



Universitat d'Alacant
Universidad de Alicante

Ecological effects of *Caulerpa racemosa* var. *cylindracea* on amphipod assemblages (Amphipoda, Crustacea) associated with shallow water habitats in the Mediterranean Sea

Maria Teresa Vázquez Luis



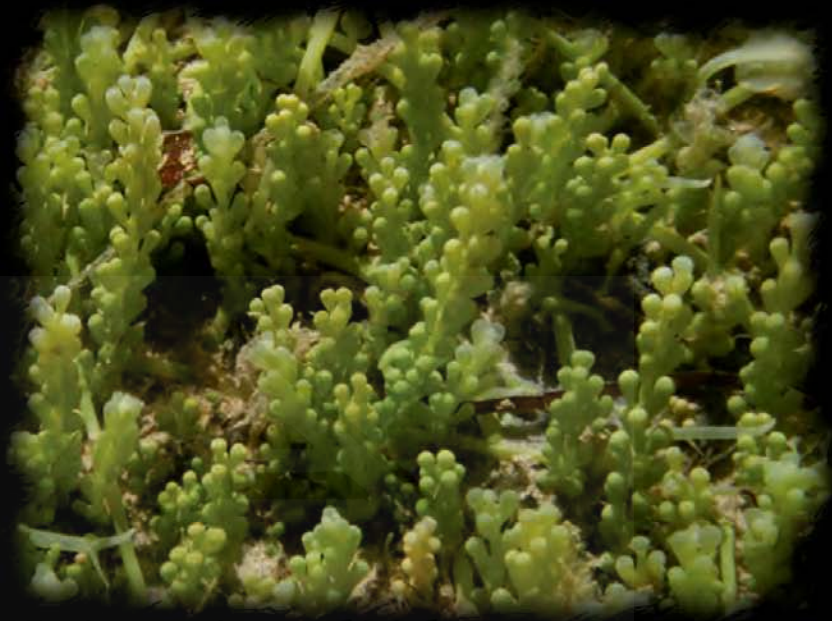
Tesis

Doctorales

www.eltallerdigital.com

UNIVERSIDAD de ALICANTE

Ecological effects of *Caulerpa racemosa* var. *cylindracea* on amphipod assemblages (Amphipoda, Crustacea) associated with shallow water habitats in the Mediterranean Sea



Universitat d'Alacant
PhD Thesis
Universidad de Alicante

Maite Vázquez Luis

March 2011





Universitat d'Alacant
Universidad de Alicante

Dpt. de Ciències del Mar i Biologia Aplicada
Dpto. de Ciencias del Mar y Biología Aplicada

**Ecological effects of *Caulerpa racemosa* var. *cylindracea* on amphipod assemblages
(Amphipoda, Crustacea) associated with shallow water habitats
in the Mediterranean Sea**

**Efectos ecológicos de *Caulerpa racemosa* var. *cylindracea* en los poblamientos
de anfípodos (Amphipoda, Crustacea) asociados a hábitats someros
en el Mar Mediterráneo**

Universitat d'Alacant
Universidad de Alicante

Memoria presentada para optar al grado de Doctora, mención de Doctora
Europea, en la Universidad de Alicante por

MARIA TERESA VÁZQUEZ LUIS

ALICANTE, Marzo 2011



Universitat d'Alacant
Universidad de Alicante

Dpt. de Ciències del Mar i Biologia Aplicada
Dpto. de Ciencias del Mar y Biología Aplicada

TESIS DOCTORAL

**Ecological effects of *Caulerpa racemosa* var. *cylindracea* on amphipod assemblages
(Amphipoda, Crustacea) associated with shallow water habitats
in the Mediterranean Sea**

**Maria Teresa Vázquez Luis
Marzo 2011**

VºBº Directores de la Tesis Doctoral

Dr. Pablo J. Sánchez Jerez

Dr. Just T. Bayle Sempere



Universitat d'Alacant
Universidad de Alicante

Dpt. de Ciències del Mar i Biologia Aplicada
Dpto. de Ciencias del Mar y Biología Aplicada

TESIS DOCTORAL

**Ecological effects of *Caulerpa racemosa* var. *cylindracea* on amphipod assemblages
(Amphipoda, Crustacea) associated with shallow water habitats
in the Mediterranean Sea**

Maria Teresa Vázquez Luis
Marzo 2011

VºBº Director del Departamento de Ciencias del Mar y Biología Aplicada

Universitat d'Alacant
Universidad de Alicante

Dr. Jose Luis Sánchez Lizaso



Universitat d'Alacant Universidad de Alicante

Dpt. de Ciències del Mar i Biologia Aplicada
Dpto. de Ciencias del Mar y Biología Aplicada

Los doctores **PABLO J. SÁNCHEZ JEREZ** y **JUST T. BAYLE SEMPERE**,
Profesores Titulares del Área de Zoología de la Universidad de Alicante,

CERTIFICAN:

Que la memoria de Tesis doctoral titulada "**Ecological effects of *Caulerpa racemosa* var. *cylindracea* on amphipod assemblages (Amphipoda, Crustacea) associated with shallow water habitats in the Mediterranean Sea**", presentada por **MARIA TERESA VÁZQUEZ LUIS**, ha sido realizada bajo su dirección en el Departamento de Ciencias del Mar y Biología Aplicada de la Universidad de Alicante, y en el Centro de Investigación Marina de Santa Pola (CIMAR). Y para que conste a los efectos oportunos, firman en Alicante a 31 de Enero del año dos mil once.

Universitat d'Alacant
Universidad de Alicante

Fdo: Dr. Pablo J. Sanchez Jerez

Fdo: Dr. Just T. Bayle Sempere

Agradecimientos- Acknowledgements

Al fin llegó el momento de agradecer a todos aquellos que me han ayudado en este camino de un modo u otro. Lo cierto es que no sé muy bien hasta dónde remontarme en el tiempo para agradecer a todos aquellos que han hecho que llegue este momento. La verdad es que desde que era pequeña sentí un profundo amor por la naturaleza en general, y por el mar en especial. A esto contribuyó en gran medida mi padre ya que como amante del mar me enseñó montones de cosas y siempre hizo que tuviera una estrecha relación con él. Más tarde, en el instituto, la motivación y energía que mi profe de biología, José Luis León Nicolás, ponía en sus clases, me hizo saber sin ninguna duda que quería estudiar biología. Ya en la Universidad tuve la suerte de disfrutar de una beca Erasmus, y fue en Montpellier donde pasé una de las mejores épocas de mi vida, pero además Jean-Pierre Lumaret me abrió las puertas de su laboratorio con enorme gentileza. Esto me brindó la oportunidad de conocer el mundo de la investigación y mi jefe en aquel momento, Pierre Jay-Robert, me motivó mucho a seguir en este mundo...aunque me faltaba el mar!

Gracias a la confianza que Pablo Sánchez y Just Bayle depositaron en mí pude hacer mi sueño realidad. Siempre recordaré cuando Pablo me dijo: “así que te gusta la taxonomía y los invertebrados...podríamos hacer algo con anfípodos!”, fue el comienzo de esta tesis. Como director ha sido inmejorable, me ha enseñado prácticamente todo lo que sé: trabajar en el mar, determinación de los anfípodos, diseño experimental, tratamiento estadístico, y otras tantas cosas... Mil gracias. Muchas gracias también a Just que también depositó una gran confianza en mí y he de agradecer la oportunidad que me brindó al poder hacer lo que siempre he deseado.

En mi caso la falta de financiación, como ocurre demasiado a menudo en estos casos, empezó a ser un problema. Y fue gracias a la creación del CIMAR (Centro de Investigaciones Marinas de Santa Pola; Ayuntamiento de Santa Pola y Universidad de Alicante) que Alfonso A. Ramos Esplá me dio la posibilidad de poder seguir con la tesis y participar en otros proyectos de investigación. Gracias Alfonso por haberme ayudado a seguir adelante.

También el Instituto Alicantino de Cultura “Juan Gil-Albert” de la Excelentísima Diputación Provincial de Alicante, depositó una gran confianza en este proyecto de tesis concediéndome dos ayudas de Apoyo a la Investigación que han sido de gran importancia: en la modalidad de “Ciencias Experimentales y Técnicas” (para la realización del DEA) y más tarde la “Ayuda a la realización de Tesis Doctorales”. Muchísimas gracias.

Más tarde llegaron las estancias. En Sevilla tuve el gran privilegio de trabajar con uno de los mejores especialistas de anfípodos; con el Dr. José Manuel Guerra-García aprendí un montón de cosas y me acogió de la mejor manera posible. Su entusiasmo y motivación se contagian y hacen que el trabajo más tedioso parezca verdaderamente sencillo. Muchísimas gracias. Los agradecimientos los hago extensivos a todo el grupo *Caprella* y “anexo”: Pili, Elena, Macarena, Azahara, Susana, Gina, Aurora, Isa, David, Álvaro, Víctor, Ana Mari, Juanjo y Mar, ya que siempre habéis estado ahí ayudado en todo lo posible y siempre me habéis tratado como a una más del equipo sevillano.

Después tuve la gran suerte de ir a Malta a trabajar con el Dr. Joseph A Borg. Para Joseph tengo muchísimas palabras de agradecimiento, nada más llegar me hice un esguince y le tocó hacer parte de mi trabajo! Además, le doy infinitas gracias por revisar el inglés de los manuscritos y por todos los comentarios que ayudaron a mejorar muchos aspectos. En Malta todo el equipo de Ecoserv fue muy amable conmigo: Sarah Gauci-Carlton, Mildred Vella-Magro, Julian Evans, Graziella Xuereb, Julie Tabone, Sarah Debono, Lucienne Borg y Aaron Hill. Thank you so much for everything and for all the moments that I share with you, I am really grateful!

Tampoco hubiera sido posible la realización de este trabajo sin el apoyo logístico prestado por la Unidad de Biología Marina del Departamento de Ciencias del Mar y Biología Aplicada, así como el CIMAR. Pero más importante aún ha sido el equipo humano que me ha ayudado en diversos momentos. En los muestreos: Pablo A, Dami, Celia, Mercedes, Tere, Irene, Sarah Gauci-Carlton, Joseph Borg, Julian Evans, Redsinsa S.A. y particularmente a Eduardo Minuéz director del P.N.S.G y su equipo. En la identificación de las algas: Alfonso. En la separación de algunas muestras: Lydia y Marianna. En la identificación de los contenidos estomacales: José Manuel Guerra-García y Estibaliz Berecibar, Yoana, Paqui, Vicky, Elena, José Carlos García-Gómez, Manuel Maldonado, Nicolas Sturaro y Loïc Michel. En las últimas correcciones de la tesis: Pablo A, Dami y Carlos. En papeleos varios a lo largo de este período: Ana Frutos, Marta Alonso, Ana Nuevo y Rosa San Román. Muchísimas gracias a todos y todas. A los demás de la Unidad de Biología Marina (a los que aún no he nombrado): Bea, Jose Antonio, Yolanda, José Miguel, Jose Luis, Aitor, Andrés, Cristina, Candela, Tito, Loya, Aurora, Lute, Agustín, Kilian, Esther, Tocho, Marko, Juan Carlos, Antonio, Pilar, Carmen, José Jacobo, José Vicente, Sofía, Mohamed, Isidro, Colilla, Juanma, Mari Loli,...gracias por muchas de las cosas que hemos compartido juntos (y si me olvido de alguien ruego que me perdone). Lo cierto es que de este camino me llevo una gran amistad con muchos de vosotros que espero que dure mucho tiempo.

No puedo dejar de agradecer a otras personas que me han ayudado en diversos aspectos como: Tony Underwood, Traudl Krapp-Schickel, Ed Hendrycks, Oliver Coleman, Tim Dempster, Marc Verlaque y Enric Ballesteros. Vuestra experiencia me ha resultado de gran ayuda para llevar a buen fin este trabajo.

Al resto de gente de fuera del Departamento que llevo en mi corazón, familiares y amigos (a los que están y a los que no), que me han demostrado su cariño y apoyo cada día. En especial a mis chicas: Judith, Fuen, Bea y Francián por que estáis ahí siempre y sois de las pocas que entendéis lo que hago (es lo que tiene ser endogámicos, jeje...). En cuanto a mi familia, a pesar de que no entendieran mucho a lo que me dedicaba, ni eso de las "becas" (a excepción de mi primo Marcos) siempre han mostrado apoyo y cariño; especialmente a las personas que dedico esta tesis, a mi hermana Fani y a mi bisabuela Tónica (aunque ya no esté con nosotros), porque ellas han sido los pilares fundamentales de mi vida!

Y por supuesto a la persona que ha estado conmigo día a día no sé cómo agradecerle todo su apoyo incondicional. Pablo A, millones de gracias por ayudarme en los momentos duros, compartir los buenos momentos, animarme a seguir adelante y por estar siempre a mi lado.

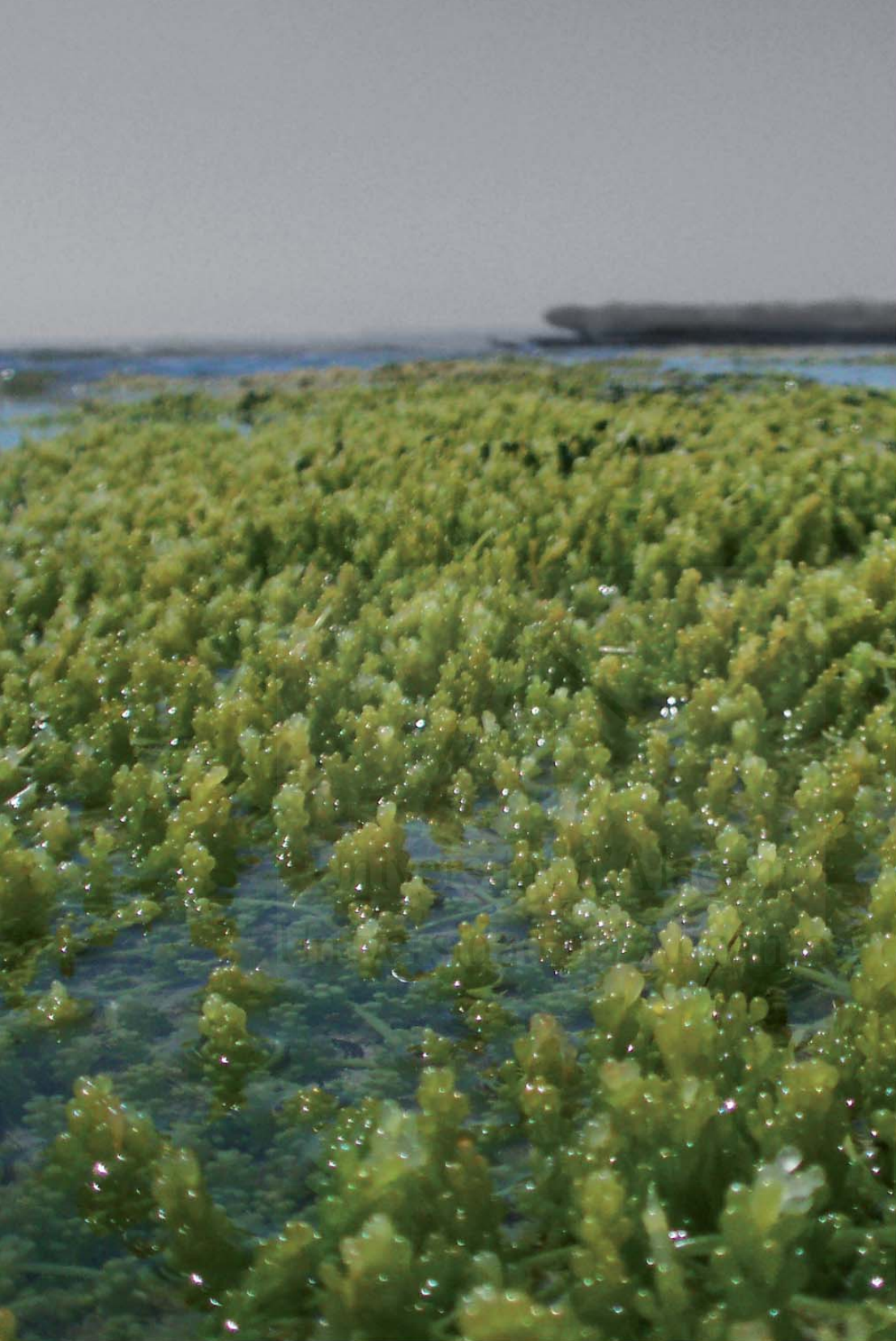
CONTENTS

Resumen.....	1
General abstract.....	11
1. General introduction.....	15
1.1. Invasive seaweeds in the Mediterranean Sea	15
1.2. The invasive alga <i>Caulerpa racemosa</i>	17
1.3. Effects of <i>C. racemosa</i> on the Mediterranean Sea fauna	19
1.4. Amphipods as important component of the Mediterranean coastal ecosystem.....	20
1.5. PhD Thesis justification: Why effects of <i>C. racemosa</i> on native communities should be studied?.....	20
1.5.1. Aims and thesis structure.....	21
1.5.1.1. Does <i>C. racemosa</i> modify the amphipod assemblage on hard bottoms? ..	21
1.5.1.2. Does <i>C. racemosa</i> change the amphipod assemblage on soft bottoms? ..	21
1.5.1.3. Does <i>C. racemosa</i> affect colonization of vegetated habitats by amphipods?	22
1.5.1.4. Does <i>C. racemosa</i> modify the feeding habits of amphipods?.....	22
1.5.1.5. Does <i>C. racemosa</i> affect amphipod prey availability to predators?	22
2. Does <i>C. racemosa</i> modify the amphipod assemblage on hard bottoms?	27
2.1. Abstract.....	27
2.2. Introduction.....	27
2.3. Materials and methods	28
2.3.1. Study area	28
2.3.2. Sampling and experimental design	28
2.3.3. Data analysis.....	29
2.3.3.1. Multivariate analysis	29
2.3.3.2. Univariate analysis	29
2.4. Results	29
2.4.1. Habitat features.....	29
2.4.2. Amphipod assemblage.....	32
2.5. Discussion.....	35
3. Does <i>C. racemosa</i> change the amphipod assemblage on soft bottoms?	41
3.1. Abstract.....	41
3.2. Introduction.....	41
3.3. Materials and methods	42
3.3.1. Study area	42
3.3.2. Sampling design	43
3.3.3. Data analysis.....	43
3.3.3.1. Univariate analysis	43
3.3.3.2. Multivariate analysis	43
3.4. Results	44
3.4.1. Habitat features.....	44
3.4.2. Amphipod assemblages	46
3.5. Discussion.....	52
4. Does <i>C. racemosa</i> affect colonization of vegetated habitats by amphipods?	59
4.1. Abstract.....	59
4.2. Introduction	59
4.3. Materials and methods	60
4.3.1. Study sites.....	60
4.3.2. Characterisation of habitat features and amphipod fauna	61
4.3.3. Experimental setup and sampling design	61
4.3.4. Data analyses	62
4.4. Results	62
4.4.1. Habitat and amphipod fauna	62
4.4.2. Colonization	63
4.5. Discussion.....	68
5. Does <i>C. racemosa</i> modify the feeding habits of amphipods?.....	73
5.1. Abstract.....	73
5.2. Introduction	73
5.3. Material and methods.....	74
5.4. Results	75
5.5. Discussion.....	79
6. Does <i>C. racemosa</i> affect amphipod prey availability to predators?.....	83
6.1. Abstract.....	83
6.2. Introduction	83
6.3. Material and methods.....	84
6.3.1. Prey availability.....	84
6.3.2. Animal collection and fish-holding conditions	84
6.3.3. Predation experiment	85
6.3.4. Data analysis.....	85
6.4. Results	85
6.4.1. Prey availability.....	85
6.4.2. Predation rate.....	86
6.5. Discussion.....	88
7. General discussion.....	93
7.1. Explaining the mechanisms of biological invasions	93
7.2. Facilitation of invasion process by human impacts.....	94
7.3. Effects of <i>C. racemosa</i> on habitat complexity and heterogeneity.....	96
7.4. Effects of <i>C. racemosa</i> on associated fauna community	96
7.5. Gaps of knowledge and future research	98
7.6. Management and mitigation of invaded ecosystems	99
Conclusions.....	103
References.....	107



*A mi yaya Tónica (D.E.P.)
y a mi hermana Fani*

Universitat d'Alacant
Universidad de Alicante



Resumen

1. Introducción

El Mediterráneo y el Mar Negro son muy diversos ecológicamente, contienen aproximadamente un 6.4% de las especies marinas a nivel mundial, a pesar de que la superficie del Mar Mediterráneo sólo representa el 0.8% de la superficie total de los océanos. Recientes estudios han estimado que existen 12.725 especies de organismos marinos macroscópicos en el Mediterráneo (11.594 metazoos y 1.131 macrofitos; Coll et al 2010). Esta alta riqueza de especies también se ve acompañada por un alto nivel de especies endémicas, como promedio el 20.2% de los metazoos y el 22.3% de los macrófitos son endémicos (Coll et al. 2010).

A pesar de poseer esta alta riqueza y endemidad de especies, el Mar Mediterráneo es la región más invadida a nivel mundial por macrófitos (Williams y Smith, 2007), con más de 125 especies introducidas, siendo 21 de ellos invasoras o potencialmente invasoras (Verlaque *com. pers.*). Según los datos disponibles, desde principios del siglo XX el número de las especies de macrófitos invasores ha aumentado exponencialmente y prácticamente se duplica cada 20 años (Ribera y Boudouresque 1995), siendo el principal área donante la región del Indo-Pacífica (Verlaque et al. 2007). En el Mediterráneo estas introducciones pueden ser el resultado de una variedad de causas (vectores), como el transporte de especies en los cascos de barco (fouling), las aguas de lastre y materias sólidas transportadas por los buques, la apertura del Canal de Suez, las importaciones de especies de acuicultura, las transferencias entre cuencas, fugas accidentales de los acuarios, liberaciones de especies utilizadas como material de embalaje de cebos para la pesca o las especies epifitas que han llegado asociadas a otra especie exótica (al Verlaque et al. 2007). En el Mediterráneo la acuicultura es el principal vector de introducción de macrófitos, seguido por el tráfico de buques y del Canal de Suez (Galil 2009). En el mar Mediterráneo dos de las algas más invasoras pertenecen la familia

Caulerpacea, y específicamente al género *Caulerpa*: *C. taxifolia* y *C. racemosa*.

Es ampliamente aceptado que la diversidad biológica mundial del medio marino y su valor como productores de recursos valiosos se ve amenazada por las actividades antropogénicas. En particular, la alteración y destrucción de hábitat, la sobrepesca, el cambio climático y la introducción de especies exóticas han sido identificados como las principales causas de estrés, especialmente en las regiones costeras (Norse 1993, Vitousek et al. 1997, Carlton 2000). En concreto, las invasiones biológicas se consideran una grave amenaza para los sistemas marinos (Ribera y Boudouresque 1995). La tasa de introducciones marinas, incluyendo la introducción de las algas marinas, se han incrementado en los últimos 20 años (Verlaque et al. 2007), sin embargo, el conocimiento actual de los impactos que algas exóticas generan en los ecosistemas marinos es muy escaso. Este hecho contrasta con la percepción de que la invasión de macroalgas presenta graves impactos potenciales, ya que pueden alterar la estructura y funcionamiento del ecosistema por monopolizar el espacio, ya que actúan como ingenieros del ecosistema y pueden, por tanto, modificar las cadenas tróficas marinas (Schaffelke y Hewitt 2007).

Uno de los grupos de invertebrados más ubicuo y abundante en la vegetación de hábitats marinos son los anfípodos, cuya densidad a menudo llegan a varios miles de individuos por m² (Brawley 1992, Tuya et al. 2010). En el Mar Mediterráneo existen alrededor de 48 familias, siendo la mayoría de ellos especies de vida libre, con un alto grado de endemidad en lo que respecta a los anfípodos bentónicos (aproximadamente el 37% de las especies; Ruffo 1998). Los anfípodos bentónicos se pueden encontrar en todos los pisos presentes en el Mar Mediterráneo, desde el adlitoral hasta el abisal, exhibiendo la mayor diversidad en la zona infralitoral, donde aproximadamente

el 69% de las especies están representadas (Ruffo et al. 1998). Estos crustáceos habitan en todo tipo de hábitats, siendo un compartimento clave en las praderas de fanerógamas marinas y actuando como importantes recicladores de materia orgánica (Sánchez-Jerez et al. 2002). También son importantes productores secundarios (Carrasco y Arcos 1984, Highsmith y Coyle 1990, Sarvala y Uitto 1991), y muestran un amplio rango de estrategias tróficas: ramoneadores, filtradores, detritívoros, depredadores, carroñeros, carnívoros, omnívoros, y herbívoros. Algunas especies pueden usar diferentes estrategias de manera simultánea, sucesivamente en el tiempo o en función de las condiciones ambientales (Ruffo et al. 1998). Además, son una gran fuente de alimento para una amplia variedad de depredadores marinos (Stoner 1979, Beare y Moore 1997, Sánchez-Jerez et al. 1999, 2002; Stål et al. 2007), y por tanto, desempeñan un papel clave en el flujo de energía a través de las redes tróficas marinas.

Los anfípodos son crustáceos peracáridos, lo que implica que tiene un desarrollo de las larvas protegidas en el marsupio de las hembras y un ciclo de vida completo asociado con el bentos. Por tanto, los anfípodos se han descrito como especies que desarrollan poblaciones locales en consonancia con las condiciones ambientales del medio debido a su bajo poder de dispersión (Virnstein 1987, Sánchez-Jerez y Ramos Esplá 1996) y responden de manera muy significativa a la modificación del hábitat (Sánchez-Jerez et al. 2000). Algunas especies tienen alta especificidad de hábitat, mientras que otros toleran un amplio rango de alteración del mismo que pueden derivarse de la contaminación, la invasión de especies exóticas y otras perturbaciones, y por consiguiente pueden ser utilizados como un indicador de los impactos ambientales sobre los hábitats con vegetación (Bellan-Santini 1980, Virnstein 1987, Conradi et al. 1997).

A pesar del hecho de que *C. racemosa* es comparable a *C. taxifolia* en términos de capacidad de colonización y modificación de comunidades autóctonas, hay una gran

disparidad en el esfuerzo invertido ante estas dos especies invasoras. La gran mayoría de los estudios realizados para evaluar el impacto de *C. racemosa* en la fauna asociada han utilizado muestreos puntuales y los resultados han sido meramente descriptivos, lo que dificulta llegar a conclusiones y a crear directrices específicas para la gestión de este tipo de invasiones. En general, las interacciones ecológicas de algas invasoras a nivel de comunidad constituyen un marco importante de investigación y, por tanto, los efectos indirectos entre distintos niveles tróficos, la movilidad de los consumidores, y las restricciones de replicación espacial representan retos actuales en el ámbito de la investigación (Williams y Smith 2007). En particular Klein y Verlaque (2008) señalaron que no existen estudios en profundidad sobre *C. racemosa*. Por tanto, es esencial incrementar el conocimiento de los efectos de *C. racemosa* sobre los ecosistemas marinos del Mediterráneo. Con este fin, la presente tesis doctoral analiza los efectos que *C. racemosa* genera sobre (i) la estructura de los poblamientos de anfípodos en diferentes hábitats someros del Mediterráneo, (ii) sus requerimientos tróficos y (iii) las relaciones depredador-presa.

2. ¿Modifica *C. racemosa* al poblamiento de anfípodos de fondos duros?

Desde los primeros estudios de la expansión de *C. racemosa* en las aguas costeras del Mediterráneo, se han llevado a cabo varios estudios sobre la ecología de esta especie se llevaron a cabo (véase una revisión en Klein y Verlaque 2008). Sin embargo, se hace patente la escasez de información sobre los cambios provocados por el alga invasora en la fauna asociada (Argyrou et al. 1999, Piazzzi y Balata 2008). Para estudiar los efectos de la invasión de *C. racemosa* sobre la estructura del hábitat del poblamiento algal en fondos rocosos someros y, por consiguiente, la afección que tienen estos cambios sobre el poblamiento de anfípodos asociados a dicha comunidad algal, se compararon dos hábitats diferentes (invadido y no invadido) localizados en el SE de la Península Ibérica a una profundidad de 0.2-0.5 m. En dichos

hábitat se llevó a cabo un muestreo en verano y en invierno (acorde con el máximo y mínimo desarrollo vegetativo del alga invasora). Para cada tipo de hábitat se llevó a cabo la caracterización de la estructura de hábitat en términos de biomasa algal, riqueza específica y cantidad de detritus, así como la identificación a nivel de especie del poblamiento de anfípodos.

Los resultados mostraron que la presencia y biomasa de *C. racemosa* tuvo un marcado efecto sobre la estructura de la comunidad de macroalgas, afectando a la composición de las especies de macrófitos y generando un aumento de detritus acumulado. Todo ello causa importantes cambios en la abundancia, riqueza y composición de especies del poblamiento de anfípodos asociado a dichos hábitats. Los efectos de la especie invasora en la estructura del hábitat fueron más importantes en el periodo más cálido, debido a la dominancia de *C. racemosa* durante este periodo. La variación estacional de *C. racemosa* observada en este estudio en términos de biomasa fue similar a la descrita en otras localidades, donde los valores máximos de desarrollo se observaron en el periodo cálido. Además, los valores de biomasa también fueron similares o incluso superiores a los registrados en otras localidades del Mediterráneo (Piazzini et al. 1999). El patrón de colonización resultante de la reproducción sexual y asexual (Panayotidis y Žuljevič 2001, Ceccherelli et al. 2001, Ceccherelli y Piazzini 2001, Renoncourt y Meinesz 2002), permiten a *C. racemosa* colonizar un hábitat de una manera muy eficiente. Esto da lugar a una fuerte modificación de la estructura del hábitat, como es el caso de las comunidades de macroalgas. En cuanto al poblamiento de anfípodos un total de 33 especies fueron identificadas, dicho poblamiento se vio influenciado por estos cambios en el hábitat, probablemente debido a sus diferentes requerimientos ecológicos. No obstante, la riqueza específica de anfípodos fue relativamente alta en ambos hábitats, mientras que la composición de las especies de anfípodos se vio modificada en gran medida. Por ejemplo, algunas especies como *Apocorophium acutum* se vio favorecida

por la expansión de *C. racemosa*, en cambio, especies como *Ampithoe ramondi* y *Hyale schmidti* fueron afectadas negativamente por la presencia del alga invasora. La invasión del hábitat por *C. racemosa* puede tener una influencia importante en hábitats rocosos someros, modificando tanto la estructura del hábitat como la fauna asociada, con efectos desconocidos sobre la totalidad del ecosistema.

3. ¿Cambia *C. racemosa* al poblamiento de anfípodo de fondos blandos?

El crecimiento peculiar de *C. racemosa*, con estolones y rizoides que se extienden rápidamente en todas direcciones, hace que pueda crecer sobre sustratos blandos arenosos compitiendo por el espacio con otros macrófitos. Esta interacción puede desencadenar efectos sobre otros hábitats y en consecuencia modificar el poblamiento de anfípodos, como se observó previamente en fondos duros. Para estudiar estos posibles cambios, se muestrearon en verano y en invierno 5 hábitats diferentes localizados entre 2-5 m de profundidad: *C. racemosa*, *Caulerpa prolifera*, *Cymodocea nodosa*, *Posidonia oceanica* y sustratos arenosos desprovistos de vegetación. Igualmente, se llevó a cabo la caracterización de la estructura de hábitat en términos de biomasa algal, riqueza específica y cantidad de detritus, así como la identificación a nivel de especie del poblamiento de anfípodos para cada tipo de hábitat.

Los resultados mostraron que los hábitats naturales más complejos, como las praderas de *P. oceanica*, presentaron las mayores abundancias y riqueza específica de anfípodos. Sin embargo, el nuevo hábitat generado por *C. racemosa* presentaba un poblamiento de anfípodos diverso y abundante en comparación con otros hábitats naturales como *C. nodosa* y *C. prolifera*. En general, la estructura de la comunidad de anfípodos fue diferente en función de la vegetación existente. En cuanto al poblamiento de anfípodos, un total de 63 especies fueron identificadas. Cabe destacar que algunas especies presentaron diferentes abundancias en

función del hábitat, como por ejemplo *Microdeutopus obtusatus* se vio favorecido por *C. racemosa*. Por el contrario la especie *Ampelisca diadema* se mostró más asociada a sustratos colonizados por *C. prolifera*, mientras que *Hyale schmidti* fue más abundante en las praderas de *P. oceanica*. La invasión del hábitat por *C. racemosa* puede ejercer una influencia importante en las comunidades bióticas asociadas a fondos blandos, modificando tanto la estructura del hábitat como la fauna de dicho ecosistema.

4. ¿Afecta *C. racemosa* a la colonización de los hábitats con vegetación por parte de los anfípodos?

Los anteriores capítulos han demostrado que *C. racemosa* cambia completamente la estructura del hábitat, ya que establece una pradera monoespecífica y homogénea en la que se acumula detritus, y además provoca cambios en el poblamiento de anfípodos. Sin embargo, dichos estudios se centraban principalmente en la fase post-invasión, por lo que se hace necesario estudiar cómo *C. racemosa* afecta a la colonización de hábitats vegetados por parte de los anfípodos. Para ello se llevó a cabo un experimento en fondos rocosos someros en dos localidades del Mediterráneo (Malta y España). Tras la caracterización del hábitat existente en cada localidad, y la caracterización del poblamiento de anfípodos, se colocaron una serie de bolsas de malla conteniendo 3 tipos de hábitats diferentes, además de bolsas de malla vacía como control del experimento: *Halopteris scoparia*, *C. racemosa* y sustrato artificial (rafía de plástico). Adicionalmente, se consideraron tres niveles de detritus para cada uno de los hábitats anteriores: sin detritus, con poco detritus y con alta cantidad de detritus. Las bolsas de malla fueron colocadas sobre sustrato rocoso a una profundidad de 4.5 - 5 m durante un período de 14 días, tras el cual se recolectaron con el fin de estudiar la comunidad de anfípodos asociada. Los resultados mostraron que: (i) la riqueza de especies de anfípodos y la abundancia fueron mayores en las bolsas que contenían *H. scoparia*, y (ii) la presencia de detritus en *C. racemosa* se relacionó con un

aumento de los valores de la riqueza específica y abundancia de los anfípodos asociados, sin embargo, la presencia de detritus en *H. scoparia* parece no ser un factor tan importante para la comunidad de anfípodos. En cambio, el hábitat formado por *H. scoparia* presentó los mayores valores de riqueza específica y abundancia de anfípodos, probablemente debido a su mayor complejidad estructural. Por último, el sustrato artificial albergó a algunas especies de anfípodos, pero no resultó ser tan atractivo como los hábitats vegetados. Algunos anfípodos, por tanto, exhiben un amplio rango de distribución y no parecen verse afectados por la propagación de *C. racemosa*, pero cabe destacar que el detritus acumulado por *C. racemosa* es un factor determinante para la selección de este hábitat para algunos anfípodos.

5. ¿Modifica *C. racemosa* los hábitos alimenticios de los anfípodos?

Algunas especies de anfípodos, como *Apocorophium acutum*, *Ampithoe ramondi*, *Elasmopus brasiliensis* y *Microdeutopus obtusatus* entre otros, están presentes en hábitats tanto autóctonos como invadidos, pero en cambio, nada se sabe sobre las adaptaciones en el uso de los recursos tróficos proporcionados por el nuevo hábitat. Se estudiaron los hábitos alimenticios de 14 especies de anfípodos mediante el análisis de los contenidos estomacales en función del tipo de hábitat donde fueron encontrados. Diez de las especies objeto de estudio estaban presentes en ambos hábitats (algas autóctonas e invadidos por *C. racemosa*), mientras que dos especies se encontraban sólo en los hábitats de algas nativas, y las otras dos especies sólo en hábitats de *C. racemosa*. Tras el análisis de los contenidos estomacales, se identificaron un total de 11 tipos diferentes de elementos: detritus, detritus vegetal, algas, tejido animal, oligoquetos, poliquetos, foraminíferos, crustáceos, sipuncúlidos, diatomeas y elementos no identificados. La expansión de *C. racemosa* en la comunidad de algas nativas produce cambios en los hábitos alimenticios de los anfípodos herbívoros, ya que su alimento preferido (algas epifitas) no está disponible en el nuevo hábitat

formado por *C. racemosa*, probablemente debido a que la presencia de caulerpinas en *C. racemosa* retarda el crecimiento de algas epífitas. Sin embargo, otras especies no se vieron afectadas o incluso se vieron ligeramente beneficiadas por la invasión, como es el caso de las especies detritívoras cuya principal hábitat y fuente de alimento sigue estando disponible. En general, se detectaron ligeros cambios en el trofodinamismo de los poblamientos de anfípodos, pero no parecen ser muy relevantes en una etapa inicial. En este sentido, se deben analizar los efectos indirectos que pueden tener estos cambios alimenticios en el metabolismo y ciclo vital de especies de interés.

6. ¿Afecta *C. racemosa* a la disponibilidad de anfípodos para los depredadores?

Tras estudiar los cambios que *C. racemosa* produce en la estructura del hábitat y en el poblamiento de anfípodos asociado a dichos hábitats, tanto en la colonización del hábitat como en los hábitos alimenticios, cabe estudiar si altera indirectamente la eficiencia de los depredadores alterando la disponibilidad de presas para estos niveles tróficos superiores. Para probar los efectos de los cambios en la estructura del hábitat como resultado de la colonización por *C. racemosa* en la disponibilidad de presas para los depredadores, se evaluó en primer lugar la importancia de dos grandes subordenes de anfípodos, gamáridos y caprélidos, como recurso trófico en el hábitat naturales e invadidos por *C. racemosa*. Posteriormente se llevaron a cabo experimentos para evaluar los efectos del alga invasora sobre la depredación de *Thalassoma pavo* sobre dos especies de anfípodos tipo: *Elasmopus brasiliensis* (Gammaridea) y *Caprella dilatata* (Caprellidea). Los experimentos se llevaron a cabo en diferentes acuarios con cinco hábitats de vegetación tipo: *Halopteris scoparia*, *Jania rubens*, *C. racemosa* sin detritus, *C. racemosa* con detritus, *Cymodocea nodosa*; y parcelas control. Primeramente, la vegetación era defaunada e introducida en un acuario junto con 30 individuos de anfípodos de la misma especie. Tras la aclimatación de los

anfípodos, se introducía un individuo de *Thalassoma pavo* (previamente aclimatado y privado de comida durante al menos 24 h) durante 1 hora, tras la cual se examinaba su contenido estomacal. Los resultados mostraron que el consumo de caprélidos fue mayor que el de gamáridos para todos los hábitats estudiados, que podría deberse al diferente uso de microhábitat por parte de estos dos grandes grupos de anfípodos, pudiendo afectar a la susceptibilidad de ser depredados. No obstante, el consumo de anfípodos también varió según el tipo de hábitat. La mayor tasa de depredación fue encontrada en el hábitat de *C. nodosa* y la menor en los hábitats de *C. racemosa* con y sin detritus. La tasa de depredación mostró un patrón similar tanto para caprélidos como gamáridos en todos los hábitats, lo que refuerza la importancia del hábitat en la depredación de anfípodos. Por tanto, se ha demostrado que las especies invasoras como *C. racemosa* pueden reducir la disponibilidad del recurso trófico de los depredadores demersales como *T. pavo*. Estos cambios en la interacción depredador-presa podrían tener consecuencias en las redes tróficas de los ecosistemas costeros del Mediterráneo.

7. Discusión

Muchas de las especies invasoras ejercen fuertes impactos sobre las comunidades y los ecosistemas invadidos (Vila 2009) y transforman las características del ecosistema (Richardson et al. 2000), lo que inevitablemente conduce a cambios en las comunidades biológicas. Si una especie introducida puede en sí misma alterar los procesos a nivel del ecosistema, tales como la productividad primaria o secundaria, la hidrología, el ciclo de nutrientes, el desarrollo del sustrato, o la frecuencia de perturbación, entonces esta claro que las propiedades de las distintas especies invasoras pueden controlar el funcionamiento del conjunto del ecosistema (Vitousek 1986).

En esta tesis se ha demostrado que la presencia y la abundancia de *C. racemosa* ejerce un marcado efecto en la estructura de la comunidad de macroalgas de fondos someros rocosos del Mediterráneo, afectando a la composición de especies y a

la biomasa de la vegetación, y aumentando a su vez la concentración de detritus. Con respecto a estos cambios en la vegetación resultantes de la presencia de *C. racemosa*, se detectó una importante reducción en la riqueza de especies durante el período más cálido, debido a la dominancia del alga invasora. En cambio, durante el período más frío, coincidiendo con el ciclo vegetativo más bajo de *C. racemosa*, la riqueza específica entre hábitats invadidos y no invadidos fue similar. Sin embargo, la biomasa algal en sitios invadidos fue inferior. Esto sugiere que cuando *C. racemosa* está aparentemente ausente, la comunidad algal se caracteriza por presentar valores altos de riqueza específica pero menos en términos de biomasa, característico de una etapa temprana de madurez sucesional del ecosistema (Klein y Verlaque 2009a). Acorde con otros estudios, las algas autóctonas pueden alterar la estructura de las comunidades nativas incluso cuando éstas se encuentran en fase de letargo (Bulleri et al 2010). Por tanto, los efectos negativos de *C. racemosa* se detectan durante todo el año, incluso durante el período invernal cuando supuestamente desaparece esta alga (Klein y Verlaque 2009b). Además, uno de los impactos remarcables de *C. racemosa* es la acumulación de detritus, la cual persiste a lo largo de todo el año, a pesar de la importante variabilidad estacional de biomasa de *C. racemosa*. El entramado generado por los estolones de esta especie favorece la retención de detritus, mientras que la estructura multicapa formada por los talos atrapa el sedimento. Esta acumulación podría alterar el ciclo biogeoquímico, modificando la disponibilidad de alimento y nutrientes y afectando a la calidad de los recursos en el ecosistema (Crooks 2002). En las poblaciones de anfípodos estudiadas se ha observado que la expansión de *C. racemosa* genera cambios en la complejidad, heterogeneidad y calidad del hábitat. Los poblamientos de anfípodos asociados a *C. racemosa* difieren de aquellas asociadas a las algas autóctonas. Diversos autores han demostrado que la modificación de la complejidad del hábitat afecta a las poblaciones de crustáceos (Stoner, 1980, Virnstein 1987, Sánchez-Jerez et al. 1999). Bellan-Santini et al.

(1995) encontró unos valores de abundancia y de riqueza específica de fauna asociada al alga invasora *C. taxifolia* muy bajos, posiblemente debido a la baja complejidad del nuevo hábitat creado por esta alga. No obstante, otros estudios encuentran mayores abundancias y riqueza específica de la macrofauna en el alga invasora *Undaria pinmatifida* comparado con las algas autóctonas (Irigoyen et al. 2010). Dichos autores sugieren que podría ser debido a causa de la mayor complejidad de hábitat creada por dicha alga invasora, la cual ofrece diferentes oportunidades de refugio y alimentación, comparado con los hábitats autóctonos morfológicamente más simples. En el caso de fondos duros de la presente tesis, tanto la riqueza específica como la abundancia de los anfípodos fueron mayores en aquellos hábitats creados por algas autóctonas. Por el contrario, ambos parámetros poblacionales en fondos blandos fueron mayores en *C. racemosa* comparados con los otros, a excepción de las praderas de *P. oceanica*. Estos resultados podrían también ser explicados por la complejidad y heterogeneidad del hábitat. El hábitat creado por *C. racemosa* en fondos rocosos es menos complejo y más homogéneo que el proporcionada por las algas nativas, las cuales ofrecen un hábitat heterogéneo y parcheado compuesto por especies más complejas, y así mismo ofrecen un mayor número de microhábitats formado por epífitos. Por el contrario, en fondos blandos el alga invasora añade complejidad estructural donde no existía previamente.

Aparte de las diferencias en la abundancia y riqueza de especies del poblamiento de anfípodos, se ha observado un gran cambio en cuanto a la composición de dichos poblamientos. En *C. racemosa* se ha detectado un aumento en la abundancia de especies detritívoras y una disminución de las herbívoras. Estos cambios, además de la estructura del hábitat, pueden ser explicados por los diferentes tipos de colonización de las distintas especies para un hábitat dado, y dicha colonización dependerá de las preferencias de hábitat de cada especie, los recursos tróficos disponibles en cada hábitat, así como por la diferente presión por depredación existente en cada hábitat.

De los experimentos de colonización de hábitat realizados en esta tesis se desprende que en general, la mayoría de las especies estaban más asociadas a las algas autóctonas que a la invasora, por lo que la preferencia por un determinado hábitat podría ser un factor determinante en la distribución de la fauna. La disponibilidad de alimento parece ser crucial para estos cambios en las especies detritívoras y herbívoras. Por un lado, el detritus acumulado por *C. racemosa* es un hábitat ideal para los detritívoros, donde el recurso trófico es ilimitado. Por otro lado, las especies herbívoras se encuentran en desventaja ya que su recurso trófico principal, las algas epífitas, no están disponibles en los hábitats creados por *C. racemosa*, y por consiguiente estas especies necesitan adaptarse a nuevos recursos tróficos.

