

DINÁMICA DE INVASIÓN DE LOS CAPRÉLIDOS (CRUSTACEA: AMPHIPODA) INTRODUCIDOS EN EL MAR MEDITERRÁNEO Y EN AGUAS ADYACENTES. DISTRIBUCIÓN GLOBAL, ECOLOGÍA Y VECTORES DE PROPAGACIÓN.

Invasion dynamic of introduced caprellids (Crustacea: Amphipoda) in the Mediterranean Sea and adjacent waters. Global distribution, ecology and vectors of spread.



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**Dinámica de invasión de los caprellidos (Crustacea: Amphipoda)
introducidos en el mar Mediterráneo y en aguas adyacentes.
Distribución global, ecología y vectores de propagación**

*Invasion dynamic of introduced caprellids (Crustacea: Amphipoda)
in the Mediterranean Sea and adjacent waters. Global distribution,
ecology and vectors of spread*

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Que esta Memoria de Investigación, titulada “*Dinámica de invasión de los caprelidos (Crustacea: Amphipoda) introducidos en el mar Mediterráneo y en aguas adyacentes. Distribución global, ecología y vectores de propagación*”, fue realizada por Macarena Ros Clemente bajo su dirección, en el Departamento de Zoología de la Universidad de Sevilla. Considerando que reúne las condiciones necesarias para constituir un trabajo de Tesis Doctoral, autorizan su defensa ante los miembros del Tribunal para optar al título de Doctor con Mención Internacional.

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A toda mi familia

*“Al carro de la cultura española
le falta la rueda de la ciencia”*

Santiago Ramón y Cajal

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Summary

Marine invasions are a fundamental component of global change and are considered one of the greatest threats from human activity in the ocean. However, knowledge of the current status of human-mediated marine species introductions is still very fragmentary. In the western Mediterranean Sea and the East Atlantic coast, especially in the Iberian Peninsula, although the interest in the topic of non-native species is increasing, most of studies are focused on alien seaweeds or ‘charismatic’ megafauna. Small and taxonomically complicated taxa are frequently overlooked, leading to a probable underestimation of the extent to which non-native species may be present.

The subphylum Crustacea includes the most successful invasive species among introduced invertebrates in marine ecosystems. Despite the number of introduced crustaceans has increased in recent decades in European seas, the number of non-native peracaridean species is still underestimated. This is particularly marked for caprellid amphipods, which often represent a challenge concerning their taxonomy. Caprellids are small marine crustaceans with an important role in marine ecosystems, where they act as trophic link between primary producers and higher trophic levels. In the present PhD thesis, caprellid amphipods are used as model group to understand the ways humans enable the transport and establishment of species with limited dispersal capabilities in new areas. Caprellids are one of the most interesting groups among marine invaders to address this issue because of: (1) the high densities they reach in artificial submerged structures (including floating objects and ship hulls); (2) their high potential for the introduction and establishment across many global regions; and (3) their limited capacity for autonomous dispersion, due to their lack of larval stage and their reduced appendages on the abdomen (which are used for swimming in other amphipod crustaceans).

Taking into account that recreational marinas are critical entry points for the introduction of non-native species and may act as reservoirs for them, the present study first explore the native and non-native caprellid species present in marinas along the Western Mediterranean and the East Atlantic coast, with

special focus on the Iberian Peninsula, and then investigate the invasion dynamic of non-natives found from a biogeographical perspective. Specifically, the likely vectors of introduction and secondary dispersal, the biotic and abiotic factors involved in their establishment success and subsequent spread, as well as the ecology of non-natives in their native vs introduced distribution ranges were investigated to understand the invasion success of non-native caprellids in southern Europe and their possible impacts on recipient communities.

Two non-native caprellids were found along the study area, *Caprella scaura* Templeton, 1936 and *Paracaprella pusilla* Mayer, 1890. The latter species was previously unknown to Europe. The presence of both species in marinas supports the role of these anthropogenic habitats as reservoirs for introduced caprellids and the effectiveness of recreational boating in their secondary dispersion. Particularly, the proliferation of arborescent biogenic substrata, such as bryozoans and hydroids, in floating pontoons and boat hulls must be considered as a high risk for establishment and expansion of non-native caprellid species.

The review of the global distribution of *C. scaura* complex and taxonomical analysis of introduced populations suggested that a single form of the complex is expanding its distribution range, while the other forms remain in a restricted distribution area. At the regional level, European distribution of *C. scaura* revealed that the species has expanded rapidly in the last decade along the Mediterranean and the Strait of Gibraltar, where it seems to displace an ecologically similar congener (*Caprella equilibra*, Say 1818). Therefore, *C. scaura* can be considered as a potential threat to native Mediterranean communities. Combination of several ecological traits, such as r-selection strategy complemented with parental care, aggressive behavior, broad environmental tolerance, ability to colonize a wide range of habitat types with preference by anthropogenic habitats (both in its native and introduced range) or trophic plasticity, are probably related with this successful spread. However, results also suggest that the low salinity and temperature of the north Atlantic coast of the Iberian Peninsula, in comparison with the Mediterranean Sea, likely limit the spread and invasive success of *C. scaura* in northern European seas, providing a competitive refuge for displaced species.

Paracaprella pusilla appeared in scattered populations in the Mediterranean and the south coast of the Iberian Peninsula, primarily associated with hydroids. Results suggest that the species is in an initial phase of colonization and is expected to appear in more Mediterranean locations. Analyses of its global distribution suggest that ship fouling is the most probable vector of its introduction, while rafting on floating objects and recreational boating are probable responsible of its secondary dispersal. The species was also recorded for the first time on the two sides of the Panama Canal, representing an indicator of the vulnerability of the area to marine introductions and highlighting the role of interoceanic canals in the biogeography of caprellid crustaceans. Several ecological aspects, analyzed for the first time in *P. pusilla*, like a broad salinity tolerance, high affinity by anthropogenic habitats, great fecundity, development of mutualistic relationships with fouling hydroids or high ability to shift between predatory and filter-feeding strategies, could be related with its widespread distribution on a global scale.

Overall, this study highlights the role that continued coastal urbanization and increasing recreational boating pressure are playing in the introduction and subsequent spread of non-native species in European coastal habitats. However, while recreational boating seems an effective vector for the secondary spread of non-native caprellids among artificial habitats, it seems to fail in their spread beyond this type of habitats. Therefore, colonization of natural habitats is considered a limiting step in the invasion dynamics of this group. Based on the native-range ecology of *C. scaura* and *P. pusilla*, they are expected to become established in sheltered and even highly polluted natural habitats in their new introduced distribution ranges, but they will be unable to colonize wave-exposed rocky shores. Further studies are necessary for a better prediction of the long-term consequences of these human-mediated introductions.

Resumen

Las invasiones biológicas en el medio marino son un componente fundamental del cambio global y se consideran una de las mayores amenazas ocasionadas por el hombre en los océanos de todo el mundo. Sin embargo, el conocimiento que se tiene sobre la introducción de especies marinas mediada por el ser humano se encuentra todavía muy fragmentado. En el Mediterráneo Occidental y en la costa Este Atlántica, especialmente en la Península Ibérica, aunque el interés por las especies exóticas está creciendo, la mayoría de los estudios se han centrado en macroalgas u organismos “carismáticos”. Los organismos de pequeño tamaño y taxonomía compleja pasan frecuentemente desapercibidos, provocando una posible infraestimación de la presencia de especies introducidas.

El subfilo Crustacea incluye las especies invasoras más prolíficas entre aquellos invertebrados introducidos en los ecosistemas marinos. A pesar de que el número de crustáceos introducidos se ha incrementado en décadas recientes en los mares europeos, el número de especies de peracáridos no-nativos está todavía infravalorado. Esto es particularmente acusado en los anfípodos caprélidos, que frecuentemente suponen un reto respecto a su taxonomía. Los caprélidos son pequeños crustáceos marinos que tienen un papel muy destacado en los ecosistemas marinos, donde constituyen un nexo trófico entre los productores primarios y los niveles tróficos superiores. En la presente Tesis Doctoral, los anfípodos caprélidos son usados como grupo modelo para entender la manera en que el ser humano promueve el transporte y establecimiento de especies con reducida capacidad de dispersión en nuevas zonas. Los caprélidos son uno de los grupos más interesantes para la consecución de este objetivo ya que: (1) alcanzan densidades extraordinarias en estructuras artificiales sumergidas (incluyendo pantalanés flotantes y cascos de barcos); (2) tienen un alto potencial para introducirse y establecerse de forma exitosa; y (3) presentan una limitada capacidad de dispersión autónoma, debido a la ausencia de fase larvaria y a la reducción de los apéndices abdominales (usados para nadar en otros crustáceos anfípodos).

Teniendo en cuenta que los puertos deportivos son puntos de entrada críticos para la introducción de especies no-nativas y que pueden actuar como reservorio para éstas, la presente Tesis se centra en primer lugar en el estudio de las especies de caprélidos nativas y no-nativas presentes en puertos deportivos a lo largo del Mediterráneo Occidental y la costa Este Atlántica, con especial énfasis en la Península Ibérica, y posteriormente investiga la dinámica de invasión de las especies no-nativas encontradas desde una perspectiva biogeográfica. Concretamente, se analizan los posibles vectores de introducción y dispersión secundaria, los factores bióticos y abióticos involucrados en el establecimiento y subsecuente propagación, así como la ecología de las especies no-nativas en áreas nativas e introducidas, con el objetivo de entender el proceso de invasión de los caprélidos no-nativos en el sur de Europa y sus posibles impactos en las comunidades receptoras.

A lo largo del área de estudio se encontraron dos caprélidos no-nativos, *Caprella scaura* Templeton, 1836 y *Paracaprella pusilla* Mayer, 1890. La última especie no había sido detectada previamente en aguas europeas. La presencia de ambas especies en puertos deportivos confirma tanto el papel de estos hábitats antrópicos como reservorios de caprélidos introducidos como la efectividad de las embarcaciones de recreo en la propagación de estas especies. Concretamente, la proliferación de sustratos biogénicos arborescentes, como briozoos e hidrozoos, en pantalanos flotantes y cascos de barcos debe considerarse como un factor de riesgo para el establecimiento y expansión de caprélidos introducidos.

La revisión de la distribución global del complejo *C. scaura* y el análisis taxonómico de las poblaciones introducidas sugirió que sólo una forma del complejo está aumentando su rango de distribución, mientras que las formas restantes permanecen en un área restringida. A nivel regional, la distribución de *C. scaura* en Europa reveló que la especie se ha expandido rápidamente en la última década a lo largo del Mediterráneo y el Estrecho de Gibraltar, donde parece estar desplazando a un congénere de características ecológicas similares (*Caprella equilibra* Say, 1818). Por todo ello, *C. scaura* puede considerarse como una amenaza potencial para las comunidades nativas. La combinación de varios aspectos ecológicos, como la selección de una estrategia tipo “r” complementada con cuidado parental, comportamiento agresivo, amplia tolerancia ambiental, capacidad de colonizar un amplio rango de hábitats con preferencia por hábitats antropogénicos (tanto en su rango nativo como en el

introducido) o plasticidad trófica, están probablemente relacionados con su fructífera expansión. Sin embargo, los resultados también sugieren que la baja salinidad y temperatura de la costa norte de la Península Ibérica, en comparación con la Mediterránea, probablemente limite la expansión de la especie y su éxito invasor en los mares Europeos del Norte, permitiendo un refugio competitivo para las especies desplazadas.

Paracaprella pusilla apareció en poblaciones dispersas en el Mediterráneo y la costa sur de la Península Ibérica, fundamentalmente asociada a hidrozoos. Los resultados sugieren que la especie se encuentra en una fase inicial de colonización y se espera que aparezca en más localidades del Mediterráneo. El análisis de su distribución global sugiere que la adhesión al *fouling* de los cascos de los barcos es el vector de introducción más probable, mientras que el *rafting* sobre objetos flotantes y las embarcaciones de recreo son probablemente los responsables de su posterior propagación. La especie también se encontró por primera vez en ambos lados del Canal de Panamá, evidenciando la vulnerabilidad de la zona a la introducción de especies marinas y resaltando el papel de los canales interoceánicos en la biogeografía de los crustáceos caprélidos. Varios aspectos ecológicos, analizados por primera vez en la especie, como una amplia tolerancia a salinidad, alta afinidad por hábitats antropogénicos, alta fecundidad, desarrollo de relaciones mutualistas con hidrozoos del *fouling* o gran capacidad para cambiar entre estrategias depredadoras y filtradoras, podrían estar relacionados con su amplia distribución a escala global.

En conjunto, este estudio destaca el papel que la modificación del litoral y el incremento de la navegación de recreo está jugando en la introducción y propagación de especies exóticas en las costas de Europa. Sin embargo, mientras que la navegación de recreo parece ser un vector eficiente en la propagación de caprélidos exóticos entre hábitats artificiales, parece fallar en la propagación de estas especies más allá de este tipo de hábitats. Por lo tanto, la colonización de ambientes naturales se considera una fase limitante en la dinámica de invasión de este grupo. A partir del estudio ecológico de *C. scaura* y *P. pusilla* en su rango nativo, se espera que se establezcan en costas protegidas e incluso altamente contaminadas, pero posiblemente no colonicen costas expuestas al oleaje. No obstante, se necesitan más estudios para predecir de forma adecuada las consecuencias a largo plazo de estas introducciones mediadas por el hombre.



1

INTRODUCCIÓN Y OBJETIVOS GENERALES

Las invasiones biológicas constituyen un fenómeno mediante el cual algunas especies se establecen, propagan y proliferan en áreas alejadas de su rango natural de distribución (Elton 1958). Aunque no es un fenómeno reciente, ni provocado en exclusiva por el hombre (Mack *et al.* 2000), su espectacular aumento no podría entenderse si no es bajo el contexto del “Cambio Global” o “Era de la Globalización” (Hobbs y Mooney 2005). En un mundo cada vez más interconectado, pocos son los rincones del planeta donde el ser humano no ha introducido (de forma accidental o intencionadamente) especies foráneas (Fridriksson y Magnusson 1992; Mack *et al.* 2000). Este proceso, en el que todavía quedan muchos aspectos por conocer, está provocando una alteración sin precedentes en los ecosistemas de todo el mundo (Carlton y Gueller 1993; Schmitz y Simberloff 1997; Ruiz *et al.* 1997; Pimentel *et al.* 2000).

1.1 LA BIOLOGÍA DE LAS INVASIONES: UNA DISCIPLINA EMERGENTE

La atención que ha recibido el fenómeno de las invasiones biológicas por parte de la comunidad científica es relativamente reciente, especialmente en el

medio marino, donde sólo se conoce una pequeña proporción de las especies introducidas y una proporción todavía menor del impacto que generan (Carlton 1979; Ruiz *et al.* 1997; Rilov y Crooks 2009).

A mediados del siglo XX Charles S. Elton puso de relieve la importancia de este proceso en su libro *The Ecology of Invasions by Animals and Plants*: “[...] estamos ante una de las mayores convulsiones históricas en la fauna y flora de todo el mundo¹” (Elton 1958). A pesar del dramatismo del mensaje, el fenómeno no atrajo la suficiente atención de los investigadores hasta hace aproximadamente dos décadas (Williamson 1996), cuando las consecuencias de la proliferación desmedida de algunas especies introducidas resultaban difícilmente aplacables sin unos planes apropiados de gestión y control de la situación. Aunque actualmente la problemática de las especies invasoras se considera un aspecto prioritario para la conservación de los ecosistemas, muchos de los aspectos ecológicos que envuelven a este proceso, incluyendo los conceptos y términos básicos que se usan para describirlo, permanecen todavía confusos (Richardson *et al.* 2000; Carlton y Ruiz 2003; Colautti *et al.* 2004).

Especies introducidas, exóticas, no-nativas o no-indígenas son ejemplos de términos empleados en la literatura para definir a aquellas especies que han sido introducidas fuera de su rango natural de distribución (rango nativo), intencionada o deliberadamente, por mediación del ser humano (IUCN 2000). Éstas especies se consideran establecidas cuando se reproducen con éxito y de forma autónoma (sin ayuda del hombre) en el lugar de introducción (Kolar y Lodge 2001). El carácter “invasor” es el término que genera mayor controversia. Por lo general, las especies invasoras son consideradas como una pequeña fracción de las especies introducidas que, una vez establecidas, causan un impacto económico y/o ecológico sobre las comunidades nativas (e.g. Davis y Thomson 2000; Mack *et al.* 2000; McNeely *et al.* 2001). Sin embargo, la falta de conocimiento sobre el impacto potencial de muchas especies y la distinta naturaleza tanto del impacto (que puede ser positivo para unas especies y negativo para otras) como de las especies que se introducen (e.g. plantas vs. animales) han llevado a algunos autores a calificar como “especie invasora” a aquella especie que una vez ha sido introducida, ha logrado establecerse y expandirse con éxito a otras localidades, independientemente del impacto que

¹ Frase original: “[...] *we are seeing one the most one of the great historical convulsions in the world's fauna and flora*” (p. 31).

pueda producir (Richardson *et al.* 2000; Pyšek *et al.* 2004). En términos legislativos, el carácter invasor suele atribuirse con frecuencia a aquellas especies introducidas que causan o han causado algún tipo de perjuicio para el hombre (pérdidas económicas, enfermedades, etc.), sin tener muchas veces en cuenta el impacto que pueden generar sobre especies nativas que carecen de interés comercial o recreativo (e.g. especies de pequeño tamaño).

Además de una terminología confusa, uno de los mayores retos a los que se enfrenta el estudio de las invasiones biológicas es la definición del rango nativo de muchas de las especies que son frecuentemente transportadas por el hombre, especialmente en el medio marino. Desde que el ser humano comenzó su proceso de expansión y colonización de nuevos territorios cruzando con navíos mares y océanos de todo el mundo, ha ido transportando y redistribuyendo millones de especies inadvertidamente a lo largo del planeta (Bax *et al.* 2003). Esto ocurrió mucho antes de que los naturalistas comenzasen a estudiar y describir muchas de las especies que habían sido transportadas durante cientos de años de unos lugares a otros. Debido a esto, surgió un problema para aquellas especies que se describían en un lugar como nativas y pocos años después eran recolectadas en áreas biogeográficas distantes: se desconocía su rango de distribución natural. Esto ocurría frecuentemente en aquellas especies asociadas a hábitats artificiales. Para clasificar estas especies, que no podían ser denominadas como nativas o no-nativas, James T. Carlton acuñó el término de “especie criptogénica” (Carlton 1996a).

1.1.1 El proceso de invasión

El proceso de invasión consiste básicamente en tres fases o etapas: una etapa inicial de introducción o dispersión inicial (en la que un organismo es introducido en una localidad alejada de su rango nativo de distribución), una segunda etapa de establecimiento (donde la especie se reproduce de forma autónoma formando poblaciones estables en el lugar de introducción) y una etapa final de expansión (en la que la especie se propaga y establece con éxito en localidades cercanas) (Williamson 1996; Kolar y Lodge 2001; Leung *et al.* 2002). Este modelo básico se puede complicar para incluir por ejemplo una etapa previa a la introducción, que sería la etapa de transporte, y una etapa final de proliferación (en la que la especie se hace dominante en las áreas donde se ha

propagado) (e.g. Colautti y MacIsaac 2004; Occhipinti-Ambrogi 2007). El éxito en cada una de las etapas no sólo depende de las características de la especie, influyen además numerosos factores como la cantidad de veces que se inocula la especie en un determinado lugar (“*propagule pressure*” o “*introduction effort*”), la estructuración de las comunidades receptoras, el nivel de contaminación o perturbación del lugar de introducción así como las condiciones climáticas o las interacciones bióticas (competencia, depredación, facilitación, etc) entre otros (Colautti y MacIsaac 2004). Todo ello hace que en la mayoría de las ocasiones el éxito en la invasión sea difícil de predecir así como sus consecuencias en los ecosistemas receptores.

Durante muchos años se ha seguido la regla del diez (“*tens rule*”) por la que se consideraba que aproximadamente sólo el 10% de las especies introducidas sobrevivían en los lugares de introducción, de éstas, sólo el 10% formaba poblaciones estables de forma autónoma, y de éstas, sólo el 10% se convertían en especies dañinas o perniciosas (Williamson 1996; Williamson y Fitter 1996). Actualmente algunos autores advierten que esta regla no se cumple en todos los casos. Por ejemplo, García-Berthou *et al.* (2005) encontraron que el porcentaje de establecimiento de 123 especies acuáticas introducidas en Europa era del 63% (muy por encima del porcentaje sugerido por la regla de Williamson y coautores).

1.1.2 Las invasiones en el medio marino

En los ecosistemas marinos, el aumento sin precedentes del tráfico marítimo (tanto comercial como recreativo) así como la construcción de las infraestructuras necesarias para facilitar este tipo de transporte (como pueden ser los canales y puertos) está provocando un incremento cada vez mayor del número de especies introducidas (Ruiz *et al.* 1997; Hulme 2009). Este proceso, clave para entender las consecuencias ecológicas de la “Era de la Globalización” en la que nos encontramos (Occhipinti-Ambrogi 2007), se considera una de las mayores amenazas derivadas de la actividad humana en mares y océanos de todo el mundo (Carlton 1996b).

Europa no ha sido una excepción a este proceso, siendo el Mar Mediterráneo uno de los mares más invadidos de todo el mundo (Galil 2000, 2012; Streftaris *et al.* 2005; Zenetos *et al.* 2010). Aunque el tráfico marítimo

(Fig. 1.1) y, en menor medida, la acuicultura y las liberaciones intencionadas han sido catalogados como importantes vías o vectores de introducción de especies en el Mediterráneo, la apertura del Canal de Suez en 1869 marcó sin duda un antes y un después en cuanto al número de especies introducidas en este mar, especialmente en su extremo más oriental (Streftaris *et al.* 2005; Galil 2009). La construcción del canal, unido al incremento constatado de la temperatura en el mar Mediterráneo (Salat y Pascual 2002), dio lugar a un fenómeno único denominado la migración Lessepsiana, por el que especies tropicales del Mar Rojo llegaron al Mar Mediterráneo atravesando el Canal de Suez y formando poblaciones estables (Por 1978). De las más de 700 especies no-nativas reconocidas actualmente en el Mediterráneo, se cree que aproximadamente la mitad han sido introducidas a través del Canal de Suez (Galil *et al.* 2014). Además, el Mediterráneo presenta otra amenaza en su extremo occidental, el Estrecho de Gibraltar, una zona que conecta el Mar Mediterráneo con el océano Atlántico y por la que transitan más de 80.000 barcos al año (Gómez 2003).

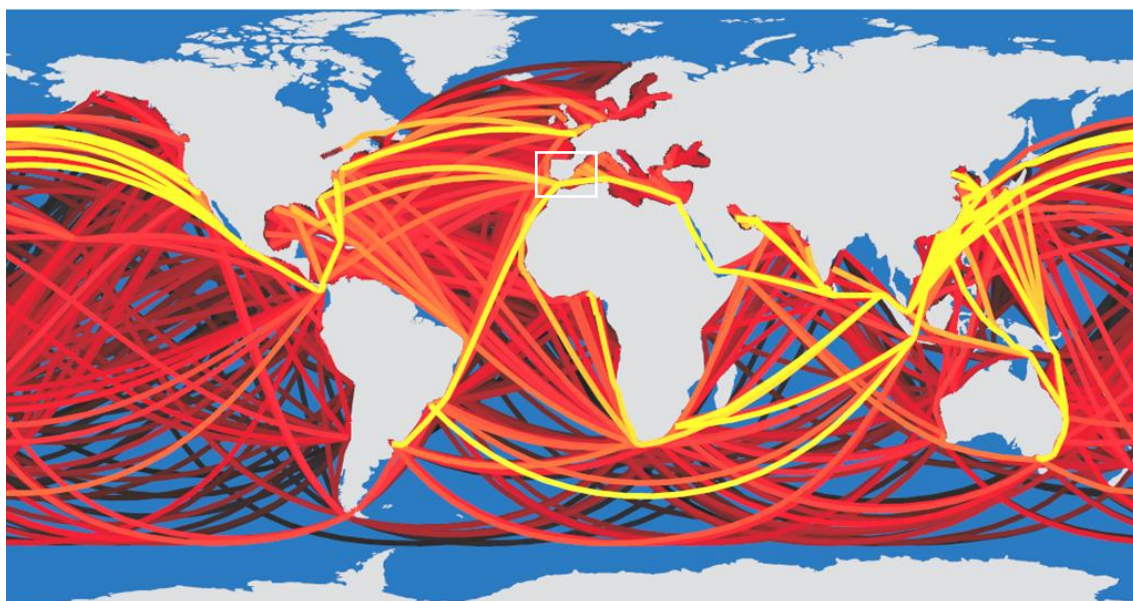


Fig. 1.1 Principales rutas de introducción de especies marinas. La figura refleja la probabilidad de invasión durante las trayectorias que conectan los principales puertos internacionales del mundo. Los colores más claros indican una mayor probabilidad de invasión mientras que los más oscuros muestran una probabilidad menor (modificado de Seebens *et al.* 2013). El recuadro blanco resalta la localización de la Península Ibérica y refleja su posición estratégica para analizar las consecuencias de las invasiones biológicas en el medio marino.

La Península Ibérica constituye un enclave único para entender las consecuencias ecológicas que el aumento del tráfico marítimo está provocando a nivel de introducción de especies. La comparativa Atlántico-Mediterránea y el continuo incremento en la construcción de estructuras portuarias (especialmente de puertos deportivos) hacen de esta región un escenario clave para estudiar los procesos ecológicos que subyacen a este fenómeno. Sin embargo, el conocimiento que se tiene sobre las especies marinas introducidas en la Península Ibérica es muy escaso. La mayoría de los estudios se han centrado en macroalgas o en especies concretas de invertebrados sésiles conocidas por su potencial invasivo en otras regiones del mundo (ICES WGITMO report 2009). Por ejemplo, las comunidades asociadas a las estructuras sumergidas que forman parte de los puertos y otras construcciones marinas, conocidas como comunidades incrustantes o comunidades del “*fouling*”, han sido muy poco estudiadas. Esto tiene al menos dos consecuencias importantes: (1) muchas especies que son introducidas a consecuencia del tráfico marítimo permanecen sin detectar, subestimándose el número de especies introducidas en la Península Ibérica, y (2) cuando se detecta una especie introducida en estas comunidades, la ausencia de estudios previos dificulta enormemente la tarea de conocer el periodo relativo de introducción así como su dinámica de invasión. Este desconocimiento se hace todavía más patente en los puertos deportivos españoles, muchos de los cuales se han construido de forma reciente y las comunidades que habitan en ellos permanecen sin explorar.

1.1.3 Puertos deportivos y distribución de especies

La susceptibilidad de las comunidades incrustantes que crecen en las estructuras portuarias para ser invadidas es bien conocida (Bulleri y Chapman 2010) y radica, en gran parte, en el hecho de que se encuentran sometidas a una gran presión de introducción de especies exóticas por parte de las embarcaciones que llegan desde otros lugares y por las condiciones particulares en las que se encuentran (protección frente al oleaje, alto nivel de perturbación antrópica, gran cantidad de sustrato artificial disponible, etc.).

Las especies exóticas son introducidas a través del tráfico marítimo mediante dos formas principalmente: (1) en el agua de lastre usada por los grandes barcos para equilibrar su carga, y (2) a través de las comunidades

incrustantes que se adhieren a las estructuras sumergidas los barcos y las pequeñas embarcaciones. Los organismos que conforman estas comunidades y que están presentes en los puertos de origen, pueden establecerse en los puertos de destino si las condiciones son las apropiadas. Todo ello ha provocado que la fauna de los puertos sea muy similar en unos lugares y otros en lo que se ha venido a llamar la “homogeneización de la biota” (McKinney y Lockwood 1999).

Si bien el agua de lastre, por la enorme capacidad de transporte de especies foráneas, ha recibido gran atención por parte de los legisladores, la introducción de especies a través de las comunidades incrustantes ha pasado prácticamente desapercibida para éstos. Así por ejemplo, en el año 2004 se adoptó el “*Convenio Internacional para el Control y Gestión del Agua de Lastre y Sedimentos de los Buques*” ratificado por España (BOE de 25 de marzo de 2008), pero no hay hasta la fecha una ley que regule las especies transportadas en el exterior de los barcos. Esto es especialmente relevante para las embarcaciones de recreo o barcos deportivos, pues pueden dispersar libremente las especies que llevan incrustadas a los lugares a donde viajen.

Los puertos deportivos, a diferencia de los comerciales, aportan una mayor cantidad de superficie disponible para ser colonizada por las comunidades incrustantes que viajan adheridas a los barcos que amarran en ellos (Minchin *et al.* 2006). Por otra parte, los barcos permanecen amarrados más tiempo que en los puertos comerciales favoreciendo la formación y dispersión de los organismos que conforman estas comunidades (Floerl 2002). A todo ello se suma el hecho de que estas embarcaciones viajan no sólo a puertos cercanos con asiduidad, sino también a enclaves marinos protegidos y calas difícilmente accesibles de otra manera. Esto hace que los puertos deportivos y las pequeñas embarcaciones de recreo formen una extensa y efectiva red de propagación de especies exóticas (Ashton *et al.* 2006; Davidson *et al.* 2010) carente de regulación.

Diferentes trabajos han estudiado la fauna que forma parte de las comunidades incrustantes asociadas a hábitats artificiales (especialmente la fauna sésil), encontrándose que la composición de especies es diferente a la que se puede encontrar en los ambientes naturales adyacentes (Connell y Glasby 1998; Glasby 1999; Connell 2000; Bulleri y Chapman 2004). Parte de estas diferencias

radica en el hecho de que hay un mayor número de especies introducidas asociadas a sustrato duro artificial en comparación con el sustrato duro natural que forma, por ejemplo, los intermareales rocosos (Glasby *et al.* 2007). Sin embargo, la mayor parte de los estudios se han focalizado en la fauna sésil, especialmente en los puertos. La fauna asociada a estos organismos sésiles (organismos epibiontes) es prácticamente desconocida (Chapman *et al.* 2005; People 2006; Marzinelli *et al.* 2009), a pesar de ser una pieza clave para entender el papel que tienen la construcción de puertos y otras formaciones artificiales en la estructuración de la fauna marina. Por este motivo, muchos de estos pequeños epibiontes, invertebrados móviles en su gran mayoría, son especies introducidas que han pasado y pasan desapercibidas en los estudios que cuantifican el nivel de invasión de una determinada zona.

1.2 LOS CAPRÉLIDOS COMO MODELO DE ESTUDIO

Uno de los grupos dominantes de invertebrados móviles marinos asociados con las comunidades incrustantes que crecen sobre sustrato duro artificial son los crustáceos caprélidos, objeto de estudio de la presente tesis doctoral.

1.2.1 ¿Qué son los caprélidos?

Los caprélidos, también conocidos como “*skeleton shrimps*” (gambas esqueleto) debido a su peculiar morfología (Fig. 1.2), constituyen un grupo exclusivamente marino con más de 350 especies (Guerra-García y Tierno de Figueroa 2009). Son pequeños crustáceos peracáridos (desde unos pocos milímetros a los dos o tres centímetros de longitud) pertenecientes al Orden Amphipoda, que además de incluir al suborden Caprellidea, también incluye a los subórdenes Gammaridea, Hiperiidea e Ingolfiellidea. Además de esta clasificación tradicional, existen otras clasificaciones para los anfípodos (e.g. Myers y Lowry 2003) que ponen de relieve la existencia de unas relaciones filogenéticas complejas, que no han terminado de resolverse de forma consensuada.

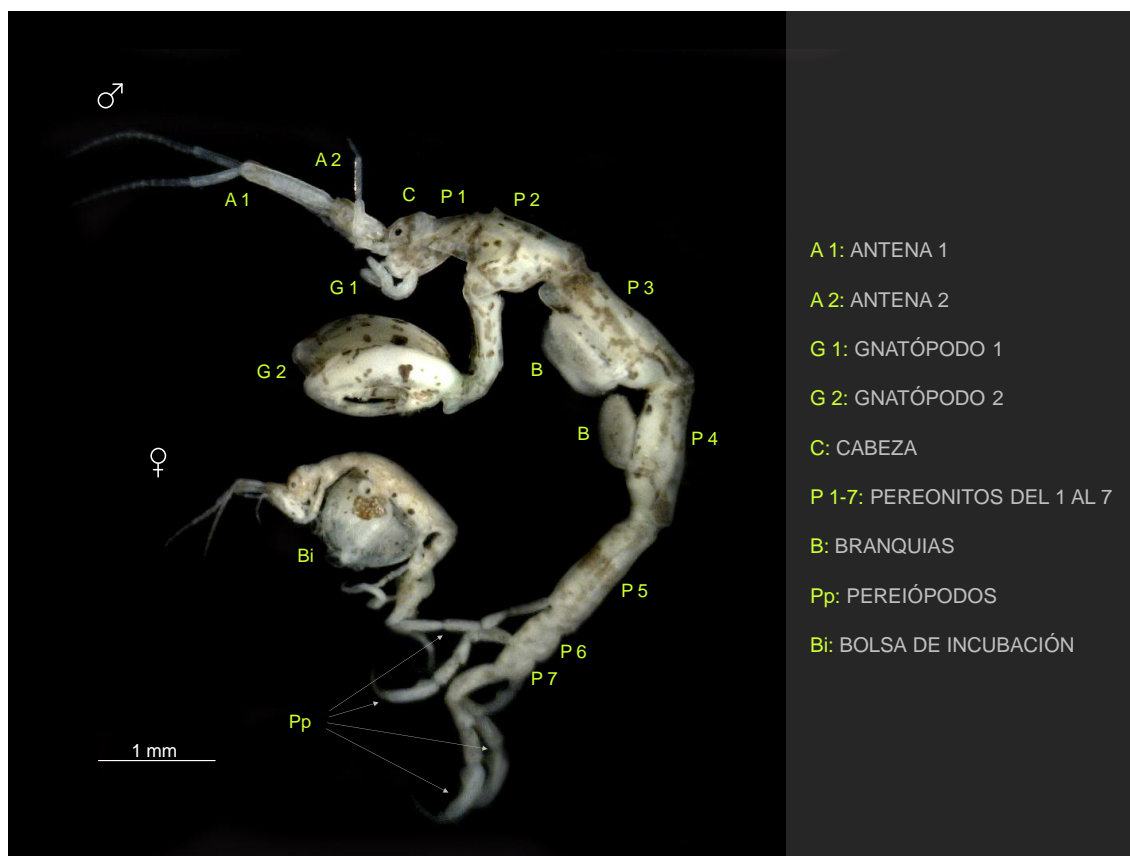


Fig. 1.2 Fotografía de un macho y una hembra de la especie *Paracaprella pusilla* recolectada en Puerto América (Cádiz) donde se muestran las partes fundamentales de la morfología externa de un caprélido.

Los caprélidos están presentes en la mayoría de las listas faunísticas de trabajos ecológicos o biogeográficos sobre el bentos, normalmente con abundancias considerables (Vázquez-Luis *et al.* 2008, 2009; Guerra-García *et al.* 2011a). Sin embargo, a pesar de su importancia en los ecosistemas marinos, las dificultades que entraña la taxonomía de estos pequeños crustáceos son la causa de que en la mayor parte de estudios, los caprélidos no se identifiquen a nivel de especie y sólo sean nombrados como suborden Caprellidea o como *Caprella* sp., o incluso que la identificación sea errónea (Guerra-García *et al.* 2014). Además de una taxonomía compleja, su pequeño tamaño unido a su capacidad para camuflarse con el sustrato, pudiendo adoptar el mismo color gracias a la presencia de cromatóforos especializados (Keith 1971), han contribuido al escaso conocimiento que se tiene sobre ellos.

Los caprélidos pueden encontrarse en ambientes muy diversos que van desde el ecuador hasta los polos y desde los intermarales rocosos hasta cientos de metros de profundidad (McCain 1968; Laubitz y Mills 1972). La mayoría son de vida libre, a excepción de un grupo parásito de cetáceos, los Cyamidae, que presentan un patrón corporal muy modificado. Aunque algunas especies se han encontrado en la columna de agua (Takeuchi y Sawamoto 2008), la mayoría son bentónicas y viven en el sedimento o como epibiontes sobre algas, fanerógamas marinas, invertebrados sésiles y otros sustratos naturales a los que permanecen aferrados con sus apéndices posteriores o pereiópodos (McCain 1968; Laubitz 1970, 1972; Caine 1978; Guerra-García 2001).

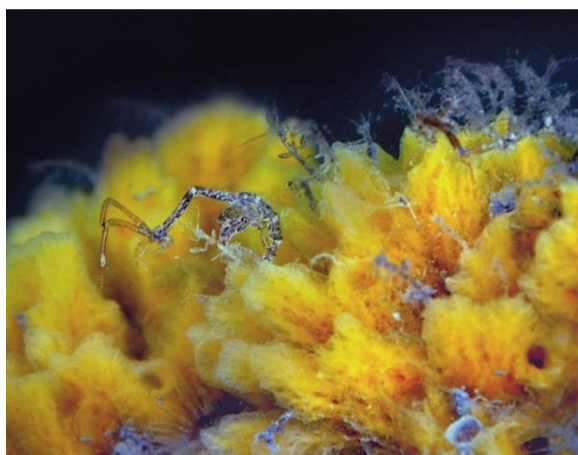


Fig. 1.3 Individuos de la especie *Caprella equilibra* asentados sobre una esponja marina asociada a un pantalán flotante en un puerto de Virginia (EEUU).

Algunas especies de caprélidos son capaces de colonizar una gran variedad de sustratos artificiales (pantalanes, cuerdas, boyas, trozos de madera flotante, plataformas petrolíferas, jaulas de acuicultura, etc.) (Thiel *et al.* 2003; Thiel y Gutow 2005; Page *et al.* 2007).

La colonización de estos sustratos, donde pueden alcanzar densidades mucho más elevadas que las presentes en los habitats naturales adyacentes (Thiel *et al.* 2003; Page *et al.* 2007), la suelen hacer de forma indirecta (Fig. 1.3), creciendo sobre los organismos que se han establecido previamente sobre la superficie de sustrato artificial. No obstante, también pueden establecerse directamente sobre la superficie que aporta el sustrato artificial y desplazarse sobre ésta (Fig. 1.4).

1.2.2 Importancia de los caprélidos en los ecosistemas marinos

Los caprélidos desempeñan un papel fundamental en la trofodinámica de los ecosistemas marinos (Caine 1987, 1991; Edgar y Aoki 1993; Dauby *et al.* 2003). Aunque la mayoría pueden considerarse oportunistas, pudiendo desarrollar diferentes estrategias tróficas en función de la disponibilidad de

alimento, estudios recientes apuntan a que la mayor parte de las especies siguen una dieta fundamentalmente detritívora (Guerra-García y Tierno de Figueroa 2009) colaborando en el reciclado y distribución de la materia orgánica del bentos. A su vez, los caprélidos constituyen una parte fundamental de la dieta de muchas especies (Caine 1987, 1989, 1991). Por ejemplo, en el intermareal y submareal somero, los caprélidos se consideran una de las presas más importantes para los peces de pequeño tamaño (< 10 cm) (Takeuchi e Hino 1997). Por este motivo, en la actualidad se está explorando su utilidad como recurso alimenticio para peces y moluscos de interés comercial (Woods 2009; Baeza-Rojano *et al.* 2010, 2014).

Por otra parte, los caprélidos son muy útiles como bioindicadores de la calidad ambiental del medio marino (Guerra-García y García-Gómez 2001; Guerra-García y Koojul 2005), incluyendo la contaminación por metales pesados (Guerra-García *et al.* 2009a, 2010) y TBTs (Takeuchi *et al.* 2001; Ohji *et al.* 2002), compuestos de tributilo de estaño (prohibidos en la actualidad pero que perduran en el medio), que fueron muy utilizados como componente de las en las pinturas “antifouling” de los barcos.



Fig. 1.4 Ejemplar de la especie introducida *Caprella scaura* desplazándose sobre la superficie lateral de un pantalán flotante en un puerto de Livorno (Italia).

A pesar de tener una enorme capacidad de colonización, los caprélidos tienen una capacidad de dispersión autónoma bastante reducida. Los pleópodos, unos apéndices abdominales utilizados por otros anfípodos para nadar, se encuentran muy reducidos, de forma que la natación se reduce a pequeños movimientos que les permiten soltarse del sustrato y desplazarse unos centímetros (Caine 1989, 1991). A todo ello se une su desarrollo directo, que se traduce en la ausencia de fase larvaria y por tanto en la ausencia de una fase pelágica que les permita dispersarse mayores distancias. Esto ha contribuido a su utilidad en los estudios biogeográficos. No obstante, algunas especies han logrado ampliar su distribución de forma espectacular, fundamentalmente en asociación con vectores antropogénicos (pero no exclusivamente). En estas especies son en las que se ha centrado la presente Tesis Doctoral.

1.2.2 El uso de los caprélidos para el estudio de las invasiones biológicas

Algunas especies de caprélidos tienen una distribución tan amplia que son consideradas hoy día como especies cosmopolitas (e.g. *Caprella equilibra*, la especie que aparece en la figura 1.3). ¿Cómo es esto posible teniendo una capacidad tan baja de dispersión autónoma? Es posible que muchas de estas especies sean en realidad un complejo de múltiples especies crípticas o de morfología extremadamente similar (e.g. Guerra-García *et al.* 2006; Watling y Carlton 2007). Esto ya se ha demostrado a nivel molecular para algunas especies, como *Caprella penantis* (Cabezas *et al.* 2013a). Sin embargo, para otras especies, las técnicas moleculares no arrojan diferencias genéticas significativas, como ocurre por ejemplo con el caprélido de origen japonés *Caprella mutica*, distribuido en regiones biogeográficas muy distantes (Ashton *et al.* 2008). Además, existe el caso intermedio: especies cosmopolitas que son un complejo de especies crípticas pero donde una de las especies ha adquirido una distribución global, tal y como ocurre con *Caprella andreae* (Cabezas *et al.* 2013b).

Parece, por tanto, que determinadas especies de caprélidos han encontrado mecanismos eficientes para dispersarse largas distancias, así como para establecerse y mantenerse con éxito en regiones muy distantes entre sí. Una característica común en estas especies ampliamente distribuidas es su capacidad para colonizar sustratos artificiales, donde pueden alcanzar grandes abundancias.

Por ejemplo, Buschbaum y Gutow (2005) encontraron densidades de *C. mutica* superiores a 3000 individuos por m² en las instalaciones de dos puertos localizados en el sudeste del Mar del Norte. De esta forma, muchos caprélidos que proliferan en las comunidades incrustantes asociadas a puertos y embarcaciones pueden ser transportados largas distancias tanto adheridos a las comunidades incrustantes que crecen en los cascos de las embarcaciones (e.g. Krapp *et al.* 2006; Montelli 2010; Ashton *et al.* 2014) como a través del agua de lastre (Carlton 1985; Gollasch *et al.* 2002). Además de estos dos vectores relacionados con el tráfico marítimo, los caprélidos también han podido ser introducidos en áreas biogeográficas distantes a través de la acuicultura. Por ejemplo, en el caso de *C. mutica*, algunos autores han sugerido su asociación con los cultivos de ostras japonesas como uno de los vectores más probables de su introducción en diferentes regiones del mundo, incluyendo Europa (Carlton 1987; Willis *et al.* 2004). En el caso de *C. andreae*, además de asociarse con objetos flotantes que viajan a la deriva (“rafting”), la vía fundamental de dispersión a largas distancias ha sido su asociación a los caparzones de las tortugas (Krapp-Schickel 1993; Sezgin *et al.* 2009; Zakhama-Sraieb *et al.* 2010; Cabezas *et al.* 2013b). Este sería uno de los pocos ejemplos de distribución cosmopolita donde el ser humano no ha jugado un papel relevante.

Una vez son introducidos en un determinado lugar, los caprélidos pueden dispersarse de forma secundaria a zonas cercanas y comenzar su propagación desde el punto de introducción. En este caso, uno de los vectores secundarios más importantes sería la adhesión a las comunidades incrustantes de los pequeños barcos o yates que se encuentran amarrados en los puertos deportivos anexos a puertos internacionales y que dispersarían los caprélidos hacia puertos deportivos cercanos (Asthon *et al.* 2006; Zabin *et al.* 2014). Otra forma importante de dispersión local (que también puede actuar a una escala espacial mayor) sería el “rafting”, a través de la asociación a sustratos, tanto naturales como artificiales, que flotan a la deriva (Thiel *et al.* 2003; Astudillo *et al.* 2009; Cabezas *et al.* 2013b). Este vector permitiría a los caprélidos dispersarse hacia ambientes naturales a los que los vectores anteriores (estrechamente relacionados con los ambientes antrópicos) apenas llegan.

Además de una gran facilidad para ser dispersados de forma pasiva y una gran plasticidad para colonizar diferentes tipos de sustratos, los caprélidos están sometidos a fuertes fluctuaciones ambientales en variables como la salinidad y la

temperatura a lo largo de su ciclo de vida (e.g. Keith 1971; Guerra-García *et al.* 2009b, 2011a). Esto hace que muchas especies de anfípodos caprélidos sean euritermas y eurihalinas pudiendo reproducirse con éxito en diferentes tipos de hábitats, inclusive estuarinos (Sconfiatti y Luparia 1995). Todo esto, unido a un ciclo de vida corto, con varias generaciones por año y desarrollo directo (Imada y Kikuchi 1984; Sakaguchi 1989; Takeuchi e Hirano 1991; Baeza-Rojano *et al.* 2013), hace que los caprélidos tengan un alto potencial para ser introducidos fuera de su rango natural de distribución y se establezcan con éxito en los ecosistemas receptores. De este modo, los caprélidos son cada vez más reconocidos como grupo modelo para entender el papel del ser humano en la distribución de los organismos marinos con baja tasa de dispersión autónoma.

Si bien el papel de los caprélidos en la dinámica de las invasiones marinas comienza poco a poco a ser reconocido, el papel de otro grupo de anfípodos, los gammáridos, ha resultado clave para entender las principales vías de introducción de especies exóticas en las aguas continentales europeas (e.g. Bij de Vaate *et al.* 2002 y referencias incluidas; Devin y Beisel 2008).

1.2.3 Caprélidos exóticos en los mares europeos

Hasta la fecha de finalización de la presente Tesis Doctoral, tres especies de caprélidos se consideran no-nativas en Europa: *Caprella mutica*, *Caprella scaura* y *Paracaprella pusilla*. De estas, *C. mutica* es la que ha recibido mayor atención tanto en Europa como a nivel mundial (e.g. Willis *et al.* 2004, 2009; Ashton *et al.* 2007, 2008; Boos 2009; Schucksmith *et al.* 2009). Es la única que se encuentra ampliamente distribuida por la costa atlántica del norte de Europa y por su afinidad hacia climas más fríos parece poco probable que se establezca con éxito en el Mediterráneo (Ashton *et al.* 2007). *Caprella scaura* fue la primera especie no-nativa de caprélido introducida en el Mediterráneo (e.g. Krapp *et al.* 2006; Guerra-García *et al.* 2011b), siendo detectada en la Península Ibérica por primera vez en 2005, en el noreste peninsular (Martínez y Adarraga 2008). Respecto a *P. pusilla*, su existencia en mares Europeos ha permanecido inadvertida para la comunidad científica hasta la publicación de parte de los capítulos que componen esta Tesis Doctoral.

A pesar de su utilidad como grupo modelo para entender el papel del ser humano en la distribución de especies marinas móviles con baja capacidad de

dispersión, los caprelidos han sido muy poco estudiados en este contexto. Muchos interrogantes sobre su ecología, patrones de distribución y posibles impactos en los ecosistemas de introducción permanecen sin resolver.

1.3 OBJETIVOS GENERALES

A lo largo de la presente tesis se persigue comprender los procesos ecológicos implicados en cada una de las etapas que conforman la dinámica de invasión de los crustáceos caprelidos introducidos en el sur de Europa y, especialmente, en la Península Ibérica. De esta forma, utilizando los caprelidos como grupo modelo, se busca contribuir al análisis del papel que ejerce el ser humano, a través del tráfico marítimo y la construcción de estructuras artificiales en el medio marino (e.g. canales y puertos), en la distribución de los organismos marinos y, consecuentemente, en la alteración de los ecosistemas litorales. Para todo ello, se van a abordar los siguientes objetivos específicos estructurados en cinco bloques principales:

- i. **DETECCIÓN Y DISTRIBUCIÓN DE CAPRELIDOS INTRODUCIDOS:** caracterizar los caprelidos nativos y no-nativos asociados a las comunidades incrustantes presentes en puertos deportivos de la costa este atlántica y del Mediterráneo occidental, con especial énfasis en la Península Ibérica y las Islas Baleares. Así mismo, estudiar los patrones de distribución y las posibles vías de introducción de las dos especies de caprelidos no-nativos encontradas (*Caprella scaura* y *Parcaprella pusilla*).
- ii. **ESTABLECIMIENTO:** analizar el proceso de establecimiento de ambas especies en la zona del Estrecho de Gibraltar y entender los factores que influyen en este proceso, incluyendo el papel que juegan los organismos del “fouling” sobre los que viven.
- iii. **IMPACTOS:** analizar el posible desplazamiento que la especie introducida *C. scaura* podría estar ejerciendo sobre una especie muy común de caprelido en la Península Ibérica, *Caprella equilibra*.
- iv. **VECTORES DE PROPAGACIÓN:** explorar el papel que ejercen los puertos deportivos y las embarcaciones de recreo en la introducción y distribución de caprelidos exóticos. Así mismo, analizar el papel del Canal de Panamá en la distribución de *P. pusilla*.

- v. **ECOLOGÍA:** estudiar aspectos ecológicos fundamentales de *C. scaura* y *P. pusilla* como la ecología trófica o el uso del hábitat, tanto en poblaciones recientemente introducidas (sur de Europa) como en aquellas que llevan establecidas cientos años (Brasil), pudiendo por tanto considerarse como poblaciones nativas en este lugar.

1.4 ESTRUCTURACIÓN DE LA TESIS

Además del capítulo introductorio que se presenta (**capítulo 1**), esta tesis se compone de cinco capítulos donde se tratan cada uno de los cinco bloques expuestos anteriormente y de dos capítulos finales donde se discuten los resultados obtenidos y se exponen las conclusiones principales. Concretamente:

- En el **capítulo 2** se describe la presencia, por primera vez, de *Paracaprella pusilla* tanto en la costa Este Atlántica (primera parte del capítulo) como en el Mar Mediterráneo (segunda parte) y se analizan los posibles patrones de introducción, su distribución global así como algunos aspectos ecológicos destacados. En la tercera parte de este capítulo se detalla la distribución global, el proceso de expansión y las posibles vías de introducción de *Caprella scaura* en el Mediterráneo.
- En el **capítulo 3** se monitoriza y analiza el proceso de establecimiento de ambas especies en el único puerto deportivo de la Península Ibérica donde ambas coexisten, Puerto América (Cádiz). Se exploran los factores bióticos y abióticos que influyen en este proceso y se hace un análisis preliminar del potencial invasor de cada especie.
- En el **capítulo 4** se analiza, a través de técnicas de modelización estadística, la influencia de *C. scaura* en el declive poblacional de una especie de caprélido muy común en la Península Ibérica, *Caprella equilibra*.
- En la primera parte del **capítulo 5** se analiza el papel de los puertos deportivos y las embarcaciones de recreo en la introducción y dispersión de caprélidos exóticos, utilizando como modelo la isla de Mallorca. En la segunda parte, además de describir por primera vez la presencia de *P. pusilla* en el Océano Pacífico, se utiliza esta especie como modelo de estudio para entender el papel del Canal de Panamá

(corredor de carácter dulceacuícola) en el transporte interoceánico de especies marinas.

- En el **capítulo 6** se profundiza en aspectos ecológicos clave para entender la dinámica de invasión de ambas especies desde un punto de vista biogeográfico, buscando la comparación entre rango nativo vs introducido. En una primera parte se analizan las preferencias en el uso del hábitat de los caprélidos del sur de Brasil (zona potencialmente nativa para *C. scaura* y *P. pusilla*) para entender el papel de las estructuras artificiales en su éxito de invasión así como para predecir los lugares que serán más susceptibles de ser invadidos por ambas especies en las zonas de reciente introducción. En la segunda parte se analiza la dieta de ambas especies en diferentes circunstancias tanto en el sur de Europa como en Brasil para entender la posible influencia de este factor en su capacidad de invasión.
- En el **capítulo 7** se lleva a cabo una discusión general con los resultados obtenidos, donde se analizan las aportaciones de la tesis así como las líneas que podrían desarrollarse en un futuro para poder entender aquellas cuestiones que permanecen sin resolver.
- En el capítulo final (**capítulo 8**) se exponen las conclusiones más relevantes.

Los capítulos y subcapítulos donde se abarcan los objetivos específicos han sido escritos en inglés con el objeto último de su publicación como artículos independientes en revistas de impacto internacional (ver pág. 307 donde se detalla la información que ya ha sido publicada y la que se encuentra en proceso de publicación). No obstante, en cada uno de estos capítulos y subcapítulos el resumen se ha escrito también en español.

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2

DETECTION & DISTRIBUTION: PART 1

On the occurrence of the tropical caprellid
Paracaprella pusilla in
Europe

- Adapted from:

Ros M, Guerra-García JM (2012) On the occurrence of the tropical caprellid *Paracaprella pusilla* Mayer, 1890 (Crustacea: Amphipoda) in Europe. Mediterranean Marine Science 13: 134–139.

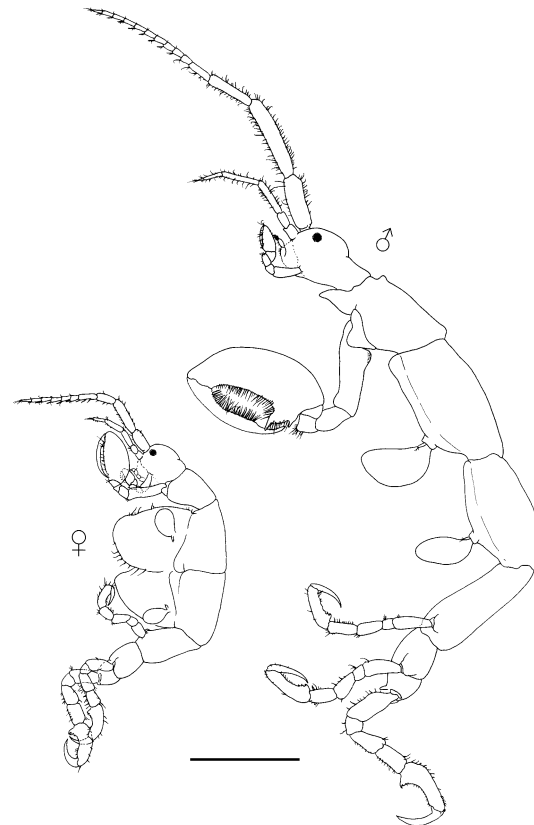
ABSTRACT. *Paracaprella pusilla* Mayer, 1980 is a tropical caprellid amphipod species. It was first described from Brazil and is found to be very common along the Atlantic coast of Central America. Since its original description, *P. pusilla* has been found at numerous widespread locations in tropical and subtropical seas around the world, and is primarily associated with fouling communities in harbours. A well established population of *P. pusilla* was recently found in Cádiz, southern Spain, which is both the northernmost collection and the first recorded finding of this species in European coastal waters. Ship fouling is the most probable vector for its introduction. The species was always found associated with the native hydroid *Eudendrium racemosum* (Cavolini, 1785) and appeared to display a mutualistic relationship with this host.

RESUMEN. *Paracaprella pusilla* Mayer, 1980 es una especie de anfípodo capreléido tropical. Fue descrita por primera vez en Brasil y es una especie muy común en la costa Atlántica de América central. Desde que fue descrita, *P. pusilla* se ha encontrado en numerosas y dispersas localidades en los mares tropicales y subtropicales del mundo, fundamentalmente asociada a las comunidades incrustantes de los puertos. Recientemente, se encontró una población estable de *P. pusilla* en Cádiz, en el sur de España, constituyendo el límite más al norte en la distribución de la especie y la primera cita para las costas Europeas. La asociación a las comunidades incrustantes de los barcos parece el vector más probable de introducción. La especie siempre se encontró en asociación con el hidrozoo nativo *Eudendrium racemosum* (Cavolini, 1785) con el que parecía desarrollar una relación mutualista.

2.1 INTRODUCTION

Caprellid amphipods, commonly known as skeleton shrimps, are small marine crustaceans that are common in many littoral habitats. Here, they form an important trophic link between primary producers and higher trophic levels (Woods 2009). *Paracaprella pusilla* (Fig. 2.1), originally described by Mayer (1890) from Brazil (type locality: Rio de Janeiro), is one of the most abundant caprellid species found along the Caribbean coast of Venezuela and Colombia (Díaz *et al.* 2005; Guerra-García 2006). The species' natural distribution is the Atlantic coast of Central and South America (Mayer 1903), with most of the records from the coasts in the Gulf of Mexico and the Caribbean (Fig. 2.2). It appears to be a strongly Caribbean species (Carlton and Elderedge 2009).

Figure 2.1 Lateral view of *Paracaprella pusilla* collected from India. Right, male; Left, female. Scale bar: 1 mm (redrawn from Guerra-García *et al.* 2010).



Paracaprella pusilla has since been reported from numerous locations in tropical and subtropical seas around the world, and is primarily associated with fouling communities in harbors (Table 2.1). Subsequent to its first reported occurrence in India (Sivaprakasam 1977), the species has become the most abundant among the caprellids found along the entire coast of India, both in

harbours and on natural intertidal rocky shores (Guerra-García *et al.* 2010). An established population of *P. pusilla* has recently been reported from northern Australia (Montelli and Lewis 2008), possibly introduced via biofouling on vessels. In spite of having direct development, caprellids can also disperse over large distances by ‘rafting’ on detached aquaculture buoys and other natural or artificial floating structures (Thiel *et al.* 2003).

Although *P. pusilla* is distributed around the globe (Fig. 2.2), to date it has only been recorded in tropical and subtropical coastal waters. This study contributes to the knowledge of the alien amphipods in the Iberian Peninsula by documenting the presence of this tropical caprellid in the Strait of Gibraltar, southern Spain, very close to the western boundary of the Mediterranean Sea. This record represents the northernmost location of the species, and the first record made in European coastal waters.

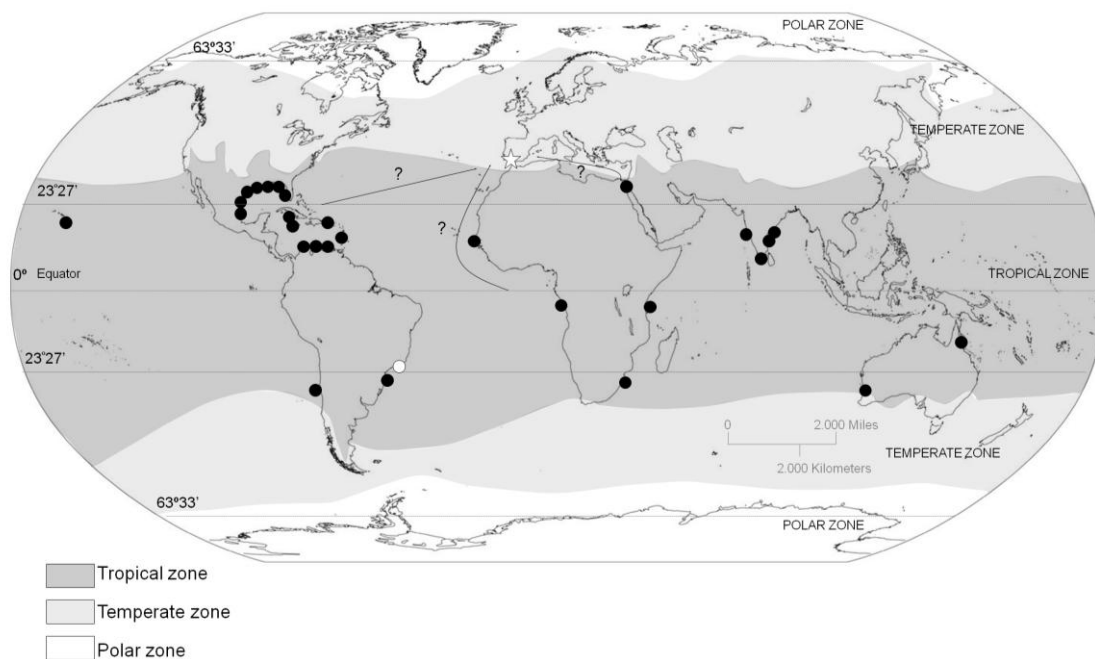


Fig 2.2 Current global distribution of *Paracaprella pusilla*. Black circles indicate the records where the species has been found (all references are shown in Table 2.1); The white circle indicates the type locality (Rio de Janeiro, Brazil); The star symbol indicates the locality that was recorded during the present study, and represents the first record for European waters. Possible pathways of introductions are indicated with question marks.

2.2 MATERIALS AND METHODS

Numerous individuals of *Paracaprella pusilla* (including mature males, ovigerous females, and juveniles) were collected from a floating pontoon at the Cadiz marina, southern Spain (36°32'29''N, 6°17'61''W) during a survey of peracarid crustaceans from harbors along the Strait of Gibraltar. This region experiences intense maritime traffic and is considered as a hot spot for biological invasions (see Drake and Lodge 2004). All specimens were found to be associated with the hydroid *Eudendrium racemosum* (Cavolini 1785).

The first discovery made was in September 2010, although it is not known how long they have been present at the site. Individuals were subsequently collected between October 2010 and July 2011 by detaching hydroid colonies at the base, and preserving them in 70% ethanol. Throughout this period, artificial substrates such as ropes, buoys and other arborescent organisms, including the bryozoans *Bugula neritina* (Linnaeus 1758), *Tricellaria inopinata*, D'hondt and Occhipinti-Ambrogi, 1985, *Zoobotryon verticillatum* (Della Chiaje 1822) and the hydroid *Halocordile* sp., were sampled and examined in the laboratory for the presence of *P. pusilla*.

In the laboratory, the epibionts on the hydrozoans were separated, identified under a stereomicroscope (Motic K-400L), photographed with a Nikon D90 digital camera and counted. Abundance of caprellids was expressed as number of individuals/1000 ml of substrate because of the different structures of the substrate species (see Pereira *et al.* 2006; Guerra-García *et al.* 2010). The volume of substrates was estimated as the difference between the initial and final volumes when placed into a graduated cylinder with a predetermined quantity of water. Measurements of the total body length (from the front of the head to the end of pereonite 7) of *P. pusilla* were taken using software Scion Image Alpha 4.0.3.2© (2000-2001 Scion Corporation).

Water temperature and salinity were measured every month at the collection site using a conductivity meter CRISON MM40. Twenty-five hydroid colonies were studied during this period. In addition, five colonies were taken to the laboratory alive, where each colony was placed in separate aerated aquaria of 2.5 l supplied with an aquarium air pump. For observation, each colony was placed in small glass containers of 120 ml with a diameter of 6.5 cm and a height

of 6 cm under a stereomicroscope to study the behavior of epibionts, especially the relationships between *P. pusilla*, *E. racemosum* polyps and the aeolids nudibranchs *Flabellina affinis* (Gmelin 1791) and *Cratena peregrina* (Gmelin 1791), which are hydroid predators associated with *E. racemosum*.

2.3 RESULTS

Seawater temperatures ranged from 13.4°C (January 2011) to 24.8°C (July 2011) and salinity was from 32.4 psu (March 2010) to 36.6 psu (July 2011) in the Cádiz marina. *Paracaprella pusilla* was present from September 2010 to November 2010 and disappeared in December 2010 along with *Eudendrium racemosum* colonies. Shortly after the first *E. racemosum* colonies reappeared in the summer of 2011, the caprellid epibiont was again recorded in July 2011. The most abundant population of *P. pusilla* was recorded in October 2010 (Mean \pm SD = 40.94 \pm 37.6 ind ml⁻¹ hydroid).

Paracaprella pusilla was only found associated with the hydroid *E. racemosum*, and not on any artificial substrates, nor on any other arborescent organisms at the Cadiz marina. Epibenthic peracarids were found to be commonly associated with *P. pusilla* within the hydroid colonies, including the amphipods *Caprella scaura* Templeton 1836, *C. equilibra* Say 1818, *Corophium acutum* Chevreux 1898 and *Stenothoe monoculoides* (Montagu 1815), the isopod *Dynamene edwardsi* (Lucas 1849) and the tanaid *Tanais dulongii* (Audouin 1826).

The morphological characteristics used to define *P. pusilla* were: (1) the large anterolateral projection of pereonite 2; (2) the small dorsal tubercle on pereonite 2; (3) the proximal knob on the basis of gnathopod 2; (4) the lateral pleura in pereonites 3 and 4, more specially developed in pereonite 3 (shown in plate 2, figures 36 and 37 from Mayer 1903). The specimens collected in Cádiz were found to display these features. The largest total body length for mature males was 7.97 mm, and for mature females it was 6.65 mm. Drawings of *P. pusilla* from different areas of the world, as well as our own examination of the specimens taken from the Gulf of Mexico, India and the Strait of Gibraltar showed little intraspecific variation in morphology.

Behaviourally, *P. pusilla* was found to hold an ‘up-right’ posture on its native host (*E. racemosum*), and seemed to be able to switch between a predatory and filter feeding behaviour (see Takeuchi and Hirano 1995; Guerra-García *et al.* 2002). *Paracaprella pusilla* frequently grazed the settled detritus from the hydroid branches and, in some mature specimens the body was covered with detritus, possibly as a form of camouflage (Fig. 2.3).



Fig. 2.3 *Paracaprella pusilla* (with its body covered with detritus) and the nudibranch *Cratena peregrina* associated with the hydroid *Eudendrium racemosum*

We also observed that *P. pusilla* was commonly found in proximity of the polyps of *E. racemosum*. Occasionally, the caprellid placed its mouthparts into a polyp, appearing to feed on it, but a few seconds later the polyp returned to its former position with tentacles extended, without apparent damage. However, when a hydroid nudibranch predator (*Flabellina affinis* or *Cratena peregrina*) was added to the colony and moved toward the caprellid, *P. pusilla* displayed a deterrent behaviour that triggered a change in the direction of the nudibranch, which then moved away from the area where the caprellids were present. This behaviour was more common when the nudibranch was smaller than the caprellid, and we did not observe any differences between the two nudibranch species.

Table 2.1 Global distribution records of *Paracaprella pusilla* including date of reporting, site of collection (if known) and possible mechanism of introduction. Likely vectors: SF- ship fouling; BW- ballast water; R- rafting; AQ- aquaculture.

Date	Country	Localities	Collected from	Vector	Author of record
1890	Brazil	Rio de Janeiro	Ascidians		Mayer, 1890
1903	Brazil	Desterro	Unknown		Mayer, 1903
1903	Martinique	Fort de France and St.	Unknown		Mayer, 1903
1903	Jamaica	Kingston harbour	Sides of a lighter	SF	Mayer, 1903
1928	Tanzania	Dar es Salaam	Unknown		Schellenberg, 1928
1928	Egypt	Suez Canal	Unknown	SF	Schellenberg, 1928
1937	Congo	Malembe	Algae and bryozoans	RF	Schellenberg, 1939
1937	USA	Hawaii (Honolulu harbour)	Intake water pipe	SF,BW	Edmonson and Mansfield, 1948
1941	USA	Hawaii (Honolulu harbour)	Hydroid (<i>Pennaria</i>)	SF,BW	Edmonson and Mansfield, 1948
1951	Gambia	Off Bathurst	Ships and buoys	SF, RF	Reid, 1951 (as <i>Caprella nigra</i>)
1955	South Africa	Durban harbour	Ship hull fouling	SF	Barnard, 1955; Day and Morgan, 1956
1957	USA	Texas	Fishing pier	SF,BW	Steinberg and Dougherty, 1957
1968	USA and Caribbean coast	Florida, Louisiana, Texas, Virgin Islands, Guadeloupe, Margarita, Curaçao	Mangrove roots, sea grass, hydroids and ascidians		McCain, 1968
1977	India	Kerala	Algae		
1977	India	Madras harbour, Tamil Nadu, Kerala and Pondicherry	Hydroids	SF	Sivaprakasam, 1997 (as <i>Paracaprella banardi</i> ^a)
1978	USA	Gulf of Mexico	Bryozoans (<i>Amanthia</i> sp., <i>Bugula neritina</i>)		Caine, 1978
1987	USA	Gulf of Mexico	Barnacles on petroleum platforms	RF, SF	Lewbel et al., 1987

-Cont. Table 2.1-

1994	USA	Gulf of Mexico	Muddy sediments at 498 m depth		Winfield et al., 2006
1997	USA	Florida	Artificial reefs	RF	Martin and Bortone, 1997; Camp, 1998
1998	Cuba	Sabana-Camagüey	Algae and stones		Ortiz and Lalana, 1998
1998	USA	Gulf of Mexico	Muddy sediments at 21 m depth		Borja, 1998
2000	USA	Coast of Georgia	Soft bottom		Cooksey et al. 2004
2001	Chile	Coquimbo	Detached aquaculture buoys	RF,AQ	Astudillo et al., 2009
2003	USA	Alabama, Mississippi	Navigational buoy	RF	Foster et al, 2004
2005	Venezuela	Caribbean coast	Gravel bottoms, ropes, mussels, oysters, sabellariid worms, hydroids (<i>Halocordyle</i>) associated with mangrove roots		Díaz et al., 2005
2006	Colombia	Caribbean coast	Fouling communities of light-exposed pillars	RF	Guerra-García, 2006
2008	Australia	Port of Cairns	Boats	SF	Montelli and Lewis, 2008
2009	India	Vasai creek	Hydroid on an anchoring rope	SF	Bhave and Deshmukh, 2009
2009	Mexico	Gulf of Mexico	Drifting detached seaweeds	RF	Baeza-Rojano (pers. comm.)
2010	India	From Mumbai to Visakhapatnam harbour	Boats, seaweeds (<i>Gracilaria</i>) culture, coral rubbles, bryozoans (<i>B. neritina</i>) and hydroids from natural rocky shores	SF,RF, AQ	Guerra-García et al., 2010

^aSee Guerra-García et al. 2010

2.4 DISCUSSION

2.4.1 Relationships between the introduced epibiont and its native host

Paracaprella pusilla, as with many other caprellid species, is reported to be relatively unselective with respect to substratum (Table 2.1). In different locations this caprellid has been collected from *Bugula neritina* (Caine 1978; Guerra-García *et al.* 2010), a bryozoan, also commonly present in the Cadiz marina's fouling community. However, in this location, *P. pusilla* was only found within colonies of the hydroid *E. racemosum*. Indeed, the occurrence of *P. pusilla* in the Cadiz marina seems to relate directly to the presence of this substrate, which prompts us to assume the existence of a direct relationship between the introduced epibiont and its native host. In this sense, several aspects of the caprellid behaviour could favor the establishment of the species in the area.

