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Bridging gaps for preventive management of marine bioinvasions associated to the recreational boating vector: vector assessment, baseline surveys, and social-perception

Hacia una gestión preventiva de las invasiones marinas asociadas a la navegación de recreo: evaluación del vector, monitoreo, y percepción social

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INFORMAN:

Que esta Memoria de Investigación, titulada "*Bridging gaps for a preventive management of marine bioinvasions associated to the recreational boating vector: vector assessment, baseline surveys, and social-perception*", fue realizada por Gemma Martínez Laiz 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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El Director (1)　　　　La Directora (2)

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"One individual cannot possibly make a difference, alone. It is individual efforts, collectively, that make a noticeable difference—all the difference in the world!"

Dr. Jane Goodalll

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General abstract

Biological invasions remain one of the main threats to biodiversity. They damage native communities, their habitat and ecosystem services, impact all segments of society, and result in economic losses of billions of dollars/year. In the marine environment, introduction events have exponentially increased in the last decades, and predictive models suggest this trend will only continue unless effective management actions are enforced. It is known that shipping is the main pathway responsible, with the hull-fouling vector accounting for 60-70% of the marine introduced species established worldwide. While management of ballast water has recently been implemented at international level, only certain jurisdictions (New Zealand, Australia and California state) have enforced regulations for biofouling. Especially, the recreational boating sector was long overlooked, despite posing a high risk of secondary spread, and infection of vulnerable/protected areas. Experts agree that management solutions need to be based on a precautionary approach, as prevention and early detection (addressing the early stages of invasion) have proven to be the most cost-effective strategies. In order to achieve this, efforts need to be placed on certain bases: vector assessment and regulations, baseline surveys and monitoring, and community participation and awareness. This thesis aims to fill in some gaps of knowledge for preventive management of marine bioinvasions associated to recreational boating. Peracarid crustaceans, one main component of the fouling epifauna, are used as model taxa; and most of the thesis takes place in the Iberian Peninsula and the Mediterranean Sea, hotspots for nautical tourism and marine bioinvasions at global scale, and where implementation is still lacking. Firstly, evidence is provided that recreational boating acts as a high-risk vector in the Mediterranean Sea at basin-scale, and that boaters behaviors are influential variables for the probability of carrying potential invaders in their vessel hull-fouling. This warrants developing mandatory measures to be integrated in regulations for recreational boating. Moreover, laboratory simulations empirically proved that sessile fouling basibionts act as efficient refugia for invasive mobile epifauna during the vessel transport phase. In here, the invasive species model exhibited higher boldness than the native one; which implies a risk during translocation but an advantage in other phases of invasion. This supports the idea that a fouling level higher than a slime (microfouling) incurs a risk for biosecurity; and that cleaning of this macrofouling – especially from niche areas - prior to translocation would be a desirable maintenance practice. Secondly, baseline survey efforts in marinas were carried out to raise information on several cases of hidden-invaders (small-size and/or taxonomically challenging taxa that usually remain overlooked), resulting in a substantial increase of their known distribution range. This illustrated several lessons to reduce lags in detection of hidden-invaders: 1) taxonomic expertise is the foundation and needs to be promoted, 2) willingness of expert groups to communicate and disseminate new records to raise the alarm is key; and 3) we are

in need of an effective standardized monitoring methodology for mobile fouling epifauna in ports and marinas. Together with this, the use of molecular tools proved to be useful in monitoring the post-establishment phase of the invasion process, identifying source populations that pose a risk for propagule input and detecting vulnerability periods (low diversity, population size or connectivity) of the introduced population. This outlines once again the urgency of managing the vector, as diminishing propagule pressure is determinant to weaken these unstable populations inhabiting marinas. Finally, social-perception of Spanish stakeholders on the issue was, for the first time, explored and compared with that in the only two countries with nationwide regulations (Australia and New Zealand). Preliminary results indicate that stakeholders in our region are far more overconfident than in the other countries, and that their risk perception was significantly lower and associated to the perceived responsibility of good vessel maintenance practices. Baseline knowledge, connection feeling with the environment and risk perception were the main factors driving attitudes towards management of marine exotic species. There is a potential support of the International Maritime Organization’s recommended guidelines for biofouling control in recreational vessels, but boaters were ambiguous when asked about cleaning practices. As this warrants the implementation of environmental education strategies, preliminary recommendations are provided for better orientation of these campaigns. Finally, in the light of this PhD’s research, future directions are identified, mainly based on the following ideas: 1) mapping of invasion risk in the Spanish territory and establishment of long-term monitoring in areas/polygons of interest; 2) feasibility of future management actions, both from an ecological perspective (*e.g.* evaluation of vessel in-water cleaning and treatment solutions) and from a social perspective (*e.g.* researching intended behavior and compliance of stakeholders); and 3) increasing public awareness via outreach campaigns and citizen science initiatives.