En cuanto a los efectos que *C. racemosa* ejerce en términos de depredación sobre la población de anfípodos, en esta tesis se ha evidenciado que dicha alga invasora reduce la disponibilidad de las presas para determinados peces depredadores demersales como *T. pavo*, pese a las altas abundancias de anfípodos encontradas en *C. racemosa*. Además, la presencia de *C. racemosa* podría afectar al flujo de materia y energía de los invertebrados a mayores niveles tróficos, aspecto propuesto por Crooks (2002) como uno de los posibles efectos que podrían causar las especies invasoras en los ecosistemas. Si *C. racemosa* coloniza grandes extensiones de sustrato, podrían darse fenómenos de “bottom-up” que fueren a los depredadores a utilizar nuevas áreas en busca de recursos tróficos. No obstante, estudios con *Undaria pinmatifida* especulan que en algunos casos las algas invasoras podrían producir un efecto “bottom-up” positivo en las cadenas tróficas locales, debido a un aumento de la abundancia de presas para una amplia variedad de depredadores (Irigoyen et al. 2010), pero se desconoce la disponibilidad de dichos individuos para los depredadores. Por tanto, las algas invasoras pueden producir efectos de “bottom-up” positivos o negativos dependiendo de las características de las comunidades receptoras y de los

efectos indirectos sobre las relaciones tróficas que se generan.

Comprender los impactos de las especies invasoras en los ecosistemas naturales es un componente importante para desarrollar estrategias de gestión adecuadas. Esta tesis ha llenado lagunas importantes en el conocimiento de los efectos de la invasión de *C. racemosa* var *cylindracea* en los ecosistemas receptores del litoral Mediterráneo, pero ha abierto más vías de investigación. Por ejemplo, los efectos que *C. racemosa* está causando en otros importantes taxones de invertebrados bentónicos (poliquetos, moluscos y otros crustáceos) debe ser investigado con el fin de evaluar este tipo de efectos en el conjunto de los invertebrados bentónicos. Los efectos indirectos causados por las invasiones biológicas pueden ser más importantes que los efectos directos (Gilman et al. 2010). Por tanto, las consecuencias que genera indirectamente *C. racemosa* a la comunidad de anfípodos debe ser investigado. A pesar de que hemos encontrado una modificación en la dieta de anfípodos herbívoros que viven en *C. racemosa*, se desconocen las consecuencias para la condición biológica de la fauna autóctona. Son necesarios futuros experimentos para analizar los efectos de esta modificación, como por ejemplo, estudios basados en el crecimiento, la reproducción y la supervivencia de las especies afectadas. En esta tesis se ha demostrado una alta disponibilidad de detritus como alimento para los anfípodos, pero la calidad nutricional de este detritus es desconocida. También se desconoce si la producción secundaria de anfípodos u otros taxones bentónicos se ve modificada por la propagación del alga invasora y en consecuencia sus efectos ecológicos, y se hace necesaria más investigación básica enfocada a los requerimientos tróficos y hábitos alimenticios de los anfípodos. Además, son de especial interés futuros estudios sobre las relaciones tróficas entre diversos taxones de los ecosistemas bentónicos que permitan evaluar los cambios de flujo de materia y energía en la red trófica marina como consecuencia de la llegada especies invasoras.

Conclusiones

1. La propagación de la *Caulerpa racemosa* en hábitats someros del Mediterráneo causa un gran efecto sobre la estructura del hábitat con una marcada estacionalidad, mostrando una biomasa de 3 a 4 veces mayor en el período cálido, y en consecuencia, modifica la composición de los poblamientos de anfípodos en comparación con los hábitats nativos.
2. *C. racemosa* acumula detritus debido al desarrollo de la intrincada red de estolones y rizoides. El detritus que se encuentran en hábitats colonizados por el alga invasora fue mucho mayor que la encontrada en los hábitats formados por vegetación nativa en las mismas condiciones de hidrodinamismo, y esto afecta a la heterogeneidad del hábitat.
3. La abundancia y riqueza de especies de anfípodos en fondos duros fueron menores en los sitios colonizados por *C. racemosa*, pero estas diferencias fueron mayores en el período más frío. Sin embargo, en fondos blandos *C. racemosa* mantiene un poblamiento de anfípodos diverso y abundante en comparación con los hábitats nativos, a excepción de *P. oceanica* en el período más cálido.
4. La expansión de *C. racemosa* afecta de diferente modo en función de los grupos tróficos. Se detectó una mayor abundancia de las especies detritívoras como *Apocorophium acutum*, y una disminución de las especies herbívoras como *Ampithoe ramondi*, *Hyale schmidti* y el caprélido *Caprella hirsuta*.
5. Hemos demostrado experimentalmente que el proceso de colonización de hábitats vegetados puede verse afectado por la presencia de *C. racemosa*. Por ejemplo, algunas especies, como *Elasmopus brasiliensis*, *Gammarella fucicola*, *Lysianassa costae* y *Lysianassa longicornis*, parecen evitar el hábitat proporcionado por el alga invasora, y su abundancia total y riqueza específica fueron mayores en el alga nativas *Halopteris scoparia*.
6. En cualquier caso, las unidades experimentales de *C. racemosa* fueron colonizadas por varias especies con unos valores de abundancia relativamente altos, y la presencia de detritus parece facilitar el proceso de colonización.
7. La expansión de *C. racemosa* en la comunidad de algas autóctonas produce cambios en los hábitos alimenticios de los anfípodos herbívoros *Ampithoe ramondi* y *Dexamine spiniventris*, que dejan de comer tejidos vegetales, posiblemente debido a la falta de una comunidad de epífitos, y por el contrario, aumenta la importancia del detritus acumulado como recurso trófico. Sin embargo, como se podía esperar, los hábitos alimenticios de detritívoros, como *Apocorophium acutum*, no se vieron afectados por la expansión de *C. racemosa*.
8. La explotación trófica de anfípodos asociados a *C. racemosa* por los depredadores, como *T. pavo*, puede verse reducido en comparación con las tasas de depredación en los hábitats autóctonos, siendo muy importante el diferente uso de microhábitats por parte de las presas. Sin embargo, la presencia de detritus en el hábitat de *C. racemosa* no mostró una reducción significativa en las tasas de depredación.
9. La composición del poblamiento de anfípodos asociado a *C. racemosa* se diferencia claramente de otros hábitats naturales. El comportamiento trófico de este grupo en parte se ve modificado, así como su disponibilidad para los depredadores. Por tanto, los anfípodos son un componente clave de los ecosistemas marinos para entender los efectos de esta alga invasora.



General abstract

General abstract

Invasive species are one of the most obvious threats to biodiversity, but there is a dearth of knowledge of the effects of these species on marine ecosystems. *Caulerpa racemosa* var. *cylindracea* is one of the most notable invaders in the Mediterranean Sea, whose invasive ability and negative impacts on macrophyte assemblages are quite known. However, studies on the ecological effects that *C. racemosa* causes on macroinvertebrates associated with natural vegetated habitats, and its consequences to other trophic levels, have not been undertaken. This PhD thesis focused on the ecological effects of *C. racemosa* on amphipod assemblages in shallow-water coastal habitats in the Mediterranean Sea.

To better understand of the effects of *C. racemosa* on native assemblages, the influence that invasive species exert on habitat structure of hard and soft bottom algal assemblages and, consequently, on the assemblage structure of associated amphipods was studied. Effects of *C. racemosa* on habitat structure were demonstrated, as the alga influenced the species composition and biomass of macroalgae, and accumulating detritus. In turn, such changes in habitat features affected the associated amphipod assemblages with different ecological requirements. However, the species richness and abundance of amphipods supported by *C. racemosa* were relatively high. On one hand, the species composition of amphipods on rocky habitats changed completely, where some species such as *Ampithoe ramondi* and *Hyale schmidtii* did not colonize invaded habitats, while others such as *Apocorophium acutum* were favoured by the spread of *C. racemosa*. On the other hand, differences in the assemblage structure among the different habitats on soft bottoms were also found, *Microdeutopus obtusatus* was favoured by *C. racemosa* habitat, *Ampelisca diadema* was associated with *C. prolifera* beds, and *Hyale schmidtii* was more abundant in *P. oceanica* meadows.

On the basis of these results, we hypothesized that colonization of vegetated habitats by amphipods will be modified by the presence of *C. racemosa*, and that such an effect mainly results from accumulation of detritus by the alien alga. Overall, amphipod species richness and abundance were higher in the native alga *H. scoparia*, whose forests have a higher structural complexity than the invasive alga and serve as an important habitat for amphipod assemblages. However, some amphipods exhibit a wide distribution range and are not affected by the spread of *C. racemosa*, but the detritus accumulated by *C. racemosa* is a determining factor for colonization by some amphipod species.

Despite the fact that certain amphipod species coexist in invaded and non-invaded habitats, sharing the space as a resource, nothing is known whether these species are benefiting from the same trophic resources. Through stomach content analyses of amphipods from in both native and invaded seaweed assemblages, it has been demonstrated in this thesis that the expansion of *C. racemosa* to native algal communities changes the feeding habits of herbivorous amphipods, since their preferred food (epiphytic algae) is not available in the new habitat produced by *C. racemosa*, probably due to the presence of caulerpenynes. Nevertheless, other species were not affected or benefited by the invasion such as detritivorous species whose main food source and habitat remain available. Altogether, slight changes in the trophodynamism of amphipod assemblages have been detected, which could have unpredictable consequences for amphipod populations.

Not only does *C. racemosa* affect the habitat structure and trophic resources for some amphipod species, but it also affects higher trophic levels such as demersal fish predators. In this thesis it has been experimentally demonstrated that amphipods were less available to fish predators (*Thalassoma pavo*) where *C. racemosa* was present independently of the presence of detritus. Therefore, alteration of

habitat structure by the introduction of the alien alga may provide a better refuge for amphipods. Moreover, caprellids were more predated than gammarids in all habitat types, due to their different uses of microhabitat.

Habitat invasion by *C. racemosa* can have an important influence on biotic assemblages, modifying both habitat structure and the associated amphipod assemblages, promoting some indirect effects in the energetic budget of populations, which may affect the life history of amphipods. Furthermore, the changes detected in predator-prey interactions could have consequences for

food webs. Understanding the impacts of invasive species on natural ecosystems is an important step in developing management strategies. This thesis has filled substantial gaps in knowledge of the effects of invasive seaweed *C. racemosa* var. *cylindracea* on recipient ecosystems, but has opened more avenues of investigation of major importance. Further studies about fitness of species, other invertebrate benthic taxa and food-web interactions in the ecosystems affected by invasions are necessary for a better understanding of how invasive seaweeds affect the shallow water marine ecosystems of the Mediterranean Sea.



Universitat d'Alacant
Universidad de Alicante



General introduction

Chapter 1

1. General introduction

1.1. Invasive seaweeds in the Mediterranean Sea

The Mediterranean and the Black Seas are ecologically diverse, containing about 6.4% of the total world marine species, even though the surface of the Mediterranean only represents the 0.8% of the total surface of the oceans. An estimate of 12725 species of macroscopic marine organisms has been made for the Mediterranean (11594 metazoans and 1131 macrophytes; Coll et al. 2010).

This high species richness is also accompanied by a high level of endemic species, with an average of 20.2% for metazoans and 22.3% for macrophytes (Coll et al. 2010). Even though the Mediterranean Sea is the most heavily invaded region in the world for macrophytes (Williams and Smith 2007, Fig. 1.1), with over 125 introduced macrophytes species, 21 of them being invasive or potentially invasive (Verlaque *pers. com.*).

An **introduced species** is a species that: (i) colonizes a new area where it was not previously present, (ii) the extension of its range is linked, directly or indirectly, to human activity, (iii) there is a geographical

discontinuity between its native area and the new area, and (iv) new generations of the non-native species are propagated in situ without human assistance and constitute self-sustaining populations (Carlton 1985, Ribera and Boudouresque 1995, Williamson and Fitter 1996, Boudouresque 1999, Boudouresque and Verlaque 2002). Additionally, an **invasive species** can be considered as an introduced species that is ecologically and/or economically harmful (Williamson and Fitter 1996, Clout 1998).

The kinetics of an introduction of species has four phases (Fig. 1.2): (i) arrival; (ii) installation (settlement phase). This phase ends either by elimination of the candidate species, or by naturalization (establishment): it can then be considered introduced; (iii) the expansion phase: the species tries to occupy all biotopes and the whole geographical area that are accessible; and (iv) the phase of persistence. This last phase can take two different forms, depending on if there is or not natural decline (Fig. 1.2), during or at the end of expansion phase (Fig. 1.2; Mollison 1986, Boudouresque 1999, Wonham et al. 2000). The change from one phase to another is called transition by Kolar and Lodge (2001).

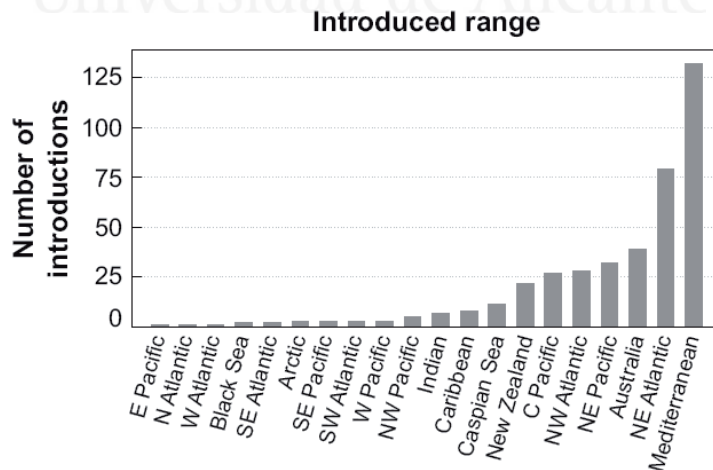


Figure 1.1. Introduced range of invasive seaweeds (from Williams and Smith 2007)

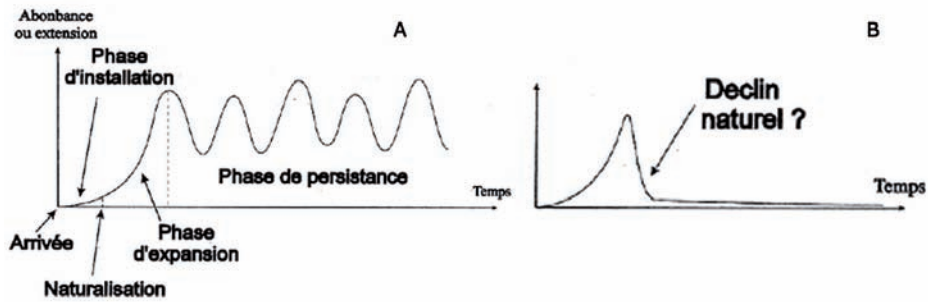


Figure 1.2. The kinetics of an introduction of species without (A) and with (B) natural decline (Boudouresque 1999)

According to available data, since the early twentieth century the number of invasive macrophyte species is considered exponential and nearly doubles every 20 years (Ribera and Boudouresque 1995), with the Indo-Pacific region being the main donor area (Fig. 1.3, Verlaque et al. 2007). Following this tendency, 80 new species are expected to be introduced in the next 20 years. In the Mediterranean Sea, those introductions may result from a variety of causes (vectors), like transportation of species on the hulls of ships and offshore structures (fouling), deballasting of waters and solid matters transported by ships, opening of the Suez Canal, importations of aquaculture species from native regions, transfers between basins, accidental escapes

from aquaria, releases of species used as packing material for fishing baits or epiphytic species that have arrived on their exotic host (Verlaque et al. 2007). Aquaculture is the main vector of introduction of macrophytes followed by ship traffic (vessels) and the Suez Canal (Fig. 1.4, Galil 2009). Once introduced, an exotic species can spread by one or several of the previous vectors (Verlaque et al. 2007). Among seaweeds, certain families contain more invaders than expected by chance as is the case of the family Caulerpacea (Williams and Smith 2007). In the Mediterranean Sea, two of the most invasive seaweeds belong to this family and specifically to the genus *Caulerpa*: *C. taxifolia* and *C. racemosa*.

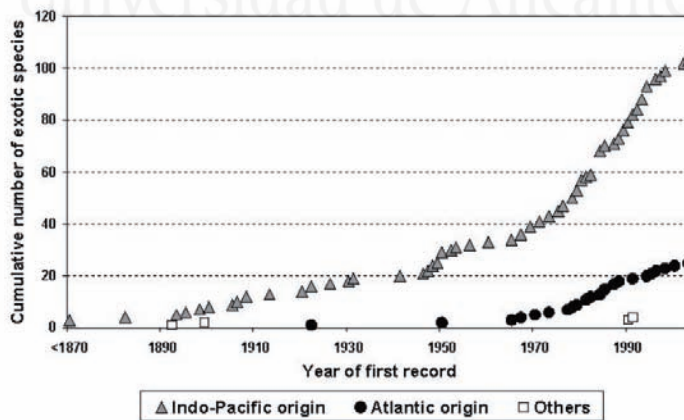


Figure 1.3. Arrivals of exotic macrophytes species in the Mediterranean and the main donor areas (from Verlaque et al. 2007)

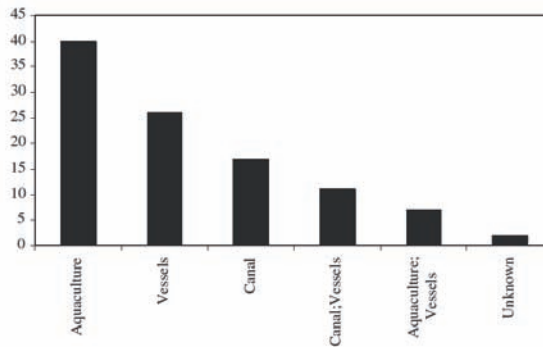


Figure 1.4. Number of alien macrophytes presented by their means of introduction (from Galil 2009)

1.2. The invasive alga *Caulerpa racemosa*

The family Caulerpacea belongs to the phylum Chlorophyta and is in the order Bryopsidales. This family contains 87 species grouped in two: *Caulerpa* with 86 species and *Caulerpella* with 1 species (Guiry and Guiry 2007). *Caulerpa racemosa* (Forsskål) J. Agardh is a species widely distributed in tropical to warm-temperate regions. Verlaque et al. (2000) suggested that three distinct taxa of *C. racemosa* were co-occurring in the Mediterranean Sea:

- An intermediate between *C. racemosa* var. *turbinata* (J. Agardh) Eubank and *uvifera* (C. Agardh) J. Agardh, known since 1926.
- *C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse f. *requienii* (Montagne) Weber-van Bosse, known since 1950s.
- Invasive variety closed to *C. racemosa* var. *occidentalis* (J. Agardh) Børgesen, known since 1990s.

Verlaque et al. (2003) identified this invasive variety as *Caulerpa cylindracea* Sonder, endemic to south-west Australia, and currently known as *C. racemosa* var. *laetevirens* f. *cylindracea* (Sonder) Weber-van Bosse. These authors, after morphological and genetic studies, classified this invasive variety as *C. racemosa* var. *cylindracea* (Sonder)

Verlaque, Huisman and Boudouresque (hereafter *C. racemosa*). The invasive *C. racemosa* is characterized by having a slender thallus fixed to the substratum by means of thin rhizoids having a length of 2-20 mm and a diameter of 0.3-0.5 that are closely arranged along the stolon. The stolon is 0.7-2.0 mm in diameter and bears simple or occasionally branched upright axes up to 19 cm and 3-10 mm across. The basal part of upright axes is slightly inflated immediately above the attachment to the stolon. Upright axes bear uncrowded vesiculate branchlets that are radially or distichously arranged (sometimes both on the same thallus) on a cylindrical rachis. The branchlets are clavate (1.5-) 2-5 (-7) mm long and 1-3 mm in greatest diameter shortly below the rounded apices, and upwardly directed (Verlaque et al. 2003).

In just 19 years, *C. racemosa* spread through the whole Mediterranean. It currently appears in 12 countries and most of the islands of the Mediterranean Sea, and also in the Canary Islands in the Atlantic Ocean. In Figure 1.5 it can be seen that most observations of *C. racemosa* in the Mediterranean Sea have been done in the northern part, on the European shores. This may be due to a higher research and sampling effort applied in these countries, but very probably *C. racemosa* is also present in the countries of the southern Mediterranean coast. Figure 1.5 it must be added the recent detection of *C. racemosa* in Ceuta (Rivera-Ingraham et al. 2010).

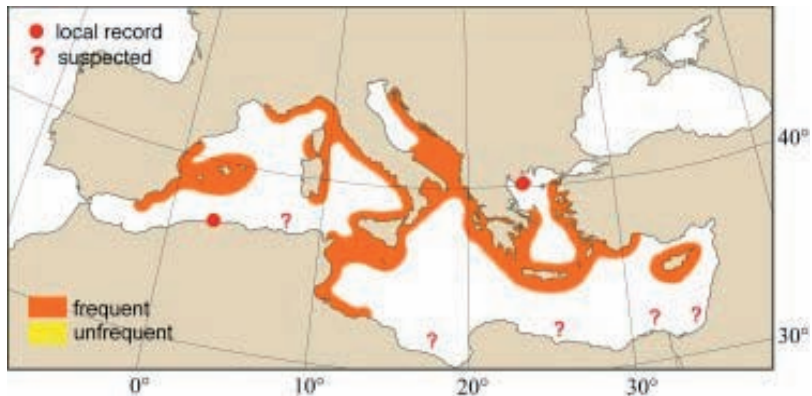


Figure 1.5. Distribution of *C. racemosa* var. *cylindracea* in the Mediterranean Sea. www.ciesm.org/atlas/macrophytes.html

There are many features that confer a high invasive capacity to this alga, one of which is its capability to complete its life cycle in the Mediterranean, being able to reproduce both sexually and asexually by stolons (Panayotidis and Žuljević 2000). It is also able to grow on any type of substrate and other benthic organisms (Piazzi et al. 1997). Another feature that contributes to this invasive capacity and characterizes all *Caulerpa* species is the production of chemicals called caulerpenynes, which are the most abundant secondary metabolites and cytotoxic substances produced by these algae. The levels of caulerpenynes found in *C. racemosa* and *C. taxifolia* are much higher than those found in other species of the genus (Guerriero et al. 1994). This substance acts as a chemical defense against several organisms; on one hand it avoids that the alga is preyed on by grazing animals and presents inhibitory effects on the growth and fouling (Lemée et al. 1993, 1997) thus decreasing the number of macrophytes that grow around, completely suppressing the presence of epiphytes on their leaves, and consequently avoiding interspecific competition.

It should be noted that there are pronounced variations in the seasonal dynamics of *C. racemosa*. Being a tropical seaweed, its distribution and annual cycle are greatly affected by changes in water temperature.

These variations are reflected in the biomass of algae, and the length and growth of the stolons. A quite pronounced regression in winter has been detected in several Mediterranean localities (Piazzi et al. 2001a, Ruitton et al. 2005, Capiomont et al. 2005, Mezgui et al. 2007), almost reaching an apparent disappearance. However, in southern Italy, this seasonal decline has not been detected (Giaccone and Di Martino 1995). According to this pattern, the maximum length of stolons reaches the highest volumes between September and November (Piazzi and Cinelli 1999). Similarly, the growth of stolons peaked in August (Piazzi and Cinelli 1999). However, a variation in summer has been also detected due to temperature fluctuations by strong north-wind episodes (Ruitton et al. 2005). Stolon growth was almost double in Livorno (1.26 cm/day) than in Marseille (0.75 cm/day). The highest number of fronds and stolon length per m² of *C. racemosa* has been observed in Croatia (Žuljević et al. 2003). In terms of biomass found for *C. racemosa*, the highest values reached up to 237.5 g dw m⁻² on rock and up to 447 g dw m⁻² on dead *Posidonia oceanica* in October (Piazzi et al. 2001b). Using the data from the studies cited above we can represent the annual cycle of *C. racemosa* in terms of biomass, length and growth of stolon (Fig. 1.6).

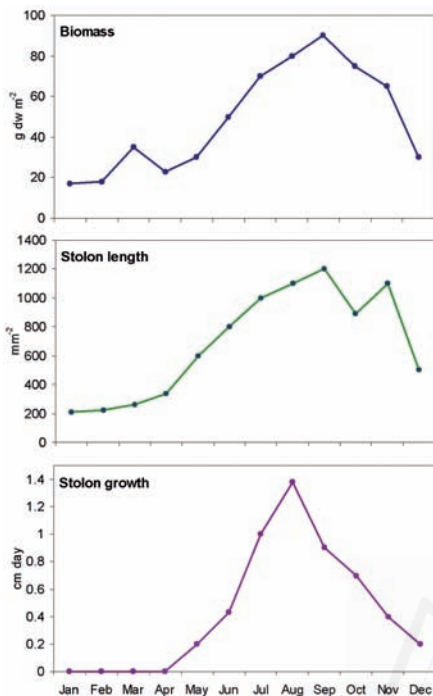


Figure 1.6. Seasonal dynamics of *C. racemosa* in terms of biomass (g dw m⁻²), stolon length (mm⁻²) and growth of stolons (cm per day). Modified from Piazzini and Cinelli 1999; Ruitton et al. 2005 and Capiomont et al. 2005.

1.3. Effects of *C. racemosa* on the Mediterranean Sea fauna

Numerous studies have dealt with the introduction of *C. racemosa*, investigating its taxonomy, biology and ecology, distribution and spread, seasonality and dynamics, and impacts on macrophyte assemblages; which are well described in the review carried out by Klein and Verlaque (2008). However, few reliable studies have been undertaken to quantify the impact of *C. racemosa* on the fauna inhabiting the vegetated substrates. The first study dealing with the interaction between *C. racemosa* and the associated fauna in the Mediterranean Sea was carried out by Argyrou et al. (1999). They detected changes in benthic invertebrates; this was reflected by an increase in the percentage of polychaete assemblages in presence of *C.*

racemosa while crustacean assemblages decreased. Carriglio et al. (2003) observed an increase in densities, diversity and evenness of meiofauna assemblages invaded by *C. racemosa* compared to controls, resulting in increased percentages of crustaceans and annelids. However, no effect of the presence of *C. racemosa* on zoobenthic assemblages of the rocky infralittoral zone was detected by Casu et al. (2005).

Regarding the polychaeta assemblages, a higher species richness has been found in *C. racemosa* invaded areas (Cantone 1999, Box et al. 2010) suggesting positive effects on them (Box et al. 2010). Other studies have demonstrated that the decapod community of invasive *Caulerpa* species, *C. racemosa* and *C. taxifolia*, and *Posidonia oceanica* seagrass are similar in species composition. In addition, several species commonly found in *P. oceanica* meadows increase their abundances in the presence of invasive *Caulerpa* (Box 2008). Three studies on the malacofauna associated with *C. racemosa* have produced contrasting results. While a low species richness (14 species) was observed by Pandolfo and Chemello (1995), later studies (Buia et al. 2001, Box 2008) found higher species richness (42 and 37 species respectively), which fluctuated according to the seasonal biomass cycle of *C. racemosa*. Furthermore, several herbivorous molluscs have been encountered on *C. racemosa* (Gianguzza et al. 2001, 2002, Yokes and Rudman 2004, Cavas and Yurdakoc 2005, Djellouli et al. 2006), despite the secondary metabolites produced by the invasive algae. Moreover, two sea urchins, *Paracentrotus lividus* and *Sphaerechinus granularis*, consume *C. racemosa* (Ruitton et al. 2006, Cebrian et al. 2010, Tomas et al. 2010), and some other zoobenthic taxa significantly feed on detritus accumulated by *C. racemosa* (Casu et al. 2009). Furthermore, *C. racemosa* has frequently been observed creeping on various kinds of macrobenthic animals such as sponges, gorgonian, corals and sea anemones (Žuljevič et al. 2004, Tsirika et al. 2006, Kruzic et al. 2008).

Concerning the interactions with fish assemblages, some studies have detected no

significant differences in abundance, biomass and species richness of fish assemblages between invaded and non-invaded areas, but suggested that *Symphodus rostratus* and *S. mediterraneus* tend to decrease in abundance and biomass in invaded areas (Martínez-Garrido et al. 2010). Nevertheless, other studies have demonstrated that some fish species, such as *Sarpa salpa* and *Spondyliosoma cantharus*, consume *C. racemosa* (Box et al. 2009, Tomas et al. 2010, Tomas et al. 2011); although the first one suggested that this ingestion might be unintentional. Some other fish species have also been observed grazing on *C. racemosa* stands, such as *Boops boops*, *Pagellus acarne*, *Sarpa salpa* and *Siganus luridus* (Nizamuddin 1991, Lundberg et al. 1999, Azzurro et al. 2004, Ruitton et al. 2006).

1.4. Amphipods as important component of the Mediterranean coastal ecosystem

One of the most ubiquitous and abundant invertebrate groups in marine vegetated habitats are the amphipods, whose densities often reach several thousands individuals per square meter (Brawley 1992, Tuya et al. 2010). There are around 48 families present in the Mediterranean, most of them free-living species. In the Mediterranean Sea there is a high degree of endemism with respect to benthic amphipods (approximately 37% of species; Ruffo 1998). Benthic amphipods can be found in all the zones present in Mediterranean Sea (adlittoral, supralittoral, midlittoral, infralittoral, circalittoral, bathyal and abyssal), exhibiting the highest diversity in the infralittoral zone where approximately 69% of the species are represented (Ruffo et al. 1998). They populate all types of habitats, being a key faunal component in the seagrass beds and act as important recyclers of organic matter (Sanchez-Jerez et al. 2002). They also are important secondary producers (Carrasco and Arcos 1984, Highsmith and Coyle 1990, Sarvala and Uitto 1991) and exhibit a wide range of feeding strategies -grazers, filter and detritic feeders, predators, scavengers, carnivores, omnivores and phytophages (both macro and microphages)- which are sometimes

used simultaneously, successively in time or according to environmental conditions (Ruffo et al. 1998). Additionally, they serve as a food source for a large variety of marine predators (Stoner 1979, Beare and Moore 1997, Sanchez-Jerez et al. 1999, 2002; Stål et al. 2007), hence playing a key role in energy flow through food webs.

Amphipods are Peracarid crustaceans, which involve having protected larval development in the marsupium of females and a life cycle completely associated with the benthos. Thus, amphipods have been described as species with local population development in line with the environmental conditions of the medium due to their low dispersal power (Virnstein 1987, Sanchez-Jerez and Ramos-Esplá 1996) and significantly responding to habitat modification (Sanchez-Jerez et al. 2000). Some species exhibit high habitat specificity while others tolerate a range of habitat alteration that may result from pollution, invasion by alien species and other disturbances, and can therefore be used as an indicator of environmental impacts on vegetated habitats (Bellan-Santini 1980, Virnstein 1987, Conradi et al. 1997).

1.5. PhD Thesis justification: Why effects of *C. racemosa* on native communities should be studied?

It is widely accepted that global marine biodiversity and its value as producer of valuable resources is threatened by anthropogenic activities. In particular, overfishing, habitat alteration and destruction, global climate change and the introduction of alien marine species has been identified as the main stressors, especially in coastal regions (Norse 1993, Vitousek et al. 1997, Carlton 2000). Specifically, biological invasions are considered a major threat to marine systems (Ribera and Boudouresque 1995). The rate of marine introductions, including introductions of seaweeds, have increased over the last 20 years (Verlaque et al. 2007), nonetheless, the current knowledge of impacts of alien macroalgae is even sparser than for other taxonomic groups of

aliens. This contrasts to the perception that invading macroalgae present serious potential impacts, because they may alter ecosystem structure and function by monopolizing space, developing into ecosystem engineers, and altering foodwebs (Schaffelke and Hewitt 2007).

Despite the fact that *C. racemosa* is comparable to *C. taxifolia* in terms of their capacity to colonize and alter native assemblages, there is a wide disparity in the effort and means used to attempt to cope with these two invasive *Caulerpa* species. The vast majority of studies carried out to assess the impact of *C. racemosa* on the associated fauna have used punctual sampling and results were merely descriptive, which present difficulties reaching specific conclusions and guidelines for the management of such an invasion event. Overall, community-level ecological interactions involving introduced seaweeds constitute a major research gap; and the indirect effects between trophic levels, the mobility of consumers, and restrictions on replications represent current research challenges (Williams and Smith 2007). Klein and Verlaque (2008) particularly indicated the lack of in-depth rigorous studies concerning *C. racemosa*, specifically on its impacts. Moreover, much of the speculation remains to be tested and the number of localities studied is insufficient. More rigorous studies (as far as experimental design is concerned) are needed, in order to properly evaluate the impact of *C. racemosa* in different habitats and regions of the Mediterranean Sea. This is essential for a better understanding of how *C. racemosa* can modify Mediterranean marine ecosystems, as well as the potential extent of this invasion.

1.5.1. Aims and thesis structure

This PhD thesis will focus on the general aim of identifying the ecological effects that *C. racemosa* causes on macroinvertebrates associated with natural vegetated habitats, focusing on amphipods because of their importance as key components of Mediterranean marine ecosystems. To reach this purpose, this work is structured in seven parts, the first corresponding to the

general introduction, followed by five research chapters and ending with a general discussion which, analyzes and compares with other studies all the gathered information in order to better understand the ecological effects of *C. racemosa*.

The following points of this introduction present the issues considered in each of the five research chapters.

1.5.1.1. Does *C. racemosa* modify the amphipod assemblage on hard bottoms?

Since the first records of spread of *C. racemosa* along Mediterranean coastal areas, several studies concerning the ecology of this species have been undertaken (see a review in Klein and Verlaque 2008). However, information on changes on the associated fauna brought about by the invasive alga is lacking (Argyrou et al. 1999; Piazzini and Balata 2008). The main aim of this chapter is to assess the effects of *C. racemosa* on amphipod assemblages associated with a shallow-water rocky bottom habitat in the south-western Mediterranean Sea, by comparing natural rocky algal assemblages invaded and non-invaded by *C. racemosa*. The hypothesis tested in this study was that the invasion of rocky habitats invaded by *C. racemosa* should influence the habitat structure of algal assemblages and, consequently, the assemblage structure of associated epifauna such as amphipods, in terms of species richness and abundance.

1.5.1.2. Does *C. racemosa* change the amphipod assemblage on soft bottoms?

On soft bottoms, *C. racemosa* grows on sandy substrates, either unvegetated areas or covering dead mat of *Posidonia oceanica*, competing with other macrophytes for space. The aims of this chapter were to compare the structure of amphipod assemblages associated with *C. racemosa* stands on soft bottoms with those associated with unvegetated habitat and habitat vegetated by native species (*Caulerpa prolifera*, *Cymodocea nodosa* and *Posidonia oceanica*). The hypothesis tested was that *C. racemosa* stands on soft

bottoms may increase spatial complexity, thus differing from the amphipod assemblages that can be found associated with unvegetated habitats or those having native macroalgae and seagrasses.

1.5.1.3. Does *C. racemosa* affect colonization of vegetated habitats by amphipods?

Studies dealing with the ecology of macrofauna associated with *C. racemosa* have shown that it may completely change the habitat structure since it establishes a homogeneous monospecific stand in which detritus accumulates (Piazzi et al. 2007). However, such studies have mainly focussed on post-invasion assessment, while there is a dearth of data on the situation before invasion by the alien algae compared to that following invasion. We undertook an experiment to test the hypothesis that *C. racemosa* may affect colonization of vegetated habitats by amphipods, and that such effect may result from accumulation of detritus by the alien algae. Therefore, colonization processes among *C. racemosa*, native algae and artificial substrate with different levels of detritus were compared.

1.5.1.4. Does *C. racemosa* modify the feeding habits of amphipods?

From results obtained in previous chapters it is known that some amphipod species coexist in both native and invaded habitats, using the space and maybe the available trophic resources. However, it is still unknown if these species change their diet and adapt to the potential new resources resulted from the presence of *C. racemosa*. The main objective of this chapter was to assess if the feeding habits of amphipods associated with coastal plant communities

are affected by the spread of the invasive *C. racemosa*, through the analysis of the stomach contents of amphipods living in both native and invaded seaweeds assemblages.

1.5.1.5. Does *C. racemosa* affect amphipod prey availability to predators?

In spite of the importance of the spread of *C. racemosa*, data on the potential consequences on the trophic web are unavailable. Therefore, the effect of the presence of this species on predation of invertebrates by fishes was assessed. This is needed for a proper assessment of how introduced algae can affect predation rates of invertebrates by fishes and for a better understanding of how invasive species can alter the flow of matter and energy through ecosystems. Thus, the general aim of the present chapter is to experimentally determine whether invasion of Mediterranean vegetated habitats by *C. racemosa* generates any potential effects on prey availability for predators. This work also considered the importance of detritus accumulation by *C. racemosa*, since this could affect prey availability. Consequently, the predatory labrid fish *Thalassoma pavo* and two amphipod species with different living strategies -one gammarid and one caprellid- were selected. It was hypothesized that (i) the rate of predation of amphipods by *T. pavo* is affected in vegetated habitat invaded by *C. racemosa*, compared to native vegetated habitats, (ii) predation rates in *C. racemosa* are reduced because of the presence of accumulated detritus (compared to uninvasion vegetated habitat, where no detritus is accumulated) and (iii) different use of microhabitats by different prey species affects predation.

Material published or under review**- Chapter 2 and 3:**

Vázquez-Luis M, Sanchez-Jerez P, and Bayle-Sempere JT (2008) Changes in amphipod (Crustacea) assemblages associated with shallow-water algal habitats invaded by *Caulerpa racemosa* var. *cylindracea* in the western Mediterranean Sea. *Marine Environmental Research* 65:416-426

Vázquez-Luis M, Guerra-García JM, Sanchez-Jerez P, and Bayle-Sempere JT (2009) Caprellid assemblages (Crustacea: Amphipoda) in shallow waters invaded by *Caulerpa racemosa* var. *cylindracea* from southeastern Spain. *Helgoland Marine Research* 63:107-117

Vázquez-Luis M, Sanchez-Jerez P, and Bayle-Sempere JT (2009) Comparison between amphipod assemblages associated with *Caulerpa racemosa* var. *cylindracea* and those of other Mediterranean habitats on soft substrate. *Estuarine Coastal and Shelf Science* 84:161-170

- Chapter 4:

Vázquez-Luis M, Borg JA, Sanchez-Jerez P, Bayle-Sempere JT Habitat association by amphipods: a comparison between native and alien algae. *Biological Invasions*, submitted

- Chapter 5:

Vázquez-Luis M, Sanchez-Jerez P, and Bayle-Sempere JT Does the invasion of *Caulerpa racemosa* var. *cylindracea* affect the feeding habits of amphipods (Crustacea: Amphipoda)? *Helgoland Marine Research*, submitted

- Chapter 6:

Vázquez-Luis M, Sanchez-Jerez P, and Bayle-Sempere JT (2010) Effects of *Caulerpa racemosa* var. *cylindracea* on prey availability: an experimental approach to predation by *Thalassoma pavo* (Labridae) on amphipods. *Hydrobiologia* 654:147-154



Does *C. racemosa* modify the amphipod assemblage on hard bottoms?

2. Does *C. racemosa* modify the amphipod assemblage on hard bottoms?

2.1. Abstract

The effects of the invasive species *Caulerpa racemosa* var. *cylindracea* (hereafter *C. racemosa*) on amphipod assemblages associated with shallow-water rocky habitats were studied. Two habitats located along the SE Iberian Peninsula were compared; invaded and non-invaded. The results showed that growth of *C. racemosa* affects habitat structure, influencing the species composition and biomass of macroalgae, and detritus accumulation. In turn, such changes in habitat features affected the associated amphipod assemblages with different ecological requirements. However, the species richness of amphipods was relatively high in both habitats, while the species composition of amphipods changed completely. For example, some species such as *Ampithoe ramondi* and *Hyale schmidtii* did not colonize invaded habitats, while others such as *Apocorophium acutum* were favoured by the spread of *C. racemosa*. Habitat invasion by *C. racemosa* can have an important influence on biotic assemblages, modifying both habitat structure and the associated fauna, with unknown effects on the overall ecosystem.

2.2. Introduction

The green alga *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque (Verlaque et al. 2003) (hereafter *C. racemosa*) was first detected in Tunisia (Mediterranean Sea) in 1926. More recently, during the 1990s, *C. racemosa* spread steadily throughout the Mediterranean region (see Piazzini et al. 2005). Recent genetic analyses have shown that the *C. racemosa* population originated from the southwest coast of Western Australia (Verlaque et al. 2003), and is not a lessepsian migrant as thought originally. A remarkable spread of *C. racemosa* throughout the Mediterranean has been recorded during the last 15 years or so, during which the alga invaded different habitats located at water depths of 0 m to more than 60 m. The recorded rate of invasion has been much faster than that for other invasive species such as *Caulerpa taxifolia* (Verlaque et al. 2003). The rapid spread of *C. racemosa* can be explained by its very efficient reproductive strategies, both sexual and asexual (via propagules), which is not the case with *Caulerpa taxifolia* (Panayotidis and Žuljević 2001, Renoncourt and Meisenz 2002).

Several studies have demonstrated that habitat heterogeneity and complexity play an important role in influencing the assemblage structure of epibenthic marine fauna (Johnson 1970, Stoner 1980, Dean

and Connell 1987, Edgar 1992, Taylor and Cole 1994, Ayala and Martín 2003). The pattern of distribution and species richness and abundance of epifauna on rocky bottoms is affected by changes in the composition of associated macroalgae (Connell 1972). Therefore, changes in habitat structure of vegetated substrates resulting from the invasion of species such as *C. racemosa* could affect the associated fauna, including amphipods.

Amphipods are one of the most important groups of invertebrates associated with vegetated habitats, and they play an important role as trophic resources for fish populations (Sanchez-Jerez et al. 1999, Stål et al. 2007). Amphipods respond to habitat alteration and can therefore be used as an indicator of environmental impacts on vegetated habitats (Bellan-Santini 1980, Virnstein 1987, Conradi et al. 1997, Sanchez-Jerez et al. 2000).

Since the first records of spread of *C. racemosa* along Mediterranean coastal areas, several studies concerning the ecology of this species have been undertaken (see Ruitton et al. 2005, Cavas et al. 2006). However, information on changes on the associated fauna brought about by the invasive alga is lacking (Argyrou et al. 1999, Piazzini and Balata 2008). The main aim of this study was to assess the effects of *C. racemosa* on amphipod assemblages associated with a shallow water rocky bottom habitat in the

southwestern Mediterranean Sea, by comparing natural rocky algal assemblages and the same habitat type that has been invaded by *C. racemosa*. The hypothesis tested in our study was: invasion by *C. racemosa* should influence the habitat structure of rocky bottom algal assemblages and, consequently, the assemblage structure of associated epifauna such as amphipods may be affected, in terms of species richness and abundance.

2.3. Materials and methods

2.3.1. Study area

Fieldwork was carried out along the Cape of Santa Pola (Alicante, south-east Spain; Fig. 2.1) on a shallow rocky platform. In Alicante, *C. racemosa* was first recorded in 2002 at a site located around ten kilometres north of our study area, where it colonised soft sediments and dead mat of *Posidonia oceanica*. Following two months, *C. racemosa* was detected on the rocky platform in our study area (Pena-Martin et al. 2003). The alien alga now occurs in extensive areas of ecologically important rocky bottoms, on sandy and muddy substrata, and on dead mat of *Posidonia oceanica*. It also occurs intermixed with *Cymodocea nodosa* in meadows of the seagrass within a wide depth range (0.2 - 1.5 m) and with patchy distribution.

2.3.2. Sampling and experimental design

Shallow rocky habitat, located within the 0.2 m - 0.5 m depth range, was sampled.

Several sampling sites, separated by hundreds of metres, with *C. racemosa* and without the alien alga, were randomly selected across the study area. All sites presented similar environmental conditions with respect to wave exposure and light (Ferrandis-Ballester and Bartolomé Pina 1985). Three 'invaded' rocky habitat sites and another three 'non-invaded' rocky habitat sites were sampled in September 2004 (average water temperature = 27.5 °C), which coincides with the period of maximum vegetative growth of the alga (Piazzi and Cinelli 1999). Other sites were sampled again in March 2005 (water temperature = 13 °C) which coincides with the period of minimum vegetative growth. At each site, three random replications were taken using a 20×20 cm quadrat by scraping the whole surface using a trowel (Edgar 1990). Samples were separated by tens of meters. A 300 µm mesh bag was attached to the quadrat to avoid loss of the motile fauna. Samples were preserved in 4% solution of formaldehyde in seawater. Each replicate was sieved in sea-water through a 500 µm mesh, obtaining the fine fraction of detritus. In the laboratory, the amphipods were separated, identified, and counted. After that, algae were sorted as well as the bigger fragments of detritus. Afterwards the macrophytes and the detritus were dried for 24 h at 80 °C, and weighed. The macrophytes were identified to the species level. Habitat structure was characterised using three attributes: species richness of macrophytes, biomass of each species (g) and quantity of detritus (g).

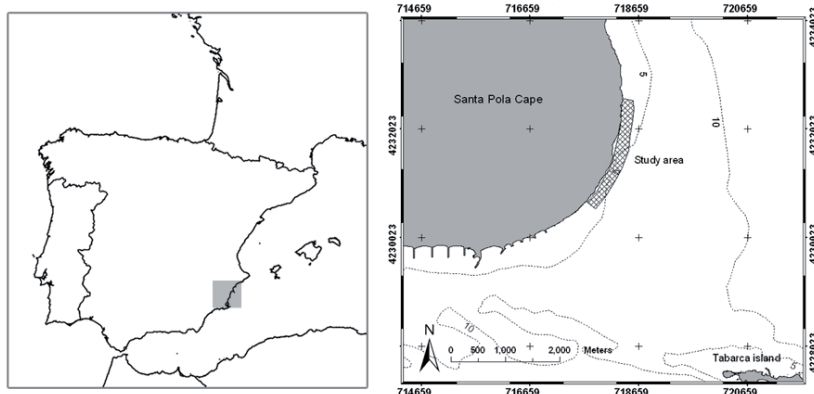


Figure 2.1. Map showing the location of Alicante on the southeastern coast of Spain, and the study area.

2.3.3. Data analysis

Two different statistical approaches were used to identify potential changes in the amphipod assemblages and habitat features, based on the null hypothesis of no change in amphipod assemblage composition between the two habitat types across different sampling times.