First, *P. pusilla* seems to display an occasionally clepto-commensalist behavior by stealing captured prey from the polyps of *E. racemosum*. This peculiar trophic strategy was also observed by Bavestrello *et al.* (1996) to exist in other caprellid species (*Pseudoprotella phasma* and *Caprella* sp) on *Eudendrium glomeratum* polyps. Second, *P. pusilla* is able to deter predator nudibranchs which feed on *Eudendrium* polyps by diverting them to areas of the colony devoid of caprellids. This mutualistic relationship was observed by Caine (1998) for *Paracaprella tenuis* on the hydrozoan *Bougainvillia rugosa* Clarke 1882. Furthermore, the caprellid actively cleaned detritus from the hydroid branches. Dewey (1970) found that *Caprella equilibra* functioned as a defouling agent that promoted the survival of the hydroid *Aglaophenia pinquis*. In summary, the caprellid receives a suitable substratum for attachment, which also serves as camouflage from predators, and access to food items including detritus, diatoms or copepods while living on the hydroid. In return, the hydroid is defouled and protected from predation.

Several studies have suggested that some caprellids may feed directly on hydroids, but when Guerra-García and Tierno de Figueroa (2009) analyzed the stomach content of *P. pusilla* they did not find any remnants of hydroids. These authors considered *P. pusilla* as a detritivore species. However, Caine (1978) did observe that ambush predation was the most frequently used strategy for

obtaining food by this species, although it did commonly utilized alternate feeding modes. *P. pusilla*'s association with the hydroid *E. racemosum* may be either facultative, since it is found on numerous substrates in others localities, or obligatory (in this new range) as it is seemingly restricted to living in association with this hydroid in this area. Di Camillo *et al.* (2008) studied the interactions between *Eudendrium* and its epibionts, and emphasized the role of the *Eudendrium* colonies as a hotspot of coevolutionary relationships and life histories. This facilitation of the non-native caprellid by the native hydroid could have contributed to the establishment success of *P. pusilla* in this new habitat.

2.4.2 Remarks of distribution and possible mechanism of introduction

Paracaprella pusilla is commonly reported from anthropogenic disturbed habitats where it has colonized a variety of artificial structures such as ropes, buoys, pontoons and oil platforms (Table 2.1). Lewbel *et al.* (1987) reports the highest population density for this species, with 7948 caprellids/m² on an oil platform in the Gulf of Mexico (Woods 2009). This ability to colonize a wide range of substrates suggests that this species may be capable of disperse by rafting on floating substrates. Thiel *et al.* (2003) reported the importance of rafting for the distribution of caprellids over long distances along the coast of Chile. Both rafting and biofouling communities on ships could explain the distribution of this species and the low morphological variation among specimens from different areas of the world. Further morphological and molecular studies are necessary to explore if the small morphological variation among populations in different geographic regions of the world correspond with low genetic structure.

One possible hypothesis in explaining the current global distribution of *P. pusilla* is that the species had spread from its natural range along the tropical eastern coast of the Americas, first to Africa and the Suez Canal (1920-30s) and then to India (1980-90s). The species has not yet been found in the Mediterranean, so the presence in the Strait of Gibraltar by entrance through the Suez Canal and the Mediterranean seems uncertain. However, knowledge of alien amphipods in this area is far from thorough (Zenetos *et al.* 2010), and it is therefore possible that the species is present in the Mediterranean but has yet to be detected there. Another possibility is that the species in Cadiz come from the

West African coasts or from the Caribbean. Cadiz is one of Europe's busiest cruise destinations, with many cruise ships from the Caribbean and West Africa arriving at the international port of Cadiz, located a few kilometers from the Cadiz marina. The first introduction of *P. pusilla* into European coastal waters has possibly been through this international port via shipping or ballast water, with local secondary spread to the Cadiz marina by rafting or recreational boating. Once introduced to a marina, there is a high probability that a species would be spread further via recreational yacht hulls; most likely to habitats of similar environmental conditions where the species can successfully establish (Ashton *et al.* 2006).

Although the abundance and species richness of caprellids in many areas of the world's oceans are still poorly known (Thiel *et al.* 2003), Laubitz (1970) pointed out that surface water temperature is an important factor determining the distribution of littoral caprellids. Evidences of changes in the geographic distribution of non-indigenous species are increasingly accompanied by observations of warming in particular areas of the sea (Occhipinti-Ambrogi 2007). Further information about the potential warming in regions within temperate latitudes, and some details of the ecology of the species, like temperature tolerance studies, is still necessary to determinate if climate change could be involved in the establishment success of this Caribbean species in temperate ecosystems.

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2

DETECTION & DISTRIBUTION: PART 2

Paracaprella pusilla: a new alien crustacean
in the Mediterranean Sea

- Adapted from:

Ros M, Vázquez-Luis M, Guerra-García JM (2013) The tropical caprellid amphipod *Paracaprella pusilla*: a new alien crustacean in the Mediterranean Sea. Helgoland Marine Research 67: 675–685.

ABSTRACT. *Paracaprella pusilla* Mayer, 1890, originally described from Brazil, is one of the most abundant caprellid amphipod species in tropical and subtropical seas around the world. During a survey of caprellid amphipods from marinas along the Balearic Island (Western Mediterranean Sea) carried out between November 2011 and August 2012, we found two established populations of *P. pusilla* in Mallorca and Ibiza, respectively. So far its occurrence in European waters was reported only from southwestern Spain in 2010. This record represents a northward range expansion of the species' distribution, which is found for the first time in the Mediterranean. This is also the first record of the genus *Paracaprella* in the Mediterranean Sea. The most probable introduction vector was ship fouling. We also found the invasive caprellid *Caprella scaura* Templeton, 1836 in Mallorca and Menorca, which is recorded for the first time in the Balearic Islands, confirming its rapid expansion along the Mediterranean. When comparing reproductive traits between both alien species, we found that *P. pusilla* has a higher fecundity than *C. scaura* for the same female size. Taking into account this evidence, the species may be expected to appear in other Mediterranean and adjacent areas.

RESUMEN. *Paracaprella pusilla* Mayer, 1890, descrita inicialmente en Brasil, es una de las especies de anfípodos caprelídeos más abundante en los mares tropicales y subtropicales de todo el mundo. Durante un muestreo centrado en los anfípodos caprelídeos presentes en puertos deportivos en las Islas Baleares (Mediterráneo Occidental), llevado a cabo entre noviembre de 2011 y agosto de 2012, encontramos dos poblaciones estables de *P. pusilla* en Mallorca e Ibiza, respectivamente. Hasta ahora, su presencia en aguas europeas sólo había sido detectada en la costa suroeste de España, en 2010. Este hallazgo implica una extensión hacia el norte en el rango de distribución de la especie, que es encontrada por primera vez en el Mediterráneo. Este es, además, el primer registro del género *Paracaprella* en el mar Mediterráneo. El vector de introducción más probable fue la adhesión de la especie a las comunidades incrustantes de los barcos. También encontramos el caprelídeo invasor *Caprella scaura* Templeton, 1836 en Mallorca y Menorca, siendo la primera vez que esta especie aparece en las Islas Baleares y confirmando su rápida expansión por el Mediterráneo. Cuando se compararon varios aspectos reproductivos entre ambas especies introducidas, encontramos que *P. pusilla* tuvo una mayor capacidad reproductora que *C. scaura* para el mismo tamaño de hembra. Teniendo en cuenta esta evidencia, es probable que *P. pusilla* aparezca en otras regiones del Mediterráneo, así como en áreas adyacentes.

2.5 INTRODUCTION

The Mediterranean Sea is one of the world areas most affected by biological invasions with about 955 introduced species, 153 of these representing crustaceans (Zenetos *et al.* 2010). Among alien crustaceans, the taxa most frequently recorded in the Mediterranean are Decapoda, followed by Calanoida and Amphipoda (Galil 2011). However, although the number of alien crustaceans has increased noticeably in the last two decades, probably reflecting both an increase in introductions and an interest in their study (Galil 2009), the number of alien crustaceans in the groups of amphipods, cirripedes, cumaceans, isopods and tanaidaceans is still underestimated (Zenetos 2010). Caprellid amphipods, commonly known as skeleton shrimps, are small marine crustaceans that are common in many littoral habitats, where they form an important trophic link between primary producers and higher trophic levels (Woods 2009). The morphology of caprellids, with reduced abdominal appendages which in other amphipods are used for swimming (Takeuchi and Sawamoto 1998) as well as a lack of a planktonic larval stage, suggests that the cosmopolitan distribution of many littoral caprellids is facilitated by the fact that they are often associated with fouling communities on floating objects and vessels (Thiel *et al.* 2003).

The Mediterranean Sea has one of the best-documented amphipod faunas in the world (Ruffo 1982, 1989, 1993, 1998), but new species are still being described, especially in the case of caprellid amphipods (e.g. *Caprella tavolarenensis* Sturaro and Guerra-García 2011, based on specimens collected from *Posidonia oceanica*), indicating that further sampling should be conducted to complete our knowledge about Mediterranean caprellids. This is particularly important in the case of fouling communities in harbors and marinas which are still scarcely sampled in some areas of the Mediterranean. Fouling communities include arborescent substrates such as bryozoans and hydroids, which may act as suitable reservoirs for introduced caprellids that have remained unrecorded as yet (Ros *et al.* 2013). Bellan-Santini and Ruffo (1998) list three caprellid species native to the Mediterranean but known for their propensity for passive dispersal and presence in Mediterranean harbor fouling communities: *Caprella acanthifera*, *C. dilatata* and *C. equilibra*. In 1994, an unusual-looking caprellid, characterized by an acute cephalic projection, was found associated to the fouling community of the wooden piles in the Lagoon of Venice (Sacchi *et al.* 1998). This caprellid, identified later as *Caprella scaura* (Templeton 1836) by Sandro

Ruffo (Krapp *et al.* 2006), represented the first and only introduced caprellid reported in the Mediterranean Sea. During the last decade, this Indopacific species has spread very fast across the Mediterranean Sea and has expanded its non-native range to the East Atlantic coast (Sconfiatti *et al.* 2005; Krapp *et al.* 2006; Galil *et al.* 2008; Martinez and Adarraga 2008; Ben Souissi *et al.* 2010; Bakir and Katagan 2011; Guerra-García *et al.* 2011; Eleftheriou *et al.* 2011). In September 2010, an established population of another alien caprellid, the tropical species *Paracaprella pusilla* Mayer 1890, was found for the first time in European waters, in the fouling community of a marina on the southwest Atlantic coast of Spain (Ros and Guerra-García 2012). This tropical/subtropical species, originally described from Rio de Janeiro, Brazil, was found associated with the native hydroid *Eudendrium racemosum*.

This study reports the result of a survey on the fouling communities of marinas of the Balearic Islands to determine the presence and quantify abundances of non-indigenous caprellids (NICs) in the Western Mediterranean region. Considering our scant knowledge about the ecology of *P. pusilla*, some reproductive biology traits were studied for the first time for the species, and its fecundity was compared with the invasive *C. scaura*. The likely vector and pattern of introduction of *P. pusilla* in the Mediterranean Sea as well as the species' current status were analyzed.

2.6 MATERIALS AND METHODS

2.6.1 Study area

The Balearic Islands, located in the centre of the western Mediterranean, are one of the most important tourist destinations in the Mediterranean Sea and are among the preferred destinations for cruise ships crossing the Mediterranean (Minchin *et al.* 2006). They are characterised by an intense maritime traffic and are a potential hot spot of marine biological invasions (see Drake and Lodge 2004). The region comprises the four main islands of Mallorca, Menorca, Ibiza and Formentera, as well as the small island of Cabrera (Fig. 2.4).

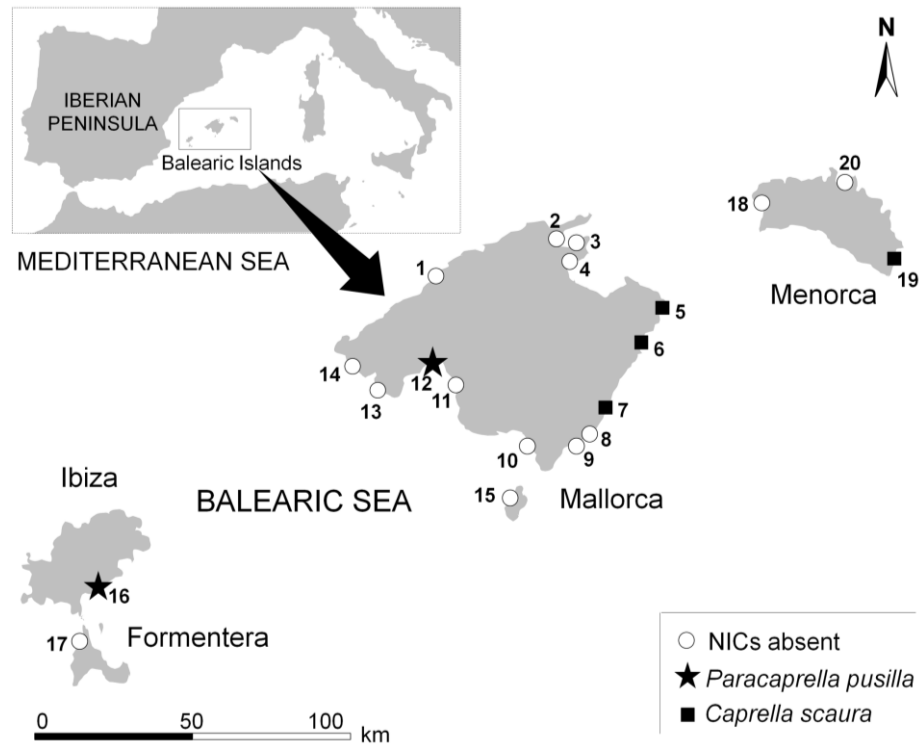


Figure 2.4 Map of the Balearic Islands showing sampling stations and presence/absence of NICs (non-indigenous caprellids). See also Table 2.2.

2.6.2 Sampling

The sampling programme was conducted between November 2011 and August 2012. A total of 20 recreational marinas along the coast of the Balearic Islands were sampled to ensure a complete review of the total fouling communities which proliferate on artificial hard substrate including pilings, floating pontoons, ropes, buoys, wheels and ship hulls (Table 2.2). When caprellids were detected in a type of fouling substrate (hydroids, bryozoans or macroalgae), three random replicates of each substrate were taken by hand and fixed in situ in 90% ethanol. Environmental parameters (water temperature, salinity and turbidity) were measured in situ at each sampling station. Three haphazard measurements were made for each parameter across the floating pontoon system and mean values and standard deviations were calculated. Salinity and temperature were measured using a conductivity meter CRISON MM40 and turbidity in nephelometric turbidity units (ntu) using a turbidimeter WTW 335 IR.

Table 2.2 Locations and environmental characteristics of marinas surveyed in the present study. Cs: *Caprella scaura*; Pp: *Paracaprella pusilla*; NICs: non-indigenous caprellids; SD: standard deviation.

Island	Marina	Locality	Coordinates	Date	Temperature °C (mean ± SD)	Salinity (mean ± SD)	Turbidity (ntu) (mean ± SD)	NICs present
MALLORCA	1 Puerto de Sóller	Sóller	39°47'N, 0.2°41'E	6 Nov 2011	17.7±0.1	23.8±0.2	18.1±4.9	-
	2 Puerto de Pollença	Pollença	39°54'N, 0.3°0.5'E	5 Nov 2011	20.3±0.1	37.9±0.0	5.8±0.7	-
	3 Es Barcarés	Alcúdia	39°52'N, 0.3°0.5'E	12 Mar 2012	17.1±0.3	37.3±0.1	1.3±0.2	-
	4 Alcudiamar	Alcúdia	39°49'N, 0.3°0.8'E	5 Nov 2011	19.4±0.2	35.3±0.5	7.4±5.1	-
	5 Club Náutico Cala Ratjada	Cala Ratjada	39°43'N, 0.3°28'E	5 Nov 2011	21.6±0.5	37.5±0.0	10.6±2.7	Cs
	6 Puerto de Cala Bona	Cala Bona	39°37' N, 0.3°23'E	6 Nov 2011	19.9±0.1	36.0±0.1	7.6±1.7	Cs
	7 Club Náutico Porto Colom	Porto Colom	39°25' N, 0.3°15'E	6 Nov 2011	16.3±0.1	37.6±0.3	36.3±3.6	Cs
	8 Marina de Cala d'Or	Cala d'Or	39°22' N, 0.3°14'E	6 Nov 2011	20.3±0.1	37.5±0.5	4.7±1.0	-
	9 Puerto de Porto Petro	Santanyí	29°21' N, 0.3°12'E	6 Nov 2011	19.8±0.1	37.6±0.3	12.7±6.0	-
	10 Puerto Colònia Sant Jordi	Colònia Sant Jordi	39°18' N, 0.2°59'E	6 Nov 2011	18.8±0.1	35.0±0.1	58.0±22.4	-
CABRERA	11 Club Náutico El Arenal	El Arenal	39°30' N, 0.3°45'E	11 Nov 2011	16.9±0.1	37.2±0.1	1.9±0.4	-
	12 Puerto de Palma	Palma de Mallorca	39°34' N, 0.2°38'E	5 Nov 2011	21.5±0.1	33.4±1.3	3.8±1.3	Pp
	13 Puerto Adriano	El Toro	39°29' N, 0.2°29'E	7 Nov 2011	20.3±0.1	36.0±0.1	5.3±1.0	-
	14 Club de Vela Puerto de Andratx	Andratx	39°33' N, 0.2°24'E	7 Nov 2011	19.9±0.1	37.7±0.3	10.7±0.1	-
IBIZA	15 Puerto natural de Cabrera	Cabrera	39°09' N, 0.2°59'E	30 Abr 2012	-	-	-	-
	16 Club náutico de Ibiza	Ibiza	38°54' N, 0.1°26'E	2 Jun 2012	20.5±0.5	35.3±0.5	2.2±0.2	Pp
FORMENTERA	17 Puerto de La Savina	La Savina	38°44' N, 0.1°25'E	2 Jun 2012	20.5±0.5	35.3±0.5	1.4±0.2	-
	18 Club náutico de Ciutadella	Ciutadella	39°58' N, 0.3°49'E	19 Jul 2012	25.6±0.1	36.0±0.4	1.3±0.4	-
MENORCA	19 Club marítimo de Mahón	Mahón	39°52' N, 0.4°18'E	20 Jul 2012	26.3±0.0	37.1±0.2	1.7±0.8	Cs
	20 Puerto de Fornells	Mahón	40°03' N, 0.4°0.8'E	19 Aug 2012	-	-	-	-

2.6.3 Laboratory processing and statistical analysis

All caprellids were sorted and identified to species level. Abundance of caprellids was expressed as number of individuals/1,000 ml of substrate because of the different structure of the substrate types (see Pereira *et al.* 2006; Guerra-García *et al.* 2010). Volume of substrates was estimated as the difference between the initial and final volume when placed into a graduated cylinder with a fixed amount of water. Subsequently, all non-native caprellids collected were photographed on a stereomicroscope Motic K-400L with a Nikon D90 digital camera. Body length of males, mature females (with the brood pouch fully developed) and premature females (with the brood pouch underdeveloped) was measured from the front of the head to the end of pereonite 7, using the PC-based digitizing software Scion Image Alpha 4.0.3.2 © (2000-2001 Scion Corporation). A total of 145 individuals of *C. scaura* and 106 of *P. pusilla* were measured.

For each non-native caprellid species found, eggs from 15 ovigerous females with the brood pouch completely closed were counted by removing them from the brood pouch with a dissecting needle. To test possible relationships between female size and number of eggs, Pearson's correlation coefficient was calculated for each species, and differences between the slopes of regression lines of both species were tested using parallelism and equality of lines tests.

2.6.4 Reproductive traits

Five reproductive traits were selected to compare the fecundity of alien species found in the present study (modified after Grabowski *et al.* 2007):

- a) mean size of ovigerous females
- b) brood size (mean number of eggs per brood pouch)
- c) maximum number of eggs
- d) partial fecundity index (mean brood size/mean size of ovigerous females)
- e) relative age at reaching maturity (minimal size/mean size of ovigerous females)

The comparisons were carried out with alien caprellids from the same region (Mallorca) and collected in the same season (November 2011) to avoid confounding factors.

2.7 RESULTS

Two non-native caprellids were found in the Balearic Islands, *Paracaprella pusilla* and *Caprella scaura*. The morphological characteristics used to define *P. pusilla* are described in the first part of this chapter (page 16). Individuals collected in Mallorca and Ibiza display these features (Fig. 2.5). Drawings of *P. pusilla* from different world areas (Guerra-García 2006 from Colombia, Guerra-García *et al.* 2010 from India, and Díaz *et al.* 2005 from Venezuela) and our own examination of specimens from the Gulf of Mexico, Brazil, India, Southern Spain and the Balearic Islands showed little intraspecific variation in morphology.



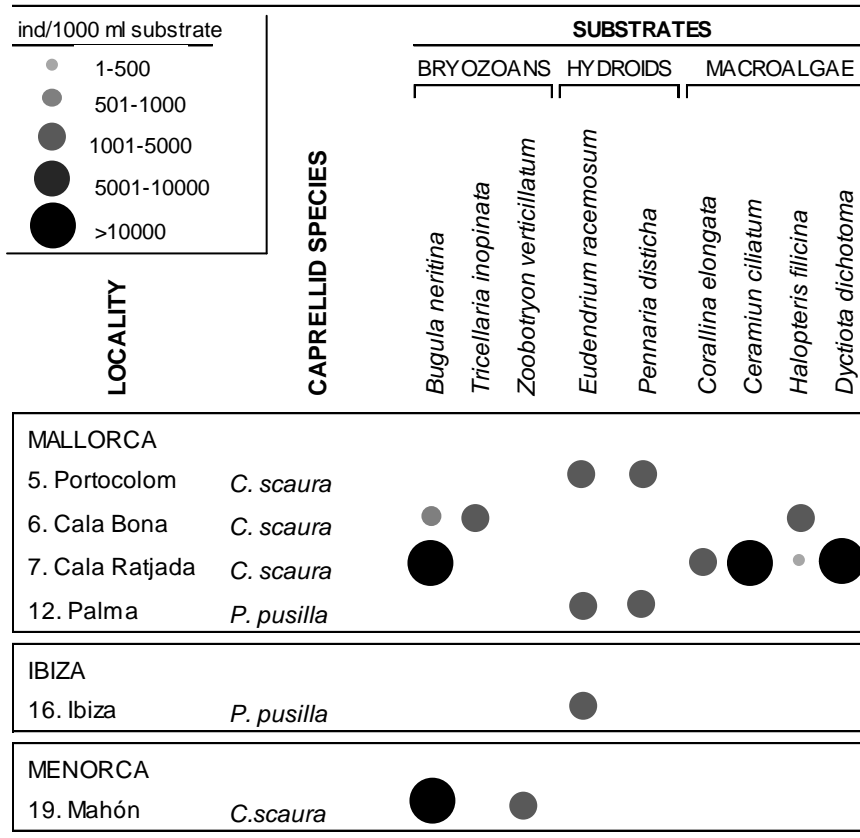
Figure 2.5 (A) Lateral view of an adult male and an adult female of *P. pusilla* collected from Spain; (B) detail of the lateral pleura in pereonite 3 (see arrow) of an adult male; (C) detail of the anterior part of an adult male showing the small dorsal tubercle, the large anterolateral projection of pereonite 2, and the proximal knob on the basis of gnathopod 2 (see arrows); (D) detail of the gnathopod 2 of an adult male.

The morphological characteristics used to identify *C. scaura* from the Mediterranean are (1) cephalon with an acute, bent forward, dorsal projection, (2) pereonites 1 and 2 elongate in males, (3) basis of gnathopod 2 long but shorter than pereonite 2 and (4) absence of ventral projection between the insertion of gnathopods 2 (Templeton 1836; Mayer 1890; Krapp *et al.* 2006). Individuals collected in Mallorca and Menorca display these features and are similar to others populations from the Iberian Peninsula, the Canary Islands, Italy and Greece, which were examined by the authors.

Alien caprellids were present in the three islands of Balearic Archipelago: Mallorca, Menorca and Ibiza; and were absent from the islands of Formentera and Cabrera (Fig. 2.4). *Paracaprella pusilla* was found in Palma marina (Mallorca; 39°34'N, 2°38'E) and Ibiza marina (Ibiza; 38°54'N, 1°26'E). The species was found in a water temperature range from 20.5°C (Ibiza) to 21.5 (Mallorca), a salinity range from 33.4 (Mallorca) to 35.3 (Ibiza), and a turbidity range from 2.2 ntu (Ibiza) to 3.8 ntu (Mallorca) (Table 2.2). In both marinas *P. pusilla* was found associated with the hydroid *Eudendrium racemosum* where it exhibited similar densities (4,611 ± 2,204 ind/1,000 ml in Mallorca and 4,100 ± 2,055 ind/1,000 ml in Ibiza, Mean ± SE). In Palma marina (Mallorca), the species was also found with the hydroid *Pennaria disticha* (Table 2.3). The maximum total length recorded for males was 8.2 mm, whereas for females, the maximum was 5.6 mm (Fig. 2.6).

Caprella scaura was found in three marinas located in the northeast coast of Mallorca: Cala Ratjada (39°43'N, 3°28'E), Cala Bona (39°37'N, 3°23'E) and Porto Colom (39°25'N, 3°15'E), and in one marina in Menorca (Mahón; 39°58'N, 4°18'E). The species was found in a water temperature range from 16.3°C (Porto Colom, Mallorca) to 26.3°C (Menorca), a salinity range from 36.0 (Cala Bona, Mallorca) to 37.6 (Porto Colom, Mallorca), and a turbidity range from 1.7 ntu (Menorca) to 36.3 ntu (Porto Colom, Mallorca) (Table 2.2). *Caprella scaura* was associated with eight different substrates, including hydroids, bryozoans and macroalgae of the marinas' fouling community (Table 2.3). The highest abundance was found in Cala Ratjada, associated with the bryozoan *Bugula neritina* (18,333 ± 8,647 ind/1,000 ml). The maximum total length recorded for males was 13.2 mm, whereas for females, the maximum was 7.9 mm (Fig. 2.6). The large individuals of both sexes were found associated with bryozoans.

Table 2.3 Density of non-indigenous caprellids (*C. scaura* and *P. pusilla*) found on different fouling species and in different locations of the Balearic Islands.



Regarding the reproductive traits, we found that the mean number of eggs per female was 29.07 for *P. pusilla* and 26.67 for *C. scaura* (Table 2.4). The partial fecundity index was 7.20 for *P. pusilla* and 5.32 for *C. scaura*, and the maturity index was 0.66 and 0.73 for *P. pusilla* and *C. scaura*, respectively.

Table 2.4 Reproductive traits for non-indigenous caprellid populations collected at Mallorca in November 2011

Species	Mean ovigerous female size (mm) ± SE	Mean brood size (n°eggs/female) ± SE	Maximun n°eggs (female size)	Partial fecundity index (brood size/mean female size)	Maturity index (min/mean female size)
<i>P. pusilla</i>	4.62±0.14	29.07±3.98	62 (5.40 mm)	7.2	0.66
<i>C. scaura</i>	5.59±0.32	26.67±5.88	72 (7.30 mm)	5.32	0.73

SE standard error

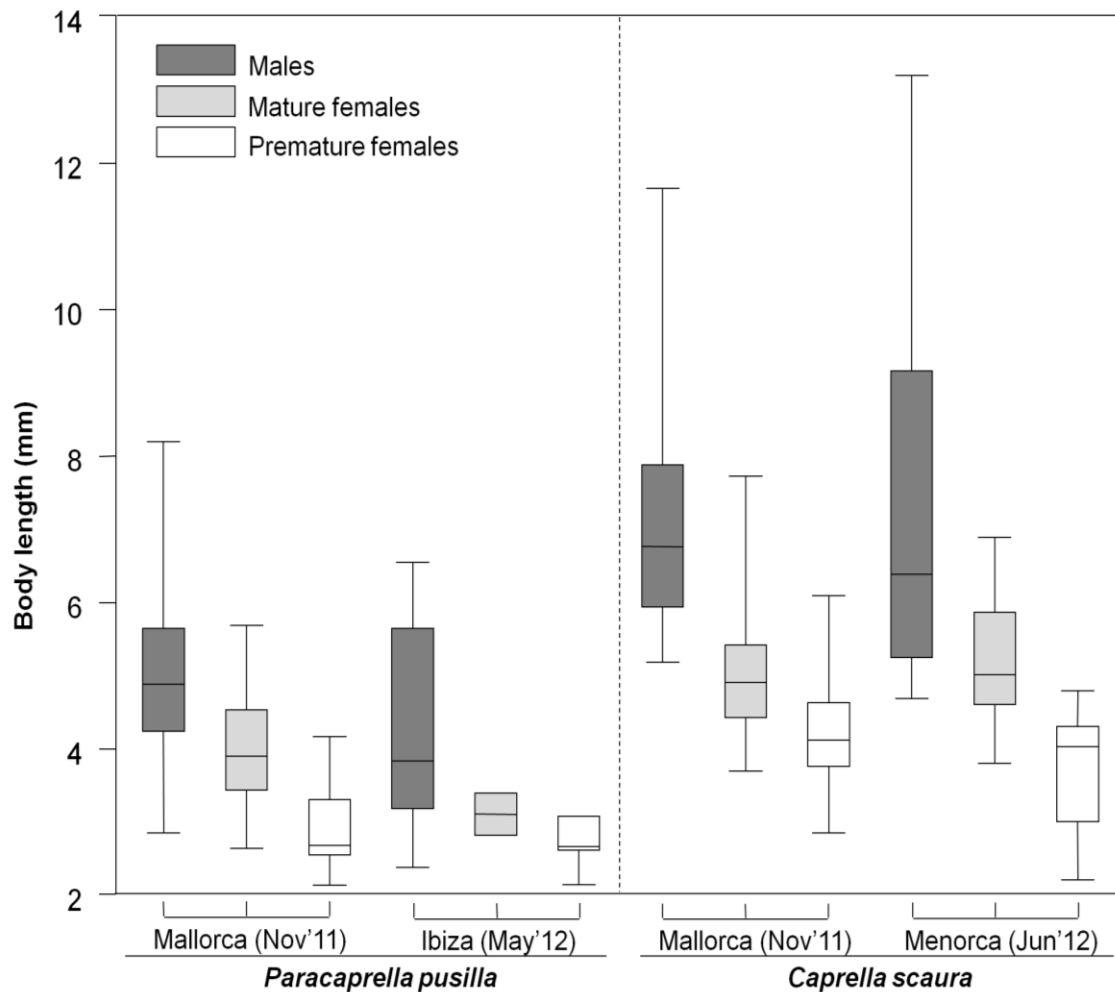
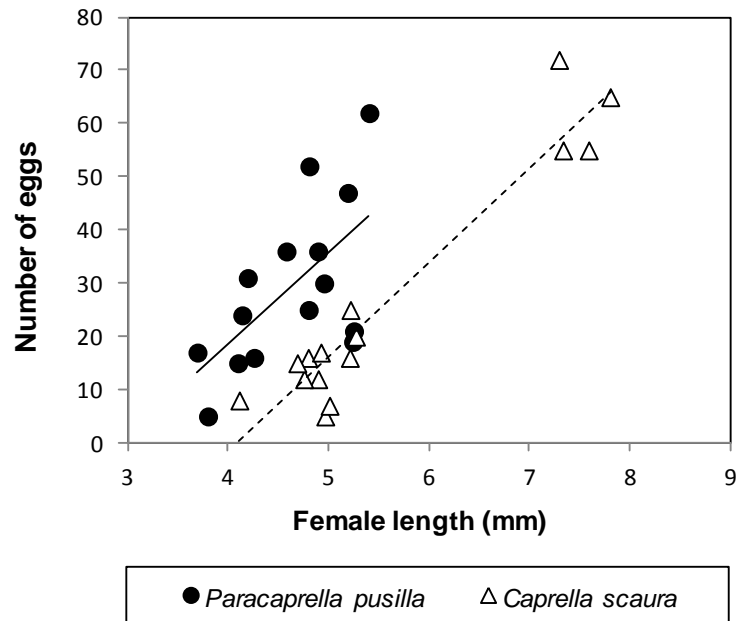


Figure 2.6 Box-and-Whisker plot for each sex/age group measured for the different populations. Median values are included; the rectangles contain values between the first and the third quartiles; the bars connect the extreme values.

A significant correlation was found between female size and number of eggs for both species (*P. pusilla*: $r=0.62$, $p<0.05$; *C. scaura*: $r=0.96$, $p<0.01$) (Fig 2.7). Although the parallelism test did not show differences between the slopes of regression lines of the two species ($F_{1,26}=0.006$, $p=0.94$), the equality of lines test showed significant differences between *C. scaura* and *P. pusilla* ($F_{2,26}=10.89$, $p=0.0004$). The graph shows that for a given body size, females of *P. pusilla* had a higher number of eggs than females of *C. scaura*.

Figure 2.7 Correlations between female size and number of eggs per brood in *P. pusilla* and *C. scaura* collected at Mallorca.



2.8 DISCUSSION

2.8.1 Non-native caprellids from the Balearic Islands and possible vectors of their introduction

We recorded, for the first time, the presence of the invasive *C. scaura* at the Balearic Islands as well as the presence of the introduced *P. pusilla* in the Mediterranean Sea, which represents the first record of the genus *Paracaprella* in the Mediterranean. These species were present at the islands of Mallorca, Menorca and Ibiza but were absent from the small islands of Formentera and Cabrera. *Caprella scaura* was found associated with a wide variety of fouling substrates, including macroalgae, hydroids and bryozoans, thus showing a high plasticity to colonize different habitat structures, while *P. pusilla* was only found associated with fouling hydroids. This pattern of habitat use was also found for both species in the fouling community of a marina in southern Spain, where presence of *P. pusilla* was associated with the native hydroid *E. racemosum* (Ros and Guerra-García 2011: Chapter 2.1), reflecting a clear preference for hydroids in the non-native area. Although *Caprella* species have been found to survive transport in ballast tanks (Carlton 1985), for a fouling species frequently recorded from ports, transport via hull fouling is assumed to be the most probable

vector (Galil 2011). As both *P. pusilla* and *C. scaura* were found associated with the fouling communities adherent to artificial hard substrates including ship hulls, ship fouling is assumed to be the most probable vector for the introduction of the species to the Balearic Islands. This may be related to the absence of both species from the islands of Formentera and Cabrera which have only few ports (two and one, respectively; FEAPDT 2011), and are therefore exposed to much lower boating pressure than the islands of Mallorca (39 ports), Menorca (9 ports) and Ibiza (8 ports).

2.8.2 Reproductive traits

Reproduction appears to be a major factor in the success of invasive amphipods (Weis, 2010). Grabowski *et al.* (2007) studied six reproductive and two additional traits (salinity tolerance and tolerance to human impacts) to compare six invasive vs. seven native gammarid species occurring in Central European waters. They found that invasive gammarids were characterized by a combination of large brood size, high partial fecundity, early maturation, and by the appearance of higher number of generations per year. In the present study we analyzed four of the six reproductive traits and an additional one (maximum number of eggs) in the newly introduced *P. pusilla* and *C. scaura*, an invasive species which has been spreading very fast across the Mediterranean and the East Atlantic coast (Guerra-García *et al.* 2011). We found that *C. scaura* and *P. pusilla* females produce a larger mean number of eggs when compared with native species from the Mediterranean Sea with similar female size such as *C. grandimana*, with an average brood size of 7.6 eggs (Baeza-Rojano *et al.* 2011). When comparing *P. pusilla* with *C. scaura*, we found that *P. pusilla* produces more eggs per brood than *C. scaura* and has a higher partial fecundity index. This implies that for a given size of the female, *P. pusilla* has a higher number of eggs than *C. scaura*. However, as females of *C. scaura* can attain larger body sizes, the maximum number of eggs per female was higher in this species. Moreover, the maturity index and thus the relative age at reaching maturity is less in *P. pusilla* than in *C. scaura*. These traits may facilitate the secondary spread of the *P. pusilla* to new areas of the Mediterranean as has already happened with *C. scaura*.

2.8.3 Current status of *P. pusilla*

Similar to other alien caprellids in Europe such as *Caprella mutica* in Scotland (Willis *et al.* 2004), the non-indigenous status of *Paracaprella pusilla* in European waters can be assessed using the criteria of Chapman and Carlton (1994): (1) previously unknown in local region; (2) post-introduction range expansion; (3) associated with a human dispersal mechanism; (4) associated with or dependent on other introduced species; (5) association with artificial environments; (6) restricted or discontinuous distribution in the region; (7) disjunct global distribution; (8) insufficient life history adaptations for natural global dispersal; and (9) exotic evolutionary origin. *Paracaprella pusilla* scores positively on criteria 1, 2, 3, 5, 6, 7, 8, and 9, suggesting it to be an alien species to the area. As this is the first record of the genus *Paracaprella* in the Mediterranean Sea, it increases the known diversity of the Caprellidea in this region.

2.8.4 Global distribution of *P. pusilla*

According to Mayer (1903), the species' natural area of distribution is the Atlantic coast of Central and South America. Most records of *P. pusilla* are from the Gulf of Mexico and the Caribbean coast (Ros and Guerra-García 2012: Chapter 2.1), and the species is one of the most abundant caprellids along the Caribbean coast of Venezuela and Colombia (Díaz *et al.* 2005; Guerra-García 2006). Therefore, the species appears to have a strong Caribbean affinity (Carlton and Eldredge 2009). Nevertheless, the origin of *P. pusilla* is unknown (Mead *et al.* 2011). Records on geographically disjunct occurrences of *P. pusilla* date back to the early 1900s, a short time after the species had been described by Mayer in 1890 (see Chapter 2.1). This, along with the facts that most of the records refer to fouling communities of harbors, and that the species may be able of travelling long distances attached to vessel hulls, has prevented a clear determination of the origin of the species. Actually, the species' global area of distribution includes the Atlantic coasts of Central and South America, tropical West Africa, East Africa, Hawaii, India, Australia and the southwest coast of Spain.

2.8.5 Introduction pattern of *P. pusilla* to the Mediterranean Sea

Due to a lack of previous studies on caprellids associated with fouling communities in the study area, the exact time of introduction to this site remains unknown for both alien species. However, extensive biological surveys in the Mediterranean over the twentieth century allow for a reasonable measure of confidence in separating alien and native biota (Galil 2009). Never recorded in the Mediterranean waters before, *P. pusilla* is not mentioned neither in the handbook of the Mediterranean amphipods fauna (Ruffo 1993) nor in the study by Guerra-García *et al.* (2011a,b) on the intertidal and shallow water caprellids of the Iberian Peninsula. Moreover, *C. scaura* and *P. pusilla* were not recorded neither in a study on amphipods of Ibiza (Ballesteros *et al.* 1998), nor in a more recent study on amphipods of Mallorca (Box 2008). Therefore, the introduction of these caprellids to the European waters of southern Spain and the Western Mediterranean Sea may have occurred during the last decade. The date of the introduction of the *inoculum* is significant for the study of the patterns and processes of invasion but is extremely difficult to ascertain for unintentional or undocumented intentional introductions (Galil 2011).

There are two main alternatives to explain the presence of *P. pusilla* in the Mediterranean Sea (Fig 2.8): The species entered (a) via the Suez Canal (Port Said) on vessels from the Indo-Pacific, or (b) through the Strait of Gibraltar, on vessels arriving from the Atlantic coast of America or from the established population in southwest Spain. The presence of *P. pusilla* in the Suez Canal was only reported by Schellenberg (1928), who recorded the species in three stations: Kantara (46 km from Port Said), Kabret (between Little Bitter Lake and Great Bitter Lake) and Port Taufiq. This seems to support hypothesis (a). However, so far the species has not been recorded neither in the Red Sea nor along the Mediterranean Sea, and recent studies on the fouling community in the Suez Canal by Emara *et al.* (2004), including Little Bitter Lake, Kabret, Great Bitter Lake and Port Taufiq, reported only the presence of the caprellid species *Caprella equilibra*, which was also the only caprellid species found in the fouling communities of the Suez Canal by El-komi (1998). Probably the population found by Schellenberg in 1928 did not succeed in adapting fast enough to the new environment and failed in spreading to adjacent areas. Moreover, the absence of *P. pusilla* in the Red Sea and its relatively recent record in the Indian Ocean (Sivaprakasam 1977) suggest that the populations recently found in

European coastal waters originate from the Atlantic coasts of Central and South America, where the species is highly abundant. In this case, an introduction through the Strait of Gibraltar (hypothesis b) would be more probable than the alternative (hypothesis a). Interestingly, many small craft of Mallorca overwinter in marinas in the south of Spain (Minchin *et al.* 2006) and thus could represent a suitable vector for the secondary spread of the species from the established population of southern Spain to the Balearic Islands. Marinas seem to provide a network of suitable habitats for the secondary spread of a species via domestic yachting activity (Ashton *et al.* 2006).

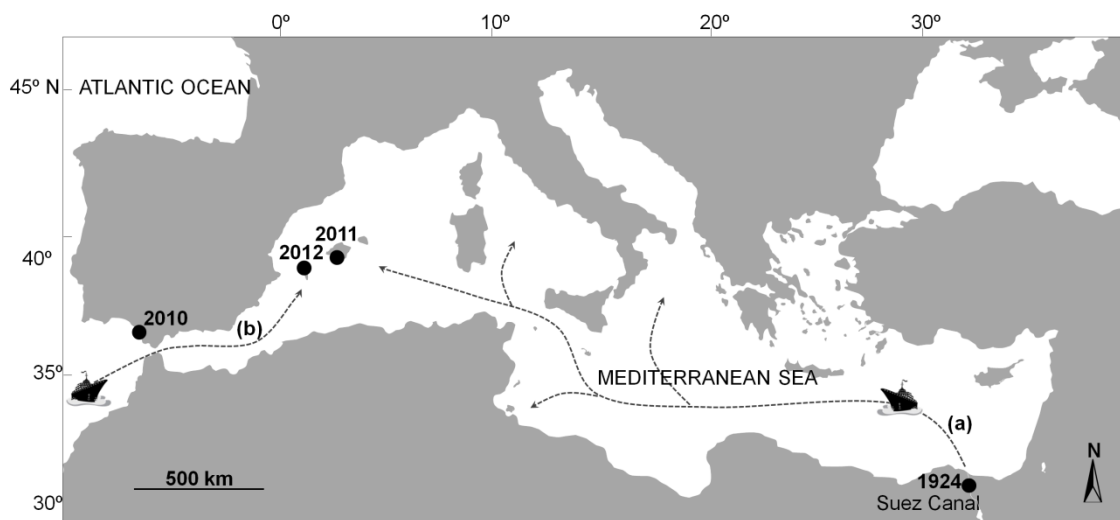


Figure 2.8 Distribution map of *P. pusilla* in the Mediterranean Sea with years of first record for the different areas. Arrows indicate two possible ways of introduction to the Mediterranean Sea (see text).

Unlike what happened when Schellenberg found the species in the Suez Canal in 1928, the last decades of the twentieth century saw pronounced thermal fluctuations and a significant increase in the average seawater-surface temperature in the Mediterranean (Nykjaer 2009). This may favor survival, growth and reproduction of tropical aliens, giving them a distinct advantage over native temperate Mediterranean taxa (Galil 2011). Along with the increasing role of the Mediterranean as a hub of international commercial shipping (Dobler 2002), this might explain the fact that *P. pusilla* has successfully reached the Western Mediterranean Sea only most recently.

The precautionary principle suggests to considering each alien species ‘guilty until proven innocent’ and calls for analyzing possible impacts on native communities (Occhipinti-Ambrogi *et al.* 2011). Taking into account that the occurrence of *P. pusilla* in the Mediterranean Sea is probably a consequence of secondary spread from the established population in the Strait of Gibraltar, and that the fraction of alien species that spread following establishment is considered one of the measures of invasion success (Galil 2011), the presence of *P. pusilla* at the Balearic Islands suggests a future invasion along marinas of the Mediterranean Sea.

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2

DETECTION & DISTRIBUTION: PART 3

Distribution patterns and taxonomical remarks of the non-native caprellid *Caprella scaura* in southern Europe and northern Africa

- Adapted from:

Ros M, Guerra-García JM, Navarro-Barranco C, Cabezas MP, Vázquez-Luis M (2014) The spreading of the non-native caprellid (Crustacea: Amphipoda) *Caprella scaura* Templeton, 1836 into southern Europe and northern Africa: a complicated taxonomic history. *Mediterranean Marine Science* 15: 145–165.

ABSTRACT. *Caprella scaura*, originally described by Templeton (1836) from Mauritius and later reported as several ‘forms’ or ‘varieties’ from numerous areas of the world, was found for the first time in the Mediterranean in 1994. Since this report, the species has been found in several Mediterranean locations. To explore the current distribution of *C. scaura* in the Iberian Peninsula and adjacent areas, we surveyed marine fouling communities from 88 marinas along the whole Iberian Peninsula and North Africa, 3 from Italy, 1 from France, 1 from Malta and 1 from Greece between June 2011 and June 2012. The results of this survey report the first confirmed record of *C. scaura* in Corsica (France), Crete (Greece) and Morocco, and confirm an extensive distribution of *C. scaura* along the Spanish Mediterranean coast and the Strait of Gibraltar. The species was absent along the north Atlantic coast of Spain and the upper distribution limit for the eastern Atlantic coast is the locality of Cascais, on the south coast of Portugal. All populations studied belong to the same morphological form, which match with the ‘varieties’ *C. scaura typica* from Brazil and *C. scaura scaura* from Mauritius, suggesting that (1) these two forms correspond to the same ‘variety’; (2) this ‘variety’ is the only one that is expanding its distribution range and (3) the remaining ‘varieties’ of *C. scaura* complex could represent distinct species with a restricted distribution.

RESUMEN. *Caprella scaura*, descrita originalmente por Templeton (1936) en las Islas Mauricio y más tarde registrada como varias ‘formas’ o ‘variedades’ en numerosas áreas del mundo, se encontró por primera vez en el Mediterráneo en 1994. Desde entonces, la especie ha sido encontrada en varias localidades Mediterráneas. Con el objetivo de explorar la distribución actual de *C. scaura* en la Península Ibérica y en zonas adyacentes, se muestrearon las comunidades incrustantes en 88 marinas repartidas a lo largo de la Península Ibérica y el norte de África, 3 marinas de Italia, 1 en Francia, 1 en Malta y 1 en Grecia entre junio de 2011 y junio de 2012. Los resultados de este muestreo confirman por primera vez la presencia de *C. scaura* en Corsica (Francia), Creta (Grecia) y Marruecos, así como una distribución muy extendida a lo largo de la costa mediterránea española y del Estrecho de Gibraltar. La especie estuvo ausente en la costa norte atlántica de España y su límite superior de distribución en la costa Este Atlántica fue la localidad de Cascais, en el sur de Portugal. Todas las poblaciones estudiadas pertenecieron a la misma forma morfológica, que coincidió con las ‘variedades’ *C. scaura typica* de Brasil y *C. scaura scaura* de Mauricio, sugiriendo esto que (1) estas dos formas corresponden a la misma ‘variedad’, (2) esta ‘variedad’ es la única que está expandiendo su rango de distribución y (3) el resto de ‘variedades’ del complejo *C. scaura* representan distintas especies con una distribución restringida.

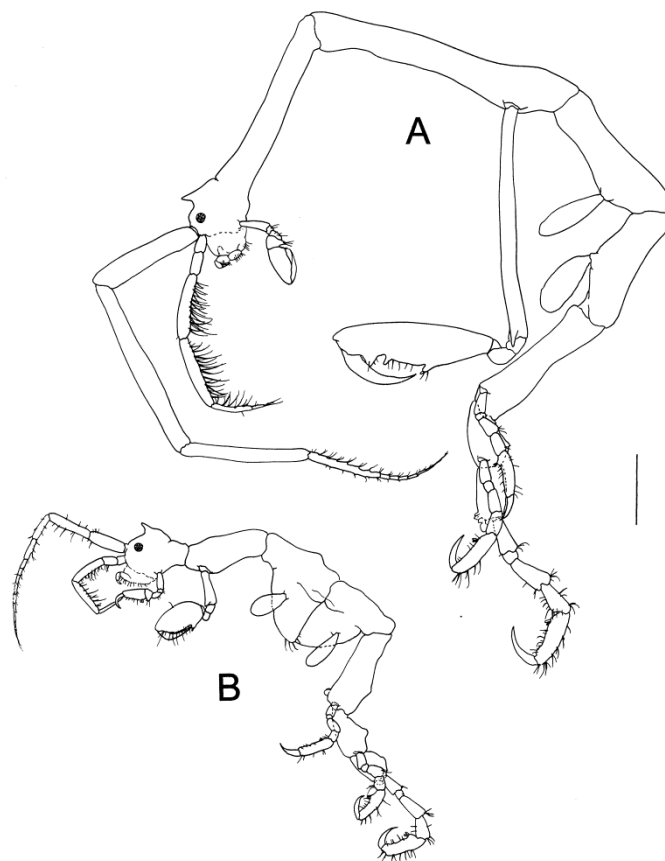
2.9 INTRODUCTION

Biological invasions are part of the anthropogenic global change and are considered one of the greatest threats to marine ecosystems (Ruiz *et al.* 2000, Occhipinti-Ambrogi 2000). The Mediterranean Sea is one of the areas in the world that is most affected by these invasions. Over 1000 introduced species have been recorded in this area, 159 of these correspond to alien crustaceans (Zenetos *et al.* 2012). The subphylum Crustacea includes the most successful species among aquatic alien invaders (Engelkes and Mills 2011). However, although the number of alien crustaceans has increased noticeably in the last two decades (Galil 2009), the number of alien species belonging to the amphipods is still underestimated (Zenetos *et al.* 2010). Caprellid amphipods, commonly known as skeleton shrimps, are small marine crustaceans that are common in many littoral habitats, where they form an important trophic link between primary producers and higher trophic levels (Woods 2009). The morphology of caprellids, with reduced appendages on the abdomen which are used for swimming in other amphipod crustaceans (Takeuchi and Sawamoto 1998), as well as the lack of a planktonic larval stage, suggest the possibility that cosmopolitan distribution of certain littoral caprellids could be facilitated by the fact that they are often associated with fouling communities on floating objects and vessels (Thiel *et al.* 2003, Astudillo *et al.* 2009). In other occasions, hidden diversity refutes cosmopolitan distribution in some caprellid species complexes traditionally considered as a single cosmopolitan species (Cabezas *et al.* 2013). This is particularly pronounced in smaller-bodied and taxonomically more challenging taxa (Carlton 2011).

In 1994, an unusual-looking caprellid characterized by an acute cephalic projection (Krapp *et al.* 2006), was found associated to the fouling community from the Lagoon of Venice, in Italy (Sconfiatti and Danesi 1996). This caprellid, identified as *Caprella scaura* Templeton, 1836 [sensu lato], was the first alien caprellid in the Mediterranean Sea. Since this record, the species was reported from several Mediterranean countries including Greece (Krapp *et al.* 2006), Spain (Martínez and Adarraga, 2008), Turkey (Bakir and Katagan 2011) and Tunisia (Ben Souissi *et al.* 2010). The species was frequently found associated with fouling communities of artificial structures such as boats, buoys, floating pontoons, aquaculture tanks or cages, but also with seagrasses and seaweeds in enclosed bays.

Caprella scaura [sensu lato] was described for the first time by Templeton in 1836 from individuals collected in Mauritius (Fig. 2.9), in the south Indian Ocean. About 50 years later, Mayer (1890, 1903) recognized 6 ‘formae’ (= varieties): *C. scaura* f. *typica* (1890), including the specimens described by Templeton and others found in Brazil and Australia, *C. s. f. diceros* (1890) from Japan, *C. s. f. cornuta* (1890) from Chile and Brazil, *C. s. f. spinirostris* (1890) from Chile, *C. s. f. californica* (1903) from California and *C. s. f. scauroides* (1903) from Hong Kong and Japan.

Figure 2.9 Lateral view of *Caprella scaura* collected from Mauritius. A, male; B, female. Scale bar: 1 mm (redrawn from Guerra-García, 2003).



According to recent rules of nomenclature, these varieties are now treated as subspecies (Krapp *et al.* 2006; Takeuchi and Oyamada 2013). Mayer (1903) grouped these subspecies in two separated groups, one for specimens without a ventral spine between the insertions of gnathopods 2, including *C. s. typica*, *C. s. diceros* and *C. s. cornuta*, and the other group for specimens with a ventral spine

between the gnathopods 2, including here the remaining subspecies. In 1947, Utinomi added a seventh subspecies without a ventral spine, *C. s. hamata*. Dougherty and Steinberg (1953) separated *C. s. californica* as a distinct species and reestablished Stimpson's (1857) name *C. californica* and McCain and Steinberg (1970) synonymized the other two subspecies with ventral spine, *C. s. scauroides* and *C. s. spinirostris*, with *C. californica* [sensu lato]. Recently, Takeuchi and Oyamada (2013) proposed *C. s. scauroides* to a species level based on differences with *C. californica* [sensu stricto] from California. In summary, at the present time, there are three valid species recognized: *C. scaura*, *C. californica* and *C. scauroides*.

Krapp *et al.* (2006) conducted a detailed revision of *C. scaura* species and found that Arimoto (1976) referred *C. scaura typica* as *Caprella scaura* (in part), probably considering that *C. scaura typica* described by Mayer was different from individuals of *C. scaura* described by Templeton, as it was pointed out by Krapp *et al.* (2006). These authors classified the individuals from Mauritius as *C. scaura scaura* to separate them from the subspecies *C. scaura typica*, according to currently valid taxonomical rules. Differences in both forms were mainly attributed to the dorsal tuberculation pattern in males. Krapp *et al.* (2006) concluded that the morphology of the Mediterranean populations of Italy and Greece matches with *C. scaura scaura*. However, differences between the subspecies *C. s. typica* and *C. s. scaura* are not clear and these authors also suggested that a more detailed observation of the growth stages in juveniles, adults and hyperadults is necessary to study the morphological variation within populations.

In the Iberian Peninsula *Caprella scaura* was recorded for the first time in 2005 by Martínez and Adarraga (2008) in Girona, located on the north east coast of Spain. These authors identified the Spanish population as *C. scaura scaura* and predicted, based on the high density of specimens found, a future invasion along the Iberian Mediterranean coast and adjacent areas. Only five years later, *C. scaura* was reported from two recreational marinas of southern Spain and one aquaculture tank in Tenerife (Canary islands, eastern Atlantic) (Guerra-García *et al.* 2011). However, there are many areas that remain unexplored and several questions that remain unresolved, for example:

1. Has *C. scaura* expanded throughout the Spanish Mediterranean coast and adjacent areas?
2. What is the northernmost distribution limit of *C. scaura* along the Atlantic coast of southern Europe?
3. Which ‘subspecies’ is invading the Mediterranean Sea?

In this chapter we study the current distribution of *C. scaura* in southern Europe and northern Africa and analyze its global distribution to address these questions and understand the invasion pattern of this species in its introduced range.

2.10 MATERIALS AND METHODS

A total of 88 marinas were surveyed along the whole Iberian Peninsula and North Africa during the summer of 2011. Additionally, 3 marinas in Italy, 1 in Corsica (France), 1 in Malta and 1 in Crete (Greece) were also surveyed in the summer of 2012 (Table 2.5). At each site, fouling communities proliferating on artificial hard substrata including floating pontoons, ropes, buoys and ship hulls were inspected for the presence of caprellids (Fig. 2.10). When caprellids were detected on a particular type of fouling substratum (hydroids, bryozoans or macroalgae), several colonies (at least three) of the host substratum were taken by hand and fixed in situ in 90% ethanol to examine the samples later in the laboratory for the presence of *Caprella scaura*.

The morphological characteristics used to identify *C. scaura* [sensu lato] males are (1) cephalon with an acute, bent forward, dorsal projection, (2) pereonites 1 and 2 elongate in males, (3) basis of gnathopod 2 long but no longer than pereonite 2 and (4) absence of ventral projection between the insertion of gnathopods 2 (adapted from Templeton 1836; Mayer 1890; Krapp *et al.* 2006). Additional morphological features were also explored to determine the possible intraspecific variation within non-native *C. scaura* populations. These features were also explored in specimens previously collected by the authors from Mauritius, the type locality of *C. scaura scaura* and Brazil, the type locality of *C. scaura typica*, to identify the subspecies which are being dispersed between marinas in the Mediterranean and the eastern Atlantic coast of Spain.

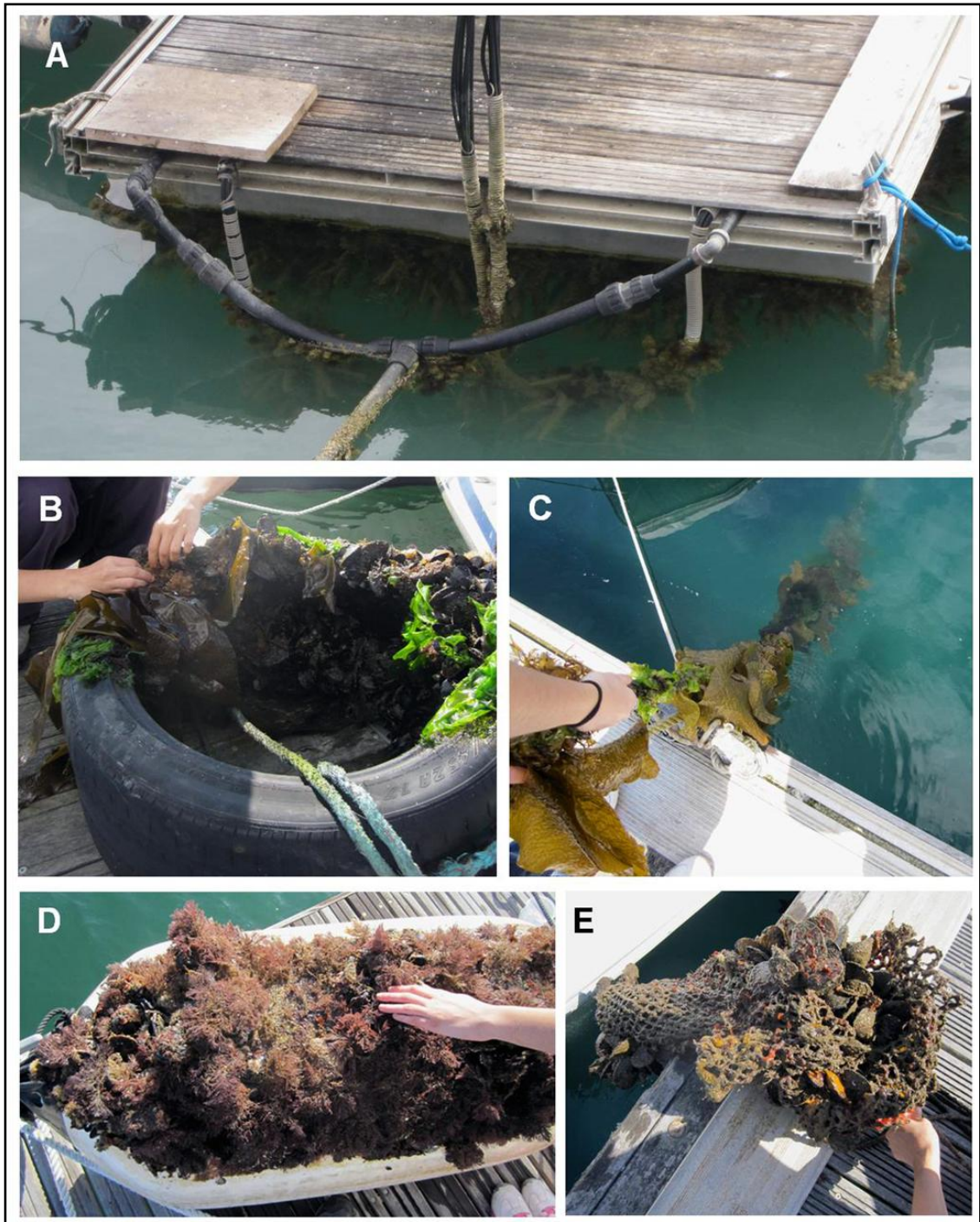


Figure 2.10 *Examples of artificial structures surveyed with their associated fouling organisms: A) floating pontoons; B) wheels; C) ropes; D) buoys; E) nets.*

Extensive study of published material on *C. scaura* records was also conducted to determine the current global distribution of this species. Descriptions, illustrations and photographs included in these publications were carefully analyzed to determine the particular subspecies of each record.

2.11 RESULTS

2.11.1 Distribution of *C. scaura* in southern Europe and northern Africa

Caprella scaura was present in thirty one marinas, being recorded for the first time on the islands of Corsica (France) and Crete (Greece), and in Morocco (Fig. 2.11, Table 2.5). The species was present in fourteen marinas distributed along the Mediterranean coast of the Iberian Peninsula and ten marinas of the Atlantic coast of the Iberian Peninsula. It was absent along the north Atlantic coast of Spain and the upper distribution limit on the eastern Atlantic coast is the locality of Cascais, on the south coast of Portugal.

As regards microhabitat use, it was found to be associated with the erect bryozoan *Bugula neritina* primarily, but also with other bryozoans such as *Zoobotryon verticillatum* and, along the southern Atlantic coast of Spain, with the invasive *Tricellaria inopinata* (Table 2.5). It was also occasionally found associated with the hydroids *Eudendrium* sp. and *Pennaria disticha*, and the seaweeds *Gelidium* sp., *Halopteris scoparia*, *Cladostephus spongiosus*, *Dictyota dichotoma* and *Corallina elongata*.

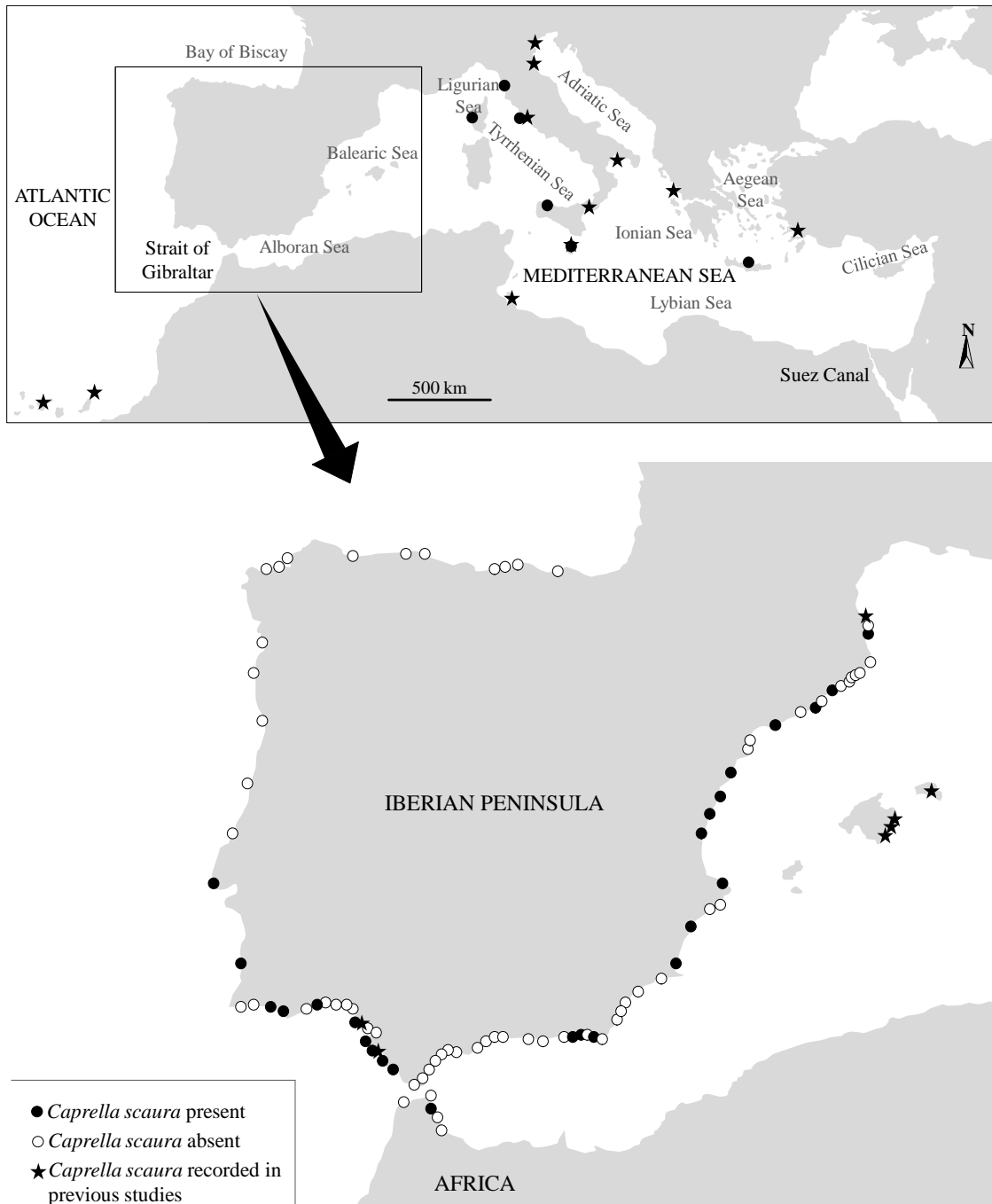


Figure 2.11 Location of marinas sampled for *C. scaura* in the Mediterranean Sea and the East Atlantic coast together with the records of *C. scaura* in the study area from previous studies (Canary Island and Southern Spain: Guerra-García et al. 2011, Lanzarote Island: Minchin et al. 2012; northeast coast of Spain: Martínez and Adarraga 2008; Balearic Islands: Ros et al. 2013a; Italy: Sconfiatti and Danesi 1996; Sacchi et al. 1998; Sconfiatti et al. 2005; Krapp et al. 2006; Galil 2008; Eleftheriou et al. 2011; Malta: Fernández et al. 2011; Greece: Krapp et al. 2006; Turkey: Bakir and Katagan 2011; Tunisia: Ben-Soussi et al. 2010).

Table 2.5 Presence/absence records of *Caprella scaura* from different fouling substrates collected in marinas from 2011 and 2012.

