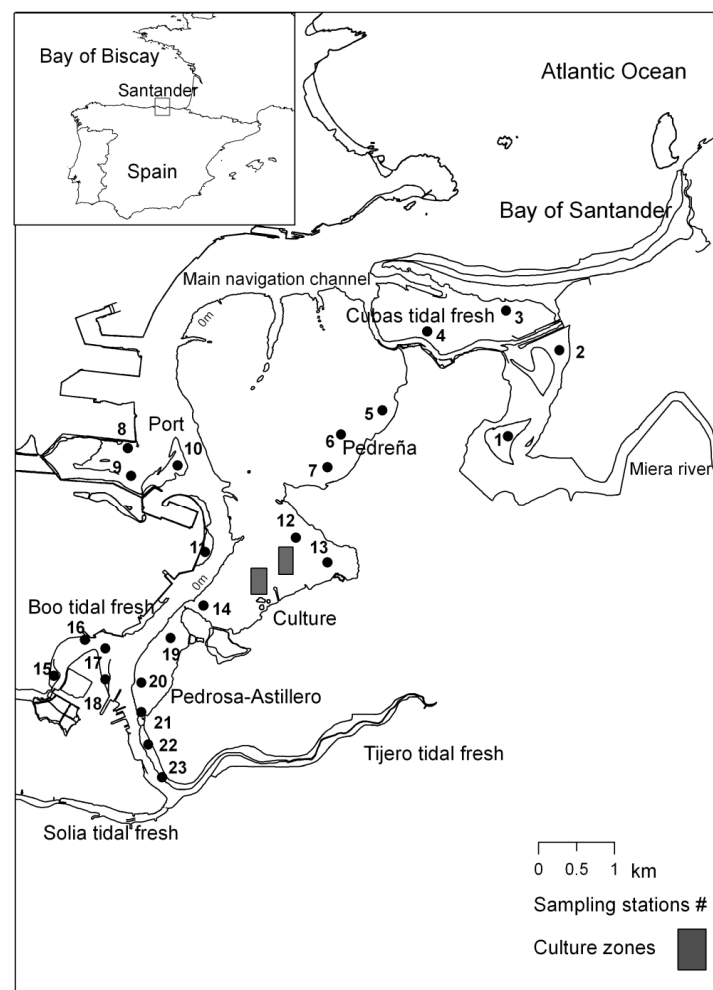


Figure 3.1 - Location of sampling stations and clam culture zones in the Bay of Santander.



Figure 3.2 – Shellfisher-woman extracting clams by hand raking of the sediment in a 10 m x 1m transect in Cubas tidal fresh flats (NE Bay of Santander).

3.2.2. Data analysis

Distribution patterns and standing stocks

Total abundance of both species of clams collected in each station (i.e. 10 m² transect) was used to describe the general distribution pattern of clams in the Bay. Further analysis of the relative abundance of each species (i.e. ratio between *R. decussatus* (*RD*) and *R. philippinarum* (*RP*) abundances) in each station was the base for establishing the criteria

for dominance ($RD/RP > 0.8$) or coexistence ($0.2 < RD/RP < 0.8$). The distribution pattern of this coexistence was used to group stations based on spatial proximity and similar characteristics in terms of species relative presence. Then, size frequency distributions density and standing stocks were analyzed by these zones.

Size frequency distributions were calculated to estimate recruitment patterns and potential effect of fisheries on clam population structure. A Kruskal-Wallis ANOVA by Ranks analysis was used to detect the effect of zone over the distribution of individuals above the minimum legal length for capture (i.e. 40 mm). The distribution pattern of the two bivalve populations was evaluated by calculating the variance-to-mean-ratio (Krebs, 1989; Schneider, 1994). Spearman Rank correlation between abundance of both species was calculated to explore whether there are indicators of a possible competition between the two bivalves (Wilson, 1983).

On the other hand, the following equation was used to calculate the stock (t) of species for each zone:

$$S = (D \times FW \times A) \times 10^{-6} \quad (1)$$

where D is the density of the species standardized to n° of ind/m², FW (g) is the mean individual fresh weight on the established area and A is the area (m²) of each fishing zone. All the information was placed in a GIS using the program ArcGis 9 (Figure 3.1), where the calculations of the surfaces of the fishing zones were carried out using the bathymetry of the Bay of Santander as the base and delimiting them by the lower and upper limit of the observed distribution of both species on the intertidal area (i.e. -0.5m to - 1.5m). Error of each variable was estimated by the standard deviation and coefficient of variation, following the Taylor's (1982) methods for products of variables in which the uncertainties are at random and there is independency. Therefore, the deviation of the coefficient of variation of the stock in each area was calculated, with an interval of confidence of 95 %,

following the proposal of Hand and Bureau (2000). The total available stock of the estuary was considered as the sum of the stocks of all fishing zones. The coefficient of variation of the standing stock (CV_s) was calculated as it is shown in equation 2 for each zone.

$$CV_s = \sqrt{CV_D^2 + CV_{FW}^2} \quad (2)$$

where CV_D is the coefficient of variation of density and CV_{FW} is the coefficient of variation of fresh weight.

A Kruskal-Wallis ANOVA by Ranks analysis was used to analyze differences in clam mean density between the different zones. Moreover, a Mann-Whitney U Test between paired of zones was done to detect those with a significantly different density.

Hydrodynamics and recruitment

Finally, the highest and lowest flow situations, which can be observed at medium and high flood tide respectively, are analyzed in order to relate the tidal currents and flow with the spatial distribution of clams considered as recruiters. For this purpose, the individuals within the size class considered as the “recruitment length to the fishing gear” were considered as recruiters. This concept has been widely used in different species fisheries management (e.g. Gordo & Molí, 1997; ICCAT, 2009) when the new recruits are not vulnerable to the fishing gear. This size class was estimated using a Spearman correlation analysis between different size classes’ abundance and total abundance in each station. Thus, the smaller size class which was correlated ($p < 0.05$) with the total abundance was selected as the recruitment length of the fishing gear. Secondly, water elevation and velocity fields were calculated using a two-dimensional hydrodynamic coastal and estuarine model, namely H2D model (Castanedo et al., 2006; Garcia et al., 2010). This model solves the two-dimensional vertically integrated hydrodynamic equations. The numerical computation was carried out on a spatial domain that represents the entire

estuary through a finite-difference and two-dimensional grid, covering Bay of Santander and its adjacent coastal zone, represented horizontally using a mesh of 199 X 253 uniform grid squares each with a length of 51 m. The hydrodynamic model was calibrated and validated against water levels and current velocities collected in a field campaign conducted by the University of Cantabria during the period from 19th to 30th May 1997, covering a full phase of spring and neap tides (López et al., 2013).

The simulation was conducted for a complete 12-h tidal event with fixed conditions of tidal wave amplitude (1.38 m) and Cubas river flow (1.16 m³/s), obtaining hourly flow (m³/s) and tidal current velocities (m/s) in each cell. These fixed conditions are the median values (percentile 50%) calculated for the time interval observed during the periods of release of *R. decussatus* and *R. philippinarum* larvae (i.e. April-November 2003-4)(Rodrigues-Carballo et al., 1992; Rodríguez-Moscoso and Arnaiz, 1998; Urrutia *et al.*, 1999; Rodríguez-Moscoso *et al.*, 1992; Ojea *et al.*, 2005) related to the cohort which in April 2005 could be within the size class considered as recruitment length of the fishing gear. From these results, different hydrodynamic zones were defined according to their flow (m³/s) and tidal current velocities (m/s) in the highest flow situation (medium flood tide) and the hydrodynamically most stable situations (high tide).

Considering that most of the larval pool is exported to the nearshore in ebb tide and the entrance of larvae in flood tide is correlated with the flow of water (Roegner, 2000), a Kruskal-Wallis ANOVA by Ranks and Mann-Whitney U-test analysis was used to explain differences of “recruitment” between previously defined hydrodynamic zones.

3.3. Results

3.3.1. Distribution patterns of clam populations

A total of 831 individuals of *R. decussatus* and 849 individuals of *R. philippinarum* were collected, giving an approximate total *R. decussatus*/*R. philippinarum* individuals ratio (RD/RP) of 1:1. The abundance of both species collected in each transect is shown in Figure 3.3. A higher abundance of total clams (i.e. sum of both species abundances) is observed in stations 4, 5, 6, 7 and 10, presenting between 100 and 300 total clams per station (10 m²). Maximums of 293 and 98 individuals of *R. decussatus* were observed at stations 4 and 6, respectively and maximums of 85 and 223 individuals of *R. philippinarum* at stations 5 and 7, respectively.

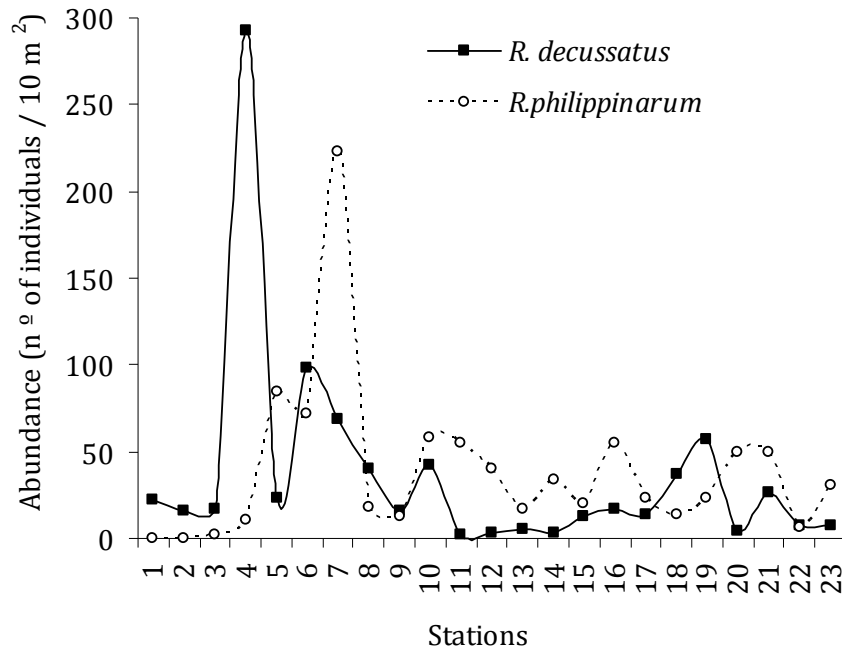


Figure 3.3 - Abundance of *R. decussatus* and *R. philippinarum* at each station.

The inner part of Cubas tidal fresh (stations 1-3), the zone around culture parks (stations 12-14), and southern inner areas (15, 22, 23) presented the lowest values of total clams (less than 50 individuals per 10 m²). Regarding the relative abundance for each station, the dominance of one species is found in two areas: *R. decussatus* (RD) dominates in Cubas tidal fresh zone (stations 1-4) and *R. philippinarum* (RP) in Culture zone (stations 11-14) (Figure 3.3), showing a mean RD/RP ratio of 0.96 ± 0.05 and 0.10 ± 0.08 , respectively. Besides, coexistence of both species was observed on both margins of the central area of the Bay (i.e. in the Port zone, stations 8-10; Pedreña zone, stations 5-7), with 0.34 ± 0.20 and 0.55 ± 0.13 mean ratios, respectively, and mainly on the southern part of the Bay. In this latter area, two zones were defined: Boo tidal fresh zone (stations 15-18), where a clear coexistence pattern of clam species is observed (0.43 ± 0.20) and Pedrosa-Astillero zone (stations 19-23), showing a mean RD/RP ratio of 0.28 ± 0.20 , although *R. philippinarum* is the predominant species in two out of five stations. Abundances of both species showed significant deviation for randomness (Chi-Square test for goodness-of-fit, $p < 0.05$) with exceedingly large variance-to-mean ratios (*R. philippinarum*: 5.53; *R. decussatus*: 10.24) which are significantly larger than 1, indicating a highly aggregated distribution of clams for both species. Spearman Rank correlation analysis between the total abundances of both clams for all stations (N=23) did not show any significant correlation ($R=0.09$, $t(N-2)=0.42$, $p=0.67$).

3.3.2. Population size structure

Based on that division of zones within the Bay, the distribution of size frequency of both species for each zone are presented in Figure 3.4. The number of individuals of *R. decussatus* encountered in Culture zone (n=11) and of *R. philippinarum* in Cubas zone (n=13) was considered too small for a reliable size frequency data analysis. The greater percentage of individuals of both species was set between 25 and 35 mm of size, falling

drastically from the intervals 30-35 m. This fall is more accused in Cubas and Boo zones for *R. decussatus* and in Pedreña for *R. philippinarum*. Although this pattern was similar for both species, 25-30 mm was the class size with a slightly greater percentage of individuals for *R. decussatus* and 30-35 mm for *R. philippinarum*.

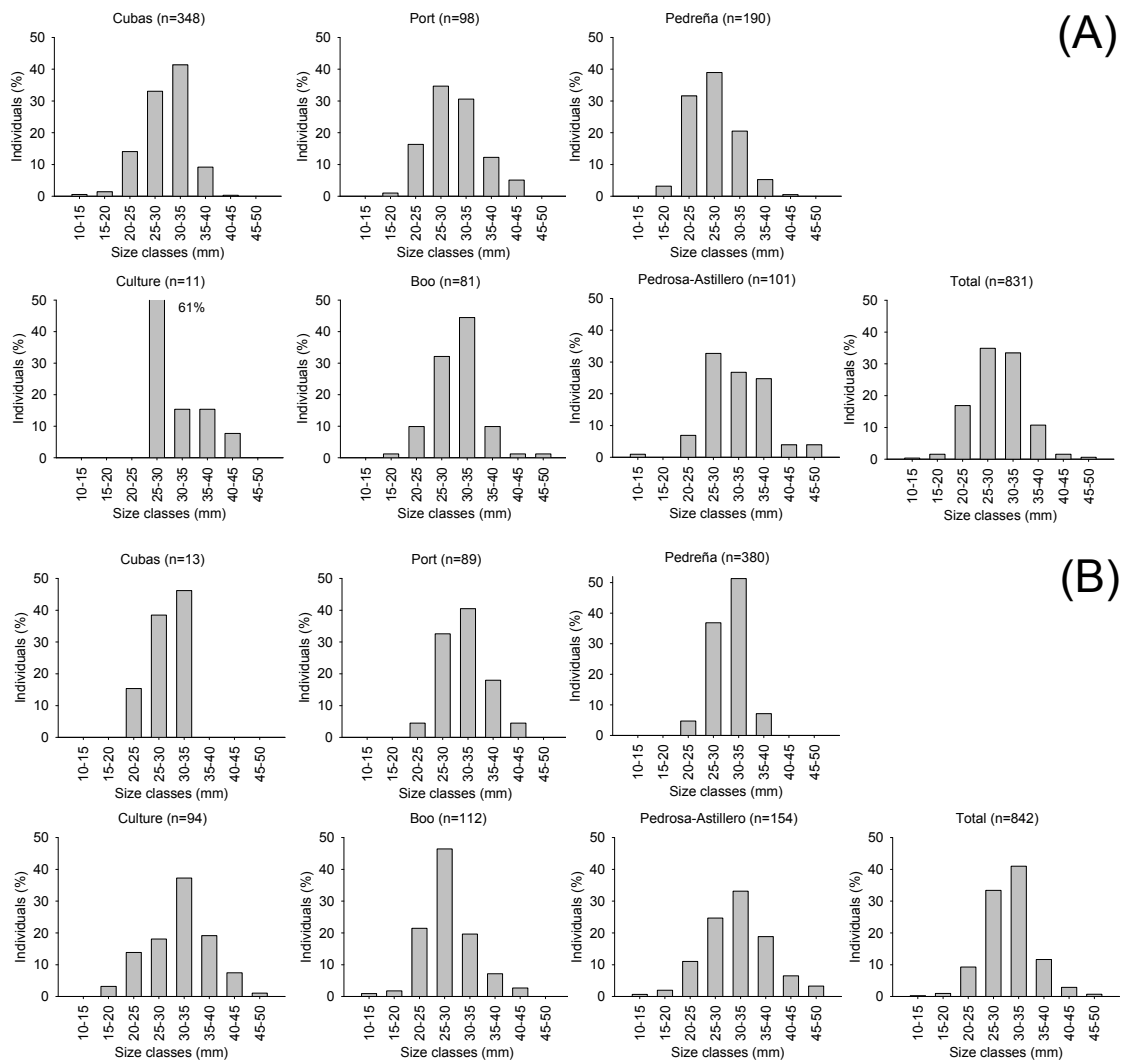


Figure 3.4 - Size frequency distribution of *Ruditapes decussatus* (A) and *Ruditapes philippinarum* (B) populations in each of the 6 fishing zones defined in the whole Bay area.

The abundance of individuals larger than 40 mm (legal size of capture) is not significantly different between zones: Kruskal-Wallis ANOVA, for >40 mm, $H(5, N=23)=3.79$ $p=0.58$ and $H(5, N=23)=6.20$ $p=0.28$, respectively for *R. decussatus* and *R. philippinarum*. Besides, it is observed a lack of individuals <20 mm and a total absence of individuals <10 m. The first size class presenting a significant percentage of individuals was 20-25 mm, which present a higher percentage of individuals for *R. decussatus* than for *R. philippinarum*. The higher percentages of this class size are observed in the north and central zones for *R. decussatus* (Cubas, Port, Pedreña) and in the southern and inner zones for *R. philippinarum* (Culture, Boo and Pedrosa-Astillero).

3.3.3. Clam densities and standing stocks

Considering results from all stations, the Mann-Whitney U-test showed that there was not a significant difference between densities of *R. decussatus* and *R. philippinarum*. Besides, considering fishing zones, Manila clam showed the highest mean density in Pedreña and the highest individual mean biomass in Pedrosa-Astillero. These highest values were higher than *R. decussatus*' ones, which showed the highest density in Cubas and Pedreña and the highest biomass in Pedrosa-Astillero (Table 1). The coefficients of variation (CV) were not very different between species except in some zones (Boo, Port). In most of the cases CV (%) were about 40-70 %. The Kruskal-Wallis ANOVA by Ranks analysis shows significant differences in density between zones for *R. philippinarum* ($H(5, N=23)=14.33$, $p=0.013$) and for *R. decussatus* ($H(5, N=23)=14.12$, $p=0.014$). For *R. decussatus* density was significantly lower in Culture zone when compared with the remainders ($p=0.02-0.04$). For *R. philippinarum* density was significantly lower in Cubas ($p=0.01-0.05$) and was significantly higher in Pedreña ($p=0.02-0.04$).

<i>Ruditapes decussatus</i>							
Fishing zone	Density (ind/m ²)			Biomass (FW, g)			Area * 10 ³ (m ²)
	Mean	SD	CV	Mean	SD	CV	
Cubas	8.70	13.74	1.58	4.53	1.82	0.40	760
Pedreña	6.33	3.78	0.60	3.35	1.72	0.51	630
Port	3.27	1.45	0.44	4.63	2.52	0.55	400
Culture	0.33	0.13	0.39	5.49	2.61	0.48	880
Boo	2.30	2.24	0.97	5.08	2.23	0.44	220
Pedrosa-Astillero	2.03	1.13	0.56	7.52	4.80	0.64	320
Total	3.91	4.65	1.19	4.56	2.44	0.54	3210

<i>Ruditapes philippinarum</i>							
Fishing zone	Density (ind/m ²)			Biomass (FW, g)			Area * 10 ³ (m ²)
	Mean	SD	CV	Mean	SD	CV	
Cubas	0.33	0.53	1.62	5.18	2.23	0.43	760
Pedreña	12.67	8.37	0.66	5.55	1.82	0.33	630
Port	2.97	2.47	0.83	7.16	3.97	0.55	400
Culture	3.65	1.57	0.43	7.25	4.35	0.60	880
Boo	3.20	1.87	0.59	6.16	3.63	0.59	220
Pedrosa-Astillero	2.80	1.84	0.66	9.49	6.49	0.68	320
Total	3.61	6.08	1.68	6.66	4.02	0.60	3210

Table 3.1 - Summary of statistical parameters (mean, SD and CV) for densities (individuals/m²) and mean individual biomass (FW,g) in each fishing zone together with their estimated areas (m²).

The standing stocks of both species for each fishing zone are presented in Figure 3.5. The coefficients of variation for density and fresh weight were likely high and this aspect implies high values of the coefficient of variation of stock for all zones. The total stock (sum of all areas' stocks) for *R. decussatus* was 58 t and 90 t for *R. philippinarum*.

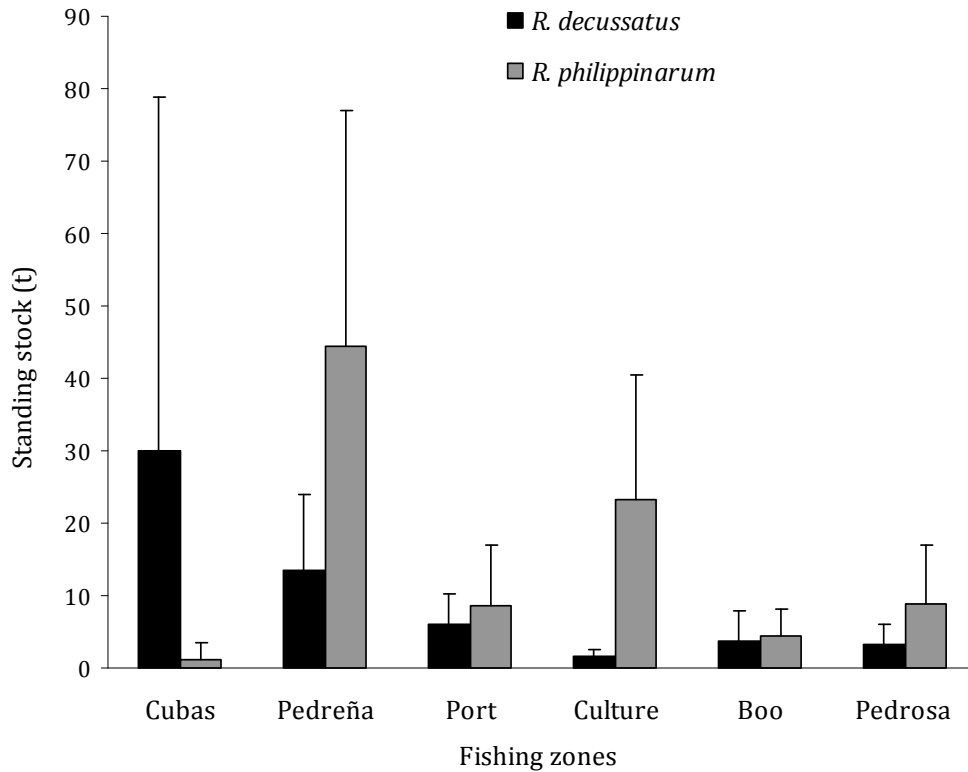


Figure 3.5 - Estimation of standing stocks of *Ruditapes decussatus* and *Ruditapes philippinarum* for each fishing zone. CV (%) of the standing stock is presented with error bars.

3.3.4. Relationship between hydrodynamics and spatial distribution of clams

Results of hydrodynamic modelling are presented in Figure 3.6. At medium flood tide a high flow of water (463 m³/s) is observed from the mouth of the estuary to the main navigation channel (main flow). This water flow is the responsible of the water input to all zones except the Cubas zone. The secondary water flow at medium flood on Cubas tidal fresh mouth is significantly lower (53.5 m³/s) (Fig. 5b). In this tidal situation the higher tidal current velocities are also located on the mouth of the Bay (0.83 m/s) and in the main

navigation channel (0.30 m/s). Moreover, in the high tide the water flow and current velocities are much reduced all across the Bay, presenting a clear circulation cell (eddy) from the central to the northern part of the estuary affecting to Port and Pedreña zones (Fig. 3.6c). Kruskal-Wallis ANOVA by Ranks analysis shows significant differences in current velocities in high tide between Cubas zone, Port+Pedreña zone and Culture+Boo+Pedrosa zone; $H(2, N=23)=11.17$, $p=0.003$, with lowest velocities encountered in this southern zone. Thus, three different zones were established in terms of observed flow and current values on clam sampling stations: Cubas zone (affected by secondary flow), Port and Pedreña zones (affected by the main flow and circulation cell or eddy in high tide), and Culture, Boo and Pedrosa zones (affected by main flow and with no eddy presence).

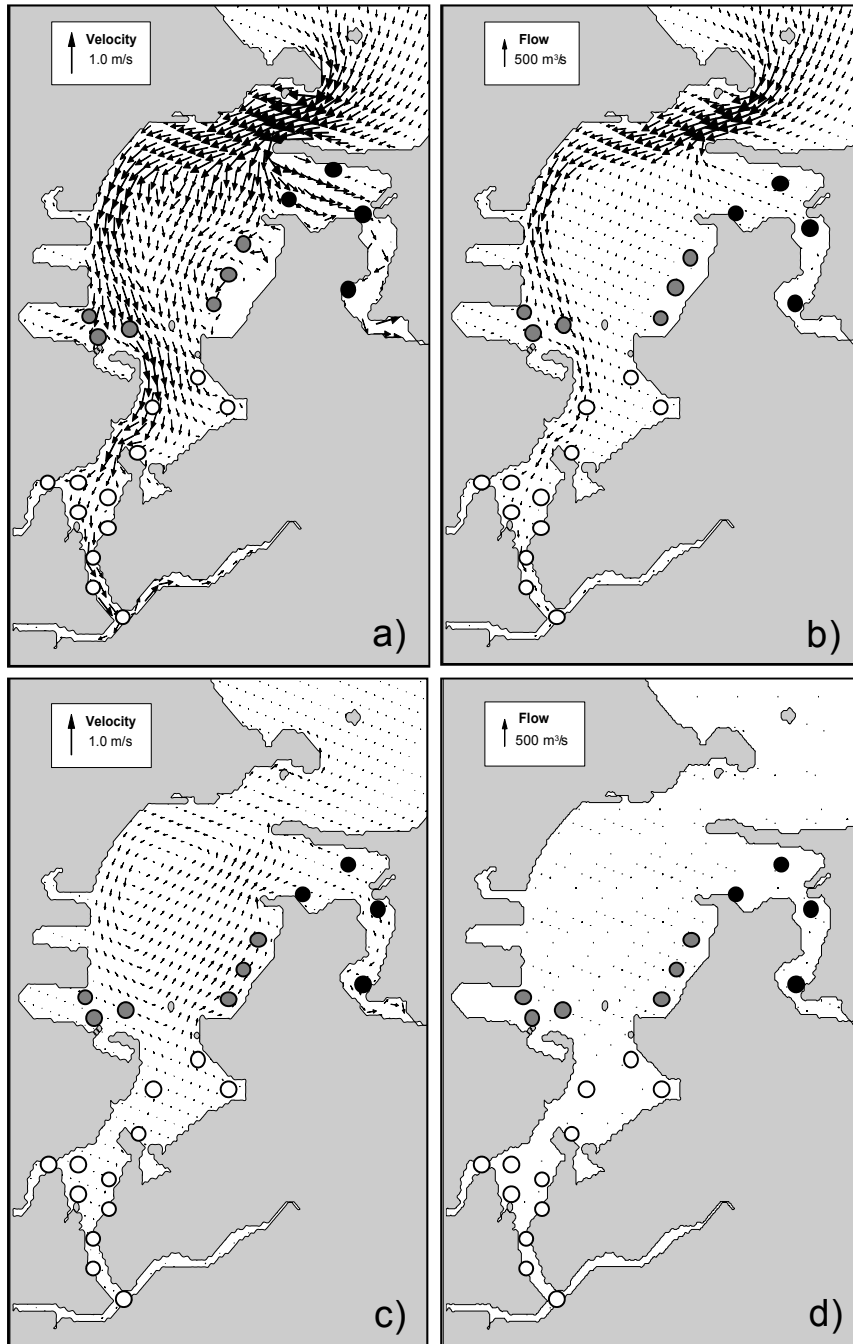


Figure 3.6 - Graphic representation of tidal current velocity (m/s) (a,c) and water flow (m³/s) (b,d) values for medium flood tide (a,b) and high tide (c,d), coinciding with the highest flow and the most stable situation, respectively. Stations are coloured according to the main influence of water flow to Cubas tidal fresh (black), main channel flow (grey) or main flow + eddy (white) on their hydrodynamic features.

According to results of the correlation analysis (Table 3.2) the abundance of 20-25 mm clams was the smaller size class abundance significantly correlated with total abundance for both species ($R=0.84$ for *R. decussatus*, $R=0.68$ for *R. philippinarum*, $p<0.05$). In consequence, this size class was selected as the recruitment length of the fishing gear.

Size (mm)	<i>R</i> (Spearman Rank)	
	<i>R. decussatus</i>	<i>R. philippinarum</i>
10-15	-0.25	0.12
15-20	0.39	0.21
20-25	0.84 *	0.68 *
25-30	0.74 *	0.87 *
30-35	0.91 *	0.91 *
35-40	0.82 *	0.55 *
40-45	0.32	0.08
45-50	0.48 *	0.09

Table 3.2 - Correlation coefficients of Spearman Rank analysis (*R*) between different size classes' abundance and total abundance for all stations ($N=23$) and both species. (* = $p<0.05$).

According to this concept, predominant recruitment of *R. decussatus* occurs in the northern area of the estuary corresponding to outsider station of Cubas (station 4), Pedreña and Port zones (5-10), with a low or null abundance of both species' recruiters in the inner stations of Cubas (1-3). Besides, a predominant recruitment of *R. philippinarum* occurs in Culture zone (11-14), Boo (15-18) and Pedrosa-Astillero (19-23), with a marked null presence of *R. decussatus* 20-25 mm individuals in Culture zone. Taking into account previously established hydrodynamic zones, the Kruskal-Wallis ANOVA by Ranks analysis showed significant differences in recruitment (abundance of 20-25 mm size clams) between these zones ($H(2, N=23)=14.40$, $p=0.006$) for *R. decussatus* and almost significant

differences ($H(2, N=23)=5.25, p=0.07$) for *R. philippinarum*. For *R. decussatus* Mann-Whitney U-test shows that abundance of 20-25 mm clams was significantly higher at stations influenced by secondary flow (Cubas zone) ($U=7.5, Z=2.09, p=0.03$) and by main flow with eddy (Pedreña + Port) ($U=7.5, Z=2.76, p=0.005$) when compared with stations influenced by main flow without eddy (Culture + Boo + Pedrosa-Astillero). For *R. philippinarum* recruitment was higher at stations influenced by main flow (with and without eddy) when compared with stations influenced by Cubas flow (Figure 3.7).

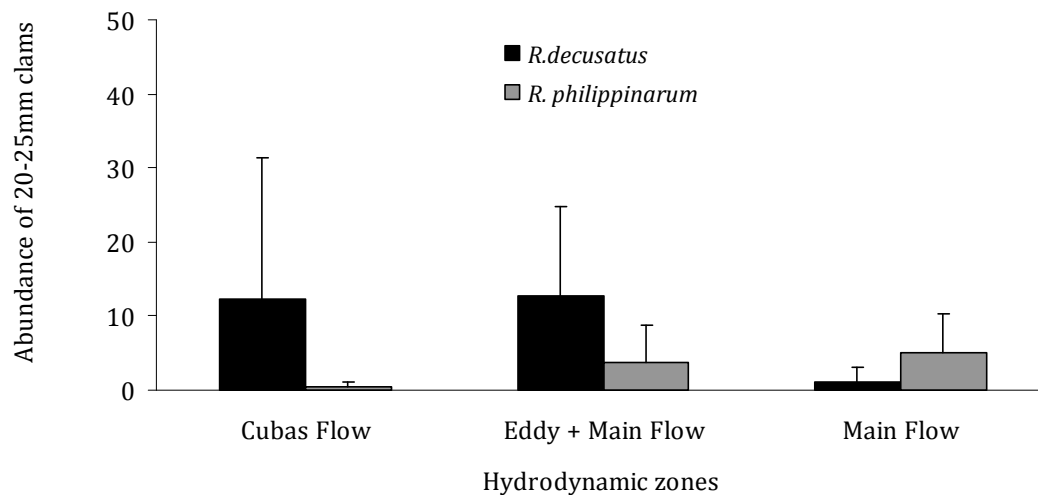


Figure 3.7 - Abundance of 20-25 mm size (recruitment length of the fishing gear) individuals of *Ruditapes decussatus* and *Ruditapes philippinarum* at the defined hydrodynamic zones (Mean + SD): Cubas Flow zone (containing Cubas zone), Main Flow+ Eddy zone (containing Predreña and Port zones) and Main Flow zone, without eddy (containing Culture, Boo and Pedrosa-Astillero zones).

3.4. Discussion

One of the possible reasons for the disappearance of native clam species in estuaries where Manila clam has been introduced (Auby, 1993; Marin, 2003; Mistri, 2004) could be the lack of a management plan based on a scientific knowledge of the resource. In this context, in the Bay of Santander, a first characterization of the grooved carpet shell clam *R.*

decussatus (native) and the Manila clam *R. philippinarum* (nonindigenous) populations was advisable in order to base the management of these commercially exploited resources in scientific data and avoid its potential failure.

Clam abundance showed a significant deviation from randomness for both species, confirming the observations of previous authors on distribution patterns of clams, who found a high spatial variability regardless of the scale or the method of sampling used (Peterson, 1982; Thompson, 1995; Lee, 1996; Bald and Borja, 2001:2005). This fact is also consistent with the aggregated patterns showed by infaunal species (Golsling, 2003). Within this high spatial variability, a higher abundance of clams was observed in the central northern area of the estuary (i.e. Pedreña and Port) and in the mouth of Cubas tidal fresh. This pattern may be related to the tidal/fresh water influence and to the hydrodynamic conditions reflecting a classical estuarine gradient, as well as to the levels of pollutants which are higher in the inner southern parts of the estuary (Puente et al., 2002) and to the mean granulometry (Bald and Borja, 2000) which is higher in open zones (*unpublished data*). In order to reduce, as much as possible, this intra-zone variability, a delimitation of environmentally homogeneous areas should be done. For this purpose the determination of habitat suitability of *R. decussatus* and *R. philippinarum* would be an useful tool as it is reported in many studies for bivalves (Soniati and Brody, 1988; Arnold et al., 2000; Peña et al., 2005; Vincenzi et al., 2006a; Vincenzi et al., 2006b; Vincenzi et al., 2007). This determination of habitat suitability for both species could be an advantage to improve a zone based management model. This aspect is investigated in detail in next Chapter IV.

This study has demonstrated that the abundance of the two species did not show any significant negative correlation, concluding that the interspecific competition for space or resource may not be present or is not intense. The same observation was detected by Peterson (1982), for the interaction between *Prothoaca staminea* and *Chione undatella* clams and by Lee (1996) for *R. philippinarum* and *Anomalocardia squamosa*. Moreover, an

interaction experimental study recently conducted in the Bay of Santander and presented in this thesis in Chapter V has confirmed this result.

The predomination of the native clam or the coexistence of both species in several areas of the Bay indicates that a drastic decline of *R. decussatus* is not observed, in contrast to occurred in other estuaries where Manila clam was introduced and where it clearly predominated over the native clams as *R. decussatus* or *Ruditapes aurea* (Auby, 1993; Marin, 2003; Mistri, 2004; Caill-Milly *et al.*, 2006). This predomination or coexistence was variable depending on the region of the bay, showing a clear dominance of *R. decussatus* in the Cubas tidal fresh (northeast) and coexistence of both species in the rest of the estuary, except in the area around the culture parks where a clear predominance of *R. philippinarum* exist. Thus, *R. decussatus* appeared to predominate in more freshwater-influenced areas of the bay pointing out that low salinity episodes due to floods may have a higher effect on the mortality of *R. philippinarum*. It should be noted that inner part of Cubas with mean salinity values between 15 and 27.2‰ (Moreno-Ventas, 1998) suffered episodes where salinity values fell below 15‰ during the heavy rainy seasons, with ensuing mortality of Manila clam according to Kim *et al.* (2001) and Coughlan *et al.* (2009). These preliminary results could help in the first establishment of closed zones based on both species distribution patterns according to management options mainly directed to the sustainability of the native species fishery.

In relation to size structure, it was unbalanced for both species, showing very low percentages of large individuals >35 mm and a deficit of juveniles. The lack of adults from this size 35 mm may agree with the removal of individuals under the minimum legal size (40 mm). These illegal extractions have been also detected by the periodical inspections of the Fisheries Service (*pers. comm.*). However, other factors such as a low growth rate and natural mortality might also be affecting to this lack of large individuals. Besides, although a higher abundance of >35 mm individuals was observed, for both species, in the southern closed zones (*i.e.* Boo and Pedrosa-Astillero), the differences in abundance are not

significant between open and closed zones. In these closed zones they were expected higher abundances of clams with sizes over the minimum legal size (40 mm) due to the reduction of the fishing effort. The high poaching activity detected by the Inspectors of the Fisheries Service (pers. comm) could be the reason of the non significant effect of the measure of closure of these zones. Moreover, the lack of juveniles or individuals <20 mm could be explained, in part, by the biased sampling towards adult sizes due to the fishing technique. Using a rake to flip the sediment and the eye detection joined to the custom of shellfishermen to focus their fishery to large sizes may be the main reason of this bias. This fact eliminates the possibility of encounter newly (i.e. autumn 2004 or summer 2005) settled recruits resulting normally on bimodal size frequency distributions of clams (Sejr et al., 2002; Dang et al., 2010). The size class to estimate recruitment (i.e. recruitment length of the fishing gear) was 20-25 mm and it may contain recruiters of 2003 for *R. decussatus* and recruiters of autumn of 2003 and spring of 2004 for *R. philippinarum*, according to the spawning season (Urrutia et al., 1999; Ojea et al., 2005) and growth (Spencer et al., 1991; Solidoro et al., 2000; Chessa et al., 2005) of these species.