2.3.3.1. Multivariate analysis

Non-parametric multidimensional scaling (MDS) was used as the ordination method for exploring changes in amphipod and macroalgal assemblages (Clarke and Warwick 1994). The similarity matrix, which was calculated using the Bray-Curtis index and double square transformation of data, was used to construct bivariate MDS plots. Calculation of percentage similarities (SIMPER) was then applied to calculate the contribution of each species to the dissimilarity between sampling times and habitats. (PRIMER software, Clarke 1993).

2.3.3.2. Univariate analysis

Algal biomass, *C. racemosa* biomass, species richness of seaweeds, biomass of detritus, total abundance of amphipods, species richness of amphipods and abundance of seven amphipod species (the most important by SIMPER) were analysed using ANOVA. To test whether the abundance of amphipods was similar across habitats and times we used an analysis of variance (ANOVA) which incorporated the following factors: 'Time of sampling', a fixed factor and orthogonal, with two treatments: September-04 and March-05; 'habitat', a fixed factor, with two treatments: macroalgal community with *C. racemosa* (invaded habitat) and without *C. racemosa* (non-invaded habitat), and 'Site', a random factor and nested with the both main factors, with three random sampling sites.

Prior to ANOVA, heterogeneity of variance was tested with Cochran's *C*-test. Data were $\sqrt{x+1}$ transformed if variances were significantly different at $p = 0.05$, and $\log(x+1)$ transformed if variance was still heterogeneous. Where variance remained

heterogeneous, untransformed data were analysed, as ANOVA is a robust statistical test and is relatively unaffected by heterogeneity of variances, particularly in balanced experiments (Underwood 1997), however, in such cases special care was taken in the interpretation of results. Furthermore, in such cases, to reduce type I error, the level of significance was reduced to < 0.01 . When ANOVA indicted a significant difference for a given factor, the source of difference was identified by applying the Student-Newman-Keul (SNK) test (Underwood 1981, Underwood 1997).

2.4. Results

2.4.1. Habitat features

A total of 24 taxa of macroalgae and one seagrass species were identified (Table 2.1). Species richness showed significant seasonal differences (Table 2.2; $T_i = P < 0.05$). During September, the invaded habitat showed a reduced number of macroalgal species: only five together with *C. racemosa*, compared with 11 species recorded from the non-invaded habitat. The most important species in terms of biomass in the non-invaded places were *Jania rubens*, *Halopteris scoparia* and *Cystoseira brachicarpa*, while in the invaded habitat *C. racemosa*, *Jania rubens* and *Padina pavonica* were most important (Table 2.1). In March, differences between invaded and non-invaded habitats were lower (14 where *C. racemosa* was present and 16 species where it was absent). The most important species in non-invaded sites during March were *Corallina elongata*, *Jania rubens* and *Dictyota fasciola*; and *C. racemosa*, *Cladophora* sp. and *Halopteris scoparia* dominated at invaded sites (Table 2.1). The MDS two-dimensional representation of macrophyte biomass clearly showed segregation of sampling sites mainly related to sampling time and habitat (Fig. 2.2).

In the case of algal biomass, excluding *C. racemosa*, significant differences were noted for the invaded habitat with respect to the non-invaded habitat (Table 2.2; Fig. 2.3). Total algal biomass recorded in September was similar between the two

different habitats (347.67 ± 117.44 g dw m⁻² for non-invaded habitat compared to 379.55 ± 64.63 g dw m⁻² for invaded habitat; Table 2.1), however, *C. racemosa* contributed a high value (74.6%), and replacing other native species. Non-invaded sites showed the highest mean biomass value, which reached a maximum value of 826.46 ± 203.15 in September (Fig. 2.3). However, total biomass (326.52 ± 79.21 g dw m⁻²) at the invaded sites, without a recovery of the community in spite of the reduction of *C. racemosa* biomass.

Differences in accumulated detritus biomass was significant between habitats ($P < 0.05$, Table 2.2). The highest values were recorded from invaded habitats during both sampling periods (472.48 g dw m⁻² in September and 466.92 g dw m⁻² in March). On the other hand, detritus biomass was relatively low at non-invaded habitats during September (5.87 ± 1.51 g dw m⁻²), and higher in March (242.22 ± 47.25 g dw m⁻²; Fig. 2.3).

Table 2.1. Mean biomass (g dw m⁻²) values \pm SE for the different species, together with total biomass, total species richness and detritus weight for the two sampling periods and habitats.

Species	SEPTEMBER - 04		MARCH - 05	
	Non-invaded habitat	Invaded habitat	Non-invaded habitat	Invaded habitat
Chlorophyta <i>Acetabularia acetabulum</i> (Linnaeus) P.C. Silva	-	-	-	0.08 \pm 0.06
<i>Caulerpa prolifera</i> (Forsskål) J.V. Lamouroux	2.23 \pm 1.02	19.4 \pm 10.57	-	0.01 \pm 0.01
<i>Caulerpa racemosa</i> (Forsskål) J. Agardh	-	259.49 \pm 65.99	-	68.91 \pm 18.71
<i>Cladophora</i> sp. Kützting, 1843	0.30 \pm 0.30	-	54.38 \pm 14.60	98.19 \pm 28.9
<i>Codium</i> (cf) F.S. Collins & Hervey, 1917	-	-	17.43 \pm 16.87	-
<i>Dasycladus vermicularis</i> (Scopoli) Krasser	4.27 \pm 1.55	0.78 \pm 0.36	-	0.99 \pm 0.63
<i>Flabellia petiolata</i> (Turra) Nizamuddin	-	-	0.13 \pm 0.11	-
<i>Halimeda tuna</i> (J. Ellis & Solander) J.V. Lamouroux	0.09 \pm 0.09	-	0.64 \pm 0.64	-
<i>Ulva</i> sp. Linnaeus	-	-	5.46 \pm 1.64	15.15 \pm 5.78
Phaeophyta <i>Cladostephus spongiosus</i> (Hudson) C. Agardh	-	-	0.15 \pm 0.15	-
<i>Colpomenia sinuosa</i> (Mertens ex Roth) Derbès & Solier	-	-	6.97 \pm 4.99	3.94 \pm 3.93
<i>Cystoseira brachicarpa</i> J. Agardh	34.75 \pm 23.07	-	-	-
<i>Cystoseira compressa</i> (Esper) Gerloff & Nizamuddin	-	-	63.92 \pm 63.92	0.71 \pm 0.71
<i>Dictyota fasciola</i> (Roth) J. V. Lamouroux	1.51 \pm 0.97	-	134.78 \pm 53.99	20.72 \pm 17.44
<i>Halopteris scoparia</i> (Linnaeus) Sauvageau	35.81 \pm 34.45	-	104.82 \pm 57.53	68.04 \pm 48.43
<i>Padina pavonica</i> (Linnaeus) Thivy	20.31 \pm 6.86	26.87 \pm 14.99	44.14 \pm 34.64	37.16 \pm 19.51
<i>Sargassum vulgare</i> C. Agardh	2.13 \pm 2.13	-	32.83 \pm 32.56	-
Rodophyta <i>Ceramium</i> spp. (Hudson) C.A. Agardh	0.11 \pm 0.11	-	10.72 \pm 8.78	-
<i>Corallina elongata</i> Ellis et Solander	-	-	186.77 \pm 175.87	0.59 \pm 0.59
<i>Halopitys incurvus</i> (Hudson) Batters	-	5.13 \pm 4.19	-	-
<i>Jania rubens</i> (Linnaeus) J.V. Lamouroux	246.16 \pm 83.17	67.87 \pm 29.74	163.12 \pm 113.76	-
<i>Laurencia pinnatifida</i> (Hudson) Lamouroux	-	-	1.24 \pm 1.24	-
<i>Peyssonnelia</i> sp. (S.G. Gmelin) Decaisne	-	-	-	9.88 \pm 9.88
<i>Asidium corallinum</i> (cf) C. Agardh	-	-	-	0.29 \pm 0.29
Seagrasses <i>Cymodocea nodosa</i> (Ucria) Ascherson	-	-	-	1.81 \pm 1.81
Total biomass	347.67 \pm 117.44	379.55 \pm 64.63	826.49 \pm 203.15	326.52 \pm 79.21
Species richness	11	6	16	15
Detritus	5.87 \pm 1.51	472.48 \pm 123.05	242.22 \pm 47.25	466.92 \pm 68.91

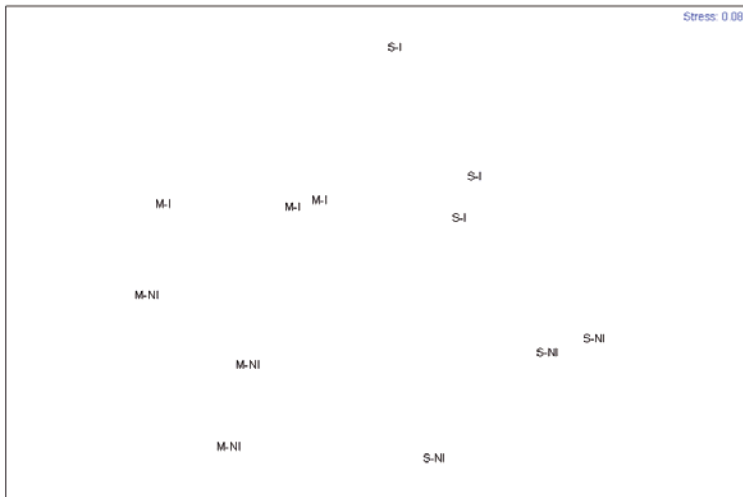


Figure 2.2. Two-dimensional MDS plot for algal biomass; S: September-04; M: March-05; I: Invaded habitat; NI: Non-invaded habitat, for each of the six sampling sites.

Table 2.2. Result of ANOVA (three-factor) for the different habitat attributes: macrophyte species richness, macrophyte biomass, macrophyte biomass without *C. racemosa*, and detritus. MS = mean square; *P* = level of probability; df = degrees of freedom; Si= site; ns = non-significant; * significant at $p < 0.05$

Source of variation	df	Species richness		Macrophyte biomass		Macrophyte biomass without <i>C. racemosa</i>		Detritus		<i>F</i> versus
		MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	
Sampling time=Ti	1	53.78	0.0158*	6.59	0.2154	6.05	0.1066	191.75	0.3812	Si(TixHa)
Habitat=Ha	1	2.78	0.5077	5.88	0.2395	10.04	0.0472*	1720.49	0.0241*	Si(TixHa)
TixHa	1	11.11	0.2029	12.34	0.1030	0.27	0.7110	210.67	0.3599	Si(TixHa)
Si (TixHa)	8	5.78	0.0043*	3.64	0.0821	1.83	0.0002*	223.34	0.0001*	Res
Residual	24	1.47		1.77		0.29		31.75		
Cochran's C-test		C=0.3585 ns		C=0.3917 ns		C=0.2616 ns		C=0.3368 ns		
Transformation		None		Sqrt(X+1)		Ln(X+1)		None		

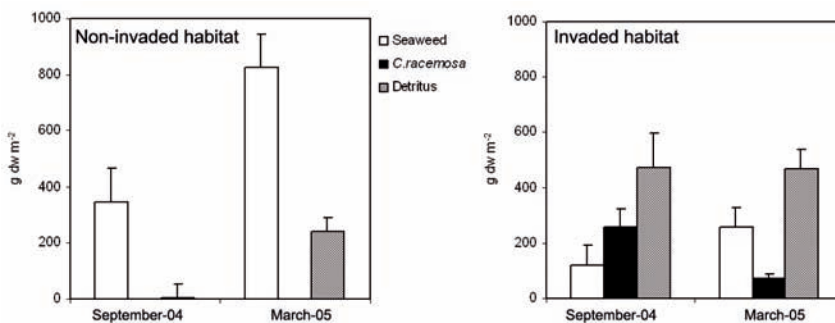


Figure 2.3. Mean seaweed biomass (\pm SE) recorded from the two different sampling periods and habitats. Seaweed = biomass of seaweeds (without *C. racemosa*); *C. racemosa* = biomass of *C. racemosa*; and Detritus = biomass of detritus.

2.4.2. Amphipod assemblage

A total of 33 amphipod taxa, belonging to 13 families, were recorded (Table 2.3). Species richness was significantly different (Ti, $P < 0.05$, Table 2.4; Fig. 2.4a) among sampling times but similar between habitats. In September, species richness recorded from the habitat dominated by *C. racemosa* was similar to that recorded from the non-invaded habitat (16 and 14 species respectively; Table 2.3), while for both habitats, values were higher in March (20 and 24 species, Table 2.3). Only ten species were present in both habitats and in both months: *Amphilochus neapolitanus*, *Ampithoe ramondi*, *Microdeutopus* spp., *Caprella hirsuta*, *Apocorophium acutum*, *Elasmopus* spp., *Lysianassa costae*, *Pereionotus testudo*, *Stenothoe monoculodes* and *Hyale schmidti*. Seven species were found only in the non-invaded habitats: *Amphilochus* sp., *Ampelisca serraticaudata*, *Ampithoe ferox*, *Lembos* sp., *Leptocheirus guttatus*, *Lysianassa* sp. and *Stenothoe tergestina*; while there seemed to be a relationship between the invaded habitat and the following six amphipods: *Ampelisca diadema*, *Corophium* spp. *Corophium insidiosum*, *Gammarella fucicola*, *Melita palmata* and *Orchomene humilis*. Total abundance was similar between habitats in September, but there were significant differences between the two habitats in March, with a maximum value of 6497.22 ind m⁻² recorded from the non-invaded habitat (Ti x Ha $P < 0.01$, Table 2.4; Fig. 2.4b).

The two-dimensional MDS plot for the amphipod assemblages showed segregation of sampling sites that was mainly related to the sampling period, while moderate segregation was noted by habitat type during March (Fig. 2.5). The main species responsible for dissimilarities in amphipod assemblages were *Caprella hirsuta*, *Ampithoe ramondi*, *Microdeutopus* spp., *Elasmopus* spp., *Apocorophium acutum*, *Hyale schmidti* and *Lysianassa costae*, for which an average dissimilarity of 64.73 % was noted between habitats. Together, these species comprised 90 % of the total amphipod abundance.

The seven most important amphipod species showed different patterns of

abundance values with respect to macroalgal assemblage. Abundance of *Ampithoe ramondi* and *Hyale schmidti* were significantly higher in non-invaded habitats (Ha, $P < 0.05$, Table 2.4; Fig. 2.6a, b). On the other hand, *Apocorophium acutum* had a significantly higher abundance in the invaded habitat (Ha, $P < 0.05$, Table 2.4; Fig. 2.6c). Significant differences were recorded for *Microdeutopus* spp. in terms of sampling times (Ti, $P < 0.05$, Table 2.4; Fig. 2.6d), while it was more abundant in September at invaded habitats but without significant differences.

With regard to differences between sampling times (Table 2.3), several species appeared only in September (*Amphilochus* sp., *Melita palmata* and *Orchomene humilis*); or in March (*Ampelisca diadema*, *Ampelisca serraticaudata*, *Lembos* sp., *Leptocheirus guttatus*, *Corophium* spp., *Corophium insidiosum*, *Dexamine spiniventris*, *Gammarella fucicola*, *Melita hergensis*, *Lysianassa* sp. and *Stenothoe tergestina*). Dissimilarity between sampling times within non-invaded sites was not very high (48.09%); the most important species contributing to this dissimilarity were *Caprella hirsuta* and *Hyale schmidti*. Dissimilarity between sampling times within invaded sites was 51.71%, the most important species contributing to this dissimilarity were *Caprella hirsuta* and *Lysianassa costae*.

The abundance of two species (*Caprella hirsuta* and *Elasmopus* spp.) was not significantly different between the two habitat types, but there was a clear pattern of higher abundances in non-invaded habitat. The abundance of *Caprella hirsuta* varied significantly with respect to season, being more abundant in March (Ti, $P < 0.05$, Table 2.4; Fig. 2.6e). *Elasmopus* spp. seemed to be more abundant on native seaweeds, especially during September, but not statistical differences were noted (Table 2.4, Fig. 2.6f). Finally, the abundance of *Lysianassa costae*, was higher in non-invaded habitats, but this was true only in March (TixHa, $P < 0.01$, Table 2.4, Fig. 2.6g); the pattern was opposite during September, but without any significant differences.

Table 2.3. Abundance of amphipod species (number of individuals per m² ± SE) recorded from the different habitats for each of the two different sampling periods, together with total abundance and species richness for the two sampling periods and habitats.

Family	Species	SEPTEMBER - 04		MARCH - 05	
		Non-invaded habitat	Invaded habitat	Non-invaded habitat	Invaded habitat
Amphilochidae	<i>Amphilochus</i> sp. Bate, 1862	5.56 ± 5.56	-	-	-
	<i>Amphilochus neapolitanus</i> Della Valle, 1893	2.78 ± 2.78	5.56 ± 5.56	50 ± 25.0	5.56 ± 3.68
Ampeliscaidae	<i>Ampelisca diadema</i> (A. Costa, 1853)	-	-	-	2.78 ± 2.78
	<i>Ampelisca serrataudata</i> Chevreux, 1888	-	-	2.78 ± 2.78	-
Amphithoidae	<i>Amphithoe ferox</i> (Chevreux, 1902)	2.78 ± 2.78	-	2.78 ± 2.78	-
	<i>Amphithoe ramondi</i> Audouin, 1826	1619.44 ± 426.08	977.78 ± 425.11	830.56 ± 262.69	108.33 ± 33.07
Aoridae	<i>Lembos</i> spp. Bate, 1856	-	-	2.78 ± 2.78	-
	<i>Leptocheirus guttatus</i> (Grube, 1864)	-	-	5.56 ± 3.68	-
	<i>Microdeutopus</i> spp. A. Costa, 1853	1130.56 ± 286.47	1433.33 ± 401.11	230.56 ± 87.28	230.56 ± 35.3
Caprellidae	<i>Caprella acanthifera</i> Leach, 1814	-	-	-	127.78 ± 127.78
	<i>Caprella grandimana</i> Mayer, 1882	-	-	477.78 ± 477.78	383.33 ± 234.59
	<i>Caprella hirsuta</i> Mayer, 1890	116.67 ± 84.68	2.78 ± 2.78	3269.44 ± 754.05	733.33 ± 364.22
Corophiidae	<i>Corophium</i> spp. Latreille, 1806	-	-	-	5.56 ± 5.56
	<i>Apocorophium acutum</i> (Chevreux, 1908)	58.33 ± 35.11	402.78 ± 108.29	27.78 ± 17.4	172.22 ± 108.45
	<i>Corophium insidiosum</i> Crawford, 1937	-	-	-	2.78 ± 2.78
	<i>Erichthonius brasiliensis</i> (Dana, 1855)	-	2.78 ± 2.78	22.22 ± 15.28	8.33 ± 5.89
Dexaminidae	<i>Atylus massiliensis</i> Bella-Santini, 1975	-	2.78 ± 2.78	105.56 ± 35.79	-
	<i>Atylus guttatus</i> (A. Costa, 1851)	2.78 ± 2.78	-	100 ± 60.42	5.56 ± 3.67
	<i>Dexamine spiniventris</i> (A. Costa, 1853)	-	-	75 ± 50.69	44.44 ± 36.03
	<i>Guerneia coalita</i> (Norman, 1868)	-	8.33 ± 5.89	41.67 ± 21.25	-
Gammaridae	<i>Elasmopus</i> spp. A. Costa, 1853	586.11 ± 241.35	272.22 ± 87.35	202.78 ± 63.25	97.22 ± 40.7
	<i>Gammarella fucicola</i> (Leach, 1814)	-	-	-	2.78 ± 2.78
	<i>Maera inaequipes</i> (A. Costa, 1857)	-	2.78 ± 2.78	11.11 ± 8.45	2.78 ± 2.78
	<i>Melita hergensis</i> Reid, 1939	-	-	16.67 ± 13.82	50 ± 44.1
	<i>Melita palmata</i> Montagu, 1804	-	2.78 ± 2.78	-	-
Lysianassidae	<i>Lysianassa</i> sp.	-	-	13.89 ± 9.42	-
	<i>Lysianassa costae</i> Milne Edwards, 1830	127.78 ± 45.54	230.56 ± 51.67	258.33 ± 64.28	33.33 ± 27.32
	<i>Orchomene humilis</i> (A. Costa, 1853)	-	25 ± 17.68	-	-
Oedicerotidae	<i>Periculodes aequimanus</i> (Kossman, 1880)	5.56 ± 3.68	-	2.78 ± 2.78	8.33 ± 5.89
Phliantidae	<i>Pereionotus testudo</i> (Montagu, 1808)	2.78 ± 2.78	2.78 ± 2.78	19.44 ± 16.55	13.89 ± 11.11
Stenothoidae	<i>Stenothoe monoculoides</i> (Montagu, 1813)	63.89 ± 30.08	5.56 ± 5.56	77.78 ± 27.15	36.11 ± 26.06
	<i>Stenothoe tergestina</i> (Nebeski, 1880)	-	-	5.56 ± 5.56	-
Talitridae	<i>Hyale schmidti</i> (Heller, 1866)	52.78 ± 38.74	2.78 ± 2.78	605.56 ± 198.55	25 ± 13.82
Unidentified		5.56 ± 5.56	-	41.67 ± 25.68	38.89 ± 30.65
Total abundance		3861.11 ± 925.18	3383.33 ± 844.11	6497.22 ± 1586.08	2130.56 ± 793.68
Species richness		14	16	25	22

Table 2.4. Results of ANOVA (three-factor) for amphipod species richness, total abundance, and abundance of the seven most abundant species. MS = mean square; *P* = level of probability; df = degrees of freedom; Si= site, ns = non-significant; * significant at *p* < 0.05, ** significant at *p* < 0.01

Source of variation	df	Species richness		Total abundance		<i>Amphithoe ramondi</i>		F versus
		MS	P	MS	P	MS	P	
Sampling time=Ti	1	78.03	0.0158*	6889.00	0.6864	13.28	0.0348*	Si(TixHa)
Habitat=Ha	1	42.25	0.0551	84487.11	0.1807	12.04	0.0420*	Si(TixHa)
TixHa	1	56.25	0.0321*	54444.44	0.0033*	2.47	0.3054	Si(TixHa)
Si (TixHa)	8	8.39	0.0481*	39278.50	0.0033 *	2.06	0.0028*	Res
Residual	24	3.53		9534.44		0.48		
Cochran's C-test		C=0.1654 ns		C=0.2210 ns		C=0.2488 ns		
Transformation		None		None		Ln(X+1)		
Source of variation	df	<i>Hyale schmidtii</i>		<i>Apocorophium acutum</i>		<i>Microdeutopus spp.</i>		F versus
		MS	P	MS	P	MS	P	
Sampling time=Ti	1	14.69	0.0104*	230.02	0.0980	23.49	0.0008*	Si(TixHa)
Habitat=Ha	1	16.63	0.0076*	890.02	0.0062*	0.82	0.3590	Si(TixHa)
TixHa	1	6.96	0.0511	132.25	0.1934	0.0003	0.9853	Si(TixHa)
Si (TixHa)	8	1.32	0.0508	65.58	0.7064	0.87	0.0943	Res
Residual	24	0.56		96.77		0.44		
Cochran's C-test		C=0.3841 ns		C=0.3720 ns		C=0.2565 ns		
Transformation		None		None		None		
Source of variation	df	<i>Caprella hirsuta</i>		<i>Elasmopus spp.</i>		<i>Lysianassa costae</i>		F versus
		MS	P	MS	P	MS	P	
Sampling time=Ti	1	54289.01	0.0045**	15.39	0.1746	16.00	0.5058	Si(TixHa)
Habitat=Ha	1	25281.01	0.0284	8.57	0.2986	53.77	0.2374	Si(TixHa)
TixHa	1	21121.78	0.0406	0.21	0.8648	386.77	0.0090*	Si(TixHa)
Si (TixHa)	8	3549.97	0.1768	6.93	0.0033*	32.97	0.5053	Res
Residual	24	2217.11		1.68		35.19		
Cochran's C-test		C=0.5620 (<i>P</i> < 0.01)		C=0.2690 ns		C=0.3228 ns		
Transformation		None		Sqrt(X+1)		None		

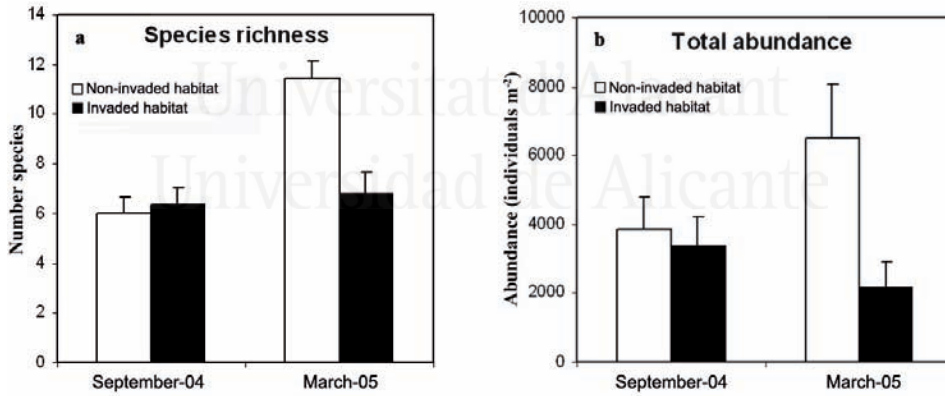


Figure 2.4. (a) Mean number of amphipod species per sample (\pm SE) and (b) total abundance (number of individuals $m^{-2} \pm$ SE), recorded from the two different sampling periods and habitats.

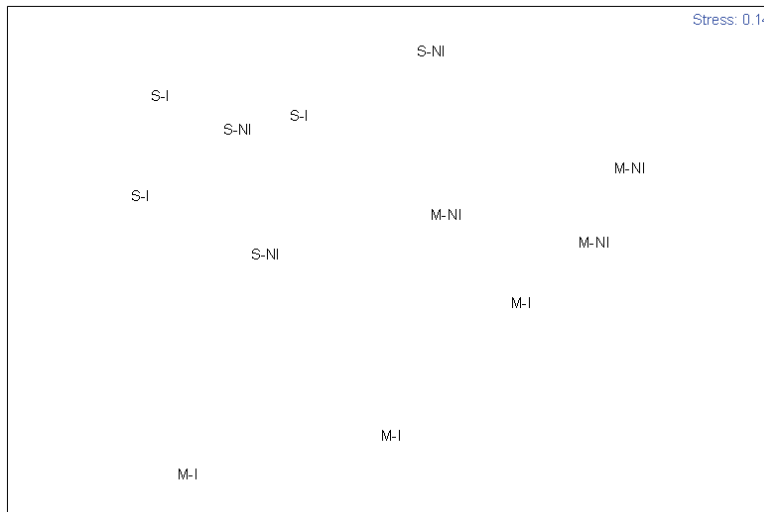


Figure 2.5. Two-dimensional MDS plot amphipod species abundance. S = September-04; M = March-05; I = Invaded habitat; NI = Non-invaded habitat for each of the six sampling sites.

2.5. Discussion

Our results show that the presence and abundance of *C. racemosa* had a marked effect on the macroalgal assemblage structure of Mediterranean shallow rocky communities, affecting the species composition of vegetation and increasing the detritus stock. This causes important changes in the associated amphipod assemblage, in terms of both abundance and species composition. The effects of the invasive species on habitat structure were more important in September because the alga was dominant at that time. The seasonal variation in abundance values for *C. racemosa* recorded from our study area was similar to that described in other localities, i.e. with maximum development in the warm season, while biomass values were similar or even greater than those recorded from other Mediterranean localities (Piazzi and Cinelli 1999). The peculiar growth of *C. racemosa*, with stolons that spread rapidly in all directions, and its colonisation pattern that results from sexual reproduction (Panayotidis and

Žuljevič 2001), together with dispersal of fragments detached from the plant (Ceccherelli et al. 2001, Ceccherelli and Piazzzi 2001) and propagules (Renoncourt and Meinesz 2002) permit *C. racemosa* to colonize a habitat very efficiently. This results in strong modification of habitat structure, particularly that of macroalgal assemblages. On the SE coast of Spain and other locations (e.g. Nervi, Italy; Modena et al. 2000), colonisation by *C. racemosa* has been mostly recorded from rocky bottom habitats located in shallow waters (0.5 m), however, on a wider scale across the Mediterranean region (e.g. France, Italy and Cyprus) more extensive spread of the alga have been recorded on soft bottom habitats in deeper waters (15-20 m) (Argyrou et al. 1999, Verlaque et al. 2004), and on dead matte of *Posidonia oceanica*. Therefore, one would expect that the spread of *C. racemosa* will extend to both rocky and soft bottom habitats, as well as to degraded *P. oceanica* meadows.

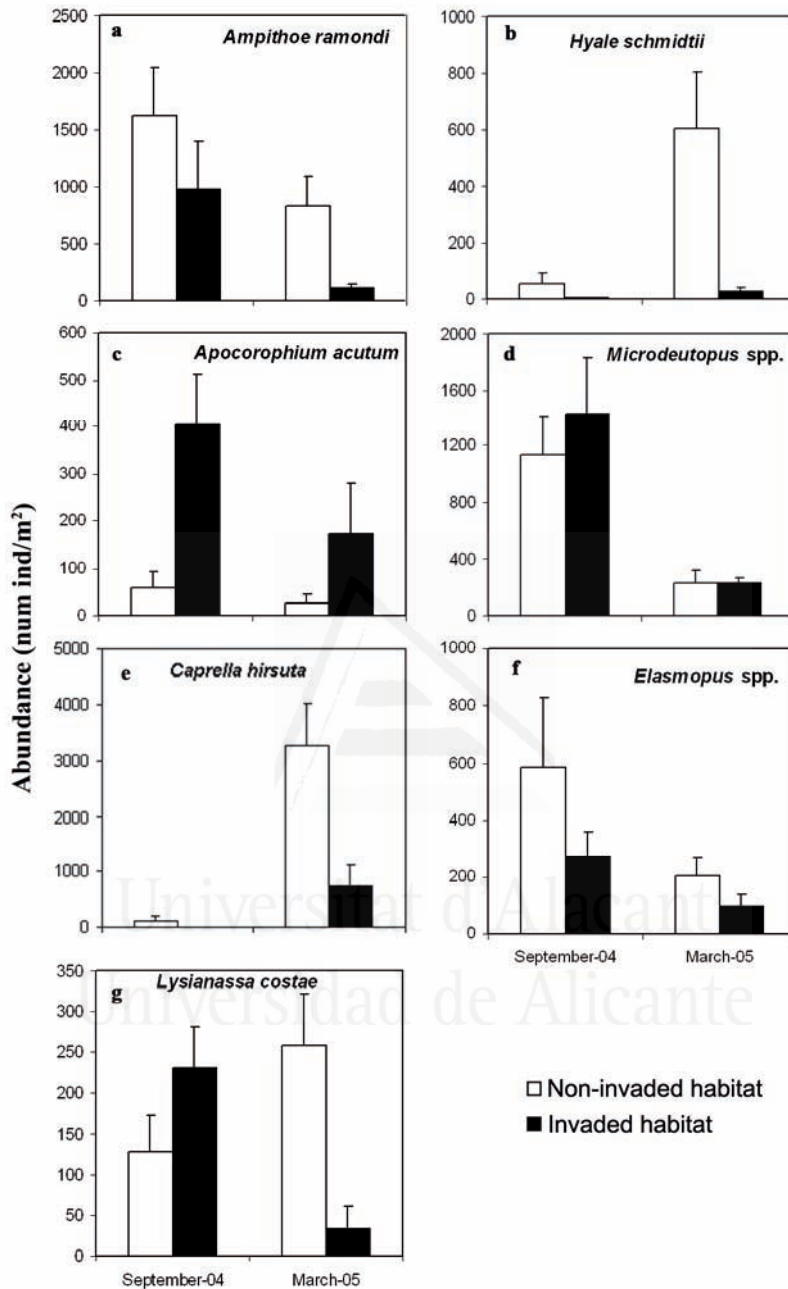


Figure 2.6. Mean abundance (number of individuals \pm SE) of the seven most abundant amphipods: *Ampithoe ramondi* (a); *Hyale schmidti* (b); *Apocorophium acutum* (c); *Microdeutopus* spp. (d); *Caprella hirsuta* (e); *Elasmopus* spp. (f) and *Lysianassa costae* (g)

With respect to changes in vegetation resulting from the presence of *C. racemosa*, an important reduction in species richness was recorded in September. On the other hand, contrary to results from other studies (Piazzi et al. 2001a), species richness was similar between invaded and non-invaded habitats in March (the colder month), which implies that some native species colonized the habitat irrespective of the different species composition and biomass of benthic vegetation. Some authors have shown that several years are necessary for the development of dense and continuous *C. racemosa* stands (Ruitton et al. 2005); possibly during the forthcoming years the effects will persist also during the coldest period at the study area, reducing the possibility of algae colonization. However, the total biomass of *C. racemosa* recorded from our study (259 g dw m^{-2}) is comparable or higher than that recorded from studies carried out on French and Italian coasts, and is also higher than values obtained for *C. taxifolia* and *C. prolifera* meadows at some localities (see a review in Capiomont et al. 2005).

One of the remarkable impacts of *C. racemosa* is the accumulation of detritus, which persists throughout the year. In spite of the important seasonal variability of *C. racemosa* biomass, detritus accumulation persists in winter. The mesh generated by the stolons of this species ensures that detritus is retained, while the multilayered structure formed by the thallus traps the sediment (Piazzi et al. 2001c). Detritus values recorded during both seasons were 2 to 6 times higher than those recorded from native seaweeds. This accumulation of detritus causes important changes in the structure of the sediment and in the granulometric composition (Walker et al. 1991), leading to anoxic conditions in which toxic substances can be possibly accumulated. On the other hand, detritus plays a very important role as a trophic resource for marine invertebrates, being one of the main trophic pathways of the marine ecosystem (Valiella 1995) and is one of the most important features of habitat structure in vegetated habitats (Allesina et al. 2005). Therefore, any modification of the detritus compartment can affect the overall trophic

web, because it could lead to a change in the faunal assemblage structure resulting from modification of trophic guilds due to different trophic requirements.

With respect to the epifauna, various studies have demonstrated that modification of habitat complexity affects crustacean assemblages (Hicks 1977, 1982; Stoner 1980, Virnstein 1987, Virnstein and Howard 1987, Edgar 1983, 1992; Sanchez-Jerez et al. 1999; Ayala and Martín 2003). In our study, the amphipod assemblages were affected by changes in habitat structure due to colonisation by *C. racemosa*. The total abundance of amphipods was greater on native seaweeds, mainly in March. However, the species richness of the amphipod assemblage did not decrease because of the spread of *C. racemosa* but because of an indirect effect; namely the increase in detritus biomass in invaded habitat resulted in an increase in the abundance of detritivores species (e.g. *Apocorophium acutum*), and decrease in the abundance of herbivorous species (e.g. *Ampithoe ramondi* and *Hyale schmidtii*), hence resulting in a large overall modification of the assemblage species composition.

The main modification in amphipod assemblage structure was manifested as an increase in abundance of *Apocorophium acutum* in habitat invaded by *C. racemosa*, which was four times higher than that of other Corophiidae species in eutrophic habitats (Guerra-García and García-Gómez 2005). Species belonging to the genus *Apocorophium* are tube-dwelling amphipods that inhabit small U-shaped tubes and are selective deposit feeders (Ciarelli et al. 1997), and feed on bacteria, algae, and diatoms adsorbed onto the surface of sediment particles (Meadows and Reid 1966). Several species of this genus are continuously exposed to toxicants in the sediment, which they ingest while feeding (Bat and Raffaelli 1998), hence they have been used for sediment toxicity tests. The accumulation of detritus in habitats invaded by *C. racemosa* seems to favour colonization by *Corophium* spp., resulting in a dramatic increase in abundance and establishment of a large population in an

altered habitat. *Microdeutopus* spp. also showed a small increase in abundance in habitat invaded by *C. racemosa*, but the effect was only recorded in September. This genus is generally associated with *Posidonia oceanica* and fine sand habitats (Ruffo 1982), but in experiments carried out recently by Roberts and Poore (2005), the family Aoridae were noted to prefer bare rock with a thin cover of sediment than an algal substratum. Furthermore, other taxonomic groups, such as the polychaetes, are favoured by accumulation of detritus amongst the *C. racemosa* stolons, as shown by Argyrou et al. (1999) from their study carried out in Cyprus.

On the other hand, the abundance of some species, such as *Ampithoe ramondi*, *Hyale schmidti* or *Caprella hirsuta*, was reduced in habitat invaded by *C. racemosa*, since these species have a preference for native seaweeds (Kocatas 1976, Russo 1997, Guerra-García and García-Gómez 2001, Roberts and Poore 2005). Other studies have also shown displacement of *Ampithoe ramondi* resulting from the presence of other *Caulerpa* species, e.g. *Caulerpa prolifera* (Sánchez-Moyano et al. 2001). The displaced species have a close relationship with seaweeds, as has been demonstrated by Viejo (1999). In the case of other species, such as *Lysianassa costae*, the effects of *C. racemosa* seem to be seasonal, inhabiting habitats that have a large algal biomass, irrespective of the species composition.

Comparing the results of the present study with ones on the effects of *C. taxifolia* on amphipod assemblages, the species richness recorded from the latter appears to be lower (Bellan-Santini 1995). Furthermore, the effects of *C. taxifolia* on the abundance of amphipods also seem to be more critical (Bellan-Santini 1995). Such differences could be due to several factors, such as the

species composition of *Caulerpa* beds. Stands of *C. taxifolia* are monospecific, while in our case; the *C. racemosa* grew intermixed with other macroalgal species during both sampling periods. Consequently, during the initial stages of invasion of a host habitat by invasive seaweed, an increment in habitat complexity will occur if the initial cover is low, hence a new and additional habitat for the local epifauna becomes available (Viejo 1999). However, if growth of the invasive species continues, leading to a monospecific and dense meadow, it could lead to impoverishment of the amphipod assemblage as has been noted for *C. taxifolia* elsewhere.

Several studies have demonstrated that consequences of *C. racemosa* in host systems are very important, particularly since it appears that this introduced species most invasive in the Mediterranean Sea (Verlaque et al. 2003). The presence of *C. racemosa* in the southeastern coast of Spain is an incipient problem. It was recorded from Alicante in 2002 and, in less than three years, important changes in the structure of shallow rocky habitat assemblages occurred, although to date the distribution of *C. racemosa* has been rather patchy, hence its effects are not very widespread locally. Several years may be necessary for development of a dense and continuous *C. racemosa* meadow (Ruitton et al. 2005); therefore in the near future, one can expect dramatic changes in rocky bottom habitats, leading to a direct impact on marine benthic communities along the western Mediterranean coast. It is therefore very important to increase our research effort to establish the ecological implications of the spread of *C. racemosa* in the Mediterranean Sea, as this will provide the necessary information and tools to develop new mitigation and eradication programs.



Does *C. racemosa* change the amphipod assemblage on soft bottoms?

3. Does *C. racemosa* change the amphipod assemblage on soft bottoms?

3.1. Abstract

The spread of the invasive alga *Caulerpa racemosa* var. *cylindracea* in shallow-water habitats can lead to a different faunal assemblage composition. We compared the amphipod assemblages associated with *C. racemosa* and natural habitats found on shallow-water Mediterranean soft substrata. Four vegetated habitats were compared: *Caulerpa racemosa*, *C. prolifera*, *Cymodocea nodosa* and *Posidonia oceanica* with unvegetated substrata. Samples were collected during two sampling periods (September 2004 and March 2005). A total of 63 amphipod species were recorded. The results showed that the vegetated habitats sampled, including *C. racemosa* stands, supported a higher abundance and species richness of amphipods. Furthermore, the assemblage structure differed between the different habitats, while the abundance of some species was significantly different, depending on habitat. For example, *Microdeutopus obtusatus* was favoured by *C. racemosa* habitat; *Ampelisca diadema* was associated with *C. prolifera* beds; and *Hyale schmidtii* was more abundant in *P. oceanica* meadows. Habitat invasion by *C. racemosa* can exert an important influence on biotic assemblages, modifying habitat structure and the associated fauna.

3.2. Introduction

Vegetated habitats allow a greater species diversity and abundance of individuals than those in unvegetated habitats (Irlandi 1994, Sanchez-Jerez et al. 1999), which is usually correlated with an increase in habitat complexity (Johnson 1970, Dean and Connell 1987, Taylor and Cole 1994). Several studies have demonstrated that habitat heterogeneity and complexity play an important role in influencing the assemblage structure of epibenthic marine fauna (Stoner 1980, Edgar 1992). The pattern of distribution and species richness and abundance of epifauna on rocky bottoms is affected by changes in the composition of associated macroalgae (Connell 1972). Therefore, changes in habitat structure of vegetated substrates resulting from the invasion of species, especially in the case of macroalgae, could affect the associated fauna (Wilkström and Kautsky 2004).

The number of introduced species outside their natural ranges is rapidly increasing, although only a very small fraction of transported and introduced species becomes invasive, the ability of these species to restructure and, hence, radically change the functioning of a recipient habitat is great (Crooks 2002). Some authors (Vitousek 1990, Crooks 2002) recognize three principal effects of invasive species on

ecosystems. First, exotics can affect the availability and flow of nutrients by altering biogeochemical cycling. Second, invasive species can affect the flow of energy through food webs. Third, invaders can affect the availability or quality of physical resources in the ecosystem (including living space or “habitat,” physical materials, sediment, light, or water). For instance, invasive plant species that generate big changes in the vegetation of an area are often regarded as highly influential ecosystem engineers (see a review in Crooks 2002). The Mediterranean Sea is the most heavily invaded region in the world for introduced seaweeds, with over 132 invasion events accounting for more than 33% of the total number of invasions (Williams and Smith 2007).

The invasive *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque (Verlaque et al. 2003) (hereafter *C. racemosa*) has spread extensively throughout the Mediterranean during the last few decades, invading habitats located in waters having a depth of 0 m to more than 60 m (Verlaque et al. 2004, Piazzini et al. 2005). The rapid expansion of *C. racemosa* is an example of how introduced species are able to modify Mediterranean habitats; this expansion can be explained by its very efficient reproductive strategies, both sexual and asexual, unlike those of *C.*

taxifolia (Panayotidis and Žuljevič 2001, Renoncourt and Meisenez 2002).

In Mediterranean coastal areas, two seagrass species, *Posidonia oceanica* and *Cymodocea nodosa*, form meadows on shallow-water sandy bottoms (Buia and Mazzella 1991). However, the two seagrasses have a contrasting meadow structure. *P. oceanica* is the largest seagrass species in the Mediterranean, with leaves sometimes exceeding one metre in length (Drew and Jupp 1976). It forms dense meadows (having a shoot density of between 300 to 1000 shoots m⁻²) with a compact root-rhizome mat and a high leaf stratum (having a leaf area index of around 7 m²), while *C. nodosa* forms meadows that have a low leaf stratum and less compact rhizome mat (leaf index of 0.4; Rull et al. 1996). Similar to seagrasses, the alga *Caulerpa prolifera* may form dense beds on soft bottoms at water depths of between 1 to 20 m, especially in areas that are characterized by a low energy environment and considerable input of organic matter (Sánchez-Moyano 2004).

Amphipods are one of the most important groups of invertebrates associated with vegetated habitats and play an important role as trophic resources for fish populations (Sanchez-Jerez et al. 1999, Stål et al. 2007). Amphipods respond to habitat alteration and can therefore be used as an indicator of environmental impacts on vegetated habitats (Bellan-Santini 1980, Virnstein 1987, Conradi et al. 1997, Sanchez-Jerez et al. 2000, Vázquez-Luis et al. 2008, Vázquez-Luis et al. 2009a).

Since the occurrence of *C. racemosa* along Mediterranean coastal areas was first recorded, several studies concerning the ecology of this species have been undertaken (see Ruitton et al. 2005, Cavas et al. 2006). It is known that fauna associated with exotic algae can differ from natives (Wikström and Kautsky 2004, Schmidt and Scheibling 2007). However, information on changes in the associated fauna brought about by this invasive *C. racemosa* is scarce (Argyrou et al. 1999, Piazzi and Balata 2008, Vázquez-Luis et al.

2008, 2009a). On soft bottoms, *C. racemosa* grows on sandy substrates, either unvegetated areas or covering dead matte of *P. oceanica*, competing with other macrophytes for the space. The aim of this study was to compare the structure of amphipod assemblages associated with a *C. racemosa* stand on soft bottom with those associated with unvegetated habitat and habitat vegetated by native species. The hypothesis tested was that the assemblages associated with *C. racemosa* stand on soft bottom may be different from unvegetated habitats and from habitats vegetated by native macroalgae and seagrasses.

3.3. Materials and methods

3.3.1. Study area

The study was carried out along the Cape of Santa Pola (Alicante, south-east Spain; Fig. 3.1) on a shallow sandy sea bed. *C. racemosa* was first recorded off Alicante in 2002, where it occurred on bare soft substrata and dead matte of *Posidonia oceanica* at a site located some 10 km north of our study area (Pena-Martin et al. 2003). It now covers large areas of rocky bottoms, bare sandy/muddy substratum, dead matte of *P. oceanica*, and also grows mixed with *Cymodocea nodosa* and *Caulerpa prolifera* within a wide depth range, showing a patchy distribution.