Resumen general

Las invasiones biológicas siguen constituyendo una de las mayores amenazas a la biodiversidad. Generan un daño en las comunidades nativas, su hábitat y sus servicios ecosistémicos, impactan en todos los segmentos de la sociedad y acarrean pérdidas económicas de billones de dólares/año. En el medio marino, la incidencia de introducciones ha aumentado exponencialmente en las últimas décadas; y los modelos predictivos sugieren la misma tendencia en el futuro si no se implementan medidas de gestión efectivas. Se sabe que la navegación es la principal vía de propagación y que el vector *biofouling* es responsable del 60-70% de la especies introducidas y establecidas a nivel mundial. Mientras que la gestión del agua de lastre ha sido recientemente implementada a nivel internacional, sólo jurisdicciones puntuales (Nueva Zelanda, Australia y el estado de California) han adoptado medidas obligatorias para el *biofouling* de embarcaciones. Concretamente, el sector de la navegación de recreo pasó inadvertido durante mucho tiempo, a pesar de suponer un alto riesgo de propagación secundaria y de infección de áreas vulnerables/protegidas. Los expertos coinciden en que las soluciones de gestión deben nacer de un principio de precaución, ya que se ha demostrado que la prevención y la detección temprana (atajando las fases tempranas del proceso de invasión) son las estratégicas con mejor coste-beneficio. Para lograr este enfoque es necesario focalizar esfuerzos en varios pilares: una regulación que obligue al control de vectores; información de base acompañada de monitorización; y la participación y concienciación de los agentes implicados. El objetivo de esta tesis es contribuir a estos tres pilares para una gestión preventiva de las invasiones marinas asociadas a la navegación de recreo. Como modelo de estudio se han usado los crustáceos peracáridos, un componente principal de la epifauna móvil del *fouling*; y la mayor parte de la tesis tiene lugar en la Península Ibérica y el Mar Mediterráneo, puntos calientes para el turismo náutico y las invasiones marinas a escala global donde falta implementación.

En primer lugar, se aporta evidencia de que la navegación de recreo está actuando como un vector de alto riesgo en el Mar Mediterráneo, y que los hábitos de los propietarios influyen en la probabilidad de transportar invasores potenciales en el casco de la embarcación. Esto justifica la integración de medidas de gestión para el control de las invasiones marinas dentro del marco de regulación de la navegación de recreo. Por otra parte, simulaciones en laboratorio probaron empíricamente que los basibiontes sésiles del *fouling* actúan como refugio para la epifauna móvil invasora durante el transporte en barco. También se observó que la especie modelo invasora mostró mayor tendencia exploratoria que la nativa; lo cual implica un riesgo durante el transporte, pero una ventaja en otras fases del proceso de invasión. Esto apoya la idea de que un nivel de *biofouling* mayor que la capa de verdina (*microfouling*) supone un riesgo para la bioseguridad; y que la limpieza de este

macrofouling – especialmente de las áreas nicho – antes del inicio del viaje en barco sería una buena práctica de mantenimiento.

En segundo lugar, se llevaron a cabo varios esfuerzos de muestreo en puertos deportivos para levantar información sobre casos de *hidden-invaders* (taxones de pequeño tamaño y alta complejidad taxonómica que suelen pasar desapercibidos); obteniéndose un aumento sustancial de los rangos de introducción conocidos hasta ahora. De esta última tarea se obtuvieron varias lecciones para minimizar los tiempos de detección de los *hidden-invaders*: 1) la base taxonómica es primordial y se debe promover; 2) la voluntad de grupos expertos en comunicarse e intercambiar nuevas citas para alertar de introducciones es fundamental; y 3) se necesita una metodología estandarizada de monitoreo para la epifauna móvil de puertos comerciales y deportivos. Junto a ello, el uso de herramientas moleculares demostró ser útil para el monitoreo temporal de la fase de post-establecimiento del proceso de invasión, identificando poblaciones fuentes que suponen un riesgo de aporte de propágulos; y detectando ventanas de vulnerabilidad (períodos de baja diversidad, tamaño poblacional o conectividad) de las poblaciones introducidas. Los resultados señalan una vez más la urgencia de controlar el vector, ya que reducir la presión de propágulos es determinante para debilitar a estas poblaciones naturalmente fluctuantes de puertos deportivos.

En tercer lugar, la percepción social de los agentes implicados en España se investigó y comparó, por primera vez, con la de los dos únicos países que cuentan con regulaciones a nivel nacional (Australia y Nueva Zelanda). Los resultados preliminares indican que en nuestra región existe un exceso de confianza en comparación con los otros dos países; y que la percepción de riesgo es significativamente más baja, estando asociada a reconocer o no la responsabilidad de buenas prácticas de mantenimiento. El conocimiento de base, el sentimiento de conexión con el medio marino, y la percepción de riesgo fueron los principales factores determinantes de las opiniones sobre regulación. Existe un apoyo potencial a las recomendaciones de la Organización Marítima Internacional para el control del *biofouling* en embarcaciones de recreo, pero los dueños de los barcos respondieron de forma ambigua cuando se les preguntó sobre sus prácticas de limpieza. Dado que todo esto justifica la implementación de estrategias de educación ambiental al respecto, se proporcionan algunas recomendaciones preliminares para orientar mejor dichas campañas. Finalmente, en base a la investigación desarrollada en esta tesis, se identifican algunas direcciones futuras basadas en las siguientes ideas: 1) mapeo del riesgo de invasión en el territorio español y monitorización a largo plazo en áreas/polígonos de interés; 2) viabilidad de medidas potenciales de gestión, desde una perspectiva tanto ecológica (e.g. evaluación de riesgo ambiental de nuevas tecnologías para la limpieza de barcos dentro del agua) como social (e.g. investigación de la acogida potencial de ciertas medidas por parte de los agentes implicados, así como del nivel de cumplimiento/curva de aprendizaje *a posteriori*); y 3) aumento de la concienciación pública mediante campañas de divulgación y puesta a punto de iniciativas de ciencia ciudadana.