In further studies, it will be necessary to discuss the need for design of newly settled clam sampling (<20mm, by sieving), without consuming much extra time and covering the whole distribution of sizes. In this manner, it would be obtained a better estimate of newly settlement specimens and of natural recruitment to understand the population dynamic and the distribution pattern in the estuary (Borsa and Millet, 1992; Olafsson et al., 1994; Chícharo and Chícharo, 2001a,b; Phillips, 2006; Humphreys et al., 2007). On the other hand, it can be considered that the subestimation of stock is acceptable as the contribution to the total weight of the smaller sizes is low. However, the fact that sampling methodology is based on the shellfishermen's resource extraction technique, can be an advantage over other sampling methodologies, to achieve a more realistic estimation of the commercial stock of these species, as the available stock will be potentially fished using this artisanal technique. This sampling method provides adequate data of abundance and density of adult (>20 mm) and commercial clams (>40 mm) and also of the

exploitation situation of different zones in a large estuary. However, the study of the early recruiter's abundance in each zone should be essential in further population assessments in order to estimate future stocks and adopt appropriate management measures.

The total stock was 58 t for *R. decussatus* and 90 t for *R. philippinarum*. While the total abundance of both clams was similar, clam-specific weights and differences in distribution patterns leads to observe a tendency of higher stock of Manila clam. This tendency could agree with the first clam specific captures data obtained by the Main Directorate of Fishing of the Government of Cantabria in 2007 (15 t of *R. decussatus* and 55 t of *R. philippinarum*) (*unpublished data*). However, density and fresh weight coefficients of variation were generally high and this implies high values of the stock coefficients of variation for all zones, similar than those observed by Caill-Milly et al. (2006). Comparing the CVs, density contributes with the highest uncertainty, being in some cases higher than 1 (e.g. for *R. decussatus* in Cubas tidal fresh and Port; for *R. philippinarum* in Cubas and Pedreña in zones). The higher values of the CVs for density observed in Cubas zone corresponds with the most heterogeneous area regarding to hydrodynamic, granulometry and salinity conditions, having stations located in inner or more estuarine areas and stations located in more oceanic areas.

Focusing on densities of both clams, it is remarkable that the mean densities of both species are low comparing to other estuaries of the Gulf of Biscay. In the estuaries of Plentzia and Mundaka, Bald and Borja (2005) recorded higher densities of *R. decussatus*, probably related to the sampling method used which detects individuals larger than 1mm and to a lower fishing pressure, as there is no a shellfish professional activity. In Arcachon Bay, Dang et al. (2010) observed higher densities of *R. philippinarum*, which may be related to a most effective naturalization of this species comparing with the occurred in the Bay of Santander.

The relative low density of *R. decussatus* near the culture parks could be due to the effects produced by high densities of cultured Manila clam. High densities of cultivated bivalves are generally considered as “sinks” of oxygen and particulate organic matter (Richard et al., 2007a,b) and may cause a food shortage for the native species with high mortality rates of juvenile clams. The ingestion of bivalve larvae by filtering organisms such as *R. philippinarum* is also known to be a significant mortality factor (Davenport et al., 2000; Lehane and Davenport, 2002). Jouffre (1989) also observed that the abundance of venerid larvae at the stations situated within intensive shellfish culture zones or at their nearest neighbours were significantly lower than the values reported at all other stations. However, the real spatial effect of this clam parks is unknown and also other settlement or post-settlement factors could also drive this pattern. Thus, to conclude cause-effect it would require a study to compare areas of both similar larval supply and environmental characteristics with and without culture parks.

The highest pool of larvae coming from near high densities of reared adults, its higher growth rate compared to that of *R. decussatus* (Spencer et al., 1991) and its high filtration velocity (Zaklan and Ydenberg, 1997) could be some of the possible reasons to explain a better adaptation of *R. philippinarum* in this zone and, hence, higher densities comparing to *R. decussatus*. With a shallower burial depth, *R. philippinarum* can filter food particles more quickly (Zaklan and Ydenberg 1997) and can invest less in the development of its siphon compared to a deeper clam with a longer siphon as *R. decussatus*. In this manner, the survival in a zone with a food shortage could be easier for this non native species.

In relation to the larval transport in estuaries, Roegner (2000) observed that it is highly correlated with the volume transport from coastal ocean during flood tide, considering that most of the larval pool is exported to the sea during ebb tide episodes. Therefore, the lowest recruitment of both species in the inner stations (1-3) of the Cubas tidal fresh may be also due to the reduced inflow during the medium flood tide, comparing to the main flow, and hence to the more limited arrival of larvae to these zone. Moreover, it should be

noted that recruitment was estimated as the “recruitment to the fishing gear”. Therefore, post-settlement mortality associated to low salinity episodes, depredation or disease may also be influencing on the distribution patterns of 20-25 mm individuals. However, in this study, in order to link the hydrodynamic regimes and larval transport with this size class, it was assumed that the effect of these factors on mortality of clams on the first 1-2 years could be of the same order of magnitude in all zones. This assumption was done taking into account that the differences in mortality between zones may be highly masked by the high variability in clam abundance within each fishing zone. However, this is a first approach for studying the effects of hydrodynamics on recruitment in the Bay of Santander and therefore, in order to reduce the uncertainty introduced by this assumption, it is essential to investigate larvae dispersal patterns, coupling a dispersion submodel to the hydrodynamic model and validating it by measuring newly recruited individuals (Ishii et al., 2001; Strasser and Günther, 2001; Siegel et al., 2003; Hinata et al., 2006). For this purpose a larval evolution model is developed in Chapter V.

The higher densities of Manila clam in Pedreña do not coincide with significant higher recruitment values in this zone. In fact, the recruitment of Manila clam is poor for the entire bay as it occurs in other estuaries as Arcachon Bay in France (Caill-Milly et al, 2006). Then, the higher density of Manila clam in Pedreña zone comparing to the inner zones could be explained by a compensation of the low recruitment by a faster growth and lower mortality (Dang et al., 2010). In this area, with more oceanic conditions, the role of the water circulation cell (eddy), helping the recirculation of larvae and the settlement-recruitment process, observed by Borsa and Millet (1992), was not detected for any species. Other aspects such as a highly suitable habitat for survival, development and reproduce could explain this densities.

In the southern zone influenced by the main flow, the significant lowest success on recruitment of *R. decussatus* could be related to the limited arrival of larvae to this zones considering that the main spawning zone of this species (i.e. higher abundances of adult

clams) were observed in the northern flats of the estuary. However, the dispersal and recruitment patterns are analyzed in detail in Chapter V as it was mentioned above.

3.5. Conclusions

The coexistence patterns of both clams in the Bay points out that the introduced nonindigenous Manila Clam has not yet supplanted the *R. decussatus* native clam by occupying entirely its ecological niche and relegating it to occupy very restricted areas as it has occurred in other estuaries or lagoons of Europe. *R. decussatus* appeared to be the dominating species in more oceanic and freshwater-influenced areas. In this line, the performed fishing activity on individuals under the minimum legal size could lead to a decline of both populations but especially of the native clam, affecting considerably the actual coexistence pattern. Establishing specific measures for each species appears to be essential to maintain this coexistence pattern stable. Although the total low density of both species is similar, the estimated higher total stock and captures of *R. philippinarum* indicates the increasing importance of the introduced species in the shellfishery, in contrast to past two decades when the native clam was the main harvested species. The sampling method provides adequate data of abundance and density of adult (> 20mm) and commercial clams (> 40 mm) and has shown its feasibility to estimate standing stocks and to know exploitation situation of different zones in a large estuary.

The coastal circulation model used in this work to study the relationship between the hydrodynamic patterns and recruitment (i.e. recruitment to the fishing gear) provides a first attempt to introduce this tool on these species fishery management models, although an estimate of newly settlement specimens and a larval evolution model seems to be essential to better understand the population dynamic and the distribution pattern in the estuary. This research line is continued in Chapter V.

To conclude, some nonspecific and specific clam management proposals are drawn out on the light of the obtained results and mainly focused on the conservation of the native species:

(i) A strict control on the minimum legal capture size should be established to avoid the actual situation of non respect of the minimum size of capture and to achieve increments in total stock. This control may be even more important for *R. decussatus* as it has a slower growth rate than *R. philippinarum* and has not the extra larval supply of Manila clam coming from the intensive cultured zones.

(ii) The current closure zones are non specific since they were established regardless the relative abundance between species. Therefore, it is important to direct policy efforts towards establishing specific closure zones in areas where the native clam population densities and recruitment are high like a conservation measure. Outside of Cubas tidal fresh and Pedreña are potentially the most important spawning and settlement areas for this species.

(iii) In the same way, they should be considered a higher control of sowings and sustainable densities of cultivated bivalves and/or a dispersion of cultivation zones locations to reduce the potential effects of high densities on surrounding natural populations of the native clams.

These management proposals require a high government involvement in enforcement and they must go hand in hand with the shellfishermen's collaboration. Therefore, the incorporation of shellfishermen in a co-management of the shellfishery should be an essential step to be taken by means of using territorial user rights for fishing, where responsibility for the exploitation of clams could be shared between fishers' guilds ("cofradías"), i.e. shell fishermen's organisations supervised by the regional government, and fishery authorities, as it has been done in the neighbour region of Galicia (N Spain).

Thus, shellfishermen would collaborate with the government fishery inspection service to avoid intrusions of illegal fishers. Furthermore, they could have external technical support to design and implement exploitation plans in their fishing grounds. Overall, co-management is supposed to increase rationality in management and create more legitimate regulations, thereby motivating user groups to follow regulations.

These proposals together with the future co-management should be integrated into an “adaptive management” process relying on systematic feedback learning and a progressive accumulation of knowledge for improved fisheries management. Thus, this process should be participatory involving both fishermen and competent authorities.

Chapter IV

**Ecological niche modeling approach to
predict the potential expansion of the
nonindigenous clam *R. philippinarum***

Chapter IV. Ecological niche modeling approach to predict the potential expansion of the nonindigenous clam *R. philippinarum*

This study has led to the submission of the research article entitled “Ecological niche modeling approach to predict the potential expansion of a nonindigenous clam” by Bidegain, G., Bárcena, J.F., García, A., Juanes, J.A. for publication in the Journal of Marine Systems.

Abstract

Habitat suitability is an essential factor regulating the expansion of an invasive or introduced species and its determination constitute a basic element in conservation strategies and fisheries management. In several estuaries of Europe the introduced Manila clam *Ruditapes philippinarum* has become a widespread and predominating species supplanting the native carpet shell clam *Ruditapes decussatus* by relegating it to occupy restricted areas. Within this context, the main goal of the present study was to determine the habitat suitability for the European native carpet shell clam and the introduced Manila clam in the Bay of Santander (N Spain) where no dominant pattern has yet been detected. In addition both clam species potential domination areas were determined. Environmental Niche Factor Analysis (ENFA) modelling approach, developed for presence-only data, was used to predict global habitat suitability for both species. Habitat-suitability maps were generated and cross-validation was used to evaluate the performance of the models. The model performed well in cross-validation. The highly suitable and potential predomination areas were considerably wider for the native species than for the introduced species, showing the important role of the habitat suitability in the regulation of the expansion of Manila clam. ENFA factors showed that (1) Manila clam habitat differs

more than that of the carpet shell clam from the mean environmental conditions over the estuary (i.e. higher marginality) and (2) Manila clam has less narrow habitat requirements (i.e. lower specialization). The environmental variables that most determined the presence of both clams in this study were bathymetry, current velocity and salinity although their importance and association (positive or negative) with high predicted habitat suitability was different between species. The model showed that, integrating new environmental variables, ENFA provides a promising technique to achieve distribution predictions of clams and explore the expansion potential of the introduced species.

4.1. Introduction

In several disturbed estuaries or lagoons of Europe as Arcachon Bay (France) or the lagoon of Venice (Italy), this species has supplanted the European native carpet shell clam *Ruditapes decussatus* by occupying almost entirely their ecological niche and relegating it to occupy restricted areas (Auby, 1993; Marin et al., 2003; Blanchet et al., 2004; Mistri, 2004; Caill-Milly et al., 2008).

In the Bay of Santander, this species was first introduced in the late 80s but until the late 90s the farming areas were testimonial and nowadays, the operational farming area is not larger than 1 ha.). In this estuary, the expansion and extreme domination pattern of the introduced bivalve species has not yet been detected since the mean ratio *R. philippinarum*/*R. decussatus* presence is approximately 1:1 and zones where (1) both species coexist, (2) nonindigenous species predominates and (3) the native species predominates have been identified in the characterization study described in Chapter III. However, being aware of the drastic decline of the native carpet shell clam in several estuaries of Europe where the Manila clam has been introduced and expanded, the determination of the potential distribution of both species in this estuary is essential as a further step beyond the characterization. This spatial information could lead to support

either the conservation measures for the native species or the appropriated fisheries management actions.

Within this context, ENFA predictive modeling was used to locate the areas that are most likely to provide suitable habitat for the native carpet shell clam *R. decussatus* and the worldwide introduced Manila clam *R. philippinarum* in the Bay of Santander. To achieve this aim the following steps were considered in this study: (1) examine the influence and compare the relative importance of 7 environmental variables in determining suitable habitat for both species (2) predict habitat suitability for both species within the Bay of Santander (3) to determine areas where they coexist or one species dominates in order to know the role of the habitat suitability regulating the expansion of Manila clam. The results of this study were intended to serve as a tool for conservation and ecosystem management of these species.

4.2. Material and methods

4.2.1. Clams presence data

Clam sampling surveys to detect presence data of *R. decussatus* and *R. philippinarum* were undertaken during spring low tides, between April and May of 2010, in the intertidal sand and muddy flats. If present, clams were collected at 39 stations by hand raking of the sediment in 1m x 10m transects by a professional shell-fisherman following the sampling methodology applied by Juanes et al. (2012). In all cases, transects were located in areas where shellfishing is routinely performed (Figure 4.2). Due to the similarities in the external shape among species, taxonomic determination of individuals was carried out in the laboratory. Both maps of species presence were transformed to a raster-based grid file (244 x 298 cells of 51m x 51m) as required to apply ENFA analysis using Biomapper 4.0

software (Hirzel et al., 2000). The raster grids were constructed as boolean maps (containing 0 and 1 only, 1 indicating the cells where the species was present and 0 the rest of cells i.e. absence cells and no data cells) as it is usually done in habitat suitability determination using ENFA (e.g. Bryan and Metaxas, 2007; Dolan et al., 2008; Tittensor et al., 2009). Data were processed using a Geographic Information System (ArcGIS 9.2 by ESRI).

4.2.2. Environmental variables

Different topographic (bathymetry), physical (salinity, current velocity and sediment characteristics such as percentage of sand, gravel and silt) and chemical environmental variables (organic matter content in sediment) were considered (Table 4.1). These variables together with the presence data were used in the environmental niche factor analysis (ENFA) performed for both species.

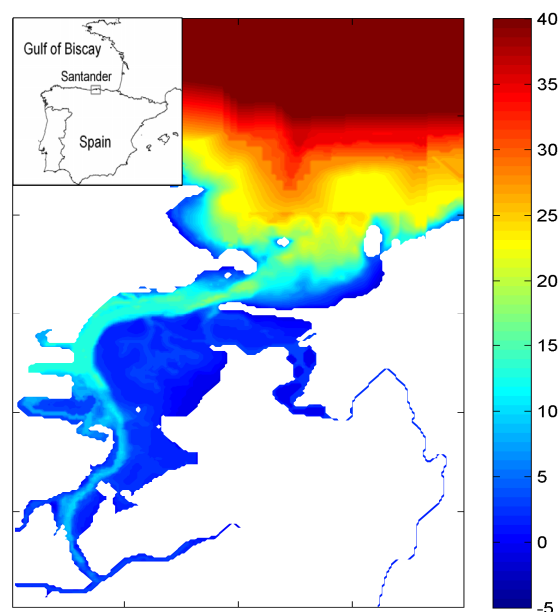


Figure 4.1 - Study area for the ENFA model: Bay of Santander estuary and adjacent waters located in the northern coast of Spain (Gulf of Biscay). Bathymetry (m) data of the modeled area is presented.

These variables were selected because they have been reported as meaningful to the ecology and distribution of these species (Laing and Child, 1996; Delalli et al., 2004; Vincenzi et al., 2006a, 2006b, Cannas, 2010; Cilenti et al., 2011) and taking into account that several authors have suggested that the physiological and biochemical responses to variation in environmental conditions are similar for both clam species studied here (e.g. Beninger & Lucas, 1984; Laing & Utting, 1994). Environmental variables data were obtained from different information sources (Table 4.1). The grid of environmental variables was identical to that of the presence data, with an extent of 244 x 298 cells (Figure 4.1) and uniform size of cells (51 m x 51 m). The variable grids obtained were transformed to raster-based grid by ArcGIS 9.2 in order to perform the ENFA analysis with Biomapper 4.0.

Variable	Source	Type of data
Bathymetry	Nautical chart IHM 940 DTM	Interpolated data derived from bathymetry data and nautical charts
Current velocity	This study	Modelled data
Salinity	This study	Modelled data
Sediment characteristics		
% of sand	Field sampling (this study) Gobierno de Cantabria (2010)	Interpolated data from 56 sampling stations (ArcGIS 9.2)
% of gravel		
% organic matter		
% of silt		

Table 4.1 - Environmental variables, sources and types of data used in the present study.

4.2.2.1. Bathymetry

A bathymetric grid of the study area was generated using the digital terrain model (DTM) of the Bay of Santander and the nautical chart IHM No. 940. The data were referred to the mean sea level (MSL). The generation of the two-dimensional grid system was built in the horizontal directions by linear interpolation using GIS.

4.2.2.2. Sediment

Sediment samples were also collected at each of the 39 stations used to obtain the presence data. Data were completed with samples obtained in June 2010 by the Littoral Water Quality Monitoring Network at 17 intertidal and subtidal stations (Gobierno de Cantabria, 2010). Sediment samples were processed in laboratory and percentage of gravel, sand, silt and organic matter content was determined following European standard methods UNE-EN 933-1:1998/A1:2006 and UNE-EN 13039-1. From these data, a raster map was obtained for each parameter, using a linear ordinary kriging interpolation with no constraints on the search ellipse (ArcGIS 9.2 by ESRI).

4.2.2.3. Current velocity and salinity

Hydrodynamics in the Bay of Santander are mainly determined by the Miera river inflows and the astronomical tides. Therefore, the current velocity in the estuary was calculated using the two-dimensional depth-averaged hydrodynamic model of coastal and estuarine circulation H2D (Bárcena et al., 2011, 2012; García et al., 2010a,b). The salinity was modeled using an Eulerian two-dimensional depth-averaged transport model (AD2D) for conservative substances and considering the wetting and drying of tidal flats. The hydrodynamic model was calibrated and validated against water levels and current velocities collected in a field campaign conducted by the University of Cantabria during the

period from 19th to 30th May 1997, covering a full phase of spring and neap tides (López et al., 2013).

In order to take into account the variability of the forcing variables, different cases of tidal amplitude and river flow were analyzed. For this purpose, the empirical cumulative distribution functions (ECDFs) calculated for (1) the astronomic tidal range from 1992 to 2007 (15 years) obtained from the tidal gauge of Santander and (2) the river discharges in the estuary from 1970 to 2003 (33 years). The 9 cases selected were a combination of 3 constant tidal amplitudes and 3 constant river flow (i.e. 25th, 50th and 75th percentiles). An equivalent weight of 33% was assigned to each percentile of tidal amplitude and current flow. Hence, it was assumed that the 25th percentile was representative of the 33 % of the values of the ECDFs (from 0th to 33th percentile). Similarly, we assumed the same equivalent weight for 50th and 75th percentiles. Thus, the probability of occurrence of each tidal amplitude and river flow is the same. Therefore, assuming a semidiurnal tidal wave, the 9 cases were simulated and 12 current fields were obtained for each case (i.e. one current field per hour). The same approach was used to assess salinity. Overall, 108 current fields and 108 salinity fields were obtained. These fields were integrated into a single current field and a salinity field as follows: (1) a tidal-averaged field was calculated from the 12 fields obtained in the numerical simulation for each case, (2) the 9 final fields obtained (i.e. a tidal average field for each case) were then averaged giving a single current field and a single salinity field. As it was done for the rest of the variables, in order to perform the ENFA analysis by Biomapper 4.0, both current and salinity grids were transformed by ArcGIS 9.2 to obtain a raster-based grid.

4.2.3. Description of habitat

The distribution of the variables in sites where species were present and in the global area was compared in order to have an improved interpretation of each variable throughout the marginality factor obtained in the ENFA model. For this purpose, minimum, maximum,

mean values and standard deviation were calculated for each variable in both species presence cells and the whole study area.

4.2.4. Ecological Niche Factor Analysis (ENFA)

4.2.4.1. Model application and factors interpretation

Ecological niche factor analysis (ENFA) is a niche-based predictive habitat suitability modeling technique for presence-only data based on multivariate ordination. The theory and equations behind the analysis are explained in detail by Hirzel (2001) and Hirzel et al., (2002). ENFA compares distributions of eco-geographical variables between the locations where the species is present (species mean) and the whole area (global mean), extracting the range of environmental conditions that the species inhabits (niche width) (Davies et al., 2008). This information is used to generate habitat suitability maps.

ENFA analysis was performed using the Biomapper 4.0 software which has implemented all procedures to conduct the analysis (Hirzel et al., 2000). Before computing the ENFA analysis the Box-Cox algorithm was applied to normalize the environmental variables (Sokal and Rohlf, 1995). Moreover, given that more than one variable were obtained from sediment samples, a covariance matrix was calculated to determine those variables that were highly correlated and remove those which had high correlation ($r > 0.8$) from the analysis (Table 4.1).

ENFA was applied to both clam species and the factors explaining most of their ecological distribution were retained for the habitat suitability maps computation. As it was mentioned above, factors produced by the model are uncorrelated and have biological significance. The first factor or axis obtained is defined as "marginality factor" (M) and is calculated by means of equation 1:

$$M = \frac{\sqrt{\sum_{i=1}^V m_i^2}}{1.96} \quad (1)$$

where the coefficients m_i of marginality factor M express the marginality of the focal species on each variable and V is the number of variables. Marginality indicates how different is niche of the species from the global area. The overall marginality M computed for all variables allows comparing marginalities of different species within a given area. A marginality value near or greater than >1 indicates that a species lives in a very specific or marginal habitat in relation to the reference set (Hirzel et al., 2001). Marginality for each variable (m_i) depends on the absolute difference between global mean (m_G , the mean of the variable in the whole study), the species mean (m_S , the mean of the variable where the species is present) and the standard deviation of the global mean (σ_G) (Hirzel et al., 2002):

$$m_i = \frac{m_{Gi} - m_{Si}}{1.96 \sigma_{Gi}} \quad (2)$$

The higher the absolute value of a coefficient m_i , the further the species departs from the mean available habitat regarding the corresponding variable. Negative coefficients indicate that the focal species prefers values that are lower than the mean with respect to the study area, while positive coefficients indicate preference for higher-than-mean values.

Similarly, the rest of the factors explaining the species distribution are defined as specialization factors (S) which indicate how restricted the niche of the species is in relation to the study area.

$$S = \frac{\sqrt{\sum_{i=1}^V \lambda_i}}{V} \quad (3)$$

The coefficients λ_i (i.e., specialization of the species in relation to each environmental variable), associated to any specialization factor, express the amount of specialization it accounts for and are defined as the ratio between the standard deviation of the global distribution and the standard deviation of the focal species on the factor axis (Hirzel, 2001) (Equation 4):

$$\lambda_i = \frac{\sigma_{Gi}}{\sigma_{Si}} \quad (4)$$

These coefficients or eigenvalues of the specialization factors have a different interpretation than that for marginality factor. Signs of the coefficients are arbitrary and therefore, only absolute values are considered. The higher the absolute value, the more restricted is the range of the focal species on the corresponding variable.

As for the M factor, the specialization of the species in relation to environmental variables (λ_i) can be combined to obtain an overall value of specialization (S) (Equation 3) which is used for among-species comparison. S ranges from 1 to ∞ , with any value greater than 1 indicating a degree of specialization with the niche becoming narrower as S increases. Tolerance (T) is the inverse of specialization and ranges from 0 to 1, indicating a wider niche as the T value is closer to 1. The first specialization factor is the one that maximizes the variance of the global distribution (orthogonal to the marginality factor). The following specialization factors are then extracted in turn, each step removing one dimension from the space, until all V factors are extracted (Hirzel, 2002). Thus, the amount of specialization accounted for by a specialization factor is necessarily lower than that due to the one previously extracted.

4.2.4.2. Habitat suitability maps

The habitat suitability (HS) map for each species was computed calculating a suitability index for each cell of the raster-based grid using the mean geometric algorithm (Hirzel and Arlettaz, 2003). A broken stick distribution was used to determine the number of factors explaining the variance of both species distribution. Each cell of the HS map was indexed with a range of 0-100, where higher values indicate more suitable habitat for the respective species (Hirzel et al., 2006).

The predicted maps were reclassified into 4 HS index (HSI) classes using GIS techniques: unsuitable ($HSI < 25$), barely suitable ($25 \leq HSI < 50$), moderately suitable ($50 \leq HSI \leq 75$), highly suitable ($HSI > 75$). The number of cells (51x51m) were counted for each HSI class and the corresponding area was calculated.

4.2.4.3. Model evaluation

The factors explaining most of the ecological distribution of the species were retained for the HS maps computation. The predictive capability of the HS model was evaluated for both species, using the Jack-knife area-adjusted frequency Cross-Validation procedure following the method described by Boyce et al. (2002). This validation method is also defined as an internal evaluation method (Valle et al., 2011) since it is based on the resampling of the same dataset that generates the model. Thus, the presence points of the species were divided into five groups. The data of four of the partitions were used to compute the HS map, whilst the fifth group was used to evaluate the result. This process was replicated five times in order to use each subset of data and hence, as a final result a mean HS index (HSI) with a confidence interval ($\pm SD$) was obtained. This index, called the Boyce Index, ranges from 0 to 1 and indicates the predicted accuracy of the habitat suitability model (e.g. Strubbe and Matthysen, 2009; Sattler et al., 2007) where values close to 1 indicate a higher predictive capability of the model.

4.2.5. Coexistence and domination patterns

The areas where (1) predomination of a species or (2) coexistence of both species without significant domination occurs were determined using GIS techniques. For this purpose, the habitat suitability raster maps obtained were treated simultaneously using the raster calculator tool (ArcGis 9.2). A coexistence-predomination pattern map was obtained as follows: (1) predomination of a species was assumed in a cell if the difference in HSI value among species was ≥ 50 and (2) coexistence was assumed in a cell if HSI value was > 25 for both species and the difference among them was < 50 . The total areas corresponding to predomination or coexistence patterns and the distribution of the variables values in these areas were calculated using zonal statistics tool (ArcGis 9.2).

4.3. Results

4.3.1. Clams presence and habitat description

Locations where the presence of both clam species was detected are shown in Figure 4.2. *R. decussatus* presence was recorded in 27 of the 39 stations while *R. philippinarum* presence was recorded in 26 stations (Figure 4.2). Presence of *R. decussatus* with no presence of *R. philippinarum* was recorded in the eastern tidal flats of Pedreña and Elechas and in the southern inner zones of Astillero, Solia and Tijero. The contrary was observed in the northeastern Cubas tidal fresh and in several central southern sand flats, between the port and Astillero.

Both clam species were often found close to the coastline likely because by the majority of the sampling was conducted in the intertidal area since a well-known relationship exists between the intertidal and shallow waters and the presence and growth of clams (Vincenzi, 2006a,b; Albentosa and Moyano, 2009).

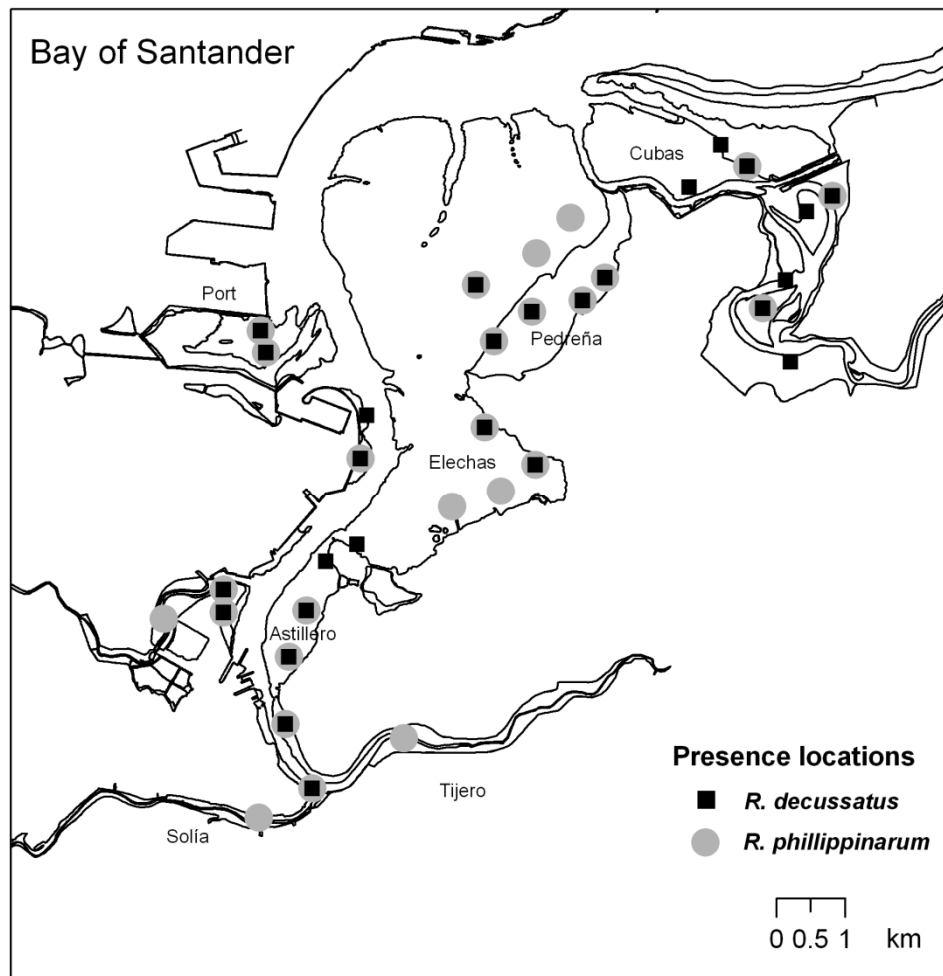


Figure 4.2 - Distribution of presence locations of *R. decussatus* (black square) and *R. philippinarum* (gray circles) in the Bay of Santander.

The distribution of the environmental variables (range, mean and standard deviation) where the presence of both species was detected is summarized in Table 4.2. For comparison, the distribution of the variables in the whole study area is also provided.

R. decussatus

R. decussatus presence was found in (1) depths between 0.6 m and 1.50 m below the MSL, which were shallower than the global mean, (2) areas with appreciable mean current

velocities, slightly higher than the mean observed in the study area ($0.29 \text{ m/s} \pm 0.09$), (3) sediments with high percentage of sand but showing an appreciable variability of substrate types inhabited ($67.60 \% \pm 31.50$), (4) water salinity values ranged between 26.0 psu and 34.1 psu with a mean, 31.5 psu, similar to the global mean and (5) sediments with an organic matter content relatively higher than the global mean.

	Max	Min	Mean	SD
Study area				
Current velocity	1.10	0.01	0.25	0.08
Sand	99.60	11.45	64.00	23.60
Salinity	34.16	0.00	31.80	3.80
Organic matter	10.70	1.20	3.57	2.40
<i>R. decussatus</i> presence locations				
Current velocity	0.40	0.05	0.29	0.09
Sand	98.60	11.70	67.60	31.50
Salinity	34.09	26.00	31.50	2.45
Organic matter	10.68	1.12	4.95	3.27
<i>R. philippinarum</i> presence locations				
Current velocity	0.35	0.01	0.20	0.10
Sand	98.30	11.45	65.20	30.80
Salinity	34.10	26.00	31.40	2.35
Organic matter	10.68	1.12	4.78	3.16

Table 4.2 - Distribution of the values of the 5 environmental variables used in ENFA analysis: bathymetry (m), current velocity (m/s), sediment sand content (%), salinity (s.u.) and sediment organic matter content (%). For each variable, minimum, maximum, mean value and standard deviation are presented for the *R. decussatus* and *R. philippinarum* presence locations and for the whole study area.

R. philippinarum

R. philippinarum presence was found in (1) depths between 0.5 m and 1.50 m below the MSL, shallower than the estuary mean and similar to *R. decussatus*, (2) areas with lower mean current velocities ($0.20 \text{ m/s} \pm 0.10$) than the global mean, (3) sediments with high percentage of sand ($65.20 \% \pm 30.80$), (4) waters with a mean salinity of 31.4 psu (as for *R. decussatus*) values ranged between 26.0 psu and 34.1 psu (5) sediments with an organic matter content relatively higher than the global mean.

The most significant difference between the two species was that Manila clam was found in more sheltered zones (lower current velocities). The other variables showed slight differences between species, with mean salinity, percentage of sand and organic matter being higher for *R. decussatus*.

4.3.2. Environmental Niche Factor Analysis

First, percentage of silt and gravel were removed since they were highly correlated ($r > 0.8$) with organic matter content (%) and percentage of sand, respectively (Table 4.1). Then, 5 environmental variables were used to compute ENFA: bathymetry, current velocity, salinity, sand percentage and sediment organic matter content.

R. decussatus

The overall marginality value of the ENFA analysis was 0.44, indicating that the native carpet shell clam environmental requirements are relatively different from the average environmental conditions of the Bay of Santander. A specialization value of 3.46 (>1) and tolerance value of 0.29 indicates that this bivalve occupies considerably narrow environmental niches. Three factors (i.e. the marginality factor (M) and two first

specialization factors(S)) were retained for the latter habitat suitability map computation, which explained the 98 % of the variance of the distribution of this species. The M factor explained 50 % of the variability. The other two S factors explained the 41% and 7% of the variability, respectively (Table 4.3).

The environmental variables that most determined the presence of the carpet shell clam (i.e., variables with the highest absolute value of coefficients along M factor) were bathymetry and current velocity, while the percentage of sand, salinity and sediment organic matter content were, in order of importance, relatively less determinant (Table 4.3). Current velocity, percentage of sand and organic matter in sediment were positively associated with high habitat suitability (i.e., variable mean at locations where species is present is higher than that for the whole study area). Furthermore, depth and salinity were negatively associated with high habitat suitability (i.e. variable mean at locations where species is present is lower than that for the whole study area) (Table 4.2 and 3).

Salinity was of critical importance in explaining species specialization (Table 4.3) as it is shown by the higher values of the coefficient of this variable along the first specialization factor. In order of importance, sediment characteristics (high percentage of sand) bathymetry (showing a narrow range of depth where the species was present) and current velocity play an appreciable role in explaining the specialization of the carpet shell clam. Regarding the organic matter content of the sediment, it appears that this variable does not have a strong effect determining the niche of these species.

R. philippinarum

For the Manila clam, the overall marginality value of the ENFA analysis was 0.65, which was slightly higher than that obtained for the native carpet shell clam. This indicates that environmental conditions required by the introduced clam are appreciably different from the mean environmental conditions of the study area (Table 4.2) and relatively different from the conditions required by the native clam.

The specialization (S) value was greater than 1 (2.12) and the tolerance value of 0.47, indicating that this species occupies appreciably narrow environmental niches though wider than those inhabited by the carpet shell clam. Four factors explained 95 % of the variance of the distribution of this species. The M factor explained 63 % of the variability. The other three S factors explained 18%, 7% and 7% of the variability, respectively (Table 4.3).

The environmental variables that most determined the presence of the Manila clam were bathymetry and current velocity, whilst percentage of salinity and percentage of sand were, in order of importance, relatively less important. Percentage of sand and organic matter content were positively associated with high habitat suitability, whilst depth, current velocity and salinity were negatively associated with high habitat suitability (Table 4.3).

<i>R. decussatus</i>				
Marginality (50 %)	Specialization 1 (41 %)	Specialization 2 (7 %)	Specialization 3 (1 %)	Specialization 4 (1 %)
Bathymetry (-0.65)	Salinity (-0.71)	Bathymetry (-0.61)	Organic matter(0.86)	Current velocity (-0.83)
Current velocity (0.45)	Sand (-0.56)	Salinity (-0.53)	Current velocity (-0.46)	Organic matter(-0.44)
Sand (0.30)	Bathymetry (0.35)	Sand (0.41)	Salinity (0.17)	Sand (0.32)
Salinity (-0.26)	Current velocity (-0.24)	Current velocity (0.35)	Sand (0.12)	Salinity (-0.09)
Organic matter (0.16)	Organic matter (-0.05)	Organic matter (-0.24)	Bathymetry (-0.09)	Bathymetry (0.08)
<i>R. philippinarum</i>				
Marginality (63 %)	Specialization 1 (18 %)	Specialization 2 (7 %)	Specialization 3 (7 %)	Specialization 4 (5%)
Bathymetry (-0.71)	Salinity (-0.89)	Bathymetry (-0.69)	Sand (0.74)	Organic matter (0.94)
Current velocity (-0.58)	Sand (-0.35)	Current velocity (-0.68)	Organic matter (0.53)	Salinity (0.32)
Salinity (-0.30)	Bathymetry (-0.22)	Salinity (-0.22)	Bathymetry (-0.36)	Current velocity (-0.14)
Sand (0.28)	Organic matter (0.16)	Sand (0.12)	Salinity (-0.22)	Bathymetry (0.03)
Organic matter (0.001)	Current velocity (0.02)	Organic matter (0.06)	Current velocity (0.02)	Sand (0.01)

Table 4.3 - Variance explained (%) by five ecological factors (marginality and specialization factors) in the ENFA model for *R. decussatus* and *R. philippinarum*. The environmental variables along the factors (bathymetry (m), current velocity (m/s), sediment sand content (%), salinity (s.u.) and sediment organic matter content (%)) are listed in order of importance (absolute coefficient value) determining the marginality and specialization of the species. The sign of the coefficient values, along the marginality factor, indicates that the species prefers higher values (+) or lower values (-) than the study area mean regarding the corresponding variable. The sign is arbitrary and has no interpretable meaning along specialization factors.