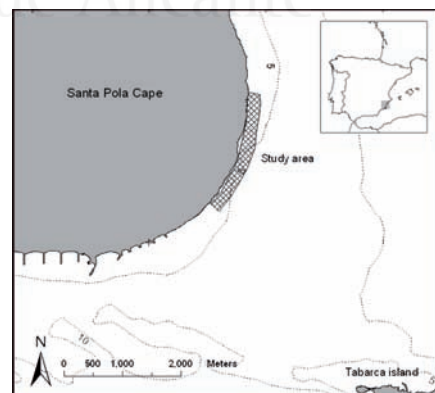


Figure 3.1. Map showing the study area in Santa Pola Cape, southeastern Spain

3.3.2. Sampling design

Five habitats occurring in shallow waters within the 2-5 m depth range, were sampled: *C. racemosa* stands, *C. prolifera* stands, *Cymodocea nodosa* meadows, *Posidonia oceanica* meadows (*P. oceanica*) and unvegetated substratum (bare sand). Several sampling sites, separated by hundreds of metres, were randomly selected across the study area. All sites had similar environmental conditions with respect to wave exposure and light (Ferrandis-Ballester and Bartolomé-Pina 1985). Three sampling sites were allocated within each of the five habitat types, which were sampled in September 2004 (average water temperature = 27.5 °C), which coincides with the period of maximum vegetative growth of *C. racemosa* (Piazzi and Cinelli 1999). Additionally, the stations were sampled again in March 2005 (water temperature = 13 °C), which coincides with the period of minimum vegetative growth. At each station, three random replicate samples were collected by SCUBA diving, using a 20 cm x 20 cm quadrat, by scraping the enclosed area using a trowel (Edgar 1990). A 300 µm mesh bag was attached to the quadrat to avoid loss of the motile fauna. Replicate samples were separated by tens of metres. The collected samples were preserved in 4% formalin. Each sample was then sieved in sea-water through a 500 µm mesh, to retain the detritus and fauna. In the laboratory, the amphipods were separated, identified, and counted. Macrophytes were sorted and identified to the lowest possible taxon, and the detritus separated. The macrophytes and detritus were dried for 24 h at 80 °C and weighed. Habitat structure was characterised using three attributes: species richness of macrophytes, biomass of each species (g), and quantity of detritus (g).

3.3.3. Data analysis

Two different statistical approaches were used to test for potential differences in the amphipod assemblage between the different habitats, based on the null hypothesis of no difference in the amphipod assemblage composition between the five habitat types and across the two sampling periods.

3.3.3.1. Univariate analysis

Plant biomass, detritus biomass, number of species and total abundance of amphipod fauna, and abundance of the most abundant amphipod species were analysed using Analysis of Variance (ANOVA). To test whether the abundance of amphipods differed significantly across habitats and time we used a three-factor ANOVA, which incorporated the following factors: (i) 'Time of sampling' a fixed factor and orthogonal with two levels: September '04 and March '05; (ii) 'Habitat' (fixed and orthogonal) with five levels: *C. racemosa*, *C. prolifera*, *C. nodosa*, *P. oceanica* and sand; and (iii) 'Site' (random and nested in 'Time of sampling' and 'Habitat') with three random sampling sites. Prior to carrying out the ANOVA, the data were tested for heterogeneity of variance using Cochran's C-test. Data were $\sqrt{x + 1}$ transformed in cases where the variances were significantly different at $p = 0.05$, and $\log(x + 1)$ transformed where the variance was still heterogeneous. Where variance remained heterogeneous, untransformed data were analysed, as ANOVA is a robust statistical test and is relatively unaffected by heterogeneity of variances, particularly in balanced experiments (Underwood 1997). However, in such cases special care was taken in the interpretation of results. Furthermore, in such cases, to reduce type I error, the level of significance was reduced to $p < 0.01$. When ANOVA indicated a significant difference for a given factor, the source of difference was identified using Student–Newman–Keul (SNK) tests (Underwood 1981, Underwood 1997).

3.3.3.2. Multivariate analysis

Non-parametric multidimensional scaling (MDS) was used as the ordination method for exploring differences in the amphipod assemblage composition (Clarke and Warwick 1994). The similarity matrix, which was calculated using the Bray–Curtis index and using double square transformed data, was used to construct bivariate MDS plots. The percentage similarities (SIMPER) procedure was then used to calculate the contribution of each species to the dissimilarity between sampling time and

habitats. (PRIMER software; Clarke 1993). A permutational multivariate ANOVA (PERMANOVA software; Anderson 2001) was used to test differences in amphipod species composition following the same experimental design than the univariate analysis; after the permutational test a pair wise test was carried out to test differences among groups.

3.4. Results

3.4.1. Habitat features

A total of 18 algal taxa and two seagrasses were identified (Table 3.1). The highest species richness was recorded from habitats colonized by *C. racemosa* in both sampling periods (Fig. 3.2). During September, habitats invaded by *C. racemosa* were characterised by a low number of associated algae (9 species plus *C. racemosa*), but a higher species richness of macrophytes was recorded in March (16 species plus *C. racemosa*).

Values of plant biomass recorded from the different habitats showed the same pattern,

with lower values in March for all habitats, except *C. prolifera* stands, for which the opposite trend was observed. ANOVA indicated a significant difference for plant biomass between habitats (Ha, $P < 0.05$; Table 3.2 and Fig. 3.2); this difference was due to significantly higher biomass recorded for *P. oceanica* meadows. In *C. racemosa* invaded areas, this species contributed in September with a high biomass value (62.3%). However, during the coldest period the biomass was only 7.62% of total, the most important habitat features being dead matte of *P. oceanica* and the red seaweed *Jania rubens*. Mean shoot density values for *P. oceanica* meadows were 1525 ± 4.6 shoots m^{-2} in September and 1147.22 ± 4.89 shoots m^{-2} in March.

The amount of detritus present in the different habitats was highest in March. Significant differences for this attribute were recorded between habitats (Ha, $P < 0.05$; Table 3.2 and Fig. 3.2). The highest value of detritus biomass was recorded from *C. prolifera* beds during both sampling periods (830.02 g dw m^{-2} in September and 1123.85 g dw m^{-2} in March)

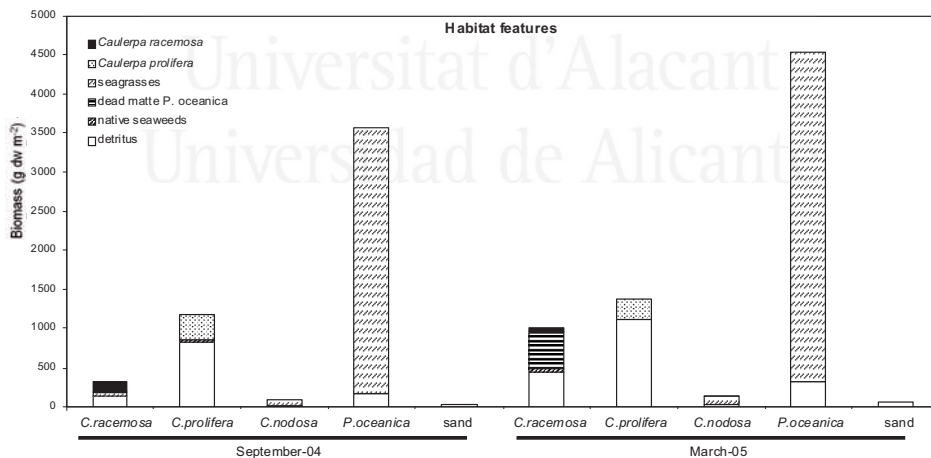


Figure 3.2. Values of mean plant biomass and detritus (g dw m^{-2}) recorded from the different habitats during the two different sampling periods.

Table 3.1. Values of mean biomass (g dw m^{-2}) \pm SE for the different species, together with total biomass, total species richness and detritus weight recorded from the two sampling periods and five habitats.

Species	SEPTEMBER - 04					MARCH - 05				
	<i>C. racemosa</i>	<i>C. prolifera</i>	<i>C. nodosa</i>	<i>P. oceanica</i>	sand	<i>C. racemosa</i>	<i>C. prolifera</i>	<i>C. nodosa</i>	<i>P. oceanica</i>	sand
Chlorophyta										
<i>Acetabularia acetabulum</i> (Linnaeus) P.C. Silva	10.07 \pm 7.729	335.47 \pm 63.94	0.82 \pm 0.7	-	-	0.06 \pm 0.05	-	-	-	-
<i>Caulerpa prolifera</i> (Forsskål) J.V. Lamouroux	122.24 \pm 24.57	-	0.69 \pm 0.65	-	-	4.34 \pm 2.89	250.07 \pm 28.15	3.01 \pm 2.27	-	-
<i>Caulerpa racemosa</i> (Forsskål) J. Agardh	1.02 \pm 0.79	-	0.11 \pm 0.11	-	-	42.49 \pm 4.98	-	-	-	-
<i>Cladophora</i> sp. Kützting, 1843	-	-	-	-	-	0.54 \pm 0.54	-	-	-	-
<i>Codium</i> sp. F.S. Collins & Hervey, 1917	0.08 \pm 0.08	16.95 \pm 16.95	-	-	-	0.19 \pm 0.13	-	-	-	-
<i>Dasycladus vermicularis</i> (Scopoli) Krasser	0.344 \pm 0.34	-	-	-	-	0.15 \pm 0.11	-	0.79 \pm 0.79	-	-
<i>Flabellia petiolata</i> (Turra) Nizamuddin	0.17 \pm 0.11	-	-	-	-	0.24 \pm 0.24	-	-	-	-
<i>Halimeda tuna</i> (J. Ellis & Solander) J.V. Lamouroux	-	-	-	-	-	-	-	-	-	-
<i>Ulva</i> sp. Linnaeus	-	-	-	-	-	0.83 \pm 0.35	-	-	-	-
Phaeophyta										
<i>Colpomenia simmisa</i> (Mertens ex Roth) Derbès & Solier	-	-	-	-	-	-	-	0.39 \pm 0.39	-	-
<i>Cystoseira compressa</i> (Esper) Gerloff & Nizamuddin	-	-	-	-	-	4.26 \pm 4.26	-	-	-	-
<i>Dichopteris membranacea</i> (Stackhouse) Batters	-	-	-	-	-	0.21 \pm 0.1	-	-	-	-
<i>Dicyota fasciola</i> (Roth) J. V. Lamouroux	-	-	-	-	-	0.91 \pm 0.62	-	0.63 \pm 0.6	-	-
<i>Halopteris scoparia</i> (Linnaeus) Sauvageau	3.35 \pm 3.35	-	3.18 \pm 2.82	-	-	1.43 \pm 1.23	-	-	-	-
<i>Padina pavonica</i> (Linnaeus) Thivy	-	-	-	-	-	5.4 \pm 4.56	-	-	-	-
<i>Corallina elongata</i> Ellis et Solander	0.37 \pm 0.37	-	0.19 \pm 0.19	-	-	0.09 \pm 0.09	-	2.35 \pm 2.35	-	-
<i>Halophys incurvus</i> (Hudson) Batters	-	-	-	-	-	1.18 \pm 1.14	-	-	-	-
<i>Jania rubens</i> (Linnaeus) J.V. Lamouroux	14.21 \pm 10.2	-	-	-	-	26.67 \pm 20.81	-	0.63 \pm 0.63	-	-
<i>Cymodocea nodosa</i> (Ucria) Ascherson	44.31 \pm 18.62	-	79.76 \pm 25.88	-	-	4.21 \pm 3.9	-	97.05 \pm 36.46	-	-
<i>Posidonia oceanica</i> (Linnaeus) Delile (leaf)	-	-	-	783.61 \pm 80.57	-	-	-	-	1257.31 \pm 132.56	-
<i>Posidonia oceanica</i> (Linnaeus) Delile (rhizome)	-	-	-	2612.22 \pm 612.98	-	-	-	-	2965.14 \pm 515.17	-
<i>Posidonia oceanica</i> (Linnaeus) Delile (dead matte)	-	-	-	-	-	464.41 \pm 137.73	-	-	-	-
Vegetal biomass	196.18 \pm 28.11	352.43 \pm 60.35	84.76 \pm 25.92	3395.83 \pm 672.16	0	557.73 \pm 121.12	250.07 \pm 28.15	104.86 \pm 39.13	4222.44 \pm 590.79	0
Species richness	10	2	6	1	0	17	1	7	1	0
Detritus	127.21 \pm 27.54	830.02 \pm 275.13	7.66 \pm 3.32	169.96 \pm 47.11	21.47 \pm 10.51	453.86 \pm 94.49	1123.85 \pm 268.57	31.55 \pm 9.71	322.28 \pm 80.49	58.63 \pm 30.19

Table 3.2. Results of the three-factor ANOVA for: macrophyte biomass and detritus biomass. MS = mean square; *P* = level of significance; df = degrees of freedom; ns = non-significant; ** = significant (*P* < 0.01).

Source of variation	df	Macrophyte biomass		Detritus biomass		<i>F</i> versus
		MS	<i>P</i>	MS	<i>P</i>	
Sampling Time =Ti	1	0.7807	0.2840	19.2287	0.0984	Si(TixHa)
Habitat=Ha	4	58.9994	0.0001**	57.5446	0.0003**	Si(TixHa)
Ti x Ha	4	0.7064	0.3853	1.8053	0.8861	Si(TixHa)
Si (Ti x Ha)	20	0.6442	0.0001**	6.3995	0.0001**	Res
Residual	60	0.1277		0.6847		
Transformation			Ln(X+1)		Sqrt(X+1)	

3.4.2. Amphipod assemblages

A total of 63 amphipod species, belonging to 20 families, was recorded (Table 3.3). Species richness was significantly higher in September (TixHa, *P*<0.05; Table 3.4 and Fig. 3.3a), with the highest values recorded from the *P. oceanica* meadows. Total abundance was similar between habitats in March, but there were significant differences in September, with the highest value (4886.11 ind m⁻²) recorded from *P. oceanica* meadows (TixHa, *P*<0.05; Table 3.4 and Fig. 3.3b). The lowest abundance values were recorded in September from *C. prolifera* stands and unvegetated bottoms (50 ± 19.54 ind m⁻² and 50 ± 21.65 ind m⁻² respectively). Of the two *Caulerpa* habitat types, *C. racemosa* had the highest abundance values, which were similar for both sampling periods (1577 ind m⁻² in September and 1672 ind m⁻² in March). On the other hand, *C. prolifera* stands had the lowest abundance values in both sampling periods, with the lowest values recorded in September (Table 3.4, Fig. 3.3b).

The two-dimensional MDS plot showed segregation of sampling stations mainly by habitat; two main groups can be distinguished: the first corresponding to *P. oceanica* meadows and the second corresponding to *C. racemosa* and *C. nodosa* habitats, while the remaining two habitats (*C. prolifera* stands and bare sand) were the most dissimilar to each other and to the each of the other three habitat types (Fig. 3.4). The multivariate response of amphipod assemblage showed significant differences among habitats regarding sampling times (PERMANOVA, TixHa, *P*<0.01, Table 3.4). After the pair wise test, in September *P. oceanica* was different to

the rest of the habitats. Nevertheless in March *C. prolifera*, *C. nodosa* and *P. oceanica* were similar with differences with sand and *C. racemosa*. The species that contributed most to the dissimilarity between amphipod assemblages were *Caprella grandimana*, *Stenothoe monoculoides*, *Microdeutopus obtusatus*, *Apocorophium acutum*, *Ampithoe ramondi*, *Ampelisca diadema*, *Hyale schmidti*, *Erichthonius brasiliensis*, *Leptocheirus guttatus* and *Elasmopus brasiliensis*. These species comprised between 60.09 % to 85.74 % of the total amphipod abundance in vegetated habitats.

The ten most abundant amphipods had different species abundance values with respect to the macrophyte habitats from which they were recorded. The abundance values of *Caprella grandimana* and *Stenothoe monoculoides* were significant higher in *C. racemosa* stands in March (TixHa, *P*<0.01; Table 3.5 and Fig. 3.5a, b). *Microdeutopus obtusatus* was significant higher at *C. racemosa* (Ha, *P*<0.01, Table 3.5, Fig. 3.5c). *Apocorophium acutum* was more abundant in *C. racemosa* stands in September but no significant differences were found (Table 3.5 and Fig. 3.5d). The abundance of *Ampithoe ramondi* was significant higher in habitats invaded by *C. racemosa* and in *C. nodosa* meadows (Ha, *P*<0.01; Table 3.5 and Fig. 3.5e). Only one species, *Ampelisca diadema*, had a significant higher abundance in *C. prolifera* stands (Ha, *P*<0.01, Table 3.5, Fig. 3.5f). On the other hand, several species seemed to be more abundant in *P. oceanica* meadows; for example, the abundance of *Hyale schmidti* is significant higher in this habitat (Ha, *P*<0.01; Table 3.5 and Fig. 3.5g). In the case of *Erichthonius*

brasiliensis these differences were detected only in September being more abundant in *P. oceanica* meadows (TixHa, $P < 0.05$, Table 3.5, Fig. 3.5h). Finally, *Leptocheirus guttatus* and *Elasmopus brasiliensis* had the

same abundance pattern as *Ericthonius brasiliensis*, although no significant differences were found, possibly to the highest variability of their abundance among sites (Table 3.5, Fig. 3.5i, j).

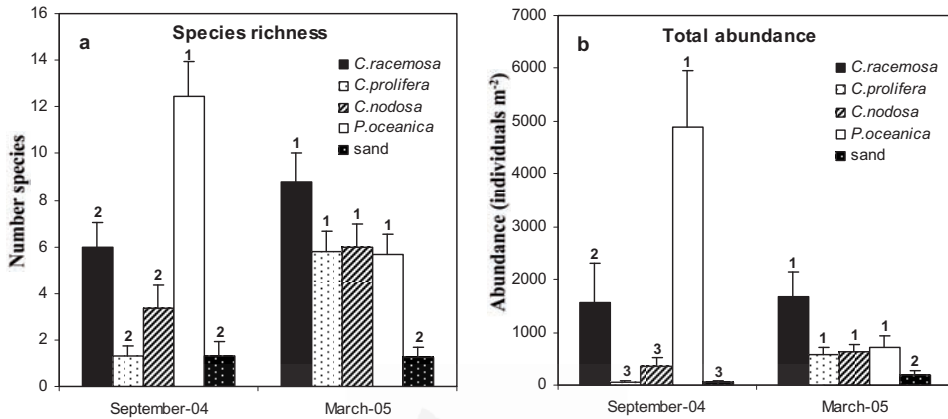


Figure 3.3. Values (\pm SE) of: (a) mean number of amphipod species recorded from each of the five stations, and (b) total abundance (individuals m^{-2}), recorded from the two sampling periods and five habitats. Groups defined by post-hoc SNK test after ANOVA were done within each time period and are showed by numbers.

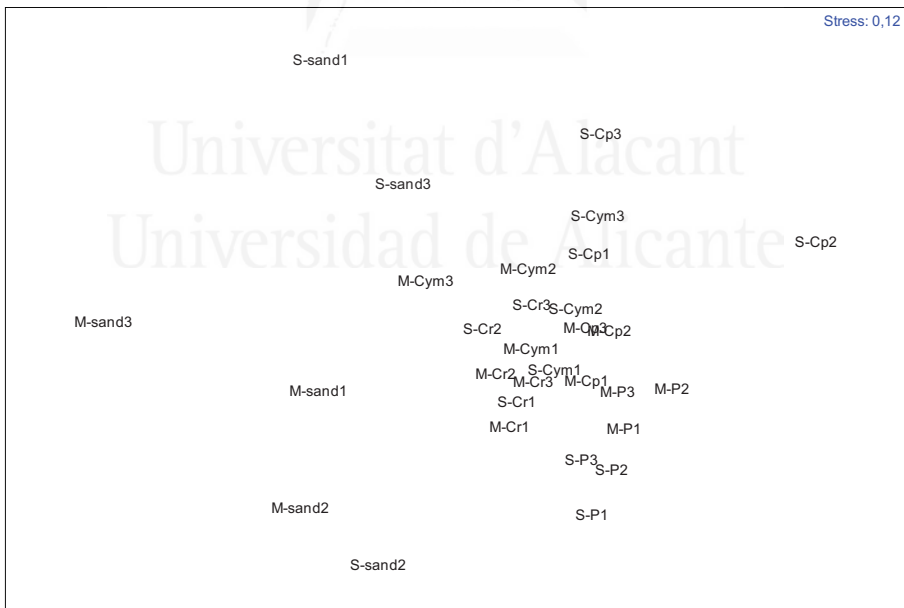


Figure 3.4. Two-dimensional MDS plot for amphipod species abundance. S = September '04; M = March '05; Cr = habitat invaded by *C. racemosa*; Cp = *C. prolifera* stands; Cym = *C. nodosa* meadows; P = *P. oceanica* meadows, and sand = unvegetated soft bottoms. The number indicates the replicate samples.

Table 3.3. Values of: Species abundance (number of individuals per m²), total abundance and species richness of amphipod fauna recorded from the different habitats and sampling periods (mean ± SE).

Family	Species	SEPTEMBER - 04				MARCH - 05						
		<i>C. racemosa</i>	<i>C. prolifera</i>	<i>C. nodosa</i>	<i>P. oceanica</i>	<i>C. racemosa</i>	<i>C. prolifera</i>	<i>C. nodosa</i>	<i>P. oceanica</i>	sand		
Amphilochoidea	<i>Amphilocheus neapolitanus</i> Della Valle, 1893	-	-	-	2.78 ± 2.78	-	-	-	8.33 ± 5.89	-	-	-
Ampeliscaidae	<i>Ampelisca antiemata</i> Bellan-Santini & Keim-Malka,	-	-	-	94.44 ± 34.3	-	-	-	-	-	-	-
	<i>Ampelisca brevicornis</i> (A. Costa, 1853)	-	-	-	-	-	-	-	-	-	-	-
	<i>Ampelisca diadema</i> (A. Costa, 1853)	-	16.67 ± 8.33	-	-	-	-	-	25 ± 14.43	2.78 ± 2.78	-	-
	<i>Ampelisca rubella</i> A. Costa, 1864	-	-	-	25 ± 25	-	-	-	-	-	-	-
Amphithoidea	<i>Ampelisca serratacaudata</i> Chevreux, 1888	13.89 ± 11.11	-	2.78 ± 2.78	102.78 ± 39.63	-	-	-	52.78 ± 49.73	2.78 ± 2.78	-	-
	<i>Amphithoe ferox</i> (Chevreux, 1902)	-	-	-	2.78 ± 2.78	-	-	-	-	-	-	-
	<i>Amphithoe helleri</i> G. Karaman, 1975	-	5.55 ± 3.67	2.78 ± 2.78	-	-	-	-	-	-	50 ± 18.63	27.78 ± 12.8
	<i>Amphithoe ramondi</i> Audouin, 1826	308.33 ± 190.3	5.55 ± 5.55	102.78 ± 65.8	75 ± 21.25	2.78 ± 2.78	-	-	94.44 ± 20.32	30.55 ± 10.01	252.78 ± 95.96	25 ± 9.32
Aoridae	<i>Aora gracilis</i> (Bate, 1857)	-	-	-	-	-	-	-	-	-	-	8.33 ± 5.89
	<i>Aora spiricornis</i> Afonso, 1976	-	-	-	63.88 ± 63.88	-	-	-	-	2.78 ± 2.78	2.78 ± 2.78	-
	<i>Lemboos spiniventris</i> (Della Valle, 1893)	-	-	-	11.11 ± 7.35	-	-	-	-	2.78 ± 2.78	-	-
	<i>Lepiocheirus guttatus</i> (Grube, 1864)	-	-	-	502.77 ± 199.32	-	-	-	47.22 ± 47.22	-	-	27.78 ± 14.7
Calliopidae	<i>Microdeutopus obesus</i> Myers, 1973	488.89 ± 242.39	5.55 ± 3.67	61.11 ± 23.6	-	-	-	469.44 ± 161.58	80.55 ± 27.36	38.89 ± 12.58	2.78 ± 2.78	-
	<i>Microdeutopus stationis</i> Della Valle, 1893	-	-	-	175 ± 88.39	-	-	-	-	-	-	-
	<i>Microdeutopus versiculatus</i> (Bate, 1856)	-	-	-	219.44 ± 121.18	-	-	-	-	-	-	-
	<i>Aphernesa chireghinii</i> Giordani-Soika, 1950	-	-	-	19.44 ± 9.1	-	-	-	-	5.55 ± 5.55	-	-
Caprellidae	<i>Caprella grandimana</i> Mayer, 1882	27.78 ± 27.78	-	-	-	-	-	488.89 ± 133.6	-	16.67 ± 9.32	-	-
	<i>Caprella hirsuta</i> Mayer, 1890	2.78 ± 2.78	-	-	-	-	-	-	-	2.78 ± 2.78	-	5.56 ± 3.67
	<i>Caprella santosrosai</i> Sanchez-Moyano, Jimenez-Martin and Garcia-Gomez, 1995	5.56 ± 3.67	-	-	-	-	-	2.78 ± 2.78	-	-	-	-
Colomastigidae	<i>Deutella schieckei</i> Cavolini, 1982	-	-	-	30.56 ± 14.89	-	-	-	-	-	-	5.56 ± 5.56
	<i>Phitsica marina</i> Slabber, 1769	-	-	-	-	-	-	-	5.56 ± 3.67	-	-	-
	<i>Pseudoprotella phasma</i> (Montagu, 1804)	-	-	-	-	-	-	-	5.56 ± 5.56	-	-	-
	<i>Colomastix pusilla</i> Grube, 1861	-	-	-	50 ± 24.65	-	-	-	-	-	-	8.33 ± 4.17

Table 3.4. Results of the multivariate analyse PERMANOVA for amphipod assemblage. MS = mean square; P = level of significance; df = degrees of freedom; ** = significant ($P < 0.01$).

Source of variation	df	Amphipod assemblage		F versus
		MS	P	
Sampling Time = Ti	1	8285.94	0.0240	Si(TixHa)
Habitat = Ha	4	21587.83	0.0040**	Si(TixHa)
Ti x Ha	4	3799.58	0.0010**	Si(TixHa)
Si (Ti x Ha)	20	9133.72	0.0010**	Res
Residual	60	2070.23		
Transformation		Fouth root		

Post-hoc test: September $C. racemosa \neq C. prolifera = C. nodosa = P. oceanica \neq$ sand;
March $P. oceanica \neq C. racemosa = C. prolifera = C. nodosa =$ sand

Table 3.5. Results of the three-factor ANOVA for species richness, total abundance, and abundance of the most important amphipod species. MS = mean square; P = level of significance; df = degrees of freedom; ns = not significant; * = significant ($P < 0.05$), ** = significant ($P < 0.01$).

Source of variation	df	Species richness		Total abundance		<i>Caprella grandimana</i>		F versus
		MS	P	MS	P	MS	P	
Sampling Time = Ti	1	9.34	0.4340	3.85	0.2053	328.71	0.0115	Si(TixHa)
Habitat = Ha	4	175.04	0.0001**	29.93	0.0001**	378.60	0.0003**	Si(TixHa)
Ti x Ha	4	84.93	0.0029**	9.66	0.0113*	301.04	0.0010**	Si(TixHa)
Si (Ti x Ha)	20	14.66	0.0027**	2.25	0.0001**	42.42	0.0247	Res
Residual	60	5.74		0.65		21.78		
Transformation		None		Ln(X+1)		None		
Source of variation	df	<i>Stenothoe monoculoides</i>		<i>Microdeutopus obtusatus</i>		<i>Apocorophium acutum</i>		F versus
		MS	P	MS	P	MS	P	
Sampling Time = Ti	1	5.38	0.0254	1.88	0.8921	214.68	0.1597	Si(TixHa)
Habitat = Ha	4	10.00	0.0001**	1210.68	0.0001**	252.21	0.0746	Si(TixHa)
Ti x Ha	4	5.38	0.0028**	11.24	0.9765	314.65	0.0377	Si(TixHa)
Si (Ti x Ha)	20	0.92	0.2567	99.48	0.7548	100.64	0.1926	Res
Residual	60	0.74		132.01		75.30		
Transformation		None		None		None		
Source of variation	df	<i>Ampithoe ramondi</i>		<i>Ampelisca diadema</i>		<i>Hyale schmidtii</i>		F versus
		MS	P	MS	P	MS	P	
Sampling Time = Ti	1	0.19	0.6991	0.54	0.3143	1.88	0.2212	Si(TixHa)
Habitat = Ha	4	7.51	0.0025**	2.26	0.0101**	11.75	0.0001**	Si(TixHa)
Ti x Ha	4	1.74	0.2753	0.51	0.2535	1.91	0.2088	Si(TixHa)
Si (Ti x Ha)	20	1.25	0.0006**	0.13	0.9063	1.18	0.8260	Res
Residual	60	0.42		0.41		1.72		
Transformation		Ln(X+1)		None		None		
Source of variation	df	<i>Erichthonius brasiliensis</i>		<i>Leptocheirus guttatus</i>		<i>Elasmopus brasiliensis</i>		F versus
		MS	P	MS	P	MS	P	
Sampling Time = Ti	1	0.51	0.4878	263.51	0.2412	86.04	0.1270	Si(TixHa)
Habitat = Ha	4	34.75	0.0001**	390.52	0.1105	71.65	0.1173	Si(TixHa)
Ti x Ha	4	6.34	0.0020**	344.26	0.1488	70.02	0.1240	Si(TixHa)
Si (Ti x Ha)	20	1.02	0.0002**	180.63	0.0001**	33.94	0.0063**	Res
Residual	60	0.31		20.77		14.60		
Transformation		Ln(X+1)		None		None		

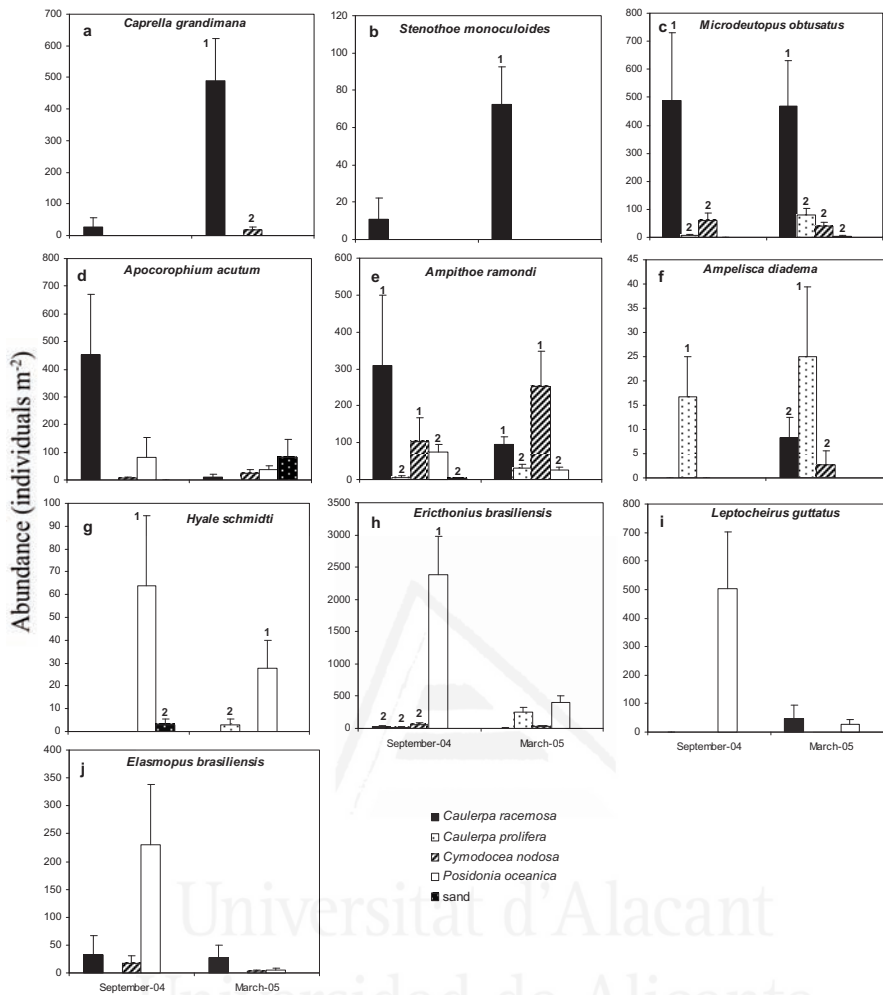


Figure 3.5. Values of mean abundance (number of individuals \pm SE) of the ten most abundant amphipods: *Caprella grandimana* (a); *Stenothoe monoculoides* (b); *Microdeutopus obtusatus* (c); *Apocorophium acutum* (d); *Ampithoe ramondi* (e); *Ampelisca diadema* (f); *Hyale schmidti* (g); *Erichthonius brasiliensis* (h); *Leptocheirus guttatus* (i) and *Elasmopus brasiliensis* (j). Groups defined by post-hoc SNK test after ANOVA were done within each time period and are showed by numbers.

3.5. Discussion

Complex natural habitats, such as *P. oceanica* meadows, hold the highest amphipod abundance and species richness. However, the new habitat generated by *C. racemosa* supports a diverse and abundant amphipod assemblage compared with other natural habitats. Overall, the amphipod assemblage structure differed between the

different vegetated habitats; this aspect can be attributed to amphipod habitat preferences, as has been shown by other authors who have demonstrated active selection of substratum (Stoner 1980, Roberts and Poore 2005), but also to differential predation among habitats (Orth 1992).

C. racemosa could affect the benthic communities in two different ways. First, adding a complex structure where none previously occurred (e.g. if this alga colonises bare substrates), such as our study case, or replacing other macrophytes. There exist many studies showing higher epifaunal abundance on vegetation compared to bare substrates (Stoner 1980, Heck and Orth 1980, Hicks 1982, Edgar et al. 1994, Sanchez-Jerez et al. 1999). The presence of vegetation enhances the heterogeneity and complexity of habitat available for the epifauna, increasing the abundance and species richness of amphipods compared to unvegetated habitats. In our study area we have not detected *C. racemosa* replacing other macrophytes in soft bottoms, but it is important to remark that *C. racemosa* in our study area was present in an incipient stage. Nevertheless, the replacements of native communities or competition with them have been detected in other studies, for instance, a study carried out by Dumay et al. (2002) reveal that *C. taxifolia* and *C. racemosa* in competition with *P. oceanica* seagrass beds affect the mean leaf longevity, leaf primary production and the sheath primary production of the seagrass. All the changes on habitat structure could affect the chemical and physical properties of the macrophytes and, in consequence, the amphipod preferences.

The heterogeneous and complex habitat structures of *P. oceanica* and *C. nodosa* meadows exert a variable influence on the abundance of amphipods, depending mainly on temporal changes in bed structure (Sanchez-Jerez et al. 1999). In our study, *P. oceanica* meadows are the most important habitat for amphipods in summer. Of the habitats found on soft sediment bottoms in the shallow waters of the Mediterranean, *P. oceanica* meadows are considered to be the most structurally complex (Pérès and Picard 1964, Duarte 2000). On the other hand, *C. nodosa* habitat did not contribute to such high amphipod abundance during summer but its importance in winter equalled that of *P. oceanica* habitat. Although *C. nodosa* meadows do not have a high structural complexity, they supported a high diversity of invertebrates; consequently the

importance of this seagrass habitat, where present on Mediterranean shallow sandy bottoms should not be undermined, especially during winter. Perhaps, for some faunal groups, high structural complexity of *P. oceanica* meadows renders them too compact and consequently there is less available habitat compared with less structurally complex meadows, such as those formed by *C. nodosa* (Sanchez-Jerez et al. 1999).

The *C. prolifera* and *C. racemosa* habitats supported very different amphipod abundances. *C. racemosa* was the second most important habitat after *P. oceanica* beds, while abundance values recorded from *C. prolifera* stands were relatively low. Nonetheless, other authors note the importance of *C. prolifera* beds as an important habitat for invertebrates (Sanchez-Moyano et al. 2007). It has been demonstrated by several studies that habitat patchiness influences total epifaunal abundance, in general, epifaunal abundance is highest in patchy or very patchy habitats compared to continuous habitats, while amphipods is one of the main groups that respond to this pattern of distribution (see a review in Boström et al. 2006). In our study, *C. racemosa* represents an initial stage of invasion, which means that it is patchily distributed and surrounded by seagrass. The observed high amphipod abundance recorded from our study area may be related to the high structural heterogeneity of *C. racemosa* habitat, while the alternative host plant habitats are affecting the amphipod assemblage (Poore 2005). On the other hand, *C. prolifera* stands are continuous, compared with those of *C. racemosa*, and this is reflected in the recorded amphipod abundance.

Regarding to species richness, seagrass habitat keeps the highest values. We note a very high species richness for *P. oceanica* meadows; 33 species in total. Other studies have reported 16, 29 and 16 amphipod species (Sanchez-Jerez et al. 1999, 2000 and Borg et al. 2006, respectively with this habitat). In the case of *C. nodosa* meadows, values of species richness recorded during the present study are similar to those from other studies (19 species, Sanchez-Jerez et

al. 1999; 18 species, Sfriso et al. 2001). Nevertheless, in the Mediterranean, *C. racemosa* meadows on soft substrata support a relatively diverse amphipod assemblage, being higher than *C. racemosa* growing on rocky substrate (Vázquez-Luis et al. 2008). This may be related to the reduced structural complexity of the latter habitat types. However, *C. prolifera* have similar species richness to other invasive *Caulerpa* species, *C. taxifolia*. Bellan-Santini (1995) reported 7 species from sampling carried out in September and 11 from sampling in March; these values are similar to those obtained for *C. prolifera* stands in our study area (6 in September and 18 in March). Sánchez-Moyano et al. (2007) reported up to 17 amphipod species from *C. prolifera* beds, which is also similar to our values recorded in March, and *C. racemosa* seems to support richer assemblages than *C. prolifera*. This could be due because of the macrophyte associated with the invasive specie while *C. prolifera* is present in a dense and continuous meadow with lower habitat complexity.

Temporal differences across the sampled habitats can be attributed to changes in habitat complexity (e.g. seasonal changes) and alteration of habitat heterogeneity (increase of epiphytes and associated algae). Seagrasses exhibit important seasonal changes in leaf index, leaf biomass and epiphytes, which ultimately affect the associated fauna (Stoner and Lewis 1985). Both *Caulerpa* species are algae having tropical and subtropical affinities and are strongly affected by seasonal fluctuations of water temperature. Both species show a considerable decrease of frond biomass during winter and remarkable growth when temperatures increase (Piazzi and Cinelli 1999). These temporal changes should affect the population dynamics of the amphipod fauna, which would explain a proportion of the temporal variability found among sampling times. Nonetheless, appropriate temporal replication is needed in order to test seasonal and intrannual patterns of amphipod community.

Nevertheless, the abundance of potential food items could result from a balance

between refuge against predation and the availability of favourable space for living (Orth 1992). Therefore, it may be necessary to consider the effects of predation to explain the changes of amphipod abundance across habitats detected on our study. Experimental studies carried out by Moksnes et al. (2008) revealed that seagrass communities are dominated by strong top-down processes that regulate the biomass and assemblage structure of both macroalgae and mobile fauna. In a manipulative experiment involving treatment with a caged goby fish predator, size-specific predation reduced the abundance of adult gammarids by 93% (Moksnes et al 2008). Caine (1991) also detected a substantial reduction of amphipod abundance due to predation. Predation pressure by fishes in temperate shallow environment is very high during warm seasons, and all habitats, except *P. oceanica* meadows, had reduced amphipod abundance during summer. Perhaps predation pressure in *P. oceanica* meadows is lower as a result of the high complexity of the leaf stratum (Orth 1992).

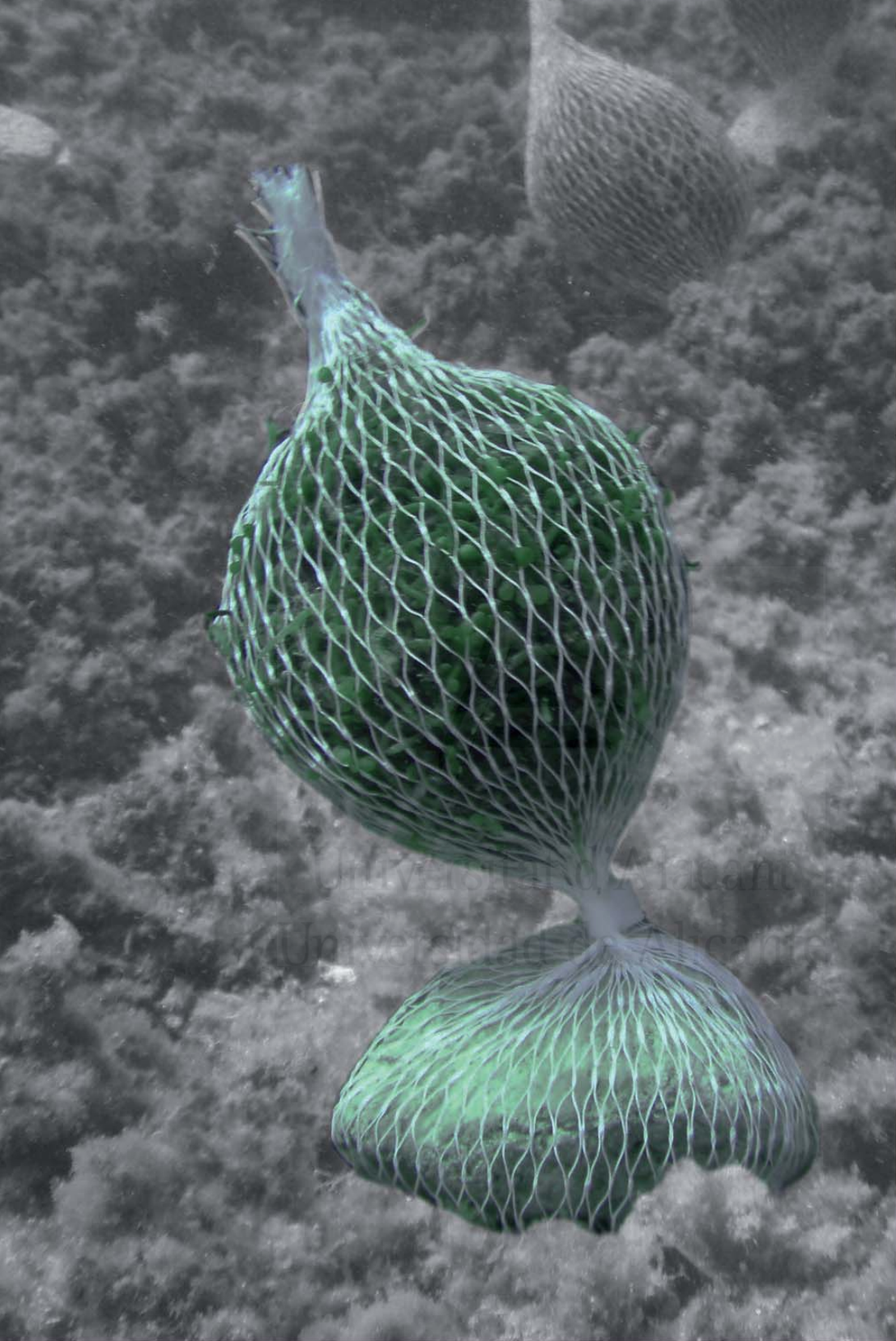
One remarkable property of the two *Caulerpa* species is the accumulation of detritus, which persisted throughout the year. In both species, the amount of detritus was higher than in seagrass meadows, but was especially high in *C. prolifera* stands, which had up to 6.5 times more detritus than *C. racemosa* beds stands. Detritus plays a very important role as a trophic resource for marine invertebrates, being one of the main trophic pathways of the marine ecosystem (Valiela 1995) and is one of the most important features of habitat structure in vegetated habitats (Allesina et al. 2005). Therefore, any modification of the detritus compartment can affect the overall trophic web, because it could lead to a change in the faunal assemblage structure resulting from modification of trophic guilds due to different trophic requirements. The detritus compartment could be supporting the high abundance of amphipods in both *C. prolifera* and *C. racemosa* stands, but perhaps the detritus also serves to make the invertebrates less available to predators (e.g. fish), unless the latter are more specialized for foraging, such as *Mullus*

surmuletus, which was frequently seen feeding in *C. racemosa* stands on soft bottoms (per. obs.).

It has been demonstrated that amphipods can actively choose substrata and show high habitat fidelity (Nelson 1979, Stoner 1980, Roberts and Poore 2005, Poore and Hill 2006). Further to this, our study shows that some species have a higher abundance in specific habitats. For example, some of the species recorded in our study, such as *Caprella grandimana*, *S. monoculoides*, *M. obtusatus* and *A. acutum*, were associated with *C. racemosa*. We think that these species are favoured by *C. racemosa* because of two main factors that are related to the stolon structure and associated algae: reduction of hydrodynamism and increased sedimentation. *Caprella grandimana* is very common in algae that inhabit the intertidal and shallow waters along the Strait of Gibraltar and other Mediterranean localities in the vicinity of our study area (Guerra-García, pers. com.) The same applies to *Stenothoe monoculoides*, which is usually associated with photophilic algae in infralittoral zone (Ruffo 1998). *Microdeutopus obtusatus*, is generally associated with *Ulva*, *P. oceanica* meadows and bare fine sand habitats (Ruffo 1982), but has also recently been recorded from sediments (Roberts and Poore 2005) and *C. racemosa* stands on rocky habitats (Vázquez-Luis et al. 2008). In our study area, *Apocorophium acutum* was very abundant in *C. racemosa* stands in September, while this species seems to prefer environments having a low hydrodynamic regime and high sedimentation rate (Conradi et al. 1997; Guerra-García and García-Gómez 2005). That *Ampelisca diadema* is closely associated with *C. prolifera*, and even with *C. racemosa* may be related to the increase of fine sediment fraction trapped. This species appears normally at sandy and muddy bottoms (Ruffo 1982), and possibly the sediment accumulation by *Caulerpa* species generates the perfect habitat in which to live. This species has been

reported by Sánchez-Moyano et al. (2007) as being typical of *C. prolifera* meadows. *Ampithoe ramondi* has a preference for native seaweeds and seagrasses (Ruffo 1982, Viejo 1999, Roberts and Poore 2005, Vázquez-Luis et al. 2008). We have detected, as Sánchez-Moyano et al. (2001), the displacement of this species resulting from the presence of *C. prolifera*. All amphipods are herbivorous, so the value of the algae or their epiphytes as food could be important factors in the distribution. *Caulerpa* species produce metabolites against epiphytes, competition and predators and studies carried out by Gollan and Wright (2006) demonstrated that *Caulerpa taxifolia* is low preference food for the amphipoid *Cymadusa setosa*. Other important species, such as *Hyale schmidtii*, *Erichthonius brasiliensis*, *Leptocheirus guttatus* and *Elasmopus brasiliensis* are highly abundant at *P. oceanica* meadows, a result consistent with other authors (Sánchez-Jerez et al. 1999, Sánchez-Jerez et al. 2000, Borg et al. 2006).