1

INTRODUCCIÓN GENERAL Y OBJETIVOS

1.1 Las invasiones marinas: contexto histórico, particularidades y vías de introducción

El ser humano ha facilitado el transporte de especies marinas a través de la navegación durante miles de años, ya sea intencionadamente o por accidente. Los viajes transoceánicos despuntaron en 1500s a raíz de las grandes expediciones europeas (Wolff 2005; Hattendorf 2007) - aunque hay evidencia de especies que habrian sido introducidas desde 1200s (Tabla 1 en Ojaveer et al., 2018) - ; y desde entonces las embarcaciones han continuado introduciendo especies en lugares del mundo que nunca hubieran alcanzado mediante corrientes marinas, venciendo así las barreras biogeográficas naturales (Galil et al., 2011). Definimos especie introducida como "aquella introducida fuera de su rango de distribución natural (pasado o presente) y fuera de su potencial de dispersión natural a causa intencional o accidental de la actividad humana" (Olenin et al., 2010). Y como especie invasora aquella que puede generar "efectos adversos en la biodiversidad, sus servicios ecosistémicos asociados y la salud y seguridad humana; así como un impacto socio-económico" (Comisión Europea, 2014, art.6 http://www.invasep.eu/ias-brochure210x210-es-web.pdf, accedido el 23/09/21). Esta forma de contaminación biológica altera profundamente las comunidades nativas, su composición genética y el funcionamiento del ecosistema (Bax et al., 2003; Bellard et al. 2016); siendo considerada por la Unión Internacional para la Conservación de la Naturaleza (UICN) como una de las principales amenazas a la biodiversidad (IPBES 2019; IUCN, 2017 https://www.iucn.org/content/marine-menace-alien-invasive-species-marine-environment-0, accedido 18/11/21) y afectando a la mayoría de grupos taxonómicos (Coll et al., 2010). A su vez, impacta en múltiples segmentos de la sociedad, acarreando daños en los servicios ecosistémicos, la salud pública y los recursos culturales e históricos; así como grandes pérdidas económicas (Lewis and Maslin, 2015; Pyšek et al., 2020; Simberloff et al., 2013; Crystal-Ornelas and Lockwood 2020; Warziniack et al. 2021). El Convenio sobre la Diversidad Biológica (*CBD*) destacó la importancia de la diversidad marina en la reunión de Jakarta en 1995 (Mandato de Jakarta, UNEP, 1995); y ya en su Plan Estratégico para 2011-2020 establecía que "para 2020, las vías de propagación [de especies invasoras] fueran identificadas y priorizadas, las especies invasoras prioritarias controladas y erradicadas, y las medidas de prevención puestas en marcha para prevenir futuras incursiones y establecimientos" (United Nations Environment Programe - UNEP, 2011). En los últimos 50 años, la incidencia de invasiones biológicas acuáticas a escala global no ha hecho más que aumentar, calculándose una tasa de detección de una especie introducida nueva cada 8.4 días de media; y se espera que continúe esta tendencia (Seebens et al., 2017; Bailey 2020; Figura 1a). El último análisis a nivel paneuropeo indica un total de 1555 especies exóticas marinas identificadas entre 1949 y 2019, de las que dos tercios han establecido poblaciones viables; y se calcula que más del 50% de las áreas costeras europeas están actualmente afectadas por aquellas con carácter invasor (EEA, 2019, https://www.eionet.europa.eu/etcs/etc-icm/products/etc-icm-report-4-2019-multiple-pressures-and-their-combined-effects-in-europes-seas, accedido el 10/11/21; Figura 1b para el período 1949-2017). Vilá et al. (2010) estimó una pérdida económica para la Unión Europea de más de 12.5 billones de euros al año y Meyerson et al. (2019) de 100 billones de dólares al año para Estados Unidos. Concretamente, los costes acarreados por las especies introducidas acuáticas se han ido incrementando exponencialmente a lo largo del tiempo, llegando a un total de 23 billones de dólares en 2020; más de lo que cuesta gestionar todas las áreas marinas protegidas el mundo (Cuthbert et al. 2021).