Salinity was dominant variable explaining this species specialization as it is indicated by the higher values of its coefficient along the first specialization factor (Table 4.3). Sediment characteristics, bathymetry and currents also play a role in explaining the specialization of the species (see factor 1 and 2 of specialization). The organic matter content of the sediment has not a strong effect determining the niche of the studied species as it occurred for the carpet shell clam.

4.3.3. Model evaluation and Habitat Suitability maps

R. decussatus

The three factors explaining most of the ecological distribution of the carpet shell clam (one marginality factor and two specialization factors) were retained in order to compute the habitat suitability map and the model validation. Cross-validation of the quality of the model resulted in a Boyce index of 0.82 ± 0.10 , indicative of the predictive power of the model.

Construction of the habitat suitability map showed that a 20 % of the surface (407 Ha) of the Bay of Santander has highly suitable environmental conditions for the development of *R. decussatus* and other 21 % (44 Ha) has a moderately suitable habitat (Table 4.4). Thus, this species suitable habitat (HS index >50) distributes mainly along the northern and outer zones in Cubas tidal fresh and along the eastern Elechas and Astillero tidal flats, with a limited presence in the southern inner zones (Figure 4.3 and 4.4).

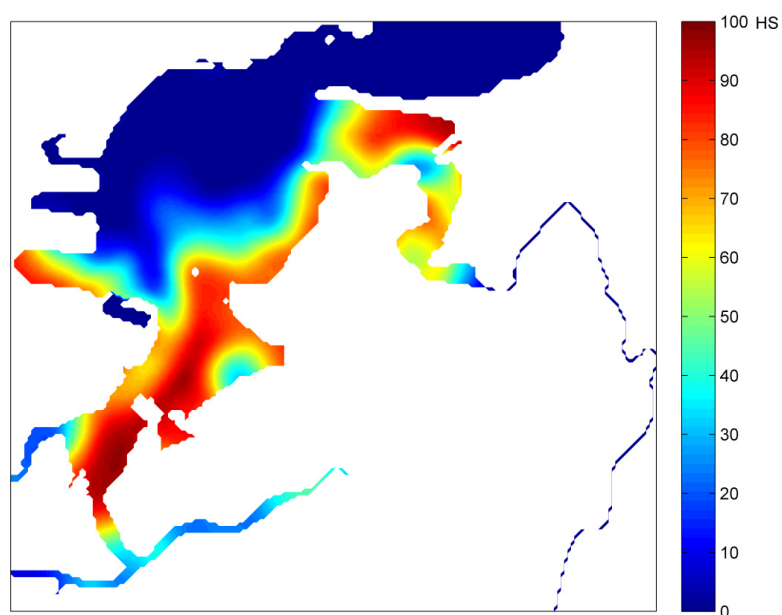


Figure 4.3 - Habitat suitability map for *R. decussatus* in the Bay of Santander.

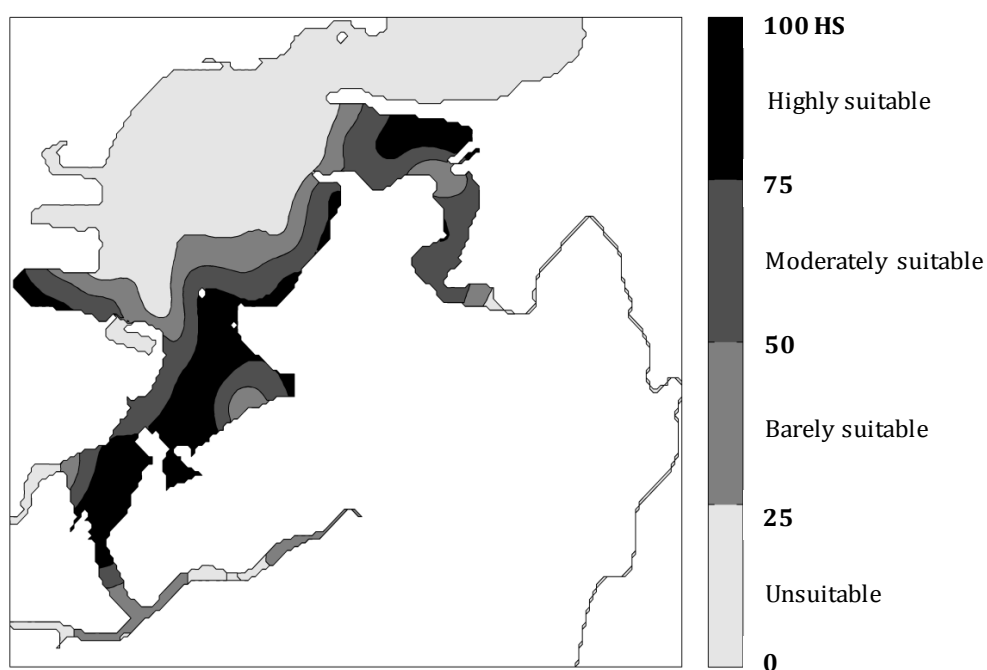


Figure 4.4 - Habitat suitability map for *R. decussatus* in the Bay of Santander, classified by into 4 habitat suitability index (HSI) classes using: unsuitable ($HSI < 25$), barely suitable ($25 \leq HSI < 50$), moderately suitable ($50 \leq HSI \leq 75$), highly suitable ($HSI > 75$).

HS	n° of cells	Relative area (%)	Area (Ha)
<i>R. decussatus</i>			
Unsuitable	3444	43	899
Barely suitable	1225	16	319
Moderately suitable	1706	21	444
Highly suitable	1565	20	407
Total	7940	100	2069
<i>R. philippinarum</i>			
Unsuitable	4414	56	1148
Barely suitable	988	12	257
Moderately suitable	1269	16	317
Highly suitable	1269	16	317
Total	7940	100	2069

Table 4.4 - Number of grid cells of 51 x 51 m (n° of cells), relative area (%) in relation to the whole Bay of Santander and area (Ha) occupied by each class of Habitat Suitability (HS): Unsuitable (HSI <25), barely suitable (25 ≤ HSI <50), moderately suitable (50 ≤ HSI <75), highly suitable (HSI >75).

R. philippinarum

4 factors (one marginality factor and 3 specialization factors) explained most of the ecological distribution of Manila clam and were used in habitat suitability map computation and the model validation.

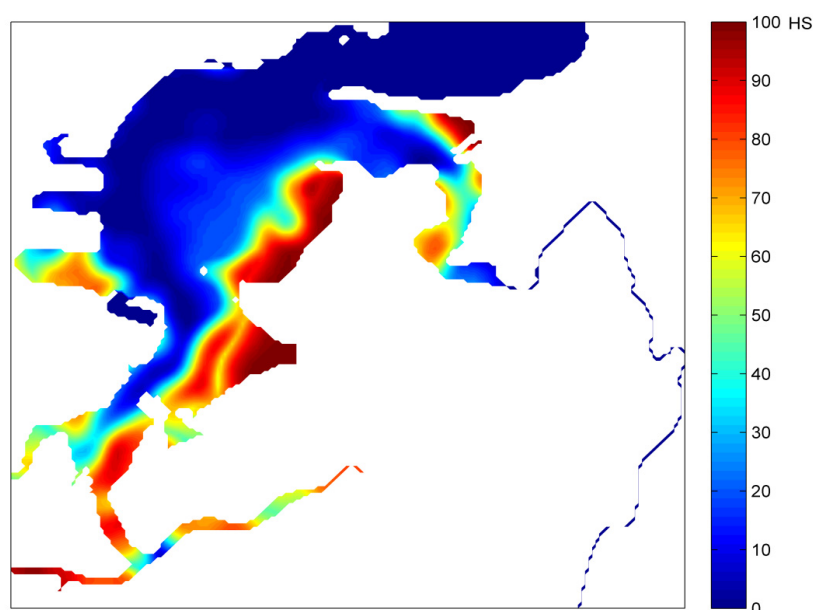


Figure 4.5 - Habitat suitability map for *R. philippinarum* in the Bay of Santander.

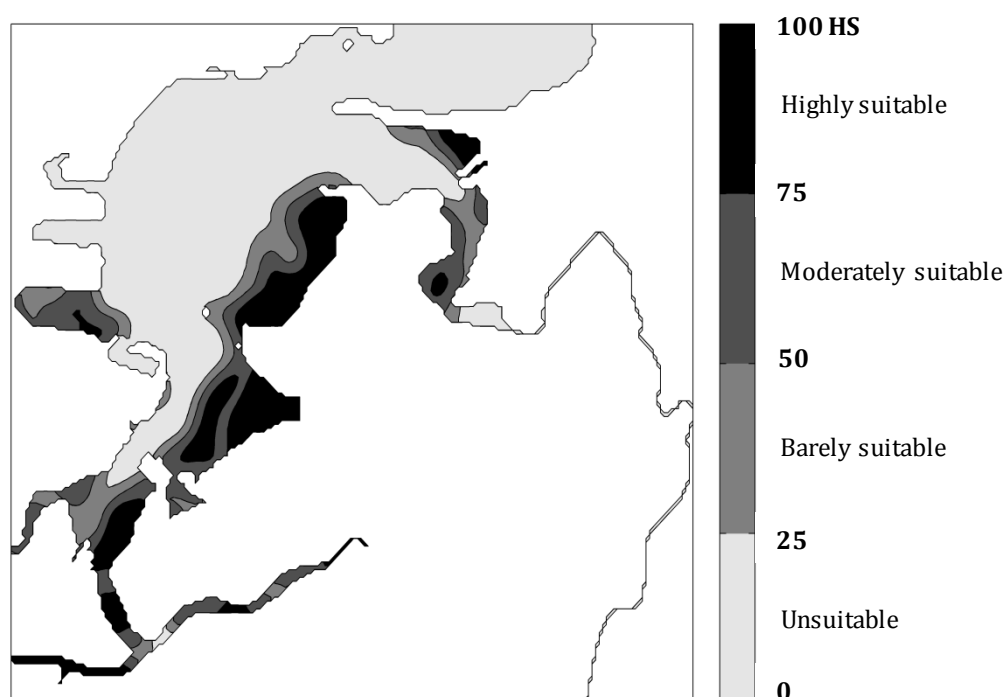


Figure 4.6 - Habitat suitability map for *R. philippinarum* in the Bay of Santander, classified by into 4 habitat suitability index (HSI) classes using: HS index value (HSI): unsuitable (HSI <25), barely suitable ($25 \leq \text{HSI} < 50$), moderately suitable ($50 \leq \text{HSI} \leq 75$), highly suitable (HS >75).

The cross-validation statistics showed the model to have a high Boyce index of 0.77 ± 0.08 , indicating the model was a good predictor of the habitat suitability of Manila clam. A 16 % of the surface of the Bay of Santander (317 Ha) has highly suitable environmental conditions for the development of this species. Moreover, other 317 Ha resulted to be moderately suitable habitats (Table 4.4). The surface with suitable habitat (HS index >50) for this species was more than 200 Ha lower than that for *R.decussatus*. The Manila clam suitable habitat distributes along the eastern tidal flats of Pedreña and Elechas and along the southern inner zones (i.e Solia and Tijero tidal fresh), with a limited presence in the Cubas tidal fresh in the northern area (Figure 4.5 and 4.6).

4.3.4. Coexistence and predomination patterns

In Figure 4.4, we present potential coexistence or predominance patterns of the native carpet shell clam and the introduced Manila clam. The coexistence pattern without potential predomination of either species is the most common pattern. In the north and in central southern zone of the estuary, the native carpet shell clam potentially predominates in an intertidal area of 153 Ha (reperesented in blue). In the southern zone of the bay, mainly in Tijero and Solía, the introduced Manila clam potentially predominates (42 ha, represented in red).

The ranges of the suitable environmental conditions found for the three analyzed patterns of relative abundance between species are presented in Table 4.5. The carpet shell native clam potentially predominates in areas with environmental conditions similar to those found in the coexistence areas. The main difference between these two areas was recorded in current velocity, being slower in carpet shell clam domination areas than in coexistence areas. The Manila clam potential predominance areas are considerably different from coexistence areas mainly because of sand and organic matter content. Low percentage of sand and high organic matter contents were found in the introduced bivalve

predomination areas compared to coexistence or carpet shell clam predomination areas. Moreover, Manila clam potential predomination areas were slightly shallower than those where the native clam potentially predominates.

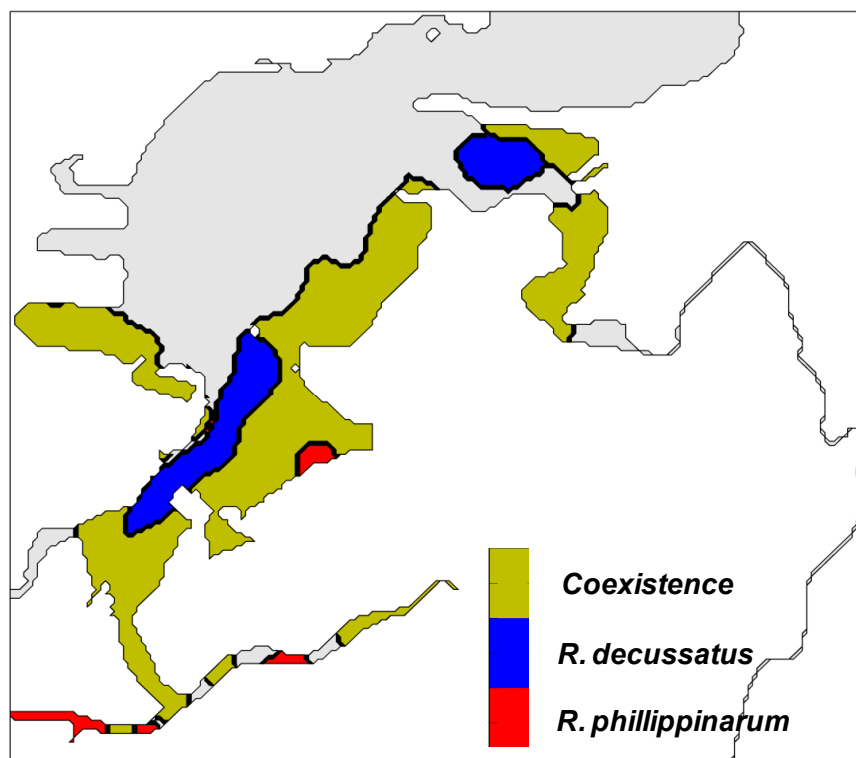


Figure 4.7 - Potential predomination and coexistence patterns of distribution of *R. decussatus* and *R. philippinarum* in the Bay of Santander.

Variable	Coexistence	<i>R. decussatus</i> predomination	<i>R. philippinarum</i> predomination
Bathymetry (m)	0.5 -1.5	1.1 -1.5	0.5 -0.8
Current velocity (m/s)	0.08 (\pm 0.07)	0.18 (\pm 0.05)	0.06 (\pm 0.04)
Sand (%)	65.1 (\pm 26.9)	65.6 (\pm 17.8)	26.5 (\pm 7.3)
Salinity (psu)	32.1 (\pm 2.2)	32.2 (\pm 1.7)	32.8 (\pm 0.3)
Organic matter (%)	4.5 (\pm 2.5)	4.7 (\pm 1.1)	8.7 (\pm 1.1)

Table 4.5 - Distribution of the values of the 5 environmental variables used in ENFA analysis. For each variable the mean value (\pm SD) or the minimum and maximum values (in the case of bathymetry) are presented for the areas where (i) potentially both species coexist, (ii) *R. decussatus* potentially dominates and (iii) *R. philippinarum* potentially dominates.

4.4. Discussion

The ENFA presence-only modelling method was used to construct habitat suitability predictions for two congeneric bivalves, the European native carpet shell clam *R. philippinarum* and the introduced Manila clam *R. philippinarum* in the Bay of Santander (North Spain). The model has considerably high predicted accuracy according to the accepted statistical test (Boyce index, Boyce et al., 2002) for internal cross validation (0.82 for *R. decussatus* and 0.77 for *R. philippinarum*). Results showed high and low suitability spatial patterns for both clam species. Moreover, the combination of the potential distribution of both species was used to analyze their potential coexistence or relative predominance patterns. The results obtained are consistent with current knowledge (e.g. Laing and Child, 1996; Vincenzi et al., 2006a,b, 2011; Cannas, 2010; Cilenti et al., 2011) and also with the differential distribution patterns of these two species recently found by Juanes et al. (2012) in the same estuary. With respect to the presence data used to construct the model, it is worth mentioning that although the density of clams was estimated at each location, the habitat suitability map was computed without attributing a

weight to the presence data. This decision was taken considering that the differential removal of clams, due to the fishery effort and the different closure regimes established in the bay (i.e. seasonal or prohibited) (Juanes et al., 2012), could lead to a significant bias in the natural densities and consequently in the determination of the habitat suitability.

The ENFA ecological marginality factor indicates that the habitat of both species differs considerably from the average conditions of the Bay of Santander. Manila clam showed a larger deviation from the average conditions of the estuary (i.e. higher marginality) than the native clam. However, the specialization factor indicates that the introduced clam is slightly less restrictive in selecting the range of conditions where it is distributed (i.e. lower specialization). This result is consistent with the possibility that Manila clam, first introduced in the late 80s in the Bay of Santander, may have behaved as a specialist during the early stages of colonization becoming more generalist than the native species as the population expanded (this is known to have occurred for several terrestrial invading species, see, for example, Hilden, 1965, and Sol et al., 1997). This result would also be consistent with the great success encountered by this species in colonizing estuaries all over the world which in many cases are governed by significantly different environmental conditions.

Extracting the environmental variables explaining the distribution of a species is complicated and requires background knowledge of the ecosystem and species biology. Variables that were strongly correlated with high habitat suitability might not necessarily be drivers, but rather they may be correlated with other important variables. In the present study, there was strong correlation between organic matter and silt content and also between gravel and sand content. This further complicates the understanding of the relative importance of each individual environmental variable (Tittensor et al., 2009). Nonetheless, the relative importance of each environmental variable in the ENFA model (Table 4.3) provide guidance on the drivers and interactions of the two species and the overall natural system.

Among the variables considered those that explain most of the variance of the distribution of both clam species were bathymetry and current velocity, with respect to the marginality, and salinity, with respect to the specialization (Table 4.3). In general, the distribution of clams is similar in terms of bathymetry, limited to a narrow intertidal zone ranges, from 0.5 m to 1.5 m below the mean sea level. This distribution seems to be associated with the most optimal conditions for growth of clams of the genus *Ruditapes* which are found in shallow rather than in deep waters (Vincenzi et al. 2006a,b; Albentosa and Moyano, 2009; Cannas, 2010). In shallow intertidal sand flats they take advantage of the tidal movement in estuaries since water currents generated by the tides continuously supply a much larger quantity of food than the amount locally available (Cilenti et al., 2011). Along this line, current velocity is positively associated with high habitat suitability for carpet shell clam whilst it is negatively associated with that for Manila clam (marginality factor, Table 4.3) which requires more sheltered areas (i.e. lower current velocities). However, it seems that since both species are filter feeders, they need a minimum current speed to produce enough food circulation and resuspension in the area they inhabit. These results are consistent with current knowledge regarding these species biology and feeding habits. Boday and Plante-Cuny (1984) found that the growth rate of *R. decussatus* is related to the primary production of the water column and to the resuspension of microphytobenthos. *R. philippinarum* seems to feed mainly on particulated organic matter resuspended from the sediment (i.e. detritus and microphytobenthos) but also on phytoplankton (Watanabe, 2009). Resuspended detritus as source of food is more abundant in inner sheltered muddy zones of the estuaries with higher organic matter inputs (Junoy and Viéitez, 1990; Mendes et al., 2006). Both species were recorded in sediments from muddy to sandy although Manila clam predominated over carpet shell clam in muddy zones with a high content of organic matter which is susceptible to resuspension in each tidal cycle.

The highly suitable habitats occupy larger areas for the native carpet shell clam than for the introduced Manila clam although both species habitat overlap over large areas. Habitat

suitability maps showed a heterogeneous pattern for both species with highly suitable intertidal areas along the northern and eastern tidal flats for *R. decussatus* and along the eastern tidal flats and southern inner zones for *R. philippinarum*. Moreover, the potential predomination pattern of this species over the native carpet shell clam occurs in restricted areas on the southern tidal flats of the estuary. These reduced areas are governed by muddy sediments with high content of organic matter and low current velocities, conditions far from those required for the optimal development of the native clam. The predomination-coexistence patterns resulting from the combination of both species habitat suitability maps are consistent with a recent study about differential patterns of these species in this estuary (Juanes et al., 2012).

The high habitat suitability, potential predomination areas and the underlying processes and interactions have potential implications for the understanding of the distribution of both species and for the development of zone-based conservation and management strategies for estuarine or lagoon ecosystems. The high values of the Boyce index indicate highly reliable maps with a high predictive power (Hirzel et al. 2006) for both species. However, these results should be treated with some caution taking into account the sampling method and the introduced species, which might have not fully occupied its fundamental niche yet. On the one hand, the locations for recording the presence of clam were placed mostly in the common fishing areas which can bias the data and could influence the final outcome of the predictive maps. In the future, it would be advisable to conduct a field campaign at more sites located also outside fishing zones so to define trends over a broader range of conditions. On the other hand, the niche factor analysis represents an estimate of both species realized niche in the study area and environmental conditions being considered. The realized niche is defined as the part of fundamental niche that an organism occupies as a result of limiting factors present in its habitat (Hutchinson, 1957; Phillips et al., 2006). The presence of competing species in an environment is one example of a limiting factor that restrains or narrows an organism's ecological niche. In a realized niche, the organism tends to occupy and play an ecological

role where it is mostly highly adapted (Rodríguez-Caval et al., 2012). If the realized and fundamental niche differ, it is impossible for any modeling algorithm to describe the species' full fundamental niche because the information is not present in the presence-only dataset (Phillips et al. 2006). With this hypothesis in mind, the results presented here could underestimate Manila clam potential distribution since it could still be expanding and, in the future, it could inhabit new areas with suitable environmental conditions. However, considering that the first introductions were realized in the 80s and, potentially, this species has importantly expanded becoming more generalist than the native species (i.e. currently, with a lower specialization value), as it is known to have occurred for several terrestrial invading species (Hilden, 1965; Sol et al., 1997), the realized niche may not be substantially different to the fundamental niche.

Therefore, considering that habitat suitability regulates the stage of development of the nonindigenous species, limiting or permitting the passage from a stage of establishment, to becoming widespread or even dominant (Occhipinti-Amborgi, 2007), it can be said that its expansion in the Bay of Santander may be regulated, in part, by the habitat suitability filter. Predation, which is considerably higher on Manila clam than on carpet shell clam, is the other main factor responsible for the regulation of the expansion and predomination of this alien species (Bidegain and Juanes, 2012c; see Chapter VI).

Apart from the suitability of the colonized area and the regulation due to predation, the number of introductions is another important factor regulating the expansion of an introduced aquaculture species. These species form widespread and dominant populations in the estuaries where they have been introduced intensively and repeatedly (Occhipinti-Amborgi, 2007). As it was mentioned above, in other estuaries in Europe (e.g. Arcachon Bay, Lagoon of Venice) Manila clam predominates dramatically over the native clams (Aubby, 1993; Marin, 2003; Blanchet et al., 2004; Mistri, 2004; Caill-Milly et al., 2008). Regarding the intensity of introductions, in Arcachon and Lagoon of Venice the farming areas are operating since the mid 80s and the farmed surface is 24 Ha and 900 Ha,

respectively (Mantovani et al., 2006; Toupoint et al., 2008). However, in the Bay of Santander the introductions have been considerably less intensive since active farming areas occupied only $\sim 1\text{Ha}$ located in a unique site (Juanes et al., 2012). Therefore, considering the intensity of introductions in the Bay of Santander, habitat suitability and predation seem to be essential in avoiding that the Manila clam reaches the predomination patterns observed in other estuaries in Europe. Yet, a drastic increase of farming areas and introduction intensities may lead to a different equilibrium of the system where the fundamental niche of Manila clam might be similar to the current one but the predomination area could be considerably larger.

Along this line, first steps given in this study should help to determine highly suitable areas for cultivation of both species and particularly for restoration of the native clam. In this regard, Vincenzi et al. (2006a,b;2011) provide the unique estimate of suitable areas for the worldwide cultivated Manila clam, using logistic and ordinary regressions, and no attempts have been made for the native carpet shell clam. However, regarding this work, the lack of information about the areas where species are absent complicates the use of common ecological modeling tools, such as logistic regression or classification and regression trees (Guisan and Zimmermann, 2000). Therefore, the ENFA approach based on presence data could provide also a promising alternative tool for scientist and managers and can be a key factor in the sustainable management of bivalve fisheries and particularly in farming site selection. Moreover, the site selection should also be conditioned by the population connectivity patterns explored by biophysical models of these species (e.g. Bidegain et al., 2012b (see Chapter V); Herbert et al., 2012). In this regard, most suitable sites (i) for the introduced *R. philippinarum* should be those from where the larval dispersion does not lead to a dramatic widespread and domination of this species over the native one and (ii) for the native *R. decussatus* could be those from where the larval dispersion lead to a potential restoration of the species in other zones of the estuary. This research line is developed in the next chapter.

4. 5. Conclusions

The Marginality and Specialization values obtained from the ENFA indicate that the presence of the European native carpet shell clam, *R. decussatus*, and the introduced Manila clam, *R. philippinarum*, is restricted to certain areas with specific environmental characteristics which differ from the mean environmental conditions. The most suitable estuarine habitat is generally sandy intertidal sediments with substantial organic matter content washed by waters with low (but non zero) current speeds and lower salinities than the open sea. Among these environmental features the Manila clam has preference for slightly lower current velocities and inner muddy estuarine zones. These differences in environmental conditions preferences are more obvious when comparing the potential predomination zones which are much reduced for the introduced Manila clam and confirm the preliminary distribution patterns observed in the clam populations characterization analyzed in Chapter III.

This is the first time that ENFA has been used to predict suitable habitat of these congeneric clam species and to explore the potential expansion of Manila clam using the habitat suitability approach. This analysis has generated 51 x 51 m resolution maps of habitat suitability which have allowed us to describe suitable clam habitats for both species by indicating the relative importance of different environmental variables. The generated models and HS maps are only the first step in determining the potential distribution of these clams and in exploring the role of habitat suitability in the regulation of the worldwide introduced Manila clam. The integration of more environmental variables with higher-resolution data and additional clam samples would allow for further refinement of the models. Thus, the habitat suitability determination could come through fine-scale predictive modelling on each tidal flat and would provide a more reliable tool for spatial aquaculture and fishery management strategies.

The results of this study suggest that, with the current cultivation intensities, Manila clam will not become a predominating species all over the Bay of Santander as it has occurred in other estuaries of Europe. Furthermore, this study provides a suitable tool for determining potential farming sites for both species.

Chapter V

LARVAHS: Predicting clam larval dispersal and recruitment using habitat suitability-based particle tracking model

Chapter V. LARVAHS: Predicting clam larval dispersal and recruitment using habitat suitability-based particle tracking model

This study has led to the submission of the research article entitled “Predicting clam larval dispersal and recruitment using habitat suitability-based particle tracking model” by Bidegain, G., Bárcena, J.F., García, A., Juanes, J.A. for publication in Ecological Modelling.

Abstract

We explore the potential larval dispersal and recruitment patterns influenced by larval behavior and hydrodynamics by means of a particle-tracking model coupled to a hydrodynamic model, for *Ruditapes decussatus* and *Ruditapes philippinarum* bivalves. For this purpose, a specific study was carried out in the Bay of Santander (N Spain), a well-mixed shallow water estuary where both species shellfishery is conducted. The model was fed with observed winds, freshwater flows and astronomical tides to obtain predictions during clams spawning period. Dispersion of larvae from 7 spawning zones was tracked subjected to three-dimensional advection, vertical turbulent diffusion and imposed vertical migration behavior parameterized from literature. A habitat suitability-based (ENFA, Environmental Niche Factor Analysis) settlement-recruitment submodel was incorporated into the model to simulate settlement behavior and post-settlement mortality. Three simulation periods (spring, summer and autumn) and 2 initial releases (spring / neap tide) were combined in 6 different modeling scenarios. The model (LARVAHS) highlights the role of specific larval swimming behavior (i.e. planktonic duration), season (i.e. predominating winds) and the spawning zone location on recruitment success together with the effect of the tidal phase at spawning. Moreover, it was proven to be a valuable tool for determining major spawning and nursery grounds

and to explore connectivity between them. These model results have important implications for shellfisheries management and aquaculture or restoration activities.

5.1. Introduction

Knowledge of the larval dispersal patterns between benthic habitat patches is critical to understand the connectivity and persistence of marine populations (e.g. Botsford et al., 2001; Hastings and Botsford, 2006; Pineda et al., 2007). Thus, during last decades, predicting the dispersion and supply of larvae has been one of the major goals on population ecology (e.g. Rougharden et al., 1988; Siegel et al., 2003, Siegel et al., 2008), specially on fisheries management and restoration activities (Kakino, 1996; Bostford et al., 1997; North et al., 2009; Savina et al., 2010; Kim et al., 2012).

The prediction of the larval supply needs to encompass (i) spawning stock abundance (Myers and Barrowman, 1996; Francis, 1997; Myers, 1997; Ye, 2000), (ii) larval dispersion, which depends largely on the swimming behavior of larvae, the duration of the planktonic stage and the hydrodynamic conditions (e.g. Thorson 1950; Roegner, 2000; Pineda et al. 2007) and (iii) settlement, which refers to where and when larvae find a suitable habitat to metamorphose (Pineda et al. 2007; North et al., 2008). The final recruitment success (i.e. the number of individuals reaching a juvenile nursery area) (North et al., 2009) is influenced by the previous settlement and the early post larval mortality (Hunt and Scheibling, 1997; Pineda et al. 2007).

Biophysical models integrating these factors are increasingly being used to predict larval transport and explore the role of different biological and physical factors on larval dispersal and settlement of marine benthic species (Metaxas and Saunders, 2009). However, only few biophysical models include a habitat suitability approach in their settlement subroutines and they are simplified subroutines and commonly do not

consider environmental variables to define the suitable habitat conditions for survival of species. The subsequent aim, beyond determining larval dispersal and simplified settlement patterns, is to move towards including habitat suitability modeling to better understand recruitment success or post settlement mortality. A better understanding of recruitment patterns will provide fundamental insights into the population dynamics, since it is determinant to predict the future stability of benthic populations.

In this study, a particle-tracking model coupled with a quasi-3D hydrodynamic model was developed, forced with observed tides, winds and freshwater flow. The model was named LARVAHS because of the integration of the habitat suitability (HS) in the larval evolution model. It was used to study the larval transport, supply and recruitment of the native *R. decussatus* and the introduced *R. philippinarum* in the Bay of Santander (N Spain, Gulf of Biscay), since further investigation in recruitment was necessary after the preliminary approximation obtained in Chapter III. For this purpose, the particle-tracking model included (1) a larval behavior submodel and (2) a settlement submodel based on the habitat suitability resulted from the Environmental Niche Factor Analysis (ENFA) previously conducted in Chapter IV. The aim of this study was to answer the questions: Where do the larvae settle? Where the settled larvae come from? To address these two questions, the specific objectives of this study were (1) investigating the effect of location of spawning zones and hydrodynamic variables (i.e. tide and wind) on larval dispersion and supply, (2) determining the most important spawning zones (i.e. mayor suppliers of successful recruits) and nursery grounds and (3) assessing the potential connectivity between the spawning and nursery grounds. These model results may have important implications for shellfisheries management and aquaculture or restoration activities.

5.2. Material and methods

5.2.1. Study area

The bathymetric grid of the Bay of Santander and the adjacent coast (Figure 5.1) used in the hydrodynamic model is defined by cells of 51x51 m and an extent of 244 x 298 cells. This grid is the same as used in the habitat suitability determination in Chapter IV in order to integrate the obtained results in the larval evolution model. Considering previous studies in neighbor areas (Rodríguez-MoscOSO et al., 1992; Rodríguez-MoscOSO and Arnaiz, 1998; Urrutia et al., 1999; Ojea et al., 2005) main spawning events may occur from spring to autumn in the Bay of Santander. Thus, these seasons were the simulation periods selected for this study. According to the results obtained in Chapter III the higher recruitment intensity may occur in central and northern zones of the Bay for the carpet shell clam and in the central and most inner southern zones for Manila clam. A more detailed description of the study area is presented in Chapter II.

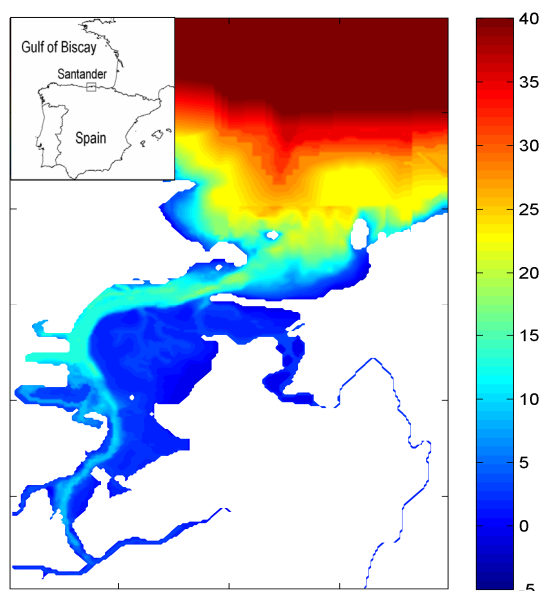


Figure 5.1 - Study area from the model: Bay of Santander estuary and adjacent waters located in the northern coast of Spain (Gulf of Biscay). Bathymetry (m) data of the modeled area is presented.

5.2.2. Model description

We constructed a larval evolution model (henceforth, LARVAHS) that incorporated predictions from a hydrodynamic model and a particle-tracking model to calculate the movement of particles that simulate larvae dynamics. In this study, the model was implemented to adequately represent larval evolution of two clam species: the native European clam (*Ruditapes decussatus*) and the nonindigenous Manila clam (*Ruditapes philippinarum*). LARVAHS tracked the trajectories of larvae in 3 dimensions and then, predicted settlement and recruitment success based on habitat suitability maps. The model was forced with tide, river and wind conditions occurred from April to November 2010 in order to capture a range of environmental variability experienced by clam larvae during the considered spawning season (see 2.1. Study area). We isolated the influence of physical conditions and organism behavior on the 3D spatial trajectories of particles to determine if specific larval swimming behavior and seasonal or tidal conditions could influence dispersal distance, encounter with suitable habitat and populations connectivity. LARVAHS was created by coupling a hydrodynamic and a particle-tracking model, and including behavior, disappearance, and settlement-recruitment sub-models. This latter sub-model is based on the habitat suitability (HS) for the studied species, giving the name to the acronym of the model.

5.2.2.1. Hydrodynamic model

Tidal current velocities were calculated by means of a two-dimensional depth-averaged hydrodynamic coastal and estuarine circulation model since vertical velocity profiles can be assumed to be almost uniform according to field measurements. This model solves the momentum equations dividing the study area into square cells. Its governing equations are presented in Bárcena et al. (2012a,b) and García et al. (2010a). A quasi three-

dimensional model that takes into account the different structure over the depth of horizontal velocities along the depth due to wind action was used (Koutitas, 1988). Its governing equations are presented in García et al. (2010b).

A similar implementation, i.e. same code, same forcing data and similar domain, has been previously calibrated and validated by López et al. (2013) against observed water levels, current velocities and salinities, covering a full phase of spring and neap tides. Comparison between in situ measurements and predicted levels provided the relative error in each of the control points (Table S1 in López et al., 2013) demonstrating the model ability to simulate different hydrodynamic conditions in the Bay of Santander. The predicted salinity results generally agreed well with the in situ measured data at four stations (Fig. S4 in López et al., 2013) confirming the ability of the model to simulate accurately the spatial pattern of salinity, and by extension, its ability to simulate the transport of passive tracers.

5.2.2.2. Larval evolution model: LARVAHS

LARVAHS was developed from a particle-tracking model performed to predict the movement of particles based on advection, sub-grid scale turbulence and larval swimming behavior. It was designed to predict the larval settlement and recruitment based on the previous habitat-suitability raster-based maps obtained by Bidegain et al., 2012 in the Bay of Santander for the study species.

Particle-tracking model

The model uses a particle-tracking approach to simulate larval advection and diffusion. Advection is computed solving the equation 1 for each particle:

$$\frac{dr}{dt} = q \quad (1)$$

where r is the position vector of the particle and q is the current vector solved in components u and v along the x and y axes. Currents are obtained by running a hydrodynamic model in advance. As a consequence, the evaluation of the tidal advective transport of larvae is very fast and is not limited by the Courant Friedrich Levy criterion (Kowlik & Murty, 1993).