Country	Locality	Date	Coordinates	Substrates										
				BRYOZOANS			HYDROIDS		MACROALGAE					
				<i>Bugula neritina</i>	<i>Tricellaria inopinata</i>	<i>Zoobotryon verticillatum</i>	<i>Eudendrium</i> sp.	<i>Pennaria disticha</i>	<i>Gelidium</i> sp.	<i>Halopteris scoparia</i>	<i>Cladosthephus spongiosus</i>	<i>Dicliota dichotoma</i>	<i>Corallina elongata</i>	
PORTUGAL	Cascais	09-May-11	38° 41' N; 9° 26' W	✓										
	Sines	09-May-11	37° 57' N; 8° 51' W	✓										
	Albufeira	10-May-11	37° 05' N; 8° 15' W	✓										
	Faro	11-May-11	37° 00' N; 7° 56' W	✓		✓								
SPAIN	Isla Canela	16-May-11	37° 11' N; 7° 20' W	✓						✓				
	Chipiona	17-May-11	36° 44' N; 6° 25' W	✓	✓	✓								
	Rota	17-May-11	36° 36' N; 6° 21' W	✓	✓	✓								
	Cádiz	17-May-11	36° 32' N; 6° 17' W	✓	✓	✓								
	Conil	17-May-11	36° 17' N; 6° 08' W	✓	✓									
	Barbate	17-May-11	36° 11' N; 5° 56' W	✓	✓									
	Almerimar	01-Jul-11	36° 41' N; 2° 47' W	✓										
	Roquetas	01-Jul-11	36° 45' N; 2° 36' W	✓										
	Almería	01-Jul-11	36° 49' N; 2° 27' W	✓		✓								
	Torreveja	29-Jun-11	37° 58' N; 0° 41' W	✓										
	Alicante	29-Jun-11	38° 20' N; 0° 29' W	✓				✓						✓
	Denia	28-Jun-11	38° 50' N; 0° 6' W	✓		✓								✓
	Valencia	28-Jun-11	39° 25' N; 0° 19' W	✓		✓								
	Borriana	28-Jun-11	39° 51' N; 0° 4' W	✓										
	Oropesa	28-Jun-11	40° 04' N; 0° 8' E	✓										
	Benicarló	27-Jun-11	40° 24' N; 0° 26' E	✓		✓								
	Tarragona	27-Jun-11	41° 06' N; 1° 15' E	✓						✓	✓	✓		
	Vilanova	27-Jun-11	41° 12' N; 1° 43' E	✓		✓								
Barcelona	26-Jun-11	41° 22' N; 2° 10' E	✓		✓									
L'Estartit	25-Jun-11	42° 03' N; 3° 12' E	✓		✓									
MOROCCO ^a	Marina Smir	30-May-11	35° 45' N; 5° 20' W	✓		✓								
ITALY	Livorno	30-May-12	43° 32' N; 10° 18' E	✓										
	Civitavecchia	29-May-12	42° 05' N; 11° 47' E	✓										
	Palermo	08-Oct-11	38° 08' N; 13° 22' E	✓		✓								
FRANCE ^a	Ajaccio	31-May-12	41° 55' N; 8° 44' E	✓			✓							
MALTA	Gzira	09-Jul-12	35° 54' N; 14° 29' E	✓		✓								
GREECE	Heraklion	18-May-12	35° 20' N; 25° 08' E	✓										

^aFirst record of *Caprella scaura* in this country

2.11.2 Intraspecific morphological variation within introduced populations

The specimens collected in the study area share the same morphology. This morphology was similar to other specimens collected by the authors in previous studies from the Canary Islands (Guerra-García *et al.* 2011) and the Balearic Islands (Ros *et al.* 2013a). However, intraspecific morphological variations, affecting the dorsal tuberculation pattern of males and females mainly, were usually found within *C. scaura* populations collected from the same locality. These variations may have some implications in the identification of the subspecies, variety or form that is spreading along the study area.

In the case of males, the dorsal pattern of tuberculation on pereonites 1-3 and especially on pereonite 5 exhibited the highest variation. The males with the most pronounced dorsal body protuberance pattern had a small distal dorsal protuberance or tubercle at the end of the pereonites 1-3, close to the junction with the adjacent pereonite, specially visible on pereonites 2 and 3 (Fig. 2.12 B and C), and two pairs of tubercles on pereonite 5 (Fig. 2.12 D). Pereonite 6 used to present a pair of knobs but in hyperadults another pair of knobs may be present. Protuberances on pereonites 1-3 were scarcely present and were only found in some small males (Fig. 2.12 A-C), with the majority of the males being dorsally smooth on these pereonites (Fig. 2.12 E-G). However, protuberances on pereonite 5 were frequently found. These tubercles gradually disappear as the male increases in length, but some hyperadult males may also have one or two pairs of tubercles on this pereonite (Fig. 2.12 H-J). The length of the cephalic projection is also variable but in general is short and acute.

The females with the most pronounced dorsal body protuberance pattern had a protuberance at the end of pereonites 1-4, a pair of protuberances in the middle of pereonite 2 and 6, a central projection on pereonites 3 and 4, and two pairs of protuberances in the middle of pereonite 5 (Fig. 2.13 A-E). These 'spiny' females coexist with smooth ones (Fig. 2.13 F), but the two pairs of tubercles on pereonite 5 are usually present in all of them. The same morphological variations in the dorsal protuberance pattern of males and females were also observed in individuals collected previously from Mauritius and Brazil. Interestingly, in all cases, pereonite 4 of males were dorsally smooth.

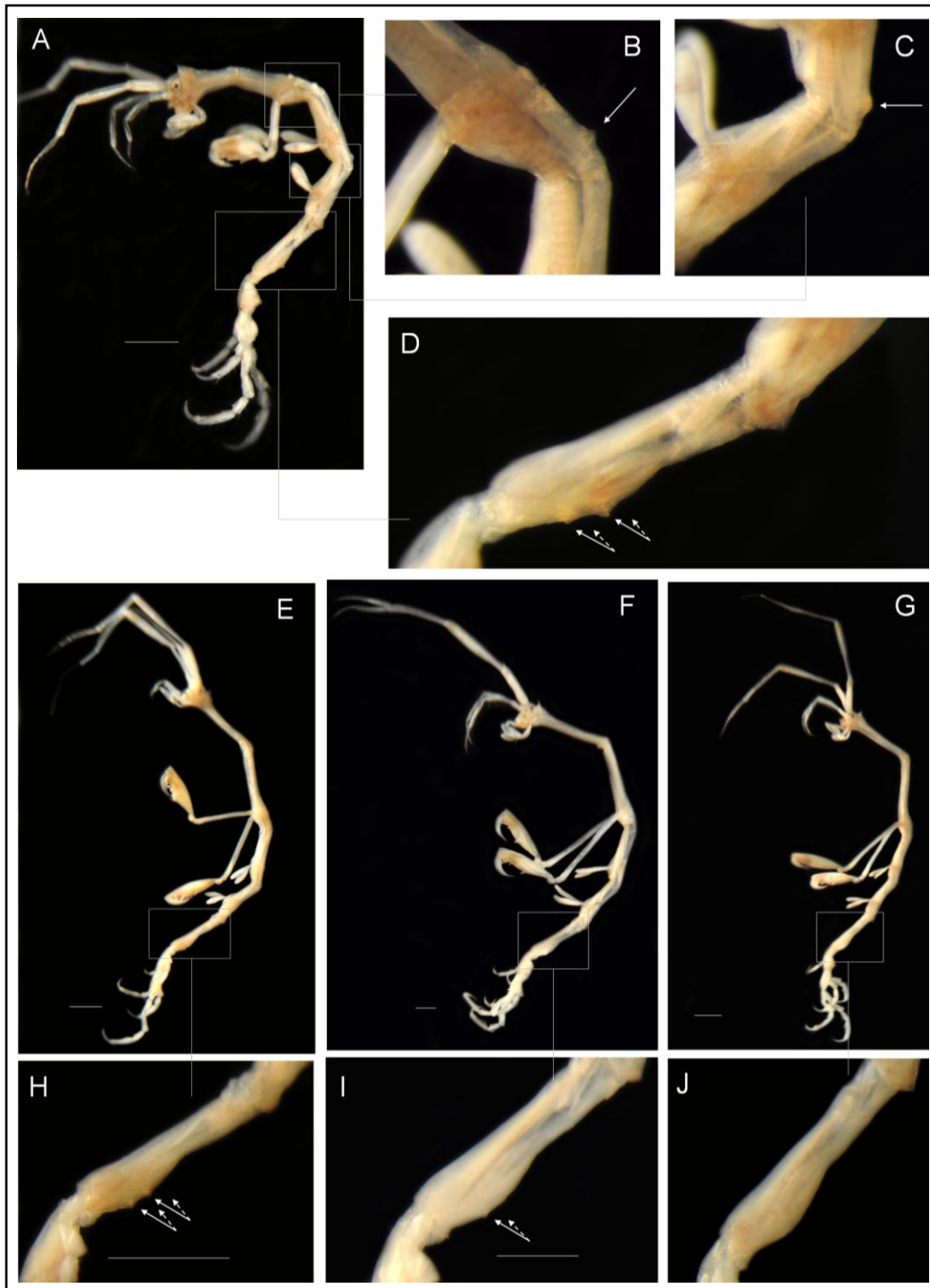


Figure 2.12 Intraspecific variations found in the dorsal tubercles of *C. scaura* males. A) Small male with a marked dorsal protuberance pattern. B-D) Detail of tubercles of pereonite 2, 3 and 5 respectively. E-G) Hyperadult males with different pattern of tubercles in pereonite 5. H-J) Variations in tubercles of pereonite 5. Dotted arrows show the presence of another tubercle, not visible in the photograph, which is part of the pair of tubercles. All individuals belong to the same locality: Cádiz, Spain. Scale bars: 1mm.

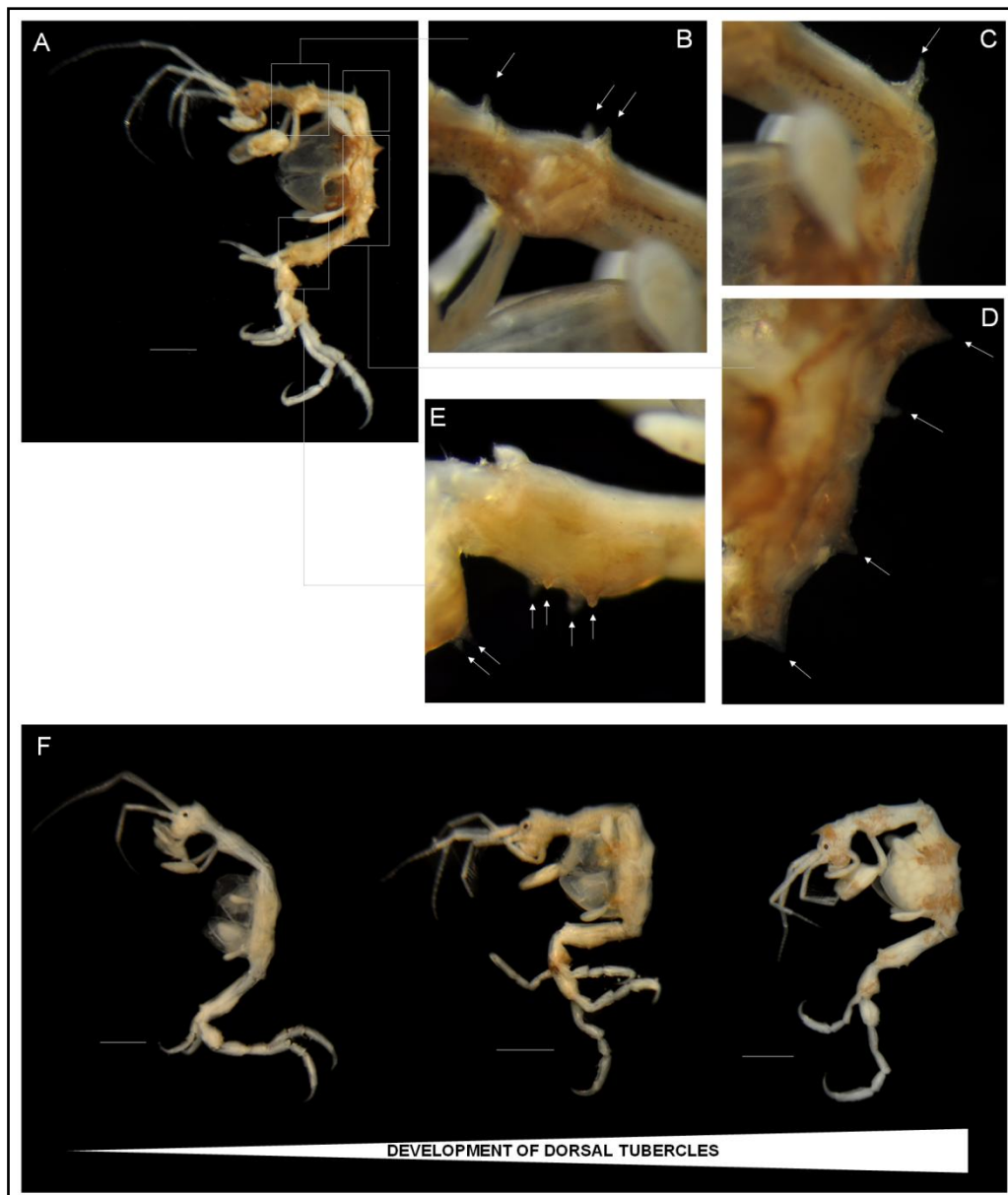


Figure 2.13 *Intraspecific variations found in the dorsal tubercles of C. scaura females.* A) Female with a marked dorsal protuberance pattern. B-E) Detail of tubercles of pereonites 1-6. F) Variations in the development of the dorsal protuberance pattern. All individuals belong to the same locality: Cádiz, Spain. Scale bars: 1mm.

A comparative analysis was carried out between the different subspecies of *C. scaura* according to these evidences (Table 2.6). We consider that the ‘form’ or ‘forms’ that better match the subspecies that are present in the marinas of southern Europe and northern Africa are *C. scaura scaura* from Mauritius, but also *C. scaura typica* from Rio de Janeiro, Brazil.

Table 2.6 Comparison of different morphological characters traditionally used to distinguish the different forms of *Caprella scaura*. The two subspecies that match with the specimens collected in the present study are highlighted in grey.

	<i>C. scaura scaura</i>	<i>C. scaura typica</i>	<i>C. scaura cornuta</i>	<i>C. scaura diceros</i>	<i>C. scaura hamata</i>	<i>C. scaura collected</i>
Type locality	Mauritius	Brazil	Brazil	Japan	Japan	
Description references	Templeton (1836)	Mayer (1890; 1903)	Dana (1853) Mayer (1890; 1903)	Stebbing (1888); Mayer (1890)	Utinomi (1947) in Arimoto (1976)	
Maximum male body length (mm)	14	21	18	35	15	23
Cephalic projection	Short, acute	Short, acute	Short, blunt	Long, acute	Long, acute	Short, acute
Flagellum antenna 1	Presence of fused articles	Presence of fused articles	Absence of fused articles	Presence of fused articles	Presence of fused articles	Presence of fused articles
Pereonite 4	Smooth	Smooth	Long, oblong, distally process in some males	Lateral tubercles and distally strong process	Dorsal and lateral tubercles and distally strong process	Smooth
Pereonite 5	"Spiny"; two pairs of dorsal processes in small males which disappear in hyper adult males (based on our examination of specimens from Mauritius)	Two pairs of dorsal processes occasionally present in males (McCain, 1968)	?	Two pairs of dorsal processes in males	Two pairs of dorsal processes in males	Two pairs of dorsal processes in males which gradually disappear in hyper adult stages

2.12 DISCUSSION

Caprella scaura was present in 33 percent of sampled marinas. This represents a successful spread of a species, which was found for the first time in the Mediterranean coastal areas just two decades before the current study, i.e. in 1994 (Sconfiatti and Danesi 1996).

2.12.1 Which ‘subspecies’ is spreading in the Mediterranean and the eastern Atlantic coastal area? A morphological and biogeographical approach

Although *C. scaura* comprises a complex of several forms or subspecies, all populations spreading in the Mediterranean and the eastern Atlantic coastal area share the same morphology. This morphology includes a number of intraspecific variations in characters that are traditionally used to distinguish two forms, *C. scaura typica* and *C. scaura scaura*. In particular, the presence of two pairs of tubercles on pereonite 5 was attributed to the form ‘*typica*’ while its absence to the form ‘*scaura*’ (see Krapp *et al.* 2006). The degree of tuberculation in females was also used to differentiate between the two forms, with the ‘extremely spinous’ females belonging to the form *C. s. typica* (Martinez and Adarraga 2008). As we found that these characters vary within the same population, both in the specimens collected from the study area and in the specimens examined from Mauritius and Brazil, we consider that this feature is not a good and stable taxonomical character to differentiate subspecies.

Based on this premise, both *C. scaura typica* and *C. scaura scaura* match quite well with the material collected and may correspond to the same subspecies, the subspecies which is spreading in the Mediterranean and the eastern Atlantic coastal area. This also suggests that the remaining subspecies, with a different morphology and a restricted distribution range (Fig. 2.14, Table 2.6), could correspond to different valid species, as has already happened with other ‘forms’ described by Mayer (e.g. Cabezas *et al.* 2012, Takeuchi and Oyamada 2013).

The form ‘*typica*’ has also been reported as the variety which was introduced along the east and west coast of North America (Marelli 1982; Foster *et al.* 2004) and Hawaii (Edmonson and Mansfield 1948). However, a molecular approach is necessary to confirm if there is only one form that is spreading by

human-mediated between different areas in the world and if the remaining forms correspond to different species with a restricted distribution.

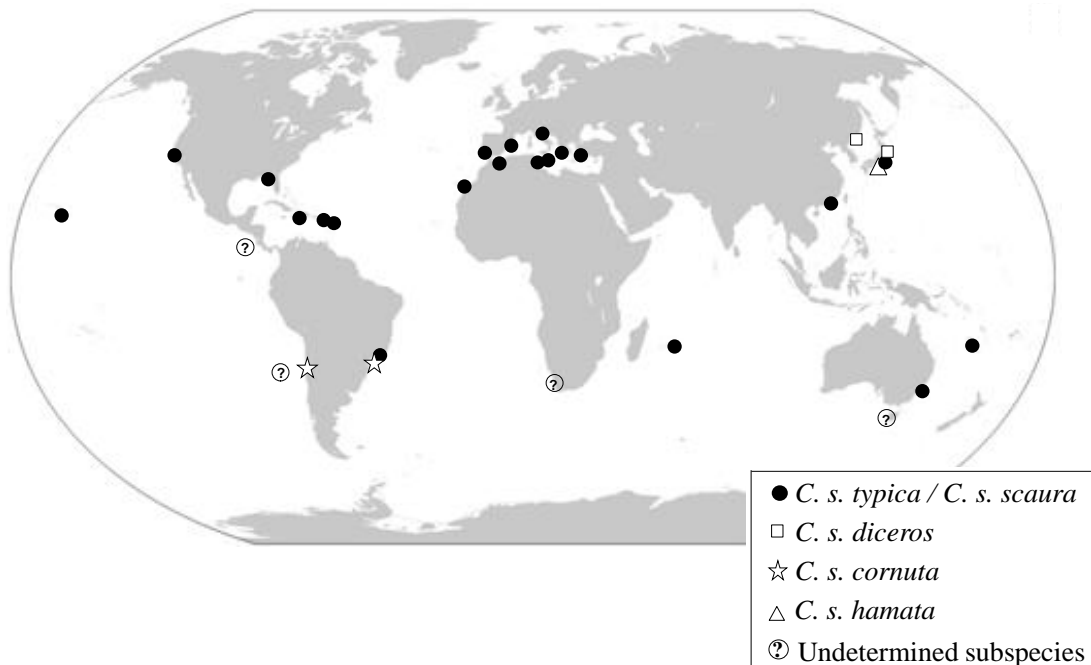


Figure 2.14 Distribution of the different subspecies of *C. scaura* without ventral spine in each region considered in Table 3. *Caprella s. typica* and *C. s. scaura* are considered as the same subspecies (see text). References in Tables 2.5 and 2.6.

2.12.2 Invasion pattern of *C. scaura* and worldwide distribution

Taking into account that specimens collected in the study area match quite well with *C. scaura typica* and *C. scaura scaura*, in order to understand the invasion pattern of *C. scaura* it is necessary analyze the worldwide distribution of both subspecies (Figure 2.14, Table 2.7).

The origin of *Caprella scaura* is unknown (Carlton and Eldredge 2009), as it occurs with other non-native species associated with fouling communities of artificial structures such as harbours and marinas. Records on geographically disjunct occurrences of *C. scaura* date back to when the species was described for the first time in Mauritius. Only two years later, the species was found in Brazil and a short time later it was found in the Caribbean Sea, Australia and Japan (references in Table 2.7). Thus, we consider that the species cannot be

demonstrably classified as native or non-native in these regions, and it must be referred to as cryptogenic (Carlton 1996). Therefore, the potential native range for *C. scaura* may include, at least, the regions of Mauritius, Brazil, Caribbean Sea, Australia and Japan.

In the early twentieth century, the species was introduced into Pearl Harbour, Hawaii, by ship fouling and/or ballast water (Carlton and Eldredge 2009). At the end of the twentieth century the species was found on the west coast of USA and a short time later on the east coast of this country. On both occasions, *C. scaura* was found associated with fouling communities in harbors, thus supporting the human-mediated introduction of the species. In 1994, it was found for the first time in the Mediterranean Sea, in Italy. Since then, *C. scaura* has spread very fast to several regions of the Mediterranean Sea and the east Atlantic Ocean.

According to our results, *C. scaura* is now widely distributed along the Mediterranean coast of the Iberian Peninsula and along the Portuguese coast, with Cascais on in the south coast of Portugal being its northernmost distribution limit.

So far its occurrence in the study area had been only reported by Martínez and Adarraga (2008) on the northeastern coast of Spain, and by Guerra-García et al. (2011) in southern Spain and the Canary Islands. As regards the Portuguese coast, there is a dubious record of a single female with a cephalic projection found by Marques and Bellan-Santini (1985) in Sines, which was classified as *Caprella cf. scaura*. However, it was found associated with algae in a natural habitat and since then, the species has not been found in Portugal until the present study. It is probable that this female corresponds with an specimen of *C. santosrosai*, a caprellid species described recently by Sánchez-Moyano et al. (1995) with a female morphology similar to that of the female of *C. scaura*. Confirmation of this report is essential to understand the most likely entry of *C. scaura* in the Mediterranean Sea. If the species was first present on the eastern Atlantic coast of the Iberian Peninsula and then in the Mediterranean, it probably entered the Mediterranean through the Strait of Gibraltar. But if the species was first present in the eastern sector of the Mediterranean, it probably entered through Suez Canal. The impossibility to review the material prevents clarification of this doubt.

Table 2.7 First records of *Caprella scaura typica* and *C. s. scaura* in each region including date of reporting, site of collection (if known), status in this region and possible mechanism of introduction. Status: C - cryptogenic; I - introduced; Q - questionable. Likely vectors: SF - ship fouling; BW - ballast water; AQ - aquaculture.

Date	Country	Localities	Collected from	Status	Vector	Author of record
1836	Mauritius	Rivière Noire	Marine Plants	C		Templeton, 1836
1838	Brazil	Rio de Janeiro	Seaweeds near the fort	C		Dana, 1853 (as <i>C. attenuata</i>)
1866	Virgin Islands	Barthelemy and St. Croix	Unknown	C		Mayer, 1903
1890	Australia	Sydney	Port Jackson	C		Mayer, 1890
1903	Japan	Tokyo Bay	Unknown	C		Mayer, 1903
1925*	South Africa	Mialagass Island (Saldanha Bay)	Sea Urchin	Q		Barnard, 1925
1929	Hawaii	Pearl harbour (Honolulu), Oahu	Algae in shallow water in Pearl Harbour; hull fouling organism	I	SF	Edmonds and Mansfield, 1948
1968	Costa Rica	Cocos island	Unknown	C		McCain, 1968
1968	Puerto Rico	Mayagüez	Unknown	C		McCain, 1968
1970*	Robinson Island (Chile)	Cumberland Bay	Unknown	Q		McCain & Steinberg, 1970
1978	USA (west coast)	San Francisco Bay, Elkhourn Sloug (California)	Steam electric power plant and yacht harbor	I	SF, BW	Marelli, 1981
1978*	Tasmania (Australia)	Fancy Point	Sargassum	Q		Guerra-García & Takeuchi, 2004
1986	China	Mirs Bay (Hong Kong)	Algal bed (14-16m deep), benthic trawl	C		Guerra-García & Takeuchi, 2003
1989	New Caledonia	Citrons Bay	Unknown (5m deep)	C		Laubitz, 1991
1994	Italy	Venice Lagoon	Wooden piles	I	SF, BW	Danesi <i>et al.</i> , 1999
1998	USA (east coast)	St. Andrew Bay (Florida) Charleston harbour (South Carolina)	Fouling organism on the jetties and channel markers	I	SF, BW	Foster <i>et al.</i> , 2004

-Cont. Table 2.7-

2002	Greece	Amvrakikos gulf	Seagrass	I	SF	Krapp <i>et al.</i> , 2006 (as <i>C. scaura scaura</i>)
2005	Spain	Roses Bay (Girona)	Fouling organisms including: bryozoans (<i>B. neritina</i>), mussels (<i>Mytilus galloprovincialis</i>) and polychaetes (<i>Ficopomatus enigmaticus</i>)	I	SF	Martinez & Adarraga, 2008 (as <i>C. s. scaura</i>)
2008	Turkey	Güllük Bay	Cages of a fish farm	I	AQ	Bakir & Katagan, 2011
2009	Tunisia	Boughrara and Bibans	Seaweeds	I	SF	Ben Souissi <i>et al.</i> , 2010
2010	Canary Islands (Spain)	Tenerife	Aquaculture tanks	I	AQ	Guerra-García <i>et al.</i> , 2011
2010	Malta	Marina Smir	Cages of a fish farm offshore	I	AQ	Fernandez <i>et al.</i> , in press
2011	Morocco	Marina Smir	Fouling bryozoans including <i>B. neritina</i> and <i>Zoobotryon verticillatum</i>	I	SF	Present study
2011	Portugal	Marinas of Cascais, Sines, Albufeira and Faro	Fouling bryozoans including <i>B. neritina</i> and <i>Z. verticillatum</i>	I	SF	Present study
2012	Corsica (France)	Ajaccio Marina	<i>B. neritina</i> and <i>Eudendrium</i> sp.	I	SF	Present study
2012	Crete (Greece)	Heraklion Marina	<i>B. neritina</i>	I	SF	Present study

* Undetermined subspecies of *C. scaura* without a ventral spine.

The presence of an established population in Morocco, Corsica (France), Crete (Greece) and the coastal waters of Malta [previously found by Fernandez-Gonzalez and Sanchez-Jerez (2014) in an offshore fish farm] confirm a rapid spreading of *C. scaura* between marinas of the Mediterranean. The expansion success of this non-native caprellid may be due to the fact that it is usually associated with the bryozoan *Bugula neritina*, in which it exhibits the highest abundance (Ros *et al.* 2013b). This bryozoan is tolerant to antifouling paints (Piola and Johnston 2006) and is able to attach easily to ship hulls, even if they are covered with fresh antifouling paints. The colonies of *B. neritina* with its associated epifaunal community are then easily transported among marinas by recreational boats, or by detached buoys or other structures (Astudillo *et al.* 2009).

However, *C. scaura* was absent on the north coast of Portugal and the north Atlantic coast of Spain, where water temperatures are lower than in the Mediterranean Sea and the Strait of Gibraltar. An analysis of the worldwide distribution of *C. scaura typica* and *C. scaura scaura* (Figure 2.14) shows that the species is also absent in the Red Sea and the northern Indian Ocean. This fact, together with the possible presence of a female of *C. scaura* on the central coast of Portugal in 1985, complicate our understanding of the introduction process of *C. scaura* in the Mediterranean Sea. Further ecological, morphological and molecular analyses are still necessary to resolve this interesting question.

In conclusion, this study reveals an important post-introduction range expansion of *C. scaura* along the Mediterranean Sea and along the South Atlantic coast of the Iberian Peninsula, representing significant improvement of the invasion dynamics of this caprellid species. Taking into account the intraspecific morphologic variations observed within populations, we also conclude that the form *C. scaura typica* and *C. s. scaura* correspond with the same variety, thus contributing to a clarification of the *C. scaura* complex and suggesting that a single form of the complex is expanding its distribution range, while the other forms remain in a restricted distribution area.

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3

ESTABLISHMENT

Influence of fouling communities on the establishment success of alien caprellids in Southern Spain

- Adapted from:

Ros M, Guerra-García JM, González-Macías M, Saavedra A, López-Fe CM (2013) Influence of fouling communities on the establishment success of alien caprellids (Crustacea: Amphipoda) in Southern Spain. *Marine Biology Research* 9: 293–305.

ABSTRACT. Recently, two non-native caprellid amphipods, *Caprella scaura* and *Paracaprella pusilla*, were found for the first time in southern Spain. In order to assess their establishment success, monitoring of the caprellid fauna associated to the marina's fouling community was carried out from summer 2010 to spring 2011 in Cádiz marina, southern Spain. Three caprellid species were found, the non-native *C. scaura* and *P. pusilla*, and the native *Caprella equilibra*. *Caprella scaura* was the dominant species with ovigerous females and juveniles throughout the whole study period, reflecting both that it reproduces all year around and that it is able to support the seasonal variations measured in water temperature, salinity and turbidity. It showed a high ability to colonize different fouling substrates, although it was significantly more abundant in the bryozoan *Bugula neritina*. *Paracaprella pusilla* was found in less abundance than *C. scaura* as it only was present in summer, associated exclusively with the hydroid *Eudendrium racemosum*. The native *C. equilibra*, very common in other nearby harbors, was scarcely represented in the study area. The results suggest first that *P. pusilla* is an introduced species with less invasiveness than *C. scaura*, and second, that fouling bryozoans, especially *B. neritina*, are suitable habitat for the establishment success of *C. scaura*, while fouling hydroids, particularly *E. racemosum*, are more suitable for the establishment success of *P. pusilla*.

RESUMEN. Recientemente, dos caprélidos no-nativos, *Caprella scaura* y *Paracaprella pusilla*, fueron detectados por primera vez en el sur de España. Con el objetivo de evaluar su establecimiento, se llevó a cabo una monitorización de la fauna de caprélidos asociada a las comunidades incrustantes del puerto deportivo de Cádiz entre el verano de 2010 y la primavera de 2011. Se encontraron tres especies: *C. scaura*, *P. pusilla* y la nativa *Caprella equilibra*. *Caprella scaura* fue la especie dominante, con hembras ovígeras y juveniles durante todo el periodo de estudio, reflejando tanto una reproducción continua a lo largo del año como la capacidad para soportar las diferencias estacionales medidas en la temperatura del agua, la salinidad y la turbidez. Esta especie mostró una gran capacidad para colonizar diferentes sustratos, aunque fue significativamente más abundante en el briozoo *Bugula neritina*. *Paracaprella pusilla* fue menos abundante que *C. scaura*, apareciendo únicamente en verano y asociada exclusivamente al hidrozoo *Eudendrium racemosum*. *Caprella equilibra*, muy común en puertos cercanos, apareció tan sólo de forma ocasional. Estos resultados sugieren que *P. pusilla* es una especie introducida con un potencial invasor menor que *C. scaura*, y que los briozoos incrustantes, especialmente *B. neritina*, son un hábitat adecuado para el establecimiento de *C. scaura*, mientras que los hidrozooos incrustantes, especialmente *E. racemosum*, son más adecuados para el establecimiento de *P. pusilla*.

3.1 INTRODUCTION

Marine bioinvasions are one of the greatest threats from human activity in the ocean (Carlton 1996a). Watercraft, both commercial and recreational, are the main vectors for the introduction and distribution of invasive species, due to encrustation and subsequent dispersal from hulls, or from ballast (Johnson *et al.* 2001; Gollasch 2002; Floerl and Inglis 2005; Somaio *et al.* 2007).

There are many terms to define non-native species such as alien, Non-indigenous species (NIS), Alien Introduced Species (AIS), exotic or allochthonous. All of these are used to define species which are introduced outside of their natural, or historically known, range of distribution and have been transported, either intentionally or accidentally, into a new area. Species with a self-sustaining population outside of its native range are classified as established (Kolar and Lodge 2001). Invasive species are the subset of introduced species that persist, reproduce, and spread rapidly into new locations, causing economic or ecosystem damage or harm to human health (Williams and Smith 2007). Species that are not demonstrably native or introduced are defined as 'cryptogenic' (Carlton 1996b).

NIS transported by international ships must find adequate substrates on which to live where they are introduced and the urban structures associated with ports and marinas may frequently provide these substrates (Bulleri and Arioldi 2005; Glasby *et al.* 2007; Rocha *et al.* 2010). Therefore, marinas can be both the first entry point for non-natives via international yachts and they can act to provide a network of suitable habitats for the secondary spread of a species via domestic yachting activity (Ashton *et al.* 2006a). It is the local, secondary spread that will ultimately determine the extent of the economic and environmental impact of a non-native species (Lodge *et al.* 1998; Ashton *et al.* 2006b). At marinas, the immersed parts of the floating pontoons are not usually treated with antifouling coatings and so can develop extensive plant and invertebrate growths (Minchin 2007). These structures are colonized first by non-native species and act as stepping stones for the colonization of natural habitats (Cangussu *et al.* 2010). Accordingly, floating structures are highly relevant for detecting newly arrived non-native species amongst the sessile biota (Connell 2001; Wasson *et al.* 2001; Lambert and Lambert 2003; Bulleri and Airoidi 2005; Arenas *et al.* 2006). Floating pontoon fouling communities include arborescent substrates such as

bryozoans or hydroids, which may act as reservoirs of introduced species, particularly in the case of small invertebrates, such as caprellid amphipods, which are well adapted to clinging to these types of substrates (e.g. Caine 1978; Takeuchi and Hirano 1995) and on which they spent their entire life as epibionts.

Caprellid amphipods, commonly known as skeleton shrimps, are small marine crustaceans that are common in many littoral habitats, where they form an important trophic link between primary producers and higher trophic levels (Woods 2009). The morphology of caprellids, with reduced appendages on the abdomen which are used for swimming in other amphipod crustaceans (Takeuchi and Sawamoto 1998), as well as the lack of a planktonic larval stage, suggests the possibility that cosmopolitan distribution of many littoral caprellids appears to be facilitated by the fact that they are often associated with fouling communities on floating objects and vessels (Thiel *et al.* 2003). Recently, two alien caprellid species were found for the first time on the Spanish coast: *Caprella scaura* Templeton, 1836, first recorded in the northeastern coast of Spain in 2005 (Martinez and Adarraga 2008) and in the southern Spain in 2010 (Guerra-García *et al.* 2011) and *Paracaprella pusilla* Mayer, 1890, recorded for the first time in European waters in 2010 in the south coast of Spain (Ros and Guerra-García 2012).

Under favourable conditions, populations of some caprellid species can reach high densities, although there may be considerable temporal and spatial variation depending upon a range of biophysical factors, such as temperature and food supply (Woods 2009; Ashton *et al.* 2010). Caprellid population cycles have also been shown to be positively correlated with seasonal biomass cycles of the biotic host substrate that they inhabit (e.g. Bavestrello *et al.* 1996; Guerra-García *et al.* 2000). Accordingly, detectability of introduced caprellid species may depend on the temporal scale of the study. In Spain, although the interest in the topic of non-natives is increasing, the number of studies conducted is very scarce, especially in the case of small invertebrates like caprellids amphipods, and most of these focus on seaweeds of the Mediterranean coast (ITCES WGITMO report 2009). In fact, there is no study about the evolution of the two non-native caprellid populations recently found in Spain.

In order to provide baseline information about control and monitoring of non-native caprellid populations in southern Spain we compared the abundance

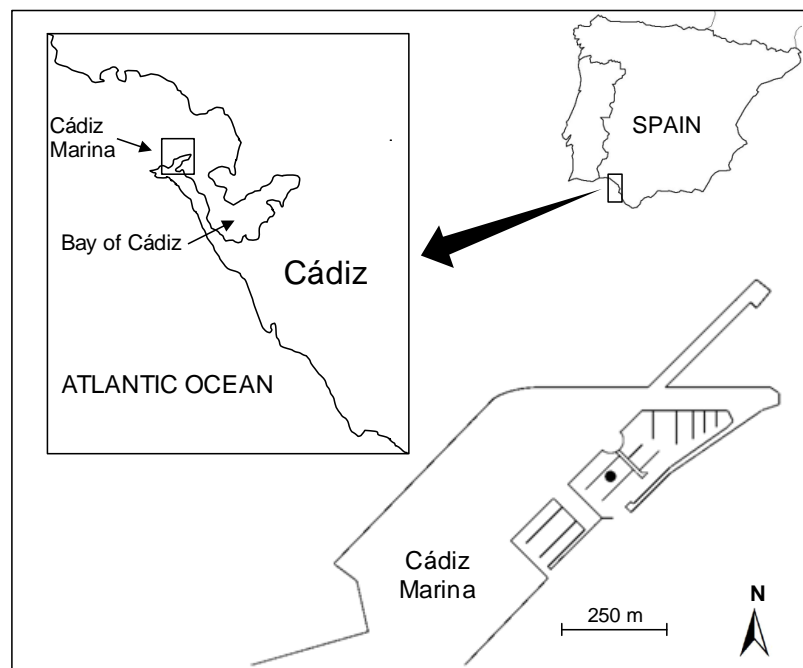
patterns of caprellids inhabiting in the Cádiz marina's fouling community both between surveys (one in each season) and between different fouling substrates. The specific aims of our study were: (1) detection of native and non-native caprellids in the study area, (2) determining the importance of temporal variation of the environmental conditions and the type of host substrate in the establishment success of non-native caprellid populations in southern Spain, and (3) assessment of the current status and the invasive potential of the non-native caprellid found in the study area.

3.2 MATERIALS AND METHODS

3.2.1 Sampling area and field procedure

The study was carried out in a recreational boating marina, Puerto América Cádiz Marina, located on the Atlantic coast of southern Spain ($36^{\circ}32'29''\text{N}$, $6^{\circ}17'61''\text{W}$) (Fig. 3.1). Four surveys were conducted (one in each season) in summer (3 September 2010), autumn (1 December 2010), winter (30 January 2011) and spring (28 March 2011).

Figure 3.1 Map of Cádiz Marina showing location on the South coast of Spain and the position of sampling site.



The following environmental parameters were measured *in situ* for each season: water temperature, pH, conductivity, dissolved oxygen and turbidity. Three haphazard measurements were made for each parameter across the floating pontoon system and mean values and standard deviation were calculated. Conductivity and pH were measured using a conductivity and pH meter CRISON MM40, temperature and oxygen concentration with an oxymeter CRISON OXI 45P, and turbidity in nephelometric turbidity units (ntu) using a turbidimeter WTW 335 IR.

During each survey we examined carefully the total fouling community for the presence of caprellid species and we only found caprellids associated with four species of the fouling community, which we have called ‘host substrate’: the bryozoans *Bugula neritina* (Linnaeus 1758), *Tricellaria inopinata* D’Hont and Occhipinti Ambrogi 1985, *Zoobotryon verticillatum* (Delle Chiaje 1828) and the hydrozoan *Eudendrium racemosum* (Cavolini 1785). Three replicates per substrate were sampled in each season. Each replicate consisted of a well developed colony of each substrate type separated by tens of meters. All samples were collected from the lateral surface of the floating pontoon, close to the water surface, directly removed by hand and preserved in 80% ethanol. Floating pontoons consisted of plastic material with a wooden boardwalk. To estimate the frequency of appearance of the dominant sessile species in the fouling community of the study area, twenty haphazard quadrats of 20 x 20 cm were used along the pontoon system. Each quadrat was placed in the lateral surface of the pontoon in contact with the water, perpendicular to the water surface. Species presence/absence in each quadrat was recorded and data were expressed as percentage frequency, which is a measure of how often a species occurs in quadrat. If a species was found in every quadrat sampled, it had a frequency of 100%. When a species only appear in one quadrat, it had a frequency of 5%. In the case where a species could not be identified ‘in situ’, a specimen of it was fixed in 80% ethanol and was taken to the laboratory for identification.

3.2.2 Laboratory processing

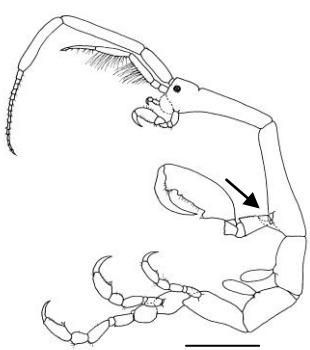
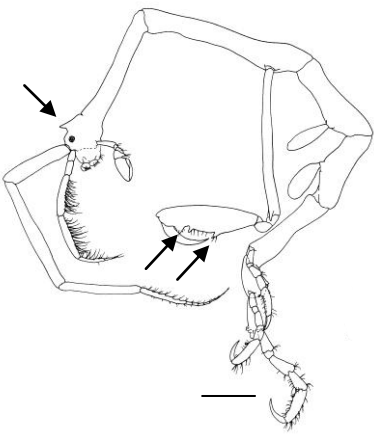
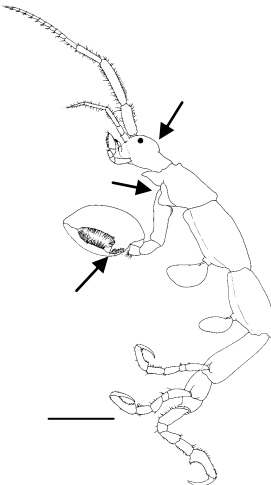
To estimate the complexity of arborescent substrate we measured the sediment retained by the substrate branches (Dubiascki-Silva and Masunari 1995). For this purpose, the sediment retained by each substrate replicate was dislodged

completely by agitating the colony of substrate in the alcohol in which it was fixed in the field. This solution was sieved using a mesh size of 0.1 mm to retain the associated macrofauna (including caprellid amphipods), even the smaller organisms (methodology modified from Satyanarayana and Ganapati 1980). This alcohol solution was then desiccated at 80° C for 48 hours. Moreover, volume of substrates was estimated as the difference between the initial and final volume when placed into a graduated narrow cylinder with a fixed amount of water, hence the sediment retained by each substrate could be expressed as grams dry weight per volume of substrate. Sediment retained may also influence in the caprellid density because marine sediment rich in organic matter attracts a large number of deposit and detritus feeders, such as amphipods among other groups (Sarma and Ganapati 1970) and may hence act as a source of food for caprellids, since they are mainly detritivores (Guerra-García and Tierno de Figueroa 2009). Furthermore, all caprellids were sorted both from the sieved alcohol solution previously mentioned and directly from the substrata branches, to ensure the collection of all individuals per sample. The specimens collected were then identified to species level under a binocular microscope and classified as native or non-native in the study area. Table 3.1 shows diagnostic characteristics used to differentiate adult males of native¹ and non-native species found. The abundance of caprellids was expressed as the number of individuals/ml of substrate because of the different structures of the substrate species (Pereira *et al.* 2006; Guerra-García *et al.* 2010).

Subsequently, all individuals of the non-native *Caprella scaura* collected, were photographed on stereomicroscope Motic K-400L with a Nikon D90 digital camera and body length were measured from the front of the head to the end of pereonite 7, using the PC-based digitizing software Scion Image Alpha 4.0.3.2 © (2000-2001 Scion Corporation). Number of adult males, mature females and juveniles per volume were determined for each sample. The criterion for determining the sex/age group of the specimens was determined according to visible signs of maturity or prematurity, respectively (Table 3.2). Characteristics used were relatively similar to those used for other species of the genus *Caprella* (see Laubitz 1970, Arimoto 1976, Boss 2009, Ashton *et al.* 2010).

¹ *Caprella equilibra* has been considered native in this chapter because it has been established in the region for hundreds of years (McCain and Steinberg 1970). However, we must take into account that it could be classified as cryptogenic based on the difficulty of determining their origin (see Carlton 1996b).

Table 3.1 Diagnostic characteristics used to distinguish native adult males of *Caprella equilibra* from non-native adult males of *Caprella scaura* and *Paracaprella pusilla*. Lateral views modified from Riera *et al.* (2003), Guerra-García (2003) and Guerra-García *et al.* (2010). Taxonomic criteria were based on the morphological descriptions provided by Templeton (1836), McCain (1968), Guerra-García and Thiel (2001), Guerra-García (2003), Krapp *et al.* (2006), Martinez and Adarraga (2008) and Bhave and Deshmukh (2009).

Character	Native		Non-native	
	<i>Caprella equilibra</i>	<i>Caprella scaura</i>	<i>Caprella scaura</i>	<i>Paracaprella pusilla</i>
				
Cephalic projection	absent	acute	absent	
Gnathopod 2	basis shorter than half the length of pereonite 2; well-developed ventral projection between gnathopods 2	basis long but shorter than pereon segment 2; propodus elongated, palm with 2 strong teeth and between them the 'poison tooth'	short basis; with proximal knob under the basis, and trapezoidal projection on the inner margin of the propodus	

3.2.3 Statistical analysis

Differences in environmental parameters at the four seasons and between total sediment retained for each substrate were analyzed using one-way ANOVA, or Kruskal Wallis when the data were not normally distributed (Kolmogorov-Smirnov test) and/or variances were not homogeneous (Cochran's C-test). To test possible relationships between environmental parameters, Pearson's correlation coefficient was used.

The influence of the season and the type of host substrate on the total caprellid abundance was tested using two-way ANOVA with the following factors: ‘Substrate’, a fixed factor, with two levels: the bryozoans *B. neritina* and *T. inopinata* (both were the only substrates that were present in all seasons) and ‘Season’, a fixed factor and orthogonal, with four levels: summer, autumn, winter and spring. Prior to ANOVA, heterogeneity of variance was tested with Cochran’s C-test. When ANOVA indicated a significant difference for a given factor, the source of difference was identified by applying the Student-Newman-Keul (SNK) test (Underwood 1997).

Possible differences in the sex/age group among seasons and type of host substrates were tested for *C. scaura* (the only caprellid species found in all seasons). For this, we used three-way ANOVA with the following factors: ‘Sex/age group’, a fixed and orthogonal factor, with three levels: mature male, mature female and juvenile; ‘Season’, a fixed and orthogonal factor, with four levels: summer, autumn, winter and spring; and ‘Substrate’, a fixed and orthogonal factor, with two levels: *B. neritina* and *T. inopinata*.

Analyses were carried out using the SPSS program and GMAV5 (Underwood and Chapman 1998).

Table 3.2 Criterion for determining the sex/age group of the specimens

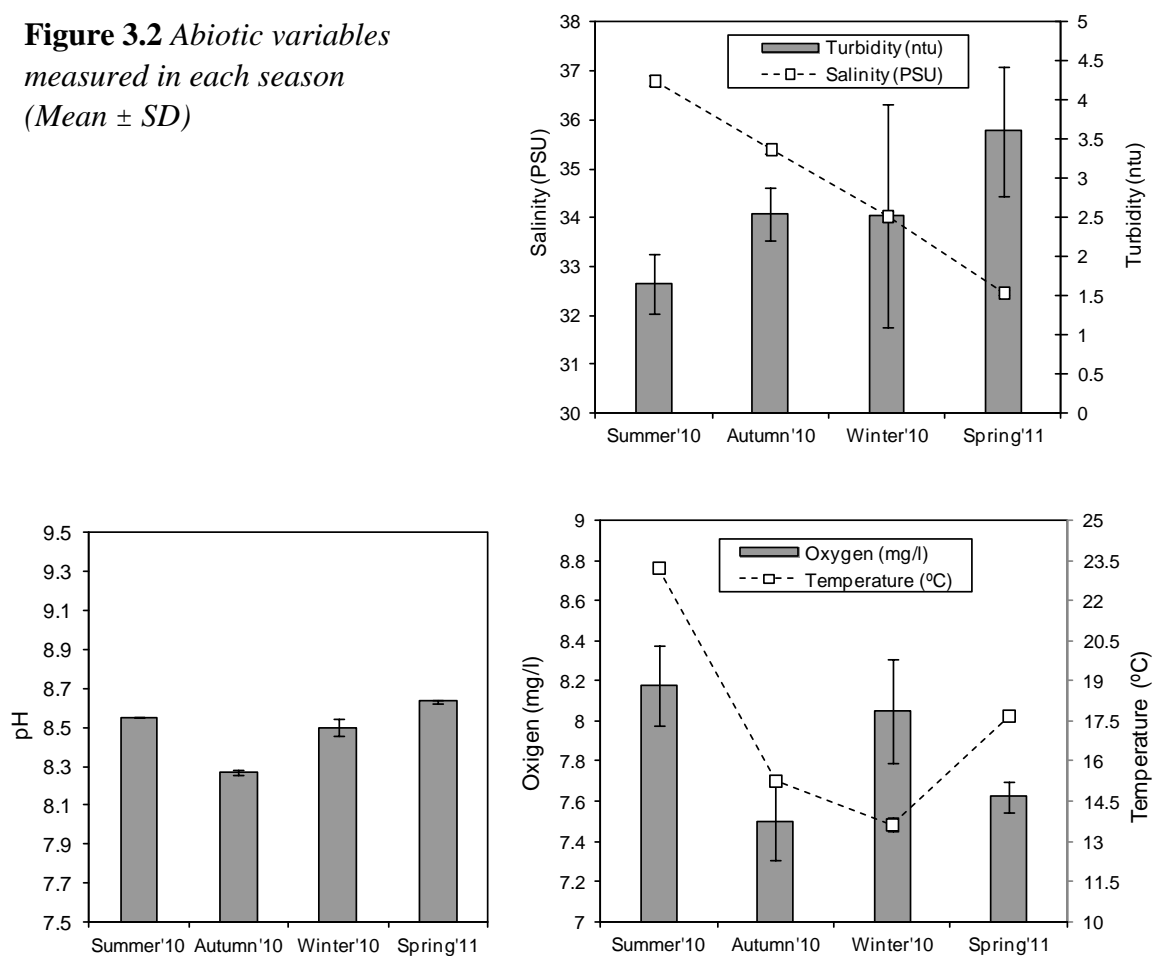
Sex/age groups	Morphological criteria
Mature Males	Gnathopods 2 clearly elongated with it basis shorter than pereonite 2 Insertion of gnathopods 2 on posterior end of pereonite 2 Pereonites 1 and 2 much longer than the rest of pereonites
Mature Females	Presence of oostegites with the brood pouch fully developed Insertion of gnathopods 2 on anterior end of pereonite 2
Juveniles	Pereonites 1 and 2 not too elongated Insertion of gnathopods 2 ranging from middle to anterior end of pereonite 2

3.3 RESULTS

3.3.1 Environmental measures and fouling community

Regarding the measures taken for the environmental parameters, seawater temperature was higher in summer (23.23 °C in September 2010) and lower in winter (13.63 °C in January 2011) (Fig. 3.2). Salinity, which was negatively correlated with turbidity ($r=-0.67$, $P<0.05$), varied between 36.8 psu in late summer 2010 and 32.47 psu in spring (March 2011), coinciding with the rainiest period. Furthermore, there were significant differences in temperature (ANOVA, $F=2348.58$, $P<0.001$) and salinity (ANOVA, $F=1126.88$, $P<0.001$) among seasons. Conversely, pH remained relatively similar among seasons ranging from 8.27 (Autumn 2010) to 8.63 (Spring 2011), also for the level of oxygen concentration, with 7.49 in Autumn 2010 and 8.18 in Summer 2010 and both were not statistically different among seasons.

Figure 3.2 Abiotic variables measured in each season (Mean \pm SD)



Percentage frequency of sessile fouling community tells us how common a species is within the marina's fouling community (Table 3.3). Non-indigenous species were dominant in the sessile fouling community, which consisted of about 50% of introduced species. This community was different among seasons but some species, like the tubeworm *Sabella spallanzani* Bloch 1784, the ascidian *Styela plicata* (Lesueur 1823) and the cirriped *Balanus perforatus* Bruguière 1789 presented relatively high frequencies throughout the whole study period. In summer, the sessile community was dominated by *S. spallanzani* and *B. perforatus*. The non-native ascidians *Ecteinascidia turbinata* Herdman 1880 was only present in this season. In autumn, the invasive bryozoan *Tricellaria inopinata* becomes the dominant species, competing for the space with other species of the community, like *Bugula neritina*, which often appeared epiphyted by *T. inopinata* (Figure 3.3). The hydroid *Eudendrium racemosum* disappears, while other ascidian, *Clavelina lepadiformis* (Müller 1776) appears in this season with an important cover. The rest of the seasons were clearly dominated by *T. inopinata*.

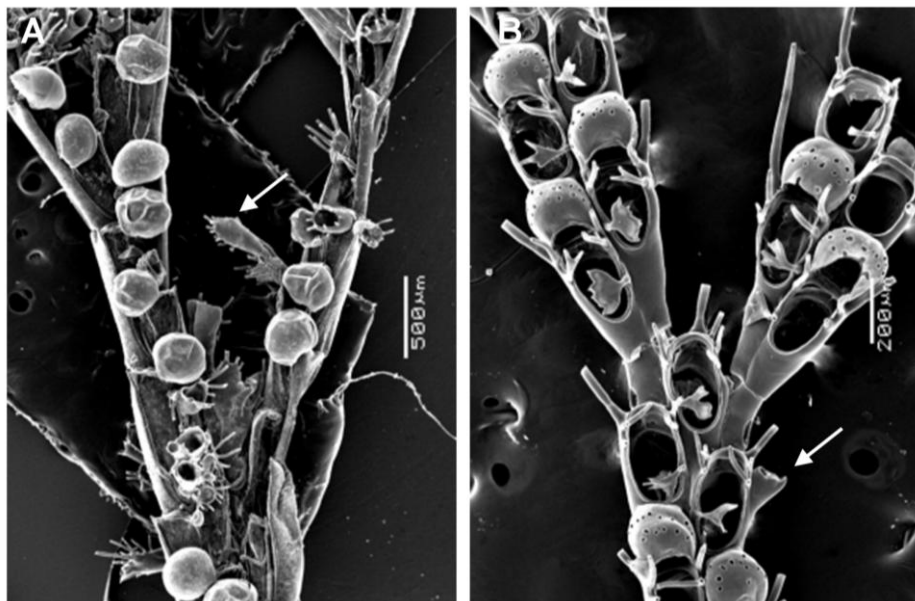



Figure 3.3 (A) SEM photography of *Bugula neritina*. The arrow shows another bryozoan, *Tricellaria inopinata*, growing on a branch of *B. neritina*; (B) SEM photography of *T. inopinata* showing defensive structures.

Table 3.3 Percentage frequency and invasive status of the dominant sessile fauna found in each season adhered to the floating pontoon lateral surface. The photograph shows an example of the floating pontoon fouling community taken in Cádiz marina (the arrows indicate the presence of *Caprella scaura* on the surface of the *Tricellaria inopinata* colonies).

frequency (%)		STATUS ^a	SEASON			
			Summer'10	Autumn'10	Winter'10	Spring'11
● 1-20 ● 21-40 ● 41-60 ● 61-80 ● 81-100						
FLOATING PONTOON FOULING COMMUNITY						
Macroalgae						
<i>Corallina elongata</i> J.Ellis & Solander, 1786		N	●		●	
Bryozoans						
<i>Bugula neritina</i> (Linnaeus, 1758)		HI	●	●	●	●
<i>Tricellaria inopinata</i> D'Hondt & Occhipinti Ambrogi, 1985		I		●	●	●
<i>Zoobotryon verticillatum</i> (Delle Chiaje, 1828)		I	●	●		
Hydrozoans						
<i>Eudendrium racemosum</i> (Cavolini, 1785)		N	●	●		
Ascidians						
<i>Clavellina lepadiformis</i> (Müller, 1776)		N	●	●	●	●
<i>Ecteinascidia turbinata</i> Herdman, 1880		I	●			
<i>Styela plicata</i> (Lesueur, 1823)		C	●	●	●	●
Crustaceans						
<i>Balanus perforatus</i> Bruguière, 1789		N	●	●	●	●
Molluscs						
<i>Mytilus sp.</i>			●	●	●	●
Polychaetes						
<i>Sabella spallanzani</i> Bloch, 1784		N	●	●	●	●

^aI= introduced, HI= historic introduction, C=cryptogenic, N= native

3.3.2 Caprellids assemblages and host substrates

In connection with caprellid assemblages, three species were recorded during the present study, the native *Caprella equilibra* Say 1818 and the non-native *Caprella scaura* and *Paracaprella pusilla*. *Caprella scaura* was the

dominant species and the only one which was present throughout the whole study period. *Paracaprella pusilla* only was present in summer but it appeared in high densities. *C. equilibra* was collected, in low abundance, in summer and autumn. *Paracaprella pusilla* only was associated with the hydroid *E. racemosum*, where it appeared in high densities; *C. equilibra* was present, in low abundance, in *E. racemosum* and *B. neritina*, while *C. scaura* was present in the four substrates in high densities (Fig. 3.4).

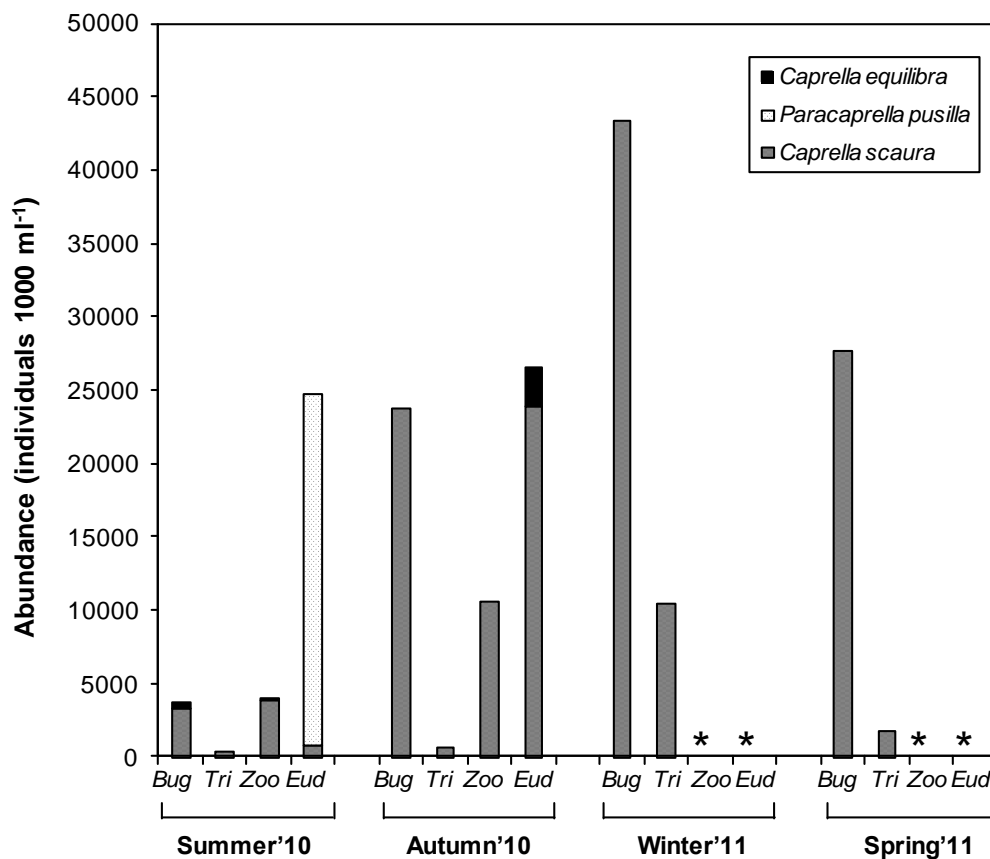


Figure 3.4 Total abundance of each caprellid species in each substrate. Bug: *Bugula neritina*, Tri: *Tricellaria inopinata*, Zoo: *Zoobotryon verticillatum*, Eud: *Eudendrium racemosum*. The asterisk indicates the absence of substrate.

Regarding percentage frequency of each host substrate, *B. neritina* and *T. inopinata* were present in all seasons whereas *Z. verticillatum* and *E. racemosum* were present only in summer and autumn (Fig. 3.5). *Bugula neritina* was the most abundant in summer following by *E. racemosum* and *Z. verticillatum*, while

the non-native bryozoan *T. inopinata*, dominated the rest of seasons. Differences in sediment retained by each host substrate shows that *T. inopinata* retained more amount of sediment than all other host substrates ($K=9.67$, $p<0.05$) following by *Z. verticillatum*, *B. neritina* and *E. racemosum* which retained very little sediment.

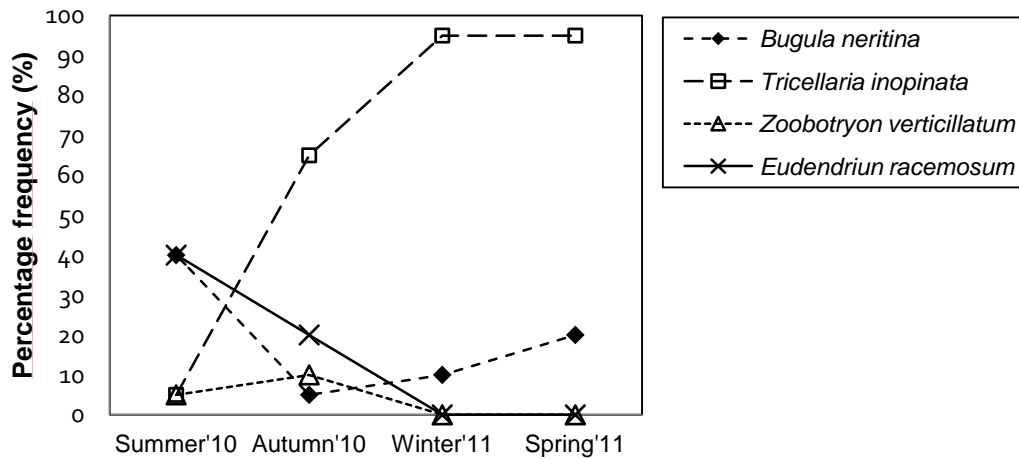


Figure 3.5 Percentage frequency of each host substrate in each season.

3.3.3 Population characteristics of *Caprella scaura* in the different seasons

Caprella scaura was the only species found in all seasons; consequently we could analyze the influence of the season and the type of substrate on the population characteristics of this caprellid species. The highest abundance of *C. scaura* was in winter in *B. neritina* (mean individuals $1,000 \text{ ml}^{-1} \pm \text{SE} = 43,333.3 \pm 1,356.3$). The maximum total length recorded for males during the study was 22.8 mm, in a *T. inopinata* sample of spring, whereas for females it was 10.7 mm, for an ovigerous female associated to *E. racemosum* in autumn (Figure 3.6). Females and juveniles dominated over males at all seasons, but their dominance was most marked in autumn and winter (Figure 3.7). Ovigerous females were found throughout the whole study period.

The total abundance of *C. scaura* was different between substrates when it was tested for the two substrates presented in all seasons: *B. neritina* and *T. inopinata* (Substrate, $P<0.05$, Table 3.4). *Bugula neritina* presented the highest caprellid density.

Figure 3.6 Total length of each sex/age groups of *Caprella scaura* (Mean \pm S.E).

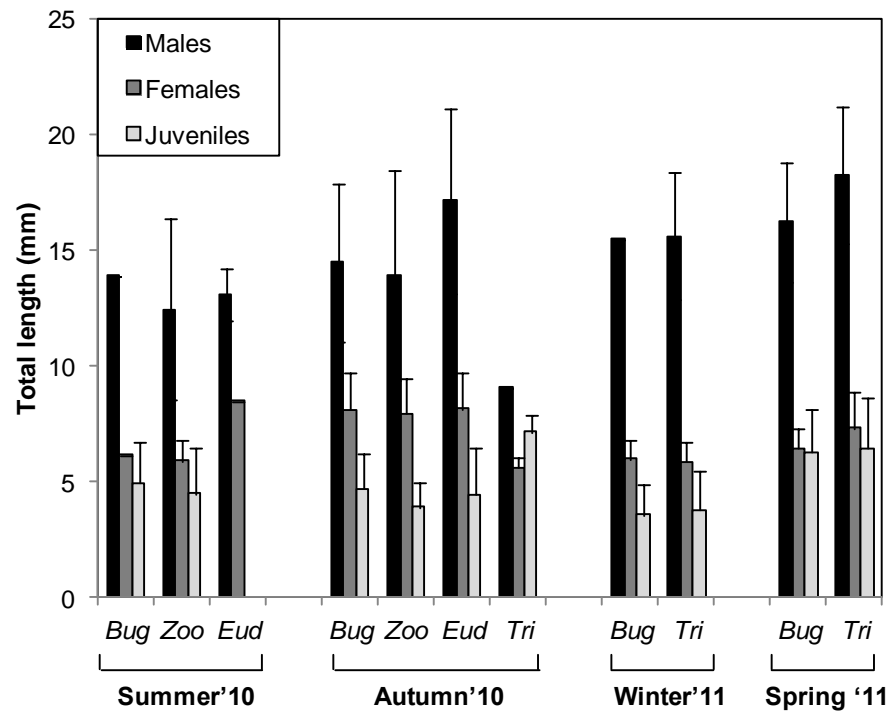


Table 3.4 ANOVA results of the influence of the type of substrate (*Bugula neritina* versus *Tricellaria inopinata*) on the abundance of *C. scaura* among seasons (* $P < 0.05$). MS, mean square; df, degrees of freedom.

Source of variation	df	Total Abundance of <i>C. scaura</i>		
		MS	F	P
Season	3	0.3354	0.61	0.6165
Substrate	1	3.1537	5.76	0.0289*
Season x Substrate	3	0.6652	1.22	0.3362
Residual	16	0.5473		
Cochran's C-test			C=0.6033	
Transformation			None	

In connection with the sex/age groups, a significant interaction was found between season and substrate (Season x Substrate, $P < 0.05$, Table 3.5) and Student Newman Keuls revealed that mature males, mature females and juveniles abundance of *C. scaura* were significantly different among seasons in the population associated with *Tricellaria inopinata*, while they were not different in *Bugula neritina*.

Figure 3.7 Total abundance of each sex/age groups of *Caprella scaura* in each host substrate.

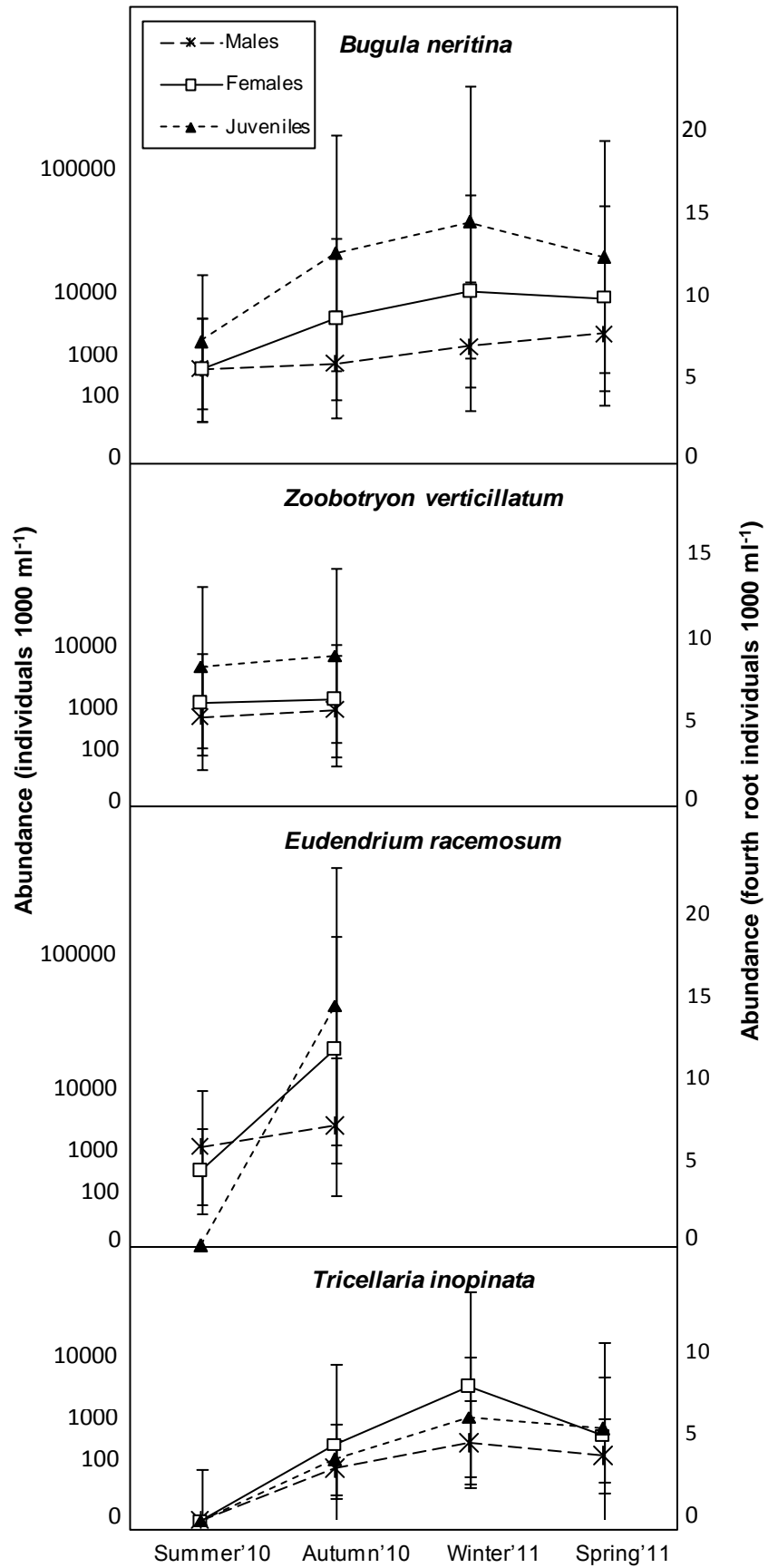


Table 3.5 ANOVA results of the influence of the type of substrate (*Bugula neritina* versus *Tricellaria inopinata*) on the abundance of sex/age groups of *C. scaura* (juvenile, mature female and mature male) among seasons (* $P < 0.05$). MS, mean square; df, degrees of freedom.