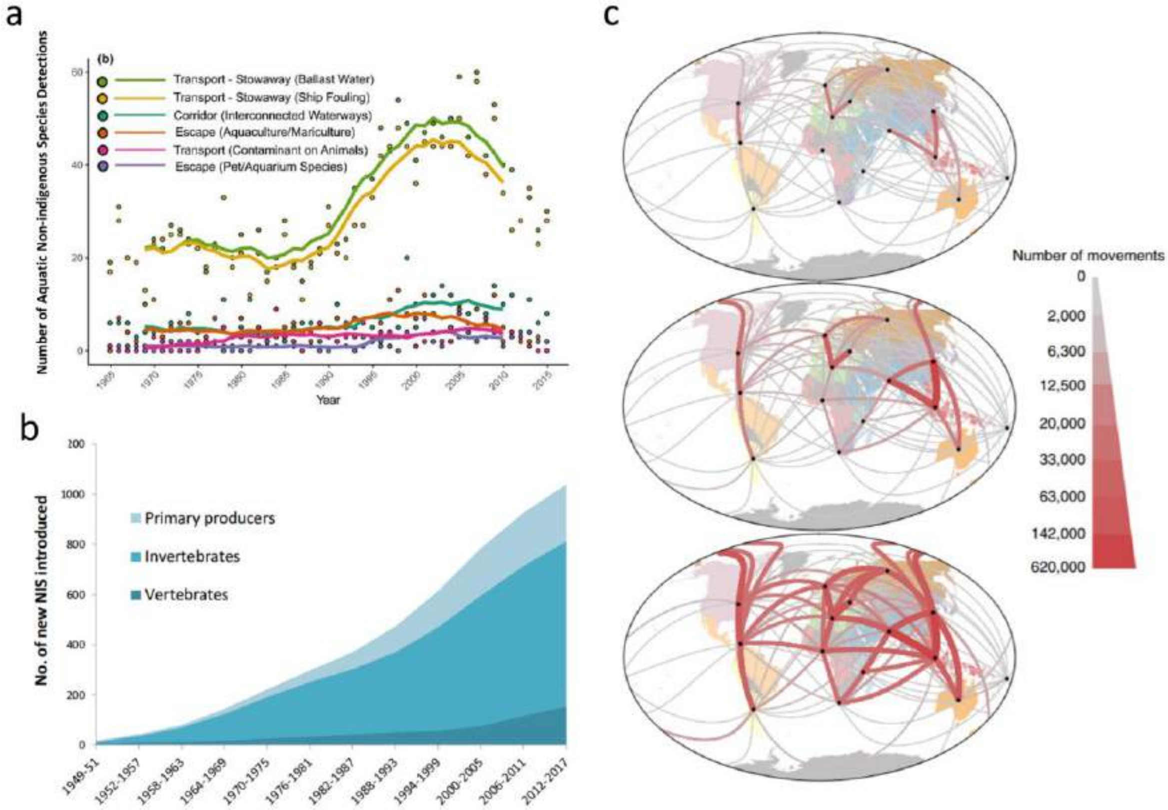

Figura 1. a) Número de primeras detecciones de especies exóticas en los últimos 50 años, con vectores indicados por colores. Extraído de Bailey et al. (2020). b) Patrón de introducciones en las costas europeas entre 1949 y 2017. Extraído de EEA, 2019. c) Tráfico marítimo internacional en 2014 (arriba) y predicciones para el 2050 (centro y abajo), teniendo en cuenta dos de los cinco escenarios de desarrollo socioeconómico (*Shared Socioeconomic Pathways - SPPs*) diseñados en el marco del Quinto Informe de Evaluación del Grupo Intergubernamental de Expertos sobre el Cambio Climático (IPCC). Estos escenarios van del 1 al 5 en una escala de desarrollo sostenible (1) a insostenible (5). La figura central ilustra la predicción de tráfico marítimo para 2050 asumiendo el mínimo crecimiento del número de movimientos de barco y mínimo margen de error del modelo (equivale a un escenario SSP3); y la de abajo lo hace asumiendo máximo crecimiento y margen de error (equivale a un escenario SSP5). Extraído de Sardain et al. (2019).

El medio marino sigue siendo un desconocido en cuanto a su diversidad en comparación con el terrestre y el dulceacuícola. Además, la gestión de invasiones biológicas se vuelve especialmente difícil debido a la cantidad de vías de propagación emergentes y las dificultades de acceso y muestreo (Townsend et al. 2018; Micheli et al., 2020). Hace más de una década se cuantificó que el 84% de las ecoregiones marinas ya albergaban especies acuáticas invasoras responsables de impacto ecológico nivel 3 ó 4 (*i.e.* afectan a múltiples especies, especies de alto valor – amenazadas o clave-, a algunas funciones del ecosistema o a todo el ecosistema) (Molnar et al. 2008). Sin embargo, se sospecha que la magnitud conocida hasta ahora es sólo la punta del iceberg (Byers et al. 2015). La era de la globalización, el crecimiento poblacional a nivel mundial, la demanda de mercado, residencia y turismo, así como el hacer frente a cambios globales (*e.g.* aumento del nivel del mar, fenómenos meteorológicos extremos) conllevan a) una mayor conectividad a nivel mundial, y con ello diversificación de redes de intercambio y movilidad de bienes y servicios (Hulme et al., 2009; Figura 1c); y b) una incesante introducción de infraestructuras en el medio costero (Bulleri and Chapman, 2010; Dafforn et al. 2015). Como resultado, el número de vectores, rutas y vías de propagación disponibles para las especies exóticas marinas también se han multiplicado (Banks et al. 2015).

El tráfico marítimo se erige como la principal vía de propagación, responsable del 70-80% de las introducciones a nivel mundial (Bailey et al. 2020; Figura 1a) , transportando especies accidentalmente en el agua de lastre (Carlton 1985; IMO 2020 https://www.imo.org/en/OurWork/Environment/Pages/BallastWaterManagement.aspx, accedido 05/11/2021) o como parte del *biofouling*, es decir, el crecimiento de organismos no deseados en la superficie sumergida de múltiples estructuras antrópicas (Catta 1876; Carlton 1994; IMO 2020 https://www.imo.org/en/OurWork/Environment/Pages/Biofouling.aspx, accedido 05/11/21). La continua creación y alteración de dichas estructuras fijas o móviles como los pantalanes, diques, cascos de embarcaciones (desde buques hasta plataformas petroleras, barcazas, y barcos recreativos), cuerdas, pilotes y boyas que encontramos en puertos comerciales y deportivos, ofrecen nuevos hábitats susceptibles de ser colonizados por especies oportunistas y exóticas - generalmente más eficientes que las nativas en este tipo de sustrato (Airoldi et al. 2015, López-Legentil et al., 2015, Megina et al. 2016; Ros et al., 2021)-; proporcionándoles así numerosos corredores y *stepping-stones* que facilitan el proceso de invasión (Glasby et al. 2007; Darbyson et al. 2009; Dafforn et al. 2009; Mineur et al. 2012). Tras el tráfico marítimo y a la par de la vía de tipo corredor (mares/cuencas/vías navegables interconectadas), la industria acuícola es la siguiente vía de propagación implicada en la introducción de especies exóticas marinas por la acción humana (Figure 3 Molnar et al., 2008; Table S3 in Bailey et al. 2020). Esta puede ser por escape de la zona de confinamiento para cultivo intencionado, por llegar accidentalmente asociadas a especies de interés comercial importadas (*e.g*. algas e invertebrados asociadas a cultivos de ostra y almeja japonesa), o facilitando la propagación mediante jaulas en mar abierto que actúan como reservorios o *stepping-stones* para el *fouling* (Minchin, 2007; Carlton, 2011, Hewitt et al. 2006; Marchini et al., 2014; Fernández-González y Sánchez-Jerez 2017; Grosholz et al., 2012, 2015). Destacan también otras vías como el mercado de acuariofilia, mediante escape o liberación intencionada (Semmens et al., 2004; Vranken et al., 2018); y otros vectores como los cebos de pesca (Haska et al., 2011; Fowler et al., 2016; Passarelli and Pernet 2019) y la basura marina (Rech et al. 2016; Carlton et al. 2017; Campbell et al. 2017); entre otros. Así mismo, en muchas ocasiones estos vectores pueden actúan conjuntamente (*polyvectic transfer*) (Carlton and Ruiz, 2005; Ruiz et al., 2011). Este escenario cobra especial relevancia en un contexto de cambio global, donde se espera que el aumento de las temperaturas altere los rangos de distribución de las especies, reduciendo aún más las barreras para la dispersión (Lord, 2017; Mahanes et al., 2019); y donde múltiples amenazas medioambientales convergen, amplificando y haciendo mucho más compleja esta problemática (Coté and Green 2012; Duarte, 2014; Chakraborty 2019; Robinson et al. 2020; Vilizzi et al., 2021). Poner en marcha medidas de gestión eficaces contra las invasiones marinas se torna hoy más urgente que nunca (Meyerson, 2019; ICES, 2019).