Because horizontal and vertical diffusivity were constant in the hydrodynamic model, three-dimensional diffusion of the turbulent particle is simulated using a random walk method (Proctor et al., 1994a; Hunter, 1987; Periañez and Elliott, 2002, Periañez, 2004). The maximum sizes of the horizontal and vertical steps, D_h and D_v respectively, are:

$$D_h = \sqrt{12K_h\Delta t} \quad (2)$$

$$D_v = \sqrt{2K_v\Delta t} \quad (3)$$

where K_h and K_v are the horizontal and vertical diffusion coefficients respectively. The model included an external time step of 10 minutes, which is the recording time step of hydrodynamic model results and an internal time-step of 30 seconds, which is the time interval of particle movement. Because of the hydrodynamic model resolution (51 m x 51 m), a given particle may take several time steps to move across a grid cell. Hence the predicted currents were interpolated in both space and time to provide 3D fine-resolution fields for advecting clam larvae according to the hydrodynamic model outputs. For particle movement due to current velocities in the x , y , and z directions, a 4th order Runge-Kutta scheme was implemented. The 4th order Runge-Kutta scheme provides the most robust estimate of the trajectory of particle motion in water bodies with complex fronts and eddy fields (Dippner 2004) like Bay of Santander.

Regarding particle movement, different boundary conditions were imposed to the particle-tracking. First, if a particle passed through the surface or bottom boundary due to turbulence or vertical advection, the particle was placed back in the model domain at the

previous time step location. Second, if a particle passed through the surface or bottom due to swimming behavior (see Behavior sub-model) it was placed just below the surface or above the bottom (i.e. it stopped near the boundary). Third, if a particle intersected a horizontal boundary, it was reflected off the boundary at an angle of reflection that equaled the angle of approach to the boundary.

Behavior sub-model

The behavior sub-model considers the larval ability to swim vertically during its life cycle following comments and results obtained by North et al. (2006;2008), Ishii et al. (2005) Kuroda (2005), and Suzuki et al (2002) for oysters such as *C. virginica* or *C. ariakensis* or Manila clam. This sub-model tries to mimic the vertical movement of larvae towards intermediate and surface water layers at early stages (trochophore, D and U larvae) and the sea-bottom, when transition to pediveliger occurs. In the simulations, for the European clam, the planktonic larval phase is considered for a period of 21 days varying its size between ~80 and ~220 microns (Pérez-Camacho, 1980; Borsa and Millet, 1992; Chícharo and Chícharo, 2001b; Vela and Moreno, 2005). Meanwhile, for the Manila clam, the planktonic larval stage is defined for a period of 15 days with a size between ~90 and ~240 microns (Young-Baek et al., 2005; Hinata and Furukawa, 2006). Table 5.1 summarizes the behavioral considerations of the two species that were implemented into the behavior sub-model. Visualization of Video 1 (See CD/LARVAL BEHAVIOR\VIDEO1) and Figure 5.7 (in results section) help understanding of the behavior of larvae during its life cycle.

Life cycle	Life days <i>R. decussatus</i>	Life days <i>R. philippinarum</i>	Swimming capability	Direction and movement probability	Swimming speed (mm/s)
Egg	0 – 1	0 – 1	No	0	0
Trochophore Larvae	1 – 2	1 – 2	Yes	90% chance of move up	0.5
D, U Larvae	2 – 14	2 – 10	Yes	Probabilities that shift their distribution from the upper layer to the lower layer as they increase in age, from a 51% chance of move up in each time step to a 51.7% chance of swimming down (linear function of particle age).	0.5-3 (speed increases linearly with age)
Pediveliger Larvae	15 – 21	10 – 15	Yes	100% chance of move down and stay within a 1 m water column from sea-bottom. In this water column, 50% chance of move up and 50% chance of move down	3

Table 5.1- Summary of larval behavior for *R. decussatus* and *R. philippinarum*. It details, for egg and larval phases, the duration (life days) and the capability and the vertical swimming behavior, adapted from Suzuki et al (2002), Kuroda (2005), Ishii et al. (2005), North et al. (2006:2008).

Settlement-recruitment sub-model

We used the habitat-suitability (HS) raster-based grid, obtained by Bidegain et al. (2012) for the study species using environmental factor analysis (ENFA), to determine if a pediveliger-stage larvae encountered suitable habitat to settle and recruit. The integration of habitat suitability maps in the model grid was automatic since extent of the study area and the cell size used were identical (244 x 298 cells of 51m x 51m). Each cell had a HS index (HSI) which ranged from 0 to 100, with higher values being more suitable for recruitment and zero being completely unsuitable. The minimum HSI value considered for recruitment to occur was 25. For every internal time step (30 s), each pediveliger-stage particle was tested to determine if it was at the sea-bottom. When it was at the bottom, the sub-model checked if the cell HSI was greater than 25 and in that case, the particle settled, stopped moving. When HSI was lower than 25 the particle continued swimming. Finally, if the particle did not encounter a cell of $HSI > 25$ at the end of the pediveliger-stage, the particle dies. Once a given particle settled, its survival probability was determined by the HSI value of the cell. If the particle survived it was successfully recruited.

5.2.3. Initial conditions and scenarios

5.2.3.1. Spawning zones

Initial conditions for the simulations were defined for the major spawning areas in the Bay of Santander. Spawning areas were considered those defined as highly suitable ($HSI > 75$) for both species by Bidegain et al.(2012a) and described in Chapter IV. Thus, the spawning zones determined were 6 for *R. decussatus* and 7 for *R. philippinarum* (Figure 5.2). The density of adult clams found in each zone adapted from Bidegain et al.(2012a) and IH Cantabria (2011) is presented in brackets.

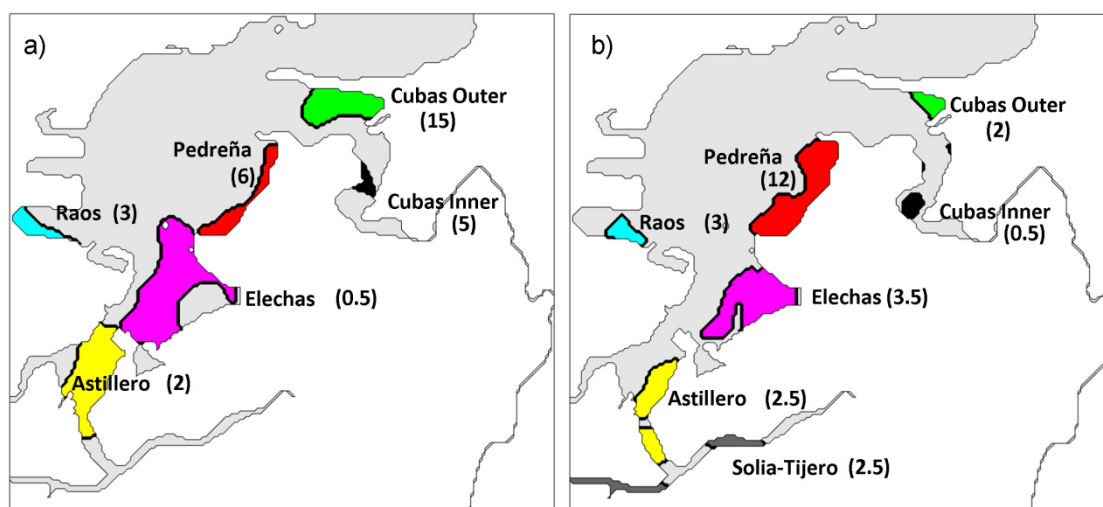


Figure 5.2 – Spawning zones for *R. decussatus* (a) and *R. philippinarum* (b) considered in the simulations, delimited by areas with habitat suitability index values greater than 75 (i.e. highly suitable areas) (Bidegain et al., 2012a, see Chapter IV). Mature clam density (individuals >20 mm/m²) in each spawning zone are presented in brackets adapted from Bidegain et al. (2012a) and IH Cantabria 2011. Different color is given to each spawning zone which is also given to the larvae coming from each of them.

5.2.3.2. Number of particles (eggs) released

The number of particles released in each spawning zone and scenario was proportional to the density of female adult clams and the number of grid cells covered by the spawning area (Figure 5.2). Considering a 1:1 ratio of males to females, it was calculated by multiplying the half of adult density (individuals/m²) in the spawning area by (2) the number of cells within the area, (3) the area of each cell (51 x 51 m) and (3) the number of eggs produced by each female adult clam. Considering previous estimations on the broodstock conditioning of this species (Yap, 1977; Chung et al., 2001; Park and Choi, 2004; Matías et al., 2009) we assume a total of 0.6×10^6 eggs released by each female clam for both species (i.e. 100000 in each scenario). A maximum of ~1 million particles were released from the most productive spawning zone due to computational constraints.

Therefore, each released particle represented 1×10^5 eggs in order to achieve the assumed egg production per female.

5.2.3.3. Simulation scenarios and environmental conditions

Three spawning seasons were tested within the identified spawning period for both species (April-November, Rodríguez-MoscOSO et al., 1992; Rodríguez-MoscOSO and Arnaiz, 1998; Urrutia et al., 1999; Ojea et al., 2005): spring, summer and autumn 2010. In each season two egg releases were tested: 15/04 and 25/04 (spring), 12/08 and 20/08 (summer), 09/10 and 16/10 (autumn) in 2010. The first date of release at each season coincided with neap tide and the second one with spring tide (Figure 5.3a). Tidal, river flow and wind conditions during simulation periods are presented in Figure 5.3 (data provided by Cantabrian Water Authority (CHC) and the Spanish Meteorological Agency, AEMET). The Miera river flow during simulation periods showed very low river flow values in general ($< 2 \text{ m}^3/\text{s}$). However, flows of $60 \text{ m}^3/\text{s}$ are reached in spring during neap tide scenario (Figure 5.3b). The two floods registered in late autumn (50 and $140 \text{ m}^3/\text{s}$) are out of the modeled periods for autumn spawning scenarios. Regarding winds, each season was mainly characterized by a predominating wind: SW winds in spring, NE winds in summer and W winds in autumn (Figure 5.3d). Wind intensities were higher in summer than in spring or autumn. In summary, 6 scenarios were tested from each spawning zone and species, corresponding to different tidal phase and season or predominating wind direction.

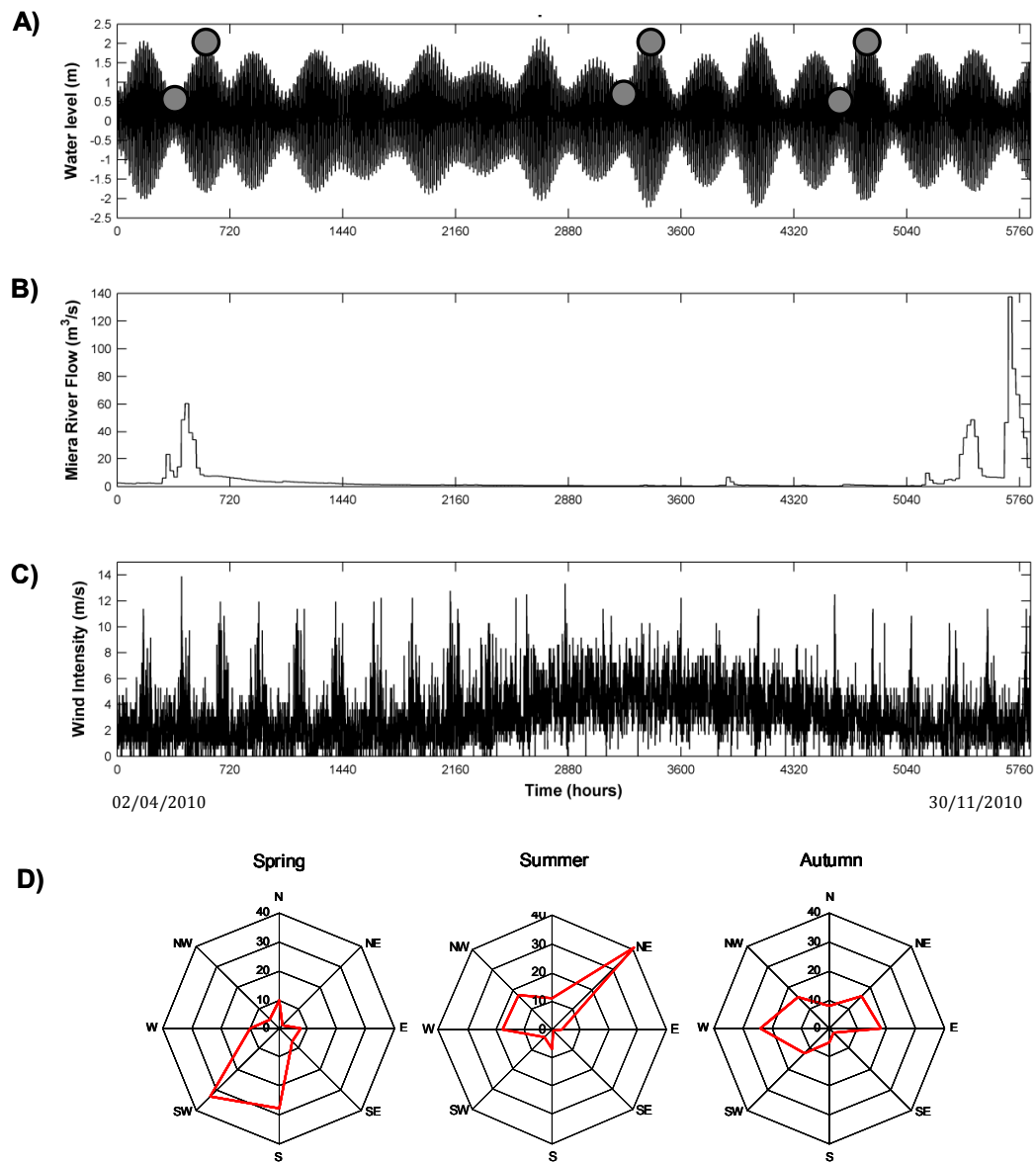


Figure 5.3 – Sea levels (a), Miera River flow (b) and wind intensity during simulation period from April (02/04/2010) to November (30/11/2010) (c) and wind roses (d) for each seasonal scenario are represented. Tidal scenarios' wind roses are not showed due to their similarity within the same season. Gray circles, in Figure 5.3.a, represent the spawning events dates at different tidal (neap and spring) and seasonal scenarios (spring, summer and autumn).

5.2.4. LARVAHS model evaluation

LARVAHS model integrates behavior and settlement-recruitment submodels in a particle transport model. In this study, a preliminary evaluation of LARVAHS model to predict recruitment of clams was conducted in two nursery grounds (Elechas and Raos). These grounds were selected because (i) they are located near each other and allows to sample both grounds in the same tide and (ii) shellfishing activity is minimal since they are located far from the coastline or out of the permitted shellfishing zones. Hence, the potential mortality of early recruiters associated to this activity (raking or pressing the sediment) was minimal.

Predicted recruitment density and observed density of early recruiters were compared and the strength of the relationship was analyzed by Spearman's rank correlation coefficient. Predicted recruitment density was calculated dividing the number of individuals successfully recruited in each nursery ground at each season (adding larvae coming from different spawning grounds at both tidal scenarios) by the nursery ground area (i.e. sum of all cells of 51 m x 51 m within the nursery ground). To obtain the observed early recruiters density, four sediment samples of 50 cm² to a depth of 15 cm were collected in each nursery ground in 29th June, 23th October and 12th December, i.e. after each spawning season modeled in the study (Spring, Summer, Autumn). All samples were passed through a 1 mm sieve and clam lengths were measured to the nearest 0.1 mm. Individuals smaller than 3 mm for *R. decussatus* and 5 mm for *R. philippinarum* were considered as early recruiters. This selected criterion followed was based on differential growth patterns described for these clam species (Arnal and Fernández-Pato, 1977, 1978; Spencer et al., 1991; Solidoro et al., 2000; Chessa et al., 2005; Dang, 2009) and trying to avoid counting early recruiters of the previous spawning season.

5.2.5. Data analysis

Model predictions were analyzed (i) to determine if spawning site location, season and tidal amplitude influence recruitment success, (ii) to determine most important spawning and nursery grounds and (iii) to assess the potential connectivity between spawning and nursery grounds.

5.2.5.1. Simulation results

For each spawning zone at each tidal and seasonal scenario the following data were recorded or calculated after each simulation: (1) number of dead larvae (i.e. not retained in the Bay or settled in cells with HSI < 25, (2) number of settled larvae within a HSI > 25 cell and (3) number of successfully recruited clam (i.e. counted after applying the survival probability associated to the habitat suitability, see within section 2.2.2. Settlement-recruitment submodel) and their associated percentages of the total eggs released.

5.2.5.2. Influential factors on recruitment success

Recruitment success, calculated as the percentage of individuals of the total eggs released that were retained in the Bay and successfully recruited, was used as response variable to compare the results obtained in different runs. Normality and homogeneity of variance were examined by Shapiro-Wilk and Levene tests, respectively, and data were transformed if these assumptions for a parametric analysis were violated. A t-test was performed to determine if recruitment success was significantly different between the studied species with different larval phase duration. In addition, to analyze the single and interactive effects of location of spawning site, tidal amplitude and season on recruitment success a multifactorial analysis of variance was conducted.

5.2.5.3. Major spawning and nursery grounds

Spawning grounds

Major current spawning grounds for both species were considered those from where, after larval dispersal, the highest number of individuals was recruited from larvae settled in cells with $HSI > 25$. To calculate all individuals recruited from a given spawning zone, individuals recruited at different tidal and seasonal scenarios were added together. Besides, most successful spawning grounds were calculated by calculating the mean percentages of recruited individuals of the total of the eggs released from a given zone at different tidal and seasonal scenarios.

Nursery grounds

Delimitation of nursery grounds considering larvae recruited in cells with HSI values greater than 25 (i.e. where recruitment occurs) was ambiguous since most of the nursery grounds were overlapped. Therefore, we consider the nursery grounds identical to the spawning grounds (i.e. highly suitable areas, $HSI > 75$) in order to have them clearly separated from each other. Thus, data analysis and interpretation of results facilitated regarding determination of major nursery grounds or connectivity between zones. Major nursery grounds were considered those where the number of recruited individuals was highest. To calculate all individuals recruited in each nursery ground, larvae coming from different spawning grounds at different tidal and seasonal scenarios were added together.

5.2.5.4. Connectivity between spawning and nursery grounds

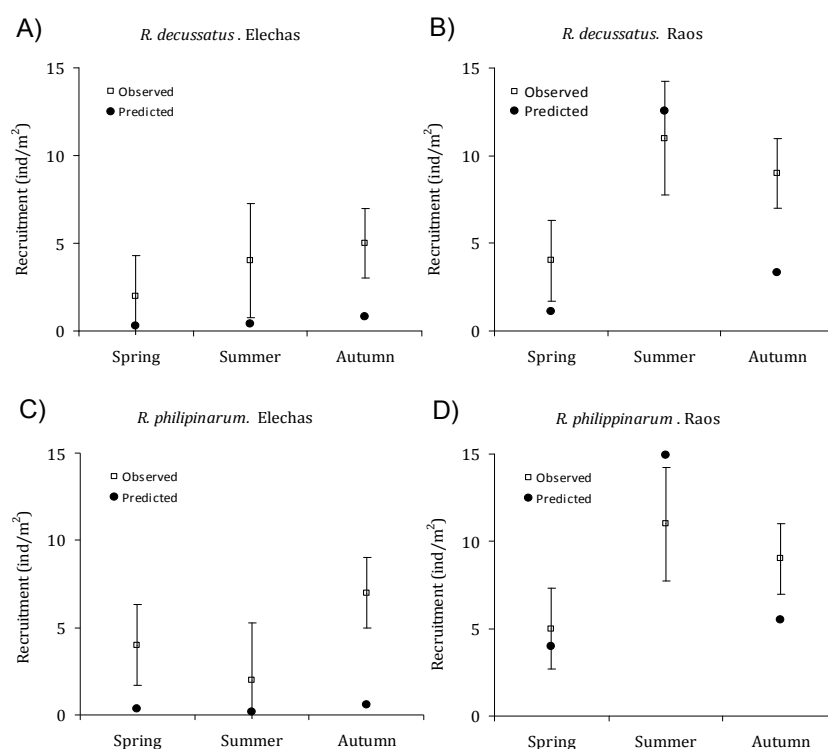
Connectivity matrices were created for each spawning season and tidal scenario. The connectivity matrices indicate which proportion of the total larvae recruited in a given nursery zone (x-axis) comes from each spawning zone (y-axis). Attending these

proportions the robustness between spawning and nursery grounds connections and the isolation and self-recruitment of grounds was analyzed.

5.3. Results

5.3.1. LARVASH model evaluation

Predicted recruitment density values were lower than mean observed values in general (Figure 5.4).



5.4 – Recruitment density (individuals/m²) predicted and observed values in Elechas and Raos sites for different seasonal scenarios.

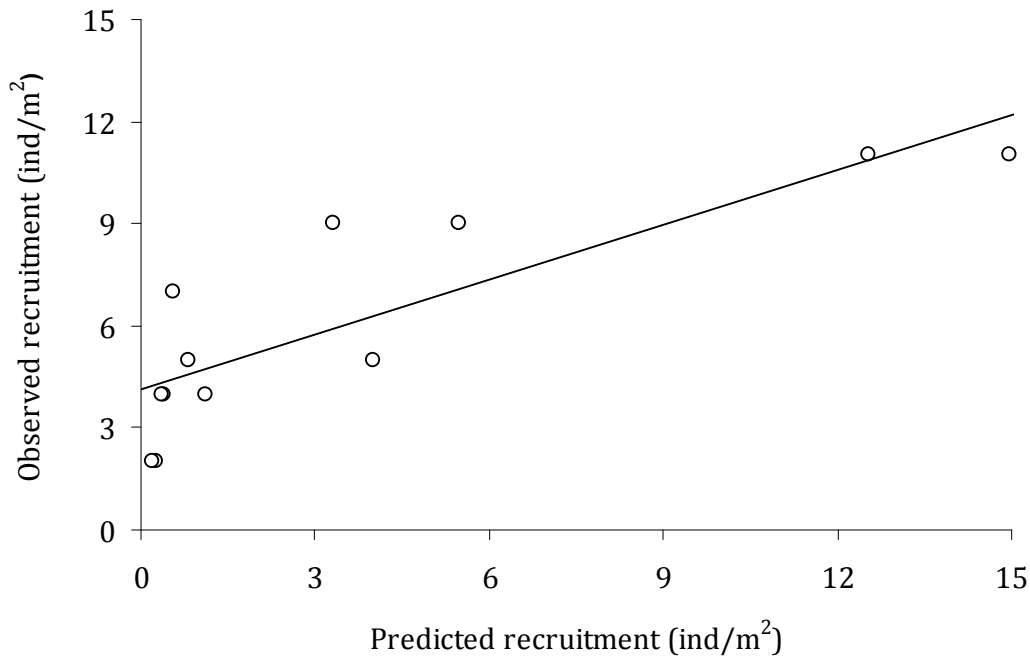


Figure 5.5 – Comparison of recruitment density (individuals/m²) obtained by modelling (predicted data, x-axis) and through in situ measurements (observed data, y-axis).

However, seasonal variability patterns were similar, obtaining a good correlation between observed and predicted recruitment density ($R^2 = 0.70$, Spearman's $R = 0.89$; $n = 12$, $t(n-2) = 6.4$, $p = 0.0001$) (Figure 4b) which involves a good qualitative fit of the model to the in situ measured data.

5.3.2. Hydrodynamic variability

In Figure 5.6, sea water circulation (mean currents) due to tidal and river forcing are presented for different seasonal scenarios, spring (a), summer (b) and autumn (c), showing a pretty similar hydrodynamic pattern since tidal force is similar and river outputs are low in general ($< 2 \text{ m}^3/\text{s}$) and not significantly different between scenarios. The punctual flood of the the Miera River observed in spring ($60 \text{ m}^3/\text{s}$, Figure 5.3b) cannot be appreciated in this Figure 5.6 a-c since the mean tidal-river currents were presented.

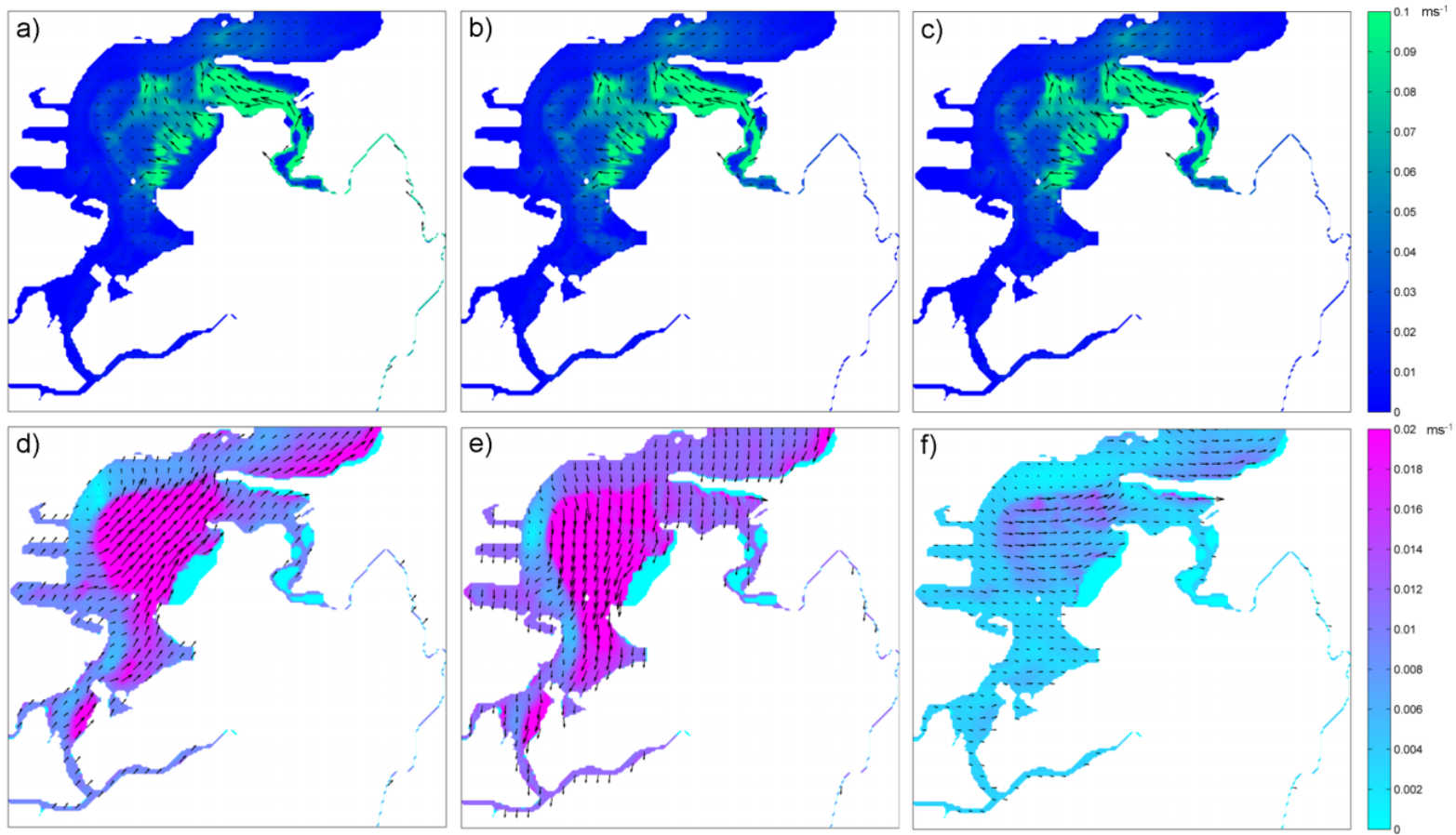


Figure 5.6 – Tidal-river currents (ms^{-1}) (a, b, c) and wind currents (d, e, f) for spring, summer and autumn scenarios,

However, the 3 seasons simulated have differentiated wind regimes: SW winds in spring, NE winds in summer and W and E winds in autumn (see Figure 5.3d). This leads to variations in the surface seawater circulation due to the wind forcing and particularly in the intensity and direction of the southward-northward or inshore-offshore transport. Northward/offshore transport is high during spring when the wind is from SW (Figure 5.6d), while in summer it is reversed and southward/inshore transport dominates helped by NE winds (Figure 5.6e). During autumn when the predominating winds are from west (Figure 5.3d), eastward transport is observed (Figure 5.6 f). The wind induced currents are superficial and smaller than tidal currents. Consequently they have a more appreciable effect in zones where the net tidal current currents are almost null, i.e., blue areas in Figure 5.6 a-c.

5.3.3. Simulation results data

In the Table 5.2, results of the 78 runs, 36 runs for *R. decussatus* (a) and 42 runs for *R. philippinarum* (b), are presented. For each spawning zone the particles released were different according to the zone extension and the density of adults clams. Thus, for *R. decussatus* Cubas Outer ground, with $\sim 800000 \times 10^5$ eggs released, was the most “egg productive” spawning zone followed by Astillero and Pedreña with $\sim 188000 \times 10^5$ and $\sim 164000 \times 10^5$ eggs, respectively. Besides, Pedreña spawning zone, with 963000×10^5 eggs released was the most productive zone for *R. philippinarum*, followed by Elechas with $\sim 333000 \times 10^5$ eggs.

<i>R. decussatus</i>										
Run	Season	Tide	Zone of release	Released eggs x 10 ⁵	Dead larvae x 10 ⁵	Settled larvae x 10 ⁵	Recruited clam x 10 ⁵	Dead larvae (%)	Settled larvae (%)	Recruited clam (%)
1	Spring	Neap	Astillero	187922	187534	388	67	99.79	0.21	0.04
2	Spring	Neap	Elechas	75924	75887	37	7	99.95	0.05	0.01
3	Spring	Neap	Raos	67704	67496	208	42	99.69	0.31	0.06
4	Spring	Neap	Pedreña	164052	164045	7	1	99.99	0.00	0.00
5	Spring	Neap	Cubas O.	813750	813696	54	43	99.99	0.01	0.01
6	Spring	Neap	Cubas I.	44485	44482	3	2	99.99	0.01	0.00
7	Spring	Spring	Astillero	187922	187763	159	26	99.92	0.08	0.01
8	Spring	Spring	Elechas	75924	75920	4	3	99.99	0.01	0.00
9	Spring	Spring	Raos	67704	67638	66	20	99.90	0.10	0.03
10	Spring	Spring	Pedreña	164052	164050	2	1	99.99	0.00	0.00
11	Spring	Spring	Cubas O.	813750	813202	548	457	99.93	0.07	0.06
12	Spring	Spring	Cubas I.	44485	44467	18	5	99.96	0.04	0.01
13	Summer	Neap	Astillero	187922	186338	1584	418	99.16	0.84	0.22
14	Summer	Neap	Elechas	75924	75673	251	26	99.67	0.33	0.03
15	Summer	Neap	Raos	67704	67446	258	60	99.62	0.38	0.09
16	Summer	Neap	Pedreña	164052	163725	327	39	99.80	0.20	0.02
17	Summer	Neap	Cubas O.	813750	813710	40	8	99.99	0.00	0.00
18	Summer	Neap	Cubas I.	44485	44443	42	4	99.91	0.09	0.01
19	Summer	Spring	Astillero	187922	187183	739	158	99.61	0.39	0.08
20	Summer	Spring	Elechas	75924	75806	118	8	99.84	0.16	0.01
21	Summer	Spring	Raos	67704	67284	420	82	99.38	0.62	0.12
22	Summer	Spring	Pedreña	164052	163902	150	22	99.91	0.09	0.01
23	Summer	Spring	Cubas O.	813750	813238	512	164	99.94	0.06	0.02
24	Summer	Spring	Cubas I.	44485	44444	41	6	99.91	0.09	0.01
25	Autumn	Neap	Astillero	187922	186995	927	196	99.51	0.49	0.10
26	Autumn	Neap	Elechas	75924	75853	71	12	99.91	0.09	0.02
27	Autumn	Neap	Raos	67704	67516	188	12	99.72	0.28	0.02
28	Autumn	Neap	Pedreña	164052	164042	10	3	99.99	0.01	0.00
29	Autumn	Neap	Cubas O.	813750	813741	9	2	99.99	0.00	0.00
30	Autumn	Neap	Cubas I.	44485	44470	15	6	99.97	0.03	0.01
31	Autumn	Spring	Astillero	187922	187645	277	40	99.85	0.15	0.02
32	Autumn	Spring	Elechas	75924	75906	18	5	99.98	0.02	0.01
33	Autumn	Spring	Raos	67704	67497	207	26	99.69	0.31	0.04
34	Autumn	Spring	Pedreña	164052	164030	22	3	99.99	0.01	0.00
35	Autumn	Spring	Cubas O.	813750	813674	76	46	99.99	0.01	0.01
36	Autumn	Spring	Cubas I.	44485	44476	9	3	99.98	0.02	0.01

Table 5.2 a - Mortality, settlement and recruitment scores for *R. decussatus* egg released (simulated particle x 10⁵) from each spawning zone (Astillero, Elechas, Raos, Pedreña, Cubas Outer, Cubas Inner) in each seasonal (spring, summer and autumn) and tidal amplitude (spring or neap tides) scenario.

<i>R. philippinarum</i>										
Run	Season	Tide	Zone of release	Released eggs $\times 10^5$	Dead larvae $\times 10^5$	Settled larvae $\times 10^5$	Recruited clam $\times 10^5$	Dead larvae (%)	Settled larvae (%)	Recruited clam (%)
37	Spring	Neap	Astillero	127912	127324	588	184	99.54	0.46	0.14
38	Spring	Neap	Elechas	332893	332574	319	35	99.90	0.10	0.01
39	Spring	Neap	Raos	50127	49992	135	18	99.73	0.27	0.04
40	Spring	Neap	Pedreña	963480	963408	72	30	99.99	0.01	0.00
41	Spring	Neap	Cubas O.	26908	26891	17	4	99.94	0.06	0.01
42	Spring	Neap	Cubas I.	5724	5722	2	1	99.97	0.03	0.02
43	Spring	Neap	Solfa-T.	100812	99541	1271	815	98.74	1.26	0.81
44	Spring	Spring	Astillero	127912	127510	402	114	99.69	0.31	0.09
45	Spring	Spring	Elechas	332893	332848	45	8	99.99	0.01	0.00
46	Spring	Spring	Raos	50127	50095	32	3	99.94	0.06	0.01
47	Spring	Spring	Pedreña	963480	963430	50	34	99.99	0.01	0.00
48	Spring	Spring	Cubas O.	26908	26808	100	80	99.63	0.37	0.30
49	Spring	Spring	Cubas I.	5724	5717	7	3	99.88	0.12	0.05
50	Spring	Spring	Solfa-T.	100812	99663	1149	681	98.86	1.14	0.68
51	Summer	Neap	Astillero	127912	126456	1456	879	98.86	1.14	0.69
52	Summer	Neap	Elechas	332893	331376	1517	667	99.54	0.46	0.20
53	Summer	Neap	Raos	50127	49952	175	32	99.65	0.35	0.06
54	Summer	Neap	Pedreña	963480	962157	1323	496	99.86	0.14	0.05
55	Summer	Neap	Cubas O.	26908	26907	1	1	99.99	0.00	0.00
56	Summer	Neap	Cubas I.	5724	5711	13	3	99.77	0.23	0.05
57	Summer	Neap	Solfa-T.	100812	99245	1567	1124	98.45	1.55	1.11
58	Summer	Spring	Astillero	127912	127148	764	333	99.40	0.60	0.26
59	Summer	Spring	Elechas	332893	332480	413	70	99.88	0.12	0.02
60	Summer	Spring	Raos	50127	49882	245	16	99.51	0.49	0.03
61	Summer	Spring	Pedreña	963480	963264	216	156	99.98	0.02	0.02
62	Summer	Spring	Cubas O.	26908	26900	8	4	99.97	0.03	0.01
63	Summer	Spring	Cubas I.	5724	5718	6	3	99.90	0.10	0.05
64	Summer	Spring	Solfa-T.	100812	99256	1556	1014	98.46	1.54	1.01
65	Autumn	Neap	Astillero	127912	126845	1067	572	99.17	0.83	0.45
66	Autumn	Neap	Elechas	332893	332327	566	137	99.83	0.17	0.04
67	Autumn	Neap	Raos	50127	49982	145	19	99.71	0.29	0.04
68	Autumn	Neap	Pedreña	963480	963377	103	37	99.99	0.01	0.00
69	Autumn	Neap	Cubas O.	26908	26905	3	2	99.99	0.01	0.01
70	Autumn	Neap	Cubas I.	5724	5718	6	2	99.90	0.10	0.03
71	Autumn	Neap	Solfa-T.	100812	99196	1616	1158	98.40	1.60	1.15
72	Autumn	Spring	Astillero	127912	127454	458	129	99.64	0.36	0.10
73	Autumn	Spring	Elechas	332893	332805	88	18	99.97	0.03	0.01
74	Autumn	Spring	Raos	50127	50040	87	20	99.83	0.17	0.04
75	Autumn	Spring	Pedreña	963480	963367	113	56	99.99	0.01	0.01
76	Autumn	Spring	Cubas O.	26908	26900	8	2	99.97	0.03	0.01
77	Autumn	Spring	Cubas I.	5724	5722	2	1	99.97	0.03	0.02
78	Autumn	Spring	Solfa-T.	100812	99426	1386	831	98.63	1.37	0.82

Table 5.2b - Mortality, settlement and recruitment scores for *R. philippinarum* egg released (simulated particle $\times 10^5$) from each spawning zone (Astillero, Elechas, Raos, Pedreña, Cubas Outer, Cubas Inner, Solfa-Tijero) in each seasonal (spring, summer and autumn) and tidal amplitude (spring or neap tides) scenario.