Source of variation	df	Total abundance of sex/age groups of <i>C. scaura</i>		
		MS	F	P
Sex/age	2	31.2845	2.25	0.1162
Season	3	48.9702	3.52	0.0218*
Substrate	1	36.2762	2.61	0.1127
Sex/age x Season	6	3.2705	0.24	0.9628
Sex/age x Substrate	2	19.2875	1.39	0.2594
Season x Substrate	3	64.1765	4.62	0.0064*
Sex/age x Season x Substrate	6	5.7452	0.41	0.8665
Residual	48	13.8951		
Cochran's C-test			C=0.1325	
Transformation			Ln (X+1)	

3.4 DISCUSSION

Although the Cádiz marina fouling community fluctuates greatly among seasons, about 50% of the dominant sessile species throughout the year are introduced. This may be due to the fact that it is situated in the Strait of Gibraltar, between the Atlantic Ocean and North Africa. This region is important for maritime traffic and is considered as a hot spot for biological invasions (see Drake and Lodge 2004). The marina is also very close to Cádiz port, a major international port in southern Spain. All of this makes Cádiz Marina an appropriate scenario for monitoring the settlement of non-native caprellid species that are likely introduced as part of hull fouling of recreational boats.

3.4.1 Caprellid assemblages

Three caprellid species, the non-native *Caprella scaura* and *Paracaprella pusilla* and the native *C. equilibra*, were found associated with the hydroid *Eudendrium racemosum* and the bryozoans *Bugula neritina*, *Zoobotryon verticillatum* and *Tricellaria inopinata* during the present study. These coincided

with the substrate types in which Guerra-García *et al.* (2011b) found *C. scaura* for the first time in southern Spain.

The tropical caprellid *Paracaprella pusilla*, first described from Brasil (Mayer 1890) and very common in the Atlantic coast of Central America, was recently reported for the first time in European waters (Ros and Guerra-García 2012). This species was found in high densities in summer associated with the hydroid *E. racemosum*. In total, thirty ovigerous females were identified, indicating that *P. pusilla* might be established in the area, with a reproductive population in this season. The occurrence of *P. pusilla* seems to be related with the high temperatures of summer months; in fact, this species has appeared for two consecutive summers since it was recorded in Spain (Ros and Guerra-García 2012), but it remains undetectable for the remaining seasons. One possible explanation is that the species is repeatedly being introduced in the study area via biofouling on the ships that arrive in the Cádiz marina. Another hypothesis is that *P. pusilla* population is able to regenerate the next summer from a few specimens that survive the cold months but remain virtually undetectable due to low abundances in the study area. In this regard, we only found one specimen of *P. pusilla* in December 2010. Molecular studies with specimens collected in both years may reveal if these populations share the same origin or are the results of multiple introductions in Cádiz marina as it has occurred with other invasive caprellid, *Caprella mutica*, in the Northern Hemisphere (Ashton *et al.* 2008). In contrast, the other non-native caprellid found, *Caprella scaura*, a native species to the western Indian Ocean and widely introduced globally (Krapp *et al.* 2006, Guerra-García *et al.* 2011b), was present throughout the whole study period and was associated with all the substrates. This species was found for the first time in the study area in June 2010 with densities of 6,650 ind/1000 ml *B. neritina* (data published by Guerra-García *et al.* 2010 as 35,945 ind/1000g *B. neritina*). It was the dominant species for most of the seasons, with highest abundances in winter and spring. Females dominated over males in all seasons, but their dominance was most marked in autumn and winter. Furthermore, juveniles and ovigerous females were found throughout the whole study period, reflecting the possibility that *C. scaura* reproduces all year around. This could be associated with its establishment success.

Caprella equilibra, the only native species found during the survey, was only present in summer and autumn. This cosmopolitan caprellid is very

common in southern Spain and exhibits an Atlantic distribution in the Iberian Peninsula coast, as Guerra-García *et al.* (2011a) showed in a recent spatial study to investigate the distribution patterns of intertidal and shallow water caprellids along the whole coast of the Iberian Peninsula. In a temporal study, Guerra-García *et al.* (2009) found *C. equilibra* in the intertidal zone of Tarifa Island, a pristine area of southern Spain, during the months of February and April. Conradi (1995) reported densities up to 10,595 ind/100g of *Caprella equilibra* associated with the bryozoan *Bugula neritina* in Algeciras Bay, Cádiz, southern Spain, a harbour area located very close to the study area. In Cádiz Marina, the abundance of this native species was low when compared with both the abundance of the non-native caprellids found in the study and the abundance of *C. equilibra* in other nearby harbours where non-native caprellids were absent. Probably, both *C. equilibra* and *C. scaura*, compete for the occupation of the substrate and is likely that *Caprella equilibra* is being displaced by *C. scaura* in the study area (Guerra-García *et al.* 2011b). Further studies will be needed to demonstrate this hypothesis conclusively.

3.4.2 Influence of host substrate in the establishment success of non-native caprellids

Many caprellid species appear to be relatively unselective with respect to their substrate within characteristic habitat types (Caine 1980; Guerra-García 2001; Thiel *et al.* 2003), although some may exhibit substrate preferences, like *Caprella californica*, a close species to *C. scaura* which shows a clear preference for *Bugula neritina* (Keith 1971) and has recently expanded around the coastline of Australia (Montelli 2010).

To test if *C. scaura* shows substrate preferences in the study area throughout the study period, we compared its abundance in the two substrates which were present in all seasons: *B. neritina* and *T. inopinata*. We rejected the other substrata, *Z. verticillatum* and *E. racemosum*, since they were not present throughout the whole study period and they cannot contribute equally to the establishment success of *C. scaura*. We found that *C. scaura* was significantly more abundant in *B. neritina* than in *T. inopinata*. If we consider the abundance of the non-native caprellid population within the host substrate as indicative of the suitability of this substrate for the establishment success of the non-native

caprellid, we may assume that *B. neritina* is more suitable for the establishment success of *C. scaura* than other substrates in which *C. scaura* was found. In this regard, Martinez and Adarraga (2008) found most of the specimens of *C. scaura* among *B. neritina* when they reported its presence along the Spanish coast and Guerra-García *et al.* (2011) found the highest density of *C. scaura* among *B. neritina* in southern Spain.



Fig. 3.8 Individuals of *C. scaura* on the surface of the bryozoan *B. neritina* collected in Cádiz. Note that the colour exhibited by *C. scaura* is very similar to the colour of the bryozoan.

This relative affinity could be attributed to the similarities in physical appearance between the two species (Fig. 3.8), related to the caprellids ability to undergo physical colour changes necessary to adapt to *B. neritina* as well as its similarities to the external morphology (Keith 1971). Although this cryptic behaviour may contribute to reduce predation and hence increase density of caprellid population, it is not necessarily restricted to *B. neritina*. Another hypothesis to explain the preference of *C. scaura* by *B. neritina* may be due to its morphological structure. Although the sediment retained by the branches of *T. inopinata* was significantly higher than in *B. neritina* showing a more complex structure, when we study the morphological structure of both

bryozoans, we could see that *T. inopinata* presents more defensive structures than *B. neritina*, such as frontal spines or lateral avicularia, which may hinder the movement of caprellids within the colony. Moreover, *T. inopinata* is more compact than *B. neritina*, and therefore caprellids may have more difficulty in penetrating the inner parts of the colony. Possibly for this reason, in the samples of *T. inopinata*, caprellids were commonly found in the surface of the colony, more exposed to predators, while in *B. neritina*, caprellids were found along the whole structure. Further studies are necessary to explain the preference of *C. scaura* by *B. neritina*, but this interaction could be another example of the phenomenon called ‘invasional meltdown’ (Simberloff and VanHolle 1999), in

which an alien species (*B. neritina*) facilitates the establishment success and occurrence of another alien species (*C. scaura*).

In spite of this, the maximum growth for *B. neritina* occurred from spring to summer while the highest abundance of *C. scaura* was in winter, coinciding with the maximum coverage of *T. inopinata*. This means that *C. scaura* is able to colonize other substrates available, such as *T. inopinata*, which is virtually absent in summer. Thus, Guerra-García (2003) reported no specific habitat selection for *C. scaura*: specimens were collected on bryozoans, red and brown algae, seagrasses (Lim and Alexander 1986; Takeuchi and Hino 1997; Guerra-García and Thiel 2001) or sponges (Serejo 1998).

Paracaprella pusilla exhibits an interesting relationship with the host substrate *Eudendrium racemosum*. Some authors collected *P. pusilla* in a high variety of substrates, including *B. neritina* (Caine 1978; Guerra-García *et al.* 2010), however in its new range, *P. pusilla* seems to prefer the native hydroid *E. racemosum* over all other substrates, since it was only recorded in this hydroid in the study area (Fig. 3.9). Indeed, the occurrence of *P. pusilla* in Cádiz marina also appears to be facilitated by the presence of this substrate. In this sense, when *P. pusilla* was recorded in southern Spain for the first time, it was observed that this species displays a mutualistic relationship with *E. racemosum*: the introduced caprellid receives a suitable substrate for attachment and camouflage from predators, and access to food items including detritus, diatoms or copepods from living on the hydroid. In return, the hydroid is defouled and protected from nudibranch predators (see Ros and Guerra-García 2012). This relationship may not be restricted to this hydroid in particular but until now, *P. pusilla* was only found among this hydroid species in southern Spain.

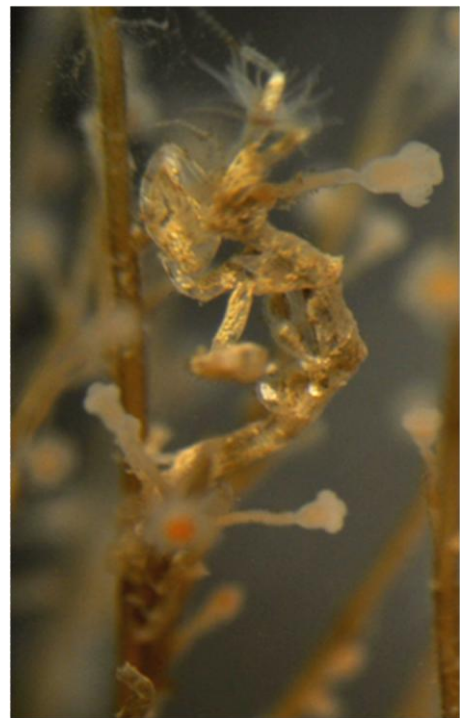


Fig. 3.9 Adult male of *P. pusilla* collected in Cádiz on the hydroid *E. racemosum*.



Fig. 3.10 Detail of the pereopods of *P. pusilla* attached to the hydroid branches.

Another hypothesis to explain the preference of *P. pusilla* on hydroids in general may be due to its morphological structure of pereopods (Fig. 3.10), which caprellids use to attach to the substrate. In this sense, Aoki and Kikuchi (1990) indicate that species in *Paracaprella* seemed to have the pereopods adapting to gripping thin things such a hydroid stems, with a serrate palm on pereopod 7. This is not the case of the other non-native found, *C. scaura*, with a propodus of pereopod 7 more setose and longer than *P. pusilla*, and therefore, well adapted to clinging to larger items like seaweeds or bryozoans.

We can conclude that arborescent bryozoans presents in marinas' fouling communities facilitate the establishment success of *C. scaura*, especially in the case of *B. neritina* in which it showed the highest density and the presence of hydroids like *E. racemosum*, facilitate the establishment success of *P. pusilla*, which is well adapted to living on this substrate.

3.4.3 Assessment of the current status: a comparative analysis of invasiveness of the two non-native caprellids found

In addition to the 'invasibility' of the recipient community (low diversity of native biota, empty niches, disturbed, polluted, etc.), traits of the introduced species are equally important in determining if they will become established in the new site (Weis 2010).

When comparing the two non-native caprellid species found in the study, we can see important morphological, behavioural and habitat use differences that may influence their establishment success. Morphologically, *Paracaprella pusilla* is clearly smaller than *Caprella scaura*, which is also provided with a dorsal acute head projection which can be used in defence, similar to that which occurs in *C. californica* (Kosman 2002; Montelli 2010). However, the advantage of this fact still needs to be determined. Recently, it has been disputed whether

larger body size implies better invasion success in marine bivalves (Miller *et al.* 2002; Roy *et al.* 2002; Ashton *et al.* 2010). On the other hand, the second gnathopod in *C. scaura* is more elongated than in *P. pusilla* and are equipped with two strong teeth and a ‘poison tooth’, which is only present in mature males (Schulz and Alexander 2001). Although the specific purpose of the poison tooth, also presents in the invasive caprellid *Caprella mutica*, has not yet been demonstrated, is known that in amphipods, the second gnathopod is used in aggression and prey capture (Lewbel 1978; Lim and Alexander 1986; Schulz and Alexander 2001). Therefore, this feature may support an advantage in agonistic encounters and the establishment success.

Behaviourally, *Caprella scaura* belongs to the group of caprellid amphipods with parental care in which females care for their young until they become large enough to disperse onto the substrate (Lim and Alexander 1986; Aoki 1999). According to Rosenblatt (1996) the evolution of parental care is an adaptation that may permit subsequent radiation. Once parental care evolves, the protection afforded immature stages may permit invasion of an environment that was formerly not suited for juvenile development (Rosenblatt 1996). Moreover, *C. scaura* males exhibit an intraspecific aggressive behaviour (Schulz and Alexander 2001). Although an interspecific aggression has not yet been demonstrated for *C. scaura*, this behaviour may be instrumental in competition for habitat and for food and appears to play a significant role for some invasive species (Weis 2010).

Regarding habitat use, *P. pusilla* prefers *E. racemosum* over the rest of the substrates, at least in its introduced range, while *C. scaura* exhibits a greater ability to colonize different substrates in its non-native area. This allows *C. scaura* to exploit a range of habitats and its cryptic behaviour may protect them from visual predators.

Furthermore, *C. scaura* is able to support the seasonal fluctuations of environmental variables. This species was present in the four seasons in spite of temperature or salinity fluctuations, showing a high tolerance for environmental variables, while the occurrence of *P. pusilla* in the present study seems to depend on high temperatures.

In conclusion, the current status of both populations in the study area indicates that *C. scaura* is an invasive species which presents a higher invasive

potential than *P. pusilla*, which is an introduced species more restrictive in the occupation of the substrate, less abundant and more sensitive to changes of environmental conditions than *C. scaura*.

Arborescent bryozoans and hydroids, which are part of the fouling community in the pontoon systems, can be used as monitoring tool for the assessment and detection of non-native caprellids since they can act as refuge for these introduced species facilitating their establishment success. These substrate species often attach to vessel hulls, allowing a subsequent secondary spread of all of its epibionts (including caprellid species). The proliferation of these types of substrates in marinas must be considered as a high risk for establishment and expansion of non-native caprellid species. Indeed, the presence of these substrates, like *E. racemosum*, in natural adjacent habitats poses a threat to spread of taxa from marina to natural coastal habitats.

Exploring the relationship within the colony of a fouling substrate between the native fauna associated and the newly arrives, are necessary to explore the possible impacts of non-natives in the native species like *C. equilibra*.

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4

POTENTIAL IMPACTS

Is *Caprella scaura* displacing an established congener along the Iberian Peninsula?

- Adapted from:

Ros M, Vázquez-Luis M, Guerra-García JM (*submitted*) Environmental factors modulating the extent of biological pollution in coastal invasions: the case of the invasive crustacean *Caprella scaura* (Amphipoda: Caprellidae) in the Iberian Peninsula.

ABSTRACT. Understanding the respective roles of environment and interspecific interactions in shaping species' distributions is a critical aspect to determine the potential impacts of newcomer species on occupied habitats. The invasive caprellid amphipod *Caprella scaura* has successfully spread along the western Mediterranean coast in a short time period, coinciding with a decline in the population of an ecologically similar congener, *Caprella equilibra*. To understand the mechanisms underlying the establishment success of *C. scaura* and its potential role in shaping *C. equilibra*'s distribution, we analyze the factors involved in the patterns of distribution and co-occurrence of both species at 42 marinas along the Iberian Peninsula and northern Africa. Results reveal that the two species are strongly spatially segregated, with a limited spatial overlap, where *C. scaura* dominates in the Mediterranean coast of the Iberian Peninsula while *C. equilibra* dominates in the Atlantic coast. Our analyses provide statistical evidence for a competitive displacement of *C. equilibra* by *C. scaura* along the Mediterranean coast of Spain, and point out the critical role of salinity in modulating this interaction and limiting the invasive success of *C. scaura* in the Iberian Atlantic coast.

RESUMEN. Entender el papel del ambiente y las interacciones específicas en la conformación de la distribución de las especies es un aspecto crítico para determinar los impactos potenciales de las especies introducidas en los hábitats ocupados. El anfípodo caprélido introducido *Caprella scaura* se ha expandido exitosamente a lo largo de la costa Mediterránea occidental en un corto espacio de tiempo, coincidiendo con el declive poblacional del congénere nativo *Caprella equilibra*. Para entender los mecanismos que subyacen al proceso de establecimiento de *C. scaura* y su papel potencial en la modelización de la distribución de *C. equilibra* hemos analizado los factores involucrados en los patrones de distribución y coexistencia de ambas especies en 42 marinas a lo largo de la Península Ibérica y el norte de África. Los resultados revelan que ambas especies están profundamente segregadas, con un escaso solapamiento espacial, donde *C. scaura* domina en la costa Mediterránea de la Península Ibérica mientras que *C. equilibra* domina en la costa Atlántica. Los modelos obtenidos indican la existencia de un desplazamiento competitivo de *C. equilibra* por parte de *C. scaura* en la costa Mediterránea y apuntan a la salinidad como factor clave en la modulación de la interacción entre ambas así como de la limitación del éxito invasivo de *C. scaura* en la costa Atlántica.

4.1 INTRODUCTION

Although non-native species are increasingly recognized as an important stressor and force of change in marine communities, the evidence and nature of these species impacts are often unclear (Ruiz *et al.* 1999). One of the major challenges to address this issue is that the impacts of an exotic species may vary along environmental gradients (MacNeil *et al.* 2001; Ricciardi 2003; Melbourne *et al.* 2007; Kestrup and Ricciardi 2009). Understanding the respective roles of environment and interspecific interactions in shaping species' distributions at broad spatial scales is necessary to determine the extent of spread, establishment success and potential impacts of newcomer species in recipient communities (Kelly *et al.* 2006; Lockwood *et al.* 2007; Piscart *et al.* 2009). As the rate of species introductions seems to have increased in recent decades (Ruiz *et al.* 2000, 2007; Mack *et al.* 2000), these interspecific interactions are become more common between new introduced species and previously established introduced species (Griffen 2009) with similar ecological requirements. This phenomenon may be particularly relevant in recreational marinas and harbors, habitats with a high rate of propagule pressure which are host to multiple introduced species through shipping related vectors (Carlton 1996a; Floerl and Inglis 2005). These habitats provide unique opportunities for assessing the role of biotic interactions in structuring marine fouling communities that may tolerate high levels of anthropogenic disturbance.

Caprellid amphipods are becoming increasingly recognized as important organisms in structuring fouling communities. These small marine crustaceans, most known for their role as trophic link between primary producers and higher trophic levels (Woods 2009), have recently attracted the attention of marine invasions researchers due to (1) the high densities they reach in artificial submerged structures (e.g. Buschbaum and Gutow 2005, Ashton *et al.* 2010); (2) their contribution to increase biotic resistance to invasive tunicates in recipient fouling communities (Collin and Johnson 2014; Rius *et al.* 2014); (3) their relatively high potential for invasions and detection across many global regions (e.g. Ashton *et al.* 2008a; Ros *et al.* 2014a). Furthermore, amphipods represent an interesting challenge with regard to studying human-mediated dispersal because they have limited dispersal capabilities due to their lack of larval stage. Although several studies have explored the likely introduction vectors (e. g. Carlton 1985; Thiel *et al.* 2003; Ashton *et al.* 2006) and distribution patterns (e.

g. Krapp *et al.* 2006; Ashton *et al.* 2008b; Cabezas *et al.* 2014) of exotic caprellids, little is known about the biotic and abiotic factors driving these species' distributions. A deeper understanding of these factors would improve both our ability to predict their potential impacts on other co-occurring species and our capacity to manage them.

Caprella scaura Templeton 1836 was the first exotic caprellid recorded in the Mediterranean Sea. Since it was detected for the first time in the lagoon of Venice (Italy) in 1994 (Sconfiatti and Danesi 1996), the species have been reported in several Mediterranean countries (Ros *et al.* 2014b and references therein; Fernandez-Gonzalez and Sánchez-Jerez 2014) and the East Atlantic Coast (Guerra-García *et al.* 2011; Minchin *et al.* 2012). In the occupied areas, *C. scaura* is very abundant in polluted or physically degraded environments, like harbors and enclosed bays. It is particularly successful in fouling communities associated with recreational marinas and aquaculture facilities (Guerra-García *et al.* 2011), even those located in off-coast areas (Fernández-Gonzalez and Sánchez-Jerez 2014). Prior to its arrival, its congener *Caprella equilibra* Say 1818, known for their propensity for passive dispersal, was one of the most commonly found caprellid species in Mediterranean harbour fouling communities (e.g. Schellenberg 1928; Brian 1938; Candela *et al.* 1983; Bellan-Santini and Ruffo 1998), reaching high densities in association with the fouling bryozoan *Bugula neritina* (Linnaeus 1758) (Conradi *et al.* 2000; Baeza-Rojano and Guerra-García 2013). After the detection of *C. scaura* in southern Spain marinas (Guerra-García *et al.* 2011) and subsequent monitoring of the species in this area, a decrease in the abundance of *C. equilibra* was observed when *C. scaura* was present (Ros *et al.* 2013). A similar pattern was observed by Fernandez-Gonzalez and Sánchez-Jerez (2014) in Mediterranean fish-farms fouling communities. Since both species occur in sympatry in the Iberian Peninsula, the existence of a niche overlap in their habitat use suggests the possibility of occurring interspecific competition (Hutchinson 1959). However, the potential role of *C. scaura* as biotic agent influencing the abundance and distribution pattern of *C. equilibra* has not been demonstrated yet. One of the major difficulties is that there may be several causative mechanisms acting alone or in concert when an invasive species arrives and previous resident species decline (Dick 2008). For example, the competitive advantage of an invasive species can differ along environmental gradients (Tilman 1982). Thus, to get better insight into processes driving the establishment success of *C. scaura* in the

Mediterranean and the nature of the potential interactions between *C. scaura* and ecologically similar congeners, we need spatial gradients ranging from sites entirely dominated by *C. scaura* to *C. scaura* free sites.

In the present study we investigate, by means of a broad scale survey of marinas along the Iberian Peninsula and northern Africa, the following questions (1) Which biotic and abiotic factors are involved in the distribution pattern of *C. scaura* and *C. equilibra*? (2) Is *C. scaura* presence a relevant factor driving the current distribution of its congener *C. equilibra*? (3) Which factor or combination of the studied factors modulates the potential interaction between the two species?

4.2 MATERIALS AND METHODS

4.2.1 Study area

The study encompassed the Atlantic and Mediterranean coasts of the Iberian Peninsula, including the area under the influx of the mixture of the two water bodies: the Strait of Gibraltar (Fig. 4.1). The circulation in the surroundings of the strait is mainly driven by the inflow/outflow coupling in the Gulf of Cadiz and by the inflow in the Alboran Sea systems (see Peliz *et al.* 2013 and references therein).

4.2.2 Sampling survey

To avoid confounding factors, sampling efforts were focused on a well-defined secondary habitat, the habitat-forming bryozoan *Bugula neritina* (hereinafter host substratum). This substratum was selected because it is a common component of the fouling communities associated with marinas and harbors in the Mediterranean Sea and the East Atlantic coast (Ryland 1967; Occhipinti Ambrogi 1981; Ryland *et al.* 2011); and it has been shown that commonly host the two species of interest: *C. scaura* and *C. equilibra* (e.g. Keith 1971, Guerra-García *et al.* 2011). The survey was carried out from May to June 2011 (late spring and early summer) as part of a general sampling program to characterize the expansion of *C. scaura* along the Iberian Peninsula and adjacent areas (see Ros *et al.* 2014b). Marinas in which *B. neritina* was absent were

discarded from the present study. Consequently, a total of 42 marinas distributed along the Iberian Peninsula and the northern coast of Morocco were selected for the present study (Fig. 4.1).

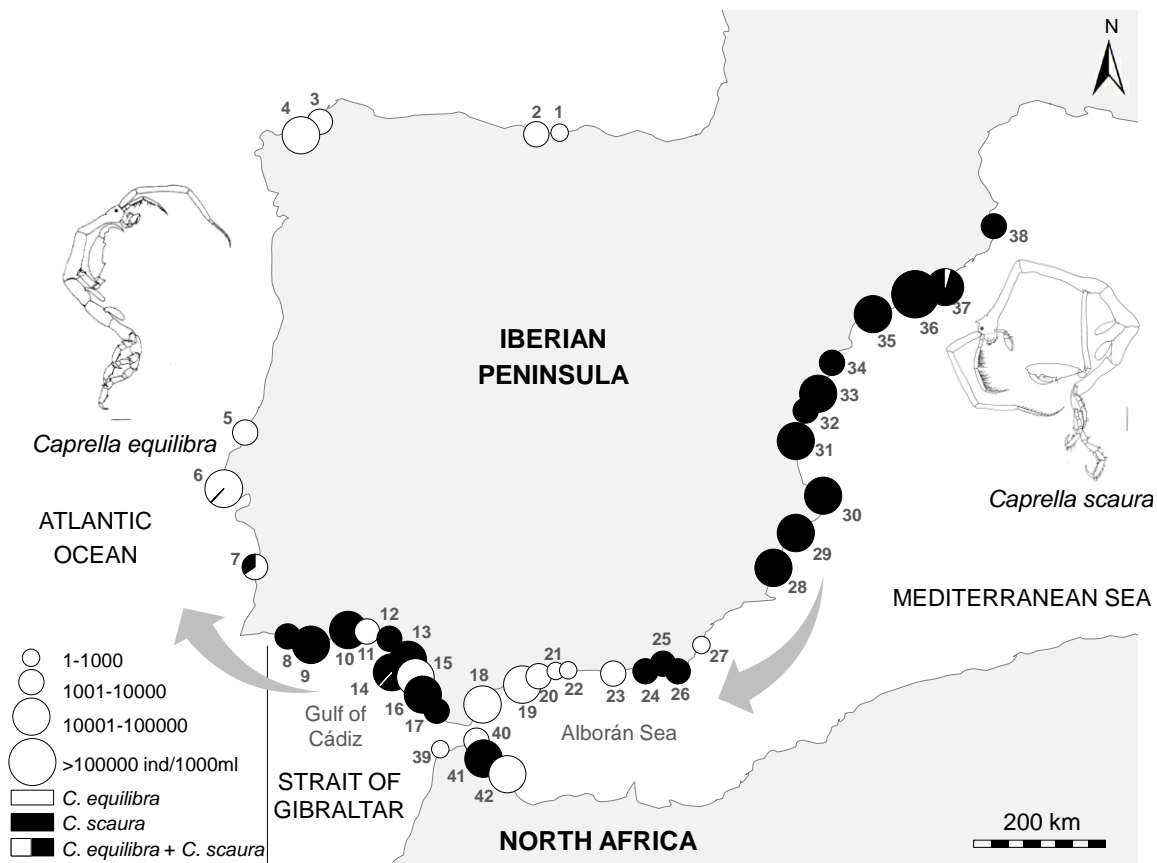


Figure 4.1 Map showing the marinas surveyed and the relative abundance of each caprellid species associated with the bryozoan *Bugula neritina* collected.

Three colonies of *B. neritina* were collected from the submerged structures at each marina, close to the water surface and directly removed by hand. Just after collection, samples were preserved in 90% ethanol. In the laboratory, all caprellids were sorted and identified to species level. The volume of the *B. neritina* clumps was estimated by placing each sample in a graduated cylinder with a fixed amount of water and measuring the volume displacement. Abundance of caprellids was expressed as number of individuals/1,000 ml of substratum. When caprellid populations exceeded 50 individuals per marina sampled, the population structure was analyzed identifying and counting the

proportion of males, mature females, premature females and juveniles (Ros *et al.* 2014a).

4.2.3 Environmental variables

Three haphazard measurements of salinity, temperature and turbidity were made *in situ* in each marina. Salinity and temperature (°C) were measured using a conductivity meter CRISON MM40 and turbidity in nephelometric turbidity units (ntu), using a turbidimeter WTW 335 IR. Additionally, three surface seawater samples were collected in each marina and kept in cold until processing in the laboratory. Analysis of: P, Cd, Cr, As, Cu, Ni, S, Zn and Pb, was performed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES Varian ICP 720-ES axially viewed) after filtration through Nylon filters (pore size = 0.45 µm) and acidification with 2% HNO₃ (30%). The carbon and nitrogen analyses were performed on a total organic carbon (TOC) auto analyzer (Shimadzu TOC-VCSH) with an attached total nitrogen measuring unit (TNM-1) after filtration through Whatman paper (pore diameter= 11 µm). The sample was injected into the analyzer by using an auto sampler (Shimadzu ASI-V).

To examine the influence of human disturbance on the distribution of fouling caprellids, human population density (mean number of people per km²) was included. Census data from 2011 for the locality to which each marina belongs were obtained from the National Statistical Systems of Spain (www.ine.es), Portugal (www.ine.pt) and Morocco (www.hcp.ma). The number of marina berths was included to explore the potential influence of the primary habitat size (artificial submerged structures provided by marinas). Data were obtained from the FEAPDT (Federación Española de Puertos Deportivos y Turísticos: www.feapdt.es) and the IPTM (Instituto Português e dos Transportes Marítimos: www.imarpor.pt). To investigate if the availability of the host substratum affects the occurrence and abundance of fouling caprellids, the frequency of appearance of *B. neritina* was also included. This measure was made following the procedure of Ros *et al.* (2013). Subsequently, data were transformed in a semiquantitative scale in which 1 includes frequencies of occurrence until 20%, 2 (>20-40%), 3 (>40-60%), 4 (>60-80%) and 5 (>80-100%) (see Table 4.1).

Table 4.1 Sampling locations, coordinates and explanatory variables measured in each location. Temp.: temperature; Turb.: turbidity; Freq.: frequency; Cs: *Caprella scaura*; Ce: *Caprella equilibra*; Cd: *Caprella dilatata*; Pm: *Ptisia marina*

Locality	Latitude, longitude	Salinity (psu)	Temp. (°C)	Turb. (ntu)	TOC (mg/L)	N (mg/L)	P (mg/L)	S (mg/L)	Cd (µg/L)
1. Santander	43.45° N, 3.82° W	34.27	18.43	0.80	1.50	<0.1	0.10	1248.61	2.41
2. Gijón	43.54° N, 5.67° W	35.57	16.97	0.33	1.60	<0.1	0.14	1282.20	0.79
3. Ferrol	43.48° N, 8.26° W	34.57	16.17	0.89	0.00	<0.1	0.13	1196.00	1.47
4. A Coruña	43.37° N, 8.40° W	35.67	15.97	0.37	3.70	<0.1	0.13	1315.91	2.07
5. Nazaré	39.59° N, 9.07° W	35.83	18.43	0.85	0.90	<0.1	0.11	1302.24	1.29
6. Cascais	38.69° N, 9.42° W	34.40	19.53	0.66	1.60	<0.1	0.17	1245.10	3.25
7. Sines	37.95° N, 8.87° W	36.27	17.63	0.55	1.70	<0.1	0.14	1317.39	<0.70
8. Albufeira	37.08° N, 8.27° W	35.80	19.87	0.34	0.90	<0.1	0.10	1303.83	1.58
9. Faro	37.01° N, 7.94° W	36.33	21.03	0.62	1.00	<0.1	0.16	1309.44	2.82
10. Isla Cristina	37.19° N, 7.34° W	36.07	23.00	6.98	1.10	<0.1	0.10	1311.73	2.34
11. El Rompido	37.22° N, 7.13° W	35.53	23.00	10.35	29.00	<0.1	0.17	1293.27	1.43
12. Chipiona	36.74° N, 6.43° W	35.37	19.27	10.05	2.10	1.10	0.13	1254.72	1.47
13. Rota	36.62° N, 6.35° W	36.37	19.53	10.11	2.00	<0.1	0.14	1290.31	2.57
14. Cádiz	36.54° N, 6.38° W	36.30	19.90	4.12	31.00	<0.1	0.07	1319.93	1.09
15. Sancti Petri	36.40° N, 6.21° W	36.70	19.93	5.83	2.70	<0.1	0.10	1331.86	2.47
16. Conil	36.29° N, 6.14° W	36.67	19.57	5.97	2.00	<0.1	0.16	1323.53	1.15
17. Barbate	36.19° N, 5.93° W	35.67	19.73	10.21	2.10	<0.1	0.12	1206.51	0.96
18. La Línea	36.16° N, 5.36° W	36.87	18.60	1.28	5.90	<0.1	0.11	1322.06	<0.70
19. Fuengirola	36.54° N, 4.62° W	36.40	20.77	0.50	1.90	<0.1	0.03	1463.47	1.46
20. Benalmádena	36.60° N, 4.51° W	36.40	20.47	1.26	2.10	<0.1	<0.03	1444.34	2.23
21. Málaga	36.72° N, 4.41° W	36.40	24.00	1.49	2.90	1.00	<0.03	1474.08	2.47
22. Caleta Vélez	36.75° N, 4.07° W	36.53	24.33	3.24	4.00	1.00	<0.03	1479.64	2.36
23. Motril	36.72° N, 3.53° W	34.97	25.07	1.91	3.00	1.00	<0.03	1286.44	1.33
24. El Ejido	36.70° N, 2.79° W	36.87	26.47	1.21	3.00	1.00	<0.03	1460.74	<0.70
25. Roquetas	36.76° N, 2.61° W	36.90	26.13	1.25	4.90	1.00	<0.03	1439.57	2.22
26. Almería	36.83° N, 2.46° W	36.77	25.70	0.92	19.60	1.00	<0.03	1417.35	1.51
27. Carbonera	36.99° N, 1.90° W	37.90	25.77	0.97	6.50	1.00	0.03	1476.06	0.84
28. Torreveja	37.97° N, 0.68° W	37.90	27.93	1.23	4.80	1.00	<0.03	1480.67	1.00
29. Alicante	38.34° N, 0.49° W	38.23	28.07	0.43	3.10	1.00	<0.03	1485.28	2.57
30. Dénia	38.85° N, 0.11° W	37.03	26.70	0.69	3.10	3.30	<0.03	1299.61	1.42
31. Valencia	39.43° N, 0.33° W	37.77	27.80	2.55	4.30	<0.1	0.04	1446.34	1.29
32. Borriana	39.86° N, 0.07° W	37.70	26.37	1.00	5.50	1.10	<0.03	1445.69	3.51
33. Oropesa Mar	40.08° N, 0.13° W	37.90	25.37	0.53	3.70	1.00	<0.03	1525.35	2.90
34. Benicarló	40.42° N, 0.43° W	36.87	26.00	0.46	7.10	1.70	0.04	1367.27	1.44
35. Tarragona	41.11° N, 1.25° W	38.00	25.83	0.46	4.30	1.00	0.07	1508.00	<0.70
36. Vilanova Geltrú	41.21° N, 1.73° W	37.80	24.43	0.98	5.10	1.00	<0.03	1441.47	2.18
37. Barcelona	41.38° N, 2.18° W	37.80	23.77	1.27	5.30	1.00	0.51	1514.66	1.01
38. L'Estartit	42.05° N, 3.21° W	36.60	22.20	0.67	3.90	1.00	0.04	1718.47	1.18
39. Tánger	35.79° N, 5.81° W	36.03	19.37	1.26	3.00	1.00	0.03	1438.09	3.12
40. Ceuta	35.89° N, 5.32° W	36.13	21.87	1.43	2.70	1.00	<0.03	1416.79	1.46
41. Tetuan	35.75° N, 5.34° W	36.37	20.23	1.06	3.60	1.00	<0.03	1451.69	2.08
42. MDiq	35.68° N, 5.31° W	36.20	21.37	0.70	3.00	1.00	<0.03	1465.83	2.64

Table 4.1 –cont.-

Locality	Cr (µg/L)	As (µg/L)	Cu (µg/L)	Zn (µg/L)	Pb (µg/L)	Freq. host substrate	Nº marina berths	Population density	Caprellids present
1. Santander	<2.00	<0.05	15.05	19.45	<9.00	2	900	5176	Ce
2. Gijón	<2.00	<0.05	11.60	<8.00	<9.00	2	779	1527	Ce
3. Ferrol	<2.00	<0.05	11.70	<8.00	<9.00	2	250	883	Ce, Pm
4. A Coruña	2.76	<0.05	10.65	12.66	<9.00	3	700	6503	Ce
5. Nazaré	<2.00	<0.05	11.05	10.47	<9.00	3	52	180	Ce
6. Cascais	<2.00	<0.05	7.82	10.13	<9.00	4	650	1832	Cs, Ce
7. Sines	2.41	<0.05	9.87	<8.00	<9.00	3	230	67	Cs, Ce, Pm
8. Albufeira	2.05	<0.05	9.83	13.96	<9.00	3	475	251	Cs
9. Faro	<2.00	<0.05	13.93	9.45	<9.00	2	300	289	Cs
10. Isla Cristina	2.41	<0.05	16.25	16.02	<9.00	2	231	448	Cs
11. El Rompido	<2.00	<0.05	11.46	22.99	<9.00	2	387	85	Ce, Cd
12. Chipiona	<2.00	<0.05	9.20	<8.00	<9.00	2	447	573	Cs
13. Rota	<2.00	<0.05	10.39	<8.00	<9.00	3	209	347	Cs
14. Cádiz	<2.00	<0.05	9.83	<8.00	<9.00	3	319	10154	Cs, Ce
15. Sancti Petri	2.42	<0.05	7.03	<8.00	<9.00	3	94	389	Ce, Cd
16. Conil	2.02	<0.05	8.62	<8.00	<9.00	2	97	245	Cs
17. Barbate	<2.00	<0.05	14.12	<8.00	<9.00	2	314	160	Cs
18. La Línea	<2.00	<0.05	9.40	<8.00	<9.00	3	624	3370	Ce
19. Fuengirola	21.89	<0.05	<7.00	9.35	<9.00	2	275	7145	Ce
20. Benalmádena	12.22	0.76	16.65	16.83	<9.00	2	1140	2373	Ce, Pm
21. Málaga	18.74	15.54	15.51	47.27	9.32	4	107	1437	Ce, Cd
22. Caleta Vélez	16.59	<0.05	18.77	27.64	10.20	1	277	488	Ce
23. Motril	9.49	8.16	15.94	14.61	20.37	3	193	555	Ce
24. El Ejido	13.11	<0.05	8.88	10.72	13.20	1	1100	371	Cs
25. Roquetas	14.59	<0.05	10.04	14.66	<9.00	1	237	1506	Cs
26. Almería	12.66	<0.05	8.06	12.17	27.47	2	277	643	Cs
27. Carbonera	20.19	25.31	8.02	14.01	14.00	1	48	86	Ce
28. Torrevieja	18.00	20.46	9.58	18.55	<9.00	2	570	1430	Cs
29. Alicante	17.77	15.61	11.13	23.09	<9.00	4	400	1661	Cs
30. Dénia	13.33	16.26	11.80	17.01	<9.00	3	300	676	Cs
31. Valencia	20.25	36.31	22.16	83.89	<9.00	3	206	5928	Cs
32. Borriana	17.66	25.51	10.98	10.02	23.78	3	713	126	Cs
33. Oropesa Mar	12.99	<0.05	14.33	19.97	<9.00	1	668	126	Cs
34. Benicarló	19.83	<0.05	9.86	8.59	<9.00	3	293	126	Cs
35. Tarragona	12.20	<0.05	23.99	59.68	17.02	3	441	2436	Cs
36. Vilanova Geltrú	14.51	<0.05	14.46	16.47	<9.00	4	812	1976	Cs
37. Barcelona	14.51	15.62	17.30	36.49	51.85	5	200	16449	Cs, Ce
38. L'Estartit	18.57	8.69	19.79	26.95	22.81	2	738	172	Cs
39. Tánger	19.28	5.74	9.11	33.31	12.33	2	500	229	Ce
40. Ceuta	14.57	7.50	20.00	41.46	<9.00	2	325	4229	Ce, Pm
41. Tetuan	14.73	<0.05	<7.00	9.92	<9.00	3	450	283	Cs
42. MDiq	21.36	4.61	11.29	15.18	9.37	2	120	283	Ce

4.2.4 Data analyses

The distribution and abundance of the non-native *C. scaura* and the native *C. equilibra*, were modeled throughout Generalized Linear Models (GLM; McCullagh and Nelder 1989). The approach developed comprised two successive steps in order to avoid problems derived from the overdispersion resulting from the presence of excessive zeros in abundance data. First, the presence/absence component was modeled through a Binomial distribution (logit link) and secondly, the abundance component was modeled using only samples with species density above zero (Stefánsson 1996; Fletcher et al. 2005), with a Gaussian distribution (identity link). Both analyses included the same set of independent variables. Each caprellid species was analysed separately.

Previous to analysis, abundance data (response or dependent variable) were square root transformed to reduce overdispersion. Additionally, environmental and demographic variables (predictor or independent variables) were tested for multicollinearity. To avoid overfitted models a conservative criterion was followed assuming collinearity when two variables had a Pearson correlation coefficient (r) greater than 0.5. Consequently, six predictor variables were excluded from the models performance: seawater temperature, S, Cr, As, P and Cu.

Model selection was carried out based on second order Akaike's information criterion (AIC) for small sample sizes (AICc, Burnham and Anderson 2002). The Akaike weight (w_i) of each model was calculated within the significant models, defined as those ones with less than 2 AICc units of difference with the model with the smallest AICc. All significant models were then used to calculate model-averaged parameter estimates and unconditional 95% confidence intervals to better represent model-selection uncertainty. Following criteria of Burnham and Anderson (2002), individual predictor variables that had a model averaged confidence intervals that did not include 0 were considered significant or well supported by the data. Besides this, the relative importance of each predictor was based on the combined Akaike weights ($\sum w_i$) for all significant models that contain a given predictor. Factors that had equal Akaike weights values were ranked in order of the magnitude of their model averaged regression coefficients. As none of the interaction terms were considered well supported by our data, all interaction terms were removed to

simplify further analysis. Variability explained by the best models was computed using Phi coefficient for binary data (Jackson *et al.* 1989) and adjusted coefficient of determination (Adjusted R^2) for abundance data. The performance of the significant presence/absence models was assessed using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. In the case of abundance models, a simple Pearson correlation between predicted and observed values was calculated to evaluate the model performance.

To ensure that spatial scale has been sufficiently captured by the best fitted models, the presence of spatial autocorrelation (a pattern in which observations are related to one another by their geographic distance) in the model's residuals was tested using the global Moran's I test (Dormann *et al.* 2007).

To explore the influence of *C. scaura* in the occurrence and distribution of *C. equilibra* along the study area the two best models developed for *C. equilibra* (presence/absence and abundance models with the lowest AICc) with and without including *C. scaura* as predictor variable were compared. An ANOVA analyses was used to test for a significant reduction in the residual deviances (portion of each observation which remains unexplained by the model) of the models after the inclusion of *C. scaura* as predictor. All statistical analyses were performed using R version 3.1.1 (R Development Core Team 2014).

4.3 RESULTS

4.3.1 Fouling caprellids found along the study area

The newcomer species *Caprella scaura* and the long established species *Caprella equilibra*, *Caprella dilatata* and *Phtisica marina*¹, were found associated with the bryozoan *Bugula neritina* along the study area. The invasive caprellid, *C. scaura*, was the dominant species, appearing in the 59.5% of marinas sampled, followed by the *C. equilibra*, which was found in the 50% of

¹ These three caprellid species have been established in the Mediterranean and the East Atlantic coast for hundreds of years (McCain and Steinberg 1970), being traditionally considered native in this region. Although in this chapter we have followed this terminology, we must take into account that they could be classified as cryptogenic based on the difficulty of determining their origin (see Carlton 1996b).

stations (Fig. 4.1). The other two caprellid species, *C. dilatata* and *P. marina*, were scarcely represented and they did not coexist with *C. scaura* (see Table 4.1). The distribution pattern of the two dominant caprellid species was disjunct and segregated. *Caprella scaura* and *C. equilibra* only coexist in four marinas. In the Mediterranean and the Gulf of Cádiz, in which *C. scaura* was the dominant species, it was more abundant than *C. equilibra* in the co-existing marinas. However, in the south coast of Portugal, the upper distribution limit of *C. scaura* for the eastern Atlantic coast, *C. equilibra* was more abundant than *C. scaura* within the same host substratum. The ‘pure’ Atlantic marinas and the ‘pure’ Mediterranean marinas harbored *C. equilibra* and *C. scaura* respectively, while the part of the coast under the influx of the Strait of Gibraltar (the Gulf of Cádiz and the Alboran Sea) were alternatively occupied by the invasive or the native species but not both, with the exception of Cádiz marina (station 14, Fig. 4.1) in which appeared together.

Regarding with the population structure, the two species were reproductively active when collected appearing both juveniles and mature females carrying with eggs in their brood pouch along the study area (Fig. 4.2). In general, *C. scaura* presented more proportion of males and mature females than *C. equilibra*, while the latter had a higher proportion of juveniles.

Tolerance ranges exhibited by *C. scaura* and *C. equilibra* occurrence (Fig. 4.3) showed that *C. scaura* was found in marinas with higher salinity, temperature and concentration of metal pollutants (arsenic, copper and zinc) than those marinas in which *C. equilibra* was found.

4.3.2 Factors involved in the distribution pattern of *Caprella scaura*

Salinity, which appeared in all significant models, was the most important factor explaining the occurrence and abundance of *C. scaura* along the Iberian Peninsula and northern Africa (Table 4.2; Fig. 4.4). Although presence/absence model with the lowest AICc only had salinity as explanatory variable, five additional models were also considered significant (Table 4.2: $\Delta_i < 2$). These models also included the following factors: turbidity, frequency of occurrence of *B. neritina* and human population density. The variability explained by significant models ranged from 35.0% to 40.01% (Table 4.2: Phi).

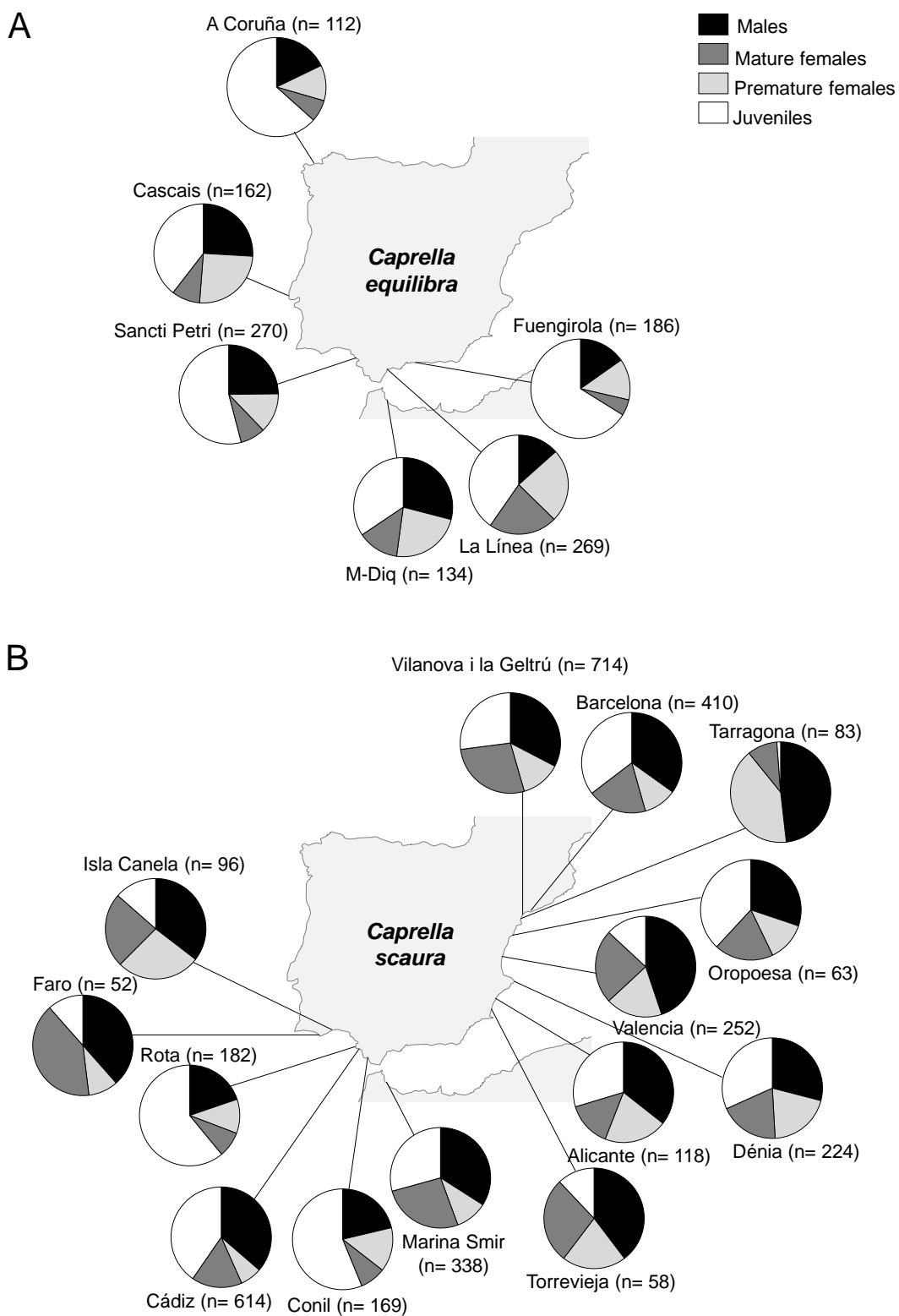


Figure 4.2 Population structure of: (A) *C. equilibra* and (B) *C. scaura* from populations with more than 50 individuals.

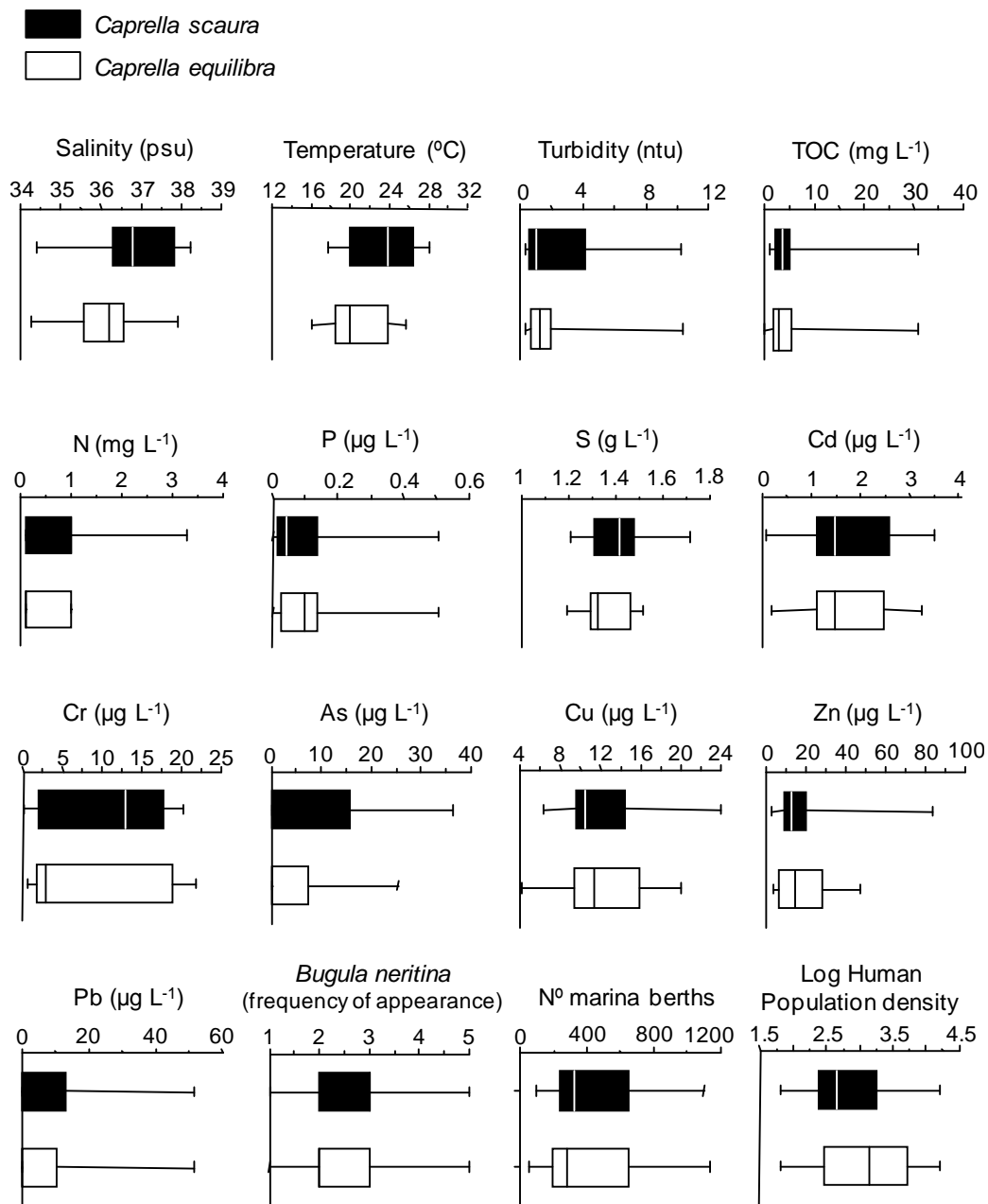


Figure 4.3 Tolerance ranges of *C. scaura* and *C. equilibra* to the environmental variables measured based on the values obtained in those marinas where the species was present. Box-and-Whisker plots include median values; the rectangles contain values between the first and the third quartiles; the bars connect the extreme values.

Model averaged coefficients (Table 4.3) showed that *C. scaura* was positively related to all four factors but the only significant relationship was between *C. scaura* occurrence and salinity ($\beta = 0.20$, $p < 0.01$; Fig. 4.5a). This

variable was the most important variable explaining the occurrence of *C. scaura* ($\sum w_i=1$) followed by turbidity, frequency of occurrence of *B. neritina* and, finally, population density. The average performance of the best significant models was good (AUC=0.79)

When the abundance of *C. scaura* (conditioned on presence) was the response variable, the nine significant models, which explained from 35.1% to 52.0% of variability (Table 4.2: Adjusted R^2), included a total of five explanatory variables (Table 4.2). These factors were, in decreased order of importance, salinity, followed by frequency of appearance of *B. neritina*, population density, lead and turbidity (Table 4.3: $\sum w_i$). *C. scaura*'s abundance was negatively related to lead and positively related with the rest of best models' factors (Fig. 4.6). However, the only variables that significantly influence the abundance of *C. scaura* were salinity, frequency of appearance of *B. neritina* and population density. The average performance of the best significant models was high ($r=0.77$, $p<0.01$).

4.3.3 Factors involved in the distribution pattern of *Caprella equilibra*

Seven models were well supported to explain the occurrence of *C. equilibra*. These models explained from 48.5% to 58.7% of variability in the presence/absence of this species and included, in decreasing order of importance, the following factors: salinity, population density, turbidity, number of marina berths, total organic carbon (TOC), lead and nitrogen (Tables 4.1 and 4.2). However, the only significant factor affecting the occurrence of *C. equilibra* was salinity ($\beta = -1.77$, $p < 0.05$; Fig. 4.5b). In contrast to *C. scaura*, *C. equilibra* was negatively related with this factor as well as with turbidity and nitrogen, while it was positively related with the rest of explanatory variables. The average performance of the best significant models was good (AUC=0.88).

The abundance of *C. equilibra* was explained, in descending order of importance, by Zn, TOC and number of marina berths (Tables 4.2 and 4.3). However, the variability explained by the significant models was low (from 13.4% to 15.1%) and none of these factors significantly affected the species' abundance. The average performance of the best significant models was fair ($r=0.57$, $p<0.01$).

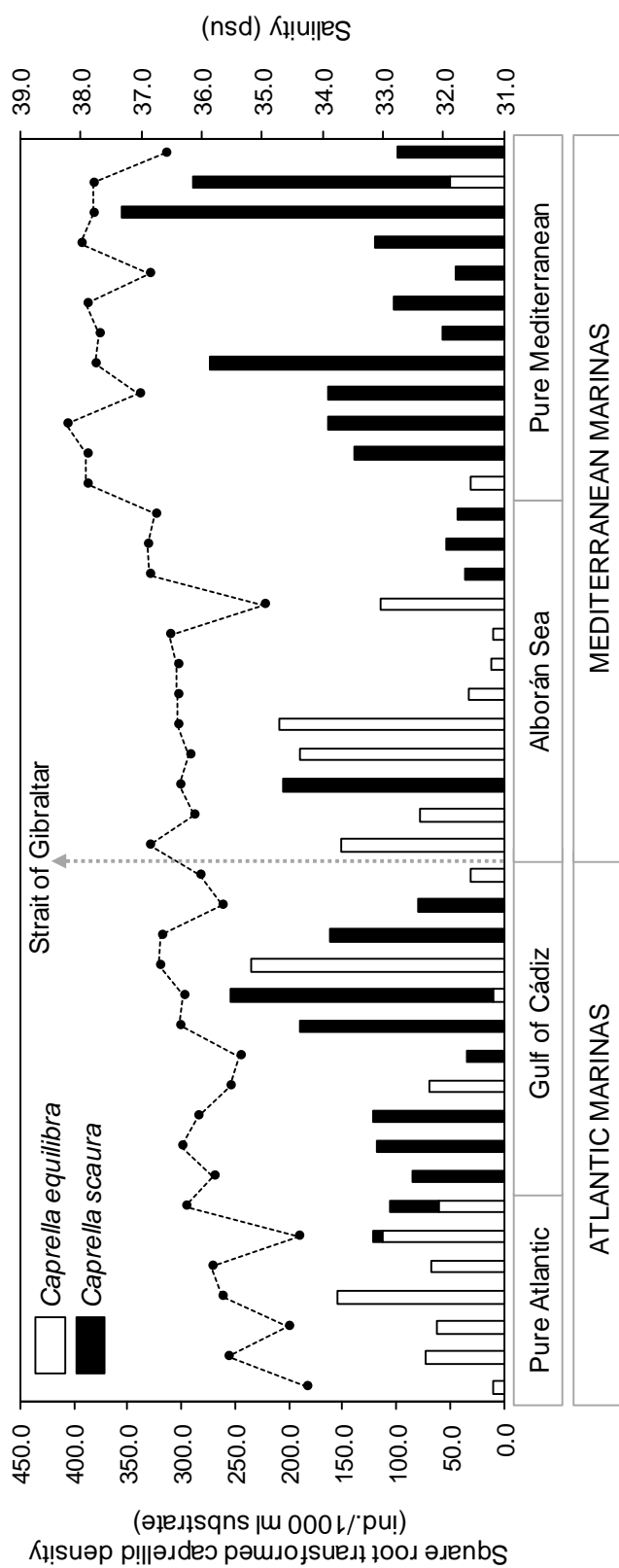


Figure 4.4 On the left axis the mean density of *C. scaura* and *C. equilibra* associated with *B. neritina* in the marinas surveyed are shown. On the right axis the mean salinity measured at each marina is shown.

Table 4.2 Model selection results explaining presence and abundance of *Caprella scaura* and *Caprella equilibra*. Akaike's Information Criterion corrected for small sample size (AICc), AICc weights (w_i) and AICc difference between the AICc of each model and the AICc of the best fitted model (Δ_i) were used for comparison. The model with the lowest Δ_i is the best AICc model. Variables: Sal, salinity; Turb, turbidity; Bug, frequency of appearance of *Bugula neritina*; Berths, number of marina berths; N, nitrogen; Zn, zinc; Pb, lead; PopD, human population density; TOC, total organic carbon.

BEST MODELS	df	AICc	Δ_i^*	w_i	LogLik	Phi	AdjR ²	Moran's I \pm SD
a) RESPONSE: <i>C. scaura</i> 's occurrence								
1a. Sal	3	58.85	0.00	0.19	-26.11	0.397	–	0.090 \pm 0.054
2a. Sal + Turb	4	59.46	0.61	0.21	-25.19	0.391	–	0.068 \pm 0.053
3a. Sal + Bug	4	60.17	1.32	0.15	-25.54	0.375	–	0.088 \pm 0.054
4a. Sal + Turb + Bug	4	60.49	1.64	0.13	-25.70	0.401	–	0.066 \pm 0.053
5a. Sal + PopD	5	60.52	1.67	0.12	-24.43	0.363	–	0.068 \pm 0.053
6a. Sal + Bug + PopD	5	60.82	1.97	0.11	-24.58	0.350	–	0.051 \pm 0.053
b) RESPONSE: <i>C. scaura</i> 's abundance								
1b. Sal + PopD + Pb + Bug	6	288.96	0.00	0.18	-136.15	–	0.475	0.082 \pm 0.082
2b. Sal + PopD + Pb + Bug + Turb	7	289.38	0.42	0.15	-134.39	–	0.520	-0.027 \pm 0.081
3b. Sal + PopD + Pb	5	289.89	0.93	0.11	-138.37	–	0.403	0.130 \pm 0.081
4b. Sal + PopD	4	289.99	1.03	0.11	-140.00	–	0.351	0.114 \pm 0.080
5b. Sal + Turb + Bug	5	290.11	1.15	0.10	-138.48	–	0.398	-0.101 \pm 0.081
6b. Sal + Turb + Bug + Pb	6	290.32	1.36	0.09	-136.83	–	0.446	-0.041 \pm 0.082
7b. Sal + Turb + Bug + PopD	6	290.34	1.38	0.09	-136.83	–	0.446	-0.081 \pm 0.081
8b. Sal + PopD + Bug	5	290.59	1.63	0.08	-138.72	–	0.386	0.055 \pm 0.082
9b. Sal + Pb + Bug	5	290.68	1.72	0.08	-138.76	–	0.384	0.092 \pm 0.082
c) RESPONSE: <i>C. equilibra</i> 's occurrence								
1c. Sal + PopD + Turb	4	46.81	0.00	0.24	-18.86	0.524	–	0.051 \pm 0.053
2c. Sal + PopD	3	47.05	0.24	0.21	-20.21	0.485	–	0.025 \pm 0.053
3c. Sal + PopD + Turb + Berths	5	47.84	1.03	0.14	-18.09	0.561	–	0.017 \pm 0.053
4c. Sal + PopD + TOC + Turb	5	48.40	1.60	0.11	-18.37	0.587	–	0.026 \pm 0.053
5c. Sal + PopD + Pb	4	48.57	1.76	0.10	-19.74	0.516	–	0.018 \pm 0.053
6c. Sal + PopD + Turb + N	5	48.60	1.80	0.10	-18.47	0.553	–	0.010 \pm 0.053
7c. Sal + PopD + Berths	4	48.71	1.91	0.09	-19.82	0.509	–	0.033 \pm 0.053
d) RESPONSE: <i>C. equilibra</i> 's abundance								
1d. Zn	3	239.16	0.00	0.43	-115.87	–	0.136	-0.187 \pm 0.092
2d. Zn + TOC	4	240.74	1.57	0.20	-115.12	–	0.151	-0.189 \pm 0.092
3d. Zn + Berths	4	241.15	1.99	0.16	-115.33	–	0.134	-0.136 \pm 0.093

Table 4.3 Model averaged coefficients (β) of explanatory variables present in the significant models for: a) *C. scaura* presence/absence, b) *C. scaura* abundance, c) *C. equilibra* presence/absence, d) *C. equilibra* abundance. Variables were ranked in order of the sum of their Akaike weights (Σw_i). Adjusted standard error (SE) of model averaged coefficients, z-values and p values are included. Star symbol represent significant coefficient values: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

PREDICTOR	β	Adjusted SE	z	P	Σw_i^a
a) <i>C. scaura</i> presence/absence:					
Intercept	-6.85	2.80	2.45	0.014*	
Sal	0.20	0.08	2.67	0.008**	1.00
Turb	0.01	0.02	0.52	0.604	0.38
Bug	0.04	0.07	0.52	0.602	0.33
PopD	0.01	0.04	0.37	0.713	0.23
b) <i>C. scaura</i> abundance:					
Intercept	-1614.08	661.54	2.44	0.015*	
Sal	42.45	18.27	2.32	0.020*	1.00
Bug	34.35	17.14	2.00	0.045*	0.78
PopD	23.36	11.88	1.97	0.049*	0.73
Pb	-2.14	1.20	1.78	0.075	0.62
Turb	7.95	4.75	1.67	0.094	0.44
c) <i>C. equilibra</i> presence/absence:					
Intercept	64.44	25.48	2.53	0.011*	
Sal	-1.77	0.70	2.55	0.010*	1.00
PopD	0.00	0.00	1.69	0.090	1.00
Turb	-0.23	0.14	1.60	0.110	0.59
Berths	0.00	0.00	1.01	0.314	0.24
TOC	0.07	0.08	0.92	0.360	0.11
Pb	0.05	0.05	0.92	0.358	0.10
N	-0.66	0.83	0.80	0.424	0.10
d) <i>C. equilibra</i> abundance:					
Intercept	119.98	31.94	3.76	0.001***	
Zn	-2.24	1.16	1.94	0.052	1.00
TOC	-1.96	1.81	1.08	0.279	0.20
Berths	-0.15	0.05	0.92	0.359	0.16

^a Σw_i values are recalculated considering only the models with $\Delta AICc \leq 2$

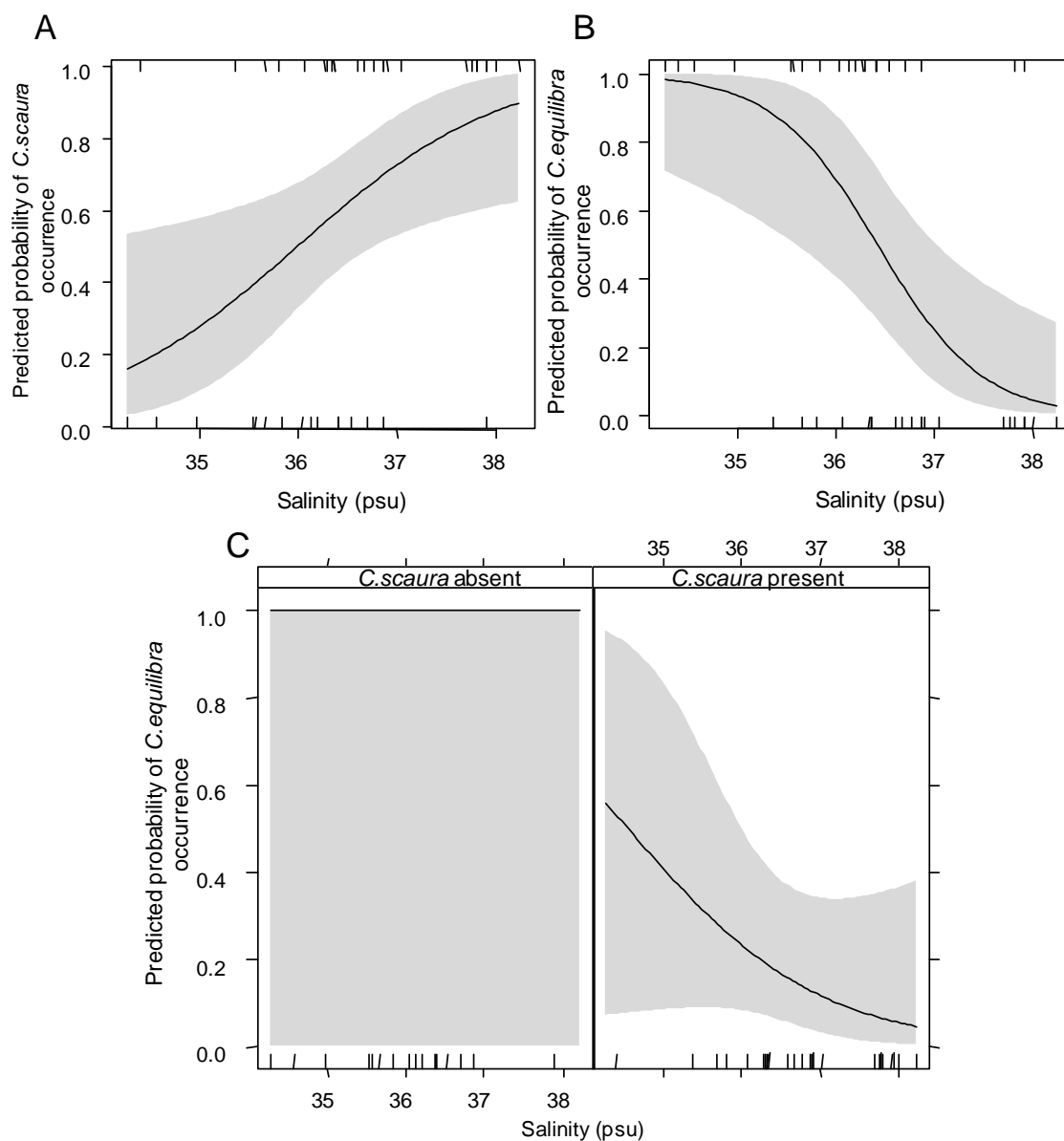


Figure 4.5 GLM plots showing of the predicted effect of salinity on: (A) the occurrence (presence/absence) of *C. scaura*; (B) the occurrence of *C. equilibra*; (C) the occurrence of *C. equilibra* without and with the presence of *C. scaura*. Shades indicate 95 % confidence limits for the fitted relationship.