1.2 La prevención y detección temprana como enfoques óptimos de gestión

Una vez que una especie invasora se establece en un nuevo ambiente, sus efectos son en la mayoría de los casos irreversibles (Streftaris et al., 2005). Del mismo modo, a medida que pasa el tiempo, su control se vuelve más difícil y costoso económicamente (Simberloff 2013, figure 1); sólo superado por el coste de los propios daños al ecosistema y recursos (Cuthbert et al., 2021). Por ello, los expertos coinciden en que la gestión de las invasiones debe estar basada en el principio de precaución; y abogan por la prevención y detección temprana como única estrategia viable y efectiva a medio-largo plazo (Keller et al. 2008; Roy et al., 2014; Finnoff et al., 2007; Hewitt and Campbell 2007; Lodge et al., 2016; Bergstrom et al., 2018). El mismo principio recoge la Unión para la Conservación de la naturaleza (IUCN 2009, https://www.iucn.org/sites/dev/files/import/downloads/marine_menace__en_1.pdf, accedido 08/11/21) y la legislación de la Unión Europea (*IAS Regulation*, Reglamento (UE) No 1143/2014; https://www.boe.es/doue/2014/317/L00035-00055.pdf, accedido el 08/11/21). Así mismo, en el Plan De Acción contra las especies invasoras de 2021 en España se hace patente la prevención "como única estrategia efectiva (…) en el medio fluvial y marino. (…) Cuando se detecta una especie, ya está asentada y los muestreos no son suficientes" (Medida 5.1 del eje coordinación y governancia; https://www.miteco.gob.es/gl/biodiversidad/temas/conservacion-de-especies/planviasdeentradadeeeilimpio12julio2021_tcm37-529319.pdf, accedido 20/11/21). La gestión en respuesta a una introducción mediante la erradicación no solo es más costosa que la planificación preventiva sino que además, en el medio marino, tiene una tasa de éxito escasa y supeditada a ciertas condiciones: que sea una invasión reciente, restringida a un área pequeña y, frecuentemente, donde exista una buena colaboración y comunicación entre instituciones, agentes implicados (*stakeholders)* y comunidad científica (ver casos revisados en Simberloff, 2021). Funcionen o no, estas estrategias son siempre limitadas, difíciles de llevar a cabo y costosas. Un ejemplo es el tunicado colonial *Didemnum vexillum* Kott, 2002, una de las especies más agresivas y de rápida colonización; introducida mediante *biofouling* y el comercio de marisco en Europa, Norteamérica y Nueva Zelanda (McKensie et al., 2017). En Nueva Zelanda, la estrategia de erradicación fallida para esta especie acarreó un coste de 650.000 dólares (Branson, 2012). En otros casos, las estrategias se mantienen durante un tiempo limitado pero acaban retirándose por considerarse insostenibles, momento en el cual ocurre una rápida reinfestación (Coutts and Forrest 2007, Forrest and Hopkins 2013), ya que los vectores siguen operando y propagando la especie de puerto en puerto (Hambrey Consulting, UK, 2011).

Gestionar las invasiones biológicas desde el punto de vista de la prevención supone un reto. De hecho, la experiencia demuestra que en general no somos capaces de responder a tiempo a las incursiones (Zenetos et al., 2019). Por una parte, entre la primera incursión y la primera recolección (detección) suelen pasar años o incluso siglos (Bailey et al. 2020). Por otra, la identificación taxonómica, reconocimiento y comunicación de la incursión conlleva más retrasos (Azzurro et al., 2016; Stanislawczyk et al., 2018). La mayoría de especies distribuidas entre los 400-500 primeros años de navegación a nivel internacional directamente han pasado desapercibidas; lo cual hace que la magnitud de las invasiones marinas siempre este infravalorada (Carlton, 2011). Byers et al., (2015) determinó también que el tiempo desde la primera introducción predice, de forma significativa, el rango de distribución de los invasores marinos; por lo que se considera que muchos aún están en expansión. Lo frecuente es detectar la incursión a raíz de la expansión poblacional o un periodo de crecimiento explosivo concreto (Harvey et al., 2009), como en el caso de la macroalga *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim, nativa del Pacífico Noroeste (Hwang, 1994). En el Mar Mediterráneo se detectó por primera vez en Francia en 2002 en la cuenca de Thau, posiblemente asociada al comercio de ostra japonesa para acuicultura, aunque por entonces no se registró carácter invasor (Verlaque et al. 2009). No se supo más hasta que en 2015 se detectó en fondos rocosos someros (ambiente natural) a ambos lados de Estrecho de Gibraltar; registrándose en tan sólo un año un crecimiento explosivo que provocó arribazones de hasta 5000 toneladas en las playas de Ceuta (Ocaña et al., 2016; Altamirano-Jeschke et al., 2016). Considerando el potencial de la especie y el impacto ya causado al fondo marino, la perspectiva de una gestión reactiva (*e.g.* erradicación, contención) en las zonas ya invadidas es inviable (Navarro-Barranco et al., 2019; Muñoz et al., 2019; García-Gómez et al. 2021; Sempere-Valverde et al., 2021).