In conclusion, our study shows that *C. racemosa* stands present on soft substrata present different amphipod assemblage than other Mediterranean habitats on soft substrate. The observed abundance and species richness of amphipods were relatively high. It is important to note that stands of *C. prolifera*, which is considered a native species of the Mediterranean Sea, had relatively low amphipod abundance and species richness compared with the invasive *C. racemosa*. Since *C. racemosa* has spread very rapidly and extensively during the last 17 years (Klein and Verlaque 2008), one predicts considerable changes, in the near future, in the composition of the faunal assemblages associated with soft-bottom habitats, given the expected colonisation of large areas of the sea bed by the species. Such a change, which may occur alongside gradual regression of seagrass meadows, may contribute to a major change in the structure of the Mediterranean benthic assemblages.



Does *C. racemosa* affect colonization of vegetated habitats by amphipods?

4. Does *C. racemosa* affect colonization of vegetated habitats by amphipods?

4.1. Abstract

Invasion of algal habitat by *Caulerpa racemosa* results in changes to the habitat structure, part of which may be due to accumulation of large amounts of detritus. In turn, this leads to alterations in the assemblage structure of associated invertebrates. Since it has been demonstrated that amphipods can actively choose a substratum, the present work addressed the question: "Do amphipods associate with the newly available habitat provided by *C. racemosa* or is the presence of detritus responsible for changes in the structure of associated invertebrate assemblages?" Field experiments on habitat colonisation were undertaken at two Mediterranean localities (Malta and Spain), and at two sites within each locality. Using mesh bags, 3 different habitat types were deployed at each site: *Halopteris scoparia*, *C. racemosa* and artificial substratum. Additionally, empty mesh bags were deployed as controls. Mesh bags containing plant material were defaunated prior to deployment. For each of the three habitat types, three treatments were applied: no detritus supplement, low detritus supplement, and high detritus supplement. The bags were placed on a rocky substratum at a water depth of 4.5 - 5 m for a period of 14 days, after which they were collected and the amphipod assemblage assessed. The results showed that: (i) overall, amphipod species richness and abundance were higher in bags containing *H. scoparia*, and (ii) the presence of detritus is related to increased values of amphipod species richness and abundance in *C. racemosa* habitat but not in *H. scoparia* habitat. We conclude that some amphipods exhibit a wide distribution range and are not affected by the spread of *C. racemosa*, but the detritus accumulated by *C. racemosa* is a determining factor for selection by some amphipods.

4.2. Introduction

Marine benthic biota depend on their substrata for physical support, whether surface or within sediments, as well as to acquire food, be it prey or other sources of nutrients (Sebens 1991). There are at least two architectural attributes that have a large influence on the diversity and abundance of species within a community. The first is the physical complexity of the substratum; the second is the structural element provided by other organisms on the substratum (Sebens 1991). Habitat structure strongly influences processes that determine the abundance and distribution of species (Orth et al. 1984, Beck 2000). It has been widely demonstrated that the variety of habitat and microhabitat types, hence available niches, affect the coexistence of species and species diversity (Huston 1979). Clearly, the greater number of different habitat types, the higher the biodiversity. In shallow rocky marine habitats, macrophytes are the most important organisms that contribute to habitat structural complexity and heterogeneity. Macrophytes act as ecosystem engineers; essentially by creating or modifying a habitat (Crooks 2002). The

effects of habitat complexity on benthic ecosystems are many. Two main aspects contribute to habitat complexity: (1) habitat heterogeneity or patchiness of habitat types across a landscape; and (2) habitat structure, the physical or architectural component of complexity (Sebens 1991). In general, Mediterranean rocky bottom algal habitats consist of mosaics and patches with different algal species that are aggregated in space at different spatial scales. Habitat complexity differs between algal stands formed by different species and is influenced by factors such as whether the species is encrusting or erect, tall or low lying (turf), or if it supports epiphytes. Hence, algal habitats can be very heterogeneous, such that availability of habitat/microhabitat space for colonisation by other species varies greatly.

Disturbance events, such as the introduction of an invasive species, can modify habitat structure and, consequently, result in large ecological effects (see review in Williams and Smith 2007). The number of introduced species outside their natural ranges is rapidly increasing, although a relatively small proportion of transported and

introduced species become invasive (Williamson and Fitter, 1996). However, the ability of such species to restructure and, hence, radically change the functioning of the recipient habitat, is high (Crooks 2002). Invasive species may: (a) modify the availability and flow of nutrients in an ecosystem by altering biogeochemical cycling; (b) contribute to changes in energy flow through a food web; and (c) affect the availability or quality of physical resources in the ecosystem, including the physical characteristics of a habitat (available space, sediment properties, light regime and water quality; see Vitousek 1990, Crooks 2002). Frequently, an invasive species acts as an ecosystem engineer, hence, determining the biota that will become associated with the habitat. When this occurs, the resulting community will have mixed characteristics that are attributed to the initial pool of species and habitat preferences, and to colonization rate of the invasive species.

Amphipods are one of the most ubiquitous and abundant invertebrate groups in marine vegetated habitats, with densities often reaching several thousands individuals per square meter (Brawley 1992, Vázquez-Luis et al. 2008). They are important secondary producers (Carrasco and Arcos 1984, Highsmith and Coyle 1990, Sarvala and Uitto 1991) and serve as a food source for a large variety of marine fauna (Stoner 1979, Beare and Moore 1997, Sanchez-Jerez et al. 1999, Stål et al. 2007), hence playing a key role in energy flow through food webs (Vázquez-Luis et al., 2010). Amphipods respond to habitat modification (Vázquez-Luis et al. 2008, 2009b); some species exhibit high habitat specificity while others tolerate a range of habitat alteration that may result from pollution, invasion by alien species, and other disturbances, and can therefore be used as an indicator of environmental impacts on vegetated habitats (Bellan-Santini 1980, Virmstein 1987, Conradi et al. 1997, Sanchez-Jerez et al. 2000).

In the Mediterranean Sea, concerns on the appearance of the alien alga *Caulerpa racemosa* var. *cylindracea* (hereafter *C. racemosa*) have resulted in several studies on the ecology of this species (see Ruitton

et al. 2005, Cavas et al. 2006). A main concern is that the fauna associated with native vegetation can change with the introduction of exotic algae (Wikstrom and Kautsky 2004, Schmidt and Scheibling 2007). Hence, several works have focussed on assessing potential changes in the faunal assemblages associated with Mediterranean vegetated habitats that may be brought about by colonisation by *C. racemosa* (Argyrou et al. 1999, Piazzini and Balata 2008, Vázquez-Luis et al. 2008, 2009a, b). Studies have shown that *C. racemosa* may completely change habitat structure, as it establishes homogeneous monospecific stands in which detritus accumulates (Vázquez-Luis et al. 2008, 2009b). However, studies on the ecology of macrofauna associated with *C. racemosa* stands have focussed on post-invasion assessment, while there is a dearth of data on the situation before invasion by the alien alga compared to that following invasion. We undertook experiments to test the hypothesis that *C. racemosa* may affect colonization of vegetated habitats by amphipods, and that such an effect mainly results from accumulation of detritus by the alien alga. Furthermore, our experiment was replicated in two Mediterranean localities, separated by thousands of kilometres, to assess occurrence of the same patterns over a large spatial scale.

4.3. Materials and methods

4.3.1. Study sites

Experiments were carried out in two Mediterranean localities: Malta and Spain. In each locality, two rocky sites with broadly similar environmental characteristics and supporting algal habitat which was not invaded by *C. racemosa*, were selected (Fig. 4.1). In Malta, the two sites were Ghallis and Pembroke, both located on the northeastern coast; in Spain, the two sites were Albir and Sierra Helada, both located on the Benidorm coast (Alicante). The four sites had similar water depths and habitat type, which comprised rocky bottom colonised by a patchy bed of native algae.

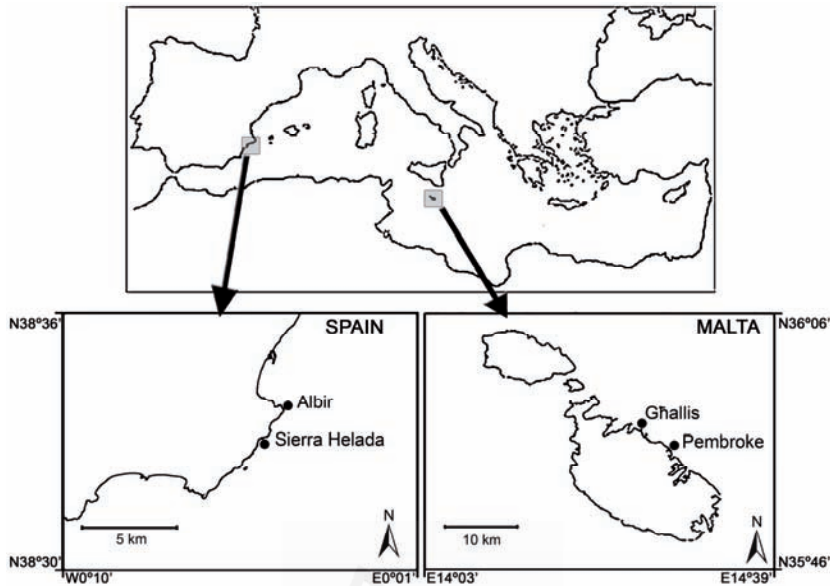


Figure 4.1. Map showing the two localities and sites within each locality where the experiments were held: Albir and Sierra Helada, located in Spain, and Ghallis and Pembroke, located in Malta.

4.3.2. Characterisation of habitat features and amphipod fauna

To characterize the algae and amphipod fauna at the study sites, three random samples were taken by SCUBA divers at each site using a 20 x 20 cm quadrat. Samples were collected by scraping off all the vegetation enclosed in the quadrat. A 300 µm mesh net was attached to the quadrat to prevent escape of motile fauna during collection. Samples were preserved in 4% formalin in seawater. Each replicate was sieved in sea-water using a 500 µm sieve. In the laboratory, the amphipods were separated, identified and counted. Algae were sorted and identified to species level and dried for 24 h in an oven at 80°C to obtain dry weight.

4.3.3. Experimental setup and sampling design

For the field experiments, two different algae were harvested: the native *H. scoparia* and *C. racemosa*. *H. scoparia* was

selected because is very common in shallow water Mediterranean benthic habitats. In the laboratory, the algae were defaunated and their wet weight recorded. Dry *Posidonia oceanica* 'balls' (Cannon 1979) were used as source for the detritus stock. Mesh bags with different treatments (plant substratum and detritus) were then prepared as follows. Bags containing algae (*H. scoparia* or *C. racemosa*) had 80 g (wet weight) per bag while those containing 'artificial algae' (garden shade fabric) had a volume equivalent to that of algae. Two different treatments were used for bags with detritus: low detritus supplement (15 g wet weight) and high detritus supplement (50 g wet weight). These detritus weights were chosen because on the basis that they were equivalent to the amount of detritus found in habitats colonised by native algae and *C. racemosa*. Accordingly, 15 g represent around 15% detritus, which is the typical quantity associated with native algae (Vázquez-Luis et al. 2008), while 50 g represent around 40% detritus, which is the quantity found associated with *C. racemosa*

(Vázquez-Luis et al. 2008, 2009a). Five replicate bags per category/treatment were prepared, such that a total of 120 bags per locality were used. The experimental design incorporated three factors: (i) 'Habitat' which was fixed and had 4 levels: *H. scoparia*, *C. racemosa*, artificial algae and control (only the mesh bag); (ii) 'Detritus', which was fixed and orthogonal and had 3 levels (without detritus, low detritus supplement and high detritus supplement); and (iii) 'Site', which was random and orthogonal, and had two levels (for each study locality).

In the field, all bags were deployed at random at each study site on a rocky bottom supporting algal habitat at a water depth of 4.5 - 5 m. Each mesh bag had a mesh size of 1.6 cm, was 30 cm long and had a large pebble (collected from a coastal beach) inside to ensure it remained stationary on the seabed, while the rest of the bag contained the particular algae/*C. racemosa*/detritus treatment, and had its upper end closed. Deployment of the mesh nets was made during summer 2008. At each site the area of seabed over which the mesh bags were spread was around 20 m². After two weeks from deployment, the mesh bags were collected. Immediately following collection, each one was placed in a plastic bag to avoid escape of macroinvertebrates. In the laboratory, the collected samples were first preserved in 4% formalin and later sieved through a 500 µm mesh sieve to retain the fauna and other material, which was then sorted. All amphipods were identified to species level and counted. An index of complexity for the habitat complexity of the two algae was calculated as the ratio between the surface area and volume provided (SA/V) by each alga. A surface-to-volume ratio (SA/V) is a simple, but practical, measure of the complexity of biogenic habitats (Schmidt-Nielson 1984, Koukouras et al. 1992). *H. scoparia* had a higher structural complexity compared to *C. racemosa* (4.38 ± 0.06 and 6.45 ± 0.19 respectively, N=4).

4.3.4. Data analyses

For each locality (Malta and Spain), differences in number of species, total

abundance of amphipods, and abundance of the most abundant amphipod species between different sites were tested using three-factor Analysis of Variance (ANOVA). Prior to carrying out the ANOVA, the data were tested for heterogeneity of variance using Cochran's C-test. Data were $\sqrt{x + 1}$ or $\log(x + 1)$ transformed in cases where the variances were significantly different at $P = 0.05$. Where variance remained heterogeneous following transformation, untransformed data were analysed, as ANOVA is a robust statistical test that is relatively unaffected by heterogeneity of variances, particularly in balanced experiments (Underwood 1997). However, in such cases, special care was taken in the interpretation of results, and to reduce type I error, α was reduced to 0.01. Where ANOVA indicated a significant difference for a given factor, the source of differences was identified using Student–Newman–Keul (SNK) tests (Underwood 1981, 1997). Affinities between the amphipod assemblage structure and treatments were explored by cluster analysis through a similarity matrix, which was calculated using the Bray–Curtis index and using double root transformed data (PRIMER software; Clarke 1993).

4.4. Results

4.4.1. Habitat and amphipod fauna

A total of 19 algal species was recorded from the Maltese sites and 11 from the Spanish ones. Values of total algal biomass were higher at the Maltese sites compared to the Spanish ones (334.34 ± 63.93 g dw m⁻² and 266.59 ± 51.21 g dw m⁻² respectively). The species that contributed most to biomass at the Maltese sites were *Cystoseira* spp. (182.04 ± 81.61 g dw m⁻²), *Halopteris scoparia* (70.64 ± 50.84 g dw m⁻²) and *Cladostephus spongiosus* (46.03 ± 46.6 g dw m⁻²), while at the Spanish sites the main contributors were *Corallina elongata* (111.27 ± 45.65 g dw m⁻²), *Halopteris scoparia* (75.5 ± 35 g dw m⁻²) and *Jania rubens* (21.41 ± 6.19 g dw m⁻²).

A total of 37 amphipod species were recorded from the Maltese sites and 34

from the Spanish ones. At both localities, the abundance of amphipods were high, with values of up to 5000 individuals per square meter being recorded, but large differences in values were noted between different sites. In Malta the most abundant species were (in order of decreasing abundance): *Elasmopus brasiliensis* (602.34 ± 152.85 ind m^{-2}), *Ampithoe ramondi* (485.38 ± 98.06 ind m^{-2}), *Dexamine spiniventris* (362.57 ± 89.65 ind m^{-2}), *Erichthonius punctatus* (347.95 ± 72.96 ind m^{-2}), *Hyale camptonyx* (301.17 ± 104.03 ind m^{-2}) and *Cymadusa crassicornis* (178.36 ± 113.01 ind m^{-2}). In Spain, the most abundant species were: *Ampithoe ramondi* (514.62 ± 119.98 ind m^{-2}), *Microdeutopus* spp. (450.29 ± 80.61 ind m^{-2}), *Dexamine spiniventris* (400.58 ± 236.91 ind m^{-2}), *Caprella grandimana* (391.81 ± 190.66 ind m^{-2}), *Caprella acanthifera* (321.64 ± 60.38 ind m^{-2}) and *Hyale schmidti* (201.75 ± 83.74 ind m^{-2}).

4.4.2. Colonization

Thirty four amphipod species colonised the mesh bags in Malta and 33 in Spain. Species richness was significantly greater in

bags containing *H. scoparia* than all other treatments at both localities (Malta: Ha, $P < 0.01$; Table 4.1, Fig. 4.2a; Spain: Ha, $P < 0.05$; Table 4.1, Fig. 4.2b). Total abundance was significantly higher at the Maltese sites for bags containing *H. scoparia* (Ha, $P < 0.05$; Table 4.1 and Fig. 4.2c); the highest values recorded were for bags having a high content of detritus (95.7 ± 11.22 ind/bag). No significant differences were detected at the Spanish sites, although a similar pattern was obtained (Table 4.1, Fig. 4.2d). Regarding the detritus treatment, significant differences in species richness were detected at the Maltese sites, where bags having low and high detritus content had a significantly higher number of species (De, $P < 0.05$; Table 4.1, Fig. 4.2a). Moreover, at one of Maltese sites total abundance was higher in bags containing high detritus content, while in the other site both high and low detritus treatments presented higher values (DexSi, $P < 0.05$; Table 4.1, Fig. 4.2c). At the Spanish sites, no significant differences were detected for the detritus treatment, but a pattern indicating an increase in amphipod abundance with an increase in the content of detritus was noted.

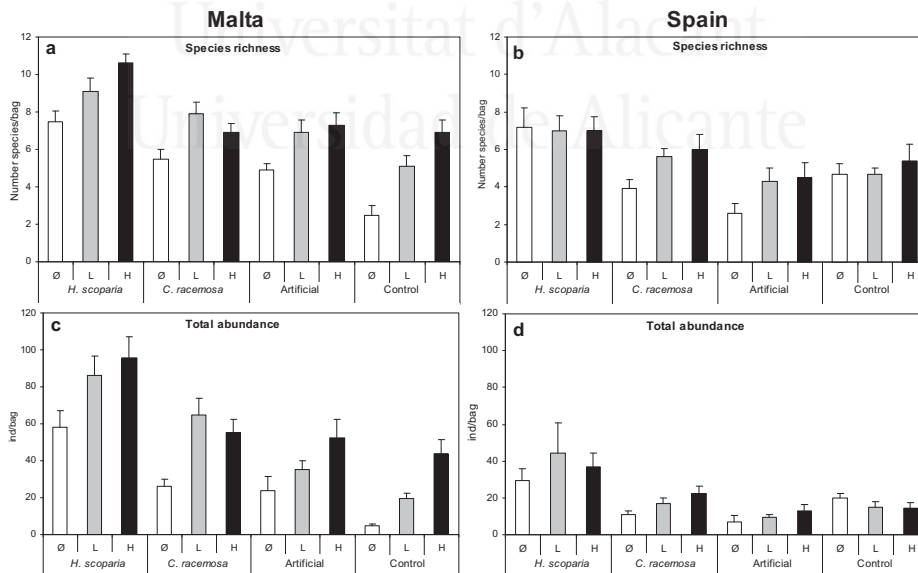


Figure 4.2. Values (\pm SE) of: mean number of amphipod species (a and b) and total amphipod abundance (individuals per bag) (c and d) recorded from each treatment in Malta (a and c) and Spain (b and d). Ø = without detritus, L = low detritus supplement and H = high detritus supplement.

The dendrogram from cluster analyses showed segregation of amphipod assemblages mainly by the combination of habitat and detritus content. In Malta, a main cluster can be distinguished at similarity of 75%, corresponding to the three detritus levels of *H. scoparia*, to low and high detritus treatment for *C. racemosa*, and to control with high detritus content (Fig. 4.3a). The artificial substratum

and the treatment without detritus were clustered at a lower similarity than the main group. In Spain, this pattern was clearer at a similarity of 70%, where *H. scoparia* with the three levels of detritus was grouped together with *C. racemosa* and control habitats with low and high detritus treatment. Also, artificial substratum and *C. racemosa* and control without detritus showed the highest dissimilarity (Fig. 4.3b).

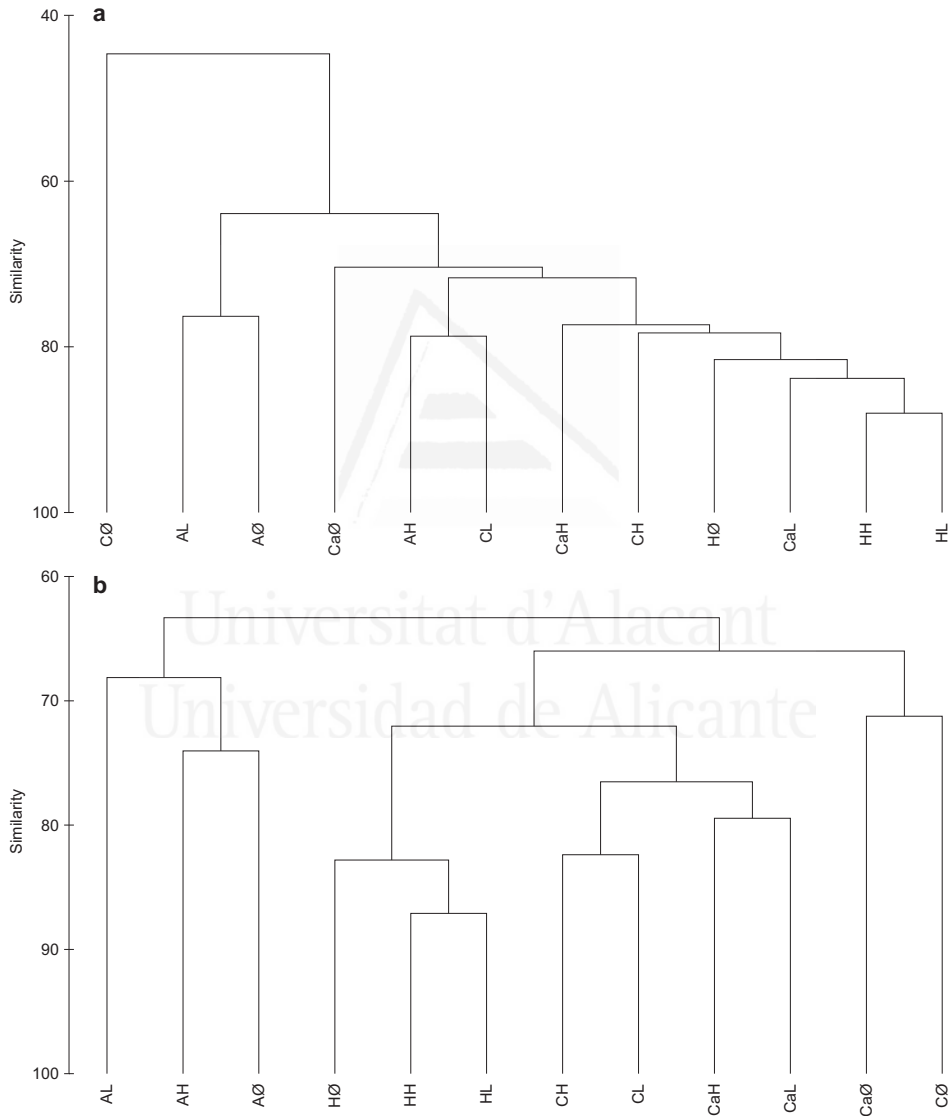


Figure 4.3. Dendrogram based on the amphipod assemblage data showing similarity between habitat types and detritus treatments in Malta (a) and Spain (b). H = *H. scoparia*, Ca = *C. racemosa*, A = artificial, C = control; Ø = without detritus, L = low detritus supplement and H = high detritus supplement.

Table 4.1. Results of the three-factor ANOVA for species richness, total abundance, and species abundance of the most abundant amphipods. MS = mean square; P = level of significance; df = degrees of freedom; ns = not significant; * = significant ($P < 0.05$); ** = significant ($P < 0.01$).

MALTA										
Source of variation	df	Species Richness		Total abundance		<i>Elasmopus brasiliensis</i>		<i>Elasmopus poecillimanus</i>		F versus
		MS	P	MS	P	MS	P	MS	P	
Habitat=Ha	3	91.88	0.0011**	17746.16	0.0221*	42.57	0.0259*	12.19	0.0516	HaxSi
Detritus=De	2	87.06	0.0170*	11781.51	0.2333	31.1	0.0156*	9.58	0.111	DexSi
Site=Si	1	20.01	0.0139*	5057.01	0.0013**	3.48	0.0693	25.52	0.0001**	Res
HaxDe	6	5.66	0.37	668.13	0.3921	2.53	0.3902	1.19	0.7662	HaxDexSi
HaxSi	3	0.69	0.883	1053.87	0.0827	2.83	0.0473*	1.35	0.1651	Res
DexSi	2	1.51	0.6241	3586.01	0.0007**	0.49	0.6208	1.19	0.2192	Res
HaxDexSi	6	4.16	0.2413	529.07	0.3385	1.99	0.0826	2.22	0.0133*	Res
Residual	96	3.18		459.16		1.03		0.78		
Cochran's C-test		C=0.0942 ns		C=0.1626 ns		C=0.1541 ns		C=0.1530 ns		
Transformation		-		-		Sqrt(x+1)		Ln(x+1)		
SNK		Ha: H>Ca=A>C De: L=H>Ø		Ha: H>Ca=A=C DexSi: 1: H=L>Ø 2: H>L>Ø		De: L=H>Ø HaxSi: 1: H>Ca>A=C 2: H>Ca>C>A				
SPAIN										
Source of variation	df	<i>Gammarella fucicola</i>		<i>Caprella acanthifera</i>		<i>Microdeutopus spp.</i>		<i>Lyssianasa costae</i>		F versus
		MS	P	MS	P	MS	P	MS	P	
Habitat=Ha	3	25.96	0.1024	1.31	0.0204*	1.29	0.4595	30.79	0.3824	HaxSi
Detritus=De	2	11.14	0.5807	0.18	0.2301	1.19	0.1329	4.13	0.2832	DexSi
Site=Si	1	5.29	0.1733	5.28	0.0001**	4.72	0.0001**	42.01	0.0001**	Res
HaxDe	6	1.57	0.9588	0.25	0.7638	0.1	0.6564	1.31	0.3297	HaxDexSi
HaxSi	3	4.91	0.1623	0.07	0.8722	1.14	0.0078**	21.14	0.0001**	Res
DexSi	2	15.43	0.0055**	0.05	0.8395	0.18	0.5111	1.63	0.1409	Res
HaxDexSi	6	7.36	0.0213*	0.46	0.1975	0.14	0.8021	0.9	0.3669	Res
Residual	96	2.81		0.31		0.27		0.82		
Cochran's C-test		C=0.1616 ns		C=0.1093 ns		C=0.1563 ns		C=0.4439 ($p < 0.01$)		
Transformation		Sqrt(x+1)		Sqrt(x+1)		Ln(x+1)		-		
SNK		DexSi: 2: H>L=Ø		Ha: Ca>H=A=C		HaxSi: 1: Ca>H>A=C		HaxSi: 1: H>Ca=A=C		
Source of variation	df	<i>Gammarella fucicola</i>		<i>Caprella acanthifera</i>		<i>Microdeutopus spp.</i>		<i>Lyssianasa longicornis</i>		F versus
		MS	P	MS	P	MS	P	MS	P	
Habitat=Ha	3	1015.84	0.0807	1.47	0.4701	4.38	0.1801	4.05	0.1566	HaxSi
Detritus=De	2	166.07	0.5125	0.29	0.7863	1.67	0.5523	0.96	0.345	DexSi
Site=Si	1	116.03	0.4549	7.79	0.0001**	23.41	0.0001**	0.95	0.0792	Res
HaxDe	6	230.42	0.1855	0.49	0.5238	0.49	0.5661	0.65	0.0922	HaxDexSi
HaxSi	3	158.46	0.5143	1.34	0.0392*	1.35	0.0294*	0.1	0.0149*	Res
DexSi	2	174.56	0.4319	1.06	0.1064	2.07	0.0106*	0.51	0.1901	Res
HaxDexSi	6	106.68	0.7937	0.51	0.3627	0.57	0.2583	0.2	0.6663	Res
Residual	96	206.12		0.46		0.43		0.3		
Cochran's C-test		C=0.8863 ($p < 0.01$)		C=0.1524 ns		C=0.1526 ns		C=0.1527 ns		
Transformation		-		Sqrt(x+1)		Sqrt(x+1)		Ln(x+1)		
SNK				HaxSi: 1: A>H=Ca=C		HaxSi: 1: H=Ca>A=C DexSi: 1: H>Ø=L		HaxSi: 1: H>Ca=A=C		

The most abundant amphipods recorded from the two study localities were *Elasmopus brasiliensis*, *E. pocillimanus*, *Gammarella fucicola*, *Caprella acanthifera*, and *Microdeutopus* spp., while 2 *Lysianassa* species, *Lysianassa costae* and *L. longicornis* were most abundant in Malta and Spain respectively. These species contribute to 82% of amphipod fauna in Malta and to 54% in Spain. The abundance values of the various species were different for different treatments of 'habitat' and 'detritus', while differences among sites were also noted. Abundance values of *Elasmopus brasiliensis* were significant higher in *H. scoparia* habitat in both Malta and Spain (Malta: HaxSi, $P < 0.05$; Table 4.1, Fig. 4.4a; Spain: Ha, $P < 0.01$; Table 4.1, Fig. 4.4b); for the factor 'detritus', the abundance of this species was significantly higher for low and high detritus content at the Maltese sites (De, $P < 0.05$; Table 4.1, Fig. 4.4a). *E. pocillimanus* appeared to be more abundant in bags having a high algal content, for both *H. scoparia* and *C. racemosa*, however, no significant differences were detected by the ANOVA (Fig. 4.4c). In Spain, this species was abundant in all habitat types except the artificial substratum (Fig. 4.4d).

G. fucicola had a high abundance in both Malta and Spain, particularly in bags containing *H. scoparia*. However, no significant differences were detected by the ANOVA, although a significant interaction between detritus and site was evident for the Maltese site, with significantly higher abundance of this amphipod being recorded from bags having a high content of detritus at one site (DexSi, $P < 0.01$; Table 4.1, Fig. 4.4e). The abundance values of this species were also high at the Spanish sites (Fig. 4.4f).

C. acanthifera showed different patterns of abundance at the two study localities. In

Malta, the abundance of this species was significantly higher on *C. racemosa* (Ha, $P < 0.05$; Table 4.1, Fig. 4.4g). For one site in Spain, a significant interaction for habitat and site was indicated; the abundance of this amphipod was higher on artificial substratum at one site (HaxSi, $P < 0.05$; Table 4.1, Fig. 4.4h), while no significant differences in abundance were detected between different habitats at the other site.

Species of the genus *Microdeutopus* were, in general, more abundant on *H. scoparia* and *C. racemosa*; in Malta *Microdeutopus* spp. was significantly more abundant on *C. racemosa* at one site (HaxSi, $P < 0.01$; Table 4.1, Fig. 4.4i). In Spain, significant differences in the abundance of these species were detected for habitat and detritus, with a significant interaction being indicated at one of the two sites. The abundance of *Microdeutopus* spp. was higher on *H. scoparia* and *C. racemosa* at one site (HaxSi, $P < 0.05$; Table 4.1, Fig. 4.4j). A significant interaction for detritus and site was also indicated for *Microdeutopus* spp.; at one of the Spanish sites, abundance was higher in the bag having a high content of detritus (DexSi, $P < 0.05$; Table 4.1, Fig. 4.4j), while no differences in abundances were evident at the other site for the different detritus content levels (DexSi, $P < 0.05$; Table 4.1, Fig. 4.4j).

Species of the genus *Lysianassa* showed overall abundance patterns whereby the amphipods were more abundant in *H. scoparia*. For *L. costae*, which was more abundant at the Maltese sites, a significant interaction of habitat and site was detected; abundance values were higher at one site on *H. scoparia* (HaxSi, $P < 0.01$; Table 4.1, Fig. 4.4k). *L. longicornis*, which was more abundant at the Spanish sites, had a higher abundance on *H. scoparia* at one site (HaxSi, $P < 0.05$; Table 4.1, Fig. 4.4l).

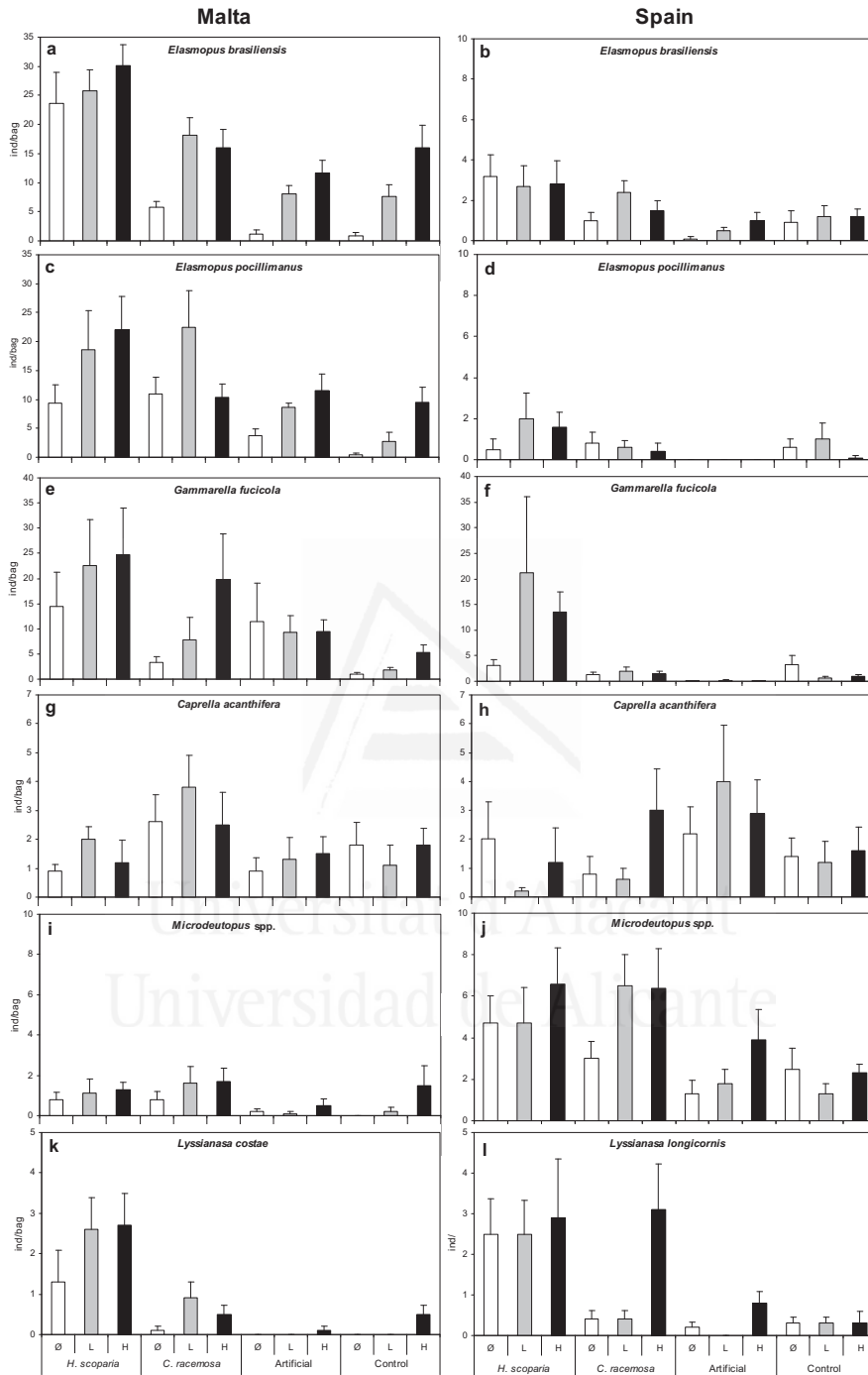


Figure 4.4. Abundance (number of individuals \pm SE per bag) of: *E. brasiliensis* (a and b), *E. pocillimanus* (c and d), *G. fucicola* (e and f), *C. acanthifera* (g and h), *Microdeutopus* spp. (i and j), *L. costae* (k) and *L. longicornis* (l) recorded from in Malta (a, c, e, g, i and k) and in Spain (b, d, f, h, j and l). Note that *E. brasiliensis* and *E. pocillimanus* present different scale bar in y axis. Ø = without detritus, L = low detritus supplement and H = high detritus supplement.

4.5. Discussion

Both species richness and abundance of amphipods were higher in bags containing *H. scoparia*. Furthermore, for *C. racemosa* habitat, the results suggest that the presence of detritus results in increased values of amphipod species richness and abundance, even though the recorded values did not reach ones recorded for the native algal species. *H. scoparia* forests have a high structural complexity and serve as an important habitat for amphipod assemblages, as shown by the highest values of species richness and abundance recorded for amphipods in the present study. Artificial substrata provide a refuge to amphipods, but natural vegetated habitats may be more attractive to this faunal group.

In general, three main processes may account for faunal distribution patterns. One is different rates of settlement/recruitment; larvae or juveniles can recruit in larger numbers to one habitat than to others (Moore and Lopez 1972, Dayton 1975). In our experiment, rate of recruitment should not be used to explain the observed patterns of species richness and abundance since the study was not carried out over a sufficiently long period, while relatively small numbers of juvenile amphipods were recorded in the mesh bags. A second alternative is that populations are distributed randomly in available habitats, but differential mortality leads to reduction in numbers in unfavourable habitats (Levins 1968, Russo 1987). The random distribution of fauna may be related to that of the host plants since neighbouring plants may affect the ability of faunal species to locate their preferred host (Poore 2004). Moreover, previous studies have demonstrated that predation rates on amphipods are different depending on species, and on the habitat where they live (Vázquez-Luis et al., 2010). Nevertheless the general pattern noted in the present study is consistent and it would seem that amphipods association with habitats results from specific selection and, possibly, similar predation pressure on replicate bags. It has been demonstrated that amphipods can actively choose substrata and show high habitat fidelity (Stoner 1980, Roberts and Poore 2005,

Poore and Hill 2006). A third alternative is that, following recruitment of juveniles, adults select favoured habitats (Underwood and Denley 1984, Crowe and Underwood 1998). In our study, the possibility that amphipods prefer *H. scoparia* compared to the other habitats may explain their observed distribution among treatments. However, since such processes are not exclusive, a combination of these may be responsible for the observed patterns of distribution of amphipods.

Several studies highlight the importance of habitat structural complexity in determining the abundance and species richness of associated mobile epifauna (Orth et al. 1984, Martin-Smith 1993, Beck 2000, Chemello and Milazzo 2001, Grabowski and Powers 2004). The present results show that some amphipod species have a higher abundance in specific habitats. For example, some of the species recorded in our study, such as *E. brasiliensis*, *G. fucicola*, *L. costae* and *L. longicornis*, were associated with *H. scoparia*. These species colonised the mesh bags with *H. scoparia* in higher numbers compared to the other treatments, possibly because of the higher structural complexity of this alga. On the other hand, the artificial plant substratum used in our experiment had a relatively high structural complexity and should therefore have resulted in a higher abundance of associated amphipods. Therefore, apart from habitat complexity, other factors play an important role in colonization of substratum by amphipods; possibly, availability of food, chemical cues, and palatability, are important factors in influencing colonisation by amphipods distribution in different vegetated habitats.

Artificial substrata usually support a community that is not representative of the local species pool (Smith and Rule, 2002). The artificial substratum used in our experiment had a relatively high structural complexity, but the presence of natural habitats would present a higher quality habitat for some species. *E. pocillimanus* and *Microdeutopus* spp. were more abundant in mesh bags with *H. scoparia* and *C. racemosa*; the plant content seems to be a determining factor for some species,

while *C. racemosa* does not appear to be an unfavourable substratum. Moreover, species of the genus *Microdeutopus* have been reported to have higher abundance values on substrata colonised by *C. racemosa* compared to ones colonised by native algae (Vázquez-Luis et al. 2008, 2009b). In contrast, *C. acanthifera* showed different patterns of abundance at the two localities; in Malta it was more abundant in *C. racemosa* habitat, while in Spain it was more abundant on artificial substratum. Several caprellid species were recorded during the present study, but only *C. acanthifera* was consistently present in the mesh bags. *C. acanthifera* has been reported as a common species on many different substrata (see Guerra-García 2001) and can tolerate stressed habitats having low water movement and high rates of sedimentation and organic matter (Guerra-García and García-Gómez 2001). The different patterns recorded from the two localities may have resulted from differences in such environmental characteristics between the two localities. It should also be noted that the mesh *per se* serves as an ideal substratum for the attachment of caprellids using their pereopods, while amphipods belonging to this group are also predominantly found attached to the more exposed parts of plants (Aoki 1999, Sudo and Azeta 2001).

Availability of food may play an important role for substratum selection by the amphipods. Our results showed that the species richness and abundance of amphipods increased with an increase in detritus content. The same applies to species abundance since values of this attribute increased with an increase in detritus content. Detritus plays a very important role as a trophic resource for marine invertebrates, and serves as one of the main trophic pathways in marine ecosystems (Valiela 1995), while it is also one of the most important features of habitat structure in vegetated habitats (Allesina et al. 2005). It is suggested that amphipods do not consume algal biomass directly, but feed on associated material such as detritus (Enequist, 1949). The detritus stock associated with *C. racemosa* appears to play an important role for

amphipod assemblages, as has been shown in other studies (Vázquez-Luis et al. 2008). It appears that gammarids are able to exploit the detritus as a microhabitat, while this is not as useful for caprellids which prefer the external canopy (Aoki, 1999). Usually, the feeding mechanisms of amphipods have been considered to depend on the morphology of mouthparts. Recent studies (Guerra-García and Tierno de Figueroa, 2009) that have assessed the diet of amphipods based on gut content analysis showed that, in caprellid species, detritus was clearly the main dietary component. Moreover, the only relationship found by these workers between the gut contents and mouthpart structural features was that a predatory mode of life of caprellids is directly related to the absence of molars. Therefore, detritus appears to play a very important role for amphipods as a source of food may serve as a very important attractant.

Some species were abundant in their natural habitat but were absent in the mesh bags. One explanation could be that the biology of a given species could be playing an important role. Herbivorous amphipods, such as those belonging to the family Ampithoidae and the genera *Hyale* and *Dexamine*, had high abundance values in their natural habitat but lower abundance values in the mesh bags at both study localities. Some species, such as *Ampithoe ramondi*, *Dexamine spiniventris*, *Hyale camptonyx* and *H. schmidti*, are strongly linked to the algal canopy, and are very sensitive to habitat alteration, and hence did not respond under experimental conditions. *A. ramondi* lives in transparent, gelatinous tubes on algae and seagrasses (Ruffo, 1982), and is therefore a sedentary species that does not displace from their host. In any case, lower abundance values of *A. ramondi* and *H. schmidti* in habitats invaded by *C. racemosa* and *C. prolifera*, have been detected in some studies (Sánchez-Moyano et al. 2001, Vázquez-Luis et al. 2008, Vázquez-Luis et al. 2009b). Moreover, the preference of amphipods for one or more habitats from a variety of potential habitats would be expected by this invertebrate group; in our experiment the amphipods may have

actively selected the natural habitats instead of the manipulated ones, for instance, by preferring the algae surrounding the mesh bags at our study sites. The results of our experiment can only be interpreted in the light of higher association or colonisation with *H. scoparia* habitat, since this is the only alga (apart from *C. racemosa*) used in our study. Therefore, it would be interesting to carry out similar experiments in which the preference of amphipods for other native plants is compared to that for *C. racemosa*. However, it is very important to carry out such experiments using appropriate experimental designs to avoid confounding influences (Olabarria et al., 2002).

In conclusion, when introduced, *C. racemosa* modifies rocky algal habitats, which results in changes to the abundance and species richness of associated amphipods. However, some amphipods fare

better in algal habitat that has been invaded by the alien species. The present findings suggest that the total abundance and species richness of amphipods is higher in algal stands formed by the native *H. scoparia*, but is also positively influenced by the presence of detritus. As a result, the presence of *C. racemosa* affects colonization of vegetated habitats by amphipods, given that this alga accumulated detritus. However, colonization of invertebrates is a complex process and seasonal changes of algal biomass and the life history of amphipod species may also be very important in determining substratum selection by amphipods. Further experiments on habitat selection and the diet of amphipods are necessary to obtain a better understanding of such potential changes to benthic ecosystem functioning that may result from the spread of invasive species.



Does *C. racemosa* modify the feeding habits of amphipods?