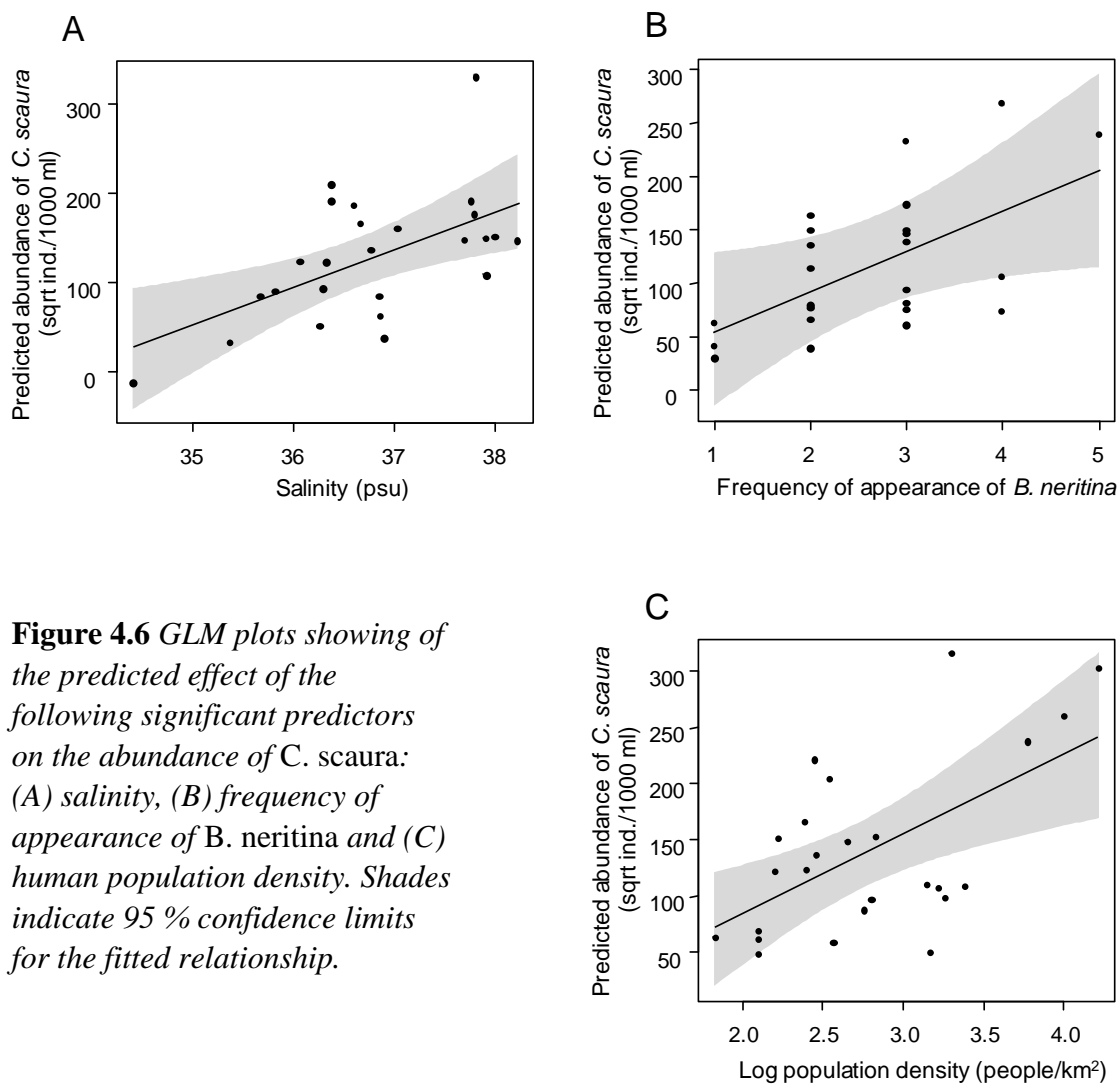


Figure 4.6 GLM plots showing of the predicted effect of the following significant predictors on the abundance of *C. scaura*: (A) salinity, (B) frequency of appearance of *B. neritina* and (C) human population density. Shades indicate 95 % confidence limits for the fitted relationship.

4.3.4 Competitive interactions

A simple correlation between the densities (not conditioned on presence) of *C. scaura* and *C. equilibra* in all marinas surveyed along the study area showed that the two dominant caprellid species associated with the same host substratum are negatively related to each other ($r_{s(n=42)} = -0.74$, $p < 0.01$) and seem to display a potential exclusion pattern. When a factor describing *C. scaura*'s occurrence was added as explanatory variable in the best fitted correlation regression model on the presence/absence of *C. equilibra*, we obtained a significant reduction in the residual deviance (i.e. the variance which could not be explained by the predictor terms) (Table 4.4). This resulted in an increase of the variability explained by the model which included *C. scaura* by 83.24% in comparison with the model without *C. scaura* as explanatory variable.

Furthermore, model performance was improved in the model that included *C. scaura* (AUC increased from 0.87 to 0.99). The inclusion of an interaction term between *C. scaura* and salinity in best fitted model on the presence/absence of *C. equilibra* revealed that *C. equilibra* was unaffected by the variations of salinity along the study area in absence of *C. scaura*. However, when *C. scaura* is present, its relationship with salinity is the same that obtained for the model without *C. scaura* as explanatory variable (Fig. 4.5c).

Table 4.4 ANOVA results examining the contribution of a term describing *C. scaura* occurrence in explaining the occurrence of *C. equilibra*.

Regression model	Residual df	Residual deviance	Change in deviance	<i>P</i>
Best environmental model	38	37.724		
Best environmental model + <i>C.scaura</i> occurrence	37	6.323	31.401	0.0001

The residual deviance of the best fitted model for the abundance of *C. equilibra* was also reduced by the addition of a predictor variable describing *C. scaura*'s abundance, but this reduction was not statistically significant. Regarding with best fitted model's performance, it also was improved after inclusion of *C. scaura*'s abundance as explanatory variable (from $r = 0.534$, $p < 0.05$ to $r = 0.622$, $p < 0.01$).

4.3.5 Spatial autocorrelation

The examination of spatial autocorrelation in the response variables showed that presence/absence of *C. scaura* and *C. equilibra* were positively autocorrelated (*C. scaura*'s occurrence: Moran's $I = 0.13$, $p < 0.01$; *C. equilibra*'s occurrence: Moran's $I = 0.16$, $p < 0.001$), while species abundance (conditioned on presence) were not spatially autocorrelated. Examination of the best fitted occurrence and abundance model residuals for the two species revealed that they were no longer spatially autocorrelated (Table 4.2), suggesting that these models sufficiently account for the spatial structuring in the response variable. Therefore, we assumed that model residuals were independent from each other and it was not necessary to include spatial scale in the models.

4.4. DISCUSSION

Although the non-native *C. scaura* and the native *C. equilibra* are similar ecologically, largely overlapping in their habitat use niche, our results represent the first field evidence that these two congeneric caprellids are strongly spatially segregated within the Iberian Peninsula, showing a limited spatial overlap. The non-native caprellid dominates in the Mediterranean coast of the Iberian Peninsula, warmer and saltier than the Atlantic coast, in which the native *C. equilibra* remains as the dominant fouling caprellid amphipod. If we disentangle the factor or combination of factors involved in this apparent exclusion pattern, we could predict which habitats will be more susceptible to be successfully occupied and impacted by the newcomer species *C. scaura*.

4.4.1 Biotic and abiotic factors involved in the distribution of *C. scaura* and *C. equilibra*

Among the variables measured, habitat use models point to salinity as the main environmental factor involved in the occurrence (presence/absence) pattern of the two species. The non-native *C. scaura* was positively related with salinity while *C. equilibra* exhibited the opposite pattern. However, it is well known that both species support a broad range of salinity conditions. Cockman and Albone (1987) found, after 24 h under laboratory conditions, LC50 values of 7.5 and 11.5 for *C. scaura* and *C. equilibra* respectively. A decade before detection of *C. scaura* in the Lagoon of Venice, Sconfiatti and Lupparia (1995) found that, although *C. equilibra* was more abundant in saltier waters at this lagoon, it was able to support partially estuarine conditions (since it tolerated salinity decreases to 15), being more euryhaline than other common fouling caprellids. Furthermore, *C. equilibra* has also commonly found in hypersaline waters (> 40 psu), like those presents along the Suez Canal (Emara and Belal 2004). This is not consistent with the spatial pattern observed for *C. equilibra* in the present study, in which the species seem to be more successful in relatively low salinity conditions. When the occurrence of *C. equilibra* in function of salinity in a scenario with absence of *C. scaura* was modeled, the probability to find *C. equilibra* in a particular marina was not correlated with salinity. In fact, salinity was not a relevant factor in the abundance models developed for this species (unlike what happens with *C. scaura*). Taking into account that *C. scaura* and *C.*

equilibra only coexists in four marinas, abundance models (conditioned on presence) virtually modeled the abundance of *C. equilibra* without strong influence of *C. scaura*. Consequently, both the shapes of *C. equilibra* responses to salinity and the positions of its ecological optima change when *C. scaura* is present. This suggests that salinity mediates the competitive interactions between *C. equilibra* and *C. scaura* for occupying the same host substratum (*B. neritina*). Thus, in the Mediterranean coast, saltier than the Atlantic coast, *C. scaura* may be a superior competitor and may displace *C. equilibra* to areas with low salinity in which *C. scaura* may be an inferior competitor. The frequency and intensity of the competitive interactions would determine the extent of *C. equilibra* displacement.

Although poorly known in marine ecosystems, the interplay of salinity and competition has been well investigated in freshwater habitats. For example, salinity is considered a critical factor modulating the invasive impact of mosquitofish, which displaces native species to saltier waters (Nordlie and Mirandi 1996; Alcaraz *et al.* 2008). In the case of freshwater amphipods, Kestrup and Ricciardi (2009) showed that conductivity mediates the rate and direction of interspecific predation between the invasive amphipod *Echinogammarus ischnus* and the native amphipod *Gammarus fasciatus* in the Great Lakes basin. In addition to salinity, temperature has been observed as another important factor driving biotic interactions among native and invasive freshwater amphipods (Poeckl and Timischl 1990). Taking into account that salinity was highly positively correlated with temperature along the Iberian Peninsula, we may assume that a combination of relatively high temperature and salinity could favor the competitive advantage and the establishment success of *C. scaura*. Wijnhoven *et al.* (2003) studied the combined effects of temperature and salinity and showed that *Gammarus tigrinus* had wider temperature tolerance range in ion rich waters, whereas *Dikerogammarus villosus* shows reduced tolerance to high temperatures in ion poor water.

However, most of these studies rely on artificial manipulations and few studies are based on field data. In a comprehensive field-based study, Grabowski *et al.* (2009) found that alien species dominated over natives at sites with raised conductivity values in Poland, but they could not distinguish between competitive interactions or different ecological preferences between natives and aliens as the cause underlying the exclusion pattern found.

Introducing competitive terms into habitat modeling can significantly increase the realism and precision of these models (Leathwick and Austin 2001; Austin 2002; Anderson *et al.* 2002; Wang and Jackson 2011). However, it is difficult to estimate whether the current absence of a particular species is due to competition or is due to an unidentified environmental variable (Guisan and Thuiller 2005; Elith and Leathwick 2009). Our approach uses statistical analysis of field data to understand first, the environmental effect on species distributions (based on a comprehensive range of potential environmental explanatory variables) and then, the potential of *C. scaura* to explain the distribution pattern observed for *C. equilibra* which is not explained by the environmental factors measured (see Leathwick and Austin 2001). Besides that we found a significant contribution of *C. scaura* term to explain the occurrence of *C. equilibra*, we suggest the existence of interspecific competition between *C. scaura* and *C. equilibra* because they overlap in a large part of its ecological niche: (1) they have been found in similar climate regions (temperate and tropical seas around the world); (2) they inhabit similar primary (e. g. artificial substratums) and secondary habitats (e. g. *B. neritina*); (3) they are trophically similar (both species are detritivores and opportunists; Guerra-García and Tierno de Figueroa 2009); (4) they are potentially dispersed through similar dispersal vectors (they have similar probabilities of introduction in a particular marina); and (5) both were reproductively active in the Atlantic and Mediterranean marinas surveyed at the moment of collection (standing well the environmental conditions at these marinas). Consequently, we assume that both species could have similar ecological preferences and that it is more probably that the exclusion pattern found relies on competitive interactions than on different ecological tolerances. Experimental studies are necessary both to confirm this hypothesis and to understand the mechanisms underlying these interspecific encounters.

In laboratory-based competition experiments, Shucksmith *et al.* (2009) found that the invader *Caprella mutica* successfully displaced two ecologically similar native European caprellids (*Caprella linearis* and *Pseudoprotella phasma*). Boos (2009) showed that the underlying mechanism of displacement of *C. linearis* by *C. mutica* was interference competition, caused by aggressive and predatory attacks by *C. mutica*. Several studies have highlighted the aggressive behavior of *C. scaura* (Aoki 1997; Lim and Alexander 1986; Schulz and Alexander 2001), which together with its parental care (Aoki 1999) have been related with its reproductive success and survival of its offspring. This may allow

the agonistic exclusion of *C. equilibra* by *C. scaura* under favorable conditions. Interestingly, although *C. equilibra* can obtain refuge in areas in which *C. scaura* cannot successfully outcompete, like the north Atlantic coast of the Iberian Peninsula, the recent detection of *C. mutica* in marinas from this area (only one year later of our sampling survey) by Almón *et al.* (2014) represents a cause for concern.

4.4.2 The Strait of Gibraltar: a coexistence area

There is an ongoing debate on whether competition is less intense in stressful environments (e.g. Brooker *et al.* 2005). Recreational marinas are highly disturbed areas which support important fluctuations of environmental parameters like salinity, temperature, organic inputs or metal pollutants in comparison with adjacent waters. Since both species are commonly found in this habitat type, we may assume that both are well adapted to these stressful conditions and that disturbance would not play a relevant role in modulate the biotic interactions between the two species. For example, both species are positively related with human population density, an indicator of the relative anthropogenic disturbance (Murray *et al.* 2014), being a significant factor determining the abundance of *C. scaura* and a relevant factor determining the occurrence of *C. equilibra*. However, marinas located under the influx of the Strait of Gibraltar are also subject to the stressful conditions provided by the mixture of waters between the Mediterranean Sea and the Atlantic Ocean. In fact, the distribution pattern of both species in southern Spain and northern Africa is less spatially structured than in the rest of the Iberian Peninsula. Within this transitional area, *C. equilibra* dominates the Mediterranean marinas located in the Alboran Sea while *C. scaura* dominates in those located in the Gulf of Cádiz (Atlantic Ocean). This apparent inverse spatial pattern reflects the environmental heterogeneity of the Strait of Gibraltar in which the Alboran Sea receives the incoming Atlantic waters while the Gulf of Cádiz receives the outgoing Mediterranean waters which flow through the Strait of Gibraltar. Consequently, although the exclusion pattern may exist in a local level (marina), this environmental heterogeneity seems to favor the coexistence of the two species in a broad context (southern Spain), limiting both the displacement of *C. equilibra* in the southern Mediterranean coast and the influence of *C. scaura* in the conformation of *C. equilibra* realized niche.

4.4.3 Current status of *C. scaura* in the Mediterranean

Taking into account both the rapid expansion of *C. scaura* along the Mediterranean (Krapp *et al.* 2006; Ros *et al.* 2014b) and its potential impacts over similar congener species, the precautionary approach suggest raising the status of *C. scaura* in the Mediterranean from non-native to invasive species. Prato *et al.* (2013), based on the low abundance of *C. scaura* associated with seaweeds in a semi-enclosed bay of Taranto (Italy), classified the species as ‘weak invader’. This contrasts with the high abundances found for *C. scaura* in other studies (Martinez and Adarraga 2008; Guerra-García *et al.* 2011; Fernández-Gonzalez *et al.* 2014; Ros *et al.* 2013) suggesting that such type of affirmations may be context-dependents and should be taken with caution. Beyond the abundant pattern of an introduced species in a particular site, the evaluation of the potential interactions with other species may give us relevant clues about its invasive potential.

4.4.4 Conclusions

Our analyses provide statistical evidence, based on field data, for a competitive displacement of the native *C. equilibra* by the invasive caprellid *C. scaura*, and point out the critical role of salinity in modulate this interaction. Although experimental studies are necessary to confirm our results and understand the mechanisms that mediate these interactions, we hypothesize that the low salinity together with the low temperature of the central and north Atlantic coast of the Iberian Peninsula, in comparison with the Mediterranean coast, limit the spread and invasive success of *C. scaura*, providing a competitive refuge for other previously established species that occupy similar habitats. This study illustrate the importance of integrating biotic and abiotic factors in modeling marine invasive species distribution and detecting potential impacts on recipient communities. Future studies are necessary for a better prediction of the long-term consequences of this interaction.

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5

VECTORS OF SPREAD: PART 1

The role of marinas and recreational boating in the occurrence and distribution of exotic caprellids in the Western Mediterranean

- Adapted from:

Ros M, Vázquez-Luis M, Guerra-García JM (2013) The role of marinas and recreational boating in the occurrence and distribution of exotic caprellids (Crustacea: Amphipoda) in the Western Mediterranean: Mallorca Island as a case study. *Journal of Sea Research* 83: 94–103.

ABSTRACT. In the Mediterranean Sea, the number of alien marine crustacean species has increased over the past two decades. However, knowledge about small alien marine crustaceans, like caprellid amphipods, is still very scarce. To understand the role of marinas and recreational boating in the early step of the invasion process by non-indigenous caprellids, we studied the recreational boating pressure and the spatial distribution of caprellid species in Mallorca Island. We collected caprellids from 14 marinas and 9 exposed intertidal rocky shores between November 2011 and April 2012 and we analysed the differences in habitat use of native and exotic caprellids. Eight caprellid species, six native and two exotic, were found. Alien caprellids were only present in marinas, reaching high densities of population. The analysis of recreational boating pressure reveals that Palma-Migjorn is the area that is subject to the highest potential risk of introduction of exotic species via ship fouling. In the secondary dispersal of alien caprellids, the study reflects that recreational boating seems effective as a secondary vector in the transport of exotic species from marinas to marinas but not from marinas to natural and exposed areas. An illustrated key of caprellids from Balearic Island is provided to differentiate native and non-indigenous species.

RESUMEN. En el mar Mediterráneo, el número de especies exóticas de crustáceos se ha ido incrementado desde hace dos décadas. Sin embargo, el conocimiento que se tiene de las especies de menor tamaño, como los anfípodos caprelídeos, es todavía muy escaso. Para entender el papel de los puertos deportivos y las embarcaciones de recreo en una etapa temprana del proceso de invasión de caprelídeos introducidos, se estudió la presión ejercida por las embarcaciones de recreo y la distribución espacial de las especies de caprelídeos en la isla de Mallorca. Se recolectaron los caprelídeos presentes en 14 marinas y 9 zonas de intermareal rocoso entre noviembre de 2011 y abril de 2012 y analizamos las diferencias en el uso del hábitat entre caprelídeos nativos e introducidos. Se encontraron un total de 8 especies, 6 nativas y 2 exóticas. Los caprelídeos introducidos se encontraron exclusivamente en puertos deportivos, donde alcanzaron grandes densidades. El análisis de la presión por embarcaciones de recreo reveló que el area de Palma-Migjorn es la que tiene un mayor riesgo de introducción de especies exóticas a través de la asociación de estas especies a los cascos de los barcos. Respecto a la dispersión secundaria de los caprelídeos exóticos, el estudio reflejó que las embarcaciones de recreo son efectivas en el transporte de especies entre puertos deportivos pero no entre éstos y los hábitats costeros naturales. Se incluye una clave ilustrada de los caprelídeos de las Islas Baleares con el objeto de diferenciar entre las especies nativas y no-nativas.

5.1 INTRODUCTION

Human-mediated biological invasions involve the transport of a species to an area where it does not naturally occur. Reducing the threat of new invasions requires a focus on the ways humans enable the transport and establishment of species in new areas (Floerl and Inglis 2005). Despite the large number of private and commercial recreational boats in coastal locations worldwide, the role of recreational boating in the transport of non-indigenous species (NIS) has received little attention to date (Minchin *et al.* 2006). A recent study showed that recreational boats represent a high-risk vector both for primary introduction and secondary spread of marine NIS (Murray *et al.* 2011). Small recreational boats can travel long distances, and their relatively low speeds (compared to commercial ships) make them ideal vectors for fouling species (Minchin *et al.* 2006). These fouling assemblages reflect in part the composition of biotic assemblages within the marina in which small boats were moored (Floerl and Inglis 2005).

Marinas are critical entry points for NIS and may act as reservoirs for introduced species (e.g. Glasby *et al.* 2007). This is particularly important in the case of small invertebrates, like caprellid amphipods, because marinas' fouling communities include arborescent substrates such as bryozoans or hydroids which may act as habitat for non-indigenous caprellids (Ros *et al.* 2013a). These small marine crustaceans, commonly known as skeleton shrimps, are very successful colonizers of artificial hard substrate, reaching high densities in harbors and marinas (Buschbaum and Gutow 2005; Ashton *et al.* 2010) including biofouling on ship hulls (Frey *et al.* 2009). They are also common in many littoral habitats being an important trophic link between primary producers and higher trophic levels (Woods 2009). The morphology of caprellids, with reduced abdominal appendages which in other amphipods are used for swimming (Takeuchi and Sawamoto 1998) as well as a lack of a planktonic larval stage, suggests that the cosmopolitan distribution of many littoral caprellids is facilitated by the fact that they are often associated with fouling communities on floating objects and vessels (Thiel *et al.* 2003). Marinas can also act as stepping stones for the colonization of natural habitats (Cangussu *et al.* 2010). Therefore, a high recreational boating pressure may involve a high risk of dispersion of exotic species. However, although marinas can act as reservoirs of non-indigenous

caprellids, it is still unclear if these exotic caprellids can colonize natural and preserved intertidal rocky shores.

In the Mediterranean Sea, the number of alien marine crustacean species has increased over the past two decades (Galil 2011). However, invasions are rarely reported amongst smaller-bodied and taxonomically more challenging taxa (Carlton 2011) such as caprellid amphipods, sometimes difficult to identify to species level. Recently, two non-indigenous caprellids were found for the first time in marinas of the Balearic Islands, in the western Mediterranean Sea: *Caprella scaura* Templeton, 1836 and *Paracaprella pusilla* Mayer, 1890. The widespread species *Caprella scaura* was first recorded on the Northeastern coast of Spain in 2005 (Martinez and Adarraga 2008), in southern Spain in 2010 (Guerra-García *et al.* 2011a) and on the islands of Mallorca and Menorca in 2011 (Ros *et al.* 2013b). *Paracaprella pusilla* was recorded for the first time in European waters of the South coast of Spain in 2010 (Ros and Guerra-García 2012), and only one year later was detected for the first time in the Mediterranean Sea, on the islands of Mallorca and Ibiza (Ros *et al.* 2013b).

This study intends to provide new data to contribute to the knowledge of the diversity and distribution of crustacean caprellids along the coast of Mallorca comparing artificial habitat (marinas) with natural and exposed areas (intertidal rocky shores), including the detection of native and exotic species which have not been found previously in the Balearic Islands. We also compare the habitat use of native and exotic caprellids in the island to identify the habitats that are more susceptible to harbor introduced species and to understand the most probable vector for their introduction. Considering that the majority of exotic caprellids are introduced into a new area via ship fouling (Krapp *et al.* 2006) and dispersed secondarily via recreational boating (Ashton *et al.* 2006), this study will estimate the pressure of recreational boating in the different coastal regions of Mallorca to understand its role as a secondary vector in the dispersion of this group and to identify areas with a higher risk of introduction of alien species through this vector. In order to provide a monitoring tool for the early detection of exotic caprellids by non-taxonomic experts, we provide an illustrated key for the caprellidea in the Balearic Island including the species detected in the present study in Mallorca and those detected previously for the Balearic Islands.

5.2 MATERIALS AND METHODS

5.2.1 Study area

The survey was carried out on Mallorca Island, located in the centre of the western Mediterranean. Mallorca is the largest of the Balearic Islands with 719 km of coastline (Fig. 5.1). In spite of having a large number of marine protected areas (MPAs) in comparison with other Mediterranean regions, it is characterized by intense maritime traffic and is the location with the highest number of moorings in Spain (FEAPDT, 2011). All of this characteristics makes this island a potential hot spot of biological invasions via shipping traffic.

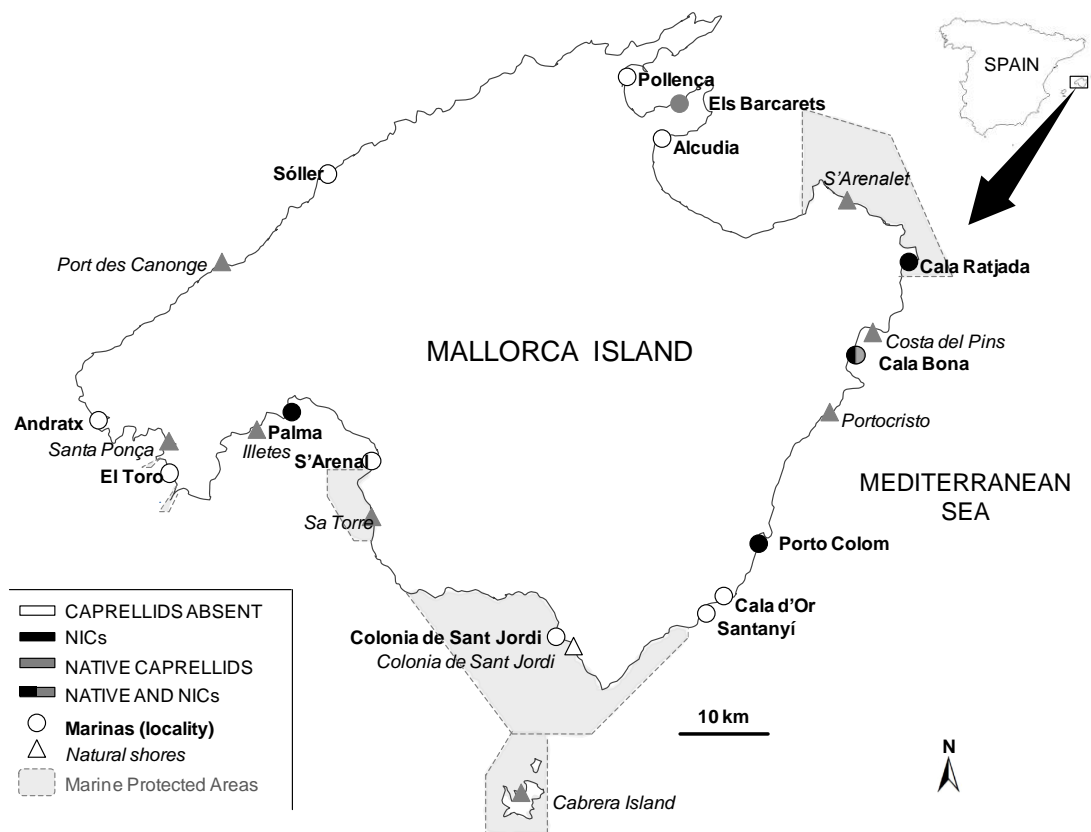


Figure 5.1 Study area showing marinas and intertidal rocky shores sampled and the presence/absence of native and non-indigenous caprellids (NICs) in the sampling stations.

5.2.2 Sampling

The sampling programme was conducted between November 2011 and April 2012 and was divided in two parts: an artificial (anthropogenic) hard substrate survey, carried out in marinas (recreational ports), to detect presence and quantify abundance of native and non-indigenous caprellids; and a natural hard substrate survey, carried out in natural rocky shores, with two aims (1) detect the presence of alien caprellids in natural habitats and (2) characterize the diversity of caprellid fauna in natural hard substrata due to scarce studies about this group in the study area.

In the artificial hard substrate survey, a total of 14 recreational marinas along the whole coast of Mallorca were sampled to ensure a complete review of the total fouling communities which proliferate on artificial hard substrate including floating pontoons, ropes, buoys, wheels and ship hulls. When caprellids were detected in a type of fouling substrate (hydroids, bryozoans or macroalgae), three random replicates of each substrate were taken by hand and fixed in situ in 90% ethanol.

In the natural hard substrate survey, we selected 9 natural rocky shores well exposed to wave action and located in relatively undisturbed areas with a low anthropic pressure. In this case, we collected hydroids and macroalgae from the intertidal zone directly by hand at low tide, and subtidal snorkeling provided algal samples from shallow waters (1-5 metres deep). Each substrate was collected individually from different rocks to avoid a patchy effect and to adequately sample caprellid diversity, until a volume of approximately 150 ml of substrata was completed (see Thiel *et al.* 2003; Guerra-García *et al.* 2011b). Samples were preserved in 90% ethanol.

5.2.3 Laboratory processing

All caprellids were sorted and identified to species level. Abundance of caprellids was expressed as number of individuals/1000ml of substrate because of the different structure of the substrate types (see Pereira *et al.* 2006). Volume of substrates was estimated as the difference between the initial and final volume when placed into a graduated cylinder with a fixed amount of water.

5.2.4 Statistical analysis

The affinities among native and non-native caprellid species based on their presence/absence in the sampling stations were established through cluster analysis using the UPGMA (unweighted pair group method using arithmetic averages), based on the Bray-Curtis similarity index.

To characterize the frequencies of small craft movement in the different coastal regions of Mallorca, we use the Index of Recreational Port Capability (RPCI). This index refers to the number of moorings/marina berths available within a given region of coastline in kilometres (Occhipinti-Ambrogi 2002). In order to simplify the subsequent analysis, the anchoring zones have been grouped according to five HEMUs (Homogeneous Environmental Management Units) of Mallorca (Balaguer *et al.* 2011). Concentrations of marinas and berthing space (high RPCI) are likely to be associated with high frequencies of small craft movements (Minchin *et al.* 2006). However, not all boats located in marinas operate at the same time or with the same frequency, and there is a significant proportion that hardly ever, or never sail (Balaguer *et al.* 2011).

To estimate the secondary dispersal potential of exotic species by recreational boating, we developed a simple but useful index for comparison between different areas: the Recreational Boating Dispersal Index (RBDI), which consists of the number of boats that sail regularly within a given region of coastline in kilometres. To estimate the number of boats that sail regularly on Mallorca Island we use the study by Balaguer *et al.* (2011), which estimated that the percentage of boats leaving their marina to navigate on the busiest day of the high season on Mallorca Island was 49% with 87% of these vessels staying in the waters around Mallorca.

5.3. RESULTS

5.3.1 Caprellids assemblages and habitat use

A total of eight caprellid species were found during the sampling programme, 6 native: *Caprella acanthifera* Leach 1814, *Pseudoprotella phasma* (Montagu 1804), *C. hirsuta* (Mayer 1890), *C. liparotensis* Haller 1879, *C.*

danilevskii Czerniavski 1868 and *C. grandimana* Mayer 1882; and 2 non-native: *Caprella scaura* and *Paracaprella pusilla*. On the basis of their presence/absence in the sampling stations, caprellids found in the present study were classified in three groups (Fig. 5.2): species found in intertidal exposed rocky shores (group I), species found both in marinas and intertidal exposed rocky shores (group II) and species found exclusively in marinas (group III). All native species belonged to group I except *C. acanthifera* which was found in both intertidal exposed rocky shores and marinas, belonging to group II. All non-native caprellids belonged to group III.

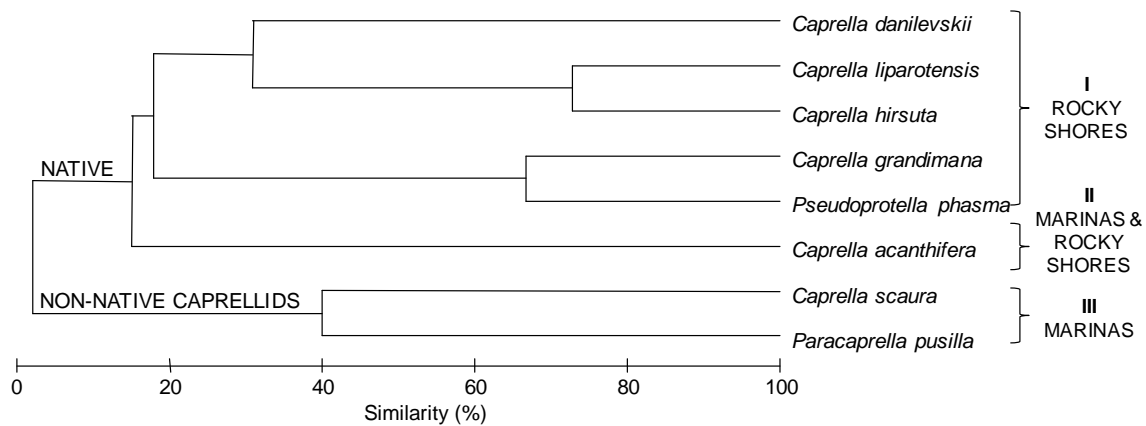
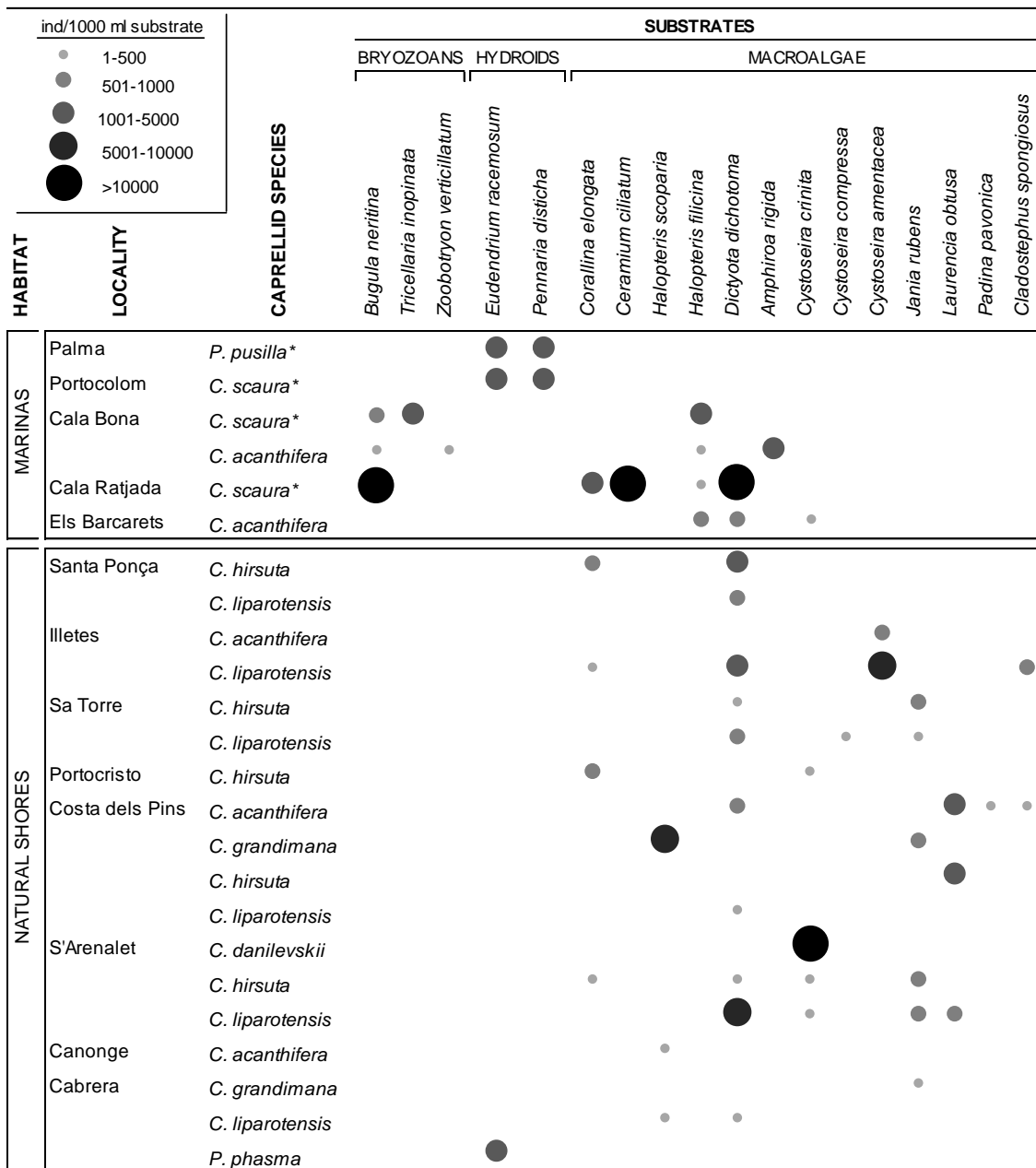


Figure 5.2 Dendrogram of similarity between the different caprellid species based on their presence/absence in the sampling stations.

Eighteen different substrate species hosted caprellids (Table 5.1) and four of these, *Eudendrium racemosum*, *Corallina elongata*, *Dictyota dichotoma* and *Cystoseira crinita* were present both in artificial hard substrate (marinas) and in natural hard substrate (platforms of intertidal rocky shores). *Caprella acanthifera*, the only species that belonged to group II, was found in two marinas and three intertidal rocky shores and exhibited relatively similar abundances in the two types of habitat. This native caprellid was found associated with the highest variety of host substrates, being found in up to ten different ones, followed by the invasive caprellid *C. scaura*.

Table 5.1 Species composition of the caprellidea collected from Mallorca Island by substrates and sampling stations.



* Non-native species

5.3.2 Non-indigenous caprellid populations

Caprella scaura was found in three marinas located in the east coast of the island: Cala Ratjada (39° 43' N 3° 28'E), Cala Bona (39° 37' N 3° 23'E) and Porto Colom (39° 25' N 3° 15'E) associated with eight different host substrates including hydroids, bryozoans and macroalgae of the marinas' fouling

community. It was the caprellid species that exhibited the highest abundance in the present study, particularly in Cala Ratjada marina, associated with the bryozoan *Bugula neritina* and the macroalgae *Ceramium ciliatum* and *Dictyota dichotoma* (Table 1). The other non-indigenous species, *Paracaprella pusilla*, was only found in Palma marina (39° 34' N 2° 38' E) associated with two hydroid species.

Regarding the habitat use of exotic caprellids in the Mediterranean Sea and adjacent areas, we analyzed the habitats in which they were found (Table 5.2) and we observed that the majority of records are from artificial hard substrate, dominated by marinas and harbours and followed by buoys and fish farm cages (Fig. 5.3). Only a few percentages of records are from natural habitats, including seagrass and seaweeds from enclosed bays with anthropogenic influence. No records were found in relation to exposed and preserved intertidal rocky shores.

Table 5.2 Habitat distribution of non-native caprellids in the Mediterranean Sea and Strait of Gibraltar.

Caprellid	Country	Locality	Habitat	Date of record	Source
<i>C. scaura</i>	Italy	Lagoon of Venice	Wooden piles	1994	Sacchi <i>et al.</i> 1998; Mizzan 1998
	Italy	Ravenna	Harbour	2004	Sconfiatti <i>et al.</i> 2005
	Italy	Lagoon of Venice	Buoys	2004	Krapp <i>et al.</i> 2006
	Italy	Sicily	Buoys	2004	Krapp <i>et al.</i> 2006
	Italy	Livorno	Harbour	2004	Galil <i>et al.</i> 2008
	Italy	Mar Piccolo of Taranto	Seaweeds	2007	Eleftheriou <i>et al.</i> 2011
	Greece	Amvrakikos gulf	Seagrass	2002	Krapp <i>et al.</i> 2006
	Spain	Roses Bay	Artificial hard substrata	2005	Martinez and Adarraga 2008
	Spain	Cádiz and Chipiona	Marina	2009	Guerra-García <i>et al.</i> 2011
	Spain	Mallorca	Marina	2011	Ros <i>et al.</i> 2013b; present study
	Turkey	Güllük Bay	Cages of a fish farm	2008	Bakir and Katagan 2011
	Tunisia	Bouhrara and Bibans	Seaweeds	2009	Ben Souissi <i>et al.</i> 2010
<i>P. pusilla</i>	Spain	Cádiz	Marina	2010	Ros and Guerra-García 2012
	Spain	Mallorca	Marina	2011	Ros <i>et al.</i> 2013b; present study

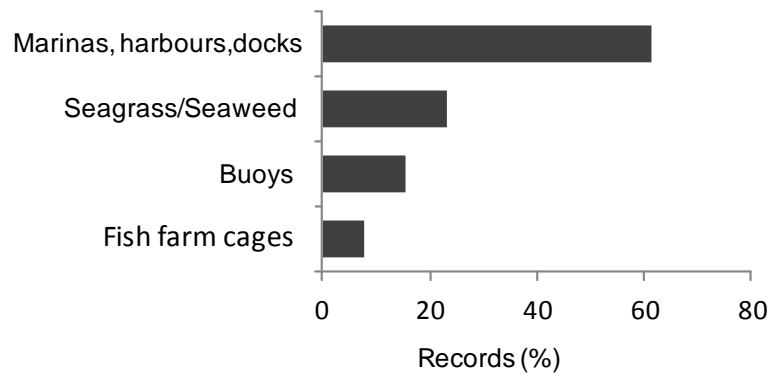


Figure 5.3 Reported distribution of non-indigenous caprellids among types of habitat/substrates in the Mediterranean Sea and the Strait of Gibraltar.

5.3.3 Recreational boating movements

The average RPCI for Mallorca was 17.4, with the highest concentrations of berths occurring in the HEMU of Palma-Migjorn, located on the southwest coast of the Island, with a RPCI of 60.3 (Table 5.3). This region is also subject to the highest potential risk of secondary dispersal of exotic species, with a RBDI four to five times higher than other regions (Fig. 5.4).

Table 5.3 Recreational Port Capabiliti Index (RPCI) and Recreational Boating Dispersal Index (RBDI) for Mallorca island.

Anchoring Zones (HEMUs)	Coastline kilometres ^a	Number of recreational marinas berths ^a	RPCI	Boats sails regularly ^a	RBDI
Ponent	146	2377	16.3	1013	6.9
Llevant	188	3203	17.0	1365	7.3
Nord	197	3057	15.5	1303	6.6
Tramuntana	104	568	5.5	242	2.3
Palma-Migjorn	84	5065	60.3	2159	25.7
Total results	719	14270	19.8	6082	8.5

^aData obtained from Balaguer *et al.* (2011)

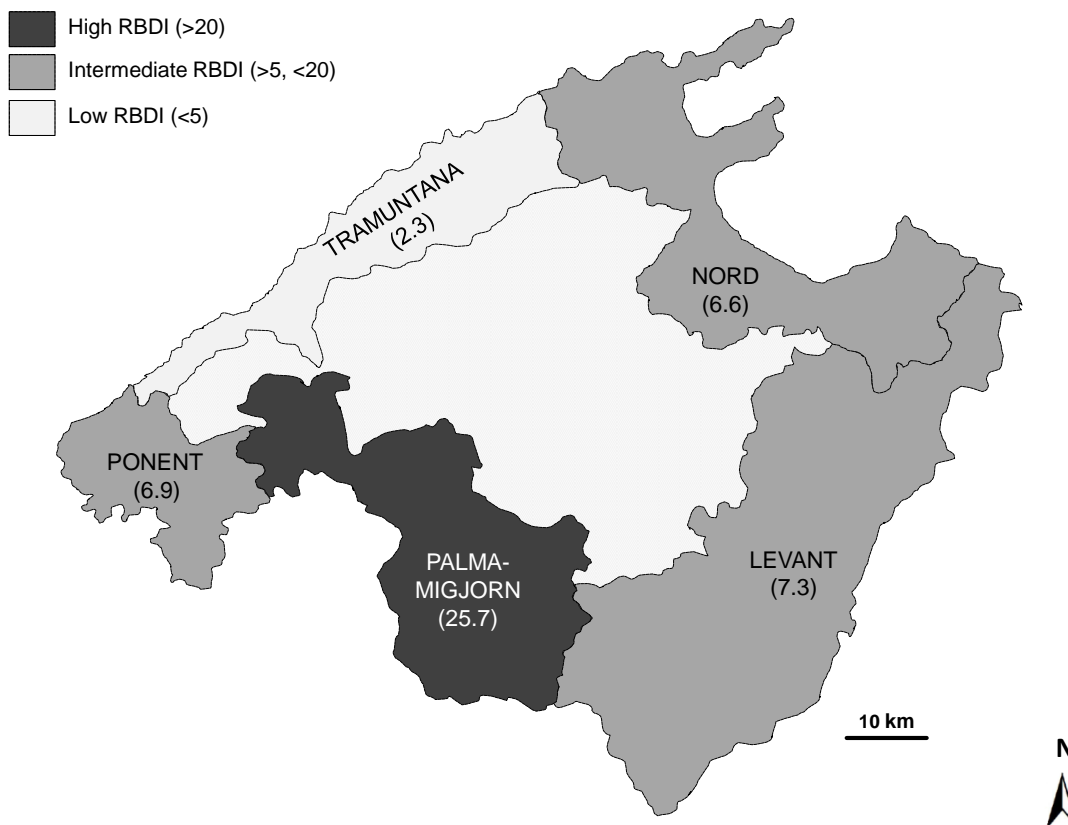


Figure 5.4 Map of Mallorca showing the different Homogeneous Environmental Management Units (HEMUs) and the Recreational Boating Dispersal Index (RBDI) calculated for each HEMU.

5.3.4 Illustrated key of caprellids from Balearic Islands

Accurate caprellid species identification, the first step in detecting non-native species, can be difficult due to the small size of specimens and, in some cases, the need to dissect the mouthparts. We provide a key for easy identification of native and non-native caprellids recorded on the Balearic Islands (Table 5.4) based on adult male specimens. The key is based on external characteristics to be used without need of dissection (see Fig. 1.2 in chapter 1 and Fig. 5.5).

1. a Pereopods 3 and 4 fully articulate *Phthisica marina*
b Pereopods 3 and 4 absent or reduced 2
2. a Head with acute dorsal projection 3
b Head smooth..... 4
3. a Dorsal projections on head and pereonites 1-2.....*Pseudoprotella phasma*
b Pereonites 1-2 without dorsal projections and more elongated than the rest of pereonites *Caprella scaura*
4. a Propodus of gnathopod 2 setose 5
b Propodus of gnathopod 2 smooth 6
5. a Propodus of gnathopod 2 dorsally with long setae. Antennae and pereopods with abundant short setae *Caprella hirsuta*
b Propodus of gnathopod 2 dorsally with short setae. Antennae and pereopods with scarce short setae *Caprella grandimana*
6. a Body with distinct dorsal projections on pereonites 3-7 *Caprella acanthifera*
b Dorsal projections absent or tiny 7
7. a Body with a few rounded humps *Caprella cavediniae*
b Body smooth..... 8
8. a Pereopods 3 and 4 present, reduced to 2-articles.....*Deutella shieckei*
b Pereopods 3 and 4 absent 9
9. a Suture between head and pereonite 1 absent *Caprella rapax*
b Suture between head and pereonite 1 present..... 10
10. a Trapezoidal projection on pereonite 2 *Paracaprella pusilla*
b Without trapezoidal projection on pereonite 2 11
11. a Pereopod 5 reduced.....*Pariambus typicus*
b Pereopod 5 with 7-articles 12
12. a Head without rostrum. *Caprella danilevskii*
b Head with rostrum. *Caprella liparotensis*

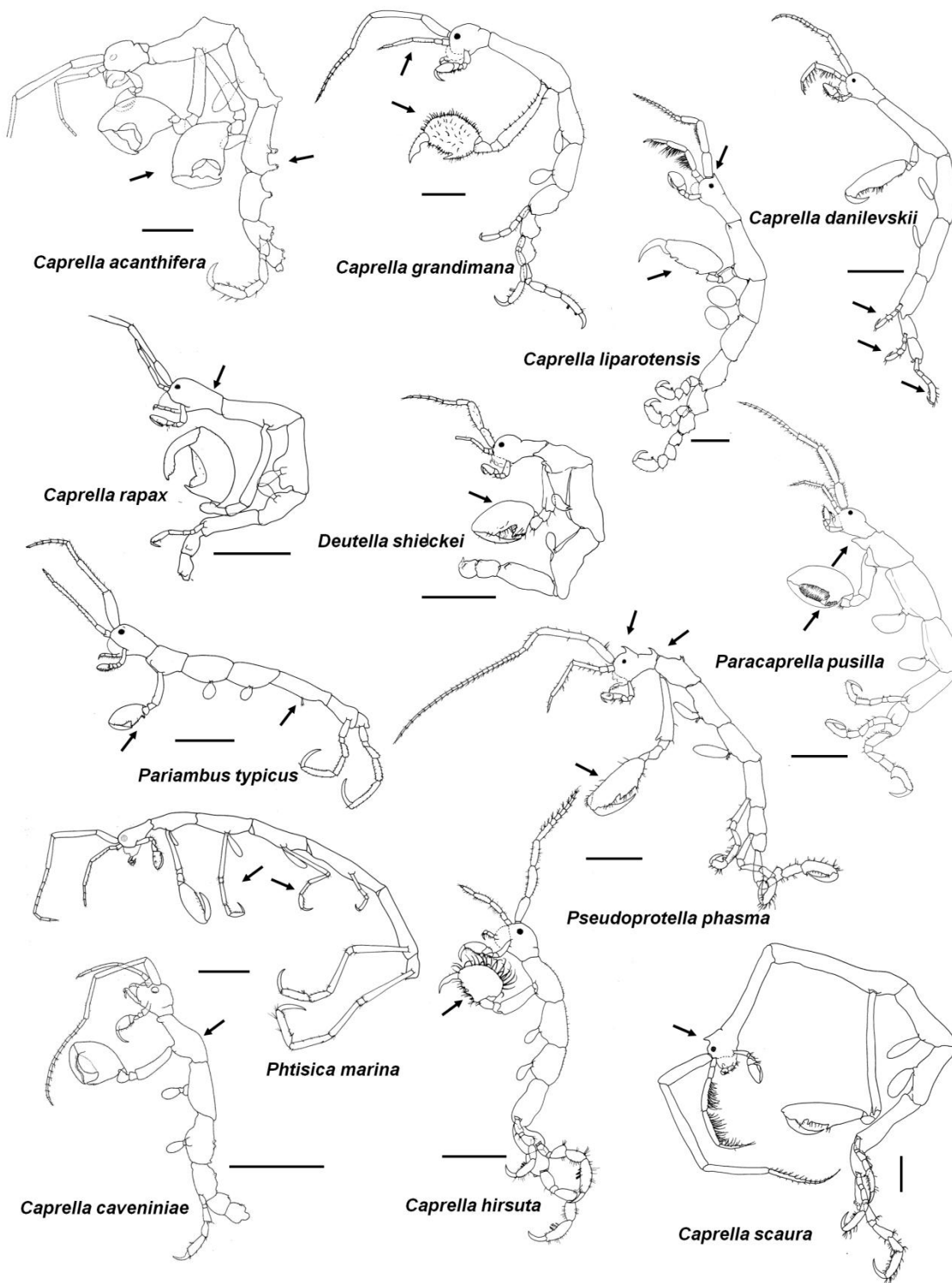


Figure 5.5 Male lateral view figures of caprellids inhabiting Balearic Islands. Refigured from Krapp-Shickel (1993), Krapp-Shickel et al. (1998), Laubitz and Sorbe (1996), Guerra-García 2003, Guerra-García et al. (2000, 2001, 2010), Guerra-García and Takeuchi (2002), Riera et al. (2003). Arrows indicate the most distinctive characteristics of each species.

Table 5.4 Caprellids recorded in Balearic Islands waters.

Name of Species	Distribution*	Status	Vector	First recorded by
<i>Caprella acanthifera</i> Leach, 1814	M, A	Native	–	Isern 1977
<i>Caprella cavediniae</i> Krapp-Schickel & Vader, 1998	E	Native	–	Box 2008
<i>Caprella danilevskii</i> Czerniavski, 1868	M, A, P, I	Native	–	present study
<i>Caprella grandimana</i> Mayer, 1882	E	Native	–	present study
<i>Caprella hirsuta</i> Mayer, 1890	E	Native	–	Ballesteros <i>et al.</i> 1987
<i>Caprella liparotensis</i> Haller, 1879	M, A	Native	–	Ballesteros <i>et al.</i> 1988
<i>Caprella rapax</i> Mayer, 1890	M, A	Native	–	Box 2008
<i>Caprella scaura</i> Templeton, 1836	M, A, P, I	Invasive	SF	Ros <i>et al.</i> 2013b
<i>Deutella schiekei</i> Cavedini, 1982	E	Native	–	Box 2008
<i>Paracaprella pusilla</i> Mayer, 1890	M, A, P, I	Introduced	SF	Ros <i>et al.</i> 2013b
<i>Pariambus typicus</i> (Krøyer, 1844)	M, A	Native	–	Box 2008
<i>Phtisica marina</i> Slabber, 1749	M, A, P	Native	–	Box 2008
<i>Pseudoprotella phasma</i> (Montagu, 1804)	M, A	Native	–	Isern <i>et al.</i> 1983

*E: Mediterranean Endemic; M: Mediterranean Sea; A: Atlantic Ocean; P: Pacific Ocean; I: Indian Ocean

5.4 DISCUSSION

5.4.1 Habitat use of native and non-native caprellids

The comparison of assemblages between artificial and natural habitats is a fundamental step toward understanding the effects of marine invasions on natural assemblages of epibenthic organisms (Bulleri and Chapman 2004). However, the effect of habitat on marine invasions has received little attention to date (Ruiz *et al.* 2009) and it remains unclear if new artificial structures are in general more likely to be colonized by alien marine species (Mineur *et al.* 2012). In our study, native caprellids were found in natural hard substrate consisting of macroalgae and hydroids that proliferate in the rocky platform of intertidal rocky shores exposed to wave action, and non-natives were only found in the fouling communities which proliferate in the artificial hard substrate provided by marinas, habitats sheltered from the wave action with a low hydrodynamism.

These results show that variation in species composition of caprellid assemblages is based on the interaction of habitat type and native and non-indigenous status. Only one caprellid species occurs in the two habitats, intertidal exposed rocky shores and marinas, the native *Caprella acanthifera*. This species exhibited the highest plasticity to colonize different host substrates, including bryozoans, hydroids and macroalgae, so this feature may play an important role in its distribution.

Guerra-García (2001) found that the composition of caprellidean communities in Ceuta (northern Africa) was different between exposed and sheltered areas. He found that *C. danilevskii*, *C. liparotensis* and *C. grandimana* were adapted to live in exposed habitats; *Pseudoprotella phasma* and *C. acanthifera* were adapted to live in both habitats (exposed and sheltered) and *C. hirsuta* was adapted to live in sheltered habitats. This coincides with our results differing only in the case of *C. hirsuta*, because we only found *C. hirsuta* in exposed habitats along with the other species that appear in intertidal rocky shores, well adapted to live in exposed areas. Takeuchi and Hirano (1995) found that the species of the exposed areas developed a ‘parallel posture’ attaching strongly to the substrate in areas subject to heavy wave action and strong currents, and species inhabiting environments with little wave action exhibited an ‘up-right posture’. For instance, *C. danilevskii*, found in exposed habitat, exhibited a parallel posture (Takeuchi and Hirano 1995) and *C. acanthifera*, found in sheltered and exposed habitat, exhibited an up-right posture (Guerra-García 2001). Regarding the exotic caprellids found in the present study, both, *C. scaura* and *P. pusilla*, exhibited an up-right posture when observed inhabiting the fouling communities of marinas (Ros and Guerra-García 2012), therefore, they seem to be more adapted to live in sheltered habitats than in exposed environments.

It appears that not all habitats are equally important as sites for colonization by non-indigenous caprellids. Artificial structures can provide suitable habitats for non-indigenous marine species and function as corridors for their expansion (Bulleri and Airoidi 2005). In North America, the vast majority of non-native species occur at high salinity in relatively sheltered waters of bays and estuaries with a smaller number reported on more exposed areas of outer coastal habitats, often near the entrance of bays or surrounding coastal harbors (Ruiz *et al.* 2009). Furthermore, within bays and estuaries, it appears that hard

bottom communities and especially artificial hard substrata, such as docks and pilings, are often a focal point for colonization (Cohen and Carlton 1995; Wasson *et al.* 2001).

In connection to the habitat distribution of *C. scaura* and *P. pusilla* along the Mediterranean Sea and the Strait of Gibraltar, we found that they have usually been reported inhabiting enclosed habitats associated to artificial hard substrate, particularly with fouling communities in harbors and marinas. They have never been found associated with natural hard substrate in the outer coast, the habitat in which we can find the highest diversity of native caprellid species. Therefore, native and non-native caprellid populations found in the present study differ in their habitat use, showing a spatial segregation associated with the type of hard substrate in which they live. However, there are some native species that can coexist with alien caprellids, like *C. acanthifera* in Mallorca, or *C. equilibra* and *C. dilatata* in the lagoon of Venice (Sacchi *et al.* 1998). These caprellid species have in common that they are able to live in the fouling communities associated with the artificial hard substrates of marinas.

All of this may have some implications in the invasion dynamics of non-indigenous caprellids: (1) constructions of artificial hard substrates are responsible for the presence of exotic caprellids (Marchini *et al.* 2007); and (2) caprellid species more adapted to live in exposed areas are less susceptible to be displaced by non-native caprellids and the opposite case occurs with the species more adapted to live in sheltered areas. This is the case of the native *C. equilibra*, well adapted to live in marinas and sheltered areas, which is being replaced in some marinas of the Iberian Peninsula by *C. scaura* (Guerra-García *et al.* 2011a; chapter 4). Further investigation into the specific mechanisms behind the apparent loss of advantage for native species on artificial substrate types is necessary.

5.4.2 Recreational boating and distribution of caprellids in Mallorca Island

Non-native caprellids found in the present study present a discontinuous distribution, occurring in multiple marinas that are separated by outer rocky coast where the species were absent. This pattern was especially pronounced in the case of the invasive caprellid *C. scaura* which was found in three adjacent marinas of the HEMU of Levant. The other non-indigenous species, *P. pusilla*,

was found in Palma marina, the only marina that receives transcontinental commercial ship traffic. This marina is located in the HEMU of Palma-Migjorn, the area with the highest pressure of recreational boating and the highest potential risk of dispersal by recreational boating in the island, followed by the HEMU of Levant. In recent surveys, the invasive *C. scaura* has also been found in Palma marina, associated with the bryozoan *B. neritina* (Ros, unpublished data). Since the probability for establishment of non-native species is directly related to propagule supply (Ruiz *et al.* 2000), regions with a high RPCI may have a higher chance of harboring or aiding in the spread of NIS compared with those regions with lower RPCI index (Minchin *et al.* 2006).

The absence of non-indigenous caprellids in areas of the Balearic Islands with a scarce number of recreational ports, like the islands of Formentera and Cabrera, and their presence on islands with a high number of marinas, like Mallorca, Menorca and Ibiza (Ros *et al.* 2013b) also support this idea. Although an accurate calculation of the recreational boating pressure is subject to many more variables than those available in this study, these results provide a first indication of the level of pressure by recreational boating, allowing comparison between different coastal zones. The average RPCI index for Italy, a country with a high number of marinas and recreational boaters, is 16 (Minchin *et al.* 2006). In Mallorca the average is even greater, with a RPCI index of 19.8.

A recent study carried out by Murray *et al.* (2011) showed that over one-quarter of the boating community of coastal British Columbia (Canada) was fouled by NIS. The proportion of boats fouled by the bryozoan *Bugula neritina*, the substrate type that hosted the highest abundance of the invasive caprellid *C. scaura* in Mallorca, is even higher, since this bryozoan is tolerant to antifouling paints (Piola and Johnston 2006). Floerl and Inglis (2005) concluded that local abundance of *B. neritina* in the source marina was a more important determinant of transport probability than the age of the antifouling paint. Therefore, local abundance of alien caprellids associated with *B. neritina* may be an important factor to quantify the recreational transport probability of this group. Furthermore, we observe that alien caprellids were associated with different fouling substrates which were present in different parts of the recreational boats, including ropes and buoys and not only ship hulls. Ropes and buoys are components of the recreational boats that are not covered by antifouling protection and are not usually taken into account in studies about biofouling on ships. Therefore, the caprellid transport probability by recreational boats may be

more than one-quarter of the boating community that sails in the waters of Mallorca.

The secondary spread marina-marina seems to be the cause of the current distribution of *C. scaura* in the three marinas of the HEMU of Levant in Mallorca. Taking into account that native species have been present on the island far longer than the exotic species, we could expect an extensive distribution of the native caprellid *C. acanthifera* in marinas of Mallorca, a species able to survive in the fouling communities present in these habitats. However, *C. acanthifera* was only found in two of the fourteen recreational ports surveyed and exhibited relatively low densities in comparison to the densities of the exotic caprellids. Therefore, recreational boating seems not effective in the secondary dispersal of all caprellid species present in marinas being more effective in the transport of alien species. Several studies (e.g. Wonham *et al.* 2001) have shown that the human transport pathway can, in itself, impose selective pressures that have a strong influence on the suite of species that subsequently survive transportation. Further studies are necessary to understand the apparent advantages for non-indigenous species in the dispersion by recreational boats.

The invasive *C. scaura* exhibited the highest density in Cala Ratjada marina, associated with the bryozoan *B. neritina* and the macroalgae *Ceramium ciliatum* and *Dictyota dichotoma*. This marina, included in a marine protected area (MPA), may act as source populations for secondary introductions to Mallorca Island through stepping-stone invasions.

The following step in the invasion dynamics would be the secondary spread from marinas to natural areas. On Mallorca Island, the recreational boating dispersal pressure is very high, with around 6082 boats that sail daily (Balaguer *et al.* 2011) and many of them anchored in exposed and preserved coastal zones. Following the study of Murray *et al.* (2011), at least, a quarter of these boats are fouled by NIS contributing to the propagule pressure of exotic species in natural and exposed areas. Furthermore, while anchoring in natural areas is regulated, transport of fouling by recreational boaters on the island is not controlled. A suitable host substrate for non-native caprellids in natural hard substrate does not represent a problem, since the host substrates *E. racemosum*, *C. elongata* and *D. dichotoma* harbored both native and non-native caprellids depending on the type of hard substrate in which they were collected. Thus, they

hosted native caprellids when they were collected in natural intertidal rocky shores and exotic ones when they were collected from the artificial structures of marinas. Nevertheless, on Mallorca Island in particular and in the Mediterranean Sea in general, exotic caprellids associated with natural hard substrate in outer areas have not yet been found.

The secondary spread marina-natural habitat and colonization of natural hard substrate of more exposed outer coasts by exotic caprellids may be limited by suitable environmental conditions and/or biotic interactions. For example, the macroalgae *D. dichotoma* hosts high densities of *C. scaura* in Cala Ratjada marina ($11,333 \pm 6,960$ ind/1,000 ml, mean \pm standard error) while the adjacent intertidal rocky shore of S'Arenalet, included in the same MPA as Cala Ratjada marina, hosts two native caprellids, *C. hirsuta* and *C. liparotensis*, but does not host *C. scaura*. It is possible that native caprellids have more advantages in this exposed environment than the invasive caprellid. Another possibility is that in enclosed water spaces, like marinas, propagule pressure on available surfaces can be several orders of magnitude greater than in adjacent coastal locations (Floerl and Inglis 2003).

Further studies are still necessary to understand the factors involved in this critical step of the invasion dynamics by non-native species. In spite of this, this topic represents a gap in our understanding of invasion risk (Ruiz *et al.* 2009). Caprellid amphipods constitute a suitable model to study this phenomenon, contributing to our understanding of the invasion process.

5.4.3 Conclusions

Caprellid assemblages attached to arborescent substrates present in artificial structures associated with recreational marinas differ substantially from those attached to arborescent substrates present in adjacent rocky shores. Understanding the causes of these differences is crucial to predicting how continued urbanization will affect the ecology of this group in coastal habitats. The high recreational boating pressure that Mallorca Island is subject to together with the subsequent increase in the constructions of artificial enclosed hard substrates is responsible for the presence of non-indigenous caprellids in the area.

Environmental conditions (enclosed water spaces), habitat suitability (available arborescent fouling substrates) and sources of propagules (high recreational boating pressure), may be important determinants of susceptibility of marinas to colonization by exotic caprellids. These artificial structures replace extensive areas of natural hard substrate (intertidal rocky shores) which host the highest diversity of native caprellids which are less susceptible to invasion by non-indigenous caprellids. However, although recreational boating seems effective in the transport of exotic species from marinas to marinas, it does not appear effective in the dispersal from marinas to natural and exposed areas.

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5

VECTORS OF SPREAD: PART 2

The Panama Canal and the transoceanic dispersal of
marine invertebrates: evaluation of the introduced
amphipod *Paracaprella pusilla*
in the Pacific Ocean

- Adapted from:

Ros M, Ashton GV, Lacerda MB, Carlton JT, Vázquez-Luis M, Guerra-García JM, Ruiz GM (2014) The Panama Canal and the transoceanic dispersal of marine invertebrates: evaluation of the introduced amphipod *Paracaprella pusilla* Mayer, 1890 in the Pacific Ocean. *Marine Environmental Research* 99: 204–211.

ABSTRACT. Although the Panama Canal is one of the major corridors for shipping and potential dispersal of marine invaders in the tropics, little is known about the effect that the Canal has had on the distribution of marine biota. In this study, we (a) document the existence of established populations of the Western Atlantic caprellid amphipod *Paracaprella pusilla*, Mayer 1890 for the first time at the Pacific entrance to the Canal, (b) review its distribution in the Pacific Ocean, and (c) evaluate possible mechanisms of introduction. The confirmed distribution of *P. pusilla* in the Pacific Ocean is limited to Australia, Hawaii, and Panama, despite earlier published reports from Chile and China. Laboratory experiments demonstrated intolerance of *P. pusilla* to freshwater, causing 100% mortality, and suggest invasion of the Pacific coast of Panama occurred through the Canal via ships' ballast water or by secondary spread via ships (ballast water or hull fouling) from another Pacific region.

RESUMEN. A pesar de que el Canal de Panamá es uno de los mayores corredores para el transporte marítimo y la dispersión potencial de especies invasoras marinas en la zona tropical, se sabe muy poco acerca del efecto que el Canal ha tenido en la distribución de las especies marinas. En este estudio, (a) documentamos la existencia de poblaciones establecidas del anfípodo caprélido del Atlántico Occidental *Paracaprella pusilla*, Mayer 1890 por primera vez en la entrada Pacífica del Canal, (b) revisamos su distribución en el Océano Pacífico, y (c) evaluamos el posible mecanismo de introducción. La distribución de *P. pusilla* en el Océano Pacífico se confirma sólo para Australia, Hawai y Panamá, a pesar de haber sido citada también en Chile y China. Experimentos llevados a cabo en condiciones de laboratorio demostraron la intolerancia de *P. pusilla* al agua dulce, causando un 100% de mortalidad y sugieren que la invasión de la costa Pacífica del Canal se llevó a cabo a través del agua de lastre de los barcos que atraviesan el Canal o bien de forma secundaria a través de barcos (ya sea en el agua de lastre o asociados al casco de las embarcaciones) desde alguna otra región del Pacífico.

5.5 INTRODUCTION

Despite the increased interest in the study of marine biological invasions, little is known about patterns and processes of marine invasions in the tropics relative to temperate regions (Ruiz *et al.* 2009a; Freestone *et al.* 2013). The Panama Canal, which created a new shipping route between the Atlantic and Pacific Oceans in 1914, provides an especially important model system for testing predictions about marine invasions in the tropics (Ruiz *et al.* 2009a).

Surprisingly, little is known about the effect that the Canal has had on the distribution of marine biota (Cohen 2006). Despite being a significant shipping corridor for 100 years, there are relatively few invasions known from Panama compared to many other global regions, and most of these are recent discoveries (Carman *et al.* 2011; Schlöder *et al.* 2013). In general, the effectiveness of the freshwater Gatun Lake as a barrier to passage of marine organisms through the Canal has been questioned and summarized by various authors (see McCosker and Dawson 1975; Cohen 2006 and references therein). Past records and studies have documented the successful transport of living marine organisms through the freshwaters of the Panama Canal, indicating that such passage occurs, especially associated with commercial ships. Bishop (1947), for example, found that three species of Pacific barnacles had survived the Canal transit, arriving alive on the hull of a vessel in Liverpool, England. Turner (1966) discovered that the Pacific Ocean shipworm *Teredo furcifera* survived the fresh waters of the Canal, arriving alive in 1962 in New York. Menzies (1968), in a short but notable experiment, towed a random mixture of subtidal and intertidal marine invertebrates through the Canal and found generally high survival. Davidson *et al.* (2008) found surviving barnacles, bryozoans, and isopods, among other hull fouling species, on two ships that passed through the Panama Canal, having originated from low salinity waters of San Francisco Bay. Chapman *et al.* (2013) found two species of barnacles and an oyster from the Atlantic that survived passage on a vessel hull bound for Oregon.