Para lograr desarrollar una gestión desde la prevención hay varios pilares a considerar: una regulación que obligue al control de vectores para limitar las introducciones; una buena información de base acompañada de monitorización; y la participación y concienciación de los agentes implicados; entre otros (Figura 2, ilustrando los tres aspectos mencionados). A continuación, se ponen en contexto dichos pilares para el tema de las invasiones marinas y la navegación de recreo como vía de propagación.

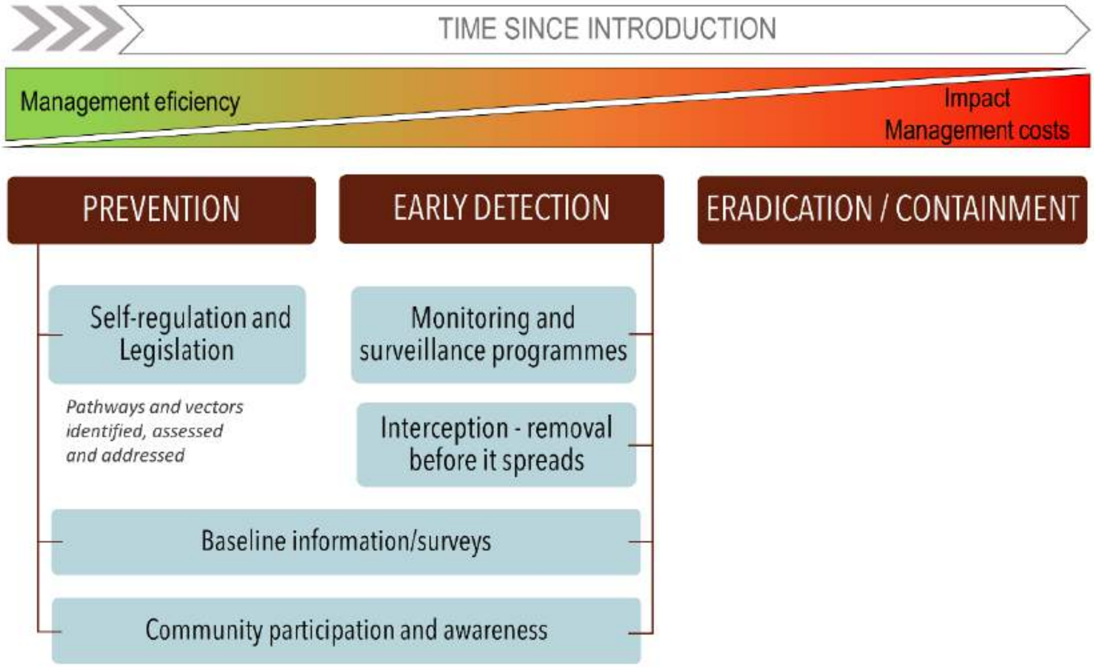

Figura 2. Estrategias de gestión contra las especies invasoras (marrón). La estrategia óptima cambia con el tiempo que pasa desde que se produce la introducción. En todo caso, a medida que pasa tiempo, la efectividad de la gestión decrece y los costes aumentan. Se destacan algunas de las bases fundamentales de la prevención y la detección temprana (azul claro). Gráfico basado en Simberloff et al. (2013) y IUCN (2009).

1.2.1 Evaluación de vías de propagación y necesidad de un apoyo legislativo

La gestión de las invasiones marinas pasa obligatoriamente por reducir la presión de propágulos; es decir, identificar, evaluar y regular los vectores y vías de introducción (Essl et al., 2015; Ojaveer et al., 2018). La inversión en medidas de bioseguridad ha demostrado ser efectiva para proteger la salud ambiental y humana, y es un eje fundamental del ecoturismo y la nueva bioeconomía (Sheppard et al., 2011; Oidtmann et al., 2011; box 1 Simberloff et al. 2013; Hall and Baird, 2013; NSMC, https://www.thensmc.com/sites/default/files/Check,%20Clean,%20Dry%20FULL%20case%20study.pdf, accedido 09/11/21). A este respecto, aunque el papel del tráfico marítimo en la propagación de especies marinas se conoce hace décadas (Carlton 1985), la puesta a punto de directivas para los diferentes vectores está siendo muy dispar. Tan sólo recientemente ha entrado en vigor a nivel internacional el Convenio sobre el agua de lastre (https://www.imo.org/en/MediaCentre/HotTopics/Pages/Implementing-the-BWM-Convention.aspx, accedido 27/10/2021), la cual establece que todos los barcos lleven instalado sistemas de tratamiento para el agua de lastre para el 2024 (*Ballast water management systems - BWMS*), y hasta entonces establece que el intercambio de agua se haga alejado de la costa. Por el contrario, la regulación del *biofouling* a nivel internacional se sigue retrasando, a pesar de considerarse la vía de propagación responsable de entre el 55.5 y el 69.2% de las especies exóticas actualmente establecidas en costas y estuarios a nivel mundial (Hewitt and Campbell 2010). No sólo supone un peligro desde el punto de vista de la bioseguridad, sino que acarrea graves costes de mantenimiento para la industria naval, recreativa y acuícola. Se calcula que la capa de *biofouling* puede acarrear un aumento de la fricción de hasta el 70%, necesitando aumento de potentica de hasta el 38% para mantener la velocidad en cargueros (Demirel et al., 2017). Esto no sólo conlleva un gasto extra de combustible, sino que aumenta las emisiones de gases de efecto invernadero (Eyring et al. 2010; Olmer et al., 2017). Junto a ello, hay que considerar los costes de mantenimiento de múltiples infraestructuras marinas a causa de las comunidades incrustantes que tapizan pantalanes, diques y pilotes, acelerando los procesos de corrosión y disminuyendo la eficacia operativa; así como en la industria acuícola (*e.g.* disminuyendo el diámetro de malla de redes y dificultando el intercambio de oxígeno en las jaulas y la limpieza de residuos, entre otros) (Fitridge et al., 2012; Flectcher et al., 2013).