For a total of the 78 runs, we obtained as final results, the number and the percentage of dead, settled and recruited larvae (Table 5.2a,b). The mortality, considered as the sum of natural mortality, larvae not retained within the Bay and settled in a cell with a HSI < 25, was very high and variable between scenarios for both species although, in general, *R. philippinarum* showed lower values ($99.62 \pm 0.4 \%$) than *R. decussatus* ($99.84 \pm 0.1 \%$). This high mortality led to very low proportions of settlement and recruitment values of the total eggs released. Differences between settlement and recruitment values were associated to the early mortality after settlement given by a probability of dead equal to the HSI value in the cell where larvae settle on.

5.3.4. Larval dynamics

The assumed vertical behavior during its life cycle (Table 5.1) was correctly simulated. It can be visualized in Video 1 (See CD/LARVAL BEHAVIOR/VIDEO01) and Figure 5.7. The results of larval spatial dynamics were produced in a video format (e.g. see CD/LARVAL SPATIAL DYNAMICS/VIDEO2-5), numerically (Table 5.2) and by fixed figures (Figure 5.8) to show final recruitment spatial patterns.

The video format helped visualization of larvae dynamic patterns in the model domain. However, differences in these patterns between species are not appreciable in this format. During the first days of simulation, a dense plume of larvae is flushed out of the Bay and a high proportion is sucked back into in a tidally synchronized movement. However, as the simulation progresses in the videos, a very high mortality and a large number of larvae remaining out of the Bay can be appreciated. The larvae flush out was quicker (within 1 day) when *R. decussatus* eggs were released from spawning zones closer to the mouth of the Bay (Cubas Outer) (Video 2, run 1, gray particles) than when spawning was initialized in inner southern grounds (Astillero) (after 4 days) (Video 2, run 5, black particles).

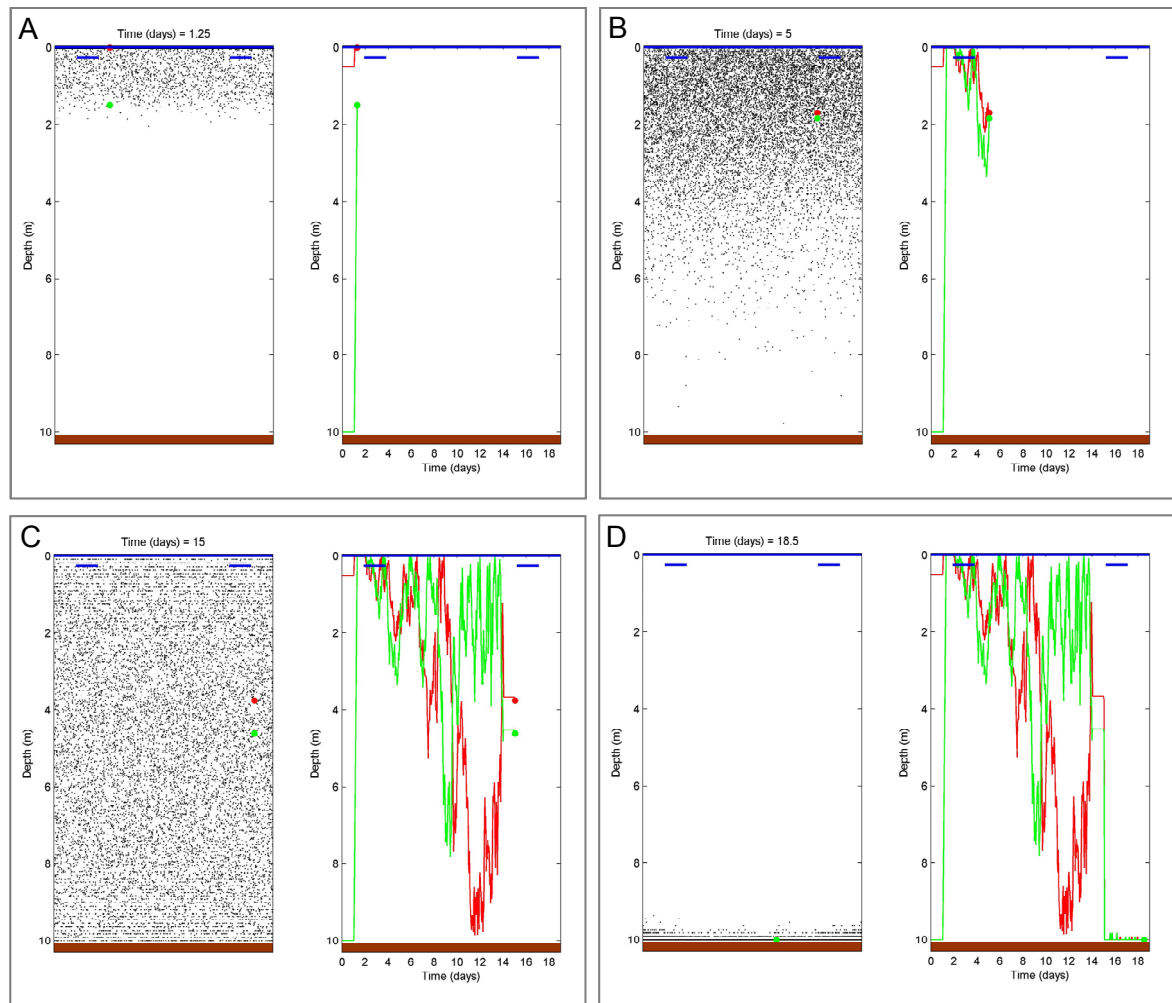


Figure 5.7 – *R. decussatus* larval dynamics regarding vertical behavior are represented in time sequential figures: (A) Day 1 (B) Day 5, (C) Day 15 and (D) Day 18. Green and red points and lines represent the particle-tracking of two randomly selected larvae.

The influence of the tide at spawning on larval dynamics is appreciable, for instance, for *R. decussatus*, in the southern zone of Astillero, since larvae released at neap tides are retained longer (Video 2, black particles) than those released at spring tides (Video 3, run 7). However, as the simulation progresses and both tidal situations (neap and spring) occurred during scenarios simulation, the differences in larval dynamics between tidal scenarios are not appreciable in the videos. Yet, winds seem to play an important role in the dynamics of larvae. For instance, when spawning occurs in the inner SW grounds

(Solía-Tijero), higher retention of larvae is observed in summer, dominated by NE winds leading to southward water currents (Figure 5.6e, Video 4, run 50) than in spring with dominating SW winds leading to northward water currents (Figure 5.6d) which flush the larvae out of the estuary more importantly (Video 5, run 64).

Although the video is an interesting format to visualize larval spatial dynamics, the larvae flush out/retention patterns affected by location and seasonal winds, the final recruitment spatial results (Figure 5.8) can be more easily interpreted and contain much more information. Thus, *R. decussatus* larvae released in Cubas outer (green rectangle) showed highest final recruitment in summer and, precisely, in the same ground (Figure 5.8b, green particles). It seems that predominating winds from NE returned them importantly (in flood) into the Cubas navigation channel and Cubas tidal flats after they were flushed out in ebb tide. However, in spring or autumn, dominated by winds from SW and W, respectively, final recruitment of this species larvae (spawned in Cubas Outer, green rectangle) was considerably lower (Figure 5.8a,c, green particles), probably because these winds avoided larvae to return to the estuary after they were flushed out in ebb tide. Moreover, important recruitment of larvae coming from several spawning zones occurred in the southern and western zones during summer for both species (Figure 5.8b,e) while a low recruitment and a great absence of connections was observed in spring with SW winds leading to northward currents (Figures 5.8a,d) or in Autumn with eastward currents (Figures 5.8c,f). Miera river flow increasing observed in Spring (Figure 5.4b) seems to not have an important negative effect in flushing larvae out of the estuary and final recruitment, since recruitment in Cubas Outer and Cubas Inner grounds was lower in early autumn simulated scenarios, with no flooding events, than in spring. The location, tide and seasonal factors effect on final recruitment and connectivity between zones are analyzed statistically or in more detail in sections 3.4. and 3.6 respectively.

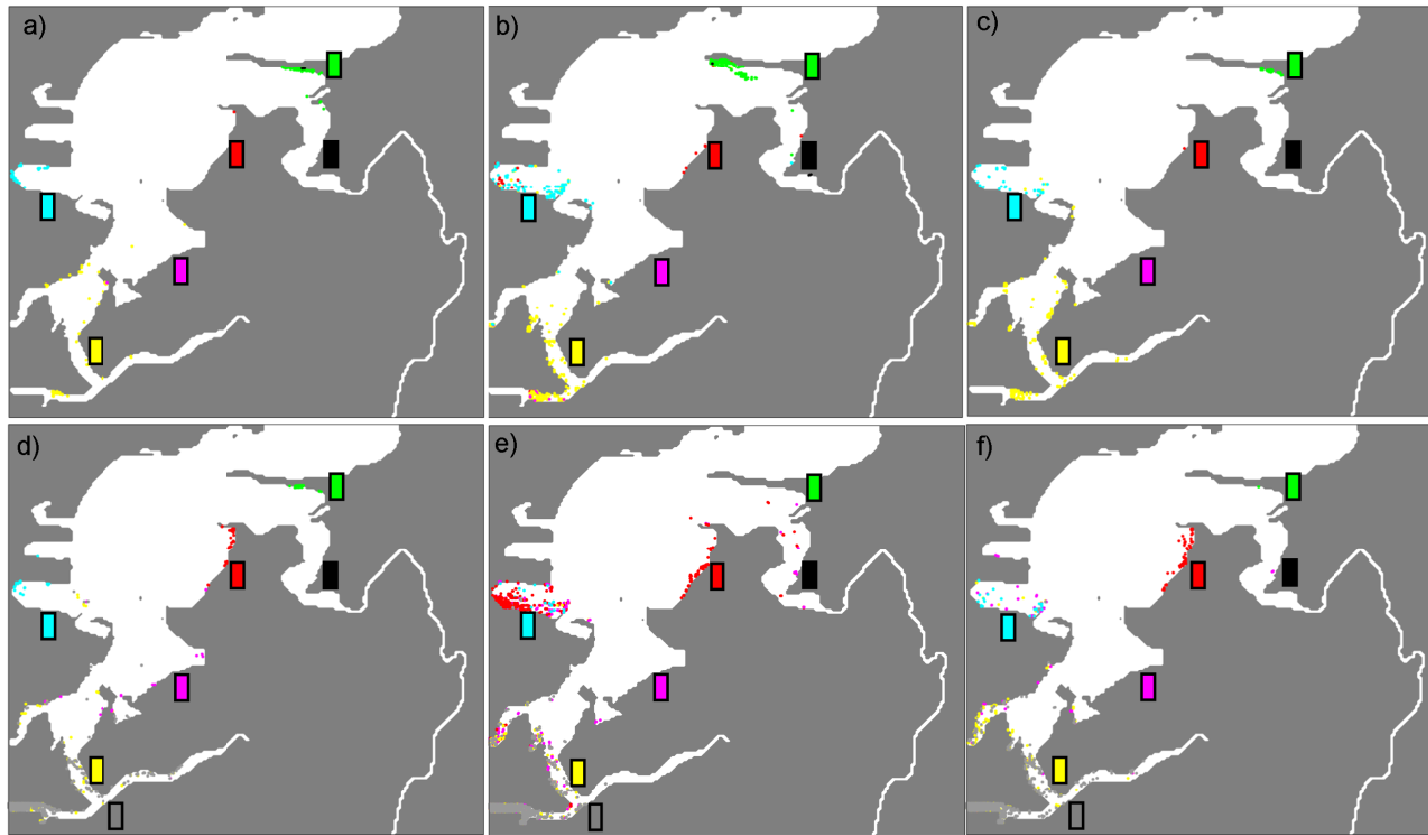


Figure 5.8 – Spatial representation of predicted recruitment for *R. decussatus* in spring (a), summer (b) and autumn (c) scenarios and for *R. philippinarum* in the same scenarios respectively (d, e, f). Rectangles represent spawning zones and dots represent larvae recruited coming from their respective same color spawning zone. Spawning zones' colors are: Astillero (yellow), Elechas (pink), Raos (blue), Pedreña (red), Cubas Outer (green), and Cubas Inner (black).

5.3.5. Influential factors on recruitment success

To analyze the factors influencing recruitment a multifactorial analysis was carried out and results are showed in Table 5.3.

<i>R. decussatus</i>	Recruitment succes (%)				
	df	SS	MS	F	p
Season	2	1.814	0.907	4.97	0.002 *
Tide	1	0.033	0.033	0.07	0.80
Spawning zone	5	8.381	1.680	9.19	0.001 *
Season x Tide	2	0.001	0.001	0.001	1.00
Season x Spawning zone	10	0.016	0.002	1.76	0.14
Tide x Spawning zone	5	2.927	0.585	2.70	0.04 *
<i>R. philippinarum</i>					
Season	2	2.140	1.068	3.90	0.04 *
Tide	1	0.258	0.258	0.37	0.55
Spawning zone	6	19.26	3.209	21.84	0.0001 *
Season x Tide	2	0.061	0.181	0.17	0.89
Season x Spawning zone	12	3.190	0.262	1.78	0.12
Tide x Spawning zone	6	1.879	0.313	1.69	0.16

Table 5.3 – Multifactorial analysis of variance observed in recruitment. Three explanatory variables are considered: Tide amplitude (at which the runs start: neap or spring tide) and Season (at which the run executes; spring, summer and autumn were the seasons considered, governed by different predominating winds) which account for different hydrodynamic conditions and the Spawning zone from where the particles or eggs are released. Df: degrees of freedom, the sum of squares (SS) and the mean sum of square (MS) are estimates of the variance attributed to the explanatory variable. F is the test used to compare the variance due to the explanatory variable considered and the total variance observed and p-value corresponds to the probability that there is no difference in means between the different levels of the explanatory variable, and therefore significant effects can be deduced from $p < 0.05$ (highlighted by an asterisk).

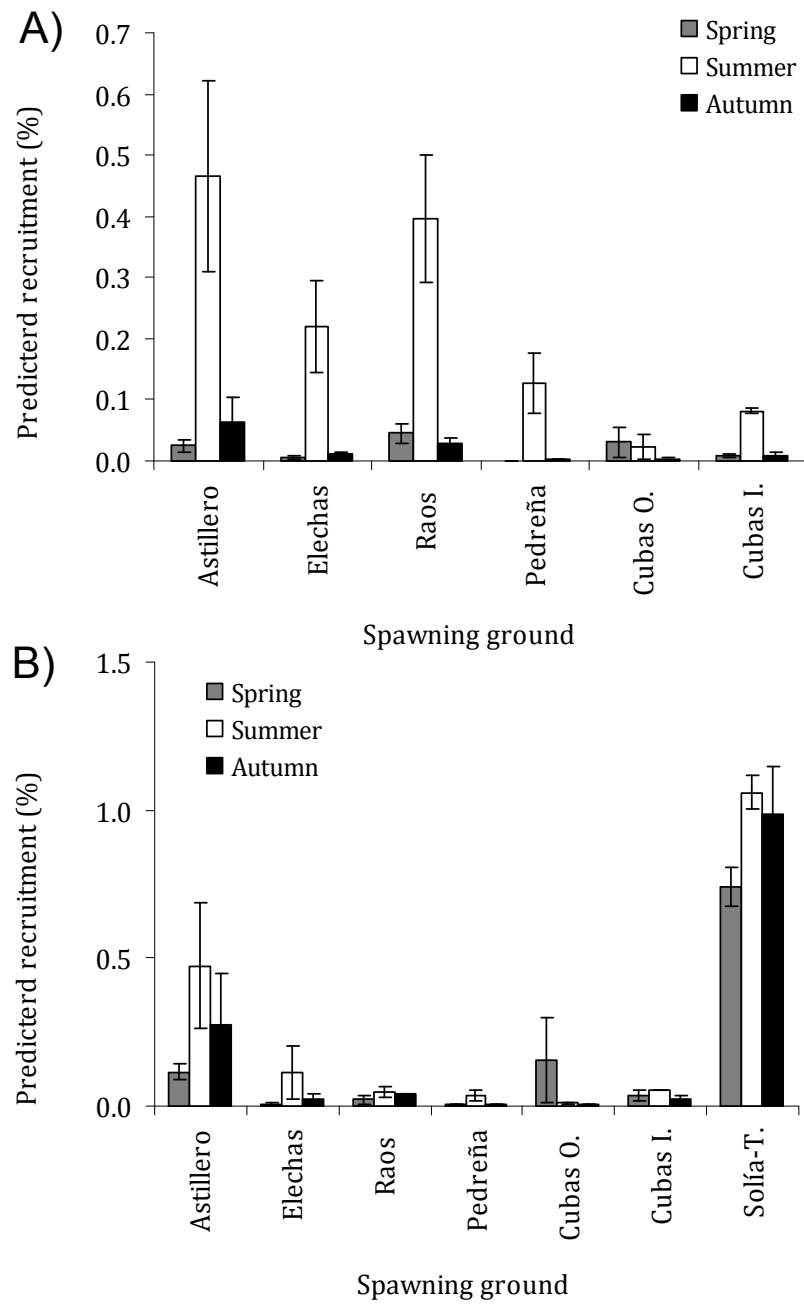


Figure 5.9 - Predicted final recruitment (%) for larvae released in each spawning ground in different seasonal scenarios (spring, summer and autumn) for (a) *R. decussatus* and (b) *R. philippinarum*. The error bars represent the \pm SE of the mean recruitment of neap and spring tide scenarios.

Recruitment data (% of individuals recruited of the total of the released eggs) were first log-transformed to achieve normality and homogeneity of variance. The t-test showed a significant difference in recruitment success between species, being significantly higher for *R. philippinarum* (0.20 ± 0.05 , mean \pm SE) with a larval phase duration 21 days than for *R. decussatus* (0.03 ± 0.01) with a larval phase duration of 15 day (df=76, $t=-3.38$, $p=0.001$). The results of the factorial ANOVA presented in Table 5.3 showed that season (i.e. predominating winds) and location of spawning zone have significant effects on final recruitment success of both clam species. Regarding spawning season, highest recruitment percentages were observed for both species when spawning occurred in summer (dominated by NE winds) for all spawning grounds except for Cubas Outer. Larvae released from this ground showed higher final recruitment in spring (Figure 5.9) governed by SW dominating winds.

Regarding zones, for *R. decussatus* larvae released from Raos and Astillero, in the southern zone of the Bay, showed the highest proportions of finally recruited individuals, whilst for *R. philippinarum* larvae released from Astillero and Solía-Tijero in the southern inner zones, showed the highest recruitment values (Table 5.2, Figure 5.11). Besides, the tidal phase (spring or neap) at the spawning moment had not significant effects on recruitment. However, the interaction between these two factors had a significant effect for *R. decussatus*. For this species, when eggs were released in Cubas Outer, the final recruitment was significantly higher at spring tide than at neap tide, while when spawning occurred in southern zones (Astillero or Elechas) recruitment was higher at neap tide than at spring tide (Table 5.2 and 5.3). For *R. philippinarum* final recruitment was also higher at spring tides when spawning occurred in Cubas Outer and at neap tides when spawning occurred in southern inner zones, yet these differences were not statistically significant (Table 5.2 and 5.3).

5.3.6. Major spawning and nursery grounds

Spawning grounds

The predicted major current spawning zones in the Bay of Santander, considering the final number of individuals recruited, are mainly Cubas Outer and Astillero for *R. decussatus* and Astillero and Solía-Tijero for *R. philippinarum* (Figure 5.10). However, the “most successful” spawning grounds, i.e. highest proportion of recruitment of the total larvae released, are the above mentioned Raos and Astillero for *R. decussatus*, showing very low recruitment percentages (Figure 5.10), whilst for *R. philippinarum*, the most successful zones were the same as the major spawning zones (Astillero and Solía Tijero), showing considerably higher recruitment success than *R. decussatus* (Figure 5.11).

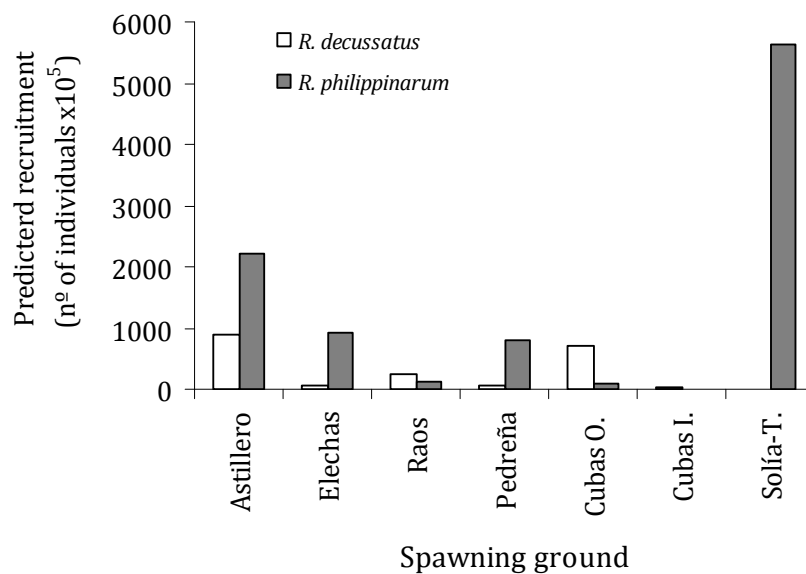


Figure 5.10 - Predicted recruitment from larvae coming from each spawning ground calculated as the sum of recruited individuals at different tidal and seasonal scenarios.

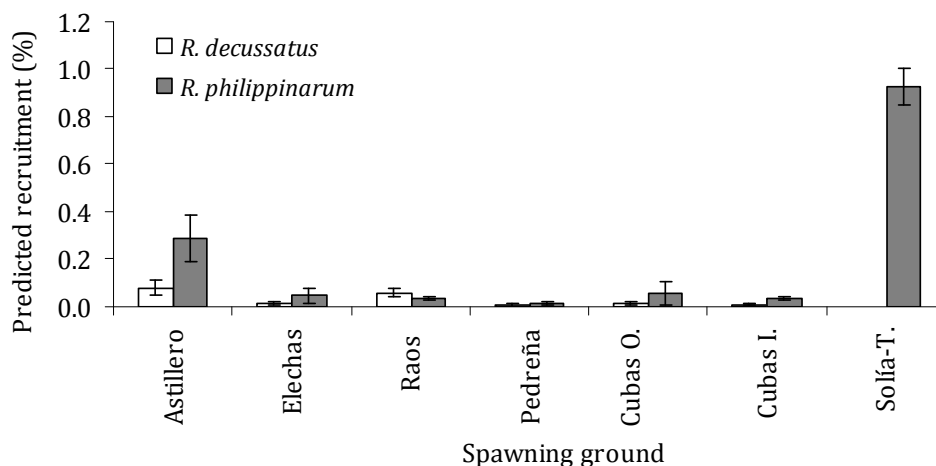


Figure 5.11 – Predicted mean recruitment (%) for larvae released in each spawning ground for *R. decussatus* and *R. philippinarum*. The error bars represent the \pm SE of the mean recruitment of different seasonal (spring, summer and autumn) and tidal (spring, tide) scenarios.

Nursery grounds

The predicted major current nursery grounds in the Bay of Santander were (1) Cubas Outer in the northeastern grounds of the estuary with ~ 80 recruiters/m² and Raos (17 recruiters/m²) in the central western flats for *R. decussatus* and (2) Solía-Tijero in the southern inner zones of the Bay (240 recruiters/m²) and Cubas Outer in the northeastern area of the bay and Astillero grounds with 50 and 40 recruiters/m², respectively, for *R. philippinarum* (Figure 5.12). The predicted recruitment density for whole the Bay in highly suitable areas (i.e. sum of individuals recruited in cells with habitat suitability index (HSI) > 75 divided by the sum of the areas of the nursery grounds together) was considerably higher for *R. philippinarum* with 50 recruiters/m² than for *R. decussatus* with 17 recruiters/m², as a result of a higher number of recruited individuals in a smaller area of suitable habitat for recruitment for *R. philippinarum* (317 Ha) comparing with *R. decussatus* (407 Ha) (Chapter IV; Bidegain et al., 2012).

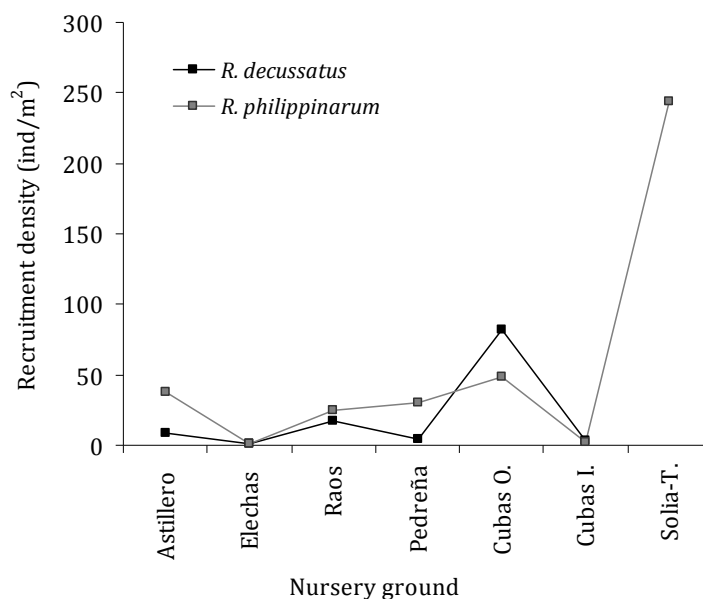


Figure 5.12 - Predicted recruitment density in nursery grounds (Habitat suitability index, HSI>75), calculated as the sum of recruitment of larvae coming from all spawning grounds at different seasons and tidal scenarios and divided by the area of the nursery ground.

5.3.7. Connectivity between spawning and nursery grounds

In the Figure 5.13 the robustness of the connectivity between spawning and nursery ground is presented. Colours represent the proportion of the total recruited larvae in a given nursery ground which is coming from each spawning site. A higher number of connections occurred for *R. philippinarum* than for *R. decussatus* (Figure 5.13). Moreover, higher number of connections occurred at neap tides than at spring tides for both species. Robust connections (i.e. more than 30 % of the total larvae recruited in a given nursery ground is coming from a given spawning site, green and black rectangles) occurred more frequently for *R. philippinarum* than for *R. decussatus*. For *R. decussatus*, more than one robust connection only occurred in autumn at spring tide scenario, while for *R. philippinarum* three or more robust connections occurred in all scenarios except in autumn at spring tide.

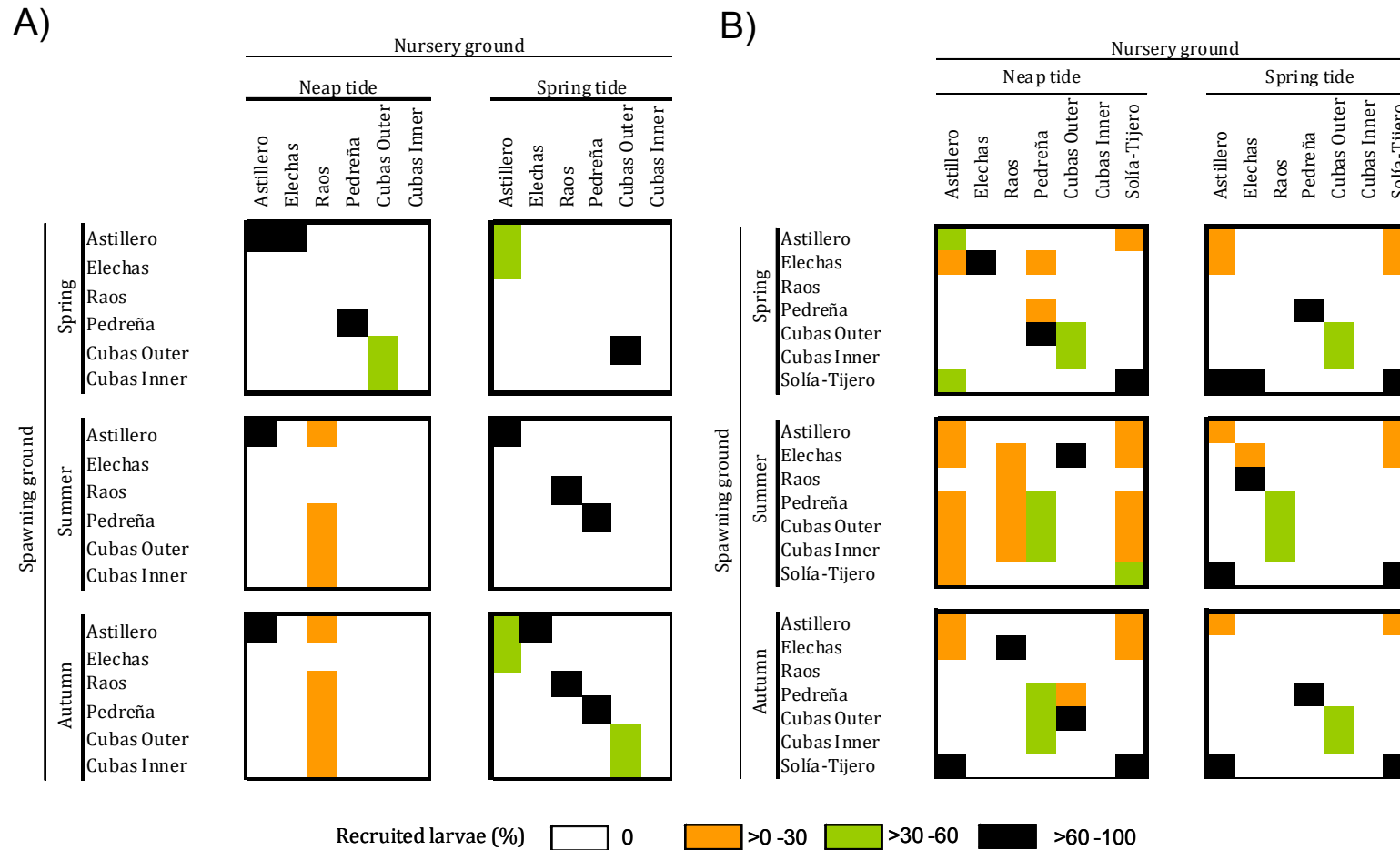


Figure 5.13 – Connectivity matrices for the 3 seasonal scenarios (spring, summer and autumn) and 2 tidal scenarios (neap and spring tides) simulated for *R. decussatus* (A) and *R. philippinarum* (B). The colour indicates the percentage of the total larvae recruited in a given nursery ground (x-axis) coming from each spawning ground (y-axis).

For *R. decussatus*, Raos nursery ground was the best connected zone in summer and autumn at neap tides, receiving larvae from Astillero, Pedreña, Cubas Outer and Cubas Inner. Astillero showed robust connections with Elechas nursery ground and Cubas Inner with Cubas Outer in spring at neap tide and in autumn at spring tide. Whilst, Elechas spawning zone was robustly connected with Astillero nursery ground in spring and autumn at spring tide.

For *R. philippinarum* the highest number of connections was found in summer at neap tide, being Astillero, Elechas and Solía-Tijero nursery grounds the best connected zones in this scenario, receiving larvae from all spawning zones except from Raos. Robust connections were found between Solía Tijero and Astillero in all scenarios except in summer at neap tides and between Cubas Outer or Inner with Pedreña at neap tides. For instance, other robust connections were also found between Solía-Tijero and Elechas and between Elechas and Cubas Outer or Raos.

Regarding the most important nursery grounds (Figure 5.12), for *R. decussatus* Cubas Outer can be considered a self-recruitment ground in spring at spring tide, since all larvae received are coming from here. However, in spring at neap tides and in autumn at spring tides this nursery ground received “allochthonous” larvae from Cubas Inner. Raos also showed a self-recruitment behavior except in summer and autumn at neap tide. For *R. philippinarum*, Solía-Tijero particularly and Astillero and Cubas Outer were the most important nursery grounds (Figure 5.12). Solía-Tijero showed robust self-recruitment behaviour, although received recruiters from Astillero and/or Elechas in all scenarios and from almost all spawning grounds in summer at neap tide. Astillero received recruiters from Astillero and Cubas Outer did from Cubas Inner in spring and autumn and also from Elechas in summer at neap tides.

Elechas, Raos and Pedreña were the zones which provide least larvae to other nursery grounds, particularly for *R. decussatus*, showing several scenarios with no connections

with any nursery grounds. The most isolated nursery was Cubas Inner for both species, considering that did not recruit larvae from any spawning site (in areas with HSI > 75).

5.4. Discussion

The incorporation of habitat suitability modelling results, i.e. HS based index maps or grids, to provide larval evolution models with cues which stimulate larval settlement (Turner et al. 1994, Tamburri et al. 1996) and an estimator of recruitment success is demonstrated to be a powerful approach. The LARVAHS model corresponds to the first attempt of development of a larval dispersal model integrating an ENFA-based (Ecological Niche Factor Analysis) settlement-recruitment submodel. This behavioral cue submodel component regulates settlement and recruitment probability trying to incorporate the fact that larvae of many taxa respond to biotic (chemical cues from living individuals) and abiotic stimuli (e.g. Turner et al., 1994; Tamburri et al., 1996; García-Lavandeira et al., 2005, Fusetani, 2004; Kingsford et al., 2002; Urrutia et al., 2004). Thus, in this study higher habitat suitability indices increase the “stimuli” to settle and recruitment probabilities. This advance follows the recommendations of the relevant ICES working group to couple larval dispersion models with additional spatial information in order to delineate the source populations, as well as the recruitment habitat, along the path of an individual particle (North et al., 2009). The prediction of recruitment is essential since it plays a determinant role in determining population size and structure (Conell, 1985; Rodríguez et al., 1993; Olafsson et al., 1994). Moreover, understanding of more successful spawning zones, larval transport processes and potential connectivity between spawning and nursery grounds is important in relation to coastal conservation, management and fisheries regulation.

In this study, LARVAHS model, designed to estimate these essential aspects for the studied species was successfully developed. The obtained predictions increase our understanding

of how physical and ecological interactions during the early life of both species influence population dispersal. Moreover, it provided interesting outcomes that quantify the effect of specific planktonic larval duration (PLD), winds, tidal amplitude and location of larval release on dispersal trajectories and final recruitment. In addition to this theoretical outcomes that has been also quantified for other benthic invertebrates and fishes (e.g., Hill, 1990; James et al., 2002; Siegel et al., 2003; Aiken et al., 2007; Fiksen et al., 2007; North et al., 2008), this model is one of the few biophysical models that has been evaluated by comparing predicted spatial patterns in dispersal destinations with spatial patterns in recruitment of benthic invertebrates (Incze and Naimie, 2000; Pfeiffer-Herbert et al., 2007). Although predicted recruitment was, in general, lower than observed they were highly correlated. Moreover, predicted recruitment density results are considerably consistent with the differential distribution patterns 20-25 mm individuals (recruitment to the fishing gear) found in this estuary in Chapter III (see Figures 5.8, 5.12 in this chapter and Figure 3.7 in Chapter II), showing higher predicted recruitment intensity and 20-25 mm individuals abundance on northern open zones for *R. decussatus* and on southern inner zones for *R. philippinarum*. The differences observed may be related with the temporal recruitment variability and other biological factors not integrated in this model such as predation. Overall, the developed larval evolution model can be considered to be reasonably suitable in its ability to forecast the dispersal and recruitment of benthic species larvae associated with oceanographic variability, taxon- specific larval behavior and habitat suitability.

The influence of current velocities at ebb tide acts significantly in the larval dispersion to offshore areas and lead to high mortalities. It is evident that larvae transport in the horizontal is importantly determined by advection (Butman, 1987) and that average bivalve speeds ($10^{-4} \text{ m}\cdot\text{s}^{-1}$) (Mann, 1986) are not sufficient to direct their 2D movement within the currents observed in the Bay of Santander (mean, $0.25 \text{ m}\cdot\text{s}^{-1}$; maximum, $1.3 \text{ m}\cdot\text{s}^{-1}$). However, the final dispersion of clam larvae is also influenced by larval behavior, i.e., vertical migration and duration of planktonic larval stage (Roegner, 2000). According to

Kuroda (2005), this vertical-down "migration" is essential to prevent all larvae to be dragged into offshore areas at ebb tide. Thus, similarly to other biophysical models (e.g. Siegel et al., 2003; Aiken et al., 2007; Herbert et al., 2012) PLD and larval behavior were parameterized to reflect development periods and vertical migration from literature. In the simulations, the PLD was considered longer for *R. decussatus* (21 days) (Pérez-Camacho, 1980; Borsa and Millet, 1992; Chícharo and Chícharo, 2001b; Vela and Moreno, 2005) than for *R. philippinarum* (15 days) (Young-Baek et al., 2005; Hinata and Furukawa, 2006). The larval behavioral considerations, i.e. swimming ability in each larval phase (Table 5.1), were similar for the two studied congeneric species and adapted from studies about *R. philippinarum* (Suzuki et al., 2002; Kuroda, 2005; Ishii et al., 2005) and other bivalves such as oysters (North et al. (2006;2008), since published literature was not found for *R. decussatus*.