Crustaceans are among the most successful aquatic invaders around the world (Carlton 2011). Among marine crustaceans, there are an increasing number of reports of introduced caprellids, suggesting this group has a relatively high potential for invasions and detection across many global regions. Caprellids, commonly known as skeleton shrimps, constitute an important trophic link

between primary producers and higher trophic levels (Woods 2009). Their reduced abdominal appendages, which in other amphipods are used for swimming (Takeuchi and Sawamoto 1998), as well as a lack of a planktonic larval stage, suggest that the wide distribution of some littoral caprellids may largely be due to rafting (Thiel *et al.* 2003; Astudillo *et al.* 2009) or to transport associated with ships' ballast water (Carlton 1985; Gollasch *et al.* 2002) or hull fouling (Montelli and Lewis, 2008; Carlton and Eldredge 2009). Established populations of introduced caprellids have been documented from subpolar waters to the tropics. However, the reported number of non-indigenous species almost certainly represent a small fraction of the actual number of introductions (Ruiz *et al.* 2009a; Zenetos *et al.* 2010), as is typical of other smaller-bodied and taxonomically challenging taxa (Carlton 2009). At higher latitudes the Asian species *Caprella mutica* Schurin, 1935 was the first non-native caprellid clearly established in coastal waters of Alaska, representing an indicator of the vulnerability of the area to marine introductions (Ashton *et al.* 2008). In the case of tropical introductions, five non-native caprellids were reported by Carlton and Eldredge (2009) in the Hawaiian Islands. Taking into account that most documented marine invasions are sessile invertebrates on hard substrate (Ruiz *et al.* 2009b), marine amphipods represent an important model group to understand marine invasions of mobile biota associated with hard substrate.

Here, we report the invasion of the Atlantic caprellid *Paracaprella pusilla* to the Pacific coast of Panama, at the entrance to the Canal. This tropical/subtropical species, originally described from Rio de Janeiro, Brazil (Mayer 1890) is likely native to the Western Atlantic Ocean (Mayer 1903; McCain 1968; Carlton and Eldredge 2009, who describe it as a 'strongly Caribbean species') and is one of the most abundant caprellids along the Caribbean coast of Venezuela and Colombia (Díaz *et al.* 2005; Guerra-García 2006). In this study, we: (1) Document the occurrence and distribution of *P. pusilla* at the Pacific and Atlantic entrances to the Panama Canal; (2) Review and clarify the distribution of previous records of *P. pusilla* in the Pacific Ocean, and (3) Test experimentally the salinity tolerances of *P. pusilla* under laboratory conditions, to evaluate its potential to survive the freshwater immersion during passage through the Canal and assess the possible vectors of introduction to Panama. To our knowledge, this is the first report of salinity tolerance of a *Paracaprella* species, and the first report of an introduced caprellid on the Panamanian coasts.

5.6 MATERIAL AND METHODS

5.6.1 Panama survey: study area and sampling collections

Panama Canal is a potential hotspot for tropical marine invasions, receiving more ship arrivals than any of the largest ports in the United States (Ruiz *et al.* 2006; Miller *et al.* 2007). Surveys were conducted in summer 2004 and 2008 at several sites within the Pacific and Atlantic entrances to the Panama Canal (Fig 5.6, Table 5.5). A standard fouling panel method was used to collect the samples of the sublittoral invertebrate community. Within each coast, 10 sites of human activity, including marina and harbor pontoons, private and public docks, and shipping terminals were surveyed. At each site, 5 PVC panels (14 × 14 cm) were deployed horizontally, surface-down at 1 m depth below low tide (mean lower low water level). The panels were deployed for 3 months to allow colonization of local marine biota. Upon retrieval, panels and associated biota were preserved in 75% ethanol. In the laboratory, the mobile component (organisms not firmly attached to the panels) of the fouling samples was analyzed for the presence of caprellids. Although Atlantic and Pacific Panama surveys were carried out in different years, identical methods were used on each coast, and panels were retrieved in the same season, allowing comparison between the two areas.

Paracaprella pusilla were identified using the following characteristics: (1) the large anterolateral projection of pereonite 2, (2) small dorsal tubercle on pereonite 2, (3) proximal knob on the basis of gnathopod 2, and (4) lateral pleura in pereonites 3 and 4, especially developed in pereonite 3 (see plate 2, figures 36 and 37 in Mayer (1903)). Although *P. pusilla* is relatively similar to *P. tenuis* Mayer, 1903 (Laubitz 1972) and can coexist in the northern Gulf of Mexico (Foster *et al.* 2004), males of the latter can be distinguished from those of *P. pusilla* by the small and triangular anteroventral margin of pereonite 2 and the absence of characteristics 2, 3 and 4 (McCain 1968; Guerra-García *et al.* 2006a, 2010; Winfield and Ortiz 2013).

All individuals of *P. pusilla* collected from the plates were photographed with a stereomicroscope Motic K-400L with a Nikon D90 digital camera. Body length was measured from the front of the head to the end of pereonite 7, using

the PC-based digitizing software Scion Image Alpha 4.0.3.2 © (2000-2001 Scion Corporation). Voucher specimens are housed at Smithsonian Environmental Research Center.

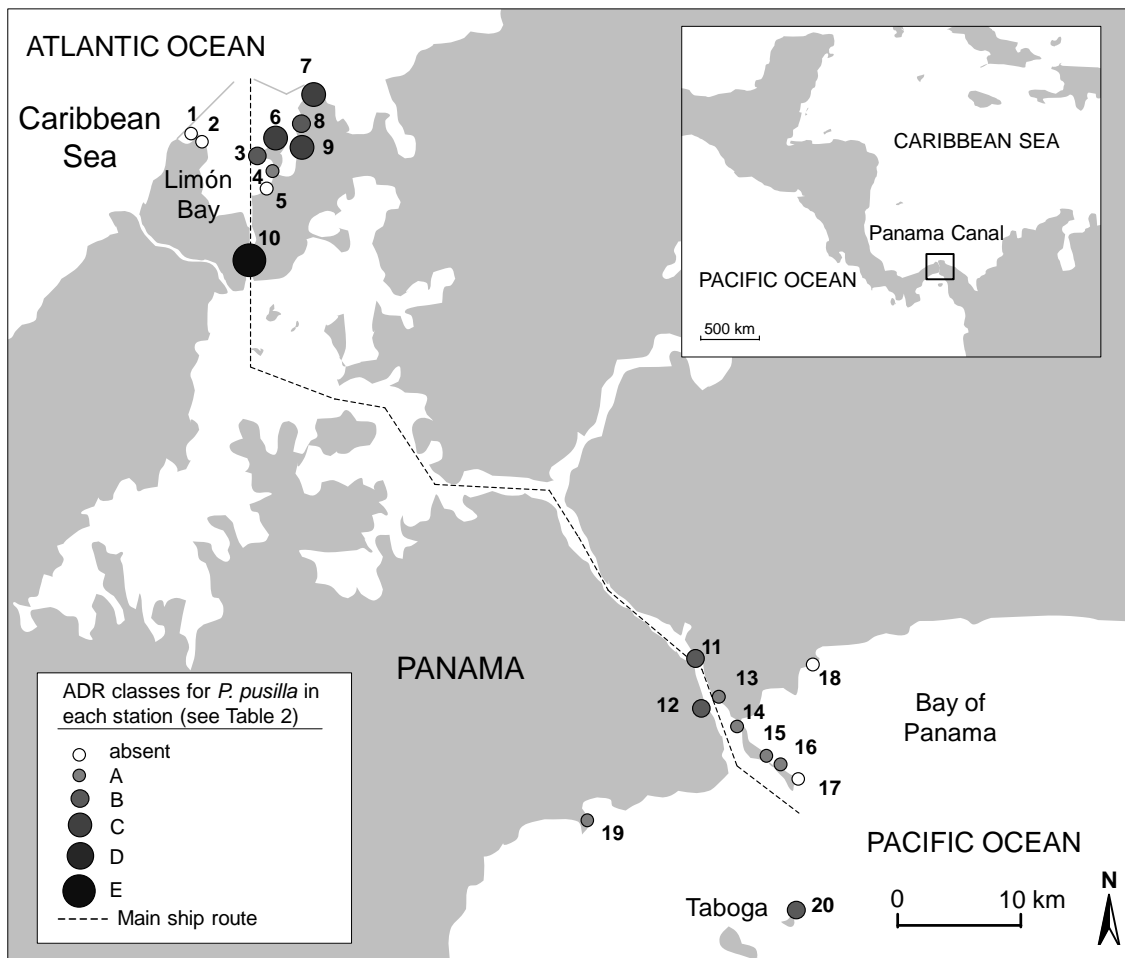


Figure 5.6 Study area showing the ADR (Abundance and Distribution Range) classes (see Table 5.5) for *Paracaprella pusilla* in each station.

5.6.2 Abundance and Distribution Range (ADR) of *Paracaprella pusilla* in Panamá

The assessment of the ADR is based on the method of Olenin *et al.* (2007) and provides a combination of the abundance and frequency of *P. pusilla* in each station (assessment units) examined. This method was modified to adapt it to the

fouling panel method in this study. The abundance of *P. pusilla* was ranked in relation to the abundance of the relevant ecological group to which the alien species belongs. In this case, we consider all the mobile fauna present on the fouling panels as the relevant ecological group, including crustaceans (caprellids, gammarids, isopods, tanaids and decapods), annelids, pycnogonids, molluscs and equinoderms. Due to the difficulty of obtaining quantitative data of mobile fauna from fouling panels, since there can be some loss of animals during removal from the water, the units of abundance for each mobile component were based on a semiquantitative scale with 5 levels: 0 (absent), 1 (1-10 individuals per panel), 2 (11-100 individuals per panel), 3 (101-1000 individuals per panel) and 4 (more than 1000 individuals per panel).

Based on the ADR method, abundance of *P. pusilla* in each station was then categorized in three levels: ‘low’ when it represented only a small portion of the mobile community; ‘moderate’ when it constitutes less than half of the abundance of the mobile community, and ‘high’ if it exceeded half the abundance and dominated. The distribution scale for *P. pusilla* was evaluated as I, where it appeared only in one panel; II, where it was present in less than half of the panels (2 panels); III, where it was found in more than half of the panels (3-4 panels) and IV, where it was found in the total of panels deployed (5 panels). Combinations of abundance and distribution provide a scale that ranges from ‘A’ few individuals in one panel to ‘E’ where a species occurs in high numbers in all panels (Table 5.6).

5.6.3 Pacific distribution data

A detailed review of the records of *P. pusilla* in the Pacific Ocean was conducted. Morphology of *P. pusilla* populations from Panama was compared with other *P. pusilla* populations, including drawings of this caprellid species from different world areas (Colombia, Guerra-García 2006a; India, Guerra-García *et al.* 2010; Venezuela, Díaz *et al.* 2005 and Hawaii, Edmonson and Mansfield, 1948) and our own examination of specimens from Chile, Australia, the Gulf of Mexico, Brazil, Southern Spain, the Balearic Islands and India, in order to establish possible morphological differences.

Table 5.5 Location of sampling stations, date of panels retrieved, caprellid species found in each station and data used to obtain the ADR class for each station. *P. pus*: *Paracaprella pusilla*; *C.eq*: *Caprella equilibra*. Semiquantitative scale: 0 (absent), 1 (1-10), 2 (11-100), 3 (101-1000) and 4 (more than 1000 individuals per panel).

Coast	Stations	Coordinates	Date of retrieved	Caprellids present	Frequency of occurrence of <i>P. pusilla</i> (%)	Abundance of <i>P. pusilla</i> per plate (semiquantitative scale)	ADR abundance (see text)				
ATLANTIC	1	9° 22' 13"N, 79° 56' 57"	Sept 2004	-	0	0	0	-			
	2	9° 22' 6"N, 79° 57' 3"	Sept 2004	-	0	0	0	-			
	3	9° 21' 14"N, 79° 54' 44"	Sept 2004	<i>P. pus.</i>	40	2	1	0	Moderate		
	4	9° 20' 57"N, 79° 54' 10"	Sept 2004	<i>P. pus.</i>	20	1	0	0	Low		
	5	9° 20' 32"N, 79° 54' 4"	Sept 2004	-	0	0	0	0	-		
	6	9° 12' 53"N, 79° 32' 2"	Sept 2004	<i>P. pus.</i>	60	2	2	0	Moderate		
	7	9° 22' 24"N, 79° 53' 2"	Sept 2004	<i>P. pus, C. eq.</i>	80	2	1	2	0	Moderate	
	8	9° 22' 14"N, 79° 53' 1"	Sept 2004	<i>P. pus.</i>	40	1	2	0	0	Moderate	
	9	9° 21' 40"N, 79° 53' 4"	Sept 2004	<i>P. pus, C. eq.</i>	100	1	1	1	1	2	Low
	10	9° 17' 23"N, 79° 55' 9"	Sept 2004	<i>P. pus.</i>	100	4	3	2	2	High	
PACIFIC	11	8° 57' 56"N, 79° 34' 24"	Sept 2008	<i>P. pus.</i>	60	1	1	1	0	0	Low
	12	8° 57' 10"N, 79° 34' 30"	Sept 2008	<i>P. pus, C. eq.</i>	60	1	1	1	0	0	Low
	13	8° 57' 19"N, 79° 33' 57"	Sept 2008	<i>P. pus.</i>	20	1	0	0	0	0	Low
	14	8° 57' 31"N, 79° 33' 20"	Sept 2008	<i>P. pus.</i>	40	1	1	0	0	0	Low
	15	8° 55' 2"N, 79° 32' 6"	Sept 2008	<i>P. pus.</i>	40	1	1	0	0	0	Low
	16	8° 55' 3"N, 79° 31' 58"	Aug 2008	<i>P. pus.</i>	20	1	0	0	0	0	Low
	17	8° 54' 47"N, 79° 31' 15"	Aug 2008	-	0	0	0	0	0	0	-
	18	8° 58' 14"N, 79° 31' 41"	Aug 2008	-	0	0	0	0	0	0	-
	19	8° 51' 47"N, 79° 40' 17"	Sept 2008	<i>P. pus, C. eq.</i>	20	1	0	0	0	0	Low
	20	8° 48' 27"N, 79° 31' 22"	Sept 2008	<i>P. pus.</i>	60	1	1	1	0	0	Low

Table 5.6 ADR classes of abundance and distribution in each station (modified from Olenin *et al.* 2007, see 5.6.2 section).

Abundance	Distribution scale			
	I	II	III	IV
Low	A	A	B	C
Moderate	B	B	C	D
High	B	C	D	E

5.6.4 Salinity tolerance experiments

The survival of *Paracaprella pusilla* at various salinities was tested experimentally. Individuals were collected from *Eudendrium* sp. hydroids attached to pontoon structures in Brazil at Paranaguá harbor (25° 30' S, 48° 30' W) in November 2012 (late dry season), at a temperature of 25°C and a salinity of 35. Paranaguá harbor is among the most important handling sites for grain and fertilizers in South America (Mizerkowski *et al.* 2012). It is located in the middle section of Paranaguá Bay, a partially mixed estuary which experienced a large seasonal variation in freshwater discharge (Mantovanelli *et al.* 2004). During the rainy season, salinity ranges from 0 to 35 and temperature from 23 to 30 °C; during the dry season, salinity ranges from 3 to 35 and temperature from 18 to 25 °C (Marone *et al.* 2005).

The collection site, close to the type locality of *P. pusilla* (Río de Janeiro, Brazil), was selected based on the abundance of *P. pusilla* due to high number of individuals needed for the experiments (a total of 570 individuals). The animals were transported in sea water (without food), still attached to the hydroid, in a 20 L container that received constant aeration with several air pumps. Animals were maintained at 25°C ± 1°C with a photoperiod regime of 12 D: 12 L throughout the tolerance experiments. Temperature was selected based on the average temperature in the collection site at the moment of collection.

Five adults of each sex were placed separately without food in plastic cylindrical containers (9 cm diameter, 12 cm height) open to the air, with 400 ml of water at different salinities. A 1 mm plastic mesh was used as substrate for attachment. Salinities were achieved by addition of artificial sea salt (Tropical Marin) to freshwater treated by reverse osmosis. Evaporation was checked, and the container was topped up with distilled water if necessary. The salinity was

measured with a conductivity meter CRISON MM40 and monitored daily to confirm its stability. Survival was checked at 24 and 48 h after the start of the experiment, and dead animals were removed. Additional measures at 1h and 12 h after the start of the range-finding test were also checked. Death was assumed if an individual did not move when stimulated with a fine needle (Ashton *et al.* 2007). The first range-finding experiment was conducted at a coarse scale with 7 treatments, using salinities of 0, 5, 15, 25, 30, 45 and 55. A control treatment was included using a similar salinity to the site of collection, 35. These salinities were selected in an effort to include mortality rates ranging from 0% to 100%.

The second experiment employed a narrower range of salinities to detect both the lower and upper lethal limit. The first was estimated to lie at around 15 at 48h, employing salinities of 13, 15, 17, 19 and 21 in the narrower test, and the second one was estimated to lie at around 45 at 48h, using salinities of 41, 43, 45, 47, 49 and 51. Consequently, each group of five individuals of the same sex was subjected to 11 treatments with three replicates by treatments and sex.

5.6.5 Statistical analysis

The 24h and 48h median lethal concentration (LC50), the concentration of salinity causing 50% mortality for each species, and associated 95% confidence limits were calculated by use of Probit analysis (Finney 1971). To test whether the data fits the Probit model, Chi-squared analysis were used. A pairwise Students't-test was used to compare differences in mortality of male and female *P. pusilla* at each salinity (see Ashton *et al.* 2009). All analyses were performed using the statistical package SPSS 15.0.

5.7 RESULTS

5.7.1 Panama survey

The mobile component associate with the panels from the Atlantic coast was dominated by annelids (mainly polychaetes), followed closely by amphipods (caprellids and gammarids), decapods, tanaids and pycnogonids. In minor proportion were molluscs, isopods and equinoderms (ophiuroids). On the

Panamanian Pacific coast the dominant group was amphipods (mainly gammarids), followed by annelids (mainly polychaetes), decapods, isopods and tanaids. Echinoderms (ophiuroids), pycnogonids and molluscs were also found, but in very low proportion. *Paracaprella pusilla* was the dominant caprellid being found for the first time on both Panamanian coasts. Another caprellid species, *Caprella equilibra* Say, 1818 (the variant with ventral projection between gnathopods 2, see McCain 1968) was also found, being present in two stations in the Atlantic coast and two stations in the Pacific coast (Table 5.5), where it co-occurred with *P. pusilla*.

Paracaprella pusilla was found in 70% of stations sampled in the Atlantic coast and 80% of stations sampled in the Pacific coast. However, ADR classes obtained by combination of ADR abundance and frequency of occurrence in each station (Table 5.5) were different on the two sides of the Panama Canal (Figure 5.6). On the Atlantic coast, stations were characterized in general terms by an ADR = B-C with moderate abundance of *P. pusilla* in several or many panels (Table 2). Only in the case of station 10, the closest station to the channel entrance, was *P. pusilla* the dominant species of the mobile fauna associated with all of the panels deployed (ADR = E). On the Pacific coast stations, *P. pusilla* also occurred in several or many panels but always in low numbers (ADR = A-B).

Ovigerous females were found on both sides of the Panama Canal. Males and females on both sides were similar in size (Fig. 5.7). No morphological differences were found between populations of the Atlantic and Pacific coast of Panama, nor between *P. pusilla* from Panama and populations from other world sites.

5.7.2 Distribution of *Paracaprella pusilla* in the Pacific Ocean

Paracaprella pusilla presents a disjunct and reduced distribution in the Pacific Ocean. The first occurrence in the Pacific was a female reported by Mayer (1890) from Amoy, China. While this record has been referenced through modern times in almost all caprellid literature, Mayer (1903) in fact later named the same material as a new species, *P. crassa*. There are thus no verified records of *P. pusilla* that we can locate from the Western Pacific.

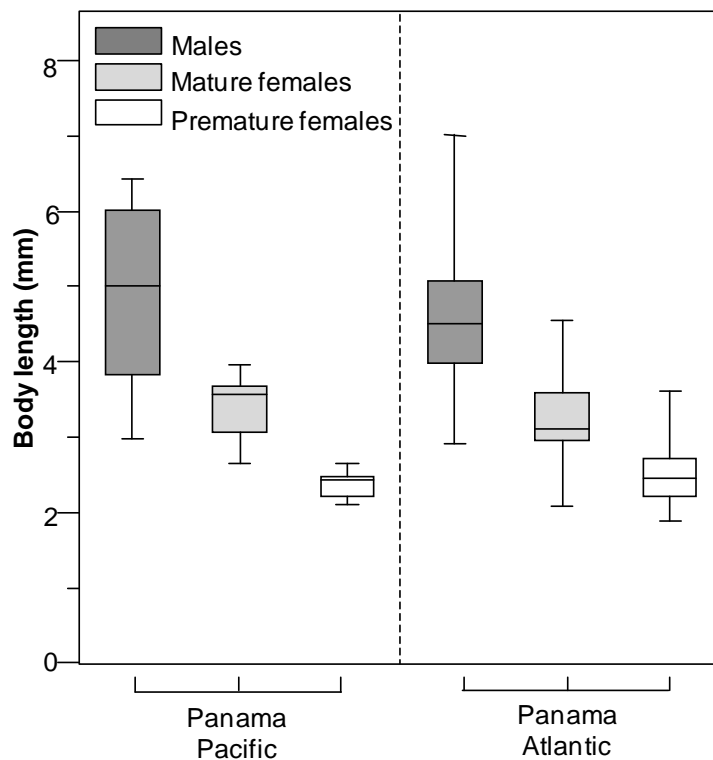
The second record of *P. pusilla* in the Pacific Ocean is in Honolulu harbor in Oahu in the Hawaiian Islands (Edmondson and Mansfield, 1948). Although we could not examine specimens from this area, their drawings show the diagnostic features of *P. pusilla*, confirming its presence there.

The next record is a juvenile caprellid reported from Coquimbo, Chile by Guerra-García and Thiel (2001). Their drawings are of a juvenile *Paracaprella*, but the specimen lacks the diagnostic characteristics of *P. pusilla* and may, therefore, be another species of this genus. A second record of *P. pusilla* from Chile is from aquaculture buoys (Astudillo *et al.* 2010). These specimens proved to be juvenile *Deutella* sp.

The last record of *P. pusilla* in the Pacific Ocean is in Australia, on hull fouling communities of local vessels (Montelly and Lewis 2008). The pictures of adult specimens provided together with our own examination of specimens from this area confirm the identification.

In summary, the presence of *P. pusilla* in the Pacific Ocean is confirmed for Hawaii and Australia (and, now, Panama). Adult *Paracaprella* will be required to confirm if *P. pusilla* is present in Chile.

Figure 5.7 Box-and-Whisker plot for each sex/age group measured for the Atlantic vs Pacific Panamanian populations of *P. pusilla*. Median values are included; the rectangles contain values between the first and the third quartiles; the bars connect the extreme values.



5.7.3 Salinity tolerance experiments

In the first range-finding test, *Paracaprella pusilla* stops all activity, measured as body movement, just after submerged in freshwater. After 1h, a mortality rate of 100% was confirmed for this treatment. In the case of caprellids exposed to a salinity of 5, a high mortality (86%) was observed after 1 h. Although some individuals were still alive, their movements were very slow. After 12 h, a mortality rate of 100% was confirmed for caprellids exposed to salinities of 5 and 55.

In the second experiment, which employed a narrower range of salinities, the low salinity 24 h LC50 (Fig. 5.8 A) was significantly lower ($P < 0.01$) for females, estimated as 10.4, than that for males, estimated as 13.3. The low salinity 24h LC50 for the total population was estimated in 11.7. Confidence intervals were unreliable at 24h because of low salinity 24 h LC50 was beyond the experimental conditions (which included salinities ranging from 13 to 21).

After 48 h (Fig. 5.8 B), the low salinity LC50 (95% confidence limits) was within the experimental conditions and was 17.8 (16.7 - 19.3) for the total population. The lower LC50 for females, which was 16.7 (15.1 - 18.4), was significantly below ($P < 0.05$) that for males, estimated as 18.7 (17.2 - 22.2).

In experiments of high salinity tolerances, no significant differences were found between males and females. The high salinity 24 h LC50 (95% confidence limits) for females was 46.4 (45.4 - 47.4), for males was 46.3 (44.8 - 47.8) and for the total population was 46.3 (45.5 - 47.1) (Fig. 5.8 A).

After 48 h, the high salinity LC 50 (95% confidence limits) for females was 43.7 (42.3 - 44.8), for males was 42.8 (41.0 - 44.0) and for the total population was 43.3 (42.2- 44.1) (Fig. 5.8 B).

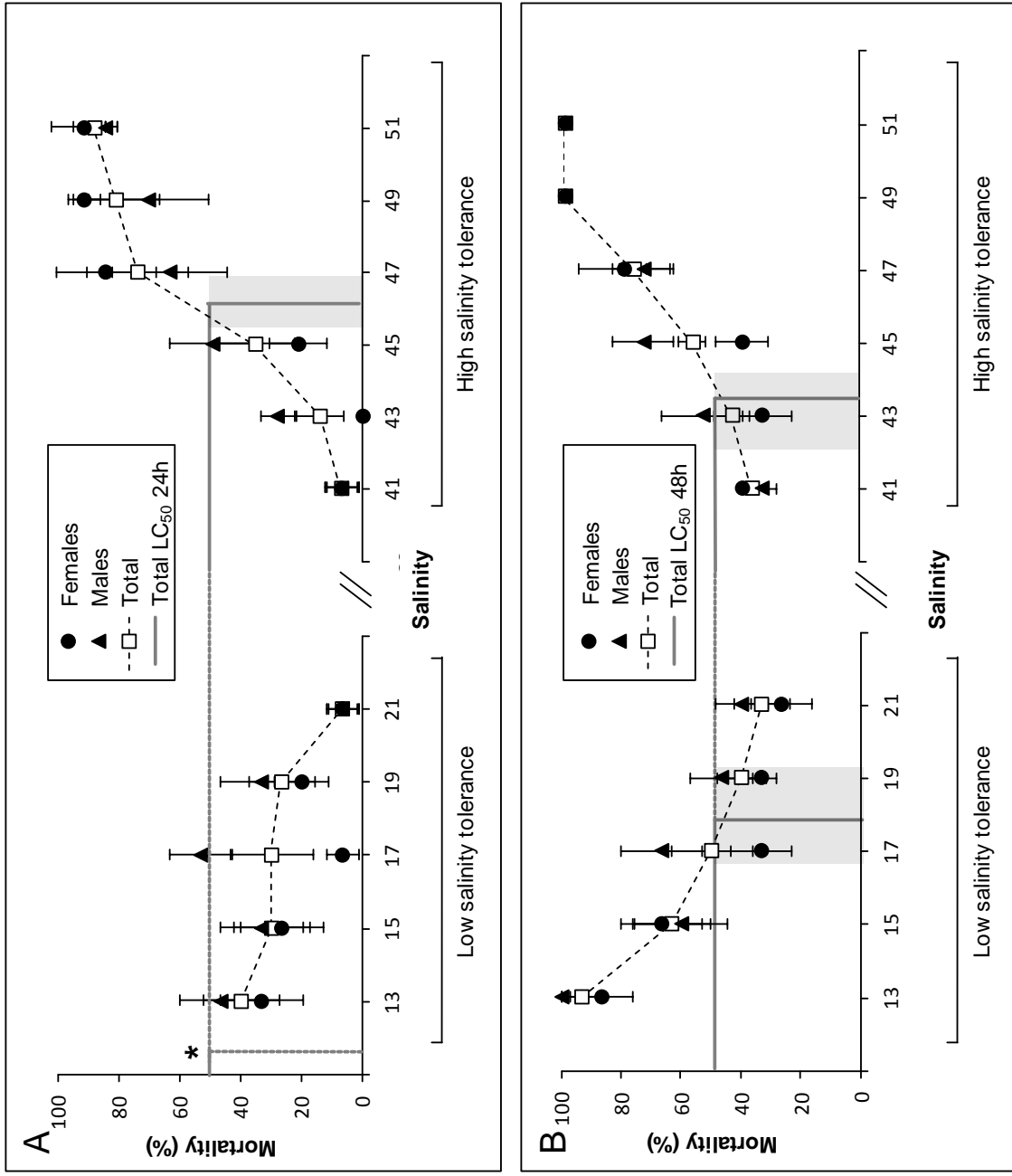


Figure 5.8 Mortality of *P. pusilla* following 24 h exposure (A) and 48 h exposure (B) at low salinities ranging from 13 to 21 and high salinities ranging from 41 to 51 (means \pm SE, $n = 3$). Gray lines indicate the high LC50 (A) and the low and high 48h LC50 (B). Gray shadows beside gray lines indicate the confidence intervals (estimated using Probit analysis). The vertical broken line with an asterisk indicates that the low 24h LC50 was beyond the experimental conditions and confidence intervals were unreliable.

5.8 DISCUSSION

5.8.1 Relative abundance of *Paracaprella pusilla* in Panama and taxonomic remarks

Our analyses indicate that the frequency of occurrence of *Paracaprella pusilla* was very high on both Panamanian coasts. However, the ADR method highlighted important differences in the abundance and local distribution range of *P. pusilla* between the Atlantic and Pacific entrances to the Panama Canal. *P. pusilla* was less abundant and its distribution per station was more limited on the Pacific side than on the Atlantic side of the Panama Canal.

The ADR method provides baseline information about the status of an alien species at a certain point in time that is independent of previous surveys (Minchin and White 2014), but it does not inform us about the potential effects of the invasion. While all alien species may have some level of impact (Carlton 2002) following colonization (e.g., through occupying space, consuming native prey, or other processes), the ecological effects of *P. pusilla* has not yet been evaluated in Panama and are poorly understood in other geographic regions.

Paracaprella pusilla from different biogeographic areas, including populations found in Panama, showed little intraspecific variation in morphology. This contrast with other caprellid species, such as *Caprella acanthifera*, *C. equilibra*, *C. penantis* or *C. scaura*, which belong to a complex of several morphological forms whose taxonomic represent a challenge (e.g. McCain 1968; Krapp-Schieckel and Vader 1998; Krapp *et al.* 2006; Cabezas *et al.* 2013). In the case of *P. pusilla*, there are no different forms described or morphological variations that remain constant among populations. Furthermore, Cabezas (2014) found low levels of mitochondrial cytochrome c oxidase subunit I (COI) genetic divergence between geographically distant populations, confirming the absence of cryptic species and clearly separating those *P. pusilla* populations from the closely-related species *P. tenuis*. As expected, preliminary genetic analyses indicate that Pacific Panama, Gulf of Mexico, and Brazilian populations are the same species (M. Ros and P. Cabezas, unpublished data). Thus, morphological and molecular evidences support the usefulness of Mayer's diagnostic characters (see Material and Methods) to properly identify specimens

belonging to *P. pusilla* and to differentiate them from similar species like *P. tenuis*.

5.8.2 On the introduction of *Paracaprella pusilla* in the Pacific Ocean

The first confirmed record of *Paracaprella pusilla* in the Pacific Ocean was about two decades after the opening of the Panama Canal, in 1937, in Honolulu harbor (Edmondson and Mansfield 1948). Curiously, there are no additional verified reports of the species in the Pacific until 2003, when it was found on the hulls of local vessels in Australia (Montelli and Lewis 2008). In 2008, as noted here, it was found at the Pacific entrance to the Panama Canal. In all of these locations – Hawaii, Australia, and Panama – *P. pusilla* may have been present years or decades earlier. No caprellid studies preceded those of Edmondson and Mansfield in Hawaii, and *P. pusilla* may have been overlooked as well in Australia. However, in the last case, none of the previous extensive studies focused on the caprellid fauna of Australia, including Tasmania (Guerra-García 2004 and 2006b; Guerra-García and Takeuchi 2004) reported the presence of *P. pusilla* in the area. In contrast, previous caprellid studies in Panama are limited to isolated records of a few species, such as *Caprella equilibra*, which was found at Taboga Island (McCain and Steinberg 1970) and *Paracaprella barnardi* McCain 1967 from Culebra Island. More extensive surveys along the Pacific coasts of Costa Rica, Panama, Colombia, and other Central and South American shores will be of great value in establishing the full range of *P. pusilla* in the tropical Eastern Pacific. If combined with detailed genetic analyses, this may also reveal the invasion history of the region.

5.8.3 Possible vectors of its introduction to Pacific Panama

There are two possible shipping-related vectors for the appearance of *P. pusilla* on the Pacific coast of Panama. The first is transport from the Caribbean or Atlantic South America in ballast water through the Canal. Importantly, our results indicate that *P. pusilla* would not survive the time of exposure on a ship's hull to completely fresh water while passing through Gatun Lake (this study, and as further discussed below). Caprellids are common in harbors and ports drifting on small pieces of debris, seaweed, or seagrasses, and are often found in plankton tows 'free' floating in the water as members of the tycho plankton; as such, they

could easily be taken up into ballast tanks, where they have in fact been found (Carlton 1985; Gollasch *et al.* 2002). No objects are known to raft through the entire Canal, from one ocean to the other, nor would rafting permit a rapid transit of a few hours through Gatun Lake. While we cannot exclude the human-aided transfer of the caprellids associated with construction materials, fishing gear, dredges, or other objects across coasts of Panama, any such transfers would have to remain wet (but not exposed to freshwater of the Canal) and be rapidly immersed into Pacific waters to allow species survival, appearing to be a rare if not unlikely event. Reish and Barnard (1979) commented that while the Panama Canal could be a pathway for amphipod transport, they knew of no species that had yet ‘transgressed’ the Canal. If *P. pusilla* originated from the Caribbean, this caprellid would be the first example of an amphipod that was known to have successfully passed through the Canal.

The second likely source is transport associated with ballast water tanks and/or the biofouling community on ships’ hulls or underwater surfaces arriving from introduced populations in Australia or Hawaii, with caprellids dispersing from hull fouling communities while vessels from either of these regions were stationary at the Canal entrance, as vessels can wait at anchorage for days before transiting the Canal. Genetic studies may assist in identifying the origin of the Pacific Panama *P. pusilla* population, particularly if Western Atlantic, Australian, or Hawaiian populations have either private alleles, unique proportions of haplotypes, or both. Unfortunately, the limited preserved specimens in absolute ethanol from the potential source populations analyzed and the need for further intensive sampling from both more native and non-native populations of this species prevent us to address this question throughout molecular tools.

5.8.4 Salinity tolerance of *Paracaprella pusilla* and its implications for its dispersal

The salinity tolerance of *Paracaprella pusilla* is similar to that of other caprellid species. The lower 48 h LC50 (17.8) is slightly lower than that of the invasive caprellid *Caprella mutica* with 48 h LC50 values of 18.7 (Ashton *et al.* 2007). However, the 24 h LC50 of *P. pusilla* (11.6) is higher than that of another invasive caprellid, *Caprella scaura* Templeton, 1836, with 24 h LC50 values of

7.5 (Cockman and Albone 1987). The increased tolerance to lower values of salinity of *P. pusilla* females in comparison with males is just the opposite case to *C. mutica*, in which males are more tolerant than females (Ashton et al. 2007). However, a high tolerance in females has been described in other amphipods like *Bathyporeia pelagica*, *B. pilosa* (Preece 1970) and *Corophium volutator*, in which the gravid females have a wider tolerance than non-gravid adult females which in turn are more tolerant than adult males (Mills and Fish 1980). During freshwater input events, a higher potential to survive by reproductive females, with eggs in its brood pouchs, may help in the survival of their offspring.

In general terms, the wide salinity tolerance of *P. pusilla* may help in the survival and establishment success of the species in either side of Gatun Lake and in the Panamanian coasts, which support large freshwater inputs in the rainy season (when the species was collected). This is also frequent in anthropogenic habitats, like marinas and harbors, in which euryhaline species may be favored. In the approach channels of the Panama Canal, measurements indicate that salinity ranges between 10 and 30 (Cohen 2006). If low salinity periods are maintained over time, *P. pusilla* could reduce its distribution to those places where low salinities are not below 15. However, rapid changes in salinity could be supported if they are not below 5. Therefore, although *P. pusilla* may be associated with fouling communities of ship hulls, it would not survive the freshwater barrier of the Panama Canal. For this reason, ballast water is suggested as the introduction vector to its interoceanic introduction through the Panama Canal, if the Caribbean is the source of this population.

Relative to its high salinity tolerance to hypersaline waters, with upper salinity tolerances above 46 at 24h and 43 at 48h observed in our experiment, it is probable that *P. pusilla* would be able to survive the hypersaline waters of the Suez Canal within the fouling communities attached to ship hulls that transit Suez. In fact, the presence of *P. pusilla* in the Suez Canal was recorded by Schellenberg (1928), although since this report, no evidence of established populations of *P. pusilla* has been found in this area or surroundings (Ros *et al.* 2013). Different factors other than salinity tolerance may be involved if *P. pusilla* failed to establish in the Suez Canal.

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6

NATIVE-RANGE ECOLOGY: PART 1

Studying exotics in their native range: can introduced caprellids expand beyond anthropogenic habitats?

- Adapted from:

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(*submitted*) Studying exotics in their native range: can introduced caprellid
amphipods expand beyond anthropogenic habitats?

ABSTRACT. The ecology of small mobile invertebrates inhabiting biofouling assemblages (secondary habitat) provided by artificial structures (primary habitat) has been poorly studied. A biogeographic approach focused in the native-range ecology of introduced and common fouling caprellid amphipods of southern Europe was used to examine the influence of habitat use patterns on the distribution of widespread marine mobile epibionts and understand the factors that may limit or facilitate their colonization of natural habitats. Specifically, abundance and composition of caprellid populations were compared among artificial (floating pontoons), sheltered and wave-exposed rocky shores along the southeastern coast of Brazil. Results showed that artificial habitats act as reservoir for globally distributed species in both their native and introduced ranges, while endemic species are more scarcely represented. Environmental conditions provided by primary habitats were more important in structuring caprellid assemblages than secondary substratum characteristics. Moreover, *Caprella scaura* and *Paracaprella pusilla*, recently introduced in southern Europe where they are virtually restricted to artificial habitats, are expected to become established in sheltered and even highly polluted natural habitats but they will be unable to colonize wave-exposed rocky shores.

RESUMEN. La ecología de los invertebrados móviles de pequeño tamaño que viven asociados al fouling (hábitat secundario) que crece sobre estructuras artificiales (hábitat primario) ha sido poco estudiada. Para examinar la influencia del uso del hábitat en la distribución de los epibiontes marinos de amplia distribución y entender los factores que limitan o favorecen su establecimiento en hábitats naturales, se estudió la ecología de las especies de anfípodos caprelidos introducidas y comúnmente asociadas al *fouling* en el sur de Europa desde una perspectiva biogeográfica focalizada en su rango nativo. Concretamente, se comparó la abundancia y composición de las poblaciones de caprelidos entre hábitats artificiales (pantales flotantes), costas rocosas protegidas y costas rocosas expuestas al oleaje a lo largo de la costa sureste de Brasil. Los resultados mostraron que los hábitats artificiales actúan como reservorios de especies de amplia distribución tanto en su rango nativo como introducido, mientras que las especies endémicas tuvieron escasa representación. Las condiciones ambientales de los hábitats primarios influyeron más en la estructuración de los caprelidos que las características del sustrato secundario. *Caprella scaura* y *Paracaprella pusilla*, recientemente introducidas en el sur de Europa, donde se encuentran prácticamente restringidas a hábitats artificiales, probablemente colonizarán hábitats naturales protegidos del oleaje, incluso estando altamente contaminados, pero serán incapaces de colonizar costas expuestas al oleaje.

6.1 INTRODUCTION

Understanding the mechanisms involved in the invasion success of non-indigenous species (NIS) beyond artificial structures is a key aspect for managing invasive species and preserving biodiversity (Priestler 2009; Ruiz *et al.* 2009; Simkanin *et al.* 2012; Davis *et al.* 2014). However, this is one of the major gaps in the field of marine bioinvasions (Ruiz *et al.* 2000; Streftaris *et al.* 2005; Simkanin *et al.* 2012).

Patterns of habitat use may have significant implications for the marine invasions dynamics (Ruiz *et al.* 2009) and can provide major insights for predicting which types of habitats will be more susceptible to be invaded by newly introduced species. Exploring the ecological preferences of these species in their native range, or in areas where they have been introduced for a long time, may contribute to understanding both the ability of these species to successfully colonize natural habitats and the factors that may help or hinder the achievement of this phase (Daehler and Gordon 1997; Hierro *et al.* 2005; Hejda *et al.* 2009; van Kleunen *et al.* 2010). While this approach shows interesting, the ecology of marine NIS is frequently ignored in their home range (Chavanich *et al.* 2006; Parker *et al.* 2013) and native-invaded comparisons are extremely rare (Lohrer *et al.* 2000).

Several studies have shown that intertidal assemblages supported by artificial habitats and those on nearby rocky shores are different (Connell and Glasby 1998; Glasby 1999; Connell 2000; Bulleri and Chapman 2004). Part of these differences lies on the higher abundance of NIS in anthropogenic habitats in comparison with the natural surrounding habitat (Glasby *et al.* 2007; Marins *et al.* 2010). However, surveys of artificial structures (primary habitats) are mostly focused on sessile benthic invertebrates, seaweeds or ‘charismatic’ macrofauna. Smaller mobile organisms inhabiting the secondary habitat provided by fouling animals and seaweeds (hereafter secondary substratum) are generally overlooked (Chapman *et al.* 2005; People 2006; Wilhelmsson and Malm 2008; Marzinelli *et al.* 2009). Furthermore, the respective roles of the primary and secondary habitat in structuring epifouling assemblages remain unclear. This is particularly relevant in groups such as caprellid amphipods, because although most caprellids can inhabit different substrata, the existence of substrata affinities has been shown for many species (e.g. Keith 1971; Lacerda and Masunari 2011). These

small crustaceans often dominate the mobile invertebrate fauna associated with artificial structures of harbours and marinas, where they can reach densities of >3000 individuals per m² (Buschbaum and Gutow 2005). Within these habitats, non-indigenous caprellids can contribute to increase the biotic resistance of fouling communities through hinder the settlement of larvae of invasive sessile species (Rius *et al.* 2014; Collin and Johnson 2014), but they also have the ability to displace ecologically similar congeners (Boos 2009; Shucksmith *et al.* 2009; Chapter 4), posing a risk to native communities. Despite not having a larval stage, introduced caprellids can be anthropogenically dispersed long distances in association with ship-related vectors (such as ballast water and hull fouling). In a local scale, while recreational boating seems effective in the transport of exotic caprellids among marinas, it fails to spread them from these habitats to intertidal rocky shores (Ros *et al.* 2013a). Thus, introduced caprellids seem virtually restricted to artificial habitats in their invaded range, reflecting the existence of a limiting step in their invasion dynamic whose causes are still unknown.

It has been suggested that anthropogenic vectors are more likely to be used as long dispersal mechanism by species that are better adapted to artificial habitats, such as those that are sheltered (Ruiz *et al.* 2009) or frequently disturbed by human activity (Byers 2002; Bulleri and Airoidi 2005; Piola and Johnston 2008). Wave exposure and patterns of water transport are major determinants of community composition in general (Southward and Orton 1954; Lewis 1964; Underwood 1981; Bulleri and Chapman 2004) and amphipod community in particular (Fenwick *et al.* 1976; Takeuchi *et al.* 1990; Guerra-García and García-Gómez 2001). This is especially important for caprellid species, many of which obtain their food from suspended material in the water column (Guerra-García and Tierno de Figueroa 2009). However, the role of this factor in the invasion dynamic of introduced caprellids has not yet been investigated.

The southeastern coast of Brazil is considered as part of the potential native range of the two caprellid species recently introduced in the Mediterranean: *Caprella scaura* Templeton, 1836 and *Paracaprella pusilla* Mayer, 1890. *Paracaprella pusilla* was described in Brazil and *C. scaura*, although it was described in Mauritius, was also recorded in Brazil only two years later (Dana 1853). Thus, both species have long been established in the area, like other wide-ranging caprellids, such as *Caprella dilatata* Krøyer, 1843 (also described in Brazil) or *Caprella equilibra* Say, 1818 (described in the North

West Atlantic coast). Along with a wide distribution, all of these species have in common that they are much more abundant in artificial than in natural environments in the Mediterranean Sea.

In this study, we used a biogeographic approach focused in the native-range ecology of introduced and common fouling caprellid species in the Mediterranean to understand the role that habitat use patterns has in their current distribution and the factors that may favor or hinder their successfully establishment in natural habitats. Additionally, we analyze the invasive potential of *P. pusilla* and *C. scaura* through a standardized invaded-native range comparison. Specifically, we tested the following hypotheses:

1. Caprellid assemblages differ among habitat types in Brazil. Particularly, wide-ranging species (*C. scaura*, *P. pusilla*, *C. dilatata* and *C. equilibra*) are more abundant in artificial than in natural habitats and, within natural habitats, in those protected from the wave action.
2. The influence of the primary habitat (artificial vs natural wave-exposed vs natural wave-protected) is more important in the distribution of caprellid species than the influence of the basibiont species (secondary substratum). Thus, the distribution and abundance patterns of caprellid species that inhabit the same secondary substratum, in the same locality, differ among the habitat types investigated in Brazil.
3. Considering Williamson's tens rule (Williamson 1996; Williamson and Fitter 1996), most of NIS occurs at similar or even lower densities in recipient communities compared with their native range (Hierro *et al.* 2005). Accordingly, within the same primary habitat (marinas) and the same secondary substratum, the abundance of *C. scaura* and *P. pusilla* is relatively similar in their invaded and in their home range.

6.2 MATERIAL AND METHODS

6.2.1 Field survey and laboratory processing

Native-range survey

The sampling survey was carried out in October and November 2012 (spring/summer). Four localities, separated from each other by hundreds of kilometers (L1: Arraial do Cabo, L2: São Sebastiao, L3: Paranaguá Bay, L4: Bombinhas-Porto Belo), were surveyed along the Southeast (Rio de Janeiro and São Paulo) and South (Paraná and Santa Catarina states) coasts of Brazil (Fig. 6.1, Table 6.1). In each locality, three habitat types, separated by thousands of meters, were selected for comparison: artificial habitats, consisting in floating pontoons present in marinas (or in close proximity, as it was the case of locality 4); wave-protected natural habitats, consisting in sheltered rocky shores; and wave-exposed natural habitats, consisting of rocky shores well exposed to wave action. Localities were specifically selected based on the presence of the three habitat types considered at a certain distance from each other (thousand of meters) and with the aim to cover a regional scale of comparison. In each habitat type, three sites separated by tens of meters were surveyed, and three replicates were taken per site. A total of nine replicates were collected per habitat type. All three habitat types were sampled in each locality on the same or consecutive days.

The sampling methodology was selected according with three criteria: (1) adequately sample caprellid diversity, (2) avoid the effects of patchiness (aggregated distribution) and (3) make the sampling comparative among the three habitat types. Hence, within each site, three patches were haphazardly selected preferentially in vertical surfaces to collect three replicates. Then, arborescent substrates present within each patch, including hydroids, bryozoans and seaweeds, were haphazardly collected until a volume of approximately 750 ml was completed (see Thiel *et al.* 2003; Guerra-García *et al.* 2010; Ros *et al.* 2013a for method adequacy).

To investigate the role of host substratum type in structuring caprellid assemblages (hypothesis 2), sampling efforts were focused on a well-defined secondary substratum, the hydroid *Eudendrium* sp. This hydroid was selected

due to its versatility to grow in different habitat types (e.g. Bavestrello *et al.* 1996; Navarro-Barranco *et al.* 2014). When this hydroid was present, three well developed colonies were separately collected from each site. A total of nine colonies were collected per habitat type.

For the native-invaded range comparison (hypothesis 3), artificial structures (floating pontoons) were selected as the primary habitat (since *P. pusilla* and *C. scaura* are present in these structures in the two distribution ranges but are absent in the other habitats in their invaded range). The secondary substratum species was *Eudendrium* sp. in the case of *P. pusilla* (because it is considered the preferred basibiont species for this species in the Mediterranean Sea and nearby areas; Ros and Guerra-García 2012; Ros *et al.* 2013b) and the bryozoan *Bugula neritina* for *C. scaura* (for the same reason as in *P. pusilla*; see Ros *et al.* 2014). All samples were collected in the same season (spring/summer).

Samples were collected directly by hand from the lateral surface of the floating pontoons (close to the water surface) and from intertidal rocky shores at low tide, while snorkeling provided samples at high tide. Just after collection, samples were preserved in 70% ethanol. At the laboratory, all caprellids were sorted and identified to species level. Total volume of substratum per replicate was estimated as the difference between the initial and final volume when placed into a graduated cylinder with a fixed amount of water. Abundance of caprellids was expressed as number of individuals/1,000 ml of substratum. Species were classified in the following geographical distribution groups (modified from Guerra-García *et al.* 2009):

- I: Endemic of Brazil
- II: Atlantic
- III: Atlantic and Mediterranean
- IV: Cosmopolitan

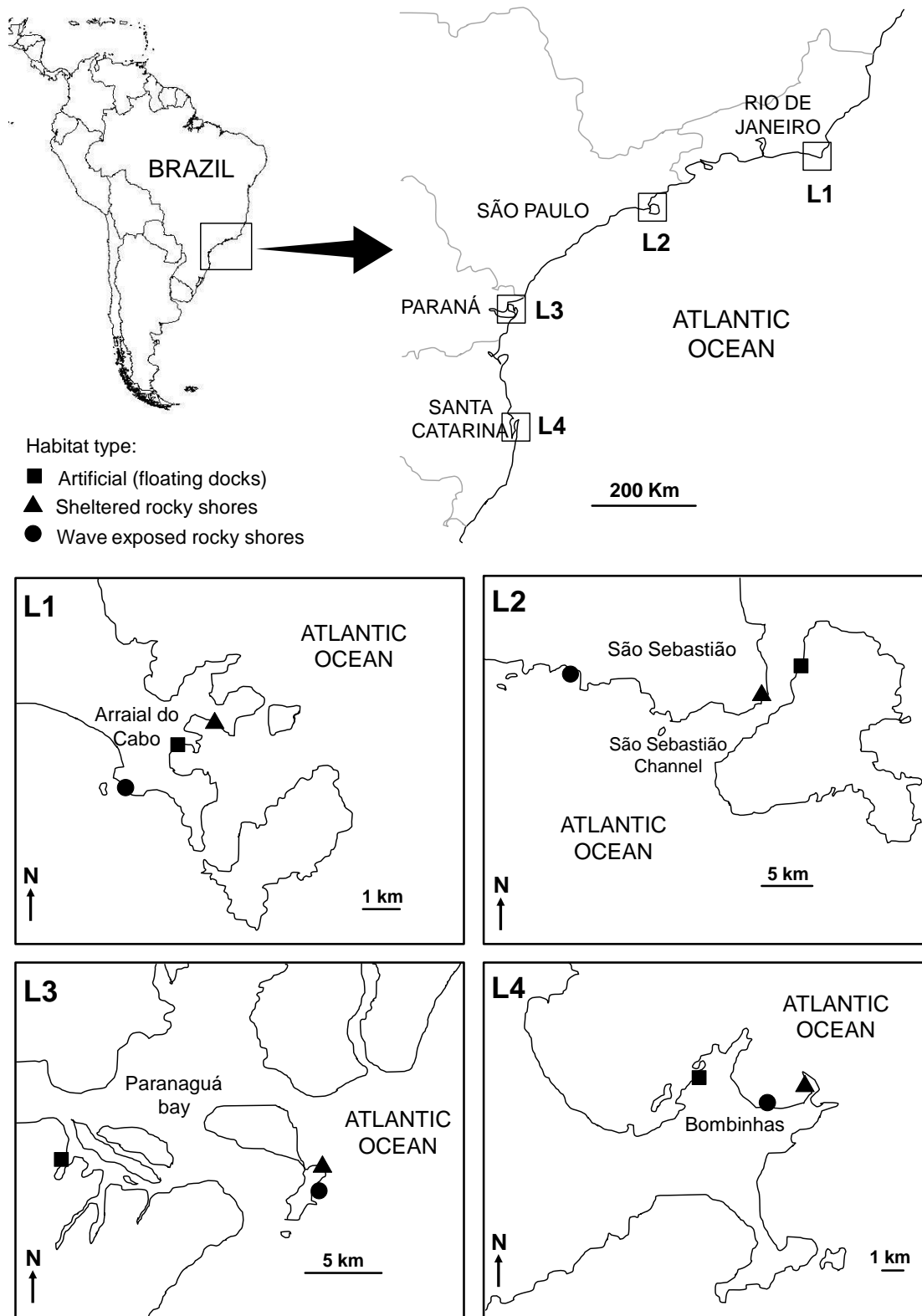


Fig. 6.1 Study area showing the four sampling localities and the habitats surveyed in each locality.

Table 6.1 Sampling localities coordinates, collection dates, wave fetch values and wave exposure class (see Table 6.2). Effect. fetch: effective fetch; Max. fetch: maximum fetch; Wave exp. class: wave exposure class.

Locality (State)	Habitat	Coordinates	Date of collection	Effect. fetch (km)	Max. fetch (km)	Wave exp. class
Arraial do Cabo (RJ)	A	22° 58' 13" S, 42° 01' 1" O	Oct 2012	0,58	3,04	VP
	P	22° 57' 58" S, 42° 00' 25" O	Oct 2012	1,70	4,02	P
	E	22° 58' 47" S, 42° 01' 57" O	Oct 2012	687,50	<1000	E
São Sebastiao (SP)	A	23° 46' 23" S, 42° 21' 23" O	Oct 2012	0,48	0,38	VP
	P	23° 46' 04" S, 45° 24' 23" O	Oct 2012	1,37	4,66	P
	E	23° 47' 00" S, 45° 38' 18" O	Oct 2012	707,64	<1000	E
Paranaguá Bay (PN)	A	25° 30' 57" S, 48° 29' 58" O	Nov 2012	0,18	0,24	VP
	P	25° 32' 22" S, 48° 17' 22" O	Nov 2012	3,79	10,26	SP
	E	25° 33' 20" S, 48° 17' 59" O	Nov 2012	707,38	<1000	E
Bombinhas-Porto Belo (SC)	A	27° 08' 55" S, 48° 32' 1" O	Oct 2012	1,24	6,14	P
	P	27° 08' 24" S, 48° 28' 43" O	Oct 2012	1,54	1,42	P
	E	27° 08' 43" S, 48° 29' 43" O	Oct 2012	707,54	<1000	E

Wave exposure analysis

Quantification of wave exposure in each station was based in a fetch model index developed by Howes *et al.* (1994). Fetch models provide good quantitative approximations of wave exposure to predict marine community patterns (e.g. Burrows *et al.* 2008; Hill *et al.* 2010). This model relies on two indices of fetch: modified effective fetch and maximum fetch. Combination of the two indices allows determining the wave exposure class of each station (Table 6.2) and is calculated from the following equation:

$$Fe = [\sum (\cos\theta_i) \times F_i] / \sum \cos\theta_i$$

where F_e is the effective fetch in km, θ_i is the angle between the shore-normal and the direction (0° , 45° to the left and 45° to the right), and F_i is the fetch distance in km along the relevant vector. Maximum fetch is defined as the maximum fetch distance in km measured from the point of interest. A value of 1000 km is conventionally used when open-ocean fetches occur. The mean value in km of modified-effective fetch and maximum fetch (hereafter average fetch) for each station was used as continuous variable in subsequent analysis.

Table 6.2 Wave exposure classes based on the modified-effective fetch and maximum fetch matrix (after Howes *et al.* 1994). VP: very protected; P: protected; SP: semi-protected; SP: semi-exposed; E: exposed.

Max Fetch (km)	Modified-effective fetch (km)				
	< 1	1 - 10	10 - 50	50 - 500	> 500
< 10	VP	P	-	-	-
10 - 50	-	SP	SP	-	-
50 - 500	-	SE	SE	SE	-
> 500	-	-	SE	E	E

Invaded-range survey

A field survey was conducted in five marinas from the Western Mediterranean coast from May to July 2012 (spring/summer) to test the last hypothesis. Palma marina in Mallorca Island (Spain, $39^\circ 34' N$, $2^\circ 38' E$) and Ibiza Yacht Club in Ibiza Island (Spain, $38^\circ 54' N$, $1^\circ 26' E$) were selected for the native *vs* introduced range comparison of *P. pusilla* (since they are the only marinas where the species has been found in the Mediterranean). In the case of *C. scaura*, Mahón maritime Club in Menorca Island (Spain, $39^\circ 52' N$, $4^\circ 18' E$), Civitavecchia marina (Italy, $42^\circ 05' N$, $11^\circ 47' E$) and Gzira marina (Malta, $35^\circ 54' N$, $14^\circ 29' E$) were selected in order to cover a wide range of its distribution in the Mediterranean (the number of marinas was selected based on the number of marinas in which *C. scaura* was present in Brasil to develop a balanced analyses). Colonies of *B. neritina* and *Eudendrium* sp. for *C. scaura* and *P. pusilla* respectively were collected in the same way as in Brazil.

6.2.2 Statistical analysis

Differences in caprellid community composition among habitat types were tested by the use of a permutational multivariate analysis of variance (PERMANOVA) with three factors: ‘habitat’, fixed with three levels (artificial, natural wave-protected and natural wave-exposed); ‘locality’, fixed and orthogonal with ‘habitat’, with four levels (L1: Arraial do Cabo, L2: São Sebastiao, L3: Paranaguá Bay, L4: Bombinhas-Porto Belo); and ‘site’, random and nested with habitat and locality, with three levels. Analysis was based on Bray-Curtis dissimilarities. Significance *P*-values were obtained by computing 9999 permutations of residuals under a reduced model (Anderson and Legendre 1999). Pairwise comparisons were then used between factors to determine where significant differences occurred within the caprellid community. To test differences of caprellid community multivariate dispersions among habitats a PERMDISP was applied and further illustrated with nMDS (based on Bray-Curtis dissimilarities).

Univariate ANOVA analysis was used to explore the abundance patterns of the target caprellid species (*P. pusilla*, *C. scaura*, *C. dilatata*, *C. equilibra*) along the study area. Experimental design was the same as it was employed in the PERMANOVA analyses. Prior to ANOVA, heterogeneity of variance was tested using Cochran's C-test. To reduce type I error, the level of significance was reduced to <0.01 when variances were significantly different (Underwood 1997). Where ANOVA indicated a significant difference for a given factor, the source of difference was identified using Student–Newman–Keul (SNK) tests. To better understand the relationship between the abundance of each target species and wave exposure, a Spearman rank correlation was used.

To test the second hypothesis, a two-way ANOVA with two factors was used for each caprellid species associated with the hydroid *Eudendrium* sp.: ‘habitat’, fixed with three levels (artificial, natural wave-protected and natural wave-exposed); and ‘site’, random and nested with habitat, with three levels. The factor ‘locality’ could not be included in this analysis because *Eudendrium* sp. was only present in the three habitat types within one locality (L3). No further substratum species was present in the three habitat types within a particular locality.

Finally, a two-way ANOVA was also performed to test the third hypothesis with the following factors: ‘range’, fixed factor with two levels (native and introduced); and ‘locality’, fixed and nested with range with two levels in *P. pusilla* (São Sebastiao y Paranaguá Bay in the native range, and Palma and Ibiza islands in the introduced range) and three levels in *C. scaura* analyses (Arraial do Cabo, São Sebastiao and Bombinhas-Porto Belo in the native range, and Menorca island, Malta island and Civitavecchia, in the introduced range). Pooling of non-significant sources of variation was done at $P > 0.25$ (Winer 1971). The factor ‘site’ could not be included in these analyses because in the invaded range not sufficient substrata were found in all sites. Eventually, three random colonies per marina were selected for comparison in order to make a balanced analysis.

Univariate analyses were conducted with SPSS 15.0 and GMAV5 (Underwood *et al.* 2002) and multivariate analysis were carried out using the PRIMER v.6 plus PERMANOVA package (Clarke and Gorley 2006).

6.3 RESULTS

6.3.1 Testing hypothesis 1

Caprellid assemblages

Across the four Brazilian localities, we collected a total of 11327 caprellids belonging to 5 genera and 10 species (Table 6.3). Seven species were recorded in artificial habitats (floating pontoons), nine on sheltered intertidal rocky shores and four on wave-exposed intertidal rocky shores. Abundance and composition of caprellid assemblages were different among habitats with few exceptions (see *a posteriori* tests in Table 6.4). PERMDISP analyses revealed significant differences in variation of the mean caprellid abundance and composition among habitats (Table 6.4). These differences were higher in artificial and natural protected habitats (PERMDISP, mean \pm standard error: 57.21 ± 1.38 and 62.36 ± 0.65 respectively) than in natural exposed habitats (37.20 ± 2.32). The spatial illustration of the multivariate dispersions of caprellid assemblages among habitats (Fig. 6.2) showed that exposed shores were less dispersed than the other two habitat types.

Table 6.3 Mean abundance (individuals/1000ml substrate) of each caprellid species found in each habitat type throughout the study area. BG: biogeographical distribution groups; I, Endemic of Brazil; II, Atlantic; III, Atlantic and Mediterranean; IV, Cosmopolitan.

CAPRELLID SPECIES	BG	HABITAT																								
		Artificial								Natural protected								Natural exposed								
		L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4									
<i>Aciconula tridentata</i> Guedes-Silva and Souza-Filho, 2013	I		●																							
<i>Caprella danilevskii</i> Czerniavski, 1868	IV										●															
<i>Caprella dilatata</i> Krøyer, 1843	III	●	●			●						●														
<i>Caprella equilibra</i> Say, 1818	IV	●	●	●																				●		
<i>Caprella scaura</i> Templeton, 1836	IV	●	●	●	●									●												
<i>Paracaprella pusilla</i> Mayer, 1890	IV	●	●	●								●														
<i>Paracaprella</i> sp.	I															●										
<i>Pseudaeginella montoucheti</i> (Quitete, 1971)	I																									
<i>Pseudaeginella</i> sp.	I	●																								
<i>Phtisica marina</i> Slabber, 1769	III																									

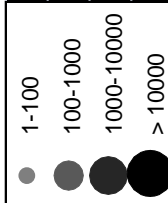
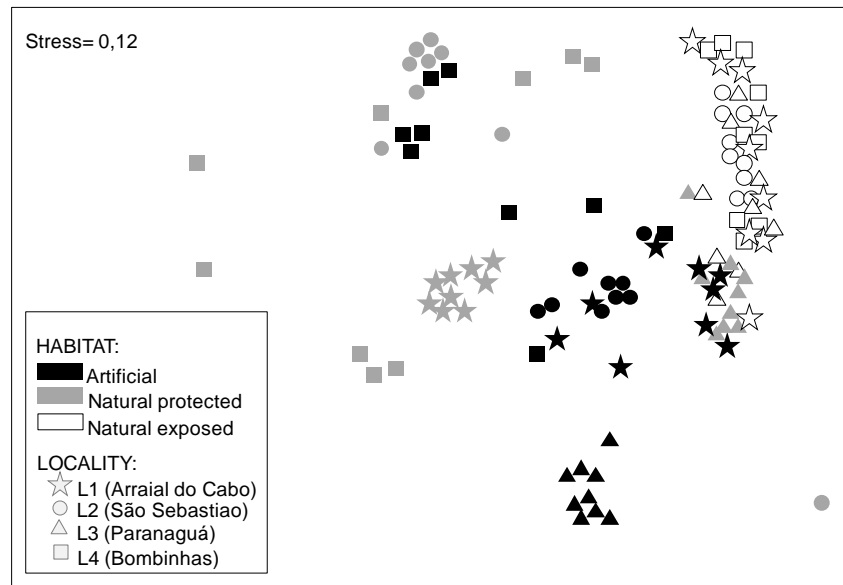


Table 6.4 Summary of PERMANOVA results examining the abundance and community composition of caprellid assemblages found in each habitat (a: artificial; p: wave-protected rocky shore; e: wave-exposed rocky shore) and each locality. Star symbol indicates significant differences: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. PERMDISP results for the factor 'habitat' were also included.

Source of variation	df	MS	Pseudo-F	$P(perm)$	MS	Pseudo-F	$P(perm)$
		Abundance (non transformed)			Composition (presence/absence)		
Habitat = Ha	2	45023,00	11,65	0,0001**	54293,00	40,33	0,0001**
Locality = Lo	3	15209,00	3,94	0,0001**	16209,00	12,04	0,0001**
Ha x Lo	6	17758,00	4,60	0,0001**	1346,00	5,53	0,0001**
Site (Ha x Lo)	24	3863,00	3,83	0,0001**	7366,90	5,47	0,0001**
Residual	72	1009,40			243,34		
PERMDISP (Ha)			F = 68,718 ($p < 0,0001$)			F = 69,588 ($p < 0,0001$)	
<i>A posteriori</i> tests ^a							
		Ha (Lo)			Ha (Lo)		
		L1: a = e ≠ p			L1, L2, L3: a ≠ p ≠ e		
		L2: a ≠ p ≠ e			L4: a = p ≠ e		
		L3: a ≠ p = e					
		L4: a = p ≠ e					

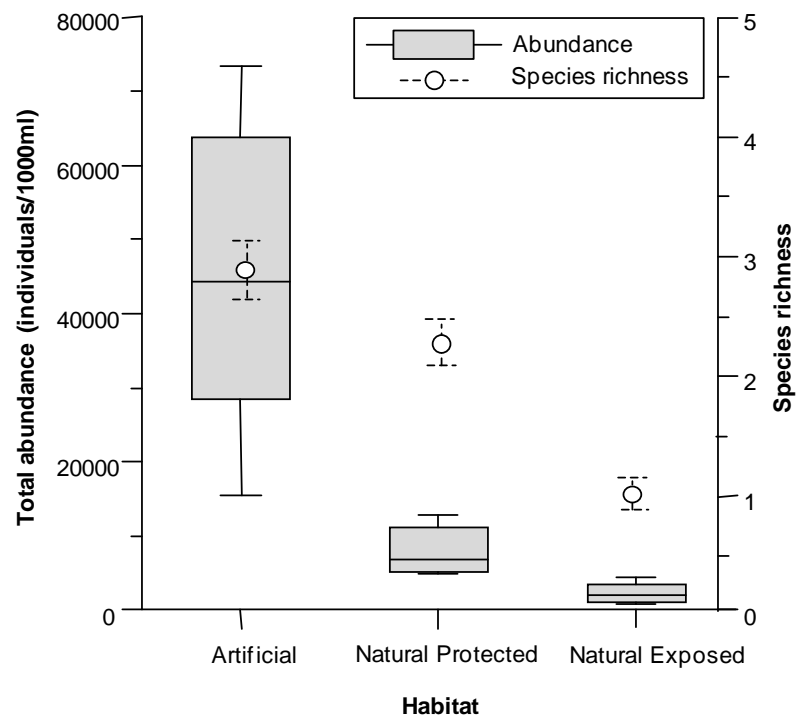
^aMonte Carlo p values were used in pair-wise test due to low number of unique permutations (Anderson, 2005)

Fig. 6.2 Non-transformed abundance nMDS plot for caprellid assemblages (habitat x locality interaction). Each point represents a replicate (n= 180).



In general terms, caprellid amphipods from southern Brasil were more abundant in artificial than in sheltered or exposed natural habitats (Fig. 6.3). The species richness was generally higher in artificial and sheltered rocky shores than in exposed habitats. However, both the species richness and the total abundance showed differences among localities (Table 6.5).

Fig. 6.3 On the left axis, boxplot for the total caprellid abundance found in each habitat; the median values are included; the rectangles contain values between the first and the third quartiles; the bars connect the extreme values. On the right axis, mean species richness in each habitat; the bars represent the Standard Error.



Distribution of target species

The wide-ranging and anthropogenically dispersed species *Paracaprella pusilla*, *Caprella scaura*, *C. dilatata* and *C. equilibra* were the dominant species in the southeastern and southern coasts of Brazil (Table 6.3). The remaining species, mostly endemic to Brazil, were restricted to only one or two habitats.

Paracaprella pusilla was more abundant in artificial than in natural habitats, being more abundant in sheltered than exposed rocky shores. This pattern was consistent among localities but it was only statistically significant in locality 3, in which *P. pusilla* exhibited its highest density (Fig. 6.4, Table 6.5). *Caprella scaura* was less abundant in exposed rocky shores than in the other two habitats (Fig. 6.4, Table 6.5). *Caprella dilatata* did not show a clear pattern among habitats, being significantly more abundant in different habitat types depending on the locality. *Caprella equilibra* was more abundant in artificial than in the other two habitats but, as occurs with *P. pusilla*, this pattern was only significant in locality 3.

Wave exposure

According to the combination of effective and maximum fetch (Tables 6.1 and 6.2), all artificial habitats were included in the 'Very Protected' category, except for locality 4 which was classified as 'Protected'. All sheltered intertidal habitats were classified as 'Protected', except for locality 3, which belonged to 'Semi-Protected' category. In the case of exposed intertidal rocky shores, fetch values were far higher and all belonged to 'Exposed' class, characteristic of open-ocean habitats.

Spearman rank correlations indicated that abundances of *P. pusilla*, *C. scaura* and *C. equilibra* were negatively correlated with average fetch (Fig. 6.5). By contrast, abundance of *C. dilatata* was positively correlated, although the coefficient obtained was very low ($r_s = 0.284$).

Table 6.5 Summary of ANOVA results examining the caprellid species richness, total caprellid abundance, abundance of dominant species (*Paracaprella pusilla*, *Caprella scaura*, *C. dilatata* and *C. equilibra*) among habitat types through the study area. Star symbol indicates significant differences: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$; n.s.: non significant; MS: mean square.