Durante años, el papel de los barcos recreativos como vectores pasó desapercibido o fue infravalorado en comparación con el de buques comerciales (Clarke Murray et al., 2011). Hoy en día se sabe que la navegación de recreo actúa como vía de alto riesgo de propagación de especies exóticas por varias razones: 1) las embarcaciones permanecen en los puertos mucho más tiempo que las comerciales, lo cual ofrece mayor riesgo de exposición a propágulos (infección) y a su vez facilita que estos puedan ser liberados al medio receptor (Floerl y Inglis 2005; Sylvester et al, 2011); 2) suelen llevar velocidades de crucero menores, lo cual facilita que las especies exóticas que viajan como polizones en el *biofouling* puedan soportar el viaje, sobre todo refugiadas en áreas nicho de difícil acceso que se escapan a la limpieza del casco (Inglis et al., 2010; Frey et al., 2014); 2) aportan mayor superficie (horizontal, vertical, submergida y parcialmente sumergida) potencialmente colonizable que los puertos comerciales (Minchin et al. 2006); y 3) las rutas que recorren conectan lugares de alto con otras de bajo riesgo de biocontaminación, exponiendo zonas como calas y bahías de Áreas Marinas Protegidas, inaccesibles para los grandes buques (Floerl 2002; Davidson et al., 2010; Ashton et al., 2011; Zabin et al., 2014). Cada vez más estudios científicos aportan evidencias del papel de la navegación de recreo como vector de propagación en distintas regiones del mundo (Ashton et al., 2014; Simard et al., 2017; Ferrario et al., 2017; Ulman et al., 2019; Pelletier-Rousseau et al., 2019; Parretti et al., 2020). La evidencia demuestra que es el principal responsable del proceso de introducción secundaria y expansión de las poblaciones exóticas; un proceso fundamental en la dinámica de invasión. Algunos ejemplos son la propagación del alga japonesa *Sargassum muticum* (Yendo) Fensholt por las cosas francesas hacia otros países europeos (Gollasch et al., 2009); el 50% de las introducciones en el Mar Báltico provenientes del Mar del Norte (Ojaveer et al., 2017); o numerosos casos de especies que fueron inicialmente introducidas en el Mar Mediterráneo, como el alga verde *Caulerpa taxifolia* (M.Vahl) C. Agardh 1817 y *Caulerpa taxifolia* var. distichopylla (Sonder) Verlaque, Huisman & Procaccini 2013; los briozoos *Watersipora arcuata* Banta, 1969, *Amathia verticillata* (Delle Chiaje, 1822) y *Tricellaria inopinata* d'Hondt and Occhipinti Ambrogi, 1985; el anfípodo *Caprella scaura* Templeton, 1836; y la ascidia *Styela plicata* (Lesueur, 1823) (revisado en Ferrario et al., 2017).