5. Does *C. racemosa* modify the feeding habits of amphipods?

5.1. Abstract

Invasive species are one of the most obvious threats to biodiversity, but there is a great ignorance of the effects generated by these species in marine ecosystems. *Caulerpa racemosa* is one of the most notable invaders in the Mediterranean Sea. It is well known that *C. racemosa* changes the amphipod community in terms of abundance and species richness with respect to native habitats. Some species coexist in invaded and non invaded habitats, inferring that they share the space as a resource. However nothing is known regarding the common use of the same trophic resources. The aim of this study was to assess if the feeding habits of amphipods associated with vegetated habitats are affected by the spread of the invasive *C. racemosa*, through stomach content analysis of amphipods living in both native and invaded seaweed assemblages. A total of 240 specimens of 14 species of amphipods were examined. Ten species were present in both studied habitats (native and invaded), while 2 species were only found in native habitats and 2 species were only found in *C. racemosa* habitat. Ten individuals of each species and at each habitat were selected and their gut content was examined. A total of 11 different items were found in the gut contents: detritus, vegetal detritus, algae, animal tissue, oligochaeta, polychaeta, foraminifera, crustacea, sipuncula, diatoms and non identified items. The expansion of *C. racemosa* to a native algal community changes the feeding habits of herbivorous amphipods, since their preferred food (epiphytic algae) is not available in the new habitat produced by *C. racemosa*. This community change occurs because of the presence of caulerpenyne in *C. racemosa*, which retards the growth of epiphytic algae. Nevertheless, other species are not affected or benefited by the invasion, such as detritivorous species whose main food source and habitat remains available. Altogether, slight changes in the trophodynamism of amphipod assemblages have been detected, which are not seen as important in an initial stage. However, they might be promoting some indirect effects in the energetic budget of populations, which may affect the life history. Further studies on food-web interactions in the ecosystems affected by invasive species are necessary.

5.2. Introduction

Habitat structure plays a very important role in marine benthic ecosystems, where biota depends on their substrate for physical support, as well as to acquire food or provide shelter (Sebens 1991). The variety of habitat and microhabitat types, hence available trophic niches, affects the coexistence of species and species diversity (Huston 1979). The habitat structure strongly influences processes that determine the presence, abundance and distribution of species (Orth et al. 1984, Beck 2000), determining the functioning of food webs. Consumers will feed on prey depending on their trophic guilds, the abundance of each prey in their habitat, the ability to capture prey and their preferences for a particular prey. Moreover, in shallow rocky marine habitats, the particular predator-prey combinations will be strongly influenced by vegetation characteristics (Heck and Crowder 1991), as marine algae are

important contributors to structure the habitat in terms of complexity and heterogeneity. Macrophytes act as ecosystem engineers; essentially they create, destroy or modify a habitat, which influences the associated epifauna.

Amphipods are one of the most ubiquitous and abundant invertebrate groups in marine vegetated habitats, with densities often reaching several thousands of individuals per square meter (Brawley 1992, Vázquez-Luis et al. 2008). They are important secondary producers and they exhibit wide feeding strategies: grazers, filter and detritic feeders, predators, scavengers, carnivores, omnivores and phytophages (macro and microphages) (Carrasco and Arcos 1984, Highsmith and Coyle 1990, Sarvala and Uitto 1991). These various feeding modes are sometimes used simultaneously or successively in time according to ambient conditions (Ruffo et al. 1998). Moreover, amphipods serve as a food source for a

large variety of marine predators (Stoner 1979, Beare and Moore 1997, Sanchez-Jerez et al. 1999, Stål et al. 2007), hence playing a key role in energy flow through food webs (Vázquez-Luis et al. 2010).

Disturbance events, such as the introduction of an invasive seaweed species can modify habitat structure and consequently, result in large ecological effects (see a review in Williams and Smith 2007). Such species present a high ability to restructure and radically change the functioning of the recipient habitat (Crooks 2002), determining the biota that will become associated with the habitat. The Mediterranean Sea is the most heavily invaded marine region in the world with respect to introduced seaweeds (Williams and Smith 2007). *Caulerpa racemosa* var. *cylindracea* (hereafter *C. racemosa*) is one of the most invasive. Some studies have demonstrated negative effects of *C. racemosa* on native seaweeds (Piazzi et al. 2001c, 2003, Balata et al. 2004), on food choice of invertebrates (Gianguzza et al. 2002), on invertebrate assemblages (Vázquez-Luis et al. 2008, 2009a) and on prey availability for fish (Vázquez-Luis et al. 2010). Other studies have found positive effects on polychaete assemblages (Argyrou et al. 1999, Box et al. 2010). In the case of seagrass density both effects have been found (Ceccherelli and Cinelli 1997, Ceccherelli and Campo 2002). However, the possibility that non positive or negative effects happen cannot be ruled out (Dumay et al. 2002).

Amphipods respond to habitat modification (Sanchez-Jerez et al. 1999); some species exhibit high habitat specificity while others tolerate a range of habitat alteration that may result from pollution, invasion by alien species and other disturbance. Therefore amphipods are good indicators to show environmental impacts on vegetated habitats (Bellan-Santini 1980, Virnstein 1987, Conradi et al. 1997, Sanchez-Jerez et al. 2000). It is known that *C. racemosa* changes the amphipod community in terms of abundance and species richness and some species coexist in both native and invaded habitats sharing the space as resource (Vázquez-Luis et al. 2008, 2009b).

However it is still poorly known if these species are benefiting from the same trophic resources. The main objective of this study is to assess if the feeding habits of amphipods associated with coastal plant communities are affected by the spread of the invasive *C. racemosa*, through stomach content analysis of amphipods living in both native and invaded seaweed assemblages.

5.3. Material and methods

A total of 240 specimens of 14 amphipod species were examined. Ten species were present in the two studied habitats (native algae and *C. racemosa*), while 2 species were only found in algae habitats and the other 2 species were only found in *C. racemosa* habitat (for further details about the provenance and abundance of the individuals see Vázquez-Luis et al. 2008). Ten individuals of each species and habitat were selected, and their gut content was examined (see Table 1). For the gut content study, individuals were analyzed following the methodology proposed by Bello and Cabrera (1999) with slight variations (Tierno de Figueroa et al. 2006, Guerra-García and Tierno de Figueroa 2009). Each individual was introduced in a vial with Hertwig's liquid (consisting of 270 g of chloral hydrate, 19 ml of chloridric acid 1 N, 150 ml of distilled water and 60 ml of glycerin) and heated in an oven at 65°C for 3 to 6 h, depending on the cuticle thickness of the specimens. After this, they were mounted on slides for examination under the microscope, equipped with an ocular micrometer.

The relevance of stomach contents in the amphipods studied was evaluated by calculating the percentage of the absolute gut content (%GC= total area occupied by the content in the whole digestive tract), and Vacuity Index (VI=100 x [number of empty stomachs/total number of stomachs analysed]). The importance of different prey types was evaluated by calculating the relative gut content (Ab=area occupied for each component within the total gut content), and the frequency of occurrence (Oc=100 x [number of stomachs containing

prey i /total number of stomachs containing prey]) of each prey item. Finally, to evaluate the degree of overlap in the use of food between the two types of habitat within each species the Morisita's Index (modified by Horn 1966) was calculated ($M = 2x \sum(P_{pxi} \times pyi) / \sum(px_i)^2 + \sum(py_i)^2$, where px and py are the percentages of occurrence in stomachs of the prey i). This overlap index varies from 0, for no diet overlap, to 1, for complete diet similarity (an overlap equal to or above 0.6 was considered to be biologically significant, following Keast 1978). The affinities among species and habitats according to the dietary analysis were explored by cluster analysis through a similarity matrix, which was calculated using the Bray–Curtis index and using double square transformed data (PRIMER software; Clarke 1993).

5.4. Results

Two hundred and forty specimens of 14 species and two habitats were examined (Table 5.1). The values of vacuity index (VI) ranged from 0 to 30 for all the species and habitat types, where *Stenothoe monoculoides* in *C. racemosa* habitat showed the highest proportion of empty gut (VI=30), followed by *Dexamine spiniventris* and *Elasmopus brasiliensis* in *C. racemosa*; and *Atylus massiliensis* in algae (VI=20). Digestive contents were found in 229 specimens (95.4%) belonging to all species. The total area occupied by the content in the whole digestive tract ranged from 16.7% for *S. monoculoides* to 55% for *Microdeutopus obtusatus*, both in native algae. Gut contents of the studied amphipod species included 11 items: detritus (organic and inorganic thin particles), vegetal detritus (vegetal remains), algae, animal tissue, oligochaeta, polychaeta, foraminifera, crustacea (mainly copepods), sipuncula, diatoms and non identified items. Food items for each species and by habitat are described in Table 5.1. *Apocorophium acutum* in both habitats fed exclusively on detritus (Fig. 5.1). *Ampithoe ramondi* consumed mainly epiphytic algae (Fam. Rhodomelacea, genera *Polysiphonia* or *Neosiphonia*) living in algae, while in *C. racemosa* showed

higher values of detritus consumption (Fig. 5.1). *Caprella grandimana* showed similar diet across habitats feeding mainly on detritus and some algae fragments when living on algae (Fig. 5.1). *Caprella hirsuta* showed similar feeding habits among habitats, with detritus being the most important item (Fig. 5.1). *Dexamine spiniventris* consumed mainly epiphytic algae (Fam. Rhodomelacea, Fam. Ulvaceae and Fam. Sphacelariaceae) living in algae, while in *C. racemosa* showed higher values of detritus consumption (Fig. 5.1). *Elasmopus brasiliensis* and *Elasmopus pocillimanus* showed a wide diversity of items in their gut contents, feeding on detritus, algae and different animal prey (Fig. 5.2). *Lysianassa costae* was the species with a higher presence of vegetal detritus in both habitats, but also detritus, animal tissue and algae were found in their gut content (Fig. 5.2). *Microdeutopus obtusatus* fed mainly on detritus and some animal prey in both habitats (Fig. 5.2). *Stenothoe monoculoides* showed the highest values of consumed crustaceans, but fed also on algae in algae habitats and polychaeta in *C. racemosa* habitats (Fig. 5.2). Regarding the gut content of the species that appeared only in one habitat, those living on algae (*Atylus guttatus* and *A. massiliensis*) showed high percentages of consumed algae tissues; and those species living in *C. racemosa* (*Caprella acanthifera* and *Melita hergensis*) fed mainly on detritus and animal tissues (Fig. 5.3).

At similarity of 50% the group of predators can be distinguished (Fig. 5.4), represented by *S. monoculoides* in both studied habitats. At similarity of 70% three groups represented by herbivores, detritivores and omnivores can be distinguished. The dietary composition of detritivores and omnivores was relatively similar, showing a clear segregation from the herbivorous group and further from the predators group. The different values obtained from the Morisita overlap index provided information about an overlap of food resources between both habitats for all species. These values ranged from 0.79 for *A. ramondi* to 1 for *A. acutum* (Table 5.2).

Table 5.1. Gut contents of the species studied in the different habitats. Hab=habitat, VI=vacuity index, %GC=total gut content occupied in the whole digestive tract, V detritus=vegetal detritus, Ab=abundance of each item and Oc=occurrence of each item. ALG=native seaweeds and CAU=*Caulerpa racemosa*.

Species	Hab	VI	%GC	Detritus		V detritus		Algae		An. tissue		Oligochaeta		Polychaeta		Foraminifera		Crustacea		Sipuncula		Diatomea		Non ident		
				Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab	Oc	Ab
<i>Apocorophium acutum</i> (Chevreux, 1908)	ALG	0	47	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAU	0	49.5	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amphithoe ramondi</i> Audouin, 1826	ALG	0	44.7	1.5	20	-	-	98.5	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAU	0	51.5	72.5	100	-	-	27.5	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Caprella grandimana</i> Mayer, 1882	ALG	0	29.7	87.9	90	-	-	10	10	2	10	-	-	-	-	0.1	10	-	-	-	-	-	-	-	-	-
	CAU	10	35	87.5	100	-	-	-	-	2.5	22.22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Caprella hirsuta</i> Mayer, 1890	ALG	0	22.3	94	100	-	-	-	-	5	40	-	-	1	10	-	-	-	-	-	-	-	-	-	-	-
	CAU	0	34	93.5	100	-	-	-	-	6.5	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dexamine spiniventris</i> (A.Costa, 1853)	ALG	0	24.7	-	-	-	-	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAU	20	21.9	29.5	37.5	-	-	50.5	75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Elaeomopus brasiliensis</i> (Dana, 1855)	ALG	0	42.7	50	90	1.5	10	26	30	16.7	40	-	-	0.5	10	0.3	10	5	10	-	-	-	-	-	-	-
	CAU	20	35.5	30.1	87.5	-	-	10.5	50	33.3	75	-	-	-	-	5.1	25	-	-	-	-	-	-	-	-	-
<i>Elaeomopus pocillimanus</i> (Bate, 1862)	ALG	0	39.3	71.9	100	-	-	3.5	20	15.5	50	-	-	-	0.1	10	9	20	-	-	-	-	-	-	-	-
	CAU	0	42.5	37.8	80	-	-	3	10	58.5	90	-	-	-	0.7	30	-	-	-	-	-	-	-	-	-	-
<i>Lysianassa costae</i> Milne Edwards, 1830	ALG	0	20	52.5	100	7.5	30	3	10	37	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAU	0	21.7	36	90	23	80	9	40	31	80	-	-	-	-	-	-	-	-	-	1	10	-	-	-	-
<i>Microdeutopus obtusatus</i> Myers, 1973	ALG	0	55	99.9	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	10	-	-
	CAU	0	42.8	93	100	-	-	-	-	7	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Stenothoe monoculoides</i> (Montagu, 1813)	ALG	0	16.7	1	10	-	-	39.9	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAU	30	17.5	-	-	-	-	10	12.5	-	-	-	-	5	12.5	-	-	-	-	-	-	-	-	-	-	5
<i>Atylus guttatus</i> (A.Costa, 1851)	ALG	10	32.8	4	22.22	1	11.11	85	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Atylus massiliensis</i> Bellan-Santini, 1975	ALG	20	31.5	10	50	-	-	69	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Caprella acanthifera</i> (Leach, 1814)	CAU	10	22.3	82.5	100	-	-	-	-	7.5	22.22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Melita bergensis</i> Reid, 1939	CAU	0	19.1	80.5	90	-	-	10	10	7.5	20	2	10	-	-	-	-	-	-	-	-	-	-	-	-	-

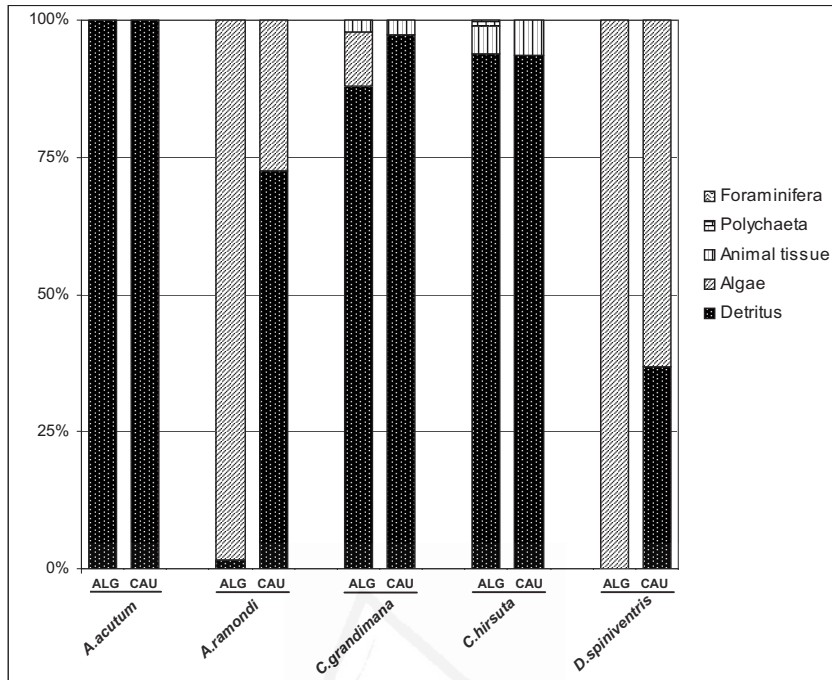


Figure 5.1. Gut contents in terms of abundance of *Apocorophium acutum*, *Amphioe ramondi*, *Caprella grandimana*, *Caprella hirsuta* and *Dexamine spiniventris* for each habitat type.

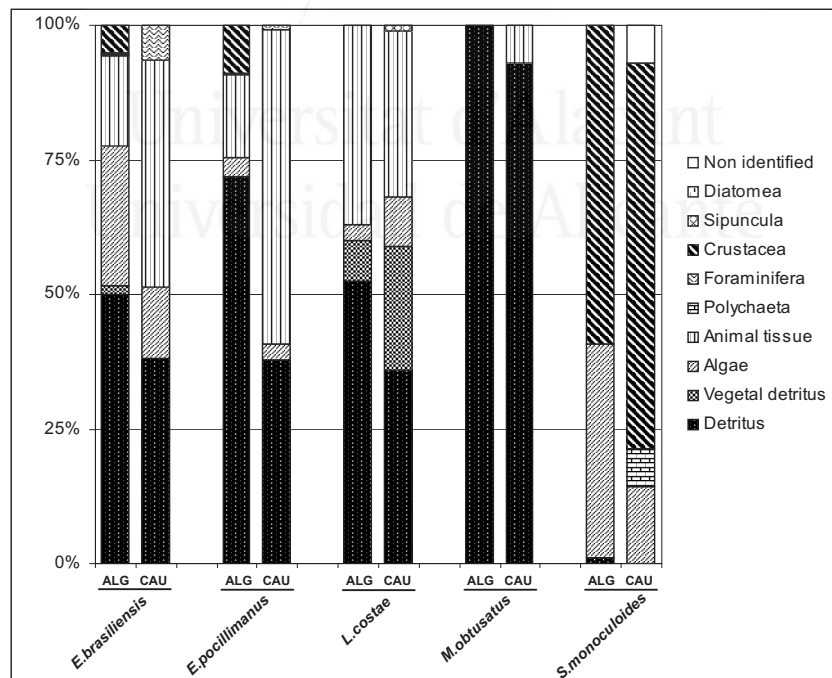


Figure 5.2. Gut contents in terms of abundance of *Elasmopus brasiliensis*, *Elasmopus pocillimanus*, *Lysianassa costae*, *Microdeutopus obtusatus* and *Stenothoe monoculoides* for each habitat type.

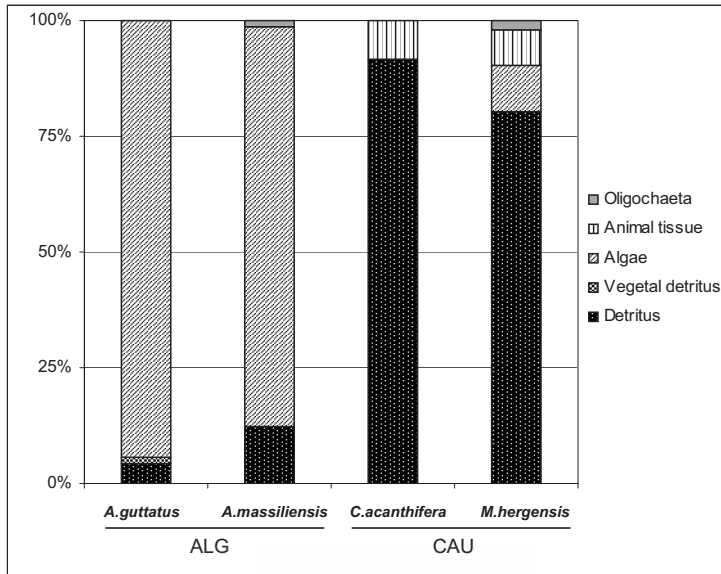


Figure 5.3. Gut contents in terms of abundance of *Atylus guttatus*, *Atylus massiliensis*, *Caprella acanthifera* and *Melita hergensis*.

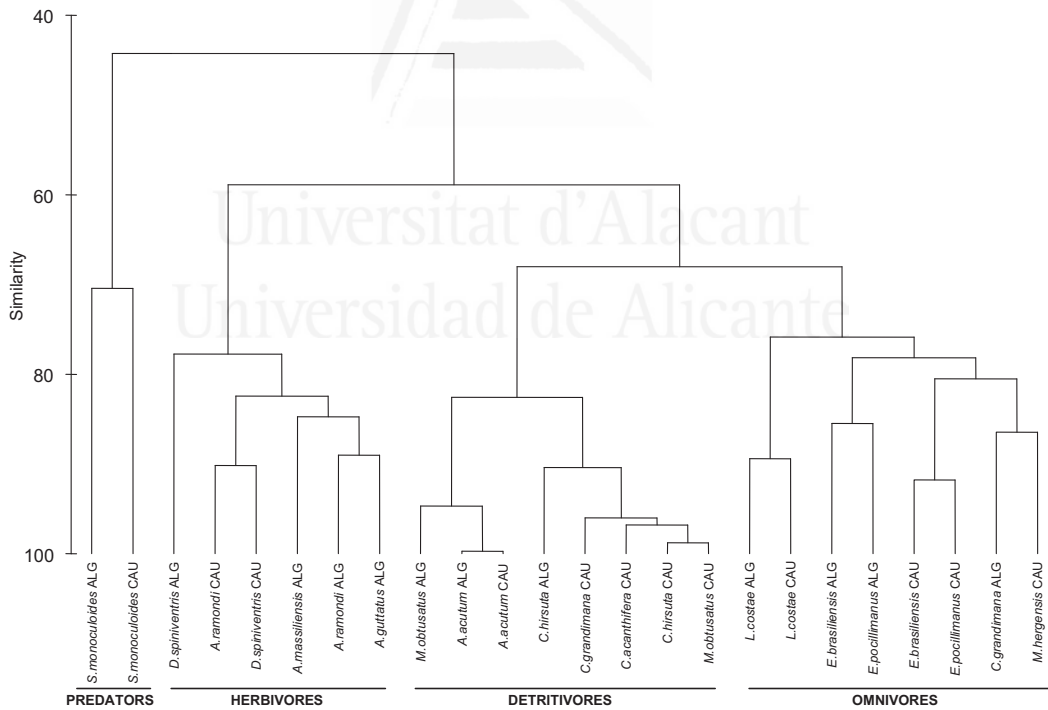


Figure 5.4. Dendrogram based on the digestive contents showing similarity between types of trophic guilds.

Table 5.2. Morisita overlap index calculated for the frequency of occurrence for different items for each species.

	Overlap index (Morisita)
	Algae/ <i>C.racemosa</i>
<i>Apocorophium acutum</i>	1
<i>Caprella grandimana</i>	0.98
<i>Microdeutopus obtusatus</i>	0.98
<i>Elasmopus brasiliensis</i>	0.92
<i>Caprella hirsuta</i>	0.92
<i>Lysianassa costae</i>	0.91
<i>Elasmopus pocillimanus</i>	0.90
<i>Dexamine spiniventris</i>	0.88
<i>Stenothoe monoculoides</i>	0.87
<i>Ampithoe ramondi</i>	0.79

5.5. Discussion

With respect to feeding habits some species are not affected by the presence of the alien algae, nevertheless other species exhibited different trophic requirements depending on the habitat they lived in. Changes of feeding habits were more significant on herbivorous amphipods, since their preferred food (epiphytic algae) was not available in the habitat colonized by *C. racemosa* and they fed on detritus as an alternative. On the other hand, detritivore species showed the least differences with respect to habitat type because their main food source and habitat remains available.

Vegetal content of *C. racemosa* has not been found in any of the analysed guts of amphipods. Our results are similar to those found on other invasive seaweeds where amphipods and other herbivores and omnivores fed very little on the invasive species, and therefore the spread of introduced seaweeds are not under herbivore control (Trowbridge 1995, Levin et al. 2002, Britton-Simmons 2004, Chavanich and Harris 2004, Conklin and Smith 2005, Davis et al. 2005, Sumi and Scheibling 2005, Gollan and Wright 2006, Box et al. 2009). Low herbivore diversity and abundance, combined with very little feeding and weak habitat preference on invasive algae result in limited grazing pressure, as has been reported for the amphipod *Cymadusa setosa* on *C. taxifolia* (Gollan and Wright 2006). Therefore,

primary production generated by the spread of *C. racemosa* appears not to be exploited by herbivores or primary consumers, but rather acts by activating the detritivore pathway. Recent experimental studies reveal that species richness and total abundance of amphipods increased with an increase in detritus content. The same applies to species abundance since values of this attribute increased with an increase in detritus content (Vázquez-Luis et al. 2009c). Detritus plays a very important role as a trophic resource for marine invertebrates and serves as one of the main trophic pathways in marine ecosystems (Valiela 1995); it is also one of the most important features of habitat structure in vegetated habitats (Allesina et al. 2005). It was already suggested that amphipods did not consume algal biomass directly, but feed on associated resources such as detritus (Enequist 1949). Caprellids feed clearly on detritus (Guerra-García and Tierno de Figueroa, 2009) and some zoobenthic taxa feed significantly on detritus accumulated by *C. racemosa* (Casu et al. 2009). Such results support the results obtained in this study. Therefore, detritus appears to play a very key role for amphipods as a food source and the detritus stock associated with *C. racemosa* is playing an important role in trophic dynamics of littoral habitats.

The changes in the ecological niche due to *C. racemosa* do not seem to affect some species, but others need to look for new trophic resources, in spite of having found trophic overlap. High abundances of the fine-particle suspension feeder *A. acutum* have been found in *C. racemosa* compared with native seaweeds habitat (Vázquez-Luis et al. 2008). The accumulated detritus on *C. racemosa* habitats seem to favour the construction of tubes where they live in and feed on this “unlimited” food resource, as has been reported for *Corophium sextonae* (Casu et al. 2009). The feeding habits of other detritivore species such as *M. obtusatus* were not highly affected by the spread of *C. racemosa*. Similar patterns have been found in three caprellid species, *C. grandimana*, *C. hirsuta* and *C. acanthifera*, which feed mostly on detritus (Guerra-García and Tierno de Figueroa

2009). Nevertheless herbivorous species, such as *A. ramondi* and *D. spiniventris*, are scarce in *C. racemosa* habitat and if they use this habitat, they should modify their feeding habits by the changes in trophic resources. They usually feed on epiphytic algae that are absent in *C. racemosa* because of the presence of caulerpenes (Léeme et al. 1997). Thus, apart from habitat structure, the availability of food is crucial for those species to live in one habitat or another. Some individuals of herbivorous species were found living in *C. racemosa* (Vázquez-Luis et al. 2008), but probably the scarce food quality for them on this habitat could generate metabolic problems and may lead to survivorship problems for the juveniles. Probably for this reason *A. guttatus* and *A. massiliensis* were not found in *C. racemosa* habitats; indeed more than 75% of their guts presented epiphytic algae. It should be noticed that within the same trophic guild, the feeding habits of some species were more similar according to the type of habitat than the species itself, such as the herbivorous *A. ramondi* and *D. spiniventris* and the omnivorous *E. brasiliensis* and *E. pocillimanus*. Regarding other omnivores, *M. hergensis* appeared only in *C. racemosa*, probably benefiting from the spread of the invasive algae. However, the abundance of this species in *C. racemosa* was very low (Vázquez-Luis et al. 2008). It must be taken into account that within the omnivorous species some, such as *L. costae*, show scavenging habits. It was the most different species in this group and seemed little affected by the spread of *C. racemosa*. The only predator found in the present study, *S. monoculoides*, does not seem to have changed or modified their feeding habits by the spread of *C. racemosa*, since it is able to find prey in both habitats.

As we can see, trophic preferences of amphipods can change if the trophic resources are modified by environmental factors, such as the establishment of an invasive species. Traditionally, species have been classified into specific trophic guilds, usually based on mouthpart morphology.

However a recent study on caprellid amphipods found no relationships between digestive contents and features of the mouthpart structure (Guerra-García and Tierno de Figueroa 2009). The only relationship that they found is that a predatory way of life is directly related with the absence of the mandibular molar. In our study we found a single predator, *S. monoculoides*, which lacks a molar on the mandible. Therefore, in most cases mouthpart structure (mostly mandibular features) by themselves are not a good tool to determine the feeding habits of amphipods. Gut content analyses are widely used to show the feeding habits of species. Moreover, the results obtained from gut contents are usually correlated with those from other analyses with higher temporal scale, such as fatty acid composition (Graeve et al. 2001) and stable isotope analyses (Kelly and Hawes 2005). However, it is necessary to include a combination of mouthpart studies with behavioural observations, gut contents, fatty acids and stable isotopes analyses to have a complete knowledge about the feeding habits of amphipods and food-web interactions in the ecosystems affected by invasive species.

We conclude that the expansion of *C. racemosa* on native algal community changes the feeding habits of herbivorous amphipods, which stop using plant tissues because of the lack of an epiphytic community. In addition, the detritus accumulated by the rhizoid network of *C. racemosa* plays an important role in the plasticity of the diet of herbivores, changing greatly their trophic strategy. Nevertheless, other species are not affected and some are benefited by the invasion, such as detritivorous species. Altogether, slight changes in the trophodynamics of amphipod assemblages have been noted, which are not detected as important in an initial stage. However, they might be promoting some indirect effects in the benthic community and in the life history of the species, with further unknown consequences in the marine trophic net.



Does *C. racemosa* affect amphipod prey availability to predators?

6. Does *C. racemosa* affect amphipod prey availability to predators?

6.1. Abstract

Alien plant species, such as *Caulerpa racemosa* var. *cylindracea*, that invade Mediterranean marine vegetated habitats, can affect habitat structure. In turn, changes in habitat structure may affect the associated invertebrate assemblages, either through changes in habitat selection or as a result of altered predation efficiency. To test for effects of changes in habitat structure resulting from colonization by *C. racemosa* on prey availability to predators, the importance of amphipods as a trophic resource in natural vegetated habitat was first assessed, and later experiments were undertaken to assess the effects of the alien algae on predation by the fish *Thalassoma pavo* on two dominant types of amphipods: *Elasmopus brasiliensis* (Gammaridea) and *Caprella dilatata* (Caprellidea). Laboratory experiments were conducted in separate aquaria with five vegetation habitat types: *Halopteris scoparia*, *Jania rubens*, *C. racemosa* without detritus, *C. racemosa* with detritus and *Cymodocea nodosa*, together with control. The vegetation was first defaunated, and then 30 amphipods were introduced to each aquarium and exposed to a single *T. pavo* individual for one hour, after which the fish's gut contents were examined. Consumption (per fish per hour) of caprellids (11.7 ± 1.4) was higher overall than that of gammarids (8.7 ± 1.5) and likely reflects different microhabitat use by amphipods, which affects susceptibility to predators. Consumption of amphipods also varied by habitat type. The highest predation rate was found in the *C. nodosa* habitat (12.7 ± 2.19) and the lowest in the *C. racemosa* habitats with detritus (4.1 ± 1.78) and without detritus (5.2 ± 0.55), which did not differ. The pattern of predation across habitats, however, was similar for both caprellid and gammarid amphipods, indicating a more general effect of habitat on amphipod predation. Our findings showed that invasive species such as *C. racemosa* can decrease feeding by predators such as *T. pavo*. Changes in predator-prey interactions could have consequences for food web support in the Mediterranean.

6.2. Introduction

Predation affects the structure and dynamics of marine populations (Conell and Vanderklift 2007). In particular, predatory fishes play an important role in regulating prey populations (Nelson 1979, Dean and Conell 1987). The efficiency of a predator is a function of detection, pursuit, and capture of the prey, all of which may be hindered by greater physical complexity of a habitat. Consequently, the abundance and diversity of marine epifauna are influenced by fish predator efficiency (Sutherland 1974, Nelson 1979, Russo 1980), while predation efficiency may be affected by habitat structural complexity (Stoner 1982, Russo 1987, Diehl 1988). In this context, several studies have shown that the degree of habitat complexity of vegetated habitats is an important factor determining the availability of prey for fish predators. As a result, changes in habitat complexity, such as those arising from alterations to habitat structure resulting from introduced algae, may affect the predation process and the

overall trophic web (Nelson 1979, Stoner 1979, Heck and Orth 1980, Williams and Smith 2007).

The number of introduced species outside their natural ranges is rapidly increasing, although only a very small fraction of transported and introduced species become invasive (Ribera and Boudouresque 1995). The ability of invasive species to restructure and, hence, radically change the functioning of a recipient habitat is high (Crooks 2002). Invasive species may: modify the availability and flow of nutrients in ecosystems by altering biogeochemical cycling; contribute to changes in the flow of energy through food webs; and affect the availability or quality of physical resources in the ecosystem, including the physical characteristics of a habitat (e.g. available space, sediment properties, light regime and water quality; see Vitousek 1990, Crooks 2002). Community-level ecological interactions involving introduced seaweeds

constitute a major research gap, and the indirect effects on the trophic web present research challenges (Williams and Smith 2007). Since the first records of occurrence of *C. racemosa* var. *cylindracea* along Mediterranean coasts, several studies concerning the ecology of this species have been undertaken (see Ruitton et al. 2005, Cavas et al. 2006). The fauna associated with exotic alga can differ from that associated with native vegetation (Wikstrom and Kautsky 2004, Schmidt and Scheibling 2007); consequently, several studies have focused on assessing the changes in faunal assemblages associated with vegetated habitats colonized by *C. racemosa* (Argyrou et al. 1999, Piazzini and Balata 2008, Vázquez-Luis et al. 2008, 2009a, b). However, data on the potential consequences that could lead to changes in the trophic web are unavailable, and an understanding of how introduced algae can affect on predation rates on invertebrates by fishes is needed for a better understanding of how invasive species can alter the flow of matter and energy through ecosystems.

Thus, the general aim of the present study was to determine experimentally whether invasion of Mediterranean vegetated habitats by *C. racemosa* has any effects on prey availability for predators. In undertaking our work, we also considered the importance of detritus accumulation by *C. racemosa* (Vázquez-Luis et al. 2008, 2009b) as this can affect prey availability. In undertaking our experiments, we selected amphipods and the predatory labrid fish *Thalassoma pavo* L. Amphipods are one of the most important groups of invertebrates associated with vegetated habitats and play an important role as a trophic resource for fishes (Sanchez-Jerez et al. 2002, Stål et al. 2007), in particular for labrids, sparids, serranids, blennids and gobids in benthic habitats (Muñoz and Ojeda 1997, Sala and Ballesteros 1997, Garrison and Link 2000). *T. pavo*, which is abundant in south-western Mediterranean coastal regions (Arechavala-López et al. 2008), feeds on small crustaceans and molluscs (Whitehead et al. 1986). First, we estimated the availability of gammarid and caprellid prey in natural uninvaded habitats and in habitats invaded by *C. racemosa*, to assess the levels of prey

stock supported by the alien alga, where present. Second, we undertook a laboratory experiment to assess whether changes in habitat structure resulting from the introduction of *C. racemosa* affect the rate of predation by demersal fish (such as *T. pavo*) on amphipods. We hypothesized that (i) the rate of predation by *T. pavo* on amphipods is affected in vegetated habitat that have been invaded by *C. racemosa*, compared to uninvaded vegetated habitats, (ii) predation rates in *C. racemosa* are reduced because of the presence of accumulated detritus (compared to uninvaded vegetated habitat, where no detritus is accumulated; (see Vázquez-Luis et al. 2008); and (iii) different use of microhabitats by different prey species affects predation.

6.3. Material and methods

6.3.1. Prey availability

To estimate amphipod abundance, three different vegetated habitats (native algae, introduced alga (*C. racemosa*) and seagrass (*Cymodocea nodosa*)) were sampled in summer and winter (for further details see Vázquez-Luis et al. 2008, 2009 a, b). We used the estimated mean abundance of caprellids and gammarids to estimate the potential trophic resource available for fish predators in the different habitats.

6.3.2. Animal collection and fish-holding conditions

Using a hand net, individuals of *T. pavo* were collected from the northern coast of Benidorm (Alicante, south-eastern Spain), where the littoral zone is characterised by extensive and heterogeneous rocky infralittoral bottoms, which are primarily characterised by boulders of different sizes, interspersed with patches of sand and *Posidonia oceanica* seagrass. The collected fish, which comprised females of a similar size, were immediately transported to the laboratory, where they were placed in a tank having a flow-through water system (25°C, salinity 38‰) exposed to an alternating 12 h light/12 h dark illumination regime. While in captivity, the fish were fed once a day with fresh food.

Algae, *Halopteris scoparia*, *Jania rubens* and *Caulerpa racemosa*, and seagrass *Cymodocea nodosa*, were harvested off the Cape of Santa Pola (Alicante, south-east Spain). The macrophytes were transported to the laboratory, where they were defaunated and attached to a metallic mesh to retain the plant structure similar to that found in the natural environment (with the algae covering 100% of the bottom in the aquaria) and at natural densities for the particular algal species. The control trials consist in the metallic mesh used before but without macrophytes.

To assess the effects of prey microhabitat use, the caprellid amphipod *Caprella dilatata* and the gammarid amphipod *Elasmopus brasiliensis* were used as the prey subjects. In their natural habitat, Caprellids are found attached to the external parts of plants using their pereopods, and exhibit low motility. On the other hand, gammarids are much more motile and live associated with those parts of the plant that are less accessible to predators. These two species were chosen since they exhibit behaviours that are quite different and potentially affect their susceptibility to predation by fish. The amphipods were collected from the fouling assemblages found at a water depth of 1 – 2 m on mooring ropes of fish farms at Guardamar, and were then transferred to aquaria in the laboratory. Different species were kept in separate aquaria each equipped with an air pump and aerator.

6.3.3. Predation experiment

Experiments were conducted using well illuminated aquarium (30 x 60 x 37 cm). Each experimental tank was divided into two compartments. One compartment contained the habitat with the amphipods, and the other contained a single fish. The two compartments were separated from each other by a removable opaque partition, so that the fish was unable to see the vegetated compartment prior to the start of the experiment. Prior to initiating the experiment, the fishes were starved for 24 h to ensure that their guts were completely empty. Thirty amphipods were introduced

to the vegetated habitat in one compartment and a single fish placed in the other compartment one hour before the start the experiment to allow acclimatisation. After this, the fish was moved to the other compartment containing the prey. Each individual of *T. pavo* was allowed a duration of 1 h in the respective aquarium, after which the fish was captured and preserved in ice. Gut contents were analyzed by counting the number of amphipods ingested and then expressed as rate of predation or feeding efficiency (in terms of mean number of amphipods consumed per fish per h). Also, the weight and the size of the fish were measured.

6.3.4. Data analysis

The effect of different fish size on predation rates by *T. pavo* was tested by examining the linear correlation (Pearson) between fish size and feeding efficiency. To test whether the predation rates of amphipods differed significantly across habitats we used ANOVA (Underwood 1981), which incorporated the following factors: (i) 'Species' (fixed) with two levels: *E. brasiliensis* and *C. dilatata*; and (ii) 'Habitat' (fixed and orthogonal) with six levels: *H. scoparia*, *J. rubens*, *C. racemosa*, *C. racemosa* with detritus, *C. nodosa* and control (only metallic mesh). For each amphipod species and habitat type, the experiment was replicated five times. Prior to carrying out the ANOVA, the data were tested for heterogeneity of variance using Cochran's C-test (Cochran 1951). When ANOVA indicated a significant difference for a given factor, the source of difference was identified using Student–Newman–Keul (SNK) tests (Underwood 1981).

6.4. Results

6.4.1. Prey availability

Amphipods were present in all natural and invaded vegetated habitat types, being especially abundant in native algal habitats. Gammarids were more abundant than caprellids in all habitat types; the highest abundance (3248.61 ± 552.48 ind m^{-2} ; Fig. 6.1) was found in uninvaded vegetated habitat. In vegetated habitat invaded by *C.*

racemosa, both amphipod species had intermediate abundances (2137.5 ± 522.8 gammarids and 619.4 ± 325.53 caprellids per square meter). The lowest abundance occurred in *C. nodosa* habitat, where caprellids were particularly scarce.

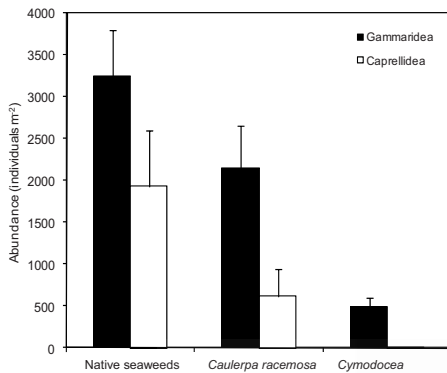


Figure 6.1. Mean abundance values (\pm SE) of amphipod prey recorded from the three habitat types.

6.4.2. Predation rate

Minor variation in fish size did not affect prey consumption across habitats as indicated by the absence of significant correlation between fish size and predation for both caprellid and gammarid species (R^2

value: 0.0401 for *E. brasiliensis* and 0.0014 for *C. dilatata*; Fig. 6.2).

Feeding efficiency (in terms of mean number of amphipods consumed per fish per h) was significantly higher for the caprellid (Sp, $P < 0.05$, Table 6.1; Fig. 6.3) in all experimental trials (consumption of caprellids: 11.7 ± 1.4 ; and that of gammarids: 8.7 ± 1.5). The highest predation rate occurred in the control units (20.6 ± 2.5 , Ha, $P < 0.01$, Table 6.1; Fig. 6.3). The next highest predation rate occurred in habitats containing *C. nodosa* (12.7 ± 2.19), *J. rubens* (11.4 ± 2.27) and *H. scoparia* (7.1 ± 1.5); while the lowest predation rate occurred in *C. racemosa* without detritus (5.2 ± 0.55) and *C. racemosa* with detritus (4.1 ± 1.78) (Ha, $P < 0.01$, Table 6.1; Fig. 6.3). In terms of relative feeding efficiency (as a percentage of the controls), predation in *C. racemosa* with detritus was 94.2% lower than the controls for the gammarid amphipod and 66% lower than the controls for the caprellid amphipod. Predation was more efficient in *C. nodosa*, where the consumption of both amphipods was less (38%) compared to the controls (Fig. 6.4).

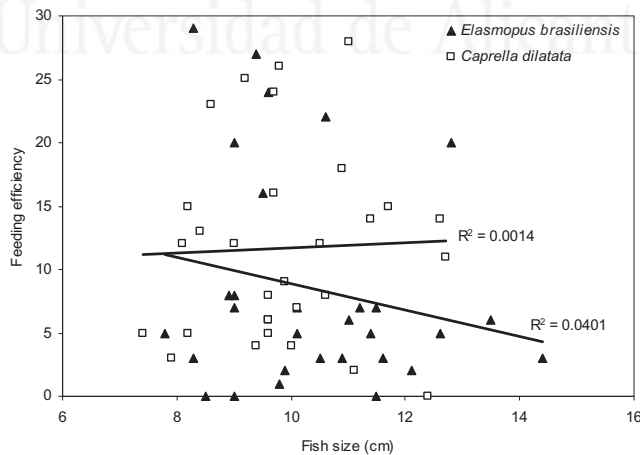


Figure 6.2. Scatter plot for the test of correlation between fish size and feeding efficiency for the two amphipod species used in the predation experiment.

Table 6.1. Results of two-factor ANOVA for predation rate. MS = mean square; P = level of significance; df = degrees of freedom; ns = not significant; * = significant ($P < 0.05$), ** = significant ($P < 0.01$).

Source of variation	df	Predation rate		F versus Res
		MS	P	
Species = Sp	1	132.02	0.0357*	Res
Habitat = Ha	5	375.34	0.0001**	Res
Sp x Ha	5	16.22	0.8162	Res
Residual	48	36.61		
Cochran's C-test		C=0.1873 ns		
Transformation		None		

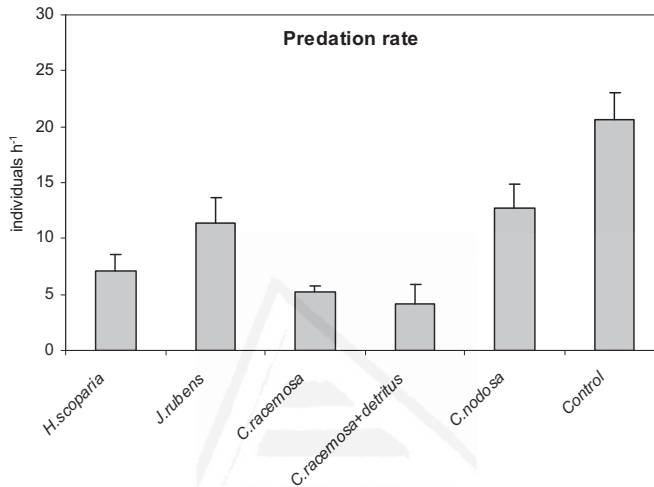


Figure 6.3. Mean values (\pm SE) per habitat of feeding efficiency (individuals per fish per hour). Groups defined by post hoc SNK tests undertaken following ANOVA are shown by the numbers.

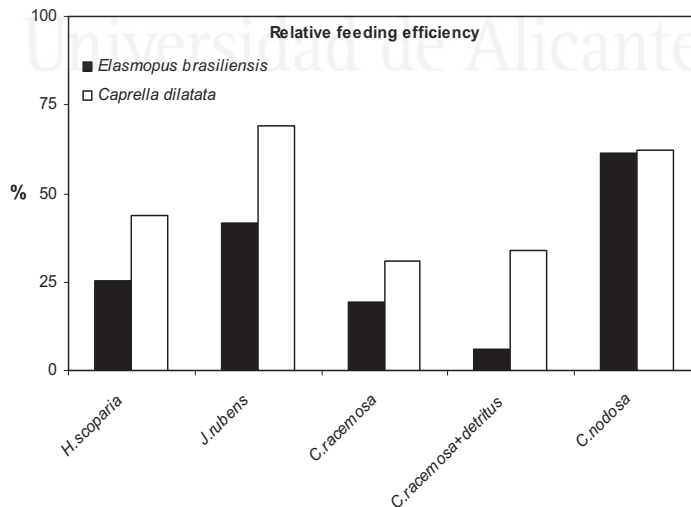


Figure 6.4. Values of relative feeding efficiency [relative frequency of consumption (individuals per fish per hour) with respect to control]

6.5. Discussion

Amphipods occurred in high abundance in both uninvaded and invaded vegetated habitats. However, this did not hold true for *C. nodosa* habitat. These results highlight that the prey stock in algal habitats is relatively important for predatory fish. It was demonstrated experimentally that amphipods were less available to *T. pavo* where *C. racemosa* was present. Therefore, alteration of habitat structure by the introduction of the alien alga may provide a better refuge for amphipods. Furthermore, predators can avoid *C. racemosa* due to other reasons, such as the presence of antifeeding chemicals that are produced by the plant. On the other hand, the lower amphipod abundance in *C. nodosa* may be explained by a higher predation rate in seagrass habitat, which offers less refuge and thus renders prey species more available to fish predators. These findings also emphasize the importance of assessing prey microhabitat in vegetated habitats to better understand predation processes.