Thus, in this study, the only difference in behavior between species, in addition to planctonic larval duration, was the duration of each larval phase with their respective vertical swimming behaviors. D and U larvae phase and also pediveliger larvae duration was longer for *R. decussatus* than for *R. philippinarum*. Importantly, the model demonstrates the potential power of these differences between species to affect patterns of retention in the estuary, settlement and recruitment. Retention in the bay is a key determinant for survival, as there is no suitable habitat outside the estuary. Longer PLD of *R. decussatus* has a significant negative effect in larval retention, obtaining higher mortality rates and lower recruitment success than *R. philippinarum* despite the highly suitable area for recruitment is considerably larger for the former (Bidegain et al., 2012). Thus, the longer PLD of *R. decussatus* over *R. philippinarum* seems to be the main reason to explain the higher larval dispersion and lower recruitment rates for this species.

This result is consistent with the outcomes of few biophysical models that have tested for it and found significant effects of PDL on larval transport (Edwards et al., 2007). For example, increasing PLD significantly of brittle star decreases the larval retention in the

natal region and increases the larval mortality (Lefebvre et al., 2003). Moreover, a reduction in the PDL of scallop larvae decreased their displacement distance (Tremblay et al., 1994). The behavioral response to salinity of *R. philippinarum* (Ishida et al., 2005) or *C. virginica* (Dekshenieks et al., 1996; Newell et al., 2005) considered by Herbert et al. (2012) and North et al. (2008) to model larval vertical migration of this bivalve species was not considered in this study. The freshwater inputs coming mainly from the Miera River through the Cubas area were generally constant and very low during the simulation periods and hence, the minimum salinity gradients required to stimulate larvae (Ishida et al., 2005) were not registered.

Once analyzed the general behavior of the model, in the next three subsections, the results of this study are discussed according to the specific objectives.

Season, spawning ground location and tidal effect

This study was targeted at the effects of location of spawning zones, season (i.e. predominating winds) and tidal amplitude on recruitment of both species. The results suggest that location of the source populations and wind-induced currents have significant effects on recruitment of both species. The complex configuration of the Bay of Santander with its different inner areas or more open tidal flats, where spawning grounds are located, appears to have important impact on the success of larval retention and recruitment. Herbert et al. (2012) found similar results when modelling *R. philippinarum* larval transport in Pool Harbour (England) which contains different embayments. Hinckley et al. (2001) highlighted the importance of spawning location and timing to successful walleye pollock *Theragra chalcogramma* larval transport to nursery areas. Moreover, Rigal et al. (2010) demonstrated that the interaction between spawning location and hydrodynamics have important effects on retention of the gastropod *Crepidula fornicata* within a coastal bay. Wind advection has been considered to have important effects in larval distribution in large (~1000 km²) and vertically stratified bays

(Hinata and Tomisu, 2005) and open coastal areas (e.g. Pineda et al., 2007; Bas et al., 2009; Ayata et al., 2010) where wind-induced currents are usually important besides water movements due to tides. Therefore, although initially it can be judged that in a little estuary such as the Bay of Santander this wind effect would not be important, our results suggest that wind-induced hydrodynamics is an important factor determining the final recruitment success.

Regarding the tidal phase (spring or neap) at the spawning moment, the model results analysis did not showed a significant effect on recruitment (Table 5.3). However, the interaction between spawning zone location and tidal phase on the spawning moment showed an effect on final recruitment, being statistically significant for *R. decussatus*. Recruitment was higher at spring tides in the outer zone of Cubas whilst was higher at neap tides in the inner southern zones. This result is consistent with other authors that suggested that tides influence larvae recruitment. Thus, recruitment of larvae retention of crabs or clam larvae is higher when spawning occurs at neap tides than at spring tides (Forward, 1987, Gove and Paula, 2000; Chícharo and Chícharo, 2000:2001a) or that ingress of crab larvae in a estuary and settlement is higher at times ranging from several days after spring tide to near the neap tide (Roegner et al., 2007).

Major spawning and nursery zones

The model ability to identify major spawning zones in terms of final recruitment density could support shellfishery management strategies such as creation of “sanctuaries” or protected areas for adults of the target species with the potential for an increased pool of larvae that can supplement populations outside the protected area both under normal conditions and in the event of unforeseen events or decline of populations (Allison et al., 1998; Lauck et al., 1998; Peterson, 2002; Jones, 2006). In this regard, results of this study suggest that, currently, the conservation strategies regarding the native clam *R. decussatus* spawning zones could be focused on the major ones (Figure 5.10). However, the

management of the nonindigenous clam *R.philippinarum* major spawning zones should be based on a different strategy and equilibrium should be achieved between guaranteeing fishery yields and regulating its expansion. Spawning sanctuaries are cited as an effective strategy when native wild stocks face pressure from excessive fishing and recruitment limitation, as well as habitat destruction, combination of reasons for the decline in shellfish stocks over time (Brumbaugh et al 2005; Schulte et al 2009). They are also cited as efficient supplements to more traditional fishery management practices such as size restrictions and catch limits (Airame et al., 2003). The potential zones estimated by the model should be properly sited considering also socio-economic suitability indicators in order to be adequate tools in an integrated approach to resource management (Airame et al., 2003; Allison et al., 1998).

Besides, the “most successful” spawning grounds, in terms of proportion of recruitment of the total larvae released, were identical to the current major spawning zones for *R. philippinarum* but not for *R. decussatus* (Figure 5.10 and 5.11). The estimation of these most successful spawning grounds based on recruitment success and highly suitable habitats for development and growth of these species could provide promising information for scientist and managers in farming site selection for this species. Therefore, in the Bay of Santander, the suitable farming sites for the native clam *R. decussatus* could be located in Cubas Outer or Astillero spawning grounds with high habitat suitability and optimum larval retention in the estuary. However, for the introduced clam *R. philippinarum* most suitable farming sites should be located in grounds from where the larval dispersion does not lead to a dramatic widespread and domination of the introduced clam and perhaps, less successful sites could be considered, such as Elechas where currently culture zone is located.

The results suggest that the variations in the larval retention due to the varying hydrodynamic and habitat suitability conditions are not consistent through space (Figure 5.12). This result is in line with observations by Ishii et al., 2001, Hinata and Furukawa

(2006) or Hebert et al. (2012) in the patterns of recruitment spatial variability. The major nursery zones in the Bay of Santander, regarding the predicted recruitment density of larvae, were Solía-Tijero for *R. philippinarum* and Cubas Outer for *R. decussatus*. This predicted major nursery zones partially coincided with the observed density of adult clams in this study (Figure 5.2) and the potential recruitment estimated in Chapter III. Non coincidences can be easily explained by (1) the temporal recruitment variability, (2) the fact that the recruitment density was predicted only in highly suitable areas and (3) other factors which importantly influence on final density of adults not integrated in the habitat suitability model such as the differential predation occurred between zones from recruitment. This last hypothesis is consistent with the high predation on clams by crabs and fishes found in Chapter V in the Bay of Santander (Bidegain and Juanes, 2012). As it was mentioned for spawning zones, seasonal protection regimes for estimated major nursery zones could be also efficient supplements to more traditional fishery management practices.

Connectivity between spawning and nursery zones

The model has also an important explanatory power in terms of connectivity given the current setting spawning and nursery grounds, providing theoretical outcomes that lead to explore the effect of different tidal and seasonal scenario. Our results suggest that there are both isolated areas or “self-recruitment nursery grounds” (i.e. >60 % of larvae coming from a unique spawning ground) and areas that are potentially well connected, where settled larvae come from distant or near spawning grounds in several scenarios. These differential connectivity patterns were different between species and also variable between scenarios (Figure 5.13). In general, a considerably higher number of connections was observed for *R. philippinarum* than for *R. decussatus*. The shorter PLD and higher retention of *R. philippinarum* larvae could be also one of the main reasons to explain this difference between species. In the same line, it seems that neap tides and dominating NE winds of summer favored connections between grounds, particularly for *R. philippinarum*,

since retention of larvae within the estuary is also favored. The connectivity between internal areas of the south to northern distant areas was less evident than between non distant zones or nearby inner grounds, being consistent with the fact that retention of larvae in inner nearby grounds is higher and connections between them more easily produced. An increased retention of larvae between inner and nearby areas and greater connectivity between open areas has been also observed recently for the Manila clam (Herbert et al., 2012)

Overall, considering all possible self-connections in all scenarios (36 for *R. decussatus* and 42 for *R. philippinarum*) a greater percentage of self-recruitment cases was found for the former (27 % over 21 %) which is, in turns, the species with lower potential recruitment success. This result suggests that self-recruitment nursery grounds, which do not receive larvae importantly from other ground, are more exposed to recruitment declines when scenarios favoring the export of larvae from this given ground out of the Bay occur. Whilst, well connected areas can compensate the larvae deficit coming from a given spawning site with other larvae pool source. The differences in connectivity between species and grounds have important implications for fisheries management, particularly for the rate of spread of the introduced populations from potential locations of farming or restoration sites (Kakino, 1996; Kim et al., 2012) as well as their capacity to exchange genetic information throughout the bay or neighbor estuaries (Munroe et al., 2012).

5.5. Conclusions

The results demonstrate the potential of the LARVAHS model to simulate larval dispersion and determine how it is influenced the spatial and environmental variables. Moreover, the potential to determine most successful spawning zones, major recruitment grounds, and connectivity between them is also demonstrated. The model may serve as a useful framework to guide quantitative investigations into the dynamics of larval dispersal and

particularly, into settlement and recruitment since it has an important focus in the habitat suitability-based recruitment submodel.

This study is a first step toward exploring spatially dependent factors that influence larvae transport and clam recruitment. LARVAHS predicted quite good seasonal recruitment variability, although, as other similar models, cannot reproduce the orders of magnitude variability since it does not include many important biological processes (e.g. specific larvae behavior, gamete fertilization success, larval mortality and growth, etc.). Future analyses should be conducted upon these results by assessing the potential contribution of these parameters. For instance, the higher overall recruitment and connectivity success of *R. philippinarum* versus *R. decussatus* does not indicate that the former species larvae would have higher survival than the latter in the Bay of Santander. Factors that would be necessary to evaluate specific survival were not parameterized in the model.

As it was mentioned above, model results have implications for fisheries and aquaculture management and also for restoration programs. However, grid resolution constrains the applicability of predictions. Higher resolution model would refine circulation predictions to better guide the location of specific management and conservation strategies.

Chapter VI

**Does expansion of the introduced Manila clam
Ruditapes philippinarum cause competitive
displacement of the European native clam
Ruditapes decussatus?**

Chapter VI. Does expansion of the introduced Manila clam *R. philippinarum* cause competitive displacement of the European native clam *R. decussatus*?

*This study has led to the publication of a research article in the Journal of Experimental Marine Biology and Ecology, vol. 445, pp. 44-52, in 2013 by Bidegain, G. and Juanes, J.A. with the title "Does expansion of the introduced Manila clam *Ruditapes philippinarum* cause competitive displacement of the European native clam *Ruditapes decussatus*?"*

Abstract

In several estuaries or lagoons of Europe the introduced Manila clam *Ruditapes philippinarum* has supplanted the native grooved carpet shell clam *Ruditapes decussatus* by occupying almost entirely its ecological niche and relegating it to restricted areas. However, it is not clear if the nonindigenous clam is the direct responsible of this predominance patterns. Within this context, the main goal of the present study was to analyze the competitive interaction between the non indigenous Manila clam and the native carpet shell clam to determine whether this interaction could impact directly growth and mortality of the native clam populations. The effect of exposure to predators on both species was also examined. For this purpose, between May 2010 and May 2011 a field experiment was conducted on an intertidal area in the Bay of Santander (N Spain) where both species coexist without an extreme predominance of the introduced species. Relative density of these species was manipulated in a randomized block experimental design. The results obtained show that (i) the increased density of Manila clam simulating species expansion scenario does not affect growth or mortality of the native clam; (ii) for densities of Manila clam substantially higher than observed in the field, predation plays an essential role in regulating both species populations, limiting their density increase. Moreover, Manila clam appears to be more negatively impacted by predation compared to

the native clam. Overall, our results indicate that predation has a more significant effect on both populations when compared to competitive interaction.

6.1. Introduction

The displacement of the native European clam *Ruditapes decussatus* occurred in several disturbed estuaries or lagoons of Europe as Arcachon Bay (France) or the lagoon of Venice (Italy) where the nonindigenous Manila clam *Ruditapes philippinarum* have been largely introduced (Aubby, 1993; Marin, 2003; Mistri, 2004; Caill-Milly et al., 2008) need a better understanding. Moreover, this extreme dominance pattern of the introduced Manila clam has not yet been detected in other estuaries of Europe such as in Bahía de Santander as it was described in Chapter III and IV. Consequently, considering the differentiated stages of invasion of Manila clam in European estuaries and taking into account the absence of studies investigating the interaction with the native carpet shell clam or other biotic interactions, nowadays, the definition of this nonindigenous species as a direct threat for the European native clam is too daring.

Few experimental studies have been designed to test directly whether competition for limited resources between Manila clam and other bivalve species is an important process (Lee, 1996; Byers, 2005; Lum, 2011) and they did not found direct effects of Manila clam on the studied species. In the Bay of Santander, the absence of negative correlation between Manila clam and carpet shell clam abundances found in Chapter III (Juanes et al., 2012) suggested that competitive interaction may not be important.

However, the assumption that an alien species is problematic until proven otherwise (Simberloff, 2005; Wittenberg and Cock, 2001) is a central tenant of conservation strategies. Therefore, it is essential to study the competitive interaction between these species in order to adopt appropriate management measures for the conservation and

sustainable exploitation of these valuable marine resources. In recognition of both an increasing pressure on managers due to the detection of zones where the introduced Manila clam predominates (Chapter III and IV) and the drastic declines of the native carpet shell clam occurred in other estuaries of Europe, a first experimental study was conducted in the Bay of Santander. This study examined the significance of the competition among these two congeneric species and to explore the impact of the nonindigenous Manila clam on the native carpet shell clam. Moreover, the role of predation in the regulation of the expansion of the nonindigenous species was explored. In order to achieve this goal, this study examines the effects of increasing density of Manila clam and predation on mortality and growth of both clam species.

6.2. Material and methods

6.2.1. Experimental site

A field experiment was conducted from May 2010 to May 2011 in an intertidal area near the semiactive Manila clam farming zone in Bahía de Santander (Figure 6. 1). The experimental site was selected because: (i) it is on a large and open area containing populations of both Manila clam and grooved carpet shell clam, (ii) it is located approximately 1 km from the nearest public access, making it effectively inaccessible to the general public and (iii) the experiment could be best safeguarded by shell fishermen over its duration. The sediment on the experimental site was composed by 25.3 % coarse, 51.2 % sand and 24.5 % silt, contained 3.74 % organic matter which represents suitable conditions for both species (Bidegain et al., 2012a, Chapter IV). Distribution and abundances of clam populations in the estuary obtained from Chapter III (Juanes et al. 2012) were used to determine the experimental densities needed to simulate both natural abundances and the effect of potential expansion of the nonindigenous species.

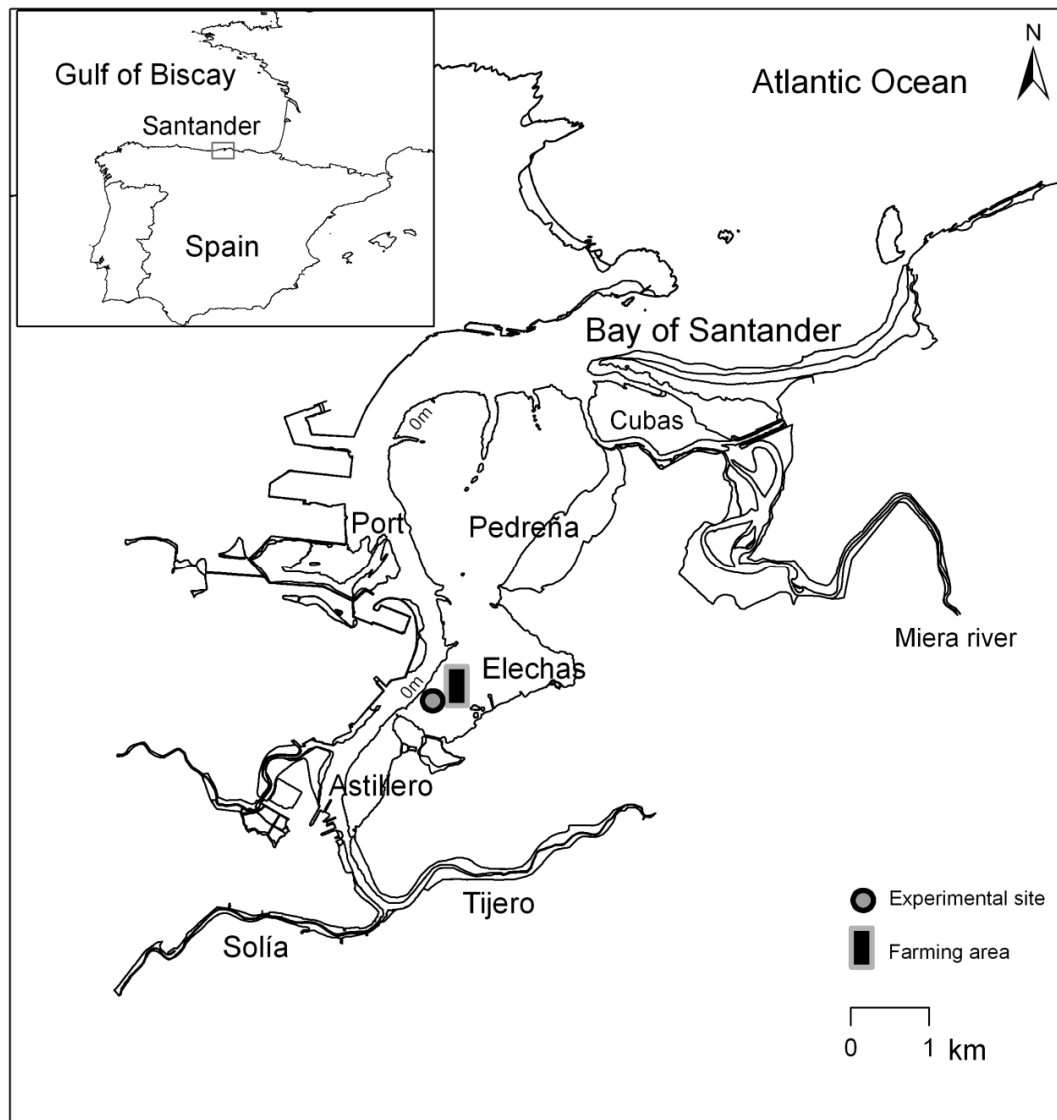


Figure 6.1 - Bay of Santander. The grey circle represents the location of the experimental site.

6.2.2. Clam collection and laboratory procedures

All clams used in this experiment were collected from the same zone in the bay, near the experimental site, to reduce the effects of potential genetic variability. As allometric relationships of studied species are slightly different, the selected adult clams sizes were higher (36.8 ± 2.8 mm) for *R. decussatus* than for *R. philippinarum* (33.5 ± 3.0 mm), in

order to have similar biomass for both species in treatments containing the same density. Bivalves were transported to the laboratory in a tank filled with sea water, and maintained at field temperature (16.5°C) in order to minimize physical and/or chemical stress. In the laboratory, they were dried, measured (maximum length) and marked with a nail polish.



Figure 6.2 –Experimental site located nearby to the Manila clam culture zone in Elechas tidal flats (Bay of Santander)



Figure 6.3 – Marked *R. decussatus* clams by nail polish.

Marked clams were transported again to the field and were allocated to the experimental units. One year after the start of the experiment, individuals collected in each enclosure were measured and counted to determine growth and mortality of clams. Additionally, mortality source was examined (1) periodically, during the year when dead individuals or cracked shells were found during enclosure nets maintenance and (2) meticulously, at the end of the experiment when enclosures were excavated. For the clams that were recovered with the identification markings, from both covered and uncovered enclosures, it was discerned the source of mortality (i.e. cracked shells=predation; empty valves intact and blackened=anoxia or disease, unrecovered clams also assumed dead by a predator).

6.2.3. Experimental design

A factorial experiment was designed with two fixed factors, relative density of clam species and predation, to examine their effect on growth and mortality of both clams. Relative density factor includes four levels as follows: (1) *R. decussatus* (*RD*) monoespecific, including 10 individuals (2) *R. philippinarum* (*RP*) monoespecific, also including 10 individuals, (3) Coexistence, with 10 individuals of *RD* + 10 individuals of *RP* and (4) predominance of Manila clam, including 10 individuals of *RD* and 20 individuals of *RP*. Whilst, predation includes two levels: exposure (uncovered) and no exposure (covered) to predation. Therefore, the experiment examined a total of eight treatments. Density levels were selected to simulate (i) zones containing only one species at natural densities, (ii) zones where both species coexist in moderate but higher density levels (augmented by ~ 50 %) than quantified in the field surveys and (iii) zones where *R. philippinarum* predominate (augmented x 4 natural densities observed in the field). The latter two levels, but in particular the Manila clam predominance scenario, simulated the effect of potential expansion of the nonindigenous population in the bay due to a hypothetical situation where it forms widespread and dominant populations in the whole

Bay after repetitive and intense introductions (see invasive stages, Occhipinti-Amborgi, 2007).

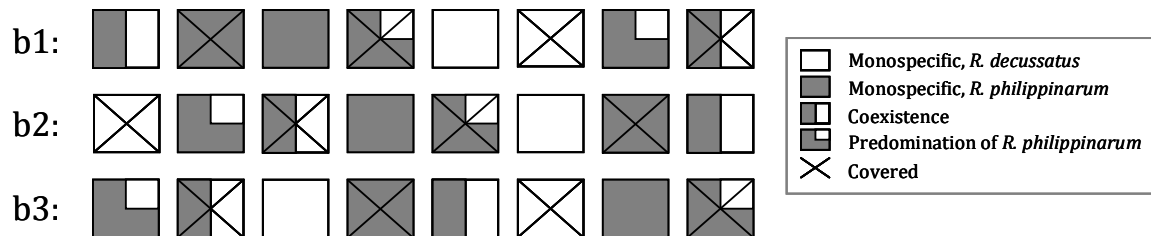


Figure 6.4 –Layout for the randomized block design (b1, b2, b3) to examine the effect of relative density of clams and predation in growth and mortality of *R. decussatus* and *R. philippinarum*. Covered and uncovered treatments were systematically interspersed within each block.

The experiment was laid out in a randomized block design where the treatments were replicated in three blocks for a total of 24 plots (Figure 6. 4). Assuming that species follow the Lotka-Volterra equations three replicas are sufficient to determine if two species compete (Tilman, 1987). Blocks were spaced 10 cm apart to also ensure homogeneity of environmental conditions between them. Although treatments were placed randomly regarding density treatments, a covered and uncovered treatments were systematically interspersed to reduce potential biases from predators (i.e. crabs) foraging in uncovered enclosures and spilling over onto an adjacent uncovered enclosure (Figure 6. 4).



Figure 6.5 - Preparation of treatments and enclosures placement procedures in the experimental site.

The treatments were examined in plots consisting of nylon net enclosures (0.25 m², height=15 cm), located along a tidal height contour of ~ 1 m above MLLW. For exposure to predator treatments, enclosures were both laterally and top covered with 8.0 mm mesh size nylon net. Enclosures were inserted in to the substrate beyond typical maximum

burial depths (~ 2 - 12 cm) for these species (Vilela, 1950; Lee, 1996; Gosling, 2003) to avoid lateral migrations of clams out of the experimental plot. Top nets tried to exclude predators (e.g. fish, crabs and birds) from the enclosure. For no exposure treatments enclosures were not top covered. Prior to initiating the experiment the sediment below each enclosure was sieved to remove predators and clams. The area was refilled and clams were placed on the surface until buried in the sand. Top nylon nets of enclosures were cleaned every two-three weeks to keep the mesh free of algae and other fouling organisms.

Additional plots were added in order to demonstrate the absence of effects of both enclosures and disturbance of the sediment on clam responses. For this purpose, three replicates for each effect analysis were located ~ 1m from the experimental array. On the one hand, for the enclosures effect analysis, the sediment was sieved from an area of 0.50 m², to remove predators and clams as it was done in the original experiment. Then the area was refilled and 20 measured and marked individuals of each species were placed in three replicates until buried. No enclosures were placed above them. This density of clams was selected to mirror the coexistence density treatment of uncovered enclosures; although a higher number of clams (x 2) were added on a larger area (x 2). This difference was assumed in order to recover individuals at the end of the experiment, considering that in absence of enclosures a certain lateral migration of clams could be expected. On the other hand, for the disturbance effect analysis, 10 marked individuals of each species were added to areas of 0.25 m² where no marks of infaunal organisms (e.g. sand crabs, polychaetes, clams, razor clams) were detected. Thus, without disturbing the sediment, clams were placed on the surface, and we waited until sure that they were buried in the sand. Then the enclosures with top nylon net were inserted above.

6.2.4. Data analysis

Growth and mortality response variables were examined at the end of the experiment (1 year). For this purpose, clams were recovered from enclosures, and then counted and measured. The difference between the initial maximum length size and the final size of individuals was used to estimate growth for each species and for each treatment. Mortality was estimated as the proportion of dead individuals in each treatment. Growth and mortality distributions normality was checked for statistical analyses. Additionally, the proportion of dead individuals corresponding to each mortality source (i.e. predation or anoxia or disease) was estimated in order to know the role of predation. Normality of the growth and mortality distributions was checked for statistical analyses. Then, two-way ANOVA was used to examine the effect of relative density and predation (fixed factors) on the response variables and, additionally, to test the effect of interaction between the two factors. In addition, t-Test was applied to analyze differences in growth and mortality between species.

In order to demonstrate the absence of both enclosures and disturbed sediment on growth and mortality, a one way ANOVA test was used. First, to analyze the effect of the enclosure, growth and mortality were compared between individuals placed in uncovered enclosures and individuals placed in patches with no enclosure. Second, to examine the effect of the sediment disturbance, response variables were compared between individuals placed in covered enclosures with disturbed sediment and individuals placed in covered enclosures with untouched sediment.

6.3. Results

6.3.1. Density effect on growth and mortality of clams

Increasing the density of *R. philippinarum* had no effect on the growth of either species (Table 6.1). The nonindigenous species grew significantly more than the native *R. decussatus* ($t = -12.9$, $df = 126$, $p < 0.0001$). Whilst *R. philippinarum* grew 5.7 mm (± 1.6 SD), *R. decussatus* grew 2.4 mm (± 1.1 SD) (Figure 6. 6a, Covered). Moreover, *R. philippinarum* density also had no effect on the mortality of none of the species (Table 6.1). Both species showed similar mean values of mortality 0.42 (± 0.12 SD) for *R. decussatus* and 0.37 (± 0.13 SD) for *R. philippinarum* ($t = 0.93$, $df = 16$, $p < 0.36$) (Figure 6. 6b, Covered). Similar specific growth patterns and absence of density effects, on both growth and mortality of both species, were also observed on uncovered plots (Figure 6. 6ab, Uncovered).

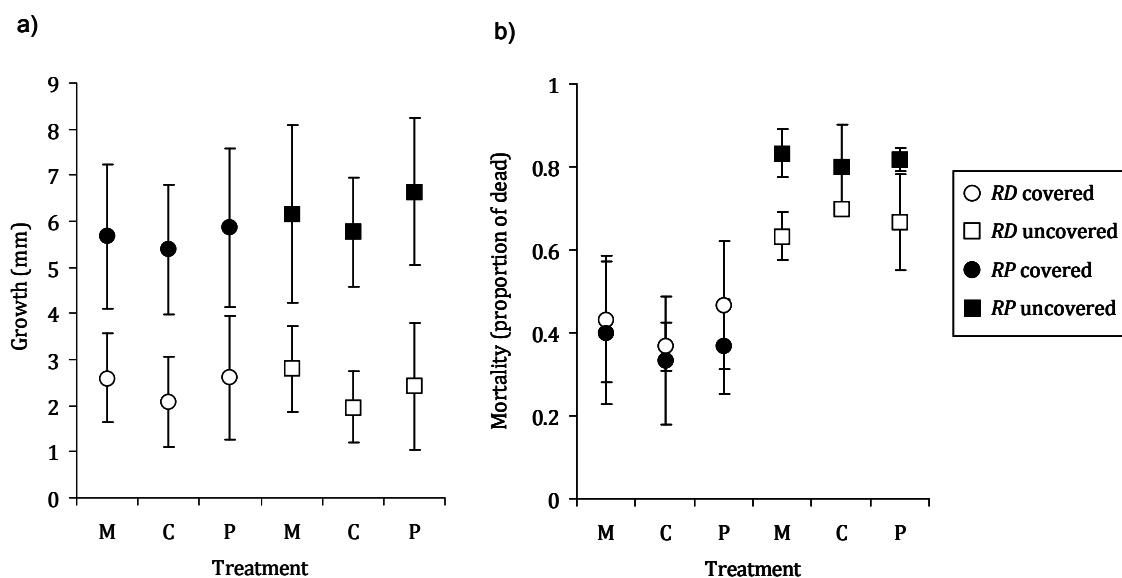


Figure 6.6 - Growth (a) and mortality (b) of *Ruditapes decussatus* (RD) and *Ruditapes philippinarum* (RP) (mean \pm SD (error bars)) during the experiment (1 year) for three density treatments in covered and uncovered plots: monoespecific (M), (2) coexistence (C), and (3) predominance of Manila clam (P).

	df	F	p
<i>R. decussatus</i>			
Growth			
<i>Density</i>	2	1.24	0.30
<i>Predation</i>	1	0.15	0.70
<i>Density x Predation</i>	2	0.16	0.85
Mortality			
<i>Density</i>	2	0.47	0.65
<i>Predation</i>	1	27.66	0.0001 *
<i>Density x Predation</i>	2	0.35	0.71
<i>R. philippinarum</i>			
Growth			
<i>Density</i>	2	0.57	0.58
<i>Predation</i>	1	3.58	0.06
<i>Density x Predation</i>	2	0.05	0.95
Mortality			
<i>Density</i>	2	0.15	0.86
<i>Predation</i>	1	85.76	0.0001 *
<i>Density x Predation</i>	2	0.03	0.97

Table 6.1 - Effects of density of the nonindigenous bivalve *Ruditapes philippinarum* and predator exposure on the mortality and shell growth of *Ruditapes decussatus* and *Ruditapes philippinarum*.

6.3.2. Predator exposure effects and source of mortality

Predator exposure affected *R. decussatus* and *R. philippinarum* differently (Figure 6. 6b, Uncovered). Whilst mortality did not show differences between species in enclosures protected from predators, it was significantly higher for *R. philippinarum* than for *R.*

decussatus in uncovered enclosures ($t = -4.8$, $df = 16$, $p = 0.0002$). This augment in percentage of dead individuals was considerably higher for *R. philippinarum* (from 37% to 82 %) than for *R. decussatus* (from 42% to 67 %) on average, considering together all density treatments. Moreover, exposure to predators had no significant effect on the growth of none of the two species (Table 6.1). The growth for no top plots was 6.4 mm (± 1.5 SD) for *R. philippinarum* and 2.5 mm (± 1.0 SD) for *R. decussatus*. Finally, interaction effects between predation and density were not detected in any of the two response variables for none of the species (Table 6.1).

Regarding the source of mortality, in covered enclosures few clams were cracked by predators or missing so that only a low percentage of *R. decussatus* (6 %) and *R. philippinarum* (16 %) died due to predation. However, in uncovered enclosures 55% of *R. decussatus* mortality was attributed to crab, fish and other macropredators, compared to a considerably higher 73 % for *R. philippinarum* (Table 6.2).

Species, treatment	Status of shells (mortality source)		
	Undamaged shells (e.g. anoxia or starvation)	Cracked shells (definitely predator killed)	Missing clams (probably predator killed)
<i>R. decussatus</i> , Covered	34	1	1
<i>R. decussatus</i> , Uncovered	27	8	25
<i>R. philippinarum</i> , Covered	37	4	3
<i>R. philippinarum</i> , Uncovered	26	11	60

Table 6.2 - Status of shells of experimental clams and the mortality source discerned following Byers (2005). Initial number of clams was 90 for *R. decussatus* and 120 for *R. philippinarum*.

6.3.3. Effect of enclosures and sediment disturbance

None of the species exhibit significant differences between no enclosure and enclosed uncovered plots neither for growth (*R. decussatus*, $F = 3.5$, $df = 1$, $p = 0.07$, *R. philippinarum*: $F = 0.12$, $df = 1$, $p = 0.74$) (Figure 6. 7a) nor for mortality (*R. decussatus*, $F = 1.0$, $df = 1$, $p = 0.37$, *R. philippinarum*: $F = 0.8$, $df = 1$, $p = 0.42$) (Figure 6. 7b).

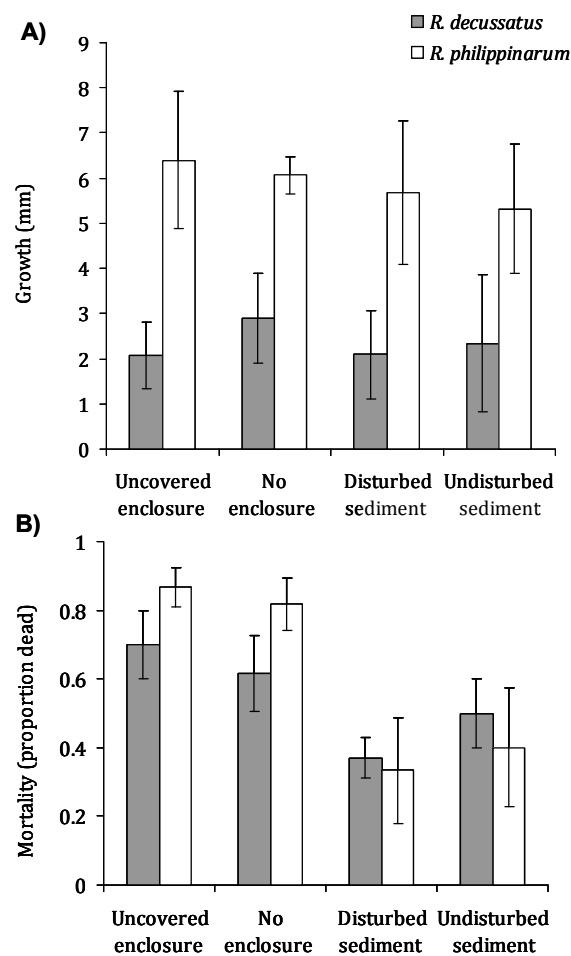


Figure 6.7 - Growth (a) and mortality rate (b) of *Ruditapes decussatus* and *Ruditapes philippinarum* during the field experiment (1 year) in (i) coexistence treatments; “Uncovered enclosures” (no top enclosures with disturbed sediment) and “Disturbed sediment” (covered enclosures with disturbed sediment) and (ii) in their respective controls; “No enclosure” to examine effect of enclosure and “Undisturbed sediment” to examine effect of sediment disturbance.

The effect of the disturbance was examined comparing response variables for both species between covered enclosures with disturbed sediment and covered enclosures with untouched sediment free of infaunal invertebrates' marks. The 1-way ANOVA test did not show significant differences in any species neither in growth (*R. decussatus*, $F = 0.5$, $df = 1$, $p = 0.48$, *R. philippinarum*: $F = 0.02$, $df = 1$, $p = 0.89$) (Figure 6. 7a) nor in mortality (*R. decussatus*, $F = 4.0$, $df = 1$, $p = 0.12$, *R. philippinarum*: $F = 0.25$, $df = 1$, $p = 0.64$) (Figure 6. 7b).

6.4. Discussion

The results of this study were necessary for two reasons: first, to test for the effects of varying density of the introduced nonindigenous species *Ruditapes philippinarum* on growth and mortality of the native clam *Ruditapes decussatus*; and second, to explore the effect of predation in the regulation of both populations, with a particular emphasis on the nonindigenous species. For this purpose, the experimental study conducted in the Bay of Santander simulates a scenario of Manila clam expansion, where it would form a widespread and dominant population. Results suggest that, for this potential scenario, the nonindigenous clam can not be defined as an ecological threat regarding competitive interaction and predation has an important effect on the regulation of the expansion of this species.

The highest density of the introduced species examined (i.e. x 4 natural density observed in the field by Juanes et al., 2012) experiencing no density mitigation by predators (covered enclosures), directly affected neither the growth nor the mortality of the confamilial native carpet shell clam. In fact, no differences were observed in response variables of outplanted Manila or carpet shell clams regardless of the density treatments examined (i.e. monoespecific, coexistence and predominance of manila clam). As density-

dependant studies are suitable to estimate resource competition between two species (e.g. Peterson 1985, Whitlatch et al. 1997; Byers, 2009 and reference therein) the results suggest that clam densities tested in this study did not result in interspecific competition. Results obtained in uncovered plots regarding density effects were similar to those found in covered enclosures, yet they should be taken with caution since density they experienced a high density mitigation effect due to predation exposure.

Enclosures can modify microenvironmental conditions (Underwood, 1985) and have an effect on results. However, the enclosures and the sediment disturbance did not show any substantial effects on response variables studied for none of the species. Byers (2005) also found that the effect of enclosures on mortality and growth of two bivalves were not significant. Although this conclusion permits to extrapolate the results and estimate natural life history properties, some caution should be taken considering that the experimental site was located near a Manila clam culture area. The location could be affecting the growth rates and survival of clams in the neighbour zones (Nizzoli et al., 2005) since high densities of cultivated bivalves are generally considered as “sinks” of oxygen and particulate organic matter (Richard et al., 2007a,b) and hence, may cause a food shortage and be attractors of macropredators. However, this potential effect was assumed to be similar for different treatments. The effects of enclosure and sediment disturbance on reproduction were not estimated, although there is no reason to expect such effects since they were not detected on species growth.