Source of variation	df	Caprellid species richness				Total caprellid abundance				<i>Paracaprella pusilla</i>			
		MS	F	P		MS	F	P		MS	F	P	F vs.
Habitat = Ha	2	33,40	40,53	0,0000***	6139419,57	17,18	0,0000***	652957,95	6,80	0,0046**			Si (Ha x Lo)
Locality = Lo	3	4,23	5,13	0,0069**	7446380,58	10,77	0,0001***	4979842,40	5,21	0,0065**			Si (Ha x Lo)
Ha x Lo	6	10,44	12,66	0,0000***	2772004,87	8,87	0,0000***	9936895,31	5,01	0,0019**			Si (Ha x Lo)
Site (Ha x Lo)	24	0,82	4,94	0,0000***	678229,22	3,81	0,0000***	7474288,00	1,41	0,1356			Res
Residual = Res	72	0,17			5150241,49			6834781,56					
Cochran's C-test				C = 0,2353 (p < 0,05)									C = 0,8021 (p < 0,01)
Transformation				None									None
													<i>C. dilatata</i>
													<i>C. equilibra</i>
Habitat = Ha	2	1211607,23	10,71	0,0005***	540361,34	1,19	0,3222	6415941,84	26,11	0,0001***			Si (Ha x Lo)
Locality = Lo	3	2140234,98	13,73	0,0001***	8159511,21	2,65	0,0717	7082171,79	16,85	0,0001***			Si (Ha x Lo)
Ha x Lo	6	8815237,13	17,08	0,0001***	4050002,52	6,38	0,0004***	2534868,51	16,42	0,0001***			Si (Ha x Lo)
Site (Ha x Lo)	24	6911966,83	1,64	0,0564	5711124,90	3,49	0,0001***	8659940,99	1,79	0,0312*			Res
Residual = Res	72	317382,06			7366981,33			1180852,23					
Cochran's C-test				C = 0,2582 (p < 0,01)									C = 0,6336 (p < 0,01)
				None									None

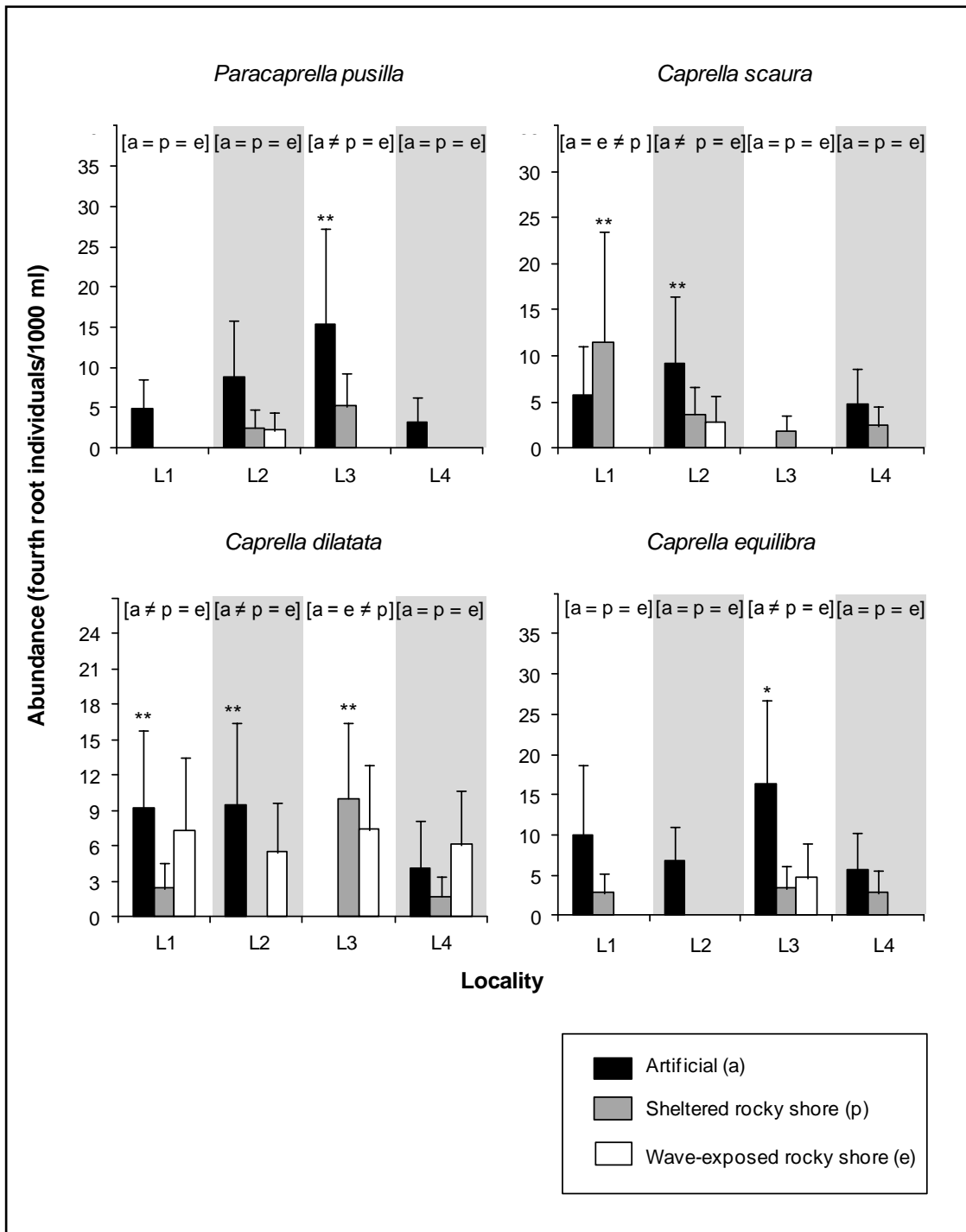


Fig. 6.4 Abundance of dominant species in each habitat type and each locality. Letters represent the SNK results of the interaction habitat x locality (see Table 6.5). Star symbol indicates significant differences: * $p < 0.05$, ** $p < 0.01$.

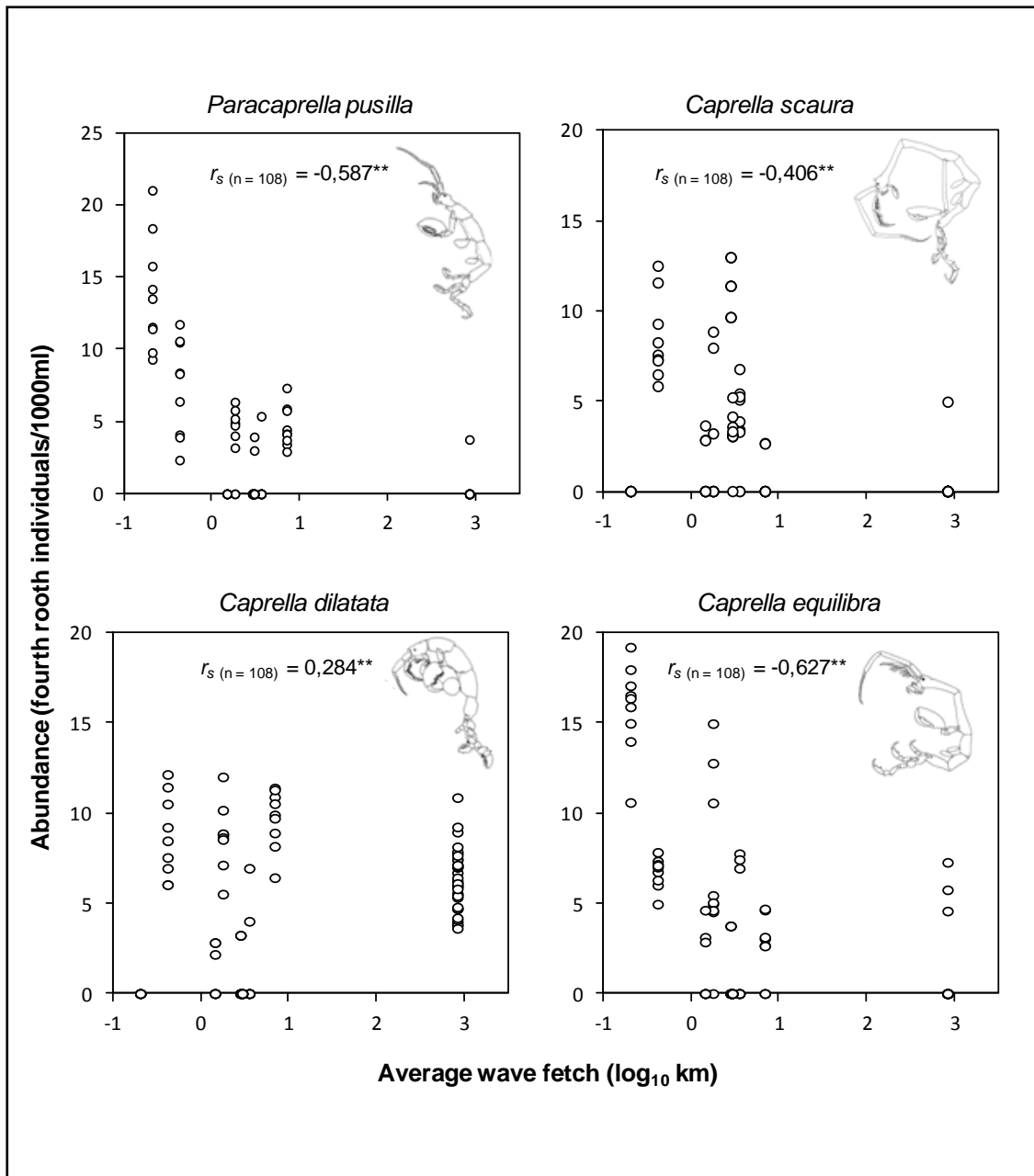


Fig. 6.5 Relationship between the total caprellid abundance and average wave fetch in km. Spearman rank order correlation coefficient is reported for the total abundance per replicate (n=180). Star symbol represent significant coefficient values: one star when $p < 0.05$ and two stars when $p < 0.01$.

6.3.2 Testing hypothesis 2

The abundance and distribution of the three caprellid species which inhabited *Eudendrium* colonies were significantly different among habitats (Fig. 6.6, Table 6.6). *Paracaprella pusilla* was only found in artificial and sheltered rocky shores, being significantly more abundant in the first one. *Caprella equilibra* was significantly more abundant in artificial than in natural (sheltered and wave-exposed) habitats, while *C. dilatata* exhibited the opposite pattern, being significantly more abundant in natural (sheltered and exposed) than in artificial habitats.

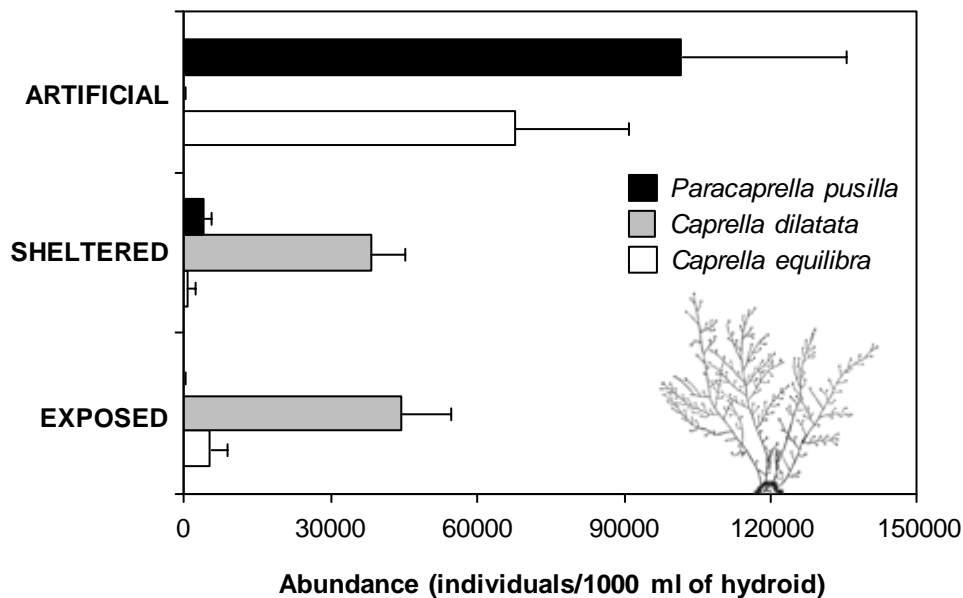


Fig. 6.6 Mean abundance of each caprellid species inhabiting the *Eudendrium* colonies collected in each habitat type from Paranaguá Bay; the bars represent the Standard Error.

Testing hypothesis 3

Abundance of *P. pusilla* and *C. scaura* between artificial habitats of their introduced (Mediterranean Sea) and native range (Brazil) were significantly different (Table 6.7). While *P. pusilla* was significantly more abundant in its native range, *C. scaura* was significantly more abundant in its introduced range.

Table 6.6 ANOVA results of the abundance patterns of caprellids inhabiting *Eudendrium* colonies among habitat types (a: artificial; p: wave-protected rocky shore; e: wave-exposed rocky shore) in Paranaguá Bay. Star symbol indicates significant differences: * $p < 0.05$, ** $p < 0.01$; n.s.: non significant.

Source of variation	df	<i>Paracaprella pusilla</i>			<i>Caprella dilatata</i>			<i>Caprella equilibra</i>			
		MS	F	P	MS	F	P	MS	F	P	
Habitat = Ha	2	1264276,93	6,41	0,0324*	4456550,48	8,85^a	0,0013**	114770,01	6,63	0,0303*	F vs. Site (Ha)
Site (Ha)	6	7081892,59	1,58	0,2100	7719891,74	1,07	0,4145	17317,96	3,50	0,0180*	Res
Residual = Res	18	7533846,85			9054044,37			4919,97			
Cochran's C-test				C = 0,6601 (p < 0,01)			C = 0,5162 (p < 0,05)				C = 0,3171 (n.s)
Transformation				None			None				Sqrt (x + 1)
SNK				Ha: a > p = e			Ha: a < p = e				Ha: a > p = e

^aF-value in bold was calculated with pooling of Site (Ha) because this source of variation showed a $p > 0,25$

Table 6.7 ANOVA results of the abundance patterns of *P. pusilla* and *C. scaura* between artificial habitats located in its introduced (Western Mediterranean) and native range (southeastern and southern Brazil). Star symbol indicates significant differences: * $p < 0.05$; n.s.: non significant.

Source of variation	df	<i>Paracaprella pusilla</i>			<i>Caprella scaura</i>			
		MS	F	P	MS	F	P	
Range = Ra	1	7534380,08	9,00^a	0,0133*	411.973.925	15,60	0,0168*	Locality (Ra)
Locality (Ra)	1	3110934,08	0,47	0,6412	26.413.749	2,46	0,1019	Res
Residual	8	3901057,08			10.737.424			
Cochran's C-test				C = 0,5290 (n.s)			C = 0,5942 (n.s)	
Transformation				None			Sqrt (x + 1)	
SNK				Ra: Native > Introduced			Ra: Native < Introduced	

^aF-value in bold was calculated with pooling of Locality (Ra) because this source of variation showed a $p > 0,25$

6.4 DISCUSSION

6.4.1 Habitat use patterns: testing hypothesis 1

Caprellid assemblages in southern and southeastern Brazil varied between artificial and natural habitats but also between natural habitats exposed to different hydrodynamic conditions. When natural and anthropogenic stress occur at the same time, it is difficult to understand the relative contribution of each factor to the observed distribution patterns. Hence, it is not the same to compare artificial habitats created by marinas (usually located in sheltered bays in Brazil) with rocky shores exposed or with those protected to wave action.

Along the coasts of Brazil, exposed rocky habitats were found to be stressed ecosystems for most of recorded species (except for *Caprella dilatata*), being characterized by the lowest caprellid abundance and species richness. This may explain its lower multivariate dispersion in comparison with the other two habitat types, in which more caprellid species were found and these species were more spatially segregated.

While the species richness was relatively similar in marinas and sheltered shores, the total caprellid abundance was far greater in marinas than in the whole of natural habitats surveyed (sheltered and exposed rocky shores). This abundance pattern is similar to that found by Thiel *et al.* (2003), who concluded that anchored buoys and ropes were the habitat with the highest abundance and species richness of littoral caprellids along the coast of Chile. This supports the role of artificial structures as a reservoir for caprellid species. Several authors have proposed that some species are able to attain spectacularly higher density in artificial vs. natural habitats because artificial structures constitute a spatial refuge from benthic predators (Dumont *et al.* 2011, Forrest *et al.* 2013, Simkaning *et al.* 2013). Although this has been shown for some invertebrate sessile NIS, the role of predation in the distribution of epifouling mobile invertebrates is still unclear. Furthermore, not all caprellid species were equally represented in artificial habitats. Although marinas harbored endemic (three species) and wide-ranging caprellids (the four target species of this study), this last group was the largest contributor to achieving such abundance in this type of habitat. Similarly, Megina *et al.* (2013) found that hydroid species optimized for low dispersal were barely represented in harbours from southern Spain while

cosmopolitan hydroids were far better represented in these habitats. This support that anthropogenic habitats are more conducive to widespread species (i.e. human-mediated globally transported species) than to those with more restricted distribution or, in other words, that human-mediated dispersed species appear to be able to take advantage of anthropogenically altered environments, as has been shown for some aquatic sessile NIS (Byers 2002; Crooks *et al.* 2011). It is possible that the shared evolutionary history of human-mediated transported caprellids with artificial structures provides these species with a competitive advantage over endemic or narrowly distributed species in these altered environments (see Byers 2002). This competitive advantage may occur due to an increased rate of inoculation of these species on artificial habitats (high propagule pressure) or because these species are better adapted to the environmental conditions provided by artificial habitats (Cohen and Carlton 1998), such as pollution and wave protection.

Exploring the habitat use patterns of the target species of this study (*Paracaprella pusilla*, *Caprella scaura*, *C. dilatata* and *C. equilibra*), we found that, with the exception of *C. dilatata*, abundance of these wide-ranging caprellids was negatively correlated with the level of wave exposure. The greatest abundance of *P. pusilla* and *C. equilibra* was recorded in Paranaguá harbour (locality 3), located in the Paranaguá Bay estuary. *Caprella scaura* exhibited the highest abundance in artificial or sheltered rocky habitats depending on the locality, but, as occurs in the other two species, it was practically absent in wave-exposed shores. *Caprella dilatata* was the only species that was found in high densities in the three habitat types, showing the highest versatility in habitat use. This species exhibited a high phenotypic plasticity and, as also documented Bynum (1980) and Caine (1989) for *C. penantis* (a closely related species), individuals collected from exposed habitats were clearly more robust than those collected from artificial or natural sheltered habitats. The ability of *C. dilatata* to occupy exposed shores, in which other species (widely distributed or endemic) failed to establish, ensures its survival and may favor its dispersal on floating objects subjected to strong currents.

Regarding with anthropogenic pollution in natural habitats, *Caprella scaura* and *Paracaprella pusilla*, considered as NIS in temperate ecosystems, were the only two species that were collected in the polluted sheltered shore of Araçá beach (locality 2). This area supports high rates of organic matter flux and

low oxygen due to the Araçá submarine outfall, affecting species richness of benthic communities (Teodoro *et al.* 2010). Thus, the two introduced species in the Mediterranean are well adapted to live in nutrient-enriched sheltered shores in their native range, in which other caprellid species are not able to survive. Furthermore, the highest abundance of *C. scaura* along the Brazilian coast was recorded in a sheltered rocky shore located in Arraial do Cabo (locality 1) which is under the influx of a floating restaurant situated very closed to the shore. In this sheltered shore, *C. scaura* was the dominant species, achieving higher densities than in surrounding artificial habitats. This implies that *C. scaura* can not only successfully establish beyond artificial structures in Brazil, but also can achieve spectacular abundances in sheltered shores as long as they are subject to anthropogenic pollution.

Considering the distribution patterns obtained for *C. scaura* and *P. pusilla* in their Brazilian range, could these species become established in natural environments in their newly introduced areas? The native-range ecology of these species suggests that while it is possible that in their European introduced range the two species will be able to establish in sheltered and even highly polluted rocky shores over time, they will fail to establish in wave-exposed habitats. In fact, introduced species are more frequently found in bays and estuaries than on exposed outer coasts and offshore localities (Carlton 1979; Wasson *et al.* 2005; Ruiz *et al.* 2009). Paradoxically, the globally distributed caprellids studied herein are usually dispersed over long distances inhabiting the fouling communities attached to vessel hulls and, consequently, they must survive a wide range of flow speeds. However, habitat use patterns are more related with post-introduction events. In this sense, caprellids adapted to sheltered conditions may survive strong currents and arrive in exposed rocky habitats, but they only became established wherever conditions are adequate.

6.4.2 Influence of secondary substratum: testing hypothesis 2

Several studies have demonstrated that host substratum characteristics are a determining factor in the composition of amphipod assemblages (Edgar 1983; Dean and Conell 1987; Hacker and Steneck 1990; Ayala and Martín 2003). Therefore, the addition of artificial structures (primary habitat) can directly or indirectly affect the caprellid composition through alterations of the secondary

habitat (host substratum composition) which they inhabit. This process is poorly understood in part because it is very difficult to separate the effect of primary and secondary habitat in structuring epibiont assemblages (Marzinelli *et al.* 2009). When we compared the caprellid assemblages inhabiting the same host substratum (*Eudendrium* colonies) among the three habitat types, we observed differences among habitats which were independent from the substratum characteristics. Thus, *P. pusilla* and *C. equilibra* were more abundant in the colonies located in artificial than in those located in natural (sheltered and exposed) habitats, while *C. dilatata* was more abundant on exposed intertidal rocky shores, where the other two species were not so successful. These differential patterns support the suggestion that environmental habitat conditions provided by artificial habitats had a direct effect on the caprellid composition and were more important in determining the distribution of caprellid species than the secondary substratum type. Similarly, Guerra-García and García-Gómez (2001) found that caprellid assemblages associated with the same seaweed species along the coast of Ceuta (North Africa) changed according to environmental factors, and People (2006) found that epibiont assemblages associated with mussels on pontoons in Sydney (Australia) differed from those on other types of structures, including natural reefs.

6.4.3 Invaded-native range comparison: testing hypothesis 3

Comparative studies to establish the degree to which species occur at different densities in their native and introduced range are needed to understand if new range conditions implement the proliferation of exotic species (Hierro *et al.* 2005; Parker *et al.* 2013). However, in the case of marine NIS, statements about different abundant pattern between introduced vs. native distribution range rely on comparisons between different habitats, mostly between artificial habitats in their introduced range and natural habitats in their native range.

When the abundance pattern of *C. scaura* and *P. pusilla* was standardized compared between native vs. introduced range, we found that while *P. pusilla* is a ‘weak invader’, *C. scaura* is a ‘strong invader’ (at least within the compared habitats: marinas). *P. pusilla* was much more abundant in their native (Brazil) than in their introduced range (Western Mediterranean), showing a limited invasive potential. By contrast, *C. scaura* was far more abundant in their introduced than in their native range, coinciding with a rapid expansion along the

whole coast of the Mediterranean (Ros et al. 2014). This caprellid is the dominant mobile epifouling species in many marinas of this region and it is considered as a potential agent of displacement of other caprellid species traditionally associated with the same spatial niche (Chapter 4). The next step is to understand why *C. scaura* and other invasive species are able to attain such dominance in recipient vs. native communities.

6.4.4 Conclusions

In conclusion, this study highlights the role of artificial habitats as reservoirs for certain caprellid species, particularly those with a widespread distribution. Most of wide-ranging caprellids were negatively correlated with the level of wave exposure, being more abundant in sheltered (artificial or natural) than in exposed habitats. Based on the native-range ecology of *Caprella scaura* and *Paracaprella pusilla*, the only two introduced caprellids recorded in the Mediterranean, they are expected to become established in sheltered and even highly polluted natural habitats but they will be unable to colonize wave-exposed rocky shores. The native-invaded range comparison for the two species indicates that while *P. pusilla* is a weak invader, *C. scaura* is a strong invader, being far more abundant in their invaded than in their native range. Additionally, we propose the use of the density of *C. scaura* and *P. pusilla* as a first diagnosis for the detection of nutrient enrichment on rocky shores.

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6

NATIVE-RANGE ECOLOGY: PART 2

Exploring trophic strategies of exotic caprellids:
Comparison between habitat types and native vs
introduced distribution ranges

- Adapted from:

Ros M, Tierno de Figueroa M, Guerra-García JM, Navarro-Barranco C, Lacerda M, Vázquez-Luis M, Masunari S (2014) Exploring trophic strategies of exotic caprellids (Crustacea: Amphipoda): comparison between habitat types and native vs introduced distribution ranges. *Estuarine, Coastal and Shelf Science* 139: 88–98.

ABSTRACT. The trophic ecology of exotic species is a key aspect to understand their invasion success and the recipient community effects. This is the first comprehensive study on the trophic strategies of two co-occurring introduced caprellid amphipods in the Spanish coasts: *Caprella scaura* and *Paracaprella pusilla*. The diet of 446 specimens of *C. scaura* and 230 of *P. pusilla* was analyzed to investigate whether there were differences in the feeding habits in relation to habitat characteristics (natural vs artificial hard substrate), type of host substrata (bryozoans and hydroids) and native vs introduced distribution ranges (Brazil vs Spain). Results revealed differences in diet preferences of the two species which may favour their coexistence in introduced areas. In general terms, *P. pusilla* is a predator species, showing preference by crustacean preys in all of its life stages, while *C. scaura* feeds mainly on detritus. Although no sex-related diet shifts were observed in either of the species, evidence of ontogenetic variation in diet of *C. scaura* was found, with juveniles feeding on more amount of preys than adults. *P. pusilla* exhibited a shift in its diet when different habitats were compared in the same distribution area, and *C. scaura* showed a flexible feeding behaviour between different host substrata in the same habitat type. This study shows that habitat characteristics at different scales can have more influence on the feeding ecology of exotic species than different distribution ranges.

RESUMEN. La ecología trófica de las especies exóticas es un factor clave para entender el éxito de la invasión y sus efectos sobre la comunidad receptora. Este es el primer estudio exhaustivo sobre las estrategias tróficas de dos caprélidos introducidos que coexisten en las costas españolas: *Caprella scaura* y *Paracaprella pusilla*. La dieta de 446 individuos de *C. scaura* y 230 de *P. pusilla* se analizó para investigar si había diferencias en los hábitos alimenticios en relación a las características del habitat (sustrato duro natural vs artificial), al tipo de sustrato hospedador (bryozoos e hidrozooos) y al rango native e introducido (Brasil vs España). Los resultados mostraron diferencias en las preferencias alimenticias de las dos especies que podrían favorecer su coexistencia en las áreas de introducción. En general, *P. pusilla* es una especie depredadora que muestra preferencia por los pequeños crustáceos mientras que *C. scaura* es fundamentalmente detritívora. Aunque no se encontraron diferencias alimenticias respecto al sexo, sí se encontraron respecto al grado de desarrollo ontogenético en *C. scaura*, donde los juveniles se alimentaban de mayor cantidad de presas que los adultos. En *P. pusilla* se observó un cambio de dieta en relación con el tipo de habitat y en *C. scaura* la plasticidad trófica se relacionó con el tipo de sustrato. Este estudio demuestra que las características del habitat a diferentes escalas influyen más en la ecología trófica de las especies exóticas que el rango de distribución.

6.5 INTRODUCTION

Biological invasions are one of the main conservation threats and have caused many species extinctions (Olden *et al.* 2004; Simberloff 2010). Accordingly, they have become an important issue at a global scale (Zenetos *et al.* 2010) and a prominent object of study. Despite being a biogeographical phenomenon, most studies on invasive species have focused exclusively on their ecology in the communities to which they have been introduced, and have ignored the ecology of these species where they are native (Hierro *et al.* 2005). The trophic ecology of invasive species, which is necessary to understand the community-wide effects of invasions (Tillberg *et al.* 2007), has traditionally focused on the relationships between the trophic niche breadth and the invasion success or on the impact of the introduced species in the native community (Olden *et al.* 2004; Piscart *et al.* 2011). Thus, for example, it is accepted that omnivorous species or those with a broad trophic niche have more success than those with a specialized diet, and often that invasive species prey on or compete with autochthonous (indigenous) taxa (Barbosa and Castellanos 2005; Simberloff 2010). However, a few studies assess the trophic changes between native and introduced distribution ranges for a particular species. For example, Tillberg *et al.* (2007) showed a shift in the diet of the ant invasive species after their establishment comparing its feeding habits between different distribution ranges.

Amphipods are a very important group in the aquatic benthic communities (Jazdzewski 1980) being a fundamental trophic link between primary producers and higher trophic levels (Woods 2009). They also are very successful colonizers of artificial hard substratum, reaching high densities in harbours and marinas (Ashton *et al.* 2010; Buschbaum and Gutow 2005) including biofouling on ship hulls (Frey *et al.* 2009). Moreover, among crustaceans, their important role as invasive taxa has been indicated (Zenetos *et al.* 2010), and an increased number of introduced species belonging to this group could be recorded (Jazdzewski *et al.* 2002). Nevertheless, the invasive amphipod species, and especially in marine environments, are still poorly studied (Zenetos *et al.* 2010). For freshwater species, a wider bibliography demonstrating their role as invasive is available (eg. Grabowski *et al.* 2007; Piscart *et al.* 2011).

In general, most invasive crustaceans are omnivorous (Karatayev *et al.* 2009) and occasionally predators (Hänfling *et al.* 2011). However, the trophic

ecology of these species in their introduced range in comparison with their native range has been almost ignored. Exploring differences in the feeding habits of exotic species in relation to habitat characteristic in different distribution areas can help to understand the factors involved in the invasion success by these species.

Caprella scaura Templeton, 1836 and *Paracaprella pusilla* Mayer, 1890 are the only two introduced caprellid amphipods in the Mediterranean Sea. Although the origin area of both species is unknown, as occur with most of fouling species which inhabits harbours (Carlton 1996), Brazil is included in the potential native range of the two species (Ros *et al.* 2013a,b). *P. pusilla* was described for the first time in Brazil and *C. scaura*, although it was described for the first time in Mauritius, was also recorded in Brazil only two years later, in 1838. Both species are well established in Spanish coastal areas co-occurring in Cádiz (south Atlantic coast of Spain) and Mallorca (Balearic Islands) (Ros *et al.* 2013a). However, *P. pusilla* has only been found in Spain, being considered a recent introduced species in Europe, while *C. scaura* is widely distributed along the Mediterranean Sea and the southern Atlantic coast of the Iberian Peninsula, including Spain and Portugal, and it appears that it is displacing other native caprellids like *Caprella equilibra* (Guerra-García *et al.* 2011; Chapter 4). Although their current distribution is well studied, little is known about their feeding strategies in their native and introduced distribution ranges. As the diet is the product of a feeding strategy (Kleppel 1993), one may gain insight into the ways in which caprellid species respond to their food environments by measuring their diets. The only data on the feeding habits of *C. scaura* and *P. pusilla*, based on its diet, were reported by Guerra-García and Tierno de Figueroa (2009) from a few individuals in a general study of the caprellid diet. Moreover, it remains unclear whether these two species differ in dietary preferences or if they are able to shift their feeding pattern according to the habitat characteristics or with the sex and the development stage.

The aims of the present study are: (1) to describe in detail the diet of *C. scaura* and *P. pusilla* according to the different sex/age groups, and (2) to analyze the differences in the feeding pattern of both species in relation with native and introduced ranges, natural and artificial habitats and different host substrata which they inhabit. Finally, taking into account that the introduced range of *C. scaura* is not only restricted to Spain, the last aim (3) is to investigate

the consistence of the diet preferences of *C. scaura* associated with the same host substratum and under similar habitat characteristics in different regions of the Mediterranean Sea.

6.6 MATERIAL AND METHODS

6.6.1 Study area

The field survey was conducted from May 2011 to November 2012 in the native (south coast of Brazil) and in the introduced range (coastal localities of southern Iberian Peninsula and Balearic Islands, Spain) of *Caprella scaura* and *Paracaprella pusilla*. In the case of *C. scaura*, additional coastal localities of central and southern Italy, Corsica, Malta and Greece were sampled to investigate the homogeneity of the *C. scaura* diet in the introduced area.

6.6.2 Sampling collection

In the introduced (Spain) and native range (Brazil), two habitats, defined by hard substratum type (artificial vs natural), were selected for comparison. Selection of sampling localities was based on the abundance of caprellid populations. As artificial habitats we considered fouling communities associated to recreational marinas while intertidal and subtidal rocky coastal shores (1-5 meters deep) were selected as natural habitats. In Brazil, both caprellid species were present in natural and artificial habitats while in Spain none of the species were found in natural habitats. In each locality, the host substrata (bryozoans and hydroids) where the caprellid species were more abundant were collected directly by hand. Each substratum was collected independently and all samples were fixed in situ in 90% ethanol. In the laboratory, caprellids were sorted and identified to species level. The studied species with the capture locations, host substrata and collection dates are given in Table 6.8.

Table 6.8 Capture locations, collection dates, habitats, substrate and caprellid species. A: artificial; N: natural; Bug: *Bugula neritina*; Zoo: *Zoobotryon verticillatum*; Eud: *Eudendrium* sp.; Caprel.: caprellid; Pp: *Paracaprella pusilla*; Cs: *Caprella scaura*. Data used for comparative analysis between the native and introduced distribution range have been highlighted in gray.

Country	Locality	Date	Coordinates	Habitat	Substrate	Caprel. species
BRAZIL	Ilhabela (Sao Paulo)	04/10/12	23° 46' S; 45° 21' O	A	<i>Bug</i>	Cs
	Ilhabela (Sao Paulo)	04/10/12	23° 46' S; 45° 21' O	A	<i>Zoo</i>	Cs
	Paranaguá Harbor (Paraná)	22/11/12	25° 30' S; 48° 30' O	A	<i>Bug</i>	Pp
	Paranaguá Harbor (Paraná)	22/11/12	25° 30' S; 48° 30' O	A	<i>Eud</i>	Pp
	Sao Sebastiao (Sao Paulo)	02/10/12	23° 46' S; 45° 24' O	N	<i>Bug</i>	Cs
	Sao Sebastiao (Sao Paulo)	02/10/12	23° 46' S; 45° 24' O	N	<i>Zoo</i>	Cs
	Paranaguá Ilha do Mel (Paraná)	22/11/12	25° 33' S; 48° 18' O	N	<i>Eud</i>	Pp
	SPAIN	Cádiz	18/05/11	36° 32' N; 6° 17' W	A	<i>Bug</i>
Cádiz		18/05/11	36° 32' N; 6° 17' W	A	<i>Zoo</i>	Cs
Mallorca (Balearic Islands)		07/11/11	39° 34' N; 2° 38' W	A	<i>Eud</i>	Pp
Mallorca (Balearic Islands)		07/11/11	39° 34' N; 2° 38' W	A	<i>Bug</i>	Pp
ITALY	Palermo	08/10/11	38° 08' N; 13° 22' E	A	<i>Bug</i>	Cs
	Civitavecchia	29/05/12	42° 05' N; 11° 47' E	A	<i>Bug</i>	Cs
GREECE	Iraklion (Creta)	18/05/12	35° 20' N; 25° 08' E	A	<i>Bug</i>	Cs
FRANCE	Ajaccio (Corsica)	31/05/12	41° 55' N; 8° 44' E	A	<i>Bug</i>	Cs
MALTA	Gzira	09/07/12	35° 54' N; 14° 29' E	A	<i>Bug</i>	Cs

6.6.3 Diet analyses

For the diet study, specimens were analyzed according to the method proposed by Bello and Cabrera (1999) and previously used in studies on Amphipoda gut content analyses (eg. Guerra-García and Tierno de Figueroa 2009; Navarro-Barranco *et al.* 2013; Vázquez-Luis *et al.* 2013). Individuals were

placed in vials with Hertwigs' liquid and heated in an oven at 70°C for ~6 h before mounting individuals on slides in Hertwigs' liquid for study under microscope (Fig. 6.7). We used a compound microscope equipped with an ocular micrometer to estimate the percentage of absolute gut content (at 40× as % total area occupied by the contents in the whole digestive tract) and the relative abundances of food items in the gut content (at 400× as % area occupied by each component of the total gut contents).

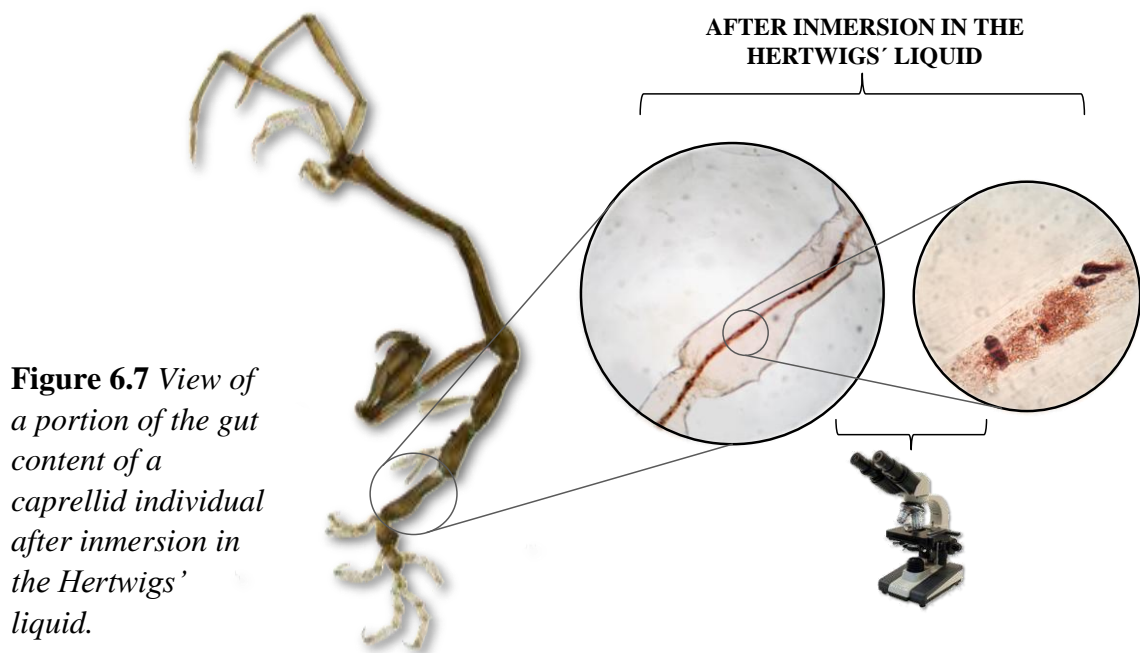


Figure 6.7 View of a portion of the gut content of a caprellid individual after immersion in the Hertwigs' liquid.

6.6.4 Statistical analyses

To explore global differences among the diet of sex/age groups for each species (considering as variable the percentage of the dominant food items), one way ANOVA was used. To test possible diet differences for each species between native and introduced ranges in different substrata associated with artificial habitats, a two-way ANOVA was used with the following factors: 'distribution range', a fixed factor with two levels: native and introduced; 'substratum', a fixed factor and orthogonal, with two levels: the hydroid *Eudendrium* sp. and the bryozoan *Bugula neritina* for *Paracaprella pusilla*, and the bryozoans *B. neritina* and *Zoobotryon verticillatum* for *Caprella scaura*.

Taking into account that none of the species has been found in natural habitats in their introduced range, to test possible differences in the diet of each species between artificial and natural habitats, only the native range was considered (Brazil). For *C. scaura*, a two-way ANOVA was used with the following factors: ‘habitat’, a fixed factor with two levels: artificial and natural; and ‘substratum’, a fixed factor and orthogonal, with two levels: the bryozoans *B. neritina* and *Z. verticillatum*. For *P. pusilla* only one substratum was available for comparison in both habitats (*Eudendrium* sp.). Consequently, to test differences among habitats for this species, one-way ANOVA was used.

The total number of specimens available with detected digestive contents was different among samples. Therefore, to properly conduct balanced ANOVA designs, we always chose the lesser sample size for each treatment and we selected randomly the same number of specimens from each sample. Prior to ANOVA, heterogeneity of variance was tested with Cochran’s C-test. Data were transformed with the $\text{Ln}(x+1)$ if variances were significantly different at $p < 0.05$. Where variances remained heterogeneous, untransformed data were analyzed, as ANOVA is a robust statistical test and is relatively unaffected by heterogeneity of variances, particularly in balanced experiments (Underwood, 1997). In such cases, to reduce type I error, the level of significance was reduced to < 0.01 . Univariate analyses were conducted with GMAV5 (Underwood et al. 2002).

The affinities among species populations according to the dietary analysis were explored by MDS analysis using UPGMA and Bray-Curtis similarity index. Data of the area occupied for each component within the total gut content were considered (Guerra-García and Tierno de Figueroa 2009). The multivariate analysis was carried out using PRIMER v.5 package (Clarke and Gorley 2001).

6.7 RESULTS

We examined a total of 446 specimens of *Caprella scaura* and 230 of *Paracaprella pusilla*. From these, digestive contents were found in 419 specimens of *C. scaura* and 168 of *P. pusilla* (Table 6.9). Gut contents of the two studied exotic species included detritus, preys (crustaceans, polychaetes and

hydroids), macroalgae, microalgae (e.g. diatoms) and dinoflagellates. The dominant component in *C. scaura* was detritus in all the sex/age groups, while crustacea (mainly harpacticoid copepods) were the dominant item in *P. pusilla* (Fig. 6.8). There were no significant differences in the diet of the different sex/age groups for both species, except for the juveniles of *C. scaura*, which showed lower values of detritus ($F=4.7$, $p<0.01$) and higher values of prey ($F=6.6$, $p<0.01$) than the remaining sex/age categories.

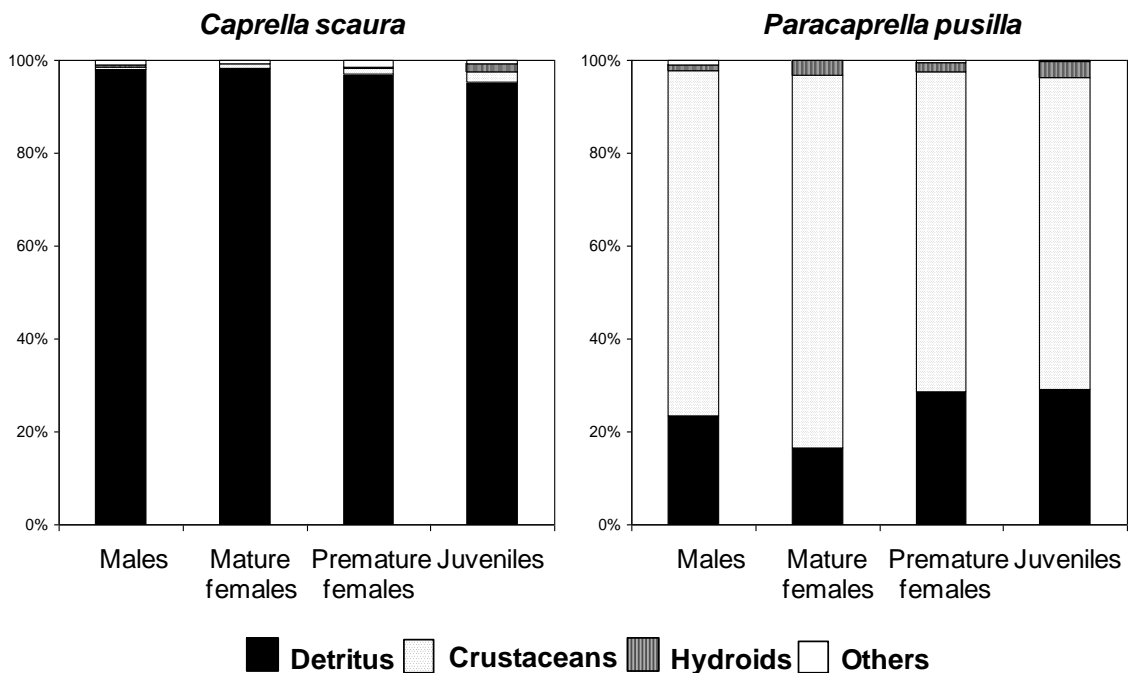


Figure 6.8 Global mean percentage of each food item for the different sex/age groups for the studied caprellid species.

The analysis of the gut contents of *C. scaura* in different localities of the Mediterranean Sea also showed that the diet was also clearly dominated by detritus, apart from the case of Malta where the percentage of crustaceans reached 45 % (Table 6.10). This suggests that *C. scaura* is a primary detritivorous species, while *P. pusilla* is a primary carnivorous taxon, with more than 50% of prey in the gut content. In *C. scaura*, the average area occupied by the content in the whole digestive tract ranged from 46.1 % to 81.9 % in *B. neritina* and from 52.5 % to 81.6 % in *Z. verticillatum*. In *P. pusilla*, mean values ranged from 22.0 % to 38.2 % in *B. neritina* and 33.2 % to 54.2 % in *Eudendrium* sp.

Table 6.9 Gut contents of *C. scaura* and *P. pusilla* in different ranges (introduced and native), habitats (artificial and natural) and substrates (Bug: *Bugula neritina*; Zoo: *Zoobotryon verticillatum*). M: males, Fm: mature females (with developed oostegites), Fp: premature females (with undeveloped oostegites), J: juveniles, T: total. N: number of specimens of each category examined, n: number of specimens with detected digestive contents. % Abs: total area occupied by the content in the whole digestive tract. Det: detritus, Cru: crustaceans, Pol: polychaetes, Hyd: hydroids, MAlg: Macroalgae; µalg: microalgae, Din: dinoflagellates.

Caprellid species	Range/habitat	Substrate	Sex/age group	N/n	Components (100%)										
					%Abs	%Det	%Cru	%Pol	%Hyd	%MAlg	%µalg	%Din			
<i>Caprella scaura</i>	Introduced/artificial	Bug	M	33/32	81.1(3.9)	98.1(1.09)	1.6(1.0)	-	0.3(0.3)	-	-	-	-	-	-
			Fm	15/15	88.3(2.7)	99.3(0.7)	0.7(0.7)	-	-	-	-	-	-	-	-
			Fp	7/7	87.1(4.7)	95.7(3.0)	4.3(3.0)	-	-	-	-	-	-	-	-
			J	64/61	80.2(2.3)	92.0(2.0)	4.3(1.3)	-	3.0(1.4)	-	-	-	-	-	0.7(0.2)
			T	119/115	81.9(1.7)	94.9(1.2)	3.1(0.8)	-	1.7(0.8)	-	-	-	-	-	0.3(0.1)
	Native/artificial	Bug	M	38/36	81.4(3.6)	100(0)	-	-	-	-	-	-	-	-	-
			Fm	8/7	85.7(3.7)	100(0)	-	-	-	-	-	-	-	-	-
			Fp	9/9	89.9(2.6)	100(0)	-	-	-	-	-	-	-	-	-
			J	50/49	79.9(3.0)	100(0)	-	-	-	-	-	-	-	-	-
			T	105/101	81.6(2.0)	100(0)	-	-	-	-	-	-	-	-	-
			M	17/17	73.5(5.4)	90.6(3.9)	1.5(1.2)	4.1(3.5)	2.4(1.3)	1.5(1.2)	-	-	-	-	-
			Fm	3/3	70.0(0)	93.3(6.7)	6.7(6.7)	-	-	-	-	-	-	-	-
			Fp	16/16	60.6(4.7)	94.4(2.5)	1.9(1.9)	-	0.3(0.3)	2.2(1.4)	1.3(0.7)	-	-	-	-
			J	10/10	77.0(2.1)	91.5(2.2)	-	2.0(2.0)	3.5(1.5)	2.5(1.3)	0.5(0.5)	-	-	-	-
			T	46/46	69.6(2.7)	92.3(1.8)	1.6(0.9)	2.0(1.4)	1.7(0.6)	1.8(0.7)	0.5(0.3)	-	-	-	-
Native/natural	Bug	M	26/25	70.8(3.3)	100(0)	-	-	-	-	-	-	-	-	-	
		Fm	12/12	69.2(3.4)	100(0)	-	-	-	-	-	-	-	-	-	
		Fp	9/8	71.3(3.5)	100(0)	-	-	-	-	-	-	-	-	-	
		J	2/2	45.0(15.0)	100(0)	-	-	-	-	-	-	-	-	-	
		T	49/47	69.4(2.2)	100(0)	-	-	-	-	-	-	-	-	-	
M	7/5	58.8(10.2)	97.0(1.2)	-	-	-	-	-	-	-	3.0(1.2)	-	-		
Fm	2/1	20	100	-	-	-	-	-	-	-	-	-	-		
Fp	2/2	45.0(5.0)	9.5(2.5)	-	-	-	-	-	2.5(2.5)	5.0(5.0)	-	-	-		
J	1/1	15	95	-	-	-	-	-	-	-	5	-	-		
T	12/9	46.1(7.9)	96.1(1.1)	-	-	-	-	-	0.6(0.6)	3.3(1.2)	-	-	-		

Table 6.9 –cont.-

Zoo	M	2/2	65.0(15.0)	100(0)	-	-	-	-	-	
	Fm	5/3	46.7(17.6)	86.7(13.3)	3.3(3.3)	-	-	10.0(10.0)	-	
	Fp	4/3	50.0(15.3)	100(0)	-	-	-	-	-	
	J	0/0	-	-	-	-	-	-	-	
	T	11/8	52.5(8.6)	95.0(5.0)	1.3(1.3)	-	-	3.8(3.8)	-	

<i>Paracaprella pusilla</i>	Introduced/artificial	Bug	M	13/7	26.4(12.0)	21.4(9.3)	77.9(9.1)	-	0.7(0.7)	-
			Fm	9/3	26.7(12.2)	30.0(21.1)	70.0(21.1)	-	-	-
			Fp	14/7	15.7(3.0)	30.0(12.6)	70.0(12.2)	-	-	-
			J	9/5	22.0(6.1)	4.0(3.3)	96.0(3.3)	-	-	-
			T	45/22	22.0(4.4)	21.4(5.7)	78.4(5.7)	-	0.2(0.2)	-
	Eud		M	20/17	53.2(5.6)	19.1(6.8)	75.3(7.4)	-	5.0(3.1)	0.6(0.6)
			Fm	16/11	56.4(6.8)	17.7(7.7)	82.3(7.7)	-	-	-
			Fp	7/6	50.0(6.3)	16.7(7.3)	74.2(8.2)	-	8.3(6.6)	-
			J	3/3	60.0(11.5)	30.0(30.0)	51.7(24.6)	-	16.7(16.7)	-
			T	46/37	54.2(3.5)	19.2(4.5)	75.3(4.7)	-	5.0(2.2)	-
	Native/artificial	Bug	M	24/18	48.3(5.5)	12.8(5.6)	86.9(5.6)	-	-	0.3(0.3)
			Fm	27/20	32.2(4.4)	6.0(3.3)	91.5(5.3)	-	2.5(2.4)	-
			Fp	7/7	30.0(30.3)	31.4(9.12)	65.7(8.4)	-	-	1.4(1.4) 2.8(2.8)
			J	4/3	36.7(8.8)	16.7(16.7)	83.3(16.7)	-	-	-
			T	62/48	38.2(3.1)	12.9(3.2)	85.5(3.6)	-	1.0(1.0)	-
	Eud		M	33/29	49.0(4.4)	25.3(5.7)	74.1(5.6)	-	-	0.2(0.2)
			Fm	9/7	47.8(4.7)	22.9(9.8)	76.4(9.6)	-	-	0.7(0.7)
			Fp	5/4	67.5(8.7)	37.5(3.7)	62.5(3.8)	-	-	-
			J	5/3	43.3(6.7)	43.3(29.7)	56.7(29.6)	-	-	-
			T	52/43	50.1(3.3)	27.3(4.6)	72.2(4.5)	-	-	0.1(0.1) 0.4(0.2)
	Native/natural	Eud	M	16/7	22.1(7.1)	57.1(15.3)	36.5(16.1)	-	0.7(0.7)	-
			Fm	17/10	42.2(6.2)	26.7(10.9)	58.3(10.3)	-	15.0(5.4)	-
			Fp	1/0	-	-	-	-	-	-
			J	1/1	30.0(-)	100.0(-)	-	-	-	-
			T	25/18	33.2(4.9)	37.7(9.3)	51.8(9.2)	-	8.2(3.3)	-
										2.3(1.3)

Table 6.10 Gut contents of *Caprella scaura* associated to *Bugula neritina* in different localities of the Mediterranean. T: total. N: number of specimens of each category examined, n: number of specimens with detected digestive contents. % Abs: total area occupied by the content in the whole digestive tract. Det: detritus, Cru: crustaceans, Pol: polychaetes, Hyd: hydroids, MAlg: Macroalgae; μ alg: microalgae, Din: dinoflagellates.

	N/n	%Abs	%Det	%Cru	Components (100%)				
					%Pol	%Hyd	%MAlg	% μ alg	%Din
Creta	19/18	56,7(6,1)	82,2(6,7)	17,2(6,8)	-	-	0,6(0,4)	-	-
Civitavechia	20/19	40,0(4,7)	98,4(1,6)	1,6(1,6)	-	-	-	-	-
Gzira	23/21	58,8(4,5)	50,7(5,7)	45,0(6,4)	-	-	1,9(1,5)	2,4(0,9)	-
Palermo	22/20	63,0(5,0)	88,5(4,8)	11,5(4,8)	-	-	-	-	-
Corsica	20/15	49,3(8,2)	66,7(8,1)	18,0(8,1)	-	-	4,0(1,6)	-	1,3(0,9)

When the influence of distribution range (native vs introduced) in the dietary composition was evaluated, no significant differences were found for any of the studied species within the same habitat characteristics (artificial habitats) (Table 6.11). However, the percentage of the main food items showed significant differences between substrata in both distribution ranges for *C. scaura*; the percentage of detritus in their gut was higher in *Z. verticillatum* (100 ± 0 , mean \pm standard error) than in *B. neritina* (93.4 ± 1.2) (Su, $p < 0.05$; Fig. 6.9, Table 6.11).

In the native range, when the influence of the habitat (artificial vs natural) was tested, we found significant differences for *P. pusilla* (Fig. 6.10; Table 6.12), with higher percentages of prey in the artificial (87.6 ± 3.7) than in natural habitat (60.0 ± 9.9) (Fig. 6.11). These differences were also observed by MDS analysis in which *P. pusilla* collected from artificial habitat are clearly separated from the specimens collected from natural one (Fig. 6.12). This analysis also showed a limited food overlap based on a clear difference between the diets of the two exotic species.

Table 6.11 Results of ANOVA test on the influence of the distribution range (native vs introduced) and substrate (*Bugula neritina* vs *Zoobotryon verticillatum* for *C. scaura* and *Eudendrium racemosum* for *P. pusilla*) in the percentage of the dominant food items. *** $P < 0.001$.

Caprellid species	Source of variation	df	%Detritus			%Prey			F versus
			MS	F	P	MS	F	P	
<i>Caprella scaura</i>	Distribution range = Ra	1	34.78	0.53	0.4661	0.01	0.01	0.9370	Res
	Substrate = Su	1	2022.28	31.02	0.0001***	29.72	34.51	0.0001***	Res
	Ra x Su	1	34.78	0.53	0.4661	0.01	0.01	0.9370	Res
	Residual	180	65.19			0.86			
	Cochran's C-test Transformation			C=0.51 (p<0.01) None			C=0.51 Ln (x+1)		
<i>Paracaprella pusilla</i>	Distribution range = Ra	1	768.18	1.69	0.1966	955.68	2.02	0.1594	Res
	Substrate = Su	1	92.04	0.20	0.6535	18.18	0.04	0.8452	Res
	Ra x Su	1	1163.63	2.57	0.1129	768.18	1.62	0.2066	Res
	Residual	84	453.38			474.16			
	Cochran's C-test Transformation			C=0.39 None			C=0.1278 None		

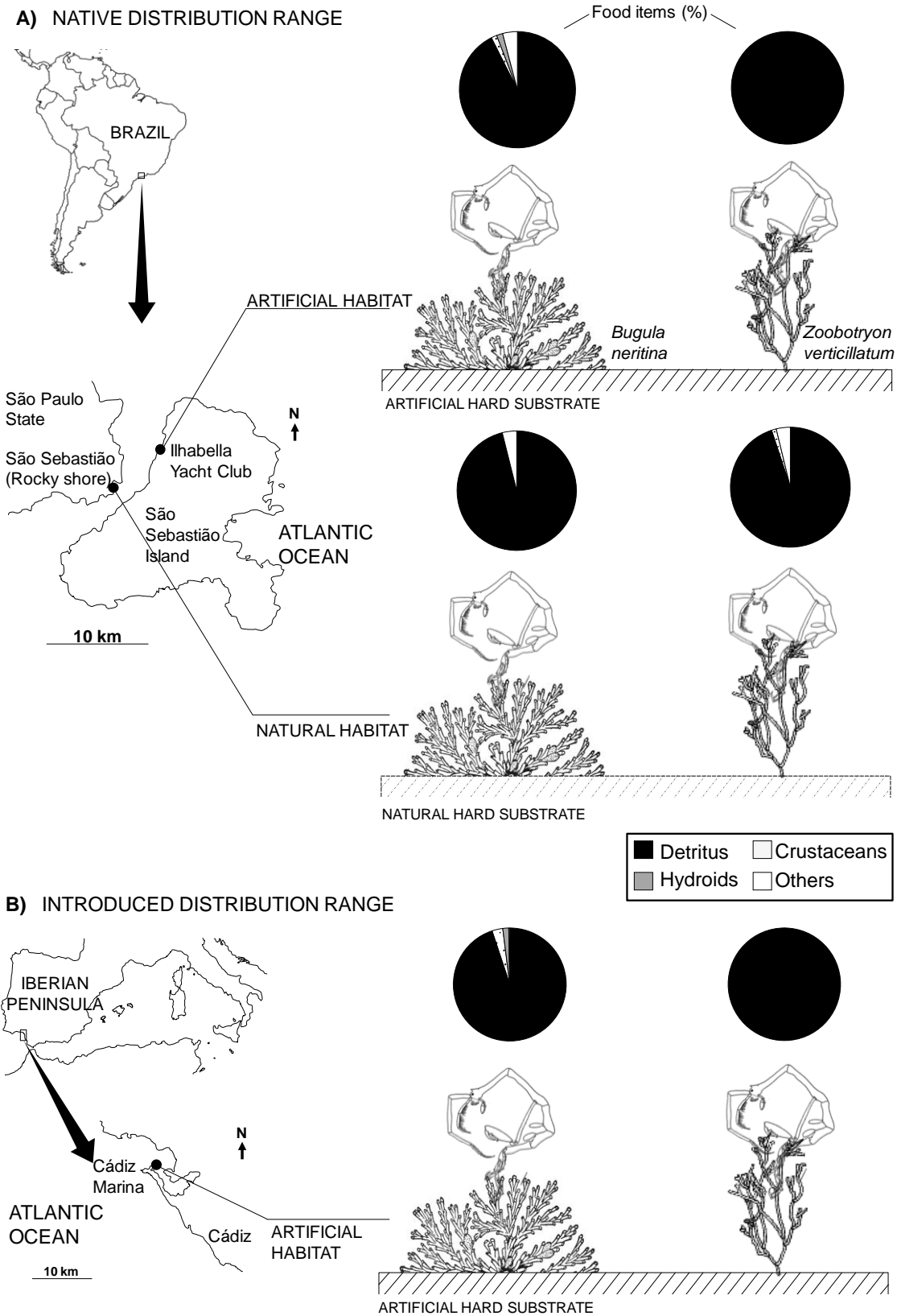


Figure 6.9 Diet preferences of *Caprella scaura* based on the percentage of each food item in populations collected in different host substrates, different habitat types and different distribution ranges.

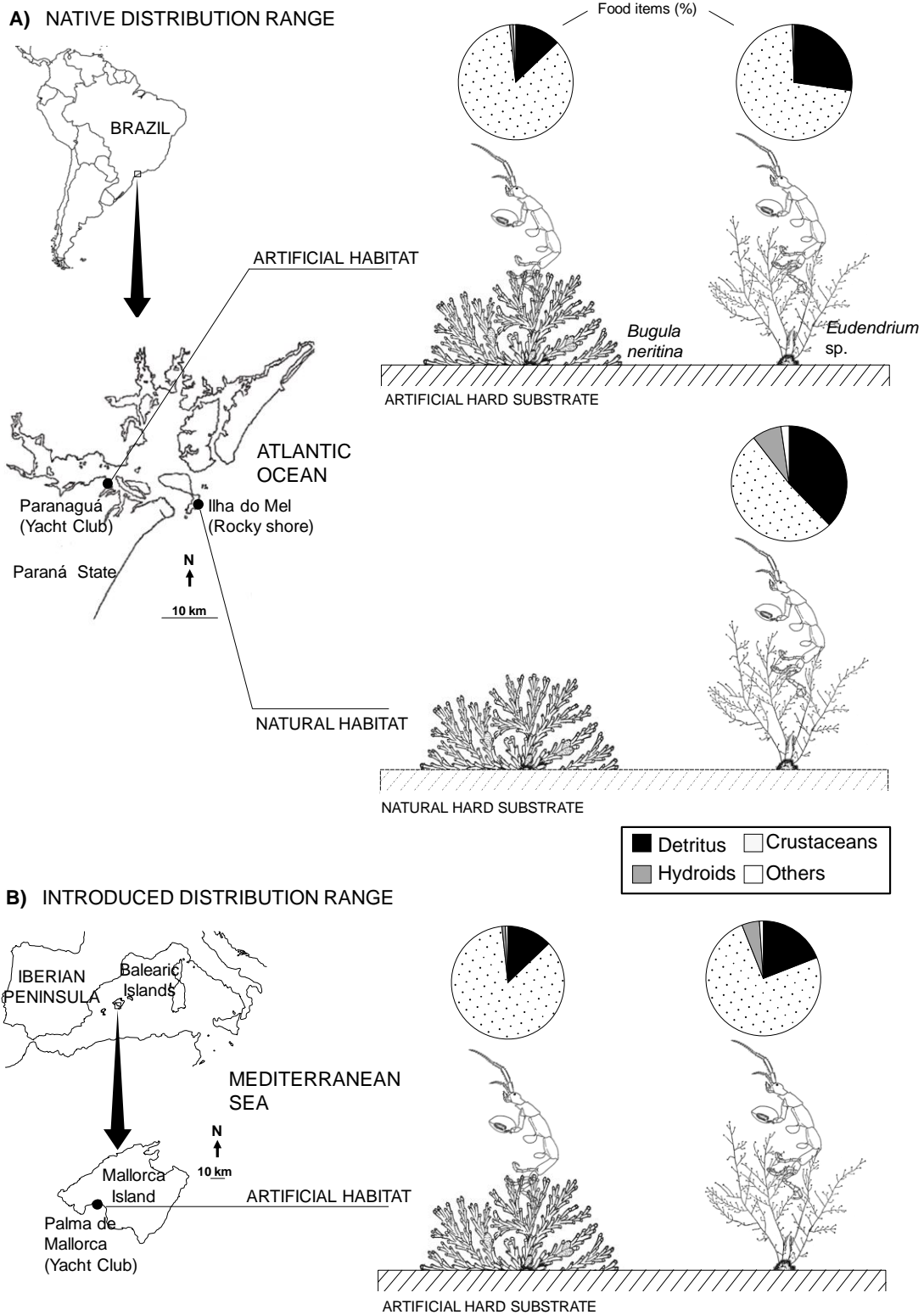


Figure 6.10 Diet preferences of *Paracaprrella pusilla* based on the percentage of each food item in populations collected in different host substrates, different habitat types and different distribution ranges.

6.8 DISCUSSION

6.8.1 Feeding strategies of *Caprella scaura* and *Paracaprella pusilla*

In general, *Caprella scaura* is mainly a detritivorous species while *Paracaprella pusilla* is a carnivorous one, based on the dominant food item found in the gut content of all of the sex/age categories considered. However, both species feed on a high variety of items being able to display different feeding strategies. Although no sex-related diet shifts were observed in either of the species, an age-related diet shift was observed in *C. scaura*, where juveniles presented higher amount of preys and lower amount of detritus than the rest of sex/age groups considered. A diet shift during the development has also been observed in other amphipods like *Talitrus saltator* (Olabarría *et al.* 2009) and has been reported as an important factor contributing to variation in diet within species (Hoeinghaus and Davis 2007). Ontogenetic shifts in diet may occur in order to overcome physiological constraints (Hentschel 1998; Rossi *et al.* 2004). For instance, when juveniles have physiological limitation in the maximum rate of food uptake, they might rely on higher quality sources of food to minimize the amount of food and maximize energy uptake (Hentschel 1998). This could explain the observation that juveniles of *C. scaura* collected from *Bugula neritina* presented a higher percentage of prey items than juveniles of *C. scaura* collected from *Zoobotryon verticillatum*, since the last substratum retained more amount of sediment than the former one (Ros *et al.* 2013b), providing an advantage to take detritus.

In addition, a biogeographical perspective is needed to establish the degree of specificity in the feeding strategy at different habitats and distribution ranges. In the case of *P. pusilla*, although we did not observe differences in its trophic habits between its native and introduced distribution ranges, we observed a clear difference in the feeding strategy of this species when the diet of populations from artificial and natural habitats was compared. In the artificial habitat, *P. pusilla* fed mainly on crustacean preys, fundamentally harpacticoid copepods (more than 80% of its gut content). In natural habitats prey represented only the 60% of its gut content, with a considerable amount of detritus. This could represent a potential adaptation to food resources. Detritus feeding may be important for carnivores when temporarily there is no available prey (Mayer *et al.* 2008), thus, the pycnogonid *Ammothella longipes* appears to be carnivore

during spring and early summer but seems to feed on detritus when availability of prey diminishes during winter (Soler-Membrives *et al.* 2011).

With regard to *Caprella scaura*, the consistency of gut content found across different habitats, host substratum and distribution ranges suggests that detritus is a food type that remains available in the different habitats studied. Vázquez-Luis *et al.* (2013) studied the influence of the habitat type in the feeding

habits on amphipods associated to macroalgae and found that detritivore species showed the least differences with respect to changes in habitats and substrata in which they inhabit. In our study, we observed a change in the proportion of detritus in the gut content of *C. scaura* when populations inhabiting different substrata were compared; particularly populations associated with *Z. verticillatum* fed practically only on detritus while populations associated with *B. neritina* fed also in other items but in low proportion. These little but significant differences could be related with the spatial structure of *B. neritina*, which host a high diversity of epiphytic fauna (Conradi 1995), and may favour the occasional ingestion of small crustaceans or polychaetes by *C. scaura*, especially in the case of juveniles of this species. Despite this, detritus was the dominant item in all populations of *C. scaura* studied, including those that are invading different countries of the Mediterranean. Only in the case of the population of Malta, a high percentage of prey in its gut content (45%) was observed, reflecting that the species exhibits a plasticity feeding behaviour in its introduced range and possibly can adapt its diet regarding to the proportion of food items available.

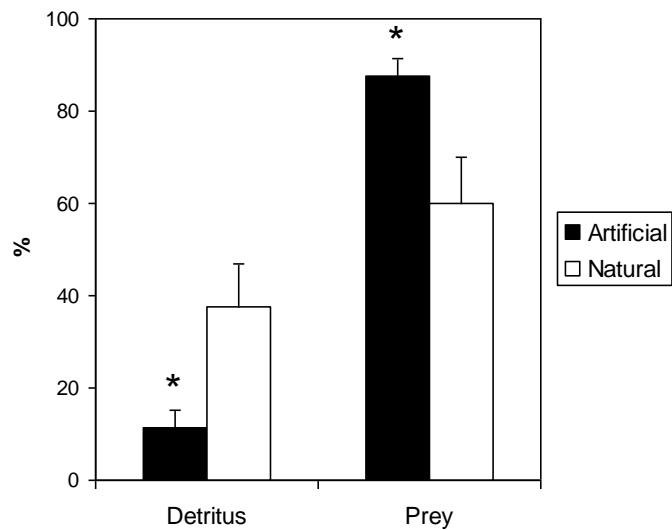


Figure 6.11 Different percentages of detritus and prey in the gut content of *Paracaprella pusilla* associated to *Eudendrium sp.* between artificial and natural habitats from its native range (Brazil). Values are mean and standard error of the mean.

Table 6.12 Results of ANOVA test on the influence of the habitat (artificial vs natural) and substrate (*Bugula neritina* vs *Zoobotryon verticillatum*) in the percentage of the dominant food items in *C. scaura* and only on the influence of the habitat (artificial vs natural) in the percentage of the dominant food items in *P. pusilla*. * $P < 0.05$.