Vegetated habitats typically support a higher abundance and species richness of macroinvertebrates compared to unvegetated habitats (e.g. Heck and Orth 1980, Peterson et al. 1984, Irlandi 1994, Heck et al. 1995). Amphipods, which serve as an important food resource for many fish species, are particularly dependent on the availability of vegetated habitat. For example, gammarid amphipods and decapod crabs were the most important groups in the diet of nine carnivorous fishes (Muñoz and Ojeda 1997). Amphipods constitute 59% of the food items present in the gut of bream *Diplodus vulgaris* (Sala and Ballesteros 1997) and they were predominant in the diet of *Limanda ferruginea* (Garrison and Link 2000); in other works an immediate disappearance of epibenthic caprellid amphipods in the field on appearance of demersal fish species was noted, while gut content analysis of *Cymatogaster aggregata* indicated that 80% of the gut contents comprised caprellid species (Caine 1991). Therefore, if typical fish prey items, such as amphipods, are less available for predators in habitats invaded by *C. racemosa*, the flow of matter and

energy from invertebrates to higher trophic levels could be altered.

Higher values of macroinvertebrate abundance and species richness recorded from benthic habitats are usually correlated with a high habitat complexity (Johnson 1970, Dean and Connell 1987, Edgar 1992, Taylor and Cole 1994, Sanchez-Jerez et al. 1999, Ayala and Martín 2003). Additionally, when habitat complexity increases, the vulnerability of prey to predation tends to decrease (Nelson 1979, Stoner 1979, Heck and Orth 1980). We expected that algae having a high structural complexity, such as *H. scoparia*, would provide a better refuge for amphipods against predators. However, a low predation rate was recorded in *C. racemosa* habitat, compared to that in the other habitats. Other authors also found that increased habitat complexity does not always provide refuge from predation (Holmlund et al. 1999, Mattila et al. 2008). It is possible that habitat complexity might be higher due to the presence of the dense rhizoids of *C. racemosa*. Other authors have hypothesized that certain small invertebrates experience less predation by fishes due to their ability to live on and consume plants that are unpalatable and which are therefore less frequented by omnivorous fishes (Hay et al. 1989, Duffy and Hay 1994). Perhaps the foraging effort of fishes and/or changes in algal palatability play an important role in the habitats studied. A change in the population structure of *Mullus surmuletus*, which authors attributed to the appearance of mats of the alien alga *Caulerpa taxifolia* has been recorded (Longepierre et al. 2005), while other works found that colonisation of shallow habitats by *C. taxifolia* resulted in a significant decrease in the abundance of benthivorous species, compared to uninvaded sites (Harmelin-Vivien et al. 2001).

Our results suggest that the different behaviour on the use of microhabitat by invertebrates can affect predation rates, as it has been demonstrated by other authors (Sudo and Azeta 2001). In our case, caprellids suffer a higher predation pressure

than gammarids, these differences can be explained because caprellids usually occur on seaweed thalli or on the epiphytes (Aoki 1999), while gammarids use different strategies, including epifaunal, shallow-burrowing, infaunal tube-dwelling and deep-burrowing ones. Other important aspect is that, contrary to the general observation that increased seagrass habitat complexity reduces predation (Stoner 1982), the predation rates in *C. nodosa* recorded in the present study were equal for both amphipod species, and were also similar to control trials (Fig. 6.3). We attribute this high predation rate to the lower refuge potential provided by *C. nodosa*, resulting from a lower habitat complexity compared to the macroalgae used in our study. Amphipods appear to be relatively available for predators in seagrass beds (Bell and Harmelin-Vivien 1983) making this habitat type important as a feeding ground for fishes. Alternatively, *C. racemosa* habitat is characterised by lower predation rates; this appears to be linked to accumulation of detritus, which favours a high abundance of amphipods. Therefore, replacement of seagrass meadows by *C. racemosa* stands can severely affect the trophic resource for fish populations. Caprellids have been shown to serve as very important food items for fish during their reproductive period (Caine 1991), and therefore constitute a relatively important dietary component (see review in Woods 2009). In contrast, some gammarid species appear to be the most important food item for red sea bream (Sudo and Azeta 2001) and very important for juveniles fish (Sanchez-Jerez et al. 2002). These results corroborate the data on abundance of gammarids and caprellids estimated in natural habitats, and the predations rates observed in the present study. It should be noted that only two species of amphipods *C. dilatata* and *E. brasiliensis* were used in the present study. In the case of *C. dilatata*, this species is quite large and is probably much more easily detected by a predator than other typical species living on the

seaweeds *H. scoparia*, *J. rubens*, such as the caprellids *Caprella hirsuta* and *C. grandimana*. Probably the latter two species, being much smaller than *C. dilatata*, are less easily detected by *T. pavo*, hence the feeding efficiency would not be so different from that on gammarids.

The Mediterranean Sea is the most heavily invaded marine region in the world with respect to introduced seaweed. The region has more than 132 species of introduced seaweed, which account for over 33% of total number of alien species recorded (Williams and Smith 2007). The potential effects of invasive plants must be determined to better understand how the ecology of natural vegetated habitats may be altered. Ecological studies of different invasive seaweeds in the Mediterranean Sea have focused mainly on changes in seaweed community structure, seagrass density, faunal assemblages, herbivorous species and fish foraging (see review in: Williams and Smith 2007, Vázquez-Luis et al. 2008, 2009a,b). However, studies that deal with more than one trophic level are unavailable, while there is a lack of information on the effects of invasive algae on the trophic web within invaded habitats. The present work has contributed to a better understanding of the effects of alien species on the ecosystems they invade.

In conclusion, the spread of *C. racemosa* to native marine benthic habitats can affect fish predation rates on invertebrates in a trophic web. Despite a high abundance of invertebrates, these food sources may not be available for higher trophic levels in habitats invaded by the alien alga. To summarize, the present study demonstrated that (i) *T. pavo* predation on amphipods was lower in habitats with *C. racemosa*, (ii) the presence of detritus in *C. racemosa* habitat did not show a significant reduction in predation, and (iii) different uses of microhabitats by amphipods affect predation by *T. pavo*.



General discussion

Chapter 7

7. General discussion

Many invasive species provoke strong impacts on invaded communities and ecosystems (Vila 2009) and transform ecosystem properties (Richardson et al. 2000), which inevitably leads to changes in biological communities. Results obtained in this thesis show that *C. racemosa*, in spite of reducing the habitat complexity by itself and generating monospecific meadows where more heterogeneous patchy habitats disappear, maintains a diverse and abundant amphipod assemblage. Nevertheless, the assemblage composition differed from those recorded from native seaweeds, due to an alteration of the natural equilibrium of the initial assemblage was altered, by an increase of detritivorous species and a reduction of herbivorous. The expansion of *C. racemosa* into native algal communities altered the food web flows in two ways. First, the feeding habits of herbivorous amphipods changed since their preferred food is not available in the presence of the invasive algae and they have to look to the new food resources. Second, predation on amphipods by higher trophic levels, such as fish was reduced because of the new habitat formed by *C. racemosa*. If an introduced species can by itself alter ecosystem-level processes such as primary or secondary productivity, hydrology, nutrient cycling, soil development, or disturbance frequency, then clearly the properties of individual invasive species can control the functioning of whole ecosystems (Vitousek 1986).

7.1. Explaining the mechanisms of biological invasions

According to some authors there are three factors that can mediate biological invasions: invasion pressure (that is the number of propagules discharged per unit of time and space), post-transport inoculant survival (propagule survival) and the opportunities that the particular invaded community provides for the invader in question (susceptibility to invasion) (Smith et al. 1999, Occhipinti-Ambrogi and Savini 2003). Once established, three principal effects of invasive species on ecosystems have been recognized (Vitousek 1990, Crooks 2002). First, exotics can affect the

availability and flow of nutrients by altering biogeochemical cycling. Second, invasive species can affect the flow of energy through food webs. Third, invaders can affect the availability or quality of physical resources in the ecosystem (including living space or “habitat,” physical materials, sediment, light, or water).

Furthermore, biological invasions may provide a useful framework for integrating population and physiological processes into ecosystem studies (Vitousek 1990). Community ecology theory can be used to better understand biological invasions by applying recent niche concepts to alien species and the communities that they invade, so the response aspect of the niche is a key aspect determining the ability of alien species to invade (Shea and Chesson 2002). The existence of an empty niche favours the development of large populations of newcomers, this means that an alien species succeeds because most of the resources and biotic interactions that it may need are freely available in the environment (Elton 1958). Temporal and spatial variation can also provide niche opportunities, which can occur through a relative nonlinearity of competition if the invader competitor had a superior colonization or local resource exploitation ability (Chesson 2000). Another factor cited by Elton (1958) notes that new exotic species should be released from competition with native species. Opportunities for development arise when natural enemies, such as diseases, predators and parasites, are in low abundance or are less effective against the new species (Settle and Wilson 1990, Torchin et al. 1996). An invader with few specialist natural enemies, such as *C. racemosa* in the Mediterranean Sea, could reach a high density, maintaining generalist natural enemies that regulate the population dynamics of the native community (Shea and Chesson 2002).

It is generally assumed that high species richness renders a habitat highly resistant to introductions, while disturbances enhance their susceptibility to be invaded. In this way, the hypothesis known as Elton’s biotic

resistance hypothesis (Elton 1958) assumes that a species rich ecosystem will be more resistant to introductions than a poor one. It has been also postulated that, in aquatic systems with high levels of human disturbance, a much wider range of invasive species can develop compared with low levels of human disturbance (Moyle and Light 1996). In accordance to the biotic resistant hypothesis, several researchers have found a negative correlation between species richness and the number of introduced species in a habitat (Lodge 1993, Ribera and Boudouresque 1995). This is usually explained by the fact that an ecosystem with many species represents a highly structured system with many complex inter-species interactions, such as competition, predation, mutualism, parasitism and transfer of disease. In contrast, it has also been found that a clear relationship does not exist among disturbances, species richness and the number of introduced macrophytes (Klein et al. 2005). Disturbance is commonly assumed to make available resources thus providing new opportunities for invaders, which frequency and magnitude determined the success of invasion (Nichols et al. 1990, Van den Brink et al. 1993). On the contrary, no clear relationship between the number of introduced macrophytes and disturbances acting on the assemblages was found in other studies (Klein et al. 2005).

Moreover, it has been considered of major importance the successional state (degree of maturity) or age of a community, proposing that it plays an important role in the vulnerability to species introductions (Crawley 1989). Certain successional advanced communities are more resistant to species introductions than younger ones, although species richness may decline during the course of succession (Meiners et al. 2002, Sax 2002). Finally, it has been also suggested that besides negative interactions between two or more introduced species in the host ecosystem, the presence of an introduced species may enhance the success of a second one and may, in this way, facilitate its establishment (Simberloff and Von Holle 1999, Richardson et al. 2000). This synergistic mechanism has been observed among

several invasive seaweeds in the Mediterranean Sea (Ceccherelli et al. 2002, Piazzini et al. 2003). Probably, as Occhipinti-Ambrogi and Savini (2003) stated, invaders may be favoured in places and times where stress is causing trouble to the existing biota and therefore vacant niches are open for colonisation.

7.2. Facilitation of invasion process by human impacts

Anthropogenic disturbance affecting in coastal ecosystems, such as human induced climate change, invasion of other introduced species, pollution, eutrophication, physical destruction of habitats and overfishing may enhance the expansion of invasive species. Expansion and intensification of different forms of human disturbance and their ecological effects on coastal ecosystems have increased and accelerated with human population growth, unchecked exploitation of biological resources, technological advance, and the increased geographic scale of exploitation through globalization of markets (Jackson et al. 2001). Moreover, the effects are synergistic, therefore creating more stressed ecosystems (Myers 1995).

Thus, climate change could dramatically alter the adaptive responses of native species, and may therefore favour the introduction and spread of invasive macrophytes as *C. racemosa*, which present tropical and subtropical affinity. Gilman et al. (2010) predicted five scenarios for describing how species interactions will influence abundances in open communities under climate change. First, the degree of ecological adaptation could provoke that obligate specialists might not effectively track changing temperatures if they are dependent upon a poorly dispersing species. In this way, an additional problem to specialist herbivorous, such as some amphipods, could be the reduction of their main food resources as a consequence of the spread of invasive macrophytes. Unfortunately, limited data exists on the dispersal abilities and degree of specialization of most species. Second, climate change could create ecological

advantages for species that track changing temperatures better than their enemies or competitors. Regarding *C. racemosa*, the absence of natural enemies in the newly colonized region might facilitate an enhanced physiological performance under extreme conditions. Third, differences in dispersion among species can exacerbate antagonistic interactions through indirect mechanisms and cause extinctions. These interactions could favour macrophyte invaders, which generally are good dispersers, and could drive to the extinction of a competitor if the macrophyte overtakes it during climate change (Davis et al. 1998a, Davis et al. 1998b). Fourth, independent range shifts could create encounters with novel species that further restrict or promote range shifts. Novel interactions can strongly affect fitness because species lack a coevolutionary history with the new partner (Reznick and Ghalambo 2001). They can cause local extinctions or hamper the ability of a species to track climate, leading to increasingly restricted ranges (Moorcroft et al. 2006, Brooker et al. 2007). Similarly, novel interactions among native and exotic species can hinder and help invasions (Colautti et al. 2004). Fifth, metacommunity dynamics could be affected by climatic changes. Inferior competitors sometimes persist because of their colonization abilities after disturbance dispersing into empty patches. Therefore, good colonizers might benefit from climate change, particularly if disturbance increases. Furthermore, an indirect effect of climate change might occur as some ecosystems become less resistant to invasive species or more resilient to their impacts under future climates. In extreme cases, climate-driven invasions could lead to completely transformed ecosystems where alien species dominate function, richness, or both, leading to reduced diversity of native species (Mack et al. 2000, Gritti et al. 2006). However, it must be taken into account that anthropogenic climate change may now be an important confounding factor but, in many cases, not the origin of the invasion (Jackson et al. 2001).

In addition to climatic changes, global environment is undergoing modifications

brought about by human increasing use of the environment, and biodiversity issues are at stake, not only because of the extinction of endangered species, but also because of new equilibria in the communities resulting from human induced changes (Occhipinti-Ambrogi and Savini 2003). There are many ways in which human activities disrupt historical patterns of resource fluctuations, for instance, habitat destruction is considered the single most important threat caused by humans to biodiversity. When habitat cascade are hierarchically organized, the destruction of basal formers will cascade to impact on intermediate habitats and focal organisms (Thomsen et al. 2010). Fishing nets and anchors have a dual role enhancing the spread of *Caulerpa* species into the Mediterranean (Occhipinti-Ambrogi and Savini 2003). On one hand, they plough the sea-bottom depleting the native seagrass meadows and preparing the ground for an easier spreading of *Caulerpa*, while on the other hand, they help dissemination of the exotic algae by fragmentation of the thallus (Ceccherelli and Cinelli 1999, Relini et al. 2000). Pollution by nutrients in marine ecosystems may at first stimulate the growth of intermediate epiphytes and seaweeds (McGlathery 2001), possibly with a net benefit for focal organisms (i.e., more habitat becomes available). However, excessive growth of the intermediate habitat formers may ultimately destroy the habitat cascade. This can occur if the seagrasses and invertebrates are affected by epiphytic shading, physical smothering of the filtering apparatus, or anoxia (McGlathery 2001, Holmer and Nielsen 2007). Moreover, experiments carried out in the Mediterranean Sea demonstrated that an invasive alga (*C. taxifolia*) is favoured by high nutrients loads in the sediment, while the seagrass *Cymodocea nodosa* is not (Ceccherelli and Cinelli 1997). Decreasing water transparency in NW Mediterranean caused a reduction of the deep edges of seagrass meadows *P. oceanica* (Duarte et al. 1999) as a result of centuries of overexploitation of the coastal area. Such phenomena could also facilitate the colonization of deep substrates by *C. racemosa*, since it has been observed

growing up to 65 m depth (Cebrian et al. 2010).

7.3. Effects of *C. racemosa* on habitat complexity and heterogeneity

Many examples of invaders affecting the physical nature of ecosystems can be found in the literature in both aquatic and terrestrial ecosystems (see a review in Crooks 2002). This physical alteration of ecosystems typically produces cascading effects on many other biota, although these biotic consequences are less well characterized because it is typically easier to measure the effects on physical ecosystem properties than to assess the many and variable ways in which other species may respond to these changes (Crooks 2002).

Results obtained in this thesis show that the presence and abundance of *C. racemosa* provoked a marked effect on the macroalgal assemblage structure of Mediterranean shallow rocky communities, affecting the species and biomass composition of vegetation and increasing the detritus stock. An important reduction in species richness was recorded during the warm period due to a strong dominance of the alga at that time. In contrast, during the coldest period (lower vegetative cycle of *C. racemosa*) species richness was similar between invaded and non-invaded habitats. However, a big reduction on algal biomass was found. This suggests that, when *C. racemosa* is apparently absent, the benthic algal assemblages are characterized by the presence of high species richness but reduced biomass, which is characteristic of an early successional stage of the assemblage (Klein and Verlaque 2009a). According to other studies, exotic seaweeds can alter the structure of native communities even when they are found at relatively low abundances and when characterized by a seasonal resting phase (Bulleri et al. 2010). The negative effects of *C. racemosa* can therefore be detected all the year, even during the winter apparent disappearance of this alga (Klein and Verlaque 2009b). Furthermore, one of the most remarkable impacts of *C. racemosa* is the accumulation of detritus, which persists

throughout the year. In spite of the important seasonal variability of *C. racemosa* biomass, detritus accumulation persists in winter. This is a consequence of the mesh generated by the stolons of this species, which ensures that detritus is retained, while the multilayered structure formed by the thallus traps the sediment. This accumulation could alter the biogeochemical cycling modifying the availability and quality of food resources in the ecosystem (Crooks 2002).

However, it is concluded that effects on ecosystem may reach more dramatic levels since *C. racemosa* was detected in an initial stage of invasion, characterized by a patchy distribution surrounded by native vegetation. During this stage of invasion of a host habitat by the seaweed, an increment in habitat complexity is expected to occur if the initial cover is low; given that a new and additional habitat for the local epifauna becomes available (Viejo 1999). Later, monospecific meadows of *C. racemosa* were detected, and possibly the homogenization and loss of habitat complexity have impoverished the associated community, as it is known that complex habitats provide different shelter and foraging opportunities than morphologically simpler habitats (Forrest and Taylor 2002, Schmidt and Scheibling 2007).

7.4. Effects of *C. racemosa* on associated fauna community

Changes generated by the spread of *C. racemosa* on complexity, heterogeneity and quality of the habitat were reflected on the amphipod community and trophic relationship, as is summarized in Fig. 7.1. Amphipod assemblages associated with *C. racemosa* differed from assemblages on native seaweeds, in accordance to other studies that have demonstrated that modification of habitat complexity affects crustacean assemblages (Stoner 1980, Virnstein 1987, Sanchez-Jerez et al. 1999). In this way, low abundance and species richness of fauna has been found associated to the invasive seaweed *C. taxifolia* (Bellan-Santini et al. 1995), possibly due to the lower habitat complexity provided by

the new alga. However, other studies reveal higher abundance and species richness of macrofauna in the invasive *Undaria pinnatifida* compared to the local native seaweeds (Irigoyen et al. 2010). These authors state that this could be a consequence of invasive alga providing a more complex habitat and offering different shelter and foraging opportunities compared to morphologically simpler native habitats. In the present study case, species richness and abundance of amphipods were higher on native seaweeds on hard bottoms. The habitat provided by *C. racemosa* was less complex and more homogeneous than that provided by native seaweeds, which offer a heterogeneous and patchy habitat composed by more complex species with higher availability of microhabitats provided by epiphytes. In contrast, on soft bottoms, both features were higher in *C. racemosa* compared to other habitats except for *P. oceanica* meadows. This could be also explained by habitat complexity and heterogeneity. As a result, the invasive alga adds a complex structure where none previously occurred. In fact, this idea is consistent regarding the other habitats, where lower complex habitats (such as *C. prolifera*, *C. nodosa* and unvegetated substratum) showed lower abundances and species richness whilst the more complex habitat (*P. oceanica* meadows) showed the highest values (Sanchez-Jerez et al. 1999).

In addition to the differences found for on abundance and species richness, results also showed a great change on amphipod assemblage composition remarkably characterize by a high increase of abundance of detritivorous species and a decrease of herbivorous on *C. racemosa*. This pattern, in addition to habitat structure, can be explained by different colonization rates and preferences of species for a given habitat, trophic resource availability and also different predation pressures among habitats. From the experiment of habitat colonization, it was demonstrated that, in general, most of the species were more closely associated to native seaweeds than

the invasive alga, therefore, preference for a given habitat could be a determining factor of fauna distribution. In addition, availability of food seems to be crucial for these changes on detritivorous and herbivorous species. On one hand, detritus accumulated by *C. racemosa* is an appropriate habitat for detritivorous where trophic resource is “unlimited” available. On the other hand, herbivorous species will not find their main food resource, epiphytic algae, therefore facing disadvantageous conditions and the need of adapting to new trophic resources. Furthermore, the refuge from predation provided by different habitats it is also important for invertebrate benthic fauna (Heck and Orth 1980), which depends on the species and the use of microhabitat by different species (Aoki 1999).

Regarding predation, it has been demonstrated that amphipods were less available to the fish *T. pavo* where *C. racemosa* was present. It is noteworthy to mention that in spite of the high abundances of amphipods found on *C. racemosa*, they are less available for predators. Therefore, the presence of *C. racemosa* could affect the flow of matter and energy from invertebrates to higher trophic levels, as it has been proposed as one of the possible effects of invasive species in ecosystems (Crooks 2002). If high extensions of substratum are colonized by *C. racemosa* stands, a bottom-up effect could be taken place forcing predators to migrate into new areas searching new for food resources. However, studies with *Undaria pinnatifida* have suggested that the invasive algae could potentially produce a positive bottom-up effect on local food chains by increasing abundance of prey for a wide variety of predators, from invertebrates to higher trophic levels (Irigoyen et al. 2010), but the availability of those individuals for predators were unknown. Hence, invasive algae could produce positive or negative bottom-up effects depending on indirect effects on trophic interactions.

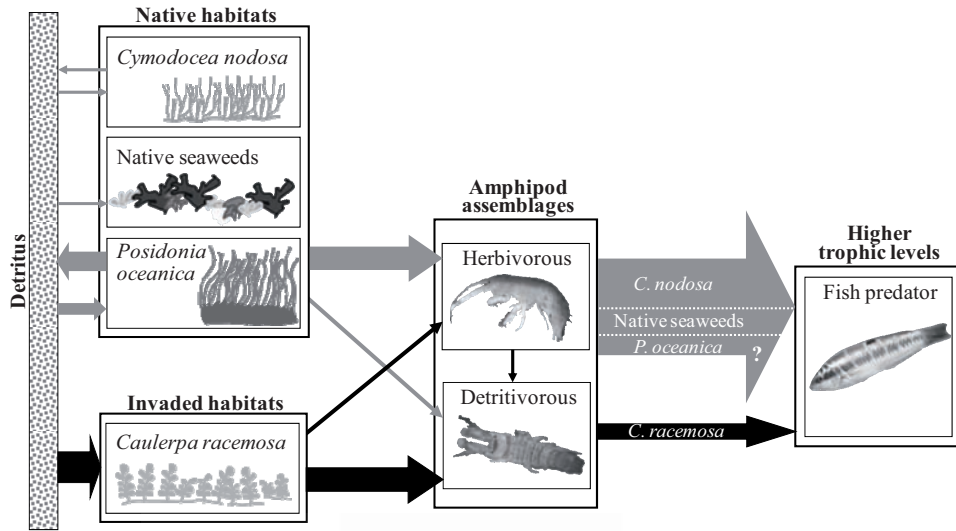


Figure 7.1. Conceptual model of ecological effects of *C. racemosa* invasion through the trophic web on shallow Mediterranean habitats and associated fauna community. Grey arrows: interactions by native habitats. Black arrow: interactions by *C. racemosa* invaded habitats. The thickness of the arrows represents the importance of the influence between compartments.

7.5. Gaps of knowledge and future research

Understanding the impacts of invasive species on natural ecosystems is an important component for the developing of management strategies. This thesis has filled substantial gaps in knowledge on the effects of the invasive seaweed *C. racemosa* var. *cylindracea* on recipient ecosystem, but has opened more avenues of investigation. The effects that *C. racemosa* is causing in other important invertebrate benthic taxa (such as polychaetes, other crustaceans or molluscs) should also be analyzed in order to evaluate such effect in the overall invertebrate benthic composition.

Specifically, the consequences that indirectly *C. racemosa* generates to the amphipod community should be tested because the side effects caused by biological invasions could be more important than direct effects (Gilman et al. 2010). Even though a modification in the diet of herbivorous amphipods living in *C.*

racemosa was found, consequences for fitness condition of this native fauna remain unknown. Further experiments are needed to analyze the effects of this modification, for instance on growth, reproduction and survival of the affected species. This thesis has shown that the availability of detritus as food for amphipods importantly increases as a result of its concentration being favoured by *C. racemosa*, but the quality of this detritus as food resource compared to others available in different habitats has not yet been analyzed. It is therefore important to examine if secondary production of amphipods or other benthic taxa are modified by the spread of the invasive algae. Finally, all of these investigations should be firmly supported by basic research, since most of the feeding habits and trophic guilds of amphipods continue to be undiscovered. Trophic relationships of this benthic fauna need to be clearly defined in order to effectively assess the effects of the invasion of *C. racemosa* on benthic communities.

Regarding bottom-up effects in ecosystems, the availability of invertebrates for other predators should be tested and future research should also focus on modelling the change on the flow change of energy and matter in the food web. Moreover, some specialised predators may obtain more benefit from the amount of invertebrates developed on *C. racemosa*. As an example, feeding of *Mullus surmuletus* on *C. racemosa* stands was observed, but it is unknown if feeding efficiency varies compared to other surrounding feeding grounds such as sand bottoms. Therefore, trophic relationships within this new habitat through higher links of the food chain are of special interest.

7.6. Management and mitigation of invaded ecosystems

In a changing world, it will be increasingly difficult to evaluate the impacts of alien species and prioritising species for removal, and it is likely that the increasing presence of “new” species and the decline of “old” ones will change successional patterns and ecosystem functioning (Harrington et al. 1999, McNeely 2001). These changes pose complex challenges for the management of biodiversity and could include implications for ecosystem functioning, especially with the addition or loss of ecosystem engineers (Jones et al. 1997). Hence, management practices with regard to the occurrence of ‘new’ species will require comprehensive evaluation of changing habitat conditions and will depend on the individual case. They could range from complete eradication to toleration and consideration of the “new” species as an enrichment of the local biodiversity as a means to facilitate ecosystem restoration or to maintain ecosystem function as native communities re-assemble and establish under the new conditions (Walther et al. 2009).

Practically, an exotic species which alters ecosystem properties would not merely compete with or consume native species, since it would alter the fundamental rules of existence for all organisms in the area (Vitousek 1990). It could have significant social or economic effects if it modifies any

of the "ecosystem services" (Ehrlich and Mooney 1983). Jackson et al. (2001) suggested that the characteristic sequence of human disturbance to marine ecosystems provides a framework for remediation and restoration that is invisible without a historical perspective. The management of population control is not based solely on consideration of the purely scientific, even those most eminently technical. The social impact that might have the tasks of control, interference with economic or recreational activities, the costs involved against the benefits obtained, the legal implications of different approaches to control or actual resources available, are only a few areas taken into account when considering the control of invasive alien species.

In this sense, Dana and Rodriguez-Luengo (2008) suggested three management process steps, first, obtention of resources, definition of general objectives and selection of participating entities. When addressing the control of invasive alien species, the first issue to be solved is the allocation of sufficient financial and human resources for the tagged purposes, which is to some extent dependent on the participating entities. Given the multifactorial nature of the process of biological invasion, cooperation between multiple sectors is often essential for achieving a set of actions really effective, as to cover as many scenarios of invasion. Second, definition of specific objectives. Outlining this objectives requires to establish, through scientific and technical criteria, the target species, the control or eradication strategy, identification of impacts, work of compensation (if applicable), diffusion and environmental education to raise awareness of citizenship, etc. Third, implementation and review of results (successes and failures). Once the work has begun, it is needed a continuous re-evaluation of the results as well as to regularly analyze the effectiveness of tasks, being monitoring of the community of major importance.

Given the ability to spread of *C. racemosa* none of the mechanisms designed for its eradication have been successful (Ceccherelli and Piazzini 2005, Institut

d'Ecologia Litoral 2006, Klein 2007). *C. racemosa* spreads up to 65 m depth, however, autonomous diving imposes strict limits both in depth and time. Eradication of species located more than 30 m depth is almost impossible if not through cutting-edge technology and high economic costs (Ballesteros 2008). Once an invader is established and dominates a community the biotic and abiotic conditions may be altered in such a degree that mitigating the causal disturbance(s) through the invader killing may not be the optimal control strategy (Firm et al. 2010). Moreover, reinstating the historical regime of disturbance does not guarantee reverting the changes faced by the biotic compartment, due to ecological resilience of the extant community or to demographic constraints of displaced populations (Suding et al. 2004). On the basis the rapid spread of *C. racemosa*, its ecological impact on habitat architecture and sediments, and the differentiation of extensive meadows in the Mediterranean Sea, it has been classified as a new keystone species (Klein and Verlaque 2008). According to Ballesteros (2008), it can only be expected that after a phase of

aggressive expansion, which is presently occurring, Mediterranean communities themselves activate response mechanisms against these invasions that can thrive native seaweeds and build new communities, although slightly modified and not losing their Mediterranean identity. Meanwhile, for further invasions scientists should learn to combat them, and society have to take steps to prevent a recurrence of such situations. To help with this task to Mediterranean communities, human disturbances on marine ecosystems should be drastically reduced, trying to keep ecosystems in good condition to face this and other biological invasions. Occhipinti-Ambrogi and Savini (2003) suggested that a robust native ecosystem can successfully fight competing newcomers. Therefore, a healthy pristine community can represent a natural impediment to bioinvasion; where and when this contrasting force is lacking, the alien species successfully outcompetes native ones. For that reason, environmental conservation plays a fundamental role in preventing further spreading of non-indigenous species.



Conclusions

Conclusions

1. The spread of *Caulerpa racemosa* in shallow Mediterranean algal habitats causes a great effect on habitat structure with a marked seasonality, increasing by 3 to 6 fold during the warm period. In consequence, it modifies the composition of amphipod assemblages when compared to native habitats.
2. *C. racemosa* accumulates detritus because the development of an intricate net of stolons and rhizoids. The detritus amount found in habitats colonized by the invasive algae was much higher than that in habitats formed by native vegetation in the same hydrodynamic conditions, which affects to habitat heterogeneity.
3. The abundance and species richness of amphipods on rocky bottoms were lower in sites colonized by *C. racemosa*, but these differences were greater during the coldest period. However, on soft bottoms, *C. racemosa* supports a diverse and abundant amphipod assemblage compared to native habitats, except for *P. oceanica* meadows in the warm period.
4. The spread of *C. racemosa* influences trophic guilds of amphipods in different ways. An increased abundance of the detritivore *Apocorophium acutum*, and a decrease of herbivorous species such as *Ampithoe ramondi* and *Hyale schmidtii*, was detected.
5. Experimentally we demonstrated that the colonization process of vegetated habitats can be affected by the presence of *C. racemosa*. Some species, such as *Elasmopus brasiliensis*, *Gammarella fucicola*, *Lysianassa costae* and *Lysianassa longicornis* seem to avoid the habitat provided by the invasive seaweed, and their total abundance and species richness were higher in algal stands formed by the native alga *H. scoparia*.
6. In experimental conditions, *C. racemosa* were colonized by several species with relatively high abundance, and the presence of detritus seems to facilitate the colonization process.
7. The expansion of *C. racemosa* to native algal community changes the feeding habits of the herbivorous amphipods *Ampithoe ramondi* and *Dexamine spiniventris*, which stop using plant tissues possibly because of the lack of an epiphytic community. In contrast, the accumulated detritus increases in its importance as their trophic resource. However, as we can expect, feeding habits of detritivorous, such as the *Apocorophium acutum*, were not affected by the spread of *C. racemosa*.
8. Trophic exploitation of amphipods associated with *C. racemosa* by predators, such as *T. pavo*, can be reduced compared to predation rates on natural habitats, different use of microhabitat by prey being important. However, the presence of detritus in *C. racemosa* habitat did not reduce significantly the predation rates.
9. The amphipod assemblage composition associated with *C. racemosa* clearly differs from that of other native habitat, being partially modified with respect to the trophic behaviour and reducing its availability for predators. Therefore, amphipods are a target component of marine ecosystems for understanding the effects of this invasive seaweed.

ISSN 0304-5714

ISSN 0304-5714

The Amphipod
of the Mediter

The Amphipoda of the Mediterranean

Editor

Sandro RUFFO



PART I

GAMMARIDEA

(ACANTHONOTOZOMATIDAE TO GAMMARIDAE)

**MEMOIRES DE L'INSTITUT
OCEANOGRAPHIQUE**
FONDATION ALBERT 1^{er}, PRINCE DE MONACO

N° 13

1982

References

- Allesina S, Bondavalli C, Scharler UM (2005) The consequences of the aggregation of detritus pools in ecological networks. *Ecological Modelling* 189: 221–232
- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32–46
- Aoki M (1999) Morphological characteristics of young, maternal care behaviour and microhabitat use by caprellid amphipods. *Journal of Marine Biological Association of the United Kingdom* 79: 629–638
- Arechavala-López P, Bayle-Sempere JT, Sánchez-Jerez P, Valle C, Forcada A, Fernández-Jover D, Ojeda-Martínez C, Vázquez-Luis M, Luna-Pérez B (2008) Biodiversity and structure of rocky reef fish assemblages in the Sierra Helada Natural Park (South-western Mediterranean Sea). *Arxius de Miscel·lània Zoològica* 6: 234–256
- Argyrou M, Demetropoulos A., Hadjichristophorou M (1999) Expansion of the macroalga *Caulerpa racemosa* and changes in softbottom macrofaunal assemblages in Moni Bay, Cyprus. *Oceanologica Acta* 22: 517–528
- Ayala Y, Martín A (2003) Relaciones entre la comunidad de anfípodos y las macroalgas a las que están asociados, en una plataforma rocosa del litoral central de Venezuela. *Boletín Instituto Español Oceanografía* 19 (1-4): 171–183
- Azzurro E, Fanelli E, Andaloro F (2004) Preliminary data on feeding habits of dusky spinefoot *Siganus luridus* in the Sicily Channel (central Mediterranean). *Biologia Marina Mediterranea* 11(3): 145
- Balata D, Piazzini L, Cinelli F (2004) A comparison among assemblages in areas invaded by *Caulerpa taxifolia* and *C. racemosa* on a subtidal Mediterranean rocky bottom. *Marine Ecology* 25: 1–13
- Ballesteros E (2008) La rápida expansión de algas del género *Caulerpa* por el litoral mediterráneo. In: Vilà MF, Valladares A, Traveset A, et al. (Eds). *Invasiones biológicas*. Madrid, Spain: CSIC-Divulgación
- Bat L, Raffaelli D (1998) Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. *Journal of Experimental Marine Biology and Ecology* 226: 217–239
- Beare DJ, Moore PG (1997) The contribution of Amphipoda to the diet of certain inshore fish species in Kames Bay, Millport. *Journal of Marine Biological Association of the United Kingdom* 77: 907–910
- Beck MW (2000) Separating the elements of habitat structure: independent effects of habitat complexity and structural components on rocky intertidal gastropods. *Journal of Experimental Marine Biology and Ecology* 249:29–49
- Bell JD, Harmelin-Vivien ML (1983) Fish fauna of French Mediterranean *Posidonia oceanica* seagrass meadows. 2. Feeding habits. *Tethys* 11: 1–14
- Bellan-Santini D (1980) Relationship between populations of amphipods and pollution. *Marine Pollution Bulletin* 11: 224–227
- Bellan-Santini D (1995) Faune d'invertébrés du peuplement à *Caulerpa taxifolia*. Données préliminaires pour les côtes de provence (Méditerranée nord-occidentale) *Biologia Marina Mediterranea* Vol 2, fasc 2 : 635–643 « Atti XXV Congresso »
- Bello CL, Cabrera MI (1999) Uso de la técnica microhistológica de Cavender y Hansen en la identificación de insectos

- acuáticos. *Boletín de Entomología Venezolana* 14:77–79
- Borg JA, Rowden AA, Attrill MJ, Schembri PJ, Jones MB (2006) Wanted dead or alive: high diversity of macroinvertebrates associated with living and « dead » *Posidonia oceanica* matte. *Marine Biology* 149: 667–677
- Boström C, Jackson E, Simenstad CA (2006) Seagrass landscapes and their effects on associated fauna: A review. *Estuarine Coastal and Shelf Science* 68: 383–403
- Boudouresque CF (1999) Introduced species in the Mediterranean: routes, kinetics and consequences. In: Proceedings of the Workshop on Invasive *Caulerpa* in the Mediterranean, Heraklion, Crete, Greece, March 18–20, 1998. UNEP, Athens, Greece, pp. 51–72
- Boudouresque CF, Verlaque M (2002) Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin* 44: 32–38
- Box A (2008) Ecología de caulerpales: fauna y biomarcadores. PhD thesis. Balearic Islands Univ., Spain 355 pp
- Box A, Deudero S, Sureda A, Blanco A, Alos J, Terrados J, Grau AM, Riera F (2009) Diet and physiological responses of *Spondyliosoma cantharus* (Linnaeus, 1758) to the *Caulerpa racemosa* var. *cylindracea* invasion. *Journal of Experimental Marine Biology and Ecology* 380:11–19
- Box A, Martin D, Deudero S (2010) Changes in seagrass polychaete assemblages after invasion by *Caulerpa racemosa* var. *cylindracea* (Chlorophyta: Caulerpaceae): community structure, trophic guilds and taxonomic distinctness. *Scientia Marina* 74(2):317–329
- Brawley SH (1992) Mesoherbivores. Pages 235–263 in: DM John, SJ Hawkins, and JH, Price, editors. Plant-animal interactions in the marine benthos. Clarendon Press, Oxford, UK
- Britton-Simmons KH (2004) Direct and indirect effects of the introduced alga *Sargassum muticum* on benthic, subtidal communities of Washington State, USA. *Marine Ecology Progress Series* 277: 61–78
- Brooker RW, Travis MJ, Clark EJ, Dytham C (2007) Modelling species' range shifts in a changing climate: the impacts of biotic interactions, dispersal distance and the rate of climate change. *Journal of Theoretical Biology* 245: 59–65
- Buia MC, Gambi MC, Terlizzi A, Mazzella L (2001) Colonization of *Caulerpa racemosa* along the southern Italian coasts: I. Distribution, phenological variability and ecological role. In: Gravez V, Ruitton S, Boudouresque CF, Le Direach L, Meinesz A, Scabbia G, Verlaque M (Eds.) Fourth International Workshop on *Caulerpa taxifolia*. GIS Posidonie Publisher, Marseille, France, pp. 352–360
- Buia MC, Mazzella L (1991) Reproductive phenology of the Mediterranean seagrasses *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Aschers and *Zostera noltii* Hornem. *Aquatic Botany* 40: 343–362
- Bulleri F, Balata D, Bertocci I, Tamburello L, Benedetti-Cecchi L (2010) The seaweed *Caulerpa racemosa* on Mediterranean rocky reefs: from passenger to driver of ecological change. *Ecology* 91(8): 2205–2212
- Caine EA (1991) Caprellid amphipods: fast food for the reproductively active. *Journal of Experimental Marine Biology and Ecology* 148: 27–33
- Cannon JFM (1979) An experimental investigation of *Posidonia* balls. *Aquatic Botany* 6: 407–410
- Cantone G (1999) Primi dati sul popolamento a Policheti associato a prati a *Caulerpa racemosa* (Forsskal) J. Agardh e *Caulerpa taxifolia* (Vahl) C. Agardh ecade *mexicana* Sonder ex

- Kutzing (Chlorophyceae, Caulerpales) lungo la costa orientale siciliana. *Bollettino dell'Accademia Gioenia di Scienze Naturali*. Catania 32(356): 151–160
- Capiomont A., Breugnot E, den Haan M, Meinesz A (2005) Phenology of a deep-water population of *Caulerpa racemosa* var. *cylindracea* in the northwestern Mediterranean Sea. Short communication. *Botanica Marina* 48: 80-83
- Carlton JT (1985) Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast waters. *Oceanography and Marine Biology: An Annual Review* 23: 313-371
- Carlton JT (2000) Global change and biological invasions in the Oceans. In: (HA Mooney and RJ Hobbs, eds) *Invasive species in a changing world*. Island Press, Washington DC. pp. 31–53
- Carrasco FD, Arcos DF (1984) Life history of a cold temperate population of the sublittoral amphipod *Ampelisca araucana*. *Marine Ecology Progress Series* 14:245-252
- Carriglio D, Sandulli R, Deastis S, Gallo d'Addabbo M, Grimaldi de Zio S (2003) *Caulerpa racemosa* spread effects on the meiofauna of the Gulf of Taranto. *Biologia Marina Mediterranea* 10(2): 509–511
- Casu D, Ceccherelli G, Palomba D, Curini-Gelletti M, Castelli A (2005) Effetto immediato della rimozione di *Caulerpa racemosa* sullo zoobenthos dell'infra-littorale superficiale roccioso di Porto Torres (Nord Sardegna). In: XV Meeting of the Italian Society of Ecology, pp. 1–3
- Casu D, Ceccherelli G, Sechi N, Rumolo P, Sarà G (2009) *Caulerpa racemosa* var. *cylindracea* as a potential source of organic matter for benthic consumers: evidences from stable isotope analysis. *Aquatic Ecology* 43: 1023-1029
- Cavas L, Yurdakoc K (2005) A comparative study: Assessment of the antioxidant system in the invasive green alga *Caulerpa racemosa* and some macrophytes from the Mediterranean. *Journal of Experimental Marine Biology and Ecology* 321: 35–41
- Cavas L, Baskin Y, Yurdakoc K, Olgun N (2006) Antiproliferative and newly attributed apoptotic activities from an invasive marine alga: *Caulerpa racemosa* var. *cylindracea*. *Journal of Experimental Marine Biology and Ecology* 339: 111-119
- Cebrian E, Ballesteros E, Linares C, Tomas (2010) Do native herbivores provide resistance to Mediterranean marine bioinvasions? A seaweed example. *Biological Invasions*, DOI 10.1007/s10530-010-9898-1
- Ceccherelli G, Campo D (2002) Different Effects of *Caulerpa racemosa* on Two Co-occurring Seagrasses in the Mediterranean. *Botanica Marina* 45:71-76
- Ceccherelli G, Cinelli (1997) Short-term effects of nutrient enrichment of the sediment and interactions between the seagrass *Cymodocea nodosa* and the introduced green alga *Caulerpa taxifolia* in a Mediterranean bay. *Journal of Experimental Marine Biology and Ecology* 217:165-177
- Ceccherelli G, Cinelli F (1999) The role of vegetative fragmentation in dispersal of the invasive alga *Caulerpa taxifolia* in the Mediterranean. *Marine Ecology Progress Series* 182: 299-303
- Ceccherelli G, Piazzì L (2001) *Caulerpa racemosa* e *Caulerpa taxifolia* in Mediterraneo: effetto della complessità dei popolamenti macroalgali autoctoni e dei loro rapporti competitivi. 328 Congresso SIBM, p 45
- Ceccherelli G, Piazzì L (2005) Exploring the success of manual eradication of *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta): the effect of habitat. *Cryptogamie Algologie* 26(3): 319-328