Competition between both species at Manila clam relatively high densities, simulating a predominance scenario, may not be an essential factor regulating the distribution and abundance of the native species. This is in good agreement with previous studies where there is no evidence that Manila clam has negative effects on native or alien species neither at natural densities (Breber, 2002, Juanes et al., 2012) nor at higher densities than observed in nature where, similarly, a lack of competition between bivalve species has been observed. Black and Peterson (1988) manipulated the densities of large suspension

feeding bivalves in three Western Australian sites to test their effect on the density and diversity of smaller bivalves and other invertebrates. The bivalves had no significant effect on small bivalves, gastropods, polychaetes, amphipods, other taxa, and all taxa combined, despite maximum treatment densities were $\times 2$ the natural densities. Byers (2005) examined the effects of Manila clam densities on the growth, mortality, and fecundity of the native littleneck clam, *P. staminea* and found that at densities 50% higher than natural ones (up to ~ 200 clams / m^2) the Manila clam had no effect on the native littleneck clam or on itself. Similarly, Lum (2011) did not observe differences in the growth rates of *R. philippinarum* and *N. obscurata* regardless of the relative densities of each species (intra-specific competition) or the density of the other species (inter-specific competition), even when clam densities doubled natural densities observed in preliminary surveys. Despite using densities larger than natural ones, these authors suggested that the experimental densities used were still not large enough to reach the critical level to trigger competition. Black and Peterson (1988) point out that their one year duration experiment may have been insufficient to permit the small infauna to colonize densely enough for competition to occur. The present experimental study could have a similar result because of densities (80 Manila clam individuals / m^2) insufficient to produce competition. However, densities were well above the average found in the Bay of Santander (i.e. 4 times the natural densities of Manila clam found under ambient conditions) and considered as sufficient to simulate a scenario of potential expansion of the nonindigenous species.

Most favoured areas of Venice Lagoon hold densities ~ 1000 individuals / m^2 (Breber 2002) whilst in Arcachon Bay the maximum density is around ~ 45 individuals / m^2 (Caill-Milly et al., 2006). Hence, the competition between the two species may not be the main reason for the drastic reductions of the native clam occurred in Arcachon Bay. In the case of the Lagoon of Venice the densities of Manila clam could have been large enough to reach the critical level to trigger competition and drastically impact the native species. However, other factors can be responsible of these reductions, such as the biogeochemical cycles altered by the farming of *R. philippinarum* (Bartoli et al., 2001) or invasions by parasites

that can caused long lasting or even irreversible consequences (Harvell et al., 2002), as in case of *Bonamia ostreae*, a disease of the European native oyster *Ostrea edulis*, which caused severe decline in their populations and, as a consequence, destruction of native oyster bed ecosystems (Wolff and Reise, 2002). Complementary possible hypotheses have been pointed out to explain the reduction of native species occurred, such as the failure of non specific management measures (e.g. identical minimum capture size) adopted during years or decades, leading to an overexploitation of the native species (Juanes et al., 2012) which has some biological disadvantages such as slower growth and less tolerant or resistant species (Spencer, 1991; Mistri, 2004 and references therein).

Three-dimensionality of infaunal species habitat (Peterson, 1979; Wilson, 1991), different feeding modes or food sources between species (Peterson 1982; Kamerans et al. 1992), filter feeding and vulnerability to predators (i.e. burial depth) (Peterson and Andre 1980; Byers, 2009) are considered to play important roles making direct interference competition ineffective at relatively high densities. In this regard, Bodooy & Plante-Cuny (1984) found that the growth rate of *R. decussatus* is mainly related to the primary production of the water column. Meanwhile, *R. philippinarum* seems to feed mainly on particulated organic matter resuspended from the sediment (i.e. detritus and microphytobenthos) (Watanabe, 2009). Both species are filter feeders of a food resource that is frequently replenished (i.e., with tidal cycle and resuspension due to currents) and whose supply is often decoupled from consumptive pressure by resident organisms. Moreover, burial depth of *R. decussatus* (~ 10-12 cm) is considerably higher than that of *R. philippinarum* (~ 2-4 cm) (Vilella, 1950; Lee, 1996; Gosling 2003). This difference in burial depth is essential in order to interpret the differences in growth and mortality between species observed in this experiment which in turns are consistent with current knowledge.

Growth of *R. philippinarum* during the experiment (1 year) was ~ 6 mm regardless of the exposure to predators. For *R. decussatus* it was considerably lower, ~ 2.5 mm. These differences are in line with findings of several authors for the large clams (~ 35 mm) used

in this study (e.g. Spencer et al., 1991; Solidoro et al., 2000; Urrutia et al., 1999; Cannas, 2010). The energetic efficiency of feeding with a siphon decreases with burial depth (Zaklan and Ydenberg, 1997) and it explains, at least partially, why the more deeply burrowing *R. decussatus* does not grow as fast as *R. philippinarum*. With a shallower burial depth Manila clam can filter particles more quickly and invests less in the development of its siphons compared to a deeper burrowing clam with a longer siphon. One of the important reasons to introduce the nonindigenous Manila clam world wide for aquaculture purposes was precisely its fast growth together with the important commercial value (Laing and Child, 1996; Usero et al., 1997). The resulting growth was not significantly different for any species between covered and uncovered enclosures. This result is in contradiction to the studies of sublethal effects of predators on clams which confirmed generally that predator presence significantly decreases clam growth (e.g. Irlandi and Peterson, 1991; Nakaoka, 2000).

Because of its high vulnerability to predation, it seems that high densities or dramatic expansion of the nonindigenous Manila clam might rarely occur in estuaries with a high exposure to macropredators such as the Bay of Santander,. Recent findings by Bidegain et al. (2012a) (see Chapter IV) suggest that the habitat suitability for this species in the Bay of Santander is also essential in regulating its potential expansion. During the year of the study, each time top nylon nets of enclosures were cleaned (i.e. every two-three weeks) the presence of gulls, oystcathers and crabs around the experimental area was detected at low tides. A considerable abundance of fishes (e.g., gill-head breams, sting rays and mullets), searching for food in the sediment, was also observed during rising tide (Bidegain pers. Obser.). These observations and the results of the predator's exposure experiment are compatible with previous studies where predation on Manila clam was considered to be strong and a great variety of macropredators were described: moonsnails (*Euspira lewisi*), sea stars (*Pisaster spp.*), a variety of birds, e.g. diving ducks (*Aythya affinis*), gulls (Famnily Laridae), crows (Family Corvidae), oystcachers, (e.g. *Haematopus ostralegus ostralegus*), scoters (Family Anatidae) (Toba et al., 1992; Gillespie et al., 2001;

Caldow et al., 2007), *Cancer sp.* crabs (Gillespie et al., 2001; Byers, 2005, Lum, 2011) and bottom fish such as rays (Peterson, 1982, Jamieson et al., 2001). These authors also described the important role of predation on regulation of the nonindigenous species expansion.

The high vulnerability of infaunal species to predation is related with the burial depth since clam survival increases with increasing burial depth (Zaklan and Ydenberg, 1997). When clams were exposed to predators the mortality was considerably higher for Manila clam than for carpet shell clam (87 % and 67 % respectively). For *R. decussates*, 55% of this mortality was attributed to crab, fish and other macropredators, whilst, for *R. philippinarum*, 73 % of the mortality was attributed to this cause. This higher vulnerability to predation of Manila clam compared with the native carpet shell clam agrees with the hypothesis of the authors mentioned above, since the typical burial depths are considerably different between species. Although, the burial depth was not measured meticulously, we noticed that at the end of the experiment, in most cases, the carpet shell clam was found relatively deeper in the sediment than Manila clam. Thus, predators may mitigate high *R. philippinarum* density preying on them more than on *R. decussatus*, presumably because crabs, birds and fish excavate the shallowly burrowing Manila clam more easily (Seitz et al., 2001). However, while deeper burial depth increases *R. decussatus* protection from predators and humans, it also exposes clams to a more anoxic environment (Byers, 2005). In fact, this species exhibited greater mortality indicative of anoxia (i.e. undamaged, blackened shells) than *R. philippinarum*.

6.5. Conclusions

The results of this study strongly suggest that neither the current distribution of populations (i.e. coexistence) nor a potential spread of the Manila clam in the Bay of Santander can be defined as an ecological threat for the native carpet shell clam. This

result confirmed the absence of negative correlation found in Chapter III regarding abundance data of both species. Moreover, predation plays an important role in regulating both species populations, but more drastically the expansion of the nonindigenous species and, hence, high densities of this clam species, are not expected in this estuary.

When extrapolating our experimental densities to other estuaries of Europe, it seems that the competitive interaction between these species is not always the main reason of the drastic decrease of the native clam. Nevertheless, additional research including experimentation on early recruiters and juveniles will help to determine the importance of competition in this more critical life phase. Besides, it should be explored how the strength of the competition relative to abiotic factors and other biotic interactions determines invasion success and impacts on the native species.

Conservation strategies of one of the most appreciated resource in many estuaries of Europe, the native carpet shell *Ruditapes decussatus*, requires several quantitative challenges including that of competitive interaction. However, these strategies might be more focused on aspects other than the potential direct impact of Manila clam, although a dramatic increase in density as it occurred in Venice Lagoon should be avoided. For this purpose, care should be taken in seeding of the nonindigenous species in culture zones i.e. control of pressure of introductions (Carlton, 1996; Lawrence and Cordell, 2010) and specific fishery management measures should be adopted considering the differences in life history attributes.

With the appropriate conservation and management measures adopted, the nonindigenous species could have beneficial aspects, including (i) enhance of biodiversity and (ii) positive socioeconomic impacts. Moreover, Manila clam could have the potential to be also of considerable benefit to several shorebird populations and other macropredators as suggested by Mieszkowska et al. (2006) and Caldow et al. (2007), since it is more readily consumed due to its shallower burial depth. As a consequence, predation on the

native clam may actually decrease. However, this food source could in turn increase predators' abundance and influence nearshore community structure. Thus, since natural systems are highly dynamic and governed by complex nonlinear interactions, conservation measures and strategies may also be re-evaluated over the years to cope with the changes resulting from species' spatio-temporal population dynamics.

Chapter VII

**Estimating minimum suitable catch size
of *Ruditapes decussatus* and *Ruditapes
philippinarum* using a kernel-based
nonparametric model**

Chapter VII. Estimating minimum suitable catch size of *Ruditapes decussatus* and *Ruditapes philippinarum* using a kernel-based nonparametric model

This study has led to the publication of a research article in Ocean and Coastal Management Journal 71, 52-63 by Bidegain, G., Sestelo, M., Roca-Pardiñas, J., Juanes, J.A. in 2013 with the title “Estimating a new suitable catch size for two clam species: Implications for shellfishery management”.

Abstract

The present study analyzes the weight gain patterns per unit of size and estimates the minimum suitable catch size of two clam species: the carpet shell clam *Ruditapes decussatus* and the Manila clam *Ruditapes philippinarum*. For this purpose, data from the two largest estuaries along the northern coast of Spain (Cantabrian Sea) were used. The length–weight relationship of both studied species was estimated using two models: a classic allometric model and a nonparametric regression model based on local linear kernel smoothers. Additionally, first derivatives were used to estimate a minimum capture size for this species, corresponding to the size at which the first derivative reached the maximum. Within this context, the models application showed (a) the nonparametric model resulted in a better fit of data for both species (b) different minimum catch sizes for each species based on maximum length (49.5 mm for *R. decussatus* and 44.7 mm for *R. philippinarum*), both considerably larger than the currently established in EU and (c) an effect of estuaries and zones on individuals weight gain patterns. This confirmed the nonparametric model as an alternative approach to analyze the length–weight relationship for the studied species and to estimate a minimum suitable catch size of capture. The application of the specific catch sizes obtained in this study could lead to an

increase in available commercial stocks of both species and positive effects on the conservation of the native species. In addition, the estimated zone-specific weight-gain patterns and minimum catch sizes could support a zone-based management. Therefore, this study provides a preliminary study and a starting point to consider the revision of the minimum legal size of the studied species in order to improve the current shellfishery management model.

7.1. Introduction

In the northern coast of Spain, Bay of Santander and Marismas de Santoña are important productive estuaries in terms of standing stocks of these species, with recorded biomasses of 90 t for *R. decussatus* and 58 t for *R. philippinarum* in the Bay of Santander and around 20 t for both species in Marismas de Santoña (see Chapter III; Juanes et al., 2012; IH Cantabria, 2011). Shellfishing is conducted on these populations and *R. philippinarum* farming areas are located on the central south-eastern sand flat of Elechas in the Bay of Santander. The stock assessment of clam populations conducted in 2010 by the Regional Government showed a considerable decrease of both species in the Bay of Santander from 2005 (IH Cantabria, 2011). The illegal extractions of clams found in Chapter III and also detected frequently by periodical inspections of the Fisheries Service (pers. comm) may be one of the important reasons of these species populations decline, particularly for the slower growing native species *R. decussatus*.

Setting a specific minimum catch size of exploited clam species is one of the most important and widely used measures in shellfishery management strategies (e.g. Berthou et al., 2005; Gilbert et al., 2006). However, the management of clam fishery in several estuaries where these species fishery is conducted, like in those in Cantabria, has been mainly based on setting the same minimum size of capture and closing areas regardless of the existing important biological differences between them. The minimum catch size of

individuals established in these estuaries and across all the north coast of Iberian Peninsula is the same (40 mm) for both species. Besides, the minimum legal size established by the European Union was 40 mm for both species to 2007 and after that it was decreased to 35 mm for Manila clam (Dang, 2010). In this regard, Juanes et al. (2012) suggested that the absence of an specific catch size could lead to an overexploitation of the native *R. decussatus*. Thus, the estimation of a specific and/or zone- or estuary-based suitable minimum catch size is essential and could have important positive effects on the conservation of the native species and on the yield of the clam fishery in the long term.

Within this context, the aims of this study were to (a) to assess the suitability of a non parametric model to study the length–weight relationship of *R. decussatus* and *R. philippinarum* and (b) to analyze the applicability of this model to estimate a minimum catch size for each species taking into account the environmental conditions integrated in different estuaries and zones of each estuary.

7.2. Materials and methods

7.2.1. Study site

This study was conducted as in the Bay of Santander as it was done for the rest of the chapters in this thesis. In addition, as an exception in this thesis, this chapter study area was also integrated by the intertidal flats of Marismas de Santoña (18.7 km²) (Figure 7. 1). Further details of these estuaries and sand flats are provided in Chapter II.

7.2.2. Data collection and laboratory procedures

Specimens of *R. decussatus* and *R. philippinarum* were collected from different sites in both estuaries where commercial operation of these resources is conducted. Sampling was performed during spring low tides in April 2010. All the individuals were extracted in stations (i.e. transects of 1m x 10m) located in the intertidal sand flats by means of the hand raking of the sediment (upper 15 cm).

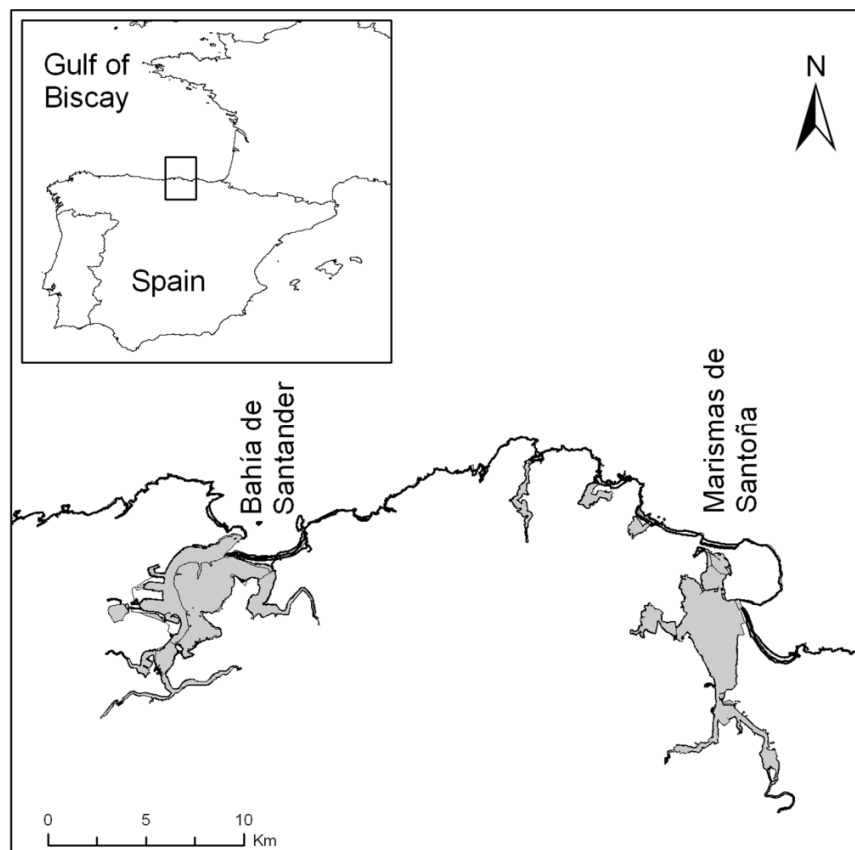


Figure 7.1 -Study area: Bay of Santander and Marismas de Santoña estuaries located in the northern coast of Spain.

This operation was similar to the artisanal shellfishing technique used to gather clams and it was conducted by an experienced shellfisher-woman supervised by scientific personnel (Juanes et al., 2012). The sediment was turned over by the rake and clams were collected by hand as they appear to the surface.

In the Bay of Santander individuals were collected from 31 stations located on three zones: Central zone, considered as a more oceanic area, and northern and southern zones considered as more estuarine or inner areas. In Marismas de Santoña individuals were gathered from 30 stations located on four zones: Central zone, considered as more oceanic open zone, and northern, southern and western zones considered as inner areas (Figure 7. 2). This grouping of stations by inner and open areas was made based on spatial proximity and similar environmental characteristics according to the requirements of the Water Framework Directive (Vincent et al., 2002) for the classification of superficial water bodies in the estuaries of Cantabria (GESHA, 2005). Taxonomic determination of each individual was carried out in the laboratory, followed by maximum shell length (ML, mm) and dry weight (DW, g) measurements. These measurements were made using a digital calliper with a precision of 0.1 mm and a 0.01 g precision balance after oven drying of individuals for 72 h at a temperature of 60°C till constant weight. A total of 2693 individuals were measured (1605 individuals of *R. decussatus* and 1088 individuals of *R. philippinarum*).

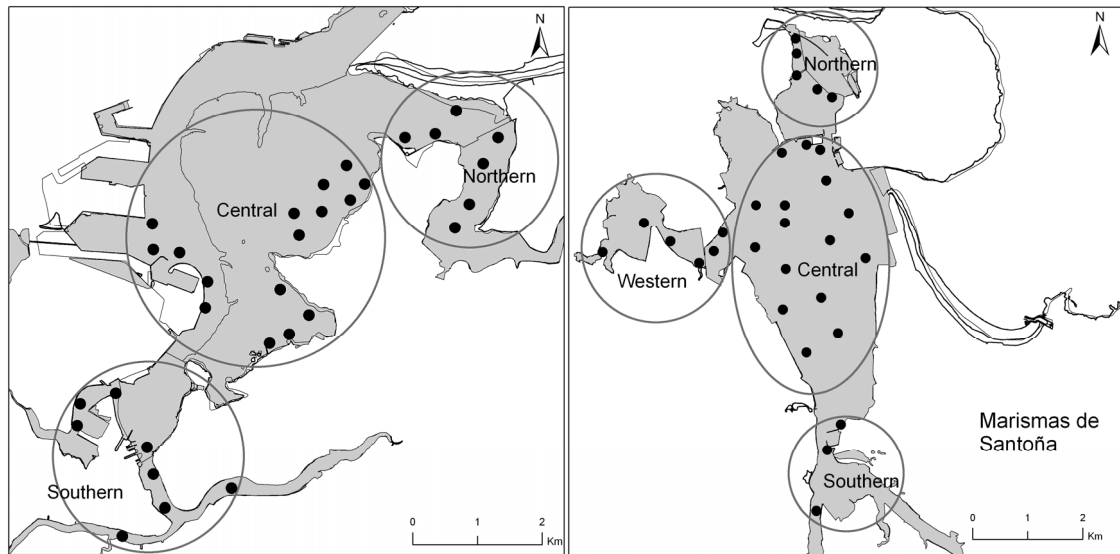


Figure 7.2 - Location of the sampling stations and zones where *R. decussatus* and *R. philippinarum* individuals were collected in Bay of Santander (left) and Marismas de Santoña (right) estuaries. Stations are represented by black circles and zones are represented by grouping circles: in Bay of Santander, Central zone, considered as a more oceanic area and northern and southern zones considered as more estuarine or inner areas; in Marismas de Santoña Central zone, considered as more oceanic open zone, and northern, southern zone and western zones considered as inner areas.

7.2.3. Statistical background

In this section we report the applied statistical methodology. Firstly, we present both the used models and the introduced test and secondly, we describe with detail the estimation algorithms and the inference procedures.

7.2.3.1. Length–weight relationship

The length–weight relationship of both species was estimated using two models: a parametric model and a more generalized model, according to Sestelo and Roca-Pardiñas (2011). Firstly, the dependence between *DW* and *ML* was estimated using a classic allometric model. As we mentioned before, this parametric model is one of the most widely used models to estimate this relationship in fish biology and fisheries. The regression curve takes the following form

$$E[DW|ML] = a ML^b \quad (1)$$

being a and b constants. This model is usually converted into its logarithmic expression, and this conversion, which is quite simple, both conceptually and mathematically, facilitates the estimation of its parameters by linear regression.

However, in certain circumstances, the assumption of a given curve on the effects of the covariate is very restrictive and it is not supported by the data at hand. Therefore and secondly, to ascertain the cited relationship, we propose the use of a more generalized nonparametric model of the type

$$DW = m(ML) + \varepsilon \quad (2)$$

where m is an unknown smooth function and ε is the error that is assumed to have mean zero and variance as function of the covariate ML . It should be note that, in contrast to allometric model, in this type of model there is no need to establish a parametric form of m .

It is important to highlight that in practice a bad specification in the model could suppose incorrect conclusions. Hence, we also propose a procedure that will help us to compare and select an adequate model to fit the data. To this end, consideration will be given to a test for the null hypothesis of an allometric model vs. a general nonparametric model. The objective is to test

$$H_0: m(ML) = a ML^b \quad (3)$$

versus H_1 with m being an unknown function.

Note that if H_0 is not rejected, then the parametric model will be suitable to the data and we will reject the use of a more general model. By contrast, if H_0 is rejected, the conclusion

to be drawn is that the specified form is not correct and it will be necessary to fit the nonparametric model proposed above.

Factor-by-curve interactions

In some circumstances, it might be interesting to compare the estimated curves between the different levels of a factor (e.g. species, estuary or zone). In this framework, a generalization of the “pure” model in (2) is the regression model with factor-by-curve interactions. In this type of model, the relationship between DW and ML can vary among subsets defined by levels of a categorical covariate F .

Particularly, in our study, we were interested in comparing first the length–weight relationship between the two species of the study. To this end, we considered the following model

$$DW = m_0 + \begin{cases} m_1(ML) + \varepsilon_1 & \text{if } F = 1 \\ m_2(ML) + \varepsilon_2 & \text{if } F = 2 \end{cases} \quad (4)$$

where ε_1 and ε_2 are the mean zero errors for each factor’s levels, m_0 represents global effect of ML on the response; and m_1 and m_2 are the specific effects of ML associated with the level 1 (*R. decussatus*) and 2 (*R. philippinarum*) of the factor F (species).

Secondly, in order to detect a possible effect of the estuary in the gain weight pattern, two alternative models were proposed, one for each species. These models are analogs to the presented in (4) but taking into account the factor F as estuary, being level 1 Bay of Santander and level 2 Marisma de Santoña.

Thirdly, to know the effect of the zone we propose again a similarly model for each species, being F the factor zone and inner zones and open zones the levels 1 and 2, respectively.

Minimum suitable catch size estimation

Based on the model in (2), we could suggest a possible size of capture for these species. The ideal size, named ml_0 , will be given for the maximizer of the first derivative of m . This point could be define as

$$ml_0 = \arg \max_{ml} m^1(ml)$$

In practice, the true $m^1 (ml)$ is not known, and decisions will be taken on the basis of its estimate $\hat{m}^1(ml)$. A natural estimator of ml_0 can be defined as the maximizer of

$$\hat{m}^1(ml_1), \dots, \hat{m}^1(ml_N)$$

with ml_1, \dots, ml_N being a grid of N equidistant points in a ranger of the ML values. We have taken an $N = 10,000$ points, so the distance between consecutive nodes is less than 0.01 mm of ML . Analogously, we can obtain ml_{01} and ml_{02} as the maximizer of $ml_1^1 (ml)$ and $ml_2^1 (ml)$, being $ml_1^1 (ml)$ and being $ml_2^1 (ml)$ the regression curves of DW on ML for the level 1 and 2 of the factor, respectively.

Additionally, to make inference about this size and compare it between the two levels of the factor (e.g. species, estuary or zone), we propose the use of a measure of association as statistical test. The proposed measure could be considered as the following difference

$$dif = ml_{02} - ml_{01}$$

where ml_{01} and ml_{02} are the maximizer of the first derivatives for *R. decussatus* and *R. philippinarum*, or for Bay of Santander and Marismas de Santoña or for inner and open zones, respectively. The confidence interval constructed for this measure will help us to know with statistical significance if the size for the two levels is the same. The general rule is that the point where the clams reach their maximum weight gain is different between levels if the zero value is not within the interval.

Additionally to the size where the first derivative reached a maximum, differences in weight gain patterns between species, estuaries and zones were analyzed descriptively by comparing the value of the curve at several sizes (e.g. 20, 30, 40, 50 mm).

The procedure that enables the confidence intervals for ml_0 or for dif to be constructed is outlined below.

7.2.3.2. Estimation and inference procedures

Here, we describe briefly the estimation of the allometric and nonparametric model. In the case of the first one (eq.1), it was converted into its logarithmic expression in (5) to estimate its parameters by linear regression

$$\log DW = \log a + b \log ML = a^* + b^* \log ML + \varepsilon \quad (5)$$

Once \hat{a}^* and \hat{b}^* have been obtained by fitting the model in (4), the parameters' original scale was returned to, $\hat{a} = \exp(\hat{a}^*)$ and $\hat{b} = \hat{b}^*$, and the estimated model, $\widehat{DW} = \hat{a}ML^{\hat{b}}$, was obtained. In addition, the estimation of the derivative of DW was then given by $\widehat{DW}' = \hat{a}\hat{b}ML^{\hat{b}-1}$.

In the case of the nonparametric model in (2), to date, several approaches to estimating the regression curve m and its first derivative m^1 have been suggested in the statistical literature, e.g. methods based on penalized regression splines (Eilers and Marx, 1996) or the Bayesian versions of these (Lang and Brezger, 2004). This paper is focused on local linear kernel smoothers (Wand and Jones, 1995). These smoothers have been chosen since, among other advantages, they enable the use of binning type acceleration techniques (Fan and Marron, 1994) to reduce computational time and so ensure that the problem can be adequately addressed in practical situations. Based on this approach and for simplicity of notation, from now on, we denote this model as KNP model (kernel-based nonparametric model).

Given the original sample $\{ML_i, DW_i\}_{i=1}^n$, the local linear kernel estimator of $m(ml)$ and its first derivative $m^1(ml)$ at a location ml are defined as $\hat{m}(ml) = \hat{\beta}_0(ml)$ and $\hat{m}^1(ml) = \hat{\beta}_1(ml)$, where $\hat{\beta} = (\hat{\beta}_0, \hat{\beta}_1)$ is the minimizer of

$$\sum_{i=1}^n (DW_i - \beta_0 - \beta_1(ML_i - ml))^2 h^{-1} K\left(\frac{ML_i - ml}{h}\right)$$

where $K(u) = 1/\sqrt{2\pi} \exp(-0.5u^2)$ is the Gaussian kernel function (a symmetric density), and $h > 0$ is the smoothing parameter or bandwidth. The nonparametric estimates obtained of m and m^1 are known to depend heavily on the bandwidth, h , which controls the trade-off between the bias and the variance of the resulting estimates. Various proposals based on some error criterion for an optimal selection have been suggested, yet the difficulty of asymptotic theory means that nowadays optimal selection is still a challenging open problem. As a practical solution to this problem, in this paper we consider that the smoothing bandwidth, h , can be selected automatically by minimizing the following cross-validation error criterion (Stone, 1977)

$$CV = \sum_{i=1}^n \left(DW_i - \hat{m}^{(-i)}(ML_i) \right)^2$$

where $\hat{m}^{(-i)}(ML_i)$ indicates the estimate at ML_i , leaving out the i^{th} element of the sample.

Bootstrap Based Confidence Intervals

The wild bootstrap procedure (see Härdle and Mammen (1993); Härdle and Marron (1991); Mammen (1992)) was used again for the construction of pointwise confidence intervals (*CI*). This resampling method is valid for heterocedastic models

where variance of ε is a function of ML . The steps for constructing these CI for a Z value obtained from the model in (2) (for instance, $Z = ml_0$, $Z = dif$, or $Z = m(ml)$ for a given ml) are the following:

Step 1. Obtain the estimated \hat{Z} from the original sample.

Step 2. For $b = 1 \dots B$ (e.g. $B=1000$), generate bootstrap samples $\{ML_i, DW_i^{\bullet b}\}_{i=1}^n$ generated the same way as in Step 2 from testing procedure presented earlier, but using, in this case, the estimations and errors of the nonparametric model, and obtain the bootstrap estimates $\hat{Z}^{\bullet b}$ the same way as in Step 1.

Finally, the $100(1 - \alpha)\%$ limits for the confidence interval of Z are given by

$$I = (\hat{Z}^{\alpha/2}, \hat{Z}^{1-\alpha/2})$$

where \hat{Z}^p represents the percentile p of bootstrapped estimates $\hat{Z}^{\bullet 1}, \dots, \hat{Z}^{\bullet B}$.

Testing for the allometric model

The objective is to test the null hypothesis in (3), $H_0: m(ML) = a ML^b$ versus general hypothesis H_1 being m an unknown nonparametric function. To test H_0 , we propose the use of the likelihood ratio test given by

$$T = \sum_{i=1}^n (DW_i - \hat{a}ML_i^{\hat{b}})^2 - \sum_{i=1}^n (DW_i - \hat{m}(ML_i))^2$$

where $\hat{a}ML_i^{\hat{b}}$ and $\hat{m}(ML_i)$ are the estimates of $m(ML_i)$ under H_0 and H_1 respectively.

The test rule based on T consists of rejecting the null hypothesis if $T > T^{1-\alpha}$, where T^p is the p -percentile of T under H_0 . Nevertheless, it is well known that, within a nonparametric regression context, the asymptotic theory for determining such percentiles is not closed, and resampling methods such as bootstrap introduced by Efron (1979) (see also Efron and Tibshirani, 1993; Härdle and Mammen, 1993; Kauermann and Opsomer, 2003) can be applied instead.

In this section we have used the wild bootstrap for determining the critical values of test T . The steps of the procedure are as follows:

Step 1. Obtain from the sample data $\{ML_i, DW_i\}_{i=1}^n$ the estimates $\hat{a}ML_i^{\hat{b}}$ and $\hat{m}(ML_i)$ obtained under H_0 and H_1 respectively, and compute the T value.

Step 2. For $b = 1 \dots B$ (e.g. $B=1000$), generate bootstrap samples $\{(ML_i, DW_i^{\bullet b})\}_{i=1}^n$ with $DW_i^{\bullet b} = \hat{a}ML_i^{\hat{b}} + \varepsilon_i^{\bullet b}$ being

$$\varepsilon_i^{\bullet b} = \begin{cases} \varepsilon_i \cdot \frac{(1 - \sqrt{5})}{2} & \text{with probability } p = \frac{5 + \sqrt{5}}{10} \\ \varepsilon_i \cdot \frac{(1 + \sqrt{5})}{2} & \text{with probability } p = \frac{5 - \sqrt{5}}{10} \end{cases}$$

where $\varepsilon_i = DW_i - \hat{a}ML_i^{\hat{b}}$ are the errors of the allometric model, and compute $\hat{T}^{\bullet b}$ the same way as in Step 1.

Finally, the test rule based on T consists of rejecting the null hypothesis if $T > T^{1-\alpha}$, where T^p is the empirical p -percentile of values $T^{\bullet b}$ ($b = 1, \dots, B$) obtained before.

7.3. Results

7.3.1. Length–weight relationship models

The fitted allometric model was $\widehat{DW} = 4.24 \times 10^{-5} ML^{3.26}$ for *R. decussatus*, whilst the model resulted for *R. philippinarum* was $\widehat{DW} = 7.34 \times 10^{-5} ML^{3.19}$ (Figure 7. 3). Under the KNP model, the initial regression curves were increasing functions for both species, very similar to the obtained with the allometric model. However, the KNP model detected variations in the final part of the regression curve which were more pronounced for *R. philippinarum* (Figure 7. 3).

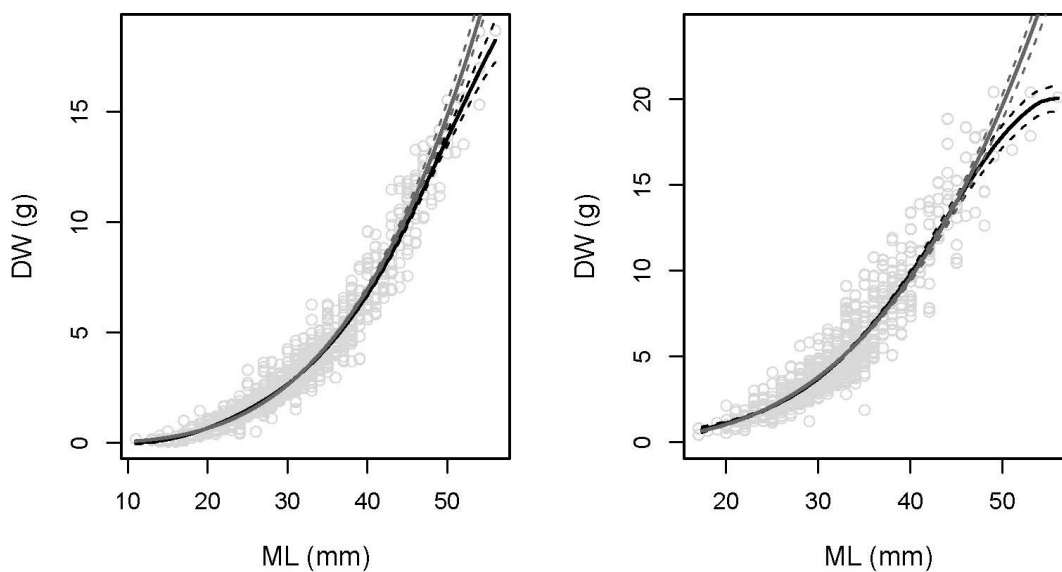


Figure 7.3 - Regression curves of the length–weight relationship (DW, dry weight and ML maximum length) for *Ruditapes decussatus* (left) and *Ruditapes philippinarum* (right). Gray solid lines refer to the allometric model and black solid lines refer to the KNP model. Bootstrap-based 95% confidence intervals are presented by broken lines. Both estuaries data set was used.

Factor	n	Statistic	p-value
Species			
<i>R. decussatus</i>	1605	0.06	0.02 *
<i>R. philippinarum</i>	1088	0.20	0.01 *
Estuary			
<i>R. decussatus</i>			
Bay of Santander	926	0.08	0.01 *
Marismas Santoña	679	0.19	0.01 *
<i>R. philippinarum</i>			
Bay of Santander	949	0.05	0.01 *
Marismas Santoña	139	1.74	0.01 *
Zone			
<i>R. decussatus</i>			
Inner	861	0.05	0.01 *
Open	744	0.09	0.02*
<i>R. philippinarum</i>			
Inner	404	0.26	0.03 *
Open	684	0.14	0.04 *

Table 7.1 - Comparison of the fit of the allometric and KNP models to the relationship between length and weight using the likelihood ratio test for each of the analysis conducted: the first analysis with species as factor (*R. decussatus* and *R. philippinarum*), the second one with estuary as factor (Bay of Santander and Marismas de Santoña) and the last one with zone as factor (inner and open areas). For values $p\text{-value} < 0.05$, marked by an asterisk, the H_0 is rejected, so the KNP model afforded better fit than the allometric model.

In the case of the KNP model the expression or formulae is omitted since in this framework there are no parameters to be estimated. Both models estimated very similar DW values until a ML value of 40 mm for *R. decussatus* and 45 mm for *R. philippinarum* was reached. Thereafter, for an ML size of 50 mm, the allometric model estimates, for *R. decussatus*, a mean DW value (95 % confidence interval) of 14.92 g (14.50, 15.53), versus 13.86 g (13.52, 14.18) estimated by the KNP model. Similarly, for *R. philippinarum*, the

allometric model estimates a DW value of 19.80 g (18.97, 20.43), versus 17.92 g (17.24, 18.57) estimated by the KNP model.

The results obtained from the likelihood ratio test (Table 7.1) to compare the fit of the models showed a better fit of data was achieved using the KNP model. The same result was obtained for both species, independently of using all data set, separating data by estuaries or by inner and open zones.

7.3.2. Estimation of the minimum size of capture

Under the allometric model, the first derivatives of the initial curves displayed increasing monotonous functions. However, under the KNP model, the first derivatives showed a maximum at a specific size after which it began to decrease (Figure 7.4).