Caprellid species	Source of variation	df	%Detritus			%Prey			F versus	
			MS	F	P	MS	F	P		
<i>Caprella scaura</i>	Habitat = Ha	1	0.78	0.01	0.9171	7.03	0.84	0.3672	Res	
	Substrate = Su	1	94.53	1.33	0.2579	7.03	0.84	0.3672	Res	
	Ha x Su	1	175.78	2.48	0.1265	38.28	4.57	0.0413	Res	
	Residual	28	70.87			8.37				
	Cochran's C-test Transformation			C=0.71 (p<0.01)			C=0.62 (p<0.01)			
			None			None				
Caprellid species	Source of variation	df	%Detritus			%Prey			F versus	
			MS	F	P	MS	F	P		
<i>Paracaprella pusilla</i>	Habitat = Ha	1	5824.26	6.78	0.0139*	6497.06	6.82	0.014*	Res	
	Residual	32	27494.11			953.31				
	Cochran's C-test Transformation			C=0.85			C=0.87			
				None			None			

6.8.2 Feeding ecology and invasion success

The different trophic strategies observed in the two caprellid species may have important consequences in their invasion success at various level of the invasion process. *C. scaura* is mainly a filter-feeder and a scraping species in all habitat types and distribution range studied, but occasionally it can display predator and herbivore behaviours, since we found preys (including polychaetes, hydroids and crustaceans) and macroalgae in its gut content. Based on the same factor, *P. pusilla* is also able to alternate between a predatory mode in artificial habitats and a combination between predatory and filter-feeding/scraping behaviour in natural ones. This agree with Caine (1978) who observed that ambush predation was the most frequently used strategy for obtaining food by this genus, although it commonly utilized other feeding modes. In contrast, Guerra-García and Tierno de Figueroa (2009) found that *P. pusilla* fed exclusively on detritus based on the gut content of few specimens which presented a low proportion of absolute gut content. This low proportion is common in carnivorous amphipod species and sometimes makes the analyses of their diet difficult (Guerra-García *et al.* 2014). With respect to prey sizes, both

species are able to use macro- and microphagous feeding modes to consume food items ranging in size from small crustaceans to fine particles of detritus. This implies high plasticity in their feeding strategies and high ability to assimilate a wide spectrum of foods, which must contribute to the ability of both species to persist and colonize new and variable habitats. *Caprella mutica*, a successful exotic caprellid in the northern hemisphere, is fundamentally a detritivorous species (Guerra-García and Tierno de Figueroa 2009), but Cook *et al.* (2010) suggested that its flexible feeding strategy play an important role in its invasion success.

The trophic niches of *P. pusilla* and *C. scaura* are segregated in their introduced range, since *P. pusilla* prefers to feed on crustacean while *C. scaura* feeds mainly on detritus. This could avoid an interspecific competition by the same food item and possibly favours the coexistence of both species in the same introduced areas and even the same substratum types. This coexistence was pointed out by Ros *et al.* (2013b) in the substrata *Eudendrium racemosum* in southern Spain.

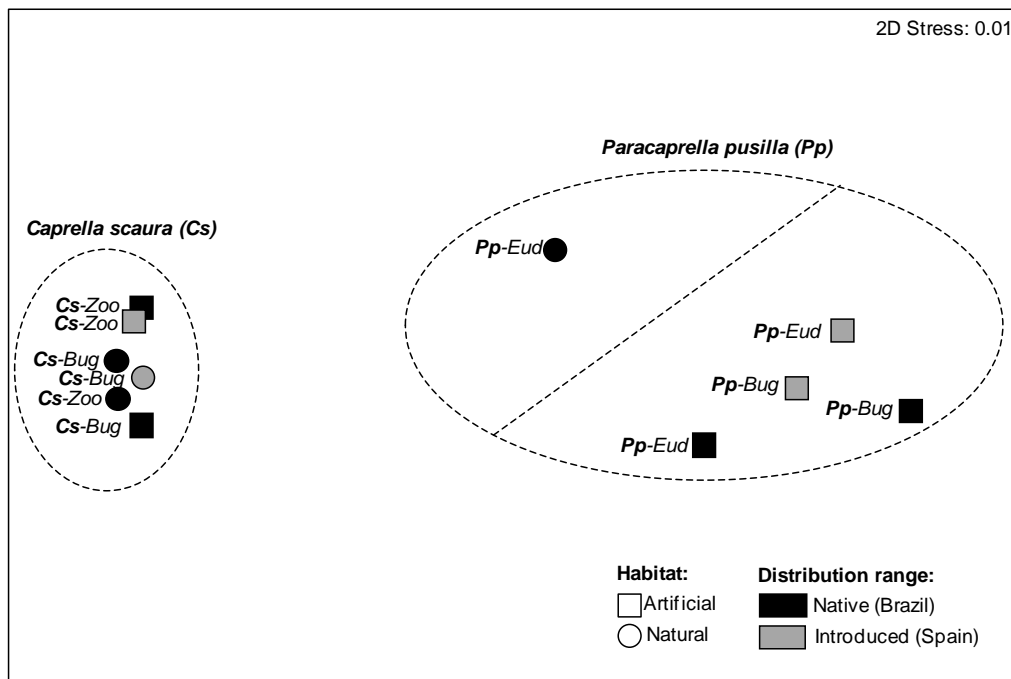


Figure 6.12 Two dimensional MDS plot based on the diet of the two species in different habitats, ranges and substrates. Data are taken from the total values of tables 2 and 3. *Eud*: Eudendrium sp.; *Bug*: Bugula neritina; *Zoo*: Zoobotryon verticillatum; *Pp*: Paracaprella pusilla; *Cs*: Caprella scaura.

Ship fouling and ballast water has been suggested as the most probable dispersal vectors for *P. pusilla* (Mead *et al.* 2011, Ros and Guerra-García 2012). Taking into account that the main crustacean prey observed in its gut content were small harpacticoid copepods, and that copepods are the most abundant metazoan in ballast waters (Smith *et al.* 1999), this feeding habit could facilitate the dispersal of this species by these means. Predation is also an important part of food acquisition in the invasive amphipod *Dikerogammarus villosus* (Sowinsky 1894), a very successful invader of freshwater ecosystems in Europe. Once in the introduced range, *P. pusilla* would need to be established in artificial habitats like harbours or artificial marinas. The eutrophication that characterizes these habitats due to anthropogenic activities may cause replacement of large copepods with small ones (Uye 1994). This could favour the increase of small harpacticoid copepod populations (the preferred diet item for *P. pusilla*) in the recipient habitat.

Paracaprella pusilla and *C. scaura*, like other fouling species, have the particularity that their dispersion usually takes place among artificial habitat, mainly ports, recreational marinas and aquaculture structures. These habitats have similar characteristics in different areas, including similar structures, fouling communities or anthropogenic food resources. This implies that species that disperse within this habitat do not need to change their feeding strategies because the characteristics of the habitat type remain similar between their native and introduced ranges, and the availability of food items is always high. Although the total gut content must be carefully considered because it may be affected by multiple factors that are not studied in this work, generally, specimens of both species collected from artificial habitats had a higher percentage of absolute gut content than specimens collected from natural ones. This could reflect a higher availability of food source in the artificial than in the natural habitats. Only when the species spread to natural areas, a shift in their diet may be necessary to adapt to the new environmental conditions, including the availability of their preferred food items or competition with other taxa. In this crucial step of the invasion dynamics it is probable that *P. pusilla*, which shows a habitat-specific trophic ecology, may be more affected than *C. scaura*, which feeds widely and without regard of the different environments in which it inhabit.

6.8.3 Trophic relationships between exotic caprellids and its preferred host substrata

We observed that individuals of *Paracaprella pusilla* collected from the hydroid *Eudendrium* sp. presented higher absolute gut content than those collected from the bryozoan *B. neritina*. This may be due to a clepto-commensalist behaviour developed by *P. pusilla* stealing captured prey from the polyps of the hydroid. This peculiar trophic strategy was observed by Ros and Guerra-García (2012) in *P. pusilla* in Southern Spain and previously by Bavestrello *et al.* (1996) in other caprellid species (*Pseudoprotella phasma* and *Caprella* sp.) on *Eudendrium glomeratum* Picard, 1952 polyps. Alarcón-Ortega *et al.* (2012) studied the feeding habits of caprellids from the west coast of Mexico and found that *Paracaprella* sp. associated to hydroids also showed a significant amount of small copepods in the digestive tract, supporting the idea of clepto-commensalism. Therefore, the presence of hydroids in their gut may be an accidental intake of polyps when *P. pusilla* is stealing the captured prey from the hydroid. Mayer (1882) and MacKay (1945) stated that caprellids parasitize or eat hydroids, and McDougal (1943) reported that caprellids invaded hydroid colonies for food and shelter and concluded that while the substrate was eaten to some extent, the major food sources were the food items occurring on the hydroid stems.

In the case of *C. scaura*, although it was found in a wide variety of substrata in its introduced range, it seems to prefer the bryozoan *B. neritina* over other type of fouling substrata (Ros *et al.* 2013b). This bryozoan is a suspension feeder which creates a current bringing microscopic plankton and organic particles toward the animal. These currents may favour the intake of detritus by *C. scaura*. In both cases, trophic relationships with its preferred substrates may favour the establishment and dispersal success of the species.

6.8.4 Functional morphology of feeding

A relationship among feeding modes, preferred food and mouthpart morphology has been noted for several feeding specialists among the amphipods (Caine 1974; McCain 1968; Mayer *et al.* 2008). Caine (1977) established that filter-feeder caprellids were those with molar and swimming setae but without mandibular palp. These are the features of *C. scaura*, as well as the other species

of the genus *Caprella*. However, Guerra-García and Tierno de Figueroa (2009) only found that, in caprellids, obligate predators were characterized by the absence of molar process and swimming setae in the antennae 2, Caine (1977) previously established that caprellid predators were those with mandibular palp and molar process but without swimming setae. *P. pusilla* has a rudimentary mandibular palp, the molar process is present but it is clearly less developed than in *C. scaura*, and it lacks the swimming setae. This implies that, in any of the cases, its morphology is not specialized for an exclusive feeding strategy. In fact, *P. pusilla* is able to prey on other items, although it is probably less efficient in the intake of detritus than *C. scaura*, which has a well-developed molar and swimming setae that create currents and act as a particle-collecting device.

6.8.5 Potential impacts on the recipient community

As we show above, substantial predation on small crustaceans, mainly harpacticoid copepods, supported a larger role in the trophic strategies of *P. pusilla*. Caine (1974) studying the predator behaviour of *Paracaprella*, asserted that: ‘*It was never observed to fail to attack a suitably-sized organism within its reach*’. With regard to the direct impact on copepods community, small copepods’ employ a variety of strategies to maximize reproduction and survival in order to overcome likely substantial losses due to predation and other factors (Turner 2004). Webb and Parsons (1991) showed, in an experimental study, that exclusion of large epibenthic predators-disturbors had little effect on harpacticoid copepod density. Based on this, it is unlikely that *P. pusilla*, even in high densities, may cause a significant impact on the copepod community. However, *P. pusilla* competes with the animals that feed on copepods, like fish larvae (Turner 1984). In turn, caprellids constitute an important food item for adult fishes (Vázquez-Luis *et al.* 2010).

In the case of *C. scaura*, the large volume of organic detritus in its gut contents suggests that it may play an important role as a vector for carbon transfer from detritus to top predators. Dense populations of *C. scaura* could both directly and indirectly impact marine food webs by changing the quantity, form and availability of these nutrients to other organisms. It is known that the introduced crayfish species *Procambarus clarkii* accumulates heavy metals and other pollutants in its organs and body tissues and transmits them to higher

trophic levels in freshwater habitats (Geiger *et al.* 2005). This also occurs with caprellid amphipods, especially detritivorous species. For example, caprellids have a high bioconcentration factor for TBT (Takeuchi *et al.* 2004), a toxic compound that was used in antifouling paintings during the 1980s and early 1990s (Stewart 1996). Taking into account that *C. scaura* reaches high densities in the introduced areas (Guerra-García *et al.* 2011) and it is able to survive throughout the year (Ros *et al.* 2013c), it plays an important role in the energy flow through food webs in the introduced areas by accumulation of pollutants and transmitting to higher trophic levels. In fact, *C. scaura* is a common prey for many fishes and crabs (Dubiaski-Silva and Masunari 2008).

6.8.6 Conclusions

Dietary analysis of *Paracaprella pusilla* and *Caprella scaura* under a biogeographical approach has revealed that exotic caprellids in the Mediterranean present a limited trophic niche overlap. In general terms, *P. pusilla* exhibits a trophic preference by crustacean prey, particularly small copepods, while *C. scaura* feeds mainly on detritus. Although no sex-related diet shifts were observed in either of the species, there is evidence of ontogenetic changes in feeding mode of *C. scaura*, with juveniles feeding more amount of preys than adults. This study supports three mechanisms that favour invasion success: (1) a switch between feeding strategies depending on the host substrata and/or habitat characteristics; (2) development of trophic relationships with the substrata in which they inhabit and are dispersed (by substrata attaching to the ship hulls), and (3) relative constancy on the diet between artificial environments in which the species spread out. It is highlight the fact that the carnivorous species, *P. pusilla*, seems to change its feeding strategy in natural environments in comparison with artificial ones, alternating between a predatory/clepto-commensalist mode and a detritivorous feeding, while *C. scaura*, a primarily detritivorous species, does not need to change its feeding strategy. Further studies are necessary to show the implications of this shift in the population fitness and the spread success to natural environments.

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7

DISCUSIÓN GENERAL y PERSPECTIVAS DE FUTURO

Los principales objetivos de esta Tesis Doctoral han sido comprender los procesos ecológicos implicados en las etapas que conforman la dinámica de invasión de los caprélidos introducidos en el Mediterráneo y en la costa Atlántica de la Península Ibérica, así como poner de relieve la utilidad de este grupo para entender determinadas piezas del puzle ecológico que conforma el fenómeno de las invasiones biológicas. Esto es especialmente necesario en el caso de la macrofauna asociada, un compartimento ecológico pocas veces contemplado en los estudios sobre ecología de las invasiones (Chapman *et al.* 2005; People 2006; Wilhelmsson y Malm 2008; Marzinelli *et al.* 2009) y donde los anfípodos en general, y los caprélidos en particular, son parte fundamental (e.g. Guerra-García *et al.* 2009, 2011a).

Para ello, se han investigado los posibles vectores de introducción de las dos especies no-nativas encontradas, *Caprella scaura* y *Paracaprella pusilla*, así como las posibles rutas por donde estas especies han podido llegar. Se ha evaluado su grado de establecimiento en la zona y se ha estudiado su proceso de expansión, así como su distribución a nivel mundial. Se ha explorado la relación de estas especies con los factores ambientales y su interacción con otras especies

de la comunidad. Además, se han buscado pistas ecológicas en su rango potencialmente nativo de distribución con el objeto de predecir qué tipo de hábitats serán más susceptibles de ser invadidos. Por otra parte, se han utilizado estas especies para evidenciar el papel que juegan determinados vectores antropogénicos en la dispersión de pequeños invertebrados marinos, como las embarcaciones de recreo y los canales interoceánicos.

Sin embargo, la necesidad de entender un proceso dinámico, y que sin duda continúa en la actualidad, ha llevado a replantear de forma constante tanto el área de muestreo como las hipótesis de trabajo. Es por ello que resulta necesario analizar los resultados obtenidos de forma conjunta para entender la dinámica del proceso con cierta perspectiva.

7.1 DINÁMICA DE INVASIÓN DE LOS CAPRÉLIDOS INTRODUCIDOS EN EL MEDITERRÁNEO Y EN AGUAS ADYACENTES

Actualmente sólo se conocen dos especies de caprélidos introducidos en el mar Mediterráneo, las especies *Caprella scaura* Templeton 1936 y *Paracaprella pusilla* Mayer 1890. Ambas especies se encuentran además en la costa sur Atlántica de la Península Ibérica (Cap. 2). A continuación se detalla el proceso de invasión de cada una en base a los resultados obtenidos, así como aquellos aspectos que han podido facilitar o dificultar este proceso.

7.1.1 Aspectos ecológicos, biogeográficos y taxonómicos del proceso de invasión de *Caprella scaura* en los mares europeos

➤ Introducción de *C. scaura* en aguas europeas (etapas 0-II)

Siguiendo el esquema y la terminología que se presentan en la figura 7.1, *Caprella scaura* fue posiblemente transportada desde una población donadora aún desconocida (situada fuera o dentro de su rango natural de distribución) hasta el mar Mediterráneo, detectándose por primera vez en la Laguna de Venecia hace dos décadas (1994-1995) (Sconfiatti y Danesi 1996).

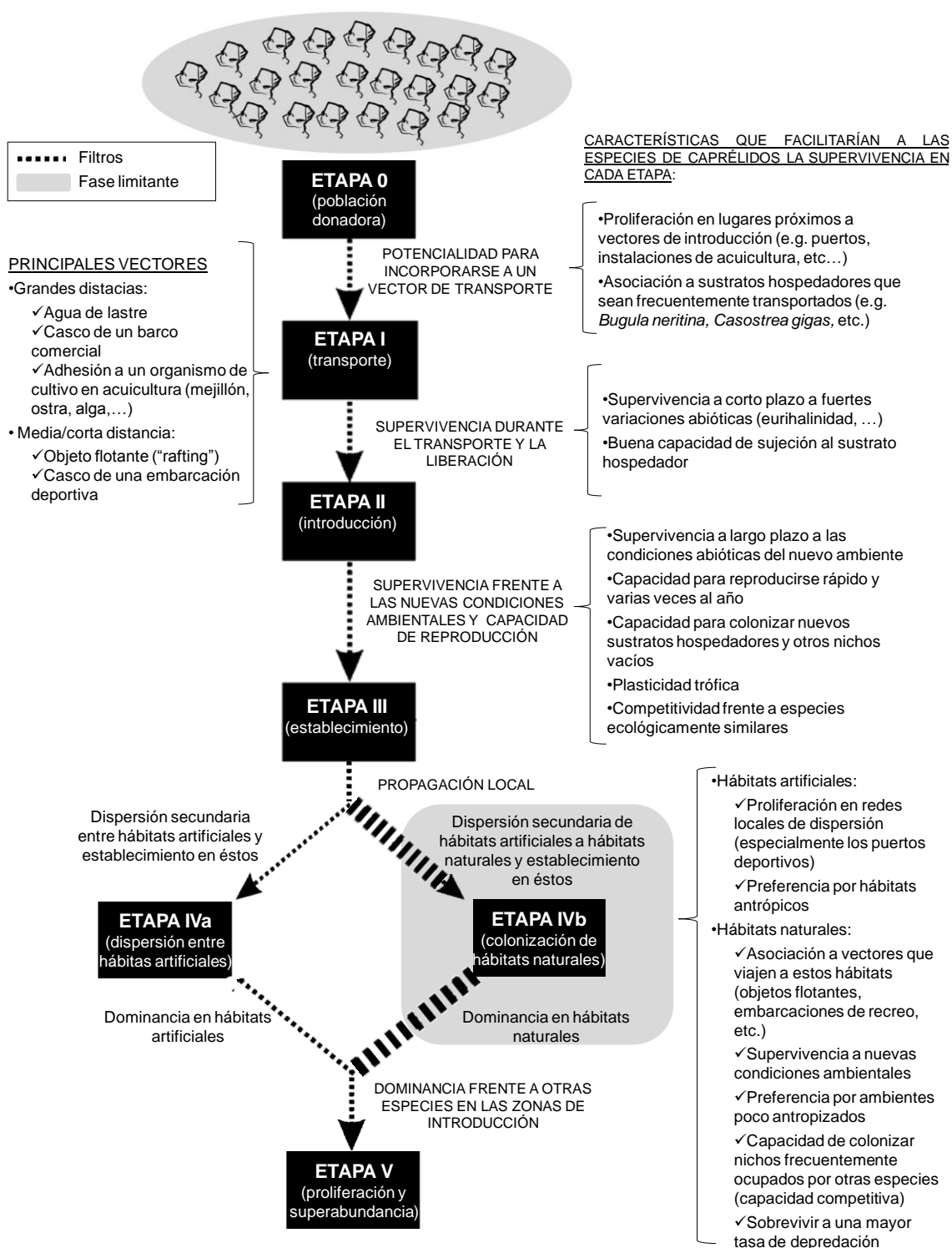


Figure 7.1 Dinámica de invasión de una especie de caprélido desde que se encuentra como invasor potencial en el área donante hasta que alcanza la dominancia en los hábitats ocupados. Se muestran los filtros que se deben superar para pasar de una etapa a otra (el grosor del filtro es proporcional a su dificultad de superación) y las características que pueden facilitar la supervivencia durante el proceso. Primera parte (etapas 0-III) modificada de Colautti y MacIsaac (2004) y Occhipinti-Ambrogi (2007).

Aunque la fecha de inoculación es prácticamente imposible de averiguar para especies introducidas de forma inadvertida (Galil 2011), el análisis de los estudios previos realizados sobre los anfípodos del mar Mediterráneo (Cap. 2, parte 3) sugieren que si *C. scaura* hubiera estado establecida con mucha anterioridad a la fecha en que se encontró por primera vez, posiblemente habría sido detectada de forma más temprana. Respecto a la vía de introducción, varios estudios apuntan a que se produjo a través del canal de Suez (Sconfiatti y Danesi 1996; Minchin *et al.* 2012; Cabezas *et al.* 2014), de forma similar a como habría ocurrido para otras especies detectadas por primera vez en el Mediterráneo en la Laguna de Venecia, tales como el picnogónido *Ammothea hilgendorfi* (Krapp y Sconfiatti 1983), el gammárido *Elasmopus pecteniscrus* o el briozoo *Tricellaria inopinata* (Occhipinti-Ambrogi 2000).

Antes de su introducción, la especie tuvo que superar dos filtros: la incorporación al vector de transporte y la supervivencia durante dicho transporte. Krapp *et al.* (2006) propusieron que el vector más probable de introducción en el Mediterráneo fue su incorporación a las comunidades incrustantes o “comunidades del *fouling*” que proliferan en las zonas sumergidas de los barcos. Algunas características de la especie pudieron facilitar este proceso, como su afinidad por los ambientes portuarios (Cap. 2, parte 3). En el capítulo 3 se observó que la especie se asociaba frecuentemente con el briozoo *Bugula neritina* (entre otros organismos del “*fouling*”), un organismo globalmente distribuido que crece en abundancia en estos ambientes (e.g. Ryland *et al.* 2011). Esta asociación, tal y como se discutió en los capítulos 3 y 4, podría representar un ejemplo de facilitación, en el que una especie introducida (*B. neritina*) favorece la dispersión de otra (*C. scaura*) así como su posterior establecimiento en las regiones de introducción.

➤ Establecimiento (etapa III)

Una vez introducida, *C. scaura* tuvo que superar una serie de barreras, tanto ambientales como bióticas (competencia, depredación, etc.) para poder establecerse con éxito en el área de introducción. Es muy probable que la especie fuera introducida múltiples veces a lo largo del tiempo hasta que las condiciones del área receptora fueron las idóneas para su establecimiento. De hecho, Cabezas *et al.* (2014) detectaron múltiples orígenes en las poblaciones de *C. scaura* presentes en la Península Ibérica, al igual que Ashton *et al.* (2008a) constataron

en las poblaciones de la especie invasora *Caprella mutica* en el norte de Europa. Es por ello que en este momento del proceso, la presión de inoculación (“*propagule pressure*”) probablemente jugó un papel decisivo. Sólo aquellas especies de caprélidos que se asocien frecuentemente con vectores de dispersión a larga distancia (ver Fig. 7.1) tendrán posibilidades de establecerse con éxito en regiones alejadas de su rango natural de distribución. De hecho, una de las características que se usan para definir si una especie es introducida o no es su asociación con hábitats artificiales (Chapman y Carlton, 1994). A pesar del papel decisivo que parece jugar la presión de inoculación en los procesos de invasión, este factor no siempre es tenido en cuenta en los estudios sobre invasiones biológicas (Ruiz *et al.* 2000; Ruesink 2005; Colautti *et al.* 2006; Occhipinti-Ambrogi 2007). Aunque pocos autores ponen en duda la relación causal entre el aumento de la presión de inoculación (tales como el incremento del tráfico marítimo, la formación de canales y puertos o el incremento de la acuicultura) y el aumento de las especies invasoras, esta relación no siempre es fácil de demostrar.

Varias características de la especie, resumidas a continuación, podrían haber facilitado su establecimiento con éxito tras su introducción:

- 1) Afinidad por hábitats artificiales relacionados con vectores de dispersión antropogénicos: altas densidades encontradas en puertos deportivos (ver Cap. 2, parte 3, Cap. 5, parte 1 y Cap. 6, parte 1, más las referencias incluidas en ellos) e instalaciones de acuicultura (en la costa: Guerra-García *et al.* 2011b; alejadas de la costa: Fernández-González y Sánchez-Jerez 2014)
- 2) En relación con lo anterior: tolerancia a contaminación por metales pesados y materia orgánica (ver Cap. 4)
- 3) Versatilidad en el uso de diferentes sustratos hospedadores en el área de introducción (ver Cap. 2, parte 3 y Cap. 3)
- 4) Plasticidad en el uso de varias estrategias tróficas (ver Cap. 6, parte 2)
- 5) Capacidad para sobrevivir a las diferentes fluctuaciones estacionales en las variables ambientales del área de introducción (ver Cap. 3)
- 6) En relación con lo anterior: eurihalinidad (Cockman y Albone, 1987)
- 7) Gran capacidad reproductora: alta fecundidad (Guerra-García *et al.* 2011b; Baeza-Rojano *et al.* 2013; Cap. 2, parte 2), varias generaciones por año

(Imada y Kikuchi 1984; Prato *et al.* 2013; Cap. 3), ciclo de vida corto con desarrollo directo (Sakaguchi 1989; Takeuchi e Hirano 1991).

- 8) Cuidado parental de los juveniles por las hembras (Aoki 1999)
- 9) Comportamiento agresivo (Lim y Alexander 1986; Schulz y Alexander 2001), incluyendo canibalismo (Baeza-Rojano *et al.* 2013).

Diferentes autores han apuntado hacia una clara relación entre el aumento de temperatura en el mar Mediterráneo y el establecimiento de especies tropicales o afines a climas cálidos (Occhipinti-Ambrogi 2007; Galil 2011, Galil *et al.* 2014). En el caso de los caprelidos que viven en climas templados se sabe que un descenso de la temperatura ralentiza la tasa de reproducción porque retrasa el periodo de muda de las hembras (Hosono 2009). Es posible que el aumento de la temperatura, unido al incremento del tráfico marítimo haya hecho posible que *C. scaura* se estableciera de forma relativamente reciente en el Mediterráneo y no en una época anterior. Sin embargo, en el Capítulo 3 (fig. 3.4) se observó que un aumento drástico de la temperatura, como el que sucede en la época de verano, no reflejó un aumento de la densidad de la especie, sino más bien todo lo contrario. Parece por tanto, que un aumento excesivo de la temperatura podría tener un efecto contraproducente en *C. scaura*, mientras que, como se aprecia en la figura 3.4, este aumento veraniego sí beneficiaría a *P. pusilla* (ver más abajo).

➤ Propagación (etapa IV)

Después de establecerse con éxito, la conjunción entre unos factores climáticos favorecedores, la presencia de nichos vacíos en los hábitats artificiales y las características de la especie anteriormente mencionadas podrían haber facilitado su extraordinaria y rápida propagación a lo largo del Mediterráneo (Cap. 2, parte 3). Las embarcaciones de recreo (Cap. 5, parte 1; Martínez y Adarraga 2008; Minchin *et al.* 2012; Guerra-García *et al.* 2011b), y en menor medida la acuicultura (Guerra-García *et al.* 2011b; Fernández-Gonzalez y Sánchez-Jerez 2014), parecen ser las vías principales de propagación local de la especie. Respecto al *rafting* en objetos flotantes, el hecho de que no se hayan registrado poblaciones de *C. scaura* adheridas a estos objetos o a sustratos naturales a la deriva en aguas europeas, sugiere que posiblemente no ha tenido un papel relevante en esta etapa.

En la actualidad, la dinámica de invasión de *C. scaura* continúa en una fase de expansión donde la especie sigue extendiendo su rango de distribución hacia la costa Este Atlántica. En dos décadas la especie se ha expandido ~3200 km hacia el sureste (si tomamos una línea recta desde la Laguna de Venecia, donde fue detectada por primera vez, hasta las Islas Canarias), y ~1500 km hacia el suroeste (si medimos la distancia desde la Laguna de Venecia hasta la bahía de Güllük, en Turquía). Los análisis moleculares aplicados a las poblaciones de la Península Ibérica sugieren que las poblaciones de la costa Atlántica se establecieron después que las poblaciones de la costa Mediterránea (Cabezas *et al.* 2014). Esto apoyaría una tendencia en el flujo de propagación que iría desde el Mediterráneo hasta la costa Este Atlántica y que se correlacionaría, además, con la temporalidad en la detección de *C. scaura* (primero en el Mediterráneo Central y Oriental, luego en el Mediterráneo Occidental y finalmente en la costa Este Atlántica Europea).

Los primeros análisis de las consecuencias de este proceso invasivo sugieren que *C. scaura* podría estar desplazando a la especie nativa *C. equilibra* (Cap. 4). Este desplazamiento parece responder a una superioridad competitiva por el uso de los recursos en la costa Mediterránea. Esta superioridad estaría condicionada por factores ambientales, como la salinidad y la temperatura, por lo que un aumento continuado de la temperatura en las costas de la Península Ibérica podría incrementar los impactos negativos de la especie sobre las poblaciones locales.

Sin embargo, existe una fase limitante que *C. scaura* parece no haber superado por el momento: su establecimiento en hábitats naturales en las zonas de introducción (Cap. 5, parte 1), especialmente en intermareales rocosos poco perturbados. Lo más cerca que ha estado la especie de establecerse en hábitats naturales ha sido en zonas rocosas de bahías sometidas a fuertes perturbaciones antrópicas como el mar Piccolo (Taranto, Italia), donde además la especie no alcanzó densidades importantes (Prato *et al.* 2013). El estudio del uso del hábitat por *C. scaura* en su región natural de distribución (Cap. 6, parte 1) demostró que la especie no es capaz de reproducir las altas densidades que alcanza en hábitats artificiales en los intermareales rocosos adyacentes, especialmente en aquellos que estaban más expuestos a la acción del oleaje. Aunque la especie se encontró también en éstos últimos, su abundancia fue similar, y en ocasiones menor, que la del resto de especies de caprélidos presentes.

La colonización de intermareales rocosos alejados de la influencia antrópica es un paso crítico para entender la potencialidad del impacto de *C. scaura* sobre las comunidades nativas del área de introducción. De hecho, la mayor parte de las especies nativas de caprélidos habitan en ambientes naturales poco perturbados (e.g. Guerra-García *et al.* 2011a) y no en ambientes portuarios y/o antropizados (Cap. 5, parte 1), por lo que esta segregación en el uso del hábitat podría evitar las relaciones de competencia entre *C. scaura* y las poblaciones nativas. En el caso de otros caprélidos invasores, como *Caprella mutica*, la superación de esta fase crítica tampoco se ha producido con éxito, a pesar de que alcance abundancias todavía mayores que *C. scaura* en hábitats artificiales (Boos 2009). De hecho, los factores que envuelven a este proceso son una de las grandes lagunas del campo de las invasiones biológicas en el medio marino (Ruiz *et al.* 2009; Simkanin *et al.* 2012). Una mayor tasa de depredación, la ausencia de nichos vacíos o un mayor estrés ambiental en los hábitats naturales han sido propuestos, entre otros, como factores limitantes para superar esta etapa en algunas especies invasoras (ver discusión del Cap. 6, parte 1).

La presencia de *C. scaura* en hábitats rocosos altamente perturbados, tanto en la zona de introducción (Mar Piccolo; Prato *et al.* 2013) como en su región natural de distribución (Araça, São Paulo; Cap. 6, parte 1), sugiere que la especie encuentra oportunidades para establecerse con éxito en hábitats naturales pero sometidos a perturbaciones antrópicas, donde otras especies encuentran dificultades para sobrevivir. La presencia y densidad poblacional de *C. scaura* en los hábitats costeros naturales en Europa podría representar, además de una amenaza para las comunidades de caprélidos que los habitan, un bioindicador de la antropización y pérdida de calidad de éstos ecosistemas.

- La problemática del rango nativo y de la cuestión taxonómica en la interpretación de los estudios sobre *Caprella scaura sensu lato*.

Aunque el estudio ecológico de la especie en Brasil, donde lleva establecida casi dos siglos, nos aporte una idea de cómo se comporta la especie en su rango natural de distribución, para entender la dinámica de invasión a nivel global es necesario entender cuál fue su zona de origen. El análisis biogeográfico de las citas globales de *C. scaura* arrojó varias regiones como potencialmente nativas (Cap. 2, parte 3). Sin embargo, es muy difícil demostrar el punto exacto de origen para una especie tradicionalmente asociada a ambientes portuarios

(Carlton 1996). En este caso además, la dificultad se incrementa porque *C. scaura* engloba en realidad a un complejo de “formas” que han sido descritas en lugares muy alejados entre sí, el complejo *C. scaura* [sensu lato]. Estas “formas” comienzan, poco a poco, a ser elevadas a nivel de especie y por tanto a ser extraídas del complejo (fig. 7.2).

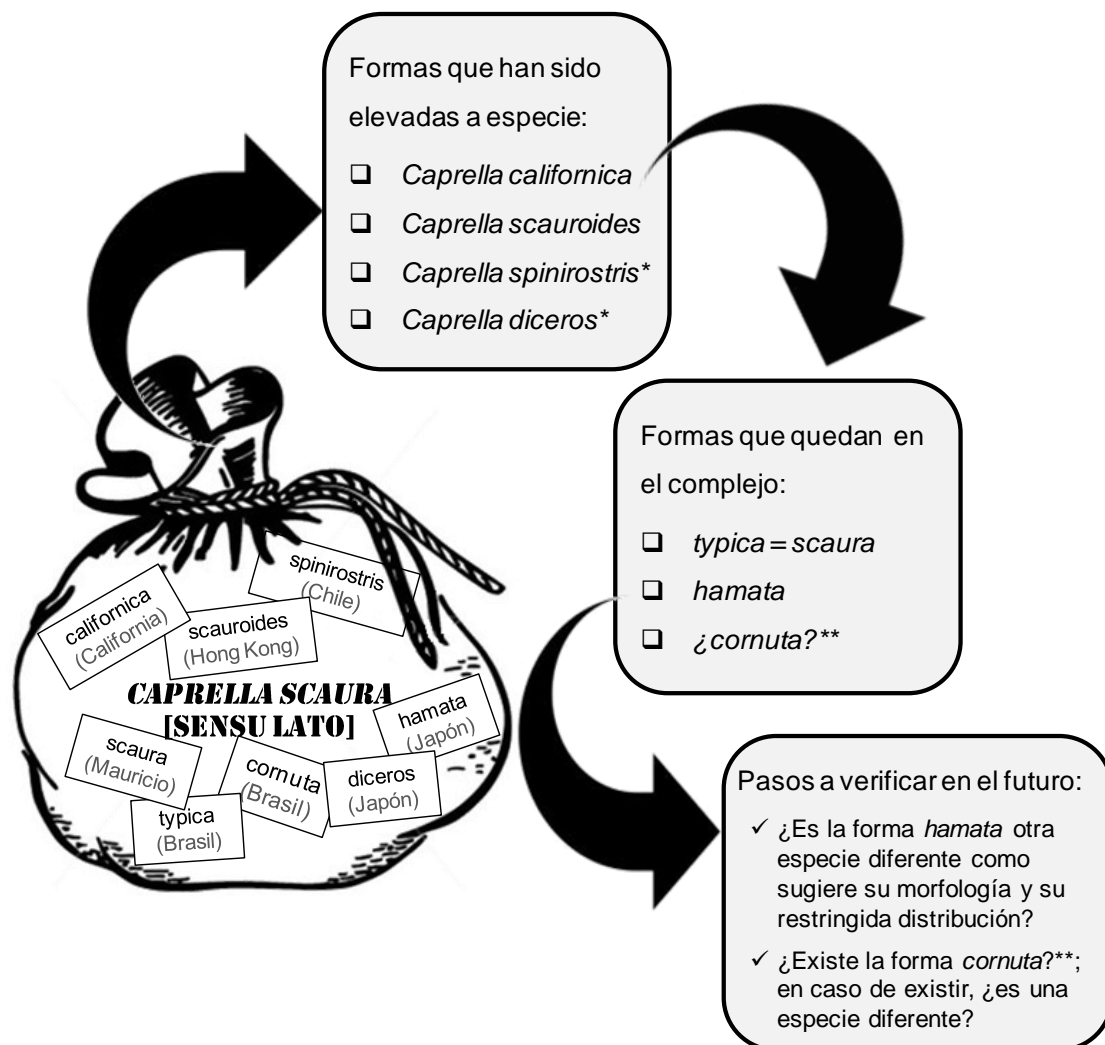


Figure 7.2 Esquema del estado actual del complejo *Caprella scaura* [sensu lato]. Los autores que describieron cada forma se encuentran detallados en el Cap. 2, parte 3. (*) Esta forma se ha elevado a especie en base a criterios moleculares (ver Cabezas et al. 2014), pero todavía requiere ser descrita. (**) Pese a los muestreos exhaustivos llevados a cabo en el estado de Río de Janeiro y otros lugares de Brasil cercanos a la localidad tipo de la forma *cornuta* (Cap. 6, parte 2), no se encontraron individuos de *C. scaura* con las características descritas para esta forma.

La exploración morfológica del complejo *C. scaura* [sensu lato], llevada a cabo en el Cap. 2, parte 3, arrojó una conclusión interesante: sólo una forma del complejo era la que se estaba dispersando de forma global. Esta forma había sido denominada como forma *typica* (inicialmente descrita en Brasil) pero también como forma *scaura* (inicialmente descrita en Mauricio). A través de análisis morfológicos se demostró que las diferencias entre ambas eran el resultado de variabilidades intrapoblacionales que no tenían validez taxonómica. Estas conclusiones fueron posteriormente ratificadas mediante técnicas genéticas por Cabezas *et al.* (2014), que concluyeron que no había evidencias moleculares para separar la forma *typica* de la forma *scaura* (Fig. 7.2). Sin embargo, estos autores encontraron diferencias entre estas formas y la forma *diceros*, afirmando que *C. s. diceros* tenía entidad de especie. El resto de formas sin proyección ventral, *hamata* y *cornuta*, no fueron analizadas.

Para terminar de resolver el complejo *C. scaura* [sensu lato] todavía es necesario confirmar a nivel molecular si las formas *hamata* y *cornuta* también tienen entidad de especie. Si esto ocurriese, la única forma que quedaría en el complejo sería la forma *typica-scaura*, que pasaría a denominarse *Caprella scaura* [sensu stricto].

Del análisis del complejo *C. scaura* [sensu lato] se deducen varios aspectos importantes para entender la dinámica de invasión de la especie:

- Sólo la forma *typica-scaura* está siendo globalmente introducida. El resto de formas que todavía quedan en el complejo (*hamata* y *cornuta*) tienen una distribución más restringida (indicativo de que podrían corresponder a especies diferentes).
- El hecho de que en Japón coexistan más variedades de *C. scaura* (concretamente *C. s. typica*, *C. s. hamata* y *C. s. diceros*; Arimoto, 1976) que en cualquier otra parte del mundo (ver Figura 2.14) podría ser un indicativo del origen de la especie. Más estudios moleculares, en la línea de los desarrollados recientemente por Cabezas *et al.* (2014), son todavía necesarios para esclarecer este punto.
- Los estudios ecológicos sobre *C. scaura* realizados en lugares donde coexistan varias formas (e.g. Japón) deben interpretarse con cautela si no aportan datos sobre la forma de *C. scaura* encontrada, pues podrían

corresponder a especies diferentes tradicionalmente denominadas como *C. scaura* [sensu lato].

7.1.2 Aspectos ecológicos, biogeográficos y taxonómicos del proceso de invasión de *Paracaprella pusilla* en los mares europeos

➤ Introducción de *P. pusilla* en aguas europeas (etapas 0-II)

El conocimiento actual que se tiene sobre la dinámica de invasión de *P. pusilla* en aguas europeas es muy escaso, entre otras razones porque se detectó por primera vez en Europa hace tan sólo 5 años (finales de 2010) (Cap. 2, parte 1). Era la primera vez, además, que una especie del género *Paracaprella* era registrada en esta región. La especie se encontró en un puerto deportivo del sur de la Península Ibérica, en Cádiz, muy cerca de un puerto internacional. La vía de entrada que se propuso como la más probable, en base a su localización, fue a través del tráfico marítimo que llegaba a este puerto internacional (bien en el agua de lastre o bien adherida a las comunidades incrustantes de los cascos de los barcos), mientras que su presencia en el puerto deportivo se asoció a un transporte secundario en asociación con los cascos de las embarcaciones de recreo. Sin embargo, el origen de esta población inicial permanece sin resolver.

Al igual que en el caso de *C. scaura*, los registros globales de la especie arrojaron una característica que podría haber facilitado su llegada a Europa: su frecuente asociación con hábitats antropogénicos y estructuras artificiales (Tabla 2.1 y referencias incluidas). Además, la alta eurihalinidad de la especie (Cap. 5, parte 2), junto con su preferencia alimenticia hacia los copépodos (Cap. 6, parte 2), habrían facilitado su supervivencia en los tanques de agua de lastre, sometidos a grandes fluctuaciones de salinidad (Santagata *et al.* 2008) y donde los copépodos son muy abundantes (Smith *et al.* 1999).

➤ Establecimiento (etapa III)

La relación de *P. pusilla* con hidrozoos del género *Eudendrium*, tanto en su rango nativo (Brasil; Cap. 6, parte 1) como en el rango de introducción, pudo haber facilitado su establecimiento en el sur de España. En Brasil, la especie se encontró en altas densidades asociada a *Eudendrium* cf. *carneum*, y en Cádiz, se encontró en abundancia sobre *Eudendrium racemosum*. Además de refugio frente

a depredadores, en el Cap. 2, parte 1 se describe una relación de tipo cleptocomensalista mediante la que la especie pudo haber visto facilitada su tarea de ingerir pequeñas presas como copépodos gracias al hidrozoo. Además, *P. pusilla* comparte con *C. scaura* las características 1 (afinidad por hábitats artificiales frecuentados por vectores de dispersión), 4 (plasticidad trófica: Cap. 6, parte 2), 6 (eurihalinidad: Cap. 5, parte 2) y 7 (gran capacidad reproductora: Cap. 2, parte 2), que también habrían podido favorecer su establecimiento.

➤ Propagación (etapa IV)

En 2011, un año después de que la especie fuera detectada en Cádiz, otra población de *P. pusilla* apareció en un puerto deportivo de la isla de Mallorca (Baleares), constituyendo la primera cita para el Mediterráneo (Cap. 2, parte 2). Aunque varias hipótesis fueron planteadas y discutidas como posibles rutas de entrada de la especie en el Mediterráneo (Fig. 2.8), su origen sigue siendo una incógnita. El hecho de que la especie fuera encontrada en un puerto deportivo sugiere que la adherencia de la especie a las comunidades incrustantes de las embarcaciones de recreo es, posiblemente, el principal vector responsable de su dispersión local.

Tres años más tarde (en 2014), la especie se encontró por primera vez en el otro extremo del Mediterráneo, concretamente en Zikim, Israel (Ros *et al.* 2015). Esto incrementó en ~3750 km hacia el este el rango de distribución de *P. pusilla* (si tomamos Cádiz como punto de referencia). La especie se encontró asociada tanto a colonias del briozoo *B. neritina* que iban flotando a la deriva como a colonias ancladas a la zona sumergida del Kurkar (un tipo de roca natural característica de la zona y formada a partir de arena fosilizada; Fishelson 2000). Este hecho ha incorporado nuevos datos al conocimiento sobre la dinámica de invasión de *P. pusilla* en aguas europeas: (1) el rafting en objetos flotantes sí juega un papel relevante en la propagación local de la especie; (2) la entrada por el canal de Suez adquiere más relevancia que cuando sólo se conocía la población de Mallorca (próxima al Estrecho de Gibraltar); (3) es muy probable que la especie se encuentre en otros lugares del Mediterráneo donde haya pasado inadvertida.

Al igual que ocurría con *C. scaura*, la colonización de ambientes naturales parece relegada (tanto en el rango de introducción como en el nativo) a zonas

sometidas a grandes perturbaciones de naturaleza antrópica, como es el caso de Zikim (Ros *et al.* 2015) o Araça (São Paulo; Cap. 6, parte 1).

Por otra parte, el aumento de temperatura del mar Mediterráneo sí podría favorecer el establecimiento y la propagación de una especie que alcanza sus mayores densidades en los meses más cálidos (ver Cap. 3). No obstante, la ecología de la especie es tan poco conocida que sería necesario estudiar su tolerancia a diferentes temperaturas para confirmar esta hipótesis.

El rango nativo de *P. pusilla* parece más claro que el de *C. scaura*. El hecho de que la mayor parte de las citas mundiales de la especie se localicen en la costa Atlántica de América Central y del Sur, coincidiendo con el lugar donde la especie fue descrita (Río de Janeiro, Brasil), sugiere que ese podría corresponder a su rango natural de distribución. Posteriormente, la especie habría llegado a las costas de África, India, Australia, Hawaii, Panamá (Cap. 5, parte 2) y, recientemente, a Europa (Cap. 2, partes 1 y 2). Si esta especie tropical, tal y como se intuye, ha sido introducida de forma relativamente reciente en climas templados, podría constituir un indicio más de las consecuencias del aumento de la temperatura en la distribución de las especies marinas. Análisis moleculares que abarquen todas estas poblaciones son todavía necesarios para esclarecer la historia de introducción global de la especie.

7.2 ANÁLISIS GLOBAL DE LAS ESPECIES DE CAPRÉLIDOS INTRODUCIDAS EN EL MUNDO

Cada vez se detectan más especies de caprélidos fuera de su rango natural de distribución. Sin embargo, no existe hasta el momento un estudio que analice la dinámica global de invasibilidad del grupo. Esta visión general es necesaria para responder a cuestiones importantes como cuáles son los principales vectores de introducción o cuáles son las áreas que están recibiendo un mayor número de especies introducidas de este grupo y por qué.

En la Tabla 7.1 y en la Figura 7.3 se recogen todas las especies de caprélidos que han sido clasificadas como introducidas (invasoras, no-nativas, no-indígenas, exóticas, etc.) en las diferentes regiones del mundo. Las especies clasificadas como criptogénicas no han sido incluidas.

Tabla 7.1 Especies de caprélidos reconocidas como introducidas en cada región del mundo. Las referencias incluyen a los autores que las han reconocido como tal por primera vez en cada zona. Vectores más probables de introducción: AQ acuicultura; SF “fouling” de los barcos; BW agua de lastre.

REGIÓN	ESPECIE	VECTOR	REFERENCIAS
O. PACÍFICO			
Alaska	<i>Caprella mutica</i>	AQ, SF	Ashton <i>et al.</i> 2008b
Canadá	<i>C. mutica</i>	SF	Ashton <i>et al.</i> 2007
Costa Pacífica de EE.UU	<i>C. monoceros</i> ¹		Chapman 2000
	<i>Phtisica marina</i>	SF	Fairey <i>et al.</i> 2002
	<i>C. acanthogaster</i>	SF	Fairey <i>et al.</i> 2002
	<i>C. equilibra</i>	SF ²	Chapman 2000
	<i>C. drepanochir</i>	SF	Watling y Carlton 2007
	<i>C. penantis</i>	SF	Fairey <i>et al.</i> 2002
	<i>C. mutica</i>	AQ	Cohen y Carlton 1995
	<i>C. simia</i>	SF	Cohen <i>et al.</i> 2005
	<i>C. scaura</i>	SF ²	Fairey <i>et al.</i> 2002
Hawái	<i>C. natalensis</i>		Fairey <i>et al.</i> 2002
	<i>C. scaura</i>	SF	Carlton y Eldredge 2009
	<i>C. penantis</i>	SF	Carlton y Eldredge 2009
	<i>C. equilibra</i>	SF	Carlton y Eldredge 2009
	<i>C. danilevskii</i>	SF	Carlton y Eldredge 2009
	<i>Paracaprella pusilla</i>	SF	Carlton y Eldredge 2009
Costa Pacífica de Panamá	<i>P. pusilla</i>	SF, BW	Ros <i>et al.</i> 2014 (Cap. 5.2)
Costa Pacífica de Australia	<i>P. pusilla</i>	SF	Montelli y Lewis 2008
	<i>C. equilibra</i>	SF	Montelli y Lewis 2008
	<i>C. penantis</i>	SF	Montelli y Lewis 2008
Tasmania	<i>C. acanthogaster</i>	AQ	Guerra-García y Takeuchi 2004
Nueva Zelanda	<i>C. mutica</i>	SF	Ashton <i>et al.</i> 2007
	<i>C. andreae</i>	AQ	Woods <i>et al.</i> 2014
O. ATLÁNTICO			
Costa Atlántica de EEUU	<i>C. mutica</i>	SF	MIT Sea Grant 2003
	<i>C. scaura</i>	SF	Foster <i>et al.</i> 2004
Europa	<i>C. scaura</i>	SF	Guerra-García <i>et al.</i> 2011b
	<i>C. mutica</i>	AQ, SF	Willis <i>et al.</i> 2004
	<i>P. pusilla</i>	SF	Ros y Guerra-García 2012
Macaronesia	<i>C. scaura</i>	AQ	Guerra-García <i>et al.</i> 2011b
Sudáfrica	<i>P. pusilla</i>	SF	J.T. Carlton (com. pers.)
M. MEDITERRÁNEO			
	<i>C. scaura</i>	SF	Sconfietti y Danesi 1996
	<i>P. pusilla</i>	SF	Ros <i>et al.</i> 2013 (Cap. 2.2)
O. ÍNDICO			
India	<i>P. pusilla</i>	SF	Ros y Guerra-García 2012
Costa Índica de Australia	<i>C. scauroides</i> ³	SF	Montelli y Lewis 2008
	<i>P. pusilla</i>	SF	Montelli y Lewis 2008
	<i>C. equilibra</i>	SF	Montelli y Lewis 2008
	<i>C. penantis</i>	SF	Montelli y Lewis 2008

¹Identificada como *C. bidentata*, que es sinonimizada con *C. monoceros* por Aoki y Kikuchi (1999).

²El vector de introducción es sugerido por Watling y Carlton (2007).

³Identificada como *C. californica* [sensu lato], que es sinonimizada posteriormente con *C. scauroides* por Takeuchi y Oyamada (2013)

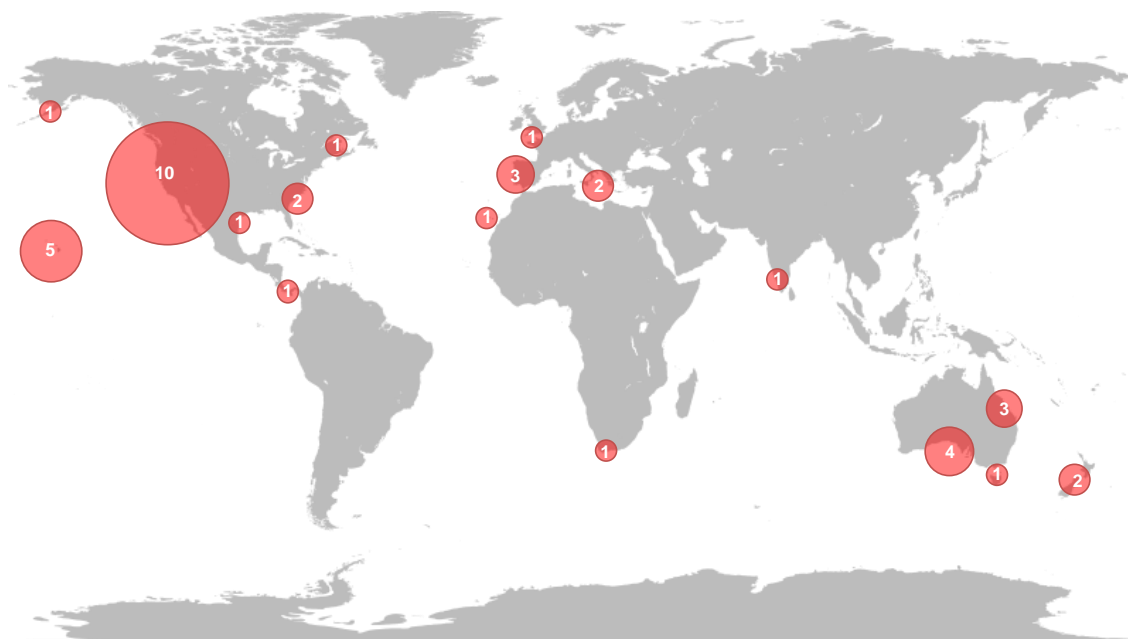


Figure 7.3 Relación mundial del número de especies de caprélidos clasificadas como introducidas a nivel global. En el interior de cada círculo aparece el número de especies introducidas por región. Las especies y las referencias de los autores que las han clasificado se encuentran en la Tabla 7.1.

7.2.1 Vectores de introducción y propagación

Hasta un total de 14 especies de caprélidos han sido introducidas mediante vectores relacionados con el ser humano en diferentes regiones del mundo. La vía principal de introducción inicial o dispersión primaria es a través del “fouling” de los barcos y, en menor medida, a través de la acuicultura y el agua de lastre.

El agua de lastre es un vector de introducción viable aunque con una menor probabilidad de introducir caprélidos en comparación con otros vectores como la asociación al “fouling” de los barcos o la acuicultura. La carencia de fase larvaria y la preferencia de los caprélidos por permanecer anclados a un sustrato (y no libremente en la columna de agua) podrían dificultar la incorporación de los caprélidos a los tanques que albergan el agua de lastre. Además, las especies introducidas en los tanques podrían experimentar dificultades para sobrevivir largos periodos si no encuentran un sustrato adecuado al que poder anclarse. No obstante, algunas especies como *Caprella equilibra* parecen tener una gran capacidad de permanencia en el plancton

durante largos periodos (Takeuchi y Sawamoto 1998) y Carlton (1985) encontró caprélidos vivos del género *Caprella* en tanques de agua de lastre.

En el caso de la acuicultura, cabe destacar la afinidad de muchas especies de caprélidos exóticos por los cultivos de ostras japonesas (*Cassostrea gigas*). Esta especie ha sido introducida deliberadamente para su cultivo como alimento a lo largo de muchas regiones del mundo (ver Figura 4 de Molnar *et al.* 2008), dispersando con ella a toda su fauna asociada. Por ejemplo, Takeuchi y Oyamada (2013) describieron la especie *C. scauroides* a partir de individuos recolectados en instalaciones de cultivo de ostras en Japón. Otras especies, como *C. scaura* [sensu lato] y *C. natalensis* también han sido encontradas en asociación con estos moluscos en Japón (Miyazaki, 1937-1938). Así mismo, la introducción de *C. mutica* a la costa pacífica de EEUU ha sido relacionada con la introducción de *Cassostrea gigas* (Carlton 1987), así como su introducción en aguas europeas (Willis *et al.* 2004).

7.2.2 Impactos en las comunidades receptoras (negativos y positivos)

Teniendo en cuenta que en la actualidad se conocen alrededor de 350 especies de caprélidos (Guerra-García y Tierno de Figueroa 2011), podría decirse que en torno al 4% de estas especies tiene potencial invasivo. Estas especies pertenecen a tres géneros, *Caprella*, *Phthisica* y *Paracaprella*, siendo el primero el que alberga un mayor número de especies de caprélidos (tanto potencialmente invasivas como sin este potencial). De estas 14 especies, al menos dos han mostrado indicios significativos de producir un impacto negativo en otras especies de caprélidos: *C. mutica* (Boos 2009; Shucksmith *et al.* 2009) y *C. scaura* (Guerra-García *et al.* 2011b; Cap. 4). En el caso de *C. mutica*, también se han encontrado impactos positivos en algunas regiones donde ha sido introducida. Collin y Johnson (2014) y Rius *et al.* (2014) han demostrado recientemente que *C. mutica* contribuye a aumentar la resistencia de las comunidades nativas a la invasión por especies exóticas de tunicados, al dificultar el asentamiento de sus larvas. Estos estudios contribuyen a resaltar el papel que pueden tener estos pequeños crustáceos en la estructuración de las comunidades bentónicas.

Cabe destacar que todos los trabajos que se citan en este apartado son recientes. La mayor parte de la ecología de las especies introducidas de

caprélidos permanece sin estudiar. En muchos casos no se conocen aspectos básicos de su ciclo de vida o su tolerancia a las variables ambientales, de modo que resulta muy difícil conocer el impacto que pueden generar. Más estudios que profundicen en la ecología de estas especies (tanto en su rango nativo como en su rango introducido) son necesarios para tener una visión más global sobre el impacto que este grupo podrían llegar a generar en los lugares de introducción.

7.2.3 Características que diferencian a un caprélido exótico de uno nativo

Aunque todas las especies que figuran en Tabla 7.1 tienen en común su afinidad a los ambientes portuarios y otros hábitats antropizados, no todas las especies que viven en estos hábitats han sido introducidas globalmente. En el Cap. 6, parte 1 pueden verse ejemplos de especies encontradas en puertos deportivos pero con una distribución restringida, como *Aciconula tridentata* o *Pseudaeginella montoucheti* (ambas endémicas de Brasil) y en el Cap. 5, parte 1 se registró la presencia de *Caprella acanthifera*, con una distribución fundamentalmente mediterránea (aunque también puede encontrarse en el Atlántico). Diferentes aspectos relacionados con la biología de la especie, especialmente aquellos involucrados en el éxito de establecimiento (ver apartado 7.1.1), probablemente sean los responsables de las diferencias entre ese 4% de especies de caprélidos con una distribución global y el 96% restante, con una distribución acorde con su baja tasa de dispersión autónoma. En el caso de los gammáridos que han invadido las aguas continentales europeas, Grabowski *et al.* (2007) encontraron que, además de una amplia tolerancia al estrés medioambiental y a la contaminación, las especies invasoras se diferenciaban de las nativas en tres aspectos fundamentales: una alta fecundidad, una maduración temprana y un número alto de generaciones por año. En definitiva, presentan una alta eficiencia reproductora (propia de un r-estratega) que facilita el establecimiento de la especie (estas y otras características son analizadas en *C. scaura* y *P. pusilla* en el Cap. 2, parte 2). Hänfling *et al.* (2011), analizando los crustáceos invasores en general, propone, además de los aspectos anteriores, la plasticidad trófica como elemento influyente en el potencial invasivo.

7.2.4 Precauciones a la hora de interpretar los datos sobre distribución de caprélidos exóticos

La región donde se ha encontrado el mayor número de especies de caprélidos introducidos es la costa Pacífica de EEUU. La mayoría de las especies citadas en esta costa fueron detectadas por primera vez en la bahía de San Francisco, una de las regiones más invadidas del mundo (Cohen y Carlton 1998). Sin embargo, existen algunas limitaciones que deben tenerse en cuenta a la hora de interpretar los datos referentes a las especies introducidas de caprélidos a nivel mundial (tales como los que se recogen en la figura 7.3 y la tabla 7.1):

- a) En muchas de estas especies el rango nativo no está del todo claro y además llevan establecidas durante siglos en lugares muy distintos entre sí. Esto provoca que en un mismo lugar estas especies sean clasificadas como nativas, criptogénicas o introducidas según el autor de la cita (e.g. *C. equilibra* y *C. penantis*).
- b) En algunos casos, la existencia de complejos de especies crípticas (morfológicamente indistinguibles), puede derivar en una sobreestimación del rango de distribución de una especie. Actualmente se han encontrado especies crípticas mediante análisis moleculares en *C. penantis* (Cabezas *et al.* 2013a) y *C. andreae* (Cabezas *et al.* 2013b).
- c) La existencia de especies morfológicamente muy similares (especies que sin ser crípticas pueden llegar a parecerlo para un taxónomo no especializado), pueden inducir a una identificación incorrecta. Por ejemplo: *C. acanthogaster* y *C. mutica* (ver Marelli 1981) o *C. natalensis* y *C. penantis* (Laubitz 1972).
- d) Existen áreas donde no se han citado especies exóticas de caprélidos, tales como la costa centroafricana, la costa Pacífica de América central o algunas regiones del Océano Índico, probablemente porque no hay estudios sobre este grupo o éstos son muy escasos.

Por todos estos motivos, el análisis en profundidad de la distribución global de cada una de estas especies (tal y como se ha pretendido desarrollar en la presente Tesis Doctoral para las especies *C. scaura* o *P. pusilla*) es necesario para poder determinar tanto su estatus en cada zona como su dinámica de introducción.

7.3 PERSPECTIVAS DE FUTURO

Los caprélidos comienzan a tener un papel cada vez más destacado en los estudios sobre invasiones en el medio marino. Gracias a especies como *C. mutica* se ha evidenciado la susceptibilidad de zonas tan aparentemente hostiles a las invasiones como Alaska (Ashton *et al.* 2008b), y la presencia de *P. pusilla* a ambos lados del canal de Panamá ha puesto de relieve el papel potencial que juega este canal dulceacuícola en la introducción de especies marinas (Cap. 5, parte 2). Sin embargo, todavía quedan muchos aspectos por estudiar en este grupo de pequeños crustáceos.

Particularmente, los resultados obtenidos sobre la dinámica de invasión de *Caprella scaura* y *Paracaprella pusilla* han abierto varias líneas donde sería interesante profundizar. Estudios en laboratorio donde se analice la interacción entre *C. scaura* y *C. equilibra* podrían ser determinantes para confirmar la relación causal entre el declive de *C. equilibra* en la costa mediterránea de la Península Ibérica y la proliferación de *C. scaura* en esta región. La realización de estos experimentos a diferentes concentraciones de salinidad y temperatura podría confirmar el rol de estos factores en esta interacción. Así mismo, la reciente detección de la especie invasora *C. mutica* en la costa Atlántica del norte de España (Almón *et al.* 2014) podría implicar otra amenaza para la especie *C. equilibra*. De ser así, *C. equilibra* tampoco encontraría refugio en esta región del Atlántico. En este caso, experimentos de competencia entre ambas especies a diferentes temperaturas (pues la salinidad no varía tanto en la costa Atlántica en comparación con lo que varía entre Atlántico-Mediterráneo) serían interesantes para confirmar una posible relación de competencia entre ambas.

En el caso de *Paracaprella pusilla*, sería de gran interés analizar la tolerancia de la especie a diferentes temperaturas para poder predecir las áreas más susceptibles para su introducción así como el papel del “cambio climático” en su dinámica de invasión. Por otra parte, análisis moleculares de las poblaciones de *P. pusilla* a lo largo del mundo podrían arrojar datos interesantes sobre el proceso de introducción global de la especie así como confirmar que su área nativa corresponde a la región central y sur de la costa este Atlántica.

Todo apunta a que un mayor esfuerzo de muestreo en los lugares susceptibles de albergar especies introducidas de caprélidos, entre los que se incluyen todo tipo de hábitats artificiales (sumergidos y flotantes) donde se

desarrollan actividades antrópicas (instalaciones de acuicultura, puertos comerciales y deportivos, bateas de mejillones, puntos de amarre fijos, etc.), arrojará un incremento en el número de caprélidos introducidos en los próximos años. Esto es especialmente relevante en el caso de España, donde a pesar de que los organismos que forman las comunidades incrustantes de este tipo de hábitats han sido muy poco estudiados, es el país de Europa con un mayor número de especies introducidas de caprélidos. Así mismo, un mayor esfuerzo de muestreo en aquellas áreas del mundo donde el conocimiento sobre los crustáceos caprélidos es muy escaso permitirá conocer el papel que el ser humano ha jugado y está jugando en la biogeografía de este grupo.

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8

GENERAL CONCLUSIONS

The main findings of the present study can be summarized as follows:

1. Two introduced caprellid species were recorded in marinas from the Mediterranean Sea and the southern Atlantic coast of the Iberian Peninsula: *Caprella scaura* Templeton 1936 and *Paracaprella pusilla* Mayer 1890.
2. Arborescent bryozoans and hydroids, which are part of the fouling community in submerged artificial structures of marinas and ship hulls, can be used as strategic points for the assessment and detection of non-native caprellids since they can act as refuge for these introduced species, facilitating their spread and subsequent establishment.
3. The tropical western Atlantic species *P. pusilla* was recorded for the first time in European coastal waters, including the Mediterranean Sea. The most probable introduction vector was ship fouling while recreational boating and rafting on floating substrata are probably responsible for its secondary spread. The species was also recorded for the first time on the

two sides of the Panama Canal, representing an indicator of the vulnerability of the area to marine introductions.

4. An important post-introduction range expansion of *C. scaura* was found along recreational marinas from the western Mediterranean Sea and the South Atlantic coast of the Iberian Peninsula, supporting the outstanding role of recreational boating in the rapid spread of the species. Furthermore, *C. scaura* was recorded for the first time in Corsica (France), Crete (Greece) and Morocco. The upper distribution limit of the species for the eastern Atlantic coast was the locality of Cascais, on the south coast of Portugal, representing the first confirmed record of *C. scaura* for this country.
5. Intraspecific morphological variations found within *C. scaura* populations, affecting the dorsal tuberculation pattern mainly, showed that the form *C. scaura typica* and *C. s. scaura* correspond with the same variety, thus contributing to a clarification of the *C. scaura* complex and suggesting that a single form of the complex is expanding its distribution range, while the other forms remain in a restricted distribution area.
6. The ecology of *P. pusilla* in its native and introduced range revealed several aspects (previously unknown to the species) that could be related with its invasion success, such as: high plasticity in the trophic strategies (with a high preference for predation on small crustaceans), euryhalinity, high tolerance to anthropogenic pollution, high fecundity and development of mutualistic relationship with its preferred host substratum in its introduced range (*Eudendrium* spp.).
7. Since *P. pusilla* is more restrictive in the occupation of the substrata, less abundant and more sensitive to changes of environmental conditions than *C. scaura*, it seems that it is an introduced species with less invasiveness than *C. scaura* in European coastal waters.
8. *Caprella scaura* is a ‘strong invader’ in artificial habitats in the Mediterranean Sea, where it reaches high densities and seems to displace ecologically similar congeners such as *Caprella equilibra*. Its high

reproductive potential (where ovigerous females are present throughout the year), high trophic plasticity (with high affinity by detritus-feeding strategy), aggressive behavior, parental care, high anthropogenic pollution tolerance, euryhalinity and high capacity to colonize different substrata, are likely related with its invasion success.

9. However, the low salinity together with the low temperature of the central and north Atlantic coast of the Iberian Peninsula, in comparison with the Mediterranean coast, limit the spread and invasive success of *C. scaura*, providing a competitive refuge for other previously established species that occupy similar habitats.
10. This study highlights the role of recreational marinas as reservoirs for wide-ranging caprellids (introduced and cryptogenic species), while endemic species were scarcely represented in these artificial habitats.
11. However, while recreational boating seems an effective vector for the secondary spread of introduced caprellids among artificial habitats, it seems to fail in their spread beyond this type of habitats. Colonization of natural environments is therefore considered a limiting step in the invasion dynamics of non-native caprellids.
12. Based on the native-range ecology of *C. scaura* and *P. pusilla*, they are expected to become established in sheltered and even highly polluted natural habitats in their new introduced distribution ranges, but they will be unable to colonize wave-exposed rocky shores. Additionally, we propose the use of the density of *C. scaura* and *P. pusilla* as a first diagnosis for the detection of nutrient enrichment on rocky shores.

List of publications resulting from this PhD Thesis

The results of the present PhD Thesis have been totally or partially published (or submitted for publication) in the following articles:

- Ros M, Guerra-García JM (2012) On the occurrence of the tropical caprellid *Paracaprella pusilla* Mayer, 1890 (Crustacea: Amphipoda) in Europe. *Mediterranean Marine Science* 13: 134–139.
- Ros M, Vázquez-Luis M, Guerra-García JM (2013) The tropical caprellid amphipod *Paracaprella pusilla*: a new alien crustacean in the Mediterranean Sea. *Helgoland Marine Research* 67: 675–685.
- Ros M, Vázquez-Luis M, Guerra-García JM (2013) The role of marinas and recreational boating in the occurrence and distribution of exotic caprellids (Crustacea: Amphipoda) in the Western Mediterranean: Mallorca Island as a case study. *Journal of Sea Research* 83: 94–103.
- Ros M, Guerra-García JM, González-Macías M, Saavedra A, López-Fe CM (2013) Influence of fouling communities on the establishment success of alien caprellids (Crustacea: Amphipoda) in Southern Spain. *Marine Biology Research* 9: 293–305.
- Ros M, Guerra-García JM, Navarro-Barranco C, Cabezas MP, Vázquez-Luis M (2014) The spreading of the non-native caprellid (Crustacea: Amphipoda) *Caprella scaura* Templeton, 1836 into southern Europe and northern Africa: a complicated taxonomic history. *Mediterranean Marine Science* 15: 145–165.
- Ros M, Tierno de Figueroa M, Guerra-García JM, Navarro-Barranco C, Lacerda M, Vázquez-Luis M, Masunari S (2014) Exploring trophic strategies of exotic caprellids (Crustacea: Amphipoda): comparison between habitat types and native vs introduced distribution ranges. *Estuarine, Coastal and Shelf Science* 139: 88–98.

- Ros M, Ashton GV, Lacerda MB, Carlton JT, Vázquez-Luis M, Guerra-García JM, Ruiz GM (2014) The Panama Canal and the transoceanic dispersal of marine invertebrates: evaluation of the introduced amphipod *Paracaprella pusilla* Mayer, 1890 in the Pacific Ocean. *Marine Environmental Research* 99: 204–211.
- Ros M, Lacerda MB, Vázquez-Luis M, Masunari S, Guerra-García JM (*submitted*) Studying exotics in their native range: can invasive caprellids expand beyond anthropogenic habitats? *Marine Ecology Progress Series*
- Ros M, Vázquez-Luis M, Guerra-García JM (*submitted*) Environmental factors modulating the extent of biological pollution in coastal invasions: the case of the invasive crustacean *Caprella scaura* (Amphipoda: Caprellidae) in the Iberian Peninsula. *Marine Pollution Bulletin*