- Ceccherelli G, Piazzì L, Balata D (2002) Spread of introduced *Caulerpa* species in macroalgal habitats. *Journal of Experimental Marine Biology and Ecology* 280:1-11
- Ceccherelli G, Piazzì L, Cinelli F (2001) The role of vegetative fragments in the recruitment process of *Caulerpa taxifolia* and *Caulerpa racemosa*. In: Gravez V, Ruitton S, Boudouresque CF, Le Direac'h L, Meinesz A, Scabbia G, Verlaque M (eds) Fourth International Workshop on *Caulerpa taxifolia*. GIS Posidonie Publ, Marseilles, France, pp 111–117
- Chavanich S, Harris LG (2004) Impact of the non-native macroalga *Codium fragile* (Sur.) Hariot ssp. *tomentosoides* (van Goor) Silva on the native snail *Lacuna vineta* (Montagu, 1803) in the Gulf of Maine. *Veliger* 47:85–90
- Chemello R, Milazzo M (2001) Effect of algal architecture on associated fauna: some evidence from phytal molluscs. *Marine Biology* 140: 981–990
- Chesson P (2000) General theory of competitive coexistence in spatially-varying environments. *Theoretical Population Biology* 58: 211–237
- Ciarelli S, Vonck WAPMA, van Straalen NM (1997) Reproducibility of spiked-sediment bioassays using the marine benthic amphipod, *Corophium volutator*. *Marine Environmental Research* 4: 329–343
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18:117–143
- Clarke KR, Warwick RM (1994) Changes in Marine Communities: an approach to statistical analysis and interpretation. Natural Environment Research Council UK, 144 pp
- Clout M (1998) And now, the Homogocene. *World Conservation, Suisse*, 97(4)-98(1): 3
- Cochran WG (1951) Testing a linear relation among variances. *Biometrics* 7: 17-32
- Colautti RI Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7: 721–733
- Coll M, Piroddi C, Steenbeek J, Kaschner K, Ben Rais Lasram F, et al. (2010) The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. *PLoS ONE* 5(8): e11842. doi:10.1371/journal.pone.0011842
- Conklin EJ, Smith JE (2005) Abundance and spread of the invasive red algae, *Kappaphycus* spp., in Kane'ohe Bay, Hawai'i and an experimental assessment of management options. *Biological Invasions* 7:1029–39
- Connell JH (1972) Community interactions on marine rocky intertidal shores. *Annual Review of Ecology and Systematics* 3: 169-192
- Connell, S.D., Vanderklift, M.A., 2007. Chapter 4: Negative Interactions: The influence of Predators and Herbivores on Prey and Ecological Systems. In: Connell, S.D., Gillanders, B.M., Part 1. Fundamental Concepts in Marine Ecology, Oxford University Press, pp. 72-100.
- Conradi M, López-González PJ, García-Gómez JC (1997) The amphipod community as a bioindicator in Algeciras Bay (Southern Iberian Peninsula) based on a spatio-temporal distribution. *Marine Ecology* 18: 97–111
- Crawley MJ (1989) Chance and timing in biological invasions. In: Drake JA, Mooney HA, Di Castri F, Groves RH, Kruger FJ, Rejmánek M, Williamson M (eds) Biological invasions: a global perspective. Scientific Committee on Problems of the Environment (SCOPE), Chichester, p 407–423
- Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: the role of

- ecosystem engineers. *Oikos* 97: 153-166
- Crowe TP, Underwood AJ (1998) Testing behavioural "preference" for suitable microhabitat. *Journal of Experimental Marine Biology and Ecology* 225: 1-11
- Dana E, Luis Rodríguez-Luengo JL (2008) Gestión del control de las especies exóticas invasoras. In: Vilà MF, Valladares A, Traveset A, et al. (Eds). *Invasiones biológicas*. Madrid, Spain: CSIC-Divulgación
- Davis AJ, Jenkinson LS, Lawton JH, Shorrocks B, Wood S (1998a) Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391, 783-786
- Davis AJ, Lawton JH, Shorrocks B, Jenkinson LS (1998b) Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. *Journal of Animal Ecology* 67: 600-612
- Davis AR, Benkendorff K, Ward DW (2005) Responses of common SE Australian herbivores to three suspected invasive *Caulerpa* spp. *Marine Biology* 146: 859-68
- Dayton PK (1975) Experimental evaluation of ecological dominance in a rocky intertidal community. *Ecol Monogr* 45: 137-159
- Dean RL, Conell JH (1987) Marine invertebrates in algal succession. II. Test of hypotheses to explain changes in diversity with succession. *Journal of Experimental Marine Biology and Ecology* 109: 217-247
- Diehl S (1988) Foraging efficiency of three freshwater fishes: effects of structural complexity and light. *Oikos* 53: 207-214
- Djellouli A, Langar H, El Abed A (2006) Mollusques Ascoglosses associés aux peuplements de *Caulerpa racemosa* en Tunisie: espèces observées et description des effets trophiques. In: United Nations Environment Programme (Ed.), *Proceedings of the Second Mediterranean Symposium on Marine Vegetation*. Regional Activity Centre for Specially Protected Areas, Tunis, Tunisia, pp. 88-92
- Drew EA, Jupp BP (1976) Some aspects of the growth of *Posidonia oceanica* in Malta. In: Drew EA, Lithgoe JN, Woods JD (eds) *Underwater research*. Academic, London, pp 357-369
- Duarte CM, Agustí S, Kennedy H, Vaqué, D (1999) The Mediterranean climate as a template for Mediterranean marine ecosystems: the example of the northeast Spanish littoral. *Progress in Oceanography* 44: 245-270
- Duarte CM (2000) Marine biodiversity and ecosystem services: an elusive link. *Journal of Experimental Marine Biology and Ecology* 250: 117-131
- Duffy JE, Hay ME (1994) Herbivore resistance to seaweed Chemicals defense: the roles of mobility and predation risk. *Ecology* 75: 1304-1319
- Dumay O, Fernandez C, Pergent G (2002) Primary production and vegetative cycle in *Posidonia oceanica* when in competition with the green algae *Caulerpa taxifolia* and *Caulerpa racemosa*. *Journal of the Marine Biological Association of the United Kingdom* 82:379-87
- Edgar GJ (1983) The ecology of South-East Tasmanian phytal animal communities. IV. Factors affecting the distribution of amphipod amphipods among algae. *Journal of Experimental Marine Biology and Ecology* 70: 205-225
- Edgar GJ (1990) The influence of plant structure on the species richness, biomass and secondary production of macrofaunal assemblages associated with Western Australian seagrass beds. *Journal of Experimental Marine Biology and Ecology* 137: 215-240
- Edgar GJ (1992) Patterns of colonization of mobile epifauna in Western Australian seagrass bed. *Journal of*

- Experimental Marine Biology and Ecology* 157: 225-246
- Edgar GJ, Shaw C, Watson GF, Hammond LS (1994) Comparisons of species richness, size-structure and production of benthos in vegetated and unvegetated habitats in Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* 176: 201-226
- Ehrlich PR, Mooney HA (1983) Extinction, substitution, and ecosystem services. *Bioscience* 33: 248-253
- Elton CS (1958) The ecology of invasions by animals and plants. Methuen & Co. University of Chicago Press
- Enequist P (1949) Studies on the soft bottoms amphipods of the Shaggerak Zool. Bidr Upps 28: 297-492
- Ferrandis-Ballester E, Bartolomé-Pina F (1985) Dulces bárbaros del este y del oeste (Análisis estadísticos de los vientos en la bahía de Alicante). In: Ramos AA (Ed.), La reserva marina de la Isla Plana o Nueva Tabarca (Alicante). Ayuntamiento de Alicante y Universidad de Alicante, Alicante pp 51-93
- Firn J, House APN, Buckley YM (2010) Alternative states models provide an effective framework for invasive species control and restoration of native communities. *Journal of Applied Ecology* 47: 96-105
- Forrest BM, Taylor MD (2002) Assessing invasion impact: survey design considerations and implications for management of an invasive marine plant. *Biological Invasions* 4: 375-386
- Galil BS (2009) Taking stock: inventory of alien species in the Mediterranean sea. *Biological Invasions* 11: 359-372
- Garrison LP, Link JS (2000) Dietary guild structure of the fish community in the Northeast United States continental shelf ecosystem. *Marine Ecology Progress Series* 202: 231-240
- Giaccone G, Di Martino V (1995) Le Caulerpe in Mediterraneo: un ritorno del vecchio bacino Tetide verso il dominio Indo-Pacifico. *Biologia Marina Mediterranea* 2(2): 607-612
- Gianguzza P, Bonaviri C, Jensen K, Riggio S (2001) Ecological relationships between the sacoglossan opisthobranch *Oxynoe olivacea* and the siphonalean alga *Caulerpa racemosa*. *Biologia Marina Mediterranea* 8 (1): 605-608
- Gianguzza P, Airoldi L, Chemello R, Todd CD, Riggio S (2002) Feeding preferences of *Oxynoe olivacea* (Opisthobranchia: Sacoglossa) among three *Caulerpa* species. *Journal of Molluscan Studies* 68: 289-290
- Gianguzza P, Airoldi L, Chemello R, Todd CD, Riggio S (2002) Feeding preferences of *Oxynoe olivacea* (Opisthobranchia: Sacoglossa) among three *Caulerpa* species. *Journal of Molluscan Studies* 68: 289-90
- Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD (2010) A framework for community interactions under climate change. *Trends in Ecology and Evolution* 25(6): 325-331
- Gollan JR and Wright JT (2006) Limited grazing pressure by native herbivores on the invasive seaweed *Caulerpa taxifolia* in a temperate Australian estuary. *Marine and Freshwater Research* 57:685-694
- Grabowski JH, Powers SP (2004) Habitat complexity mitigates trophic transfer on oyster reefs. *Marine Ecology Progress Series* 277: 291-295
- Graeve M, Dauby P, Scailteur Y (2001) Combined lipid, fatty acid and digestive tract content analyses: a penetrating approach to estimate feeding modes of Antarctic amphipods. *Polar Biology* 24:853-862
- Gritti ES, Smith B, Sykes MT (2006) Vulnerability of Mediterranean basin ecosystems to climate change and invasion by exotic plant species. *Journal of Biogeography* 33: 145-157

- Guerra-García JM (2001) Habitat use of the Caprellidea (Crustacea: Amphipoda) from Ceuta, North Africa. *Ophelia* 55: 27–38
- Guerra-García JM, García-Gómez JC (2001) The spatial Distribution of Caprellidea (Crustacea: Amphipoda): A Stress Bioindicator in Ceuta (North Africa, Gibraltar Area). *Marine Ecology* 22: 357-367
- Guerra-García JM, García-Gómez JC (2005) Oxygen levels versus chemical pollutants: do they have similar influence on macrofaunal assemblages? A case study in a harbour with two opposing entrances. *Environmental Pollution* 135: 281-291
- Guerra-García JM, Tierno de Figueroa JM (2009) What do caprellids (Crustacea: Amphipoda) feed on? *Marine Biology* 156: 1881-1890
- Guerriero A., D'Ambrosio M, Guella G, Dini F, Pietra F (1994) Secondary metabolites of the green seaweed *Caulerpa taxifolia* introduced into the Mediterranean sea, and a comparison with ciliates of the genus Euplotes, pp.171–175, in CF Boudouresque, A Meinesz, and V Gravez (eds.) *First International Workshop on Caulerpa taxifolia*. GIS Posidonie Publication
- Guiry MD, Guiry GM (2007) Family: Caulerpacae taxonomy browser. AlgaeBase version 4.2 World-wide electronic publication, National University of Ireland, Galway
- Harmelin-Vivien M, Francour P, Harmelin JG, Le Direac'h (2001) Dynamics of fish assemblage alterations caused by the introduced *Caulerpa taxifolia* near Menton (France). In: GIS Posidonie Publ. (ed) Proceedings of the Fourth International Workshop on *Caulerpa taxifolia*, Lerichi, Italy, 236–245 pp
- Harrington R, Woiwod I, Sparks T (1999) Climate change and trophic interactions. *Trends in Ecology and Evolution* 14: 146-150
- Hay ME, Pawlik JR, Duffy JE, Fenical W (1989) Seaweed-herbivore-predator interactions: host-plant specialization reduces predation on small herbivores. *Oecologia* 81: 418-427
- Heck KL Jr, Orth RJ (1980) Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. In: Kennedy V.S. (ed.), *Estuarine Perspectives*. Academic Press, New York 449–464
- Heck KL, Crowder LB (1991) Cap. 14, 281-299 pp. Habitat structure and predator-prey interactions in vegetated aquatic systems. In: Habitat structure. The physical arrangement of objects in space. Eds: Susan S Bell, Earl D McCoy and Henry R Mushinsky, Department of Biology, University of Florida
- Heck KL Jr, Able KW, Roman CT, Fahay MP (1995) Composition, abundance, biomass and production of macrofauna in a New England estuary: comparisons among eelgrass meadows and other nursery habitats. *Estuaries* 18: 379–389
- Hicks GRF (1982) Habitat structure, disturbance, and equilibrium in crustacean communities. *Marine Ecology* 3: 41-45
- Hicks GRF (1997) Observations of substrate preference of marine phytal harpacticoids (Copepoda). *Hydrobiology* 56: 7-9
- Highsmith RC, Coyle KO (1990) High productivity of northern Bering Sea benthic amphipods. *Nature* 344: 862-864
- Holme M, Nielsen RM (2007) Effects of filamentous algal mats on sulfide invasion in eelgrass (*Zostera marina*). *Journal of Experimental Marine Biology and Ecology* 353: 245-52
- Holmlund MB, Peterson CH, Hay ME (1999) Does algal morphology affect amphipod susceptibility to fish predation? *Journal of Experimental*

- Marine Biology and Ecology* 139: 65-83
- Horn HS (1966) Measurement of "overlap" in comparative ecological studies. *American Naturalist* 100: 419-424
- Huston MA (1979) A general hypothesis of species diversity. *American Naturalist* 113: 81-101
- Institut d'Ecologia Litoral (2006) Presencia de *Caulerpa racemosa* en el parque natural de Serra Gelada i su entorno natural. Anexo VII en Memoria del Proyecto Biodiversidad Marina. Memoria 2006. Línea T4855000. Conselleria de Territori i Habitatge. Generalitat Valenciana 9pp
- Irigoyen AJ, Trobbiani G, Sgarlatta MP, Raffo MP (2010) Effects of the alien algae *Undaria pinnatifida* (Phaeophyceae, Laminariales) on the diversity and abundance of benthic macrofauna in Golfo Nuevo (Patagonia, Argentina): potential implications for local food webs. *Biological Invasions* DOI 10.1007/s10530-010-9910-9
- Irlandi EA (1994) Large- and small-scale effects of habitats structure on rates of predation: how percent coverage of seagrass affects rates of predation and siphon nipping on an infaunal bivalve. *Oecologia* 98: 176-183
- Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *Science* 293: 629-638
- Johnson M (1970) Variations in diversity within benthic marine communities. *American Naturalist* 104: 285-308
- Jones CG, Lawton JH, Shachak M (1997) Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* 78: 1946-1957
- Keast A (1978) Feeding interrelationships between age groups of pumpkinseed (*Lepomis gibbosus*) and comparisons with bluegill (*L. macrochirus*). *Journal of the Fisheries Research Board of Canada* 35: 12-27
- Kelly DJ, Hawes I (2005) Effects of invasive macrophytes on littoral-zone productivity and foodweb dynamics in a New Zealand high-country lake. *Journal of the North American Benthological Society* 24(2): 300-320
- Klein J (2007) Impact of *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) on macrophyte assemblages of the north-western Mediterranean Sea. PhD thesis, University of Aix-Marseille II, France, pp 315
- Klein J, Ruitton S, Verlaque M, Boudouresque CF (2005) Species introductions, diversity and disturbances in marine macrophyte assemblages of the northwestern Mediterranean Sea. *Marine Ecology Progress Series* 290: 79-88
- Klein J, Verlaque M (2008) The *Caulerpa racemosa* invasion: A critical review. *Marine Pollution Bulletin* 56: 205-225
- Klein JC, Verlaque M (2009a) Macroalgal assemblages of disturbed coastal detritic bottoms subject to invasive species. *Estuarine, Coastal and Shelf Science* 82: 461-468
- Klein JC, Verlaque M (2009b) Macrophyte assemblage associated with an invasive species exhibiting temporal variability in its development pattern. *Hydrobiologia* 636: 369-378
- Kocatas A (1976) Note préliminaire sur les amphipodes recueillis dans les horizons supérieurs de l'étage infralittoral rocheux du Golfe d'Izmir (Turquie). *Tethys* 7 (2-3): 235-240
- Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends Ecology Evolution* 16(4): 199-204

- Koukouras A, Russo A, Voultziadoukoukoura E, Dounas C, Chintiroglou C (1992) Relationship of sponge macrofauna with the morphology of their hosts in the north Aegean Sea. *Internationale Revue Der Gesamten Hydrobiologie* 77: 609–619
- Kruzic P, Žuljević A, Nolic V (2008) The highly invasive alga *Caulerpa racemosa* var. *cylindracea* poses a new threat to the banks of the coral *Cladocora caespitosa* in the Adriatic Sea. *Coral Reefs* 27: 441
- Lemée R, Pesando D, Durand-Clement M, Dubreuil A, Meinesz A, Guerriero A, Pietra F (1993) Preliminary survey of toxicity of the green alga *Caulerpa taxifolia* introduced into the Mediterranean. *Journal of Applied Phycology* 5: 485–493
- Lemée R, Pesando D, Issanchou C, Amade P (1997) Microalgae: a Model to Investigate the Ecotoxicity of the Green Alga *Caulerpa taxifolia* from the Mediterranean Sea. *Marine Environmental Research* 44: 13-25
- Levin PS, Coyer JA, Petrik R, Good TP (2002) Community-wide effects of non indigenous species on temperate rocky reefs. *Ecology* 83: 3182–93
- Levins R (1968) Evolution in changing environments: some theoretical explorations. Princeton University Press, Princeton, NJ
- Lodge DM (1993) Biological invasions: lessons for ecology. *Trends in Ecology & Evolution* 8:133–137
- Longepierre S, Robert A, Levi F, Francour P (2005) How an invasive alga species (*Caulerpa taxifolia*) induces changes in foraging strategies of the benthivorous fish *Mullus surmuletus* in coastal Mediterranean ecosystems. *Biodiversity Conservation* 14: 365–76
- Lundberg B, Payiatas G, Argyrou M (1999) Notes on the diet of the Lessepsian migrant herbivorous fishes, *Siganus luridus* and *S. rivulatus*, in Cyprus. *Israel Journal of Zoology* 45: 127–134
- Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710
- Martínez-Garrido J, Hackradt CW, Treviño-Otón J, García-Charton JA, Marcos C, Pérez-Ruzafa A (2010) Efecto del alga invasora *Caulerpa racemosa* (Forsskål) en el poblamiento de peces demersales del área de Cabo Tiñoso (Murcia, SE España). En: Bayle Sempere JT (coord.), Valle Pérez C, Sánchez Lizaso JL, Forcada A, Sánchez Jerez P, Giménez Casalduero F, Fernández Torquemada Y, González Correa JM, Oliveira Pires S, Ramos Esplá AA (Eds.) XVI Simposio Ibérico de Estudios de Biología Marina: bases científicas para la gestión sostenible de la biodiversidad marina. Libro de resúmenes. Universidad de Alicante, Alicante. 370 pp.
- Martin-Smith KM (1993) Abundance of mobile epifauna: the role of habitat complexity and predation by fishes. *Journal of Experimental Marine Biology and Ecology* 174: 243–260
- Mattila J, Heck Jr KL, Millstein E, Millern E, Gustafsson C, Williams S, Byron D (2008) Increased habitat structure does not always provide increased refuge from predation. *Marine Ecology Progress Series* 361: 15-20
- McGlathery K (2001) Macroalgal blooms contribute to the decline in seagrasses in nutrient-enriched coastal waters. *Journal of Phycology* 37: 453-6
- McNeely JA. ed (2001) The Great Reshuffling: Human Dimensions of Invasive Alien Species, IUCN, Biodiversity Policy Coordination Division
- Meadows PS, Reid A (1966) The behaviour of *Corophium volutator*. *Journal of Zoology* London 150: 387–399

- Meiners SJ, Pickett STA, Cadenasso ML (2002) Exotic plant invasions over 40 years of field succession: community patterns and associations. *Ecography* 25:215–223
- Mezgui Y, Djellouli AS, Ben Chikh Almi I (2007) Etude biométrique (biomasse et phénologie) des populations à *Caulerpa racemosa* dans la région de Bizerte (Tunisie). In: United Nations Environment Programme (Ed.), Proceedings of the 3rd Mediterranean Symposium on Marine Vegetation, Marseille, France, 27–29 March 2007. Regional Activity Centre for Specially Protected Areas, Tunis, Tunisia, pp. 111–116
- Modena M, Matricardi G, Vacchi M, Guidetti P (2000) Spreading of *Caulerpa racemosa* (Forsskål) J. Agardh (Bryopsidaceae, Chlorophyta) along the coasts of the Ligurian Sea. *Cryptogamie Algologie* 21: 301-304
- Moksnes PO, Gullström M, Tryman K, Baden S (2008) Trophic cascades in a temperate seagrass community. *Oikos* 117: 763 – 777
- Mollison D (1986) Modelling biological invasions: chance, explanation, prediction. *Phil Trans Of the Royal Society of London*, B 314: 675-693
- Moorcroft PR, Pacala SW, Lewis MA (2006) Potential role of natural enemies during tree range expansions following climate change. *Journal of Theoretical Biology* 241: 601-616
- Moore HB, Lopez NN (1972) Factors controlling variation in the seasonal spawning pattern of *Lytechinus variegatus*. *Marine Biology* 14: 275–280
- Moyle PB, Light T (1996) Fish invasions in California: do abiotic factors determine success? *Ecology* 77: 1666-1670
- Muñoz AA, Ojeda FP (1997) Feeding guild structure of a rocky intertidal fish assemblage in central Chile. *Environmental Biology of Fishes* 49: 471–479
- Myers (1995) Environmental Unknowns. *Science* 269: 358-360
- Nelson WG (1979) An analysis of structural pattern in an eelgrass (*Zostera marina* L.) amphipod community. *Journal of Experimental Marine Biology and Ecology* 39: 213–264
- Nichols FH, Thompson JK, Schemel LE (1990) Remarkable invasion of San Francisco Bay (California, U.S.A.) by the Asian clam *Potamocorbula amurensis*. II. Displacement of a former community. *Marine Ecology Progress Series* 66: 95-101
- Nizamuddin M (1991) The Green Marine Algae of Libya. Elga Publisher, Bern.
- Norse EA (1993) Global marine biological diversity. A strategy for building conservation into decision making. Island Press, Washington, DC. pp. 383
- Occhipinti-Ambrogi A, Savini D (2003) Biological invasions as a component of global change in stressed marine ecosystems. *Marine Pollution Bulletin* 46: 542-551
- Olabarria C, Underwood AJ, Chapman MG (2002) Appropriate experimental design to evaluate preferences for microhabitat: an example of preferences by species of microgastropods. *Oecologia* 132: 159–166
- Orth RJ (1992) A perspective on plant-animal interactions in seagrasses: physical and biological determinants influencing plant and animal abundance. In: John, D.M., Hawkins, S.J., Price, J.H., (eds) Plant–animal Interactions in the Marine Benthos, Systematics Association Special, vol 46. Oxford University Press, Oxford, pp 147–164
- Orth RJ, Heck KL, von Montfrans J (1984) Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator–prey relationships. *Estuaries* 7: 339–350

- Panayotidis P, Žuljević A (2000) Sexual reproduction of the invasive green alga *Caulerpa racemosa* var. *occidentalis* in the Mediterranean Sea. *Oceanologica Acta* 24(2): 199-203
- Panayotidis P, Žuljević A. (2001) Sexual reproduction of the invasive green alga *Caulerpa racemosa* var. *occidentalis* in the Mediterranean Sea. *Oceanologica Acta* 24: 199-203
- Pena-Martín C, Cristóbal-Fernanz JC, Crespo MB (2003) *Caulerpa racemosa* (Forssk.) J Agardh (Caulerpaceae, Chlorophyceae), nueva para la flora de Alicante. *Anales Jardín Botánico de Madrid* 60: 448-449
- Pérès JM, Picard J (1964) Nouveau manuel de bionomie benthique de la mer Méditerranée. *Recueil des Travaux de la Station Marine d'Endoume* 31: 5-137
- Peterson CH, Summerson HC, Duncan PB (1984) The influence of seagrass cover on population structure and individual growth rate of a suspension-feeding bivalve, *Mercenaria mercenaria*. *Journal of Marine Research* 42: 123-138
- Piazzini L, Acunto S, Magri M, Rindi F, Balestri E (1997) Osservazioni preliminari sull'espansione di *Caulerpa racemosa* (Forsskål) J. Agardh sulle Secche della Meloria (Livorno, Italia). *Biologia Marina Mediterranea* 4 (1): 426-428
- Piazzini L, Balata D (2008) The spread of *Caulerpa racemosa* var. *cylindracea* in the Mediterranean Sea: an example of how biological invasions can influence beta diversity. *Marine Environmental Research* 65: 50-61
- Piazzini L, Balata D, Ceccherelli G, Cinelli F (2001a) Comparative study of the growth of two co-occurring introduced green algae *Caulerpa taxifolia* and *Caulerpa racemosa* along the Tuscan coast (Italy, western Mediterranean). *Cryptogamie Algologie* 22: 459-466
- Piazzini L, Ceccherelli G, Cinelli F (2001b) Expansion de *Caulerpa taxifolia* et de *Caulerpa racemosa* le long des côtes toscanes (Italie), situation en 1998. In: Gravez V, Ruitton S, Boudouresque CF, Le Direach L, Meinesz A, Scabbia G, Verlaque M (Eds.), Fourth International Workshop on *Caulerpa taxifolia*. GIS Posidonie Publisher, Marseille, France, pp. 71-77
- Piazzini L, Ceccherelli G, Cinelli F (2001c) Threat to macroalgal diversity: effects of the introduced green alga *Caulerpa racemosa* in the Mediterranean. *Marine Ecology Progress Series* 210: 149-59
- Piazzini L, Balata D, Cecchi E, Cinelli F (2003) Co-occurrence of *Caulerpa taxifolia* and *C. racemosa* in the Mediterranean Sea: Interspecific interactions and influence on native macroalgal assemblages. *Cryptogamie Algologie* 24: 233-43
- Piazzini L, Balata D, Foresi L, Cristaudo C, Cinelli F (2007) Sediment as a constituent of Mediterranean benthic communities dominated by *Caulerpa racemosa* var. *cylindracea*. *Scientia Marina* 71(1): 129-135
- Piazzini L, Ceccherelli G, Balata D, Cinelli F (2003) Early patterns of *Caulerpa racemosa* recovery in the Mediterranean Sea: the influence of algal turfs. *Journal of Marine Biological Association of the United Kingdom* 83:27-29
- Piazzini L, Cinelli F (1999) Développement et dynamique saisonnière d'un peuplement méditerranéen de l'algue tropicale *Caulerpa racemosa* (Forsskål) J. Agardh. *Cryptogamie Algologie* 20 (4): 295-300
- Piazzini L, Meinesz A, Verlaque M, Akçali B, Antolić B, Argyrou M, Balata D, Ballesteros E, Calvo S, Cinelli F, Cirik S, Cossu A, D'Archino R, Djellouli AS, Javel F, Lanfranco E, Mifsud C, Pala D, Panayotidis P, Peirano A, Pergent G, Petrocelli A, Ruitton S, Žuljević, A, Ceccherelli G (2005) Invasion of *Caulerpa racemosa* var. *cylindracea* (Caulerpaceae, Chlorophyta) in the Mediterranean Sea: an assessment of the spread. *Cryptogamie Algologie* 26 (2): 189-202

- Poore AGB (2004) Spatial associations among algae affect host use in a herbivorous marine amphipod. *Oecologia* 140: 104-112
- Poore AGB (2005) Scales of dispersal among hosts in a herbivorous marine amphipods. *Austral Ecology* 30: 219-228
- Poore AGB, Hill NA (2006) Sources of variation in herbivore preference: among individual and past diet effects on amphipod host choice. *Marine Biology* 149: 1403-1410
- Relini G, Relini M, Torchia G (2000) The role of fishing gear in the spreading of allochthonous species: the case of *Caulerpa taxifolia* in the Ligurian Sea. *ICES Journal of Marine Sciences* 57: 1421-1427
- Renoncourt L, Meinesz A (2002) Formation of propagules on a invasive strain of *Caulerpa racemosa* (Chlorophyta) in the Mediterranean Sea. *Phycologia* 5: 533-535
- Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations: what empirical studies reveal about conditions that promote adaptive evolution. *Genetica* 112-113: 183-198
- Ribera MA, Boudouresque CF (1995) Introduced marine plants, with special reference to macroalgae: mechanisms and impact. *Progress in Phycological Research* 11: 187-268
- Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta D, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* 6, 93-107
- Rivera-Ingraham GA, García-Gómez JC, Espinosa F (2010) Presence of *Caulerpa racemosa* (Forsskal) J. Agardh in Ceuta (Northern Africa, Gibraltar area). *Biological Invasions* 12: 1465-1466
- Roberts DA, Poore AGB (2005) Habitat configuration affects colonisation of epifauna in marine alga bed. *Biological Conservation* 127: 18-26
- Ruffo S (Ed.) (1982) The Amphipoda of the Mediterranean. Part 1, Gammaridea. Mémoires de l'Institut Océanographique, Monaco 13, 364 pp
- Ruffo S (Ed.) (1998) The Amphipoda of the Mediterranean. Part 4. Mémoires de l'Institut Océanographique, Monaco 13, 144 pp
- Ruitton S, Verlaque M, Aubin G, Boudouresque CF (2006) Grazing on *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) in the Mediterranean Sea by herbivorous fish and sea urchins. *Vie et Milieu* 56(1): 33-41
- Ruitton S, Verlaque M, Boudouresque CF (2005) Seasonal changes of the introduced *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its Mediterranean range. *Aquatic Botany* 82: 55-70
- Rull L, Cañizares S, Sánchez-Lizaso JL (1996) Efecto de las praderas de *Cymodocea nodosa* (Curia) Ascherson sobre el hidrodinamismo: resultados preliminares. Real Sociedad Española de Historia Natural, Tomo extraordinario, 125 Aniversario de la RSEHN pp 444-447
- Russo AR (1980) Effects of predation by fishes, competition and structural complexity of the substratum on the establishment of a marine epifaunal community. *Journal of Experimental Marine Biology Ecology* 42: 55-69
- Russo AR (1987) Role of habitat complexity in mediating predation by the gray damselfish *Abedefduf sordidus* on epiphytal amphipods. *Marine Ecology Progress Series* 36: 101-105
- Russo AR (1997) Epifauna living on sublittoral seaweeds around Cyprus. *Hydrobiologia* 344: 169-179
- Sala E, Ballesteros E (1997) Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae)

- in a Mediterranean rocky infralittoral ecosystem. *Marine Ecology Progress Series* 152: 273-283
- Sanchez-Jerez P, Barberá-Cebrián C, Ramos-Esplá AA (1999) Comparison of the epifauna spatial distribution in *Posidonia oceanica*, *Cymodocea nodosa* and unvegetated bottoms: Importance of meadow edges. *Acta Oecologica* 20: 391-405
- Sanchez-Jerez P, Barberá-Cebrián C, Ramos-Esplá AA (2000) Influence of the structure of *Posidonia oceanica* meadows modified by bottom trawling on crustacean assemblages: comparison of amphipods and decapods. *Scientia Marina* 64: 319-326
- Sanchez-Jerez P, Gillanders BM, Kingsford MJ (2002) Spatial variation in abundance of prey and diet of trumpeter (*Pelates sexlineatus*: Teraponidae) associated with *Zostera capricorni* seagrass meadows. *Austral Ecology* 27: 200-210
- Sanchez-Jerez P, Ramos-Esplá AA (1996) Detection of environmental impacts by bottom trawling on *Posidonia oceanica* (L.) Delile meadows: sensitivity of fish and macroinvertebrate communities. *Journal of Aquatic Ecosystem Health* 5: 239-253
- Sánchez-Moyano JE (2004) Las praderas de *Caulerpa prolifera*: Distribución y requerimientos ecológicos. In: Luque AA, Templado J (Eds), *Praderas y bosques marinos de Andalucía*. Conserjería de Medio Ambiente, Junta de Andalucía, Sevilla: 239-240
- Sánchez-Moyano JE, Estacio FJ, García-Adiego EM, García-Gómez JC (2001) Effect of the vegetative cycle of *Caulerpa prolifera* on the spatio-temporal variation of invertebrate macrofauna. *Aquatic Botany* 70: 163-174
- Sánchez-Moyano JE, García-Asencio I, García-Gómez JC (2007) Effects of temporal variation of the seaweed *Caulerpa prolifera* cover on the associated crustacean community. *Marine Ecology* 28: 324-337
- Sarvala J, Uitto A (1991) Production of the benthic amphipod *Pontoporeia affinis* and *P. femorata* in a Baltic archipelago. *Ophelia* 34: 71-90
- Sax DF (2002) Native and naturalized plant diversity are positively correlated in scrub communities of California and Chile. *Diversity and Distribution* 8:193-210
- Schaffelke B, Hewitt CL (2007) Impacts of introduced seaweeds. *Botanica Marina* 50: 397-417
- Schmidt AL, Scheibling RE (2007) Effects of native and invasive macroalgal canopies on composition and abundance of mobile benthic macrofauna and turf-forming algae. *Journal of Experimental Marine Biology and Ecology* 341: 110-130
- Schmidt AL, Scheibling RE (2007) Effects of native and invasive macroalgal canopies on composition and abundance of mobile benthic macrofauna and turf-forming algae. *Journal of Experimental Marine Biology and Ecology* 341:110-130
- Schmidt-Nielsen K (1984) Scaling: why is animal size so important? Cambridge University Press, Cambridge
- Sebens KP (1991). Cap. 11, 211-234 pp. Habitat structure and community dynamics in marine benthic systems. In: Habitat structure. The physical arrangement of objects in space. Eds: Susan S Bell, Earl D McCoy and Henry R Mushinsky, Department of Biology, University of Florida
- Settle WH, Wilson LT (1990) Invasion by the variegated leafhopper and biotic interactions: parasitism, competition and apparent competition. *Ecology* 71: 1461-1470
- Sfriso A, Birkemeyer T, Ghetti PF (2001) Benthic macrofauna changes in areas of Venice lagoon populated by seagrasses or seaweeds. *Marine Environmental Research* 52: 323-349

- Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17: 170-176
- Simberloff D, Von Holle B (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1: 21-32
- Smith LD, Wonham MJ, McCann DL, Ruiz GM, Hines AH, Carlton JT (1999) Invasion pressure to a ballast-flooded estuary and an assessment of inoculant survival. *Biological Invasions* 1: 67-87
- Smith SDA, Rule MJ (2002) Artificial substrata in a shallow sublittoral habitat: do they adequately represent natural habitats or the local species pool? *Journal of Experimental Marine Biology and Ecology* 277: 25-41
- Stål J, Pihl L, Wennhage H (2007) Food utilisation by coastal fish assemblages in rocky and soft bottoms on the Swedish west coast: inference for identification of essential fish habitats. *Estuarine Coastal and Shelf Science* 71: 593-607
- Stoner AW (1979) Species specific predation on amphipod Crustacea by the pinfish *Lagodon rhomboides*: Mediation by macrophyte standing crop. *Marine Biology* 55: 201-208
- Stoner AW (1980) Perception and choice of substratum by epifaunal amphipods associated with seagrasses. *Marine Ecology Progress Series* 3: 105-111
- Stoner AW (1982) The influence of benthic macrophytes on the foraging efficiency of the pinfish *Lagodon rhomboides*. *Journal of Experimental Marine Biology and Ecology* 58: 271-284
- Stoner AW, Lewis FG (1985) The influence of quantitative and qualitative aspects of habitat complexity in tropical seagrass meadows. *Journal of Experimental Marine Biology and Ecology* 94: 19-40
- Suding KN, Gross KL, Houseman GR (2004) Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology and Evolution* 19: 46-53
- Sudo H, Azeta M (2001) The microhabitat and size of gammarid species selectively predated by young red sea bream *Pagrus major*. *Fisheries Science* 67: 389-400
- Sumi CBT, Scheibling RE (2005) Role of grazing by sea urchins *Strongylocentrotus droebachiensis* in regulating the invasive alga *Codium fragile* ssp. *tomentosoides* in Nova Scotia. *Marine Ecology Progress Series* 292: 203-12
- Sutherland JP (1974) Effects of fish predation on fouling communities. *Bulletin of the Ecological Society of America* 53: 10
- Taylor RB, Cole RG (1994) Mobile epifauna on subtidal brown seaweeds in northeastern New Zealand. *Marine Ecology Progress Series* 115: 271-282
- Thomsen MS, Wernberg T, Altieri A, Tuya F, Gulbransen D, McGlathery KJ, Holmer M, Silliman BR (2010) Habitat Cascades: The Conceptual Context and Global Relevance of Facilitation Cascades via Habitat Formation and Modification. *Integrative and Comparative Biology* 50(2): 158-175
- Tierno de Figueroa JM, Vera A, López-Rodríguez MJ (2006) Adult and nymphal feeding in the stonefly species *Antarctoperla michaelsoni* and *Limnoperla jaffueli* from Central Chile (Plecoptera: Gripopterygidae). *Entomologia Generalis* 29(1): 39-45
- Tomas F, Box A, Terrados J (2010) Effects of invasive seaweeds on feeding preference and performance of a keystone Mediterranean herbivore. *Biological Invasions* doi:10.1007/s10530-010-9913-6
- Tomas F, Cebrian E, Ballesteros E (2011) Differential herbivory of invasive algae by native fish in the Mediterranean Sea. *Estuarine, Coastal and Shelf Science* doi:10.1016/j.ecss.2010.12.004

- Torchin ME, Lafferty KD, Kuris AM (1996) Infestation of an introduced host, the European green crab, *Carcinus maenas*, by a symbiotic nemertean egg predator, *Carcinonemertes epialti*. *Journal of Parasitology* 82:449-453
- Trowbridge CD (1995) Establishment of green alga *Codium fragile* ssp. *tomentosoides* on New Zealand rocky shores: Current distribution and invertebrate grazers. *Journal of Ecology* 83: 949–65
- Tsirika A, Patoucheas D, Haritonidis S (2006) Contribution to the knowledge of the benthic marine macroalgae from Mani region (Messiniakos Gulf, Greece). In: United Nations Environment Programme (Ed.), Proceedings of the Second Mediterranean Symposium on Marine Vegetation. Regional Activity Centre for Specially Protected Areas, Tunis, Tunisia, p. 245
- Tuya F, Larsen K, Platt V (2010) Patterns of abundance and assemblage structure of epifauna inhabiting two morphologically different kelp holdfasts. *Hydrobiologia* DOI 10.1007/s10750-010-0527-x
- Underwood AJ (1981) Techniques of analysis of variance in experimental biology and ecology. *Oceanography and Marine Biology. An Annual Review* 19: 513-605
- Underwood AJ, Denley EJ (1984) Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. In: Strong DR, Simberloff D, Abele LG, Thistle AB (eds) *Ecological communities: conceptual issues and the evidence*. Princeton University Press, Princeton, NJ pp 151–180
- Underwood AJ (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, 504 pp
- Valiela I (1995) *Marine Ecological Processes*. Springer-Verlag, New York, 686 pp
- Van den Brink FWB, Van der Velde G, Bij de Vaate A. (1993) Ecological aspects, explosive range extension and impact of a mass invader, *Corophium curvispinum* Sars, 1895 (Crustacea: Amphipoda), in the Lower Rhine (The Netherlands). *Oecologia* 93: 224-232
- Vázquez-Luis M, Sanchez-Jerez P, and Bayle-Sempere JT (2008) Changes in amphipod (Crustacea) assemblages associated with shallow-water algal habitats invaded by *Caulerpa racemosa* var. *cylindracea* in the western Mediterranean Sea. *Marine Environmental Research* 65: 416-426
- Vázquez-Luis M, Guerra-García JM, Sanchez-Jerez P, and Bayle-Sempere JT (2009a) Caprellid assemblages (Crustacea: Amphipoda) in shallow waters invaded by *Caulerpa racemosa* var. *cylindracea* from southeastern Spain. *Helgoland Marine Research* 63: 107-117
- Vázquez-Luis M, Sanchez-Jerez P, and Bayle-Sempere JT (2009b) Comparison between amphipod assemblages associated with *Caulerpa racemosa* var. *cylindracea* and those of other Mediterranean habitats on soft substrate. *Estuarine Coastal and Shelf Science* 84: 161-170
- Vázquez-Luis M, Borg JA, Sanchez-Jerez P, Png L, Cavallo M, Bayle-Sempere JT (2009c) Why amphipods prefer the new available habitat built by *C. racemosa*?: a field experiment in Mediterranean Sea". *International Symposium on Marine Sciences*, Vigo, Spain.
- Vázquez-Luis M, Sanchez-Jerez P, and Bayle-Sempere JT (2010) Effects of *Caulerpa racemosa* var. *cylindracea* on prey availability: an experimental approach to predation by *Thalassoma pavo* (Labridae) on amphipods. *Hydrobiologia* 654: 147–154
- Verlaque M, Alfonso-Carrillo J, Gil-Rodríguez MC, Durand C,

- Boudouresque CF, Le Parco Y (2004) Blitzkrieg in a marine invasion: *Caulerpa racemosa* var. *cylindracea* (Bryopsidales, Chlorophyta) reaches the Canary Islands (north-est Atlantic). *Biological Invasions* 6: 269-281
- Verlaque M, Boudouresque CF, Meinesz A, Gravez V (2000) The *Caulerpa racemosa* complex (Caulerpales, Ulvophyceae) in the Mediterranean Sea. *Botanica Marina* 43: 49-68
- Verlaque M, Durand C, Huisman JM, Boudouresque CF, Le Parco Y (2003) On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta). *European Journal of Phycology* 38: 325-339
- Verlaque M, Ruitton S, Mineur F, Boudouresque CF (2007) CIESM Atlas of exotic macrophytes in the Mediterranean Sea. *Rapp. Comm. int. Mer Médit.*, 38
- Viejo RM (1999) Mobile epifauna inhabiting the invasive *Sargassum muticum* and two local seaweeds in northern Spain. *Aquatic Botany* 64: 131-149
- Vilà M, Basnou C, Pyšek P, Josefsson M, Genovesi P, Gollasch S, Nentwig W, Olenin S, Roques A, Roy D, Hulme PE, DAISIE partners (2009) How well do we understand the impacts of alie species on ecological services? A pan-European cross-taxa assessment. *Frontiers in Ecology and the Environment*, doi:10.1890/080083
- Virnstein RW (1987) Seagrass-associated invertebrate communities of the Southeastern USA: a review. *Florida Marine Research Publications* 42: 89-116
- Virnstein RW, Howard RK (1987) Motile epifauna of marine macrophytes in the Indian River Lagoon, Florida. I. Comparisons among three species of seagrasses from adjacent beds. *Bulletin of Marine Sciences* 41: 1-12
- Vitousek PM (1986) Biological invasions and ecosystem properties: can species make a difference? In: Mooney HA. and Drake J (eds) *Biological invasions of North America and Hawaii*. Springer, pp. 163-176
- Vitousek PM (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57: 7-13
- Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* 21:1-16
- Walker DI, Hutchings PA, Wells FE (1991) Seagrass, sediment and infauna: a comparison of *Posidonia australis*, *Posidonia sinuosa* and *Amphibolis antarctica* in Princess Royal Harbour, south-western Australia. I. Seagrass biomass, productivity and contribution to sediments. In: Wells FE, Walker DI, Kirkman H, Lethbridge R (eds) *The Marine Flora and Fauna of Albany, W Australia*, Vol 2 W Australian Museum, Perth, pp 597-610
- Walther GR, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarošík V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semchenko V, Solarz W, Thuiller W, Vilà M, Vohland K, Settele J (2009) Alien species in a warmer world: risks and opportunities. *Trends in Ecology and Evolution* 24(12): 686-693
- Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E (1986) *Fishes of the north-eastern Atlantic and the Mediterranean*. UNESCO, Paris
- Wikström SA, Kautsky L (2004) Invasion of a habitat-forming seaweed: effects on associated biota. *Biological Invasions* 6: 141-150
- Williams SL, Smith JE (2007) A global review of the distribution, taxonomy, and impacts of introduced seaweeds.

- Annual Review of Ecology, Evolution, and Systematics* 38: 327-359
- Williamson MH, Fitter A (1996) The varying success of invaders. *Ecology* 77(6): 1661-1666
- Wonham MJ, Carlton JT, Ruiz GM, Smith LD (2000) Fish and ships: relating dispersal frequency to success in biological invasions. *Marine Biology* 136: 1111-1121
- Woods CMC (2009) Caprellid amphipods: An overlooked marine finfish aquaculture resource? *Aquaculture* 289: 199-211
- Yokes B, Rudman WB (2004) Lessepsian opisthobranchs from southwestern coast of Turkey; five new records for Mediterranean. *Rapports de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* 37: 557
- Žuljević A, Antolić B, Despalatovic M, Onofri V (2004) The spread of the invasive variety of *Caulerpa racemosa* in the Adriatic Sea. *Rapports de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* 37: 466
- Žuljević A, Antolić B, Onofri V (2003) First record of *Caulerpa racemosa* (Caulerpales: Chlorophyta) in the Adriatic Sea. *Journal of the Marine Biological Association of the UK* 83: 711-712



Universitat d'Alacant
Universidad de Alicante

Reunido el Tribunal que suscribe en el día de la fecha acordó otorgar, por _____ a la Tesis Doctoral de Dña. Maria Teresa Vázquez Luis la calificación de _____ .

Alicante de de

El Secretario,



El Presidente,

Universitat d'Alacant
Universidad de Alicante

**UNIVERSIDAD DE ALICANTE
CEDIP**

La presente Tesis de Dña. Maria Teresa Vázquez Luis ha sido registrada con el nº _____ del registro de entrada correspondiente.

Alicante ___ de _____ de _____

El Encargado del Registro,

La defensa de la tesis doctoral realizada por D^a Maria Teresa Vázquez Luis se ha realizado en las siguientes lenguas: Castellano e Inglés, por lo que unido al cumplimiento del resto de requisitos establecidos en la Normativa propia de la UA le otorga la mención de "Doctora Europea".

Alicante, ___ de _____ de _____

EL SECRETARIO

EL PRESIDENTE



Universitat d'Alacant
Universidad de Alicante



Universitat d'Alacant



Universitat d'Alacant
Universidad de Alicante

Dpt. de Ciències del Mar i Biologia Aplicada
Dpto. de Ciencias del Mar y Biología Aplicada

CIMAR - Centro de Investigación Marina de Santa Pola



INSTITUTO ALICANTINO DE CULTURA JUAN GIL-ALBERT
DIPUTACION PROVINCIAL DE ALICANTE

Apoyo a la Investigación • Ayudas a la realización de Tesis Doctorales