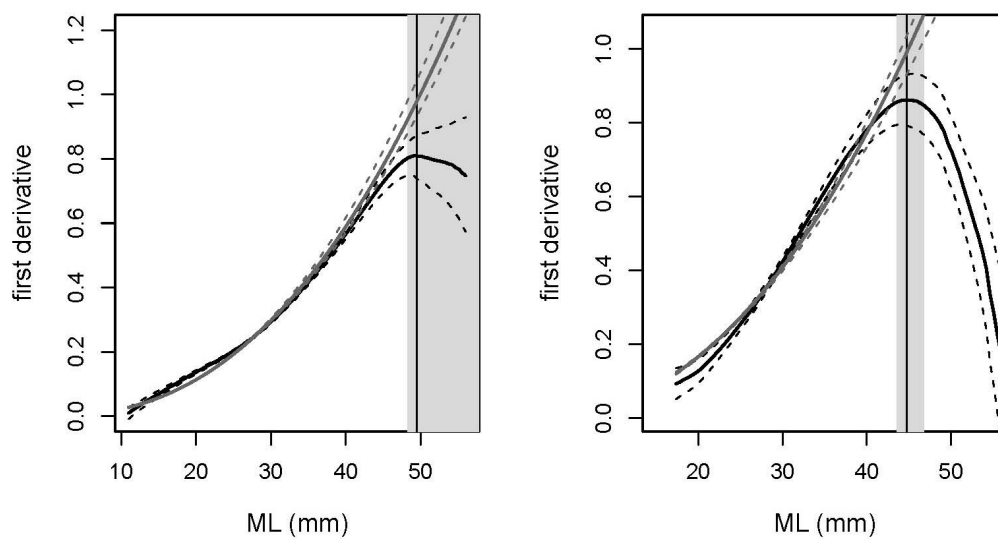


Figure 7.4 - First derivative of the initial regression curves for *Ruditapes decussatus* (left) and *Ruditapes philippinarum* (right). Gray solid lines refer to the allometric model and black solid lines refer to the KNP model. For the KNP model, solid vertical line represents the estimated mlo (size where first derivative is maximum) and the gray area represents the confidence interval constructed for the estimated mlo . Bootstrap-based 95% confidence intervals are presented by broken lines. Both estuaries data set was used.

Therefore, the first derivative of the regression curve displayed by the nonparametric model was used to estimate the minimum suitable cath size. For *R. decussatus* this minimum size (49.5 mm) was significantly larger than the one estimated for *R. philippinarum* (44.7 mm) (Table 7.2 and 7.3).

Factor	n	\widehat{ml}_0	lower CI	upper CI
Species				
<i>R. decussatus</i>	1605	49.51	48.25	—
<i>R. philippinarum</i>	1088	44.74	43.52	46.76
Estuary				
<i>R. decussatus</i>				
Bay of Santander	926	48.66	47.53	—
Marismas Santoña	679	56.00	52.71	—
<i>R. philippinarum</i>				
Bay of Santander	949	46.98	44.01	—
Marismas Santoña	139	43.58	40.69	48.46
Zone				
<i>R. decussatus</i>				
Inner zones	861	54.56	48.74	—
Open zones	744	48.88	46.90	50.77
<i>R. philippinarum</i>				
Inner zones	404	44.79	42.73	49.05
Open zones	684	44.52	41.24	50.30

Table 7.2 - Size \widehat{ml}_0 , which maximizes the first derivative of the regression curves and 95% confidence interval, for each of the analysis conducted: the first one, model with specie as factor (*R. decussatus* and *R. philippinarum*), the second models with estuary as factor (Bay of Santander and Marismas de Santoña) and the last models with zone as factor (inner and open areas).

The analysis carried out for estimating a possible size of capture in each estuary and in different studied zones (i.e. inner and open zones), showed for *R. decussatus* that this size was significantly higher in Marismas de Santoña and inner estuarine zones. On the contrary, for *R. philippinarum* this size of capture was the same in both estuaries and also in both inner and open zones (Tables 7.2 and 7.3, Figures 7.5 and 7.6).

Factor	dif	lower CI	upper CI
Species	- 4.77	- 12.03	- 2.34 *
<i>R.decussatus</i>			
Estuary	7.34	0.43	8.29 *
Zone	- 5.68	- 8.65	- 0.18 *
<i>R.philippinarum</i>			
Estuary	- 3.4	- 7.11	3.31
Zone	- 0.27	- 6.01	5.14

Table 7.3 - Results of the statistical test used to compare the size \widehat{ml}_0 between species, estuaries and zones. The difference between the maximizers of the first derivatives of the compared regression curves (*dif*) together with the 95 % confidence intervals are presented. Significant differences (i.e. zero is not included within the confidence interval) are presented by an asterisc.

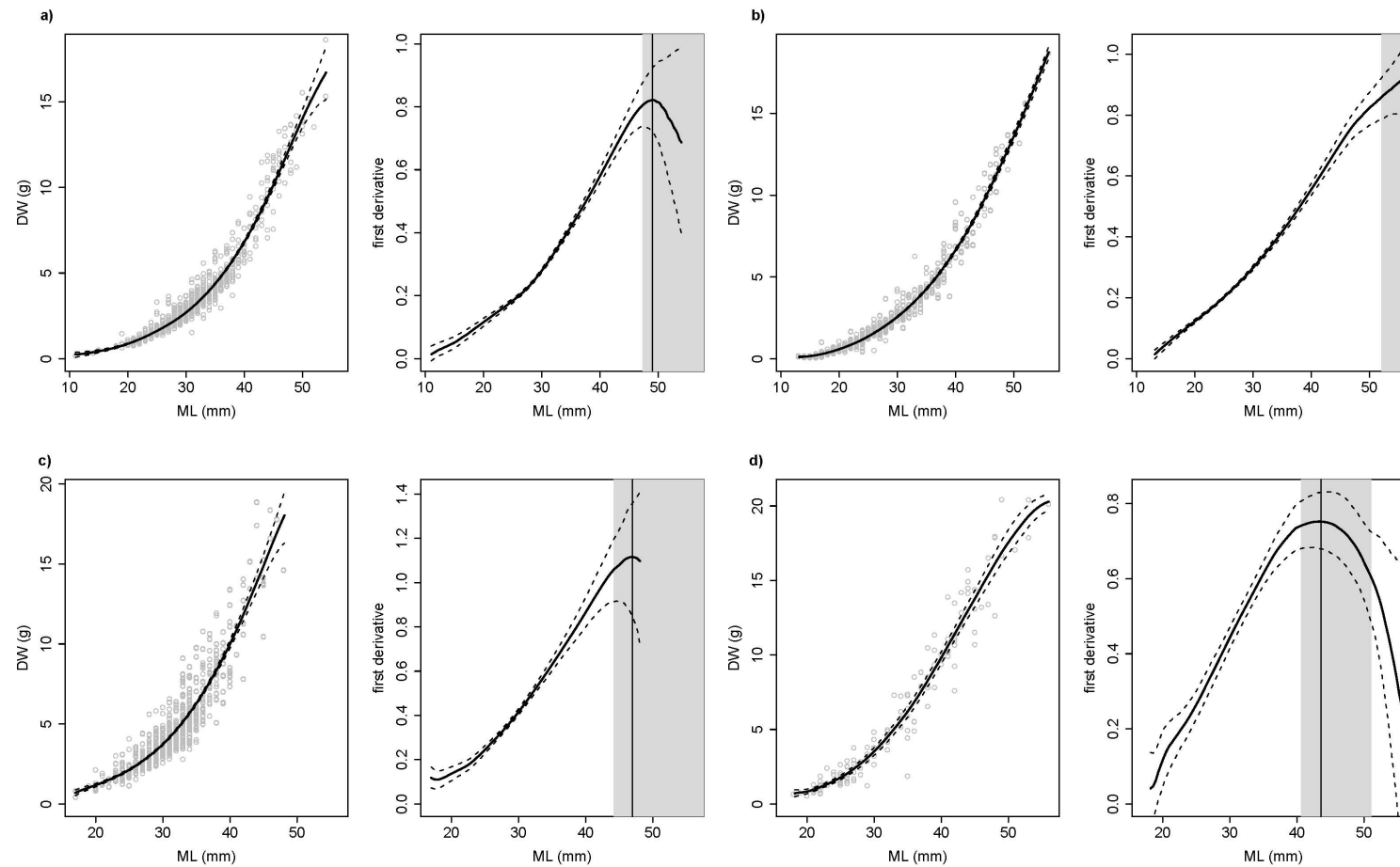


Figure 7.5 - Non parametric model regression curve and first derivative (solid curved lines) with bootstrap-based 95% confidence intervals (broken lines) for dry weight (DW) and maximum length (ML) of *Ruditapes decussatus* in Bay of Santander (a) and Marismas de Santoña (b), and for *Ruditapes philippinarum* in Bay of Santander (c) and Marismas de Santoña (d). Solid vertical lines: estimated ml_0 (size where first derivative is maximum). Gray area: confidence interval constructed for the estimated ml_0 .

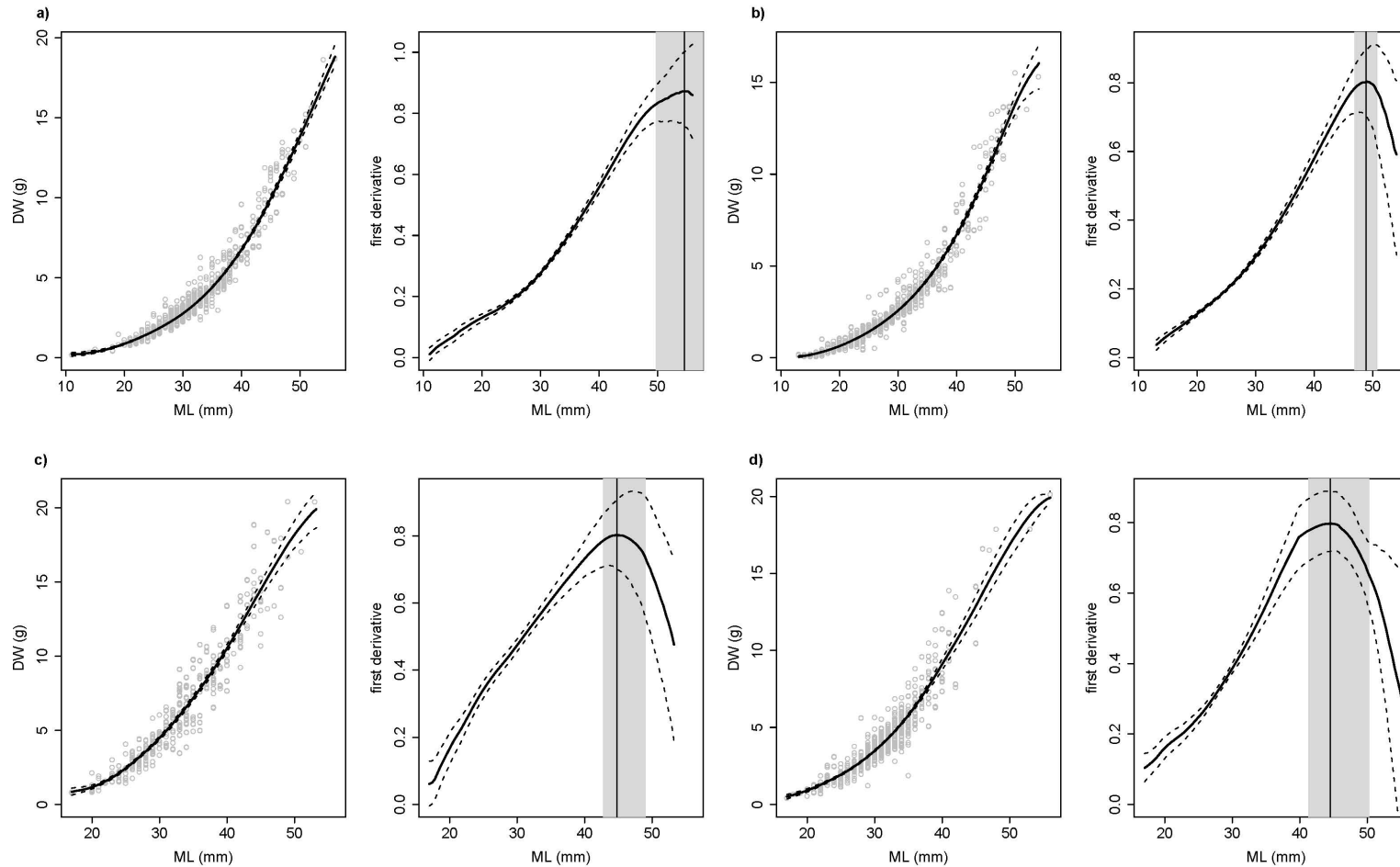


Figure 7.6 - Non parametric model regression curve and first derivative (solid lines) with bootstrap-based 95% confidence intervals (broken lines) for dry weight (DW) and maximum length (ML) of *Ruditapes decussatus* on inner (a) and open zones (b) zones and for *Ruditapes philippinarum* on inner (c) and open zones (d). Solid vertical lines: estimated ml_0 (size where first derivative is maximum). Gray area: confidence interval constructed for the estimated ml_0 .

7.3.3. Weight gain patterns

The descriptive analysis of weight gain patterns by exploring the value of the curve of the first derivative at several sizes showed that the weight gain per unit of size increased for both species to a specific size after which it began to decrease. The weight gain per unit of size was considerably higher for *R. philippinarum* to the size beyond which it began to decrease. The decreasing pattern was much pronounced for *R. philippinarum* than for *R. decussatus* (Figure 7. 4).

On the other hand, this descriptive analysis was done to explore the weight gain patterns in each estuary (Figure 7. 5) and in open and inner zones (Figure 7. 6). For *R. decussatus* in Marismas de Santoña and in inner estuarine zones, the decrease after reaching the maximum is hardly appreciable. Besides, although the weight gain pattern of *R. decussatus* was similar in both estuaries and also in both zones, the maximum weight gain per unit of size was reached at significantly smaller sizes in Bay of Santander and in open zones. However, for *R. philippinarum* the weight gain per unit of size was appreciably higher to 30 mm in Marismas de Santoña and inner zones while the maximum was reached at similar sizes in both estuaries and zones (Figures 7. 5-6 and Tables 7.2-3).

4. Discussion

The KNP model applied in this paper to analyze the length–weight relationship of *R. decussatus* and *R. philippinarum* is a suitable tool to estimate the minimum catch size of both species, considering this as the optimum size to obtain in the long term the maximum yield in weight from the fishery.

This model resulted in a better fit of data when studying the length–weight relationships of *R. decussatus* and *R. philippinarum* in the two major estuaries in northern Spain, indicating that the KNP model can be considered a viable alternative to the classic

allometric model for both species. The results coincide with those reported recently by Sestelo and Roca-Pardiñas (2011) for the gooseneck barnacle *Pollicipes pollicipes*, which is also a species that displays a differentiated weight gain pattern throughout its development.

Furthermore, the KNP model was able to record specific patterns in the data at the end of the regression curve that could not be detected by the allometric model. This suggests that some valuable biological information is lost using the allometric model (Rabaoui et al., 2007). This may be due to the fact that the nonparametric models allow for a more flexible fit of the data than the parametric regression techniques because they do not specify in advance any function that links the covariates to the response. However, the detected variations in the final part of the regression curve were slightly more pronounced for *R. philippinarum* than those of *R. decussatus*. Although this distinction could be linked to biological differences, there might be at least two possible explanations for this pattern in need of further investigation: (1) The sampling dates of this study could coincide with the beginning of the gonad development phase in *R. decussatus* (Urrutia et al., 1999; Rodrigues-Carballo et al., 1992) and consequently, some individuals might be contributing to skew the curve as they have a higher weight linked to their corresponding size than they have during the resting period; (2) the reduction in the expected number of large individuals of *R. decussatus* due to a higher fishing pressure on this species might be removing information at the last part of the curve. Therefore, in further studies it is essential a long term monitoring and sampling effort covering different seasons of the year in order to assess the influence of the gonad development on the body growth and to explore the effect of the lack of large sizes. On the other side of the curve, the absence of individuals < 20 mm is linked with the sampling technique (i.e. traditional raking of sediments carried out by fishermen, Juanes et al., 2012) but, due to the intrinsic features of the kernel estimator which use only the neighboring points to the target point to fit the model, this fact does not affect to any of the objectives of this work.

The demonstrated ability of the KNP model to analyze the length–weight relationship of this species makes it a suitable model to estimate the weight gain patterns and therefore, to estimate a minimum suitable capture size (Sestelo and Roca-Pardiñas, 2011). The first derivative of this model was analyzed for both species. The minimum suitable catch size was given by the maximizer of this derivative of the regression curve. Beyond this point the increase of weight per unit of size decreases. Thus, this size ensures that individuals smaller than this size had not yet attained the maximum yield in weight. In accordance with FAO guidelines for other species with allometric length–weight relationship, such as fishes, individuals should be not captured until they reach the “maximum yield size” (Sparre and Venema, 1997).

Based on that approach, the estimated minimum sizes were significantly higher for *R. decussatus* (49.5 mm) than for *R. philippinarum* (44.7 mm). But they were both larger than those established by both the current UE normative (40 mm and 35 mm respectively) and that one in force until 2007 (40 mm for both species) and by the actual regulations of the Regional Government in the studied Marismas de Santoña and Bay of Santander estuaries (40 mm for both species). Size limits are intended to protect exploited marine populations by allowing individuals to grow larger and spawn at least once before removal from the fishery and thereby increase long-term yield (Goodyear, 1996). In this line, considering their respective maturity sizes the specific minimum catch sizes enforced by UE and the Regional Government, allow *R. decussatus* to spawn at least once and *R. philippinarum*, with high probability, at least twice. Sexual maturation phase can begin between 10 and 20 mm shell lengths in *R. decussatus* (Lucas, 1968; Urrutia et al., 1999) while in *R. philippinarum* it can start at a 5–10 mm (Ponuvorovsky and Yakolev, 1992 and references therein). However, the major contribution to reproduction is done by large individuals since fecundity increases with size for both species (Holland and Chew, 1974; Ponuvorovsky and Yakolev, 1992; Robert et al., 1993; Laruelle et al., 1994; Urrutia et al., 1999). Therefore, in this study estimated catch sizes could avoid the exploitation of large individuals that still have a high reproduction activity allowing clams to have more

spawns than currently before they are fished. It is worth noting that growth rate decreases considerably for larger clams (>30 mm) and consequently individuals might take approximately 0.5-1 years (*R. philippinarum*) to 2-2.5 years (*R. decussatus*) (Arnal and Fernández-Pato, 1977, 1978; Spencer et al., 1991; Solidoro et al., 2000; Chessa et al., 2005; Dang, 2009) to reach the estimated minimum catch sizes starting from 40 mm (i.e. the current legal size in the region). Thus, the setting of these higher minimum legal sizes might lead to a substantial decrease of captures during the first years after the establishment of the measure and, afterwards, the available annual commercial stock together with the spawning activity and recruitment success could be considerably higher than the current ones for both species. This prediction of higher yields for the fishery is assumed based on results obtained by Bald and Borja (2002) for *R. decussatus* after modeling its exploitation and extrapolating them to our findings and to *R. philippinarum* which is a faster growing and hardier species (Breber, 1985;1991, Spencer, 1991). These authors simulated several exploitation scenarios and analyzed the evolution of the population stock including biological variables at different size classes such as fertility rate and natural mortality (Pérez-Camacho, 1979). When a scenario of exploitation of legal sizes > 40 mm was compared with a scenario of illegal fishing (21-40 mm) they found that the stock had decreased dramatically from ~3000 t to ~500 t. Regarding this result it seems that contribution of large size clams (>40 mm) to the standing stock due their high fertility rates, might be considerably more important than the negative effect associated to the increase of natural mortality at this sizes. Taking to account this author's results and the fertility and mortality rates of different size classes (Perez-Camacho, 1979) higher standing stocks and yields in the fishery could be expected in the medium term in a scenario where the minimum catch size ensures that individuals smaller than this size had not yet attained the maximum yield in weight.

This expected higher clam density scenario would be in line with the conservation of the European native clam *R. decussatus*. However, it could also lead to a dramatic expansion and predomination of the introduced Manila clam as it occurred in other European

estuaries (e.g. Arcachon Bay, Lagoon of Venice) (Auby, 1993; Marin, 2003; Mistri, 2004). However, in this hypothetical scenario a great variety of macropredators such as crabs, birds or fishes (Toba et al., 1992; Jamieson et al., 2001; Byers, 2005, Caldow et al., 2007; Lum, 2011) might mitigate the high *R. philippinarum* densities preying on them differentially more than *R. decussatus*, because crabs, birds and fish excavate the shallowly burrowing Manila clam more easily (Seitz et al., 2001). This differential mitigation effect could be highly probable in the studied estuaries both regarding to the significant higher mortality due to predation found for *R. philippinarum* comparing with *R. decussatus* in the Bay of Santander by Bidegain and Juanes (2012) (Chapter VI) and considering the high concentration of predators in Marismas de Santoña wetlands since it is an area of international importance for the passage of migratory birds. The habitat suitability for the Manila clam could also play an important role regulating a potential drastic expansion of the introduced clam (Bidegain et al., 2012a, see Chapter IV)

With respect to the study conducted by Sestelo and Pardiñas (2011), both the identification of area-specific minimum catch sizes and the statistical analysis conducted to compare these sizes are innovative aspects. The significant differences observed for *R. decussatus* when the catch size was estimated by estuary and zone could suggest the feasibility of establishing estuary or zone-specific minimum legal sizes. However, although this measure should be effective in obtaining the maximum yield in weight for each estuary or zone, it is hardly applicable in a shellfishery management plan due to the potential difficulties regarding both the control of illegal fishing and the shellfishers. However, based on these area-specific minimum catch sizes other less complicated zone-based and specific management measures could be more effective such as capture seasons, no-fishing zones, rotations strategy or sustainable locations for aquaculture (Morsan, 2007; Longdill, 2008).

Regarding the weight gain patterns analyzed by the first derivatives, higher dry weights of both soft tissue and shell documented for Manila clam (Pazos et al., 2005; Dincer, 2006) may be contributing to the observed differences between studied species. Moreover,

several environmental factors governing estuarine or zonal conditions (e.g. substrate, food availability, salinity, fluctuations of mean conditions, etc.) could be, in part, responsible for the growth, weight gain and shell structure variability of the clams (Robert et al., 1993; Gosling, 2003; Kanazawa and Sato, 2008). In line with this, the higher weight gain per unit of size observed for *R. philippinarum* (to 30-35 mm) and for *R. decussatus* (around its maximum) in Marismas de Santoña and inner zones might be related to the higher availability of an alternative source of food avoiding decreases in dry weight associated to a loss in reserve tissue when phytoplankton is scarce (Delgado and Pérez-Camacho, 2005). Page and Lastra (2003) documented that intertidal suspension-feeding bivalves primarily fed on resuspended microbenthos or detritus during periods of low phytoplankton concentration. This resuspended material is more abundant in estuaries with high organic matter inputs (river inflows) and within them in inner muddy zones (Junoy and Viéitez, 1990; Mendes et al., 2006). Thus, the higher river inflows in Marismas de Santoña estuary (twice the flow observed in Bay of Santander) (Galván et al., 2010), which result in higher concentrations of organic matter in the water as well as a higher capacity of inner muddy zones to keep the incoming organic matter (Middelburg and Herman, 2007), might have an effect in food availability and consequently, in individuals weight gain patterns. Moreover, the waste water treatment plan is still in the last phases of construction in this estuary, which leads to have an extra input of organic matter.

Although the above mentioned lack of larger sized data may be affecting the weight gain pattern results, the food availability and therefore, better growth efficiency also in large sizes could be involved in the hardly appreciable decreasing pattern observed for *R. decussatus* in Marismas de Santoña and in inner estuarine zones.

Besides, differences in growth between oceanic and inner stations resulting from differences in fluctuations of environmental conditions (e.g. temperature and salinity) observed by Robert et al. (1993) may also be contributing to these variations in weight gain, although the high water renovations rates for both estuaries (Galvan et al., 2010) are likely to minimize this potential effect. Moreover, the incorporation rates of carbon sources into shell carbonate, from dissolved inorganic carbon as well from food, has an

important role in the shell weight gain (Poulain et al., 2010) and it is expected to be different between estuaries or zones according to the environmental differences above commented which could be affecting this rate. Finally, clams higher burying capacity linked to smaller sediment grain size (i.e. in inner zones) (Nair and Ansell, 1968) leads to larger and heavier siphons development (Zwarts and Wanink, 1989). This may be another factor contributing to the differences observed in weight gain, especially for *R. decussatus* which has considerably larger siphons and burial depth than *R. philippinarum* (Mistri et al., 2004).

5. Conclusions

The results demonstrate the feasibility of using nonparametric techniques based on local linear kernel smoothers to analyze the length–weight relationship and to estimate the minimum capture size of commercial species that display differentiated weight gain patterns throughout their development. The estimated minimum suitable catch sizes are different between these species and larger than those currently considered in the normative (UE and regional). These results are in line with the biological differences between this species and may provide considerably higher yields in weight from the fishery and could have positive effects on the conservation of the native species, having important consequences in the field of management.

This study provides a preliminary study and a starting point to consider the revision of the minimum legal size of the studied species in order to improve the current management models and obtain, in the long run, an increase in available commercial stocks. Moreover, the differences in weight gain patterns observed between estuaries and zones provide insight into the growth patterns of these species and their link to the environmental conditions governing estuaries. Thus, these area-specific differences could support zone-based sustainable management models. Finally, it is important to underscore that the

potential effect of gonadal development of some individuals and the effect of the lack of large individual remain to be determined.

Chapter VIII

General conclusions and future research

Chapter VIII. General conclusions and future research

The general objective of this thesis was to advance in understanding of ecological dynamics of the European native grooved carpet shell clam *Ruditapes decussatus* and the introduced Manila clam *Ruditapes philippinarum* in order to support specific conservation strategies and shellfisheries management.

According to this general objective and the specific objectives established in the thesis, several field surveys and experimentation were carried out together with the application and development of predictive models, mainly in the tidal flats of the Bay of Santander but also in Marismas de Santoña. The results obtained permit the extraction of the following conclusions regarding the assessment and ecological dynamics of *R. decussatus* and *R. philippinarum* and also the drawing of several management proposals.

8.1. General conclusions

In this section, the general conclusions are displayed for each of the five study chapters.

Characterization of *R. decussatus* and *R. philippinarum* populations in the Bay of Santander

- The sampling method developed provides adequate data of abundance and density of adult and commercial clams, showing its feasibility to estimate standing stocks and to know exploitation situation of different zones in a large estuary.
- The species relative abundance patterns in the Bay of Santander points out that competitive interaction may not be important and the introduced nonindigenous

Manila Clam has not yet supplanted the *R. decussatus* native clam by occupying entirely its ecological niche. In addition to coexistence areas, *R. decussatus* appeared to be the dominating species in more oceanic and freshwater-influenced areas, whilst *R. philippinarum* in southern inner areas.

- Although the density of both species is similar, the estimated higher total stock and captures data of *R. philippinarum* indicates the increasing importance of the introduced species in the shellfishery, in contrast to past two decades when the native clam was the main harvested species.
- The performed fishing activity on individuals under the minimum legal size could lead to a decline of both populations but especially of the native clam, since it is a slower growing species, and might affect considerably the actual coexistence pattern.

Ecological niche modeling approach to predict the potential expansion of the nonindigenous clam *R. philippinarum*

- Environmental Niche Factorial Analysis has been proved to be a suitable tool to predict potential distribution of these species and to explore the potential expansion of the introduced clam *R. philippinarum*.
- The presence of the native *R. decussatus* and the introduced *R. philippinarum* is restricted to areas with specific environmental characteristics. The most suitable estuarine habitat for these species is generally sandy intertidal sediments with substantial organic matter content washed by waters with low current speeds and lower salinities than the open sea. Among these environmental features the *R. philippinarum* has preference for slightly lower current velocities and inner muddy estuarine zones.

- Highly suitable areas and potential predomination areas are considerably larger for *R. decussatus*. Therefore, assuming the current exploitation and cultivation intensities, habitat suitability would play an important role as a limiting factor for expansion of the introduced *R. philippinarum* in the Bay of Santander.
- The north-south predomination/coexistence patterns of these species, determined by habitat suitability, confirm the relative abundance patterns observed in the preliminary characterization of clam populations conducted in the Bay.

Predicting clam larval dispersal and recruitment using habitat suitability-based particle tracking model

- The longer planktonic larval duration of *R. decussatus* over *R. philippinarum* seems to be the main reason to explain its higher larval dispersion and lower recruitment rates.
- The location of the spawning zones and the wind-induced currents have significant effects on recruitment of both species. Moreover, the interaction between spawning zone location and tidal phase on the spawning moment also showed an effect on final recruitment, particularly for *R. decussatus*. Potential recruitment was higher at spring tides in the outer northern zones whilst was higher at neap tides in the inner southern zones.
- The model showed an important ability to identify major spawning in terms of final recruitment and major nursery grounds.
- Moreover, the model has also an important explanatory power in terms of connectivity between grounds.
- Overall, the developed LARVAHS particle-tracking model can be considered to be reasonably suitable to forecast the dispersal and recruitment of benthic species

larvae associated with taxon-specific larval behaviour, oceanographic variability and habitat suitability.

Does expansion of *Ruditapes philippinarum* cause competitive displacement of the native *Ruditapes decussatus*?

- The results suggest that neither the current distribution patterns of clam populations in the Bay of Santander nor a potential spread of the introduced *R. philippinarum* can be defined as an ecological threat for the native carpet shell clam regarding competitive interaction. This conclusion confirms preliminary findings during characterization of populations.
- The highest density of the introduced species examined, experiencing no density mitigation by predators, directly affected neither the growth nor the mortality of the confamilial native carpet shell clam.
- Predation plays an important role in regulating both species populations, but more importantly *R. philippinarum* clam populations.

Estimating minimum suitable catch size of *Ruditapes decussatus* and *Ruditapes philippinarum* using a kernel-based nonparametric model

- The results of the study demonstrate the feasibility of using nonparametric techniques based on local linear kernel smoothers to analyze the length–weight relationship and to estimate the minimum capture size of commercial species that display differentiated weight gain patterns throughout their development.
- The estimated minimum suitable catch sizes are different between these species and larger than those currently considered in the normative (UE and regional).

- Moreover, the differences in weight gain patterns observed between estuaries and zones provide insight into the growth patterns of these species and their relationship with the environmental conditions governing estuaries.

8.2. Implications for clam shellfishery management

The results of this thesis provide valuable information and tools to support the studied species fishery management models. In this section several management proposals or applications of the developed models are outlined both, in general, for the estuaries where these species dwell and are exploited and, specifically, for the Bay of Santander.

8.2.1. Global management proposals

- Sampling based on raking the sediment in transects of 1 x 10 m conducted in this study (Chapter III) could be a suitable method to evaluate clam populations exploitation situation and performance of management measures in large estuaries where intertidal shellfishing is carried out. Moreover, it is advisable that sampling is conducted by shellfishermen in order to involve them in a collaborative co-management of the fishery.
- The co-management should be integrated into an “adaptive management” process relying on systematic assessments and feedback learning in order to achieve a progressive accumulation of knowledge for improved fisheries management.
- Environmental Niche Factorial Analysis (ENFA) for the habitat suitability determination is a suitable tool for determining potential restoration sites for the native *R. decussatus* or farming sites for both species. In the same manner, setting

of closure zones or sanctuaries could be supported by the application of this model.

- LARVAHS particle-tracking model based on habitat suitability may be also powerful to support restoration strategies and aquaculture management. Thus, highly suitable habitats from where spawning achieves good larvae retention rates should be considered as potential grounds for conservation strategies and improvement of fisheries yields of the native *R. decussatus*. A better understanding of predation will also help in the location of restoration sites or sanctuaries.
- In view of the results obtained regarding competitive interaction, conservation strategies of the native *R. decussatus* might be more focused on aspects other than the potential direct impact of *R. philippinarum*, provided that an important increase in density, which can lead to potential competitive interaction effects, is avoided.
- Therefore, to control *R. philippinarum* density increasing care should be taken with placement of restoration or farming sites. In contrast to *R. decussatus*, for this species high larval retention could lead to help the expansion and predomination over the native clam. Thus, for this species, a less successful spawning zone in terms of final recruitment could be a better option to locate farming or restoration areas.
- In this study estimated minimum suitable catch sizes application could have positive effects on fishery yields of both species and conservation of the native species. Therefore, this work provides a starting point to consider the revision of the minimum legal size of the studied species.
- In summary, with the appropriate conservation and management measures adopted, the nonindigenous species could lead to beneficial socioeconomic impacts.

8.2.2. Specific management proposals for the study site

- A strict control on the minimum legal capture size should be established to avoid the actual situation, particularly for *R. decussatus* as it has a slower growth rate than *R. philippinarum* and has not the extra larval supply of the later coming from the intensive cultured zones.
- The first clam populations' assessment studies conducted in the Bay of Santander based on artisanal raking technique should be a starting point to a high government involvement in a future fishery management model, hand in hand with the shellfishers' collaboration. Therefore, the incorporation of shellfishers in a co-management of the shellfishery should increase rationality in management and create more legitimate regulations, thereby motivating user groups to follow regulations.
- The closure zones for clams in the Bay of Santander (and the rest of the estuaries of Cantabria) are non-specific. Therefore, it is important to direct policy efforts towards establishing specific closure zones as conservation measure for the native clam. The setting of these zones could be supported by the results obtained in the ENFA analysis of habitat suitability and LARVAHS particle-tracking model applied in the Bay of Santander, where highly suitable areas and major spawning and nursery grounds were defined for both species. Astillero spawning zone should be considered as a potential restoration site from where highest larvae retention and recruitment rates will be achieved leading to clam population increase in the bay.
- The Manila clam farming area is located in the tidal flat of Elechas. This study (Chapter V) did not found this spawning zone as one of the most successful spawning zones, i.e., poor larvae retention rates were achieved from here. Therefore, expansion and predomination of this species seems to be limited with the current location for Manila clam cultivation. In contrast, care should be taken locating farming sites in Solía and Astillero inner, since these spawning zones showed high retention rates and could lead to the population expansion.

- The current minimum capture size is 40 mm for both species in the Bay of Santander like in the rest of the estuaries of Cantabria. Results obtained in this study suggest a specific minimum catch size which is higher for *R. decussatus* than for *R. philippinarum*. Therefore, the revision of the minimum legal size of the studied species is advisable in the region of Cantabria in order to obtain, in the long run, the maximum yield in weight from the fishery of both species.

8.3. Future research

This thesis studies have revealed the existence of certain deficiencies in the estimation or prediction procedures described to achieve the objectives, as well as the possibility of exploring new aspects in the complex field of ecological dynamics of this species. These deficiencies and issues to explore further have been analyzed in detail in each chapter discussion section, giving possible solutions. Here, the most relevant aspects of the thesis needing future research are mentioned.

- Regarding the applied sampling methodology for clam population assessment in estuaries, particularly in those with low clam densities, the minimum transect length or sampling area should be investigated. A preliminary study conducted recently in a low clam density area showed a 1 x 5 m transect as the most suitable one. Moreover, including early recruiters and juvenile (<15 mm length) sampling without an excessive time consuming should be investigated in order to obtain an improved assessment methodology covering all size classes. Moreover, testing of this sampling method in other estuaries and other species may be essential in order to standardized sampling methodology for clam population's assessment.
- Regarding habitat suitability predictions the integration of more environmental variables with higher-resolution data and additional presence data would allow for

further refinement of the models. Thus, the habitat suitability determination could come through fine-scale predictive modelling on each tidal flat and would provide a more reliable tool for potential invasion predictions and spatial aquaculture and fishery management strategies. Moreover, predation results integration in the habitat suitability submodel must be considered in future studies.

- The LARVAHS larval evolution model may serve as a useful framework to guide quantitative investigations into the dynamics of larval dispersal and particularly, into settlement and recruitment since it has an important focus in a habitat suitability-based recruitment submodel. However, further research in higher-resolution data and many important biological processes, i.e. larvae behavior, cues, gamete fertilization success, larval mortality and growth, is essential to develop an improved model. Moreover, future analyses should be conducted towards a 3D model, together with the improving of its calculation capacity, memory management (i.e. more than 1 million particles). Calibration and validation of the model through a major effort in sampling early recruiters and also larvae is also advisable. In addition, different turbulent diffusion methods should be tested in order to select the most appropriate method for this study.
- Additional research about competitive interaction between the studied species is also required. On the one hand, despite densities used in the experiment were larger than natural ones, higher densities should be experimented since they may still not be large enough to reach the critical level to trigger competition. On the other hand, short term experimentation (i.e. clam movements, migration, etc) and covering other estuaries and different zones within estuaries is advisable in order to have a better understanding of the interaction. Finally, experimentation on early recruiters and juveniles will help to determine the importance of competition in this more critical life phase.
- Regarding the estimation of the minimum suitable catch size, it is essential a long term sampling effort covering different seasons of the year in order to assess the

influence of the gonad development on the body growth and explore the effect of the lack of large size individuals.

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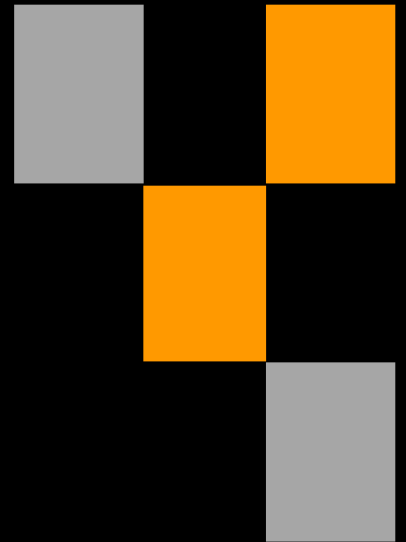
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