Las jurisdicciones que han logrado implantar una regulación son regiones o países aislados. En 2014 Nueva Zelanda se convirtió en el primer país en introducir una directiva para *biofouling* que, tras esfuerzos de divulgación implementación y mejora, fue actualizada en 2018, dando lugar a su actual marco de regulación (https://www.mpi.govt.nz/import/border-clearance/ships-and-boats-border-clearance/biofouling/biofouling-management/ , accedido en 11/08/2021). Éste exige a las embarcaciones visitantes tomar medidas preventivas para tener el casco del barco limpio antes de su llegada al país (Georgiades et al., 2018). Junto a ella, varias directivas regionales han acompañado atajando los vectores de transporte secundario (doméstico) de especies exóticas (*e.g.* embarcaciones de recreo como yates y lanchas, pesca comercial y crucero) (Cunningham et al., 2019; Northland Regional Council, 2019 https://www.nrc.govt.nz/media/echj0m4t/2019-2020-operational-report-northland-regional-pest-marine-pathways-plan.pdf, accedido 11/11/21). Australia instauró su normativa de bioseguridad a nivel nacional en 2015, dirigido también al vector *biofouling*, y que sería actualizada más adelante (MarinePestPlan 2018-2023, https://www.marinepests.gov.au/what-we-do/publications/marine-pest-plan , accedido en 27/10/2021). Actualmente ha desarrollado una normativa específica para tratar de acomodar la práctica de limpiar el barco dentro del agua y que actualmente se encuentra abierta para consulta (https://haveyoursay.awe.gov.au/in-water-cleaning , accedido en 27/10/2021). En Estados Unidos, en 2017, el estado de California también implantó una regulación tanto para el agua de lastre como para el *biofouling*, aplicable a embarcaciones visitantes de 300 toneladas o más, para minimizar la propagación de especies exóticas marinas (California State Lands Commision; https://www.slc.ca.gov/wp-content/uploads/2018/10/4_8_GuidanceDoc.pdf , accedido en 27/10/2021); actualmente en re-evaluación (Scianni et al., 2021). El estado de Washington, por su parte, decretó un plan estratégico de 6 años para desarrollar una gestión del *biofouling* (McClary et al., 2017). Por último, Canadá ha dado otro paso adelante, llevando a cabo una evaluación de riesgo a nivel nacional para determinar el papel de la navegación de recreo como vector para especies marinas exóticas (Simard et al., 2017). Por su parte, la Organización Marítima Internacional ha lanzado en 2018 la iniciativa GloFouling (https://www.glofouling.imo.org/, accedido 27/10/2021), análoga a su predecesora, a fin de implementar finalmente las directrices propuestas (IMO 2019, https://www.gob.mx/cms/uploads/attachment/file/534561/PPR_7-INF.3_-_Submissions_relating_to_the_review_of_the_2011_Biofouling_Guidelines__Australia__Finland__Japan...__.pdf, accedido el 3/12/21) para el control del *biofouling* como vector de especies exóticas a nivel internacional. Por el momento, sin embargo, sólo ofrece una serie de recomendaciones voluntarias para las embarcaciones de recreo (IMO 2012, MEPC.1/Circ. 792; https://www.transportstyrelsen.se/globalassets/global/sjofart/dokument/miljoskydd/mepc_circ.792-vagledning-bioufouling-fritidsbatar.pdf, accedido en 11/11/2021).

En 2016 la Unión Europea adopta una lista de especies exóticas invasoras (EEI) preocupantes para la Unión (Reglamento 1141/2016), que será actualizada posteriormente (Reglamentos de Ejecución 2017/1263 y 2019/126) y que determina las vías de introducción/dispersión, reconociendo tanto el agua de lastre de buques/barcos como las incrustaciones en cascos de buques/barcos como vías de tipo transporte-polizón (Reglamento de Ejecución 2017/1454). La última actualización recoge 66 especies, de las cuales 54 son terrestres, 18 dulceacuícolas y tan sólo 3 presentes en ambientes marinos o salobres; a saber: *Cabomba caroliniana* A. Gray 1837, *Eriocheir sinensis* H. Milne Edwards, 1853, y *Platosus lineatus* Thunberg, 1787. Este es el actual marco normativo para la UE en esta materia, y exige a los estados miembros un análisis exhaustivo de vías de introducción y propagación de dichas especies, identificando las que requieran acción prioritaria y estableciendo y aplicando un único plan de acción para ellas. A fin de implementar esta demanda, España elabora el Plan de acción sobre las vías de introducción y propagación de las Especies Exóticas Invasoras, aprobado el 1 de julio de este año (https://www.miteco.gob.es/gl/biodiversidad/temas/conservacion-de-especies/planviasdeentradadeeeilimpio12julio2021tcm37-529319.pdf, accedido en 19/10/2021) que se hace teniendo en cuenta 49 de las EEI preocupantes para la UE. Y en la misma línea de prevención, su nuevo Plan Nacional de Adaptación al Cambio Climático (2021-2030) contempla específicamente una línea de actuación para las EEI. En dicho plan se identifican 10 vías prioritarias del total reconocido por la UE, pero la vía "incrustación en cascos de buques y barcos" queda fuera; a pesar de haberse establecido como responsable de más introducciones que, por ejemplo, las vías 11 (agua de lastre) y 12 (polizones en barco excluyendo agua de lastre e incrustaciones), ambas incluidas (ver Figura 2 https://www.miteco.gob.es/gl/biodiversidad/temas/conservacion-de-especies/planviasdeentradadeeeilimpio12julio2021tcm37-529319.pdf). Por tanto, a día de hoy, sólo contamos en España y la UE con una guía de directrices y recomendaciones voluntarias por parte de la OMI para el vector *biofouling* (tanto para embarcaciones comerciales como de recreo). Estas medidas no implementadas son del todo insuficientes para el limitar la presión de propágulos asociados a dicho vector (ICES, 2019).

1.2.2 Muestreos para levantar información y monitorización

La mayor razón detrás del fracaso en el control de invasiones marinas es la incapacidad de actuar a tiempo antes de que se propaguen. Esto suele ser debido a un fallo en la detección temprana, un fallo en la identificación como especie exótica y/o una ausencia de bibliografía adecuada suficiente que evidencie las posibles consecuencias de llegar a establecerse la población (Locke y Hanson, 2009). Dicha incapacidad de detección temprana y dichos *gaps* de conocimiento están directamente asociados a la falta o irregularidad de esfuerzo de muestreo y vigilancia necesarios (Bailey et al. 2020). Los muestreos de base son imprescindibles para mapear los puntos calientes (*hotspots*) o de riesgo de introducciones (Lyons et al., 2020; Polaina et al., 2020; Blanco et al., 2021); y una vez establecidos, la monitorización es clave para conseguir la detección temprana (Lehtiniemi et al., 2015; Woods 2018; Verlaque and Breton 2019). De hecho, Latombe et al. (2017) explica que los registros de presencia de especies exóticas repetidos y prolongados en el tiempo son la base para desarrollar un buen sistema de observación y monitoreo a nivel nacional, que a la vez nutra la gestión a nivel global (ver Figura 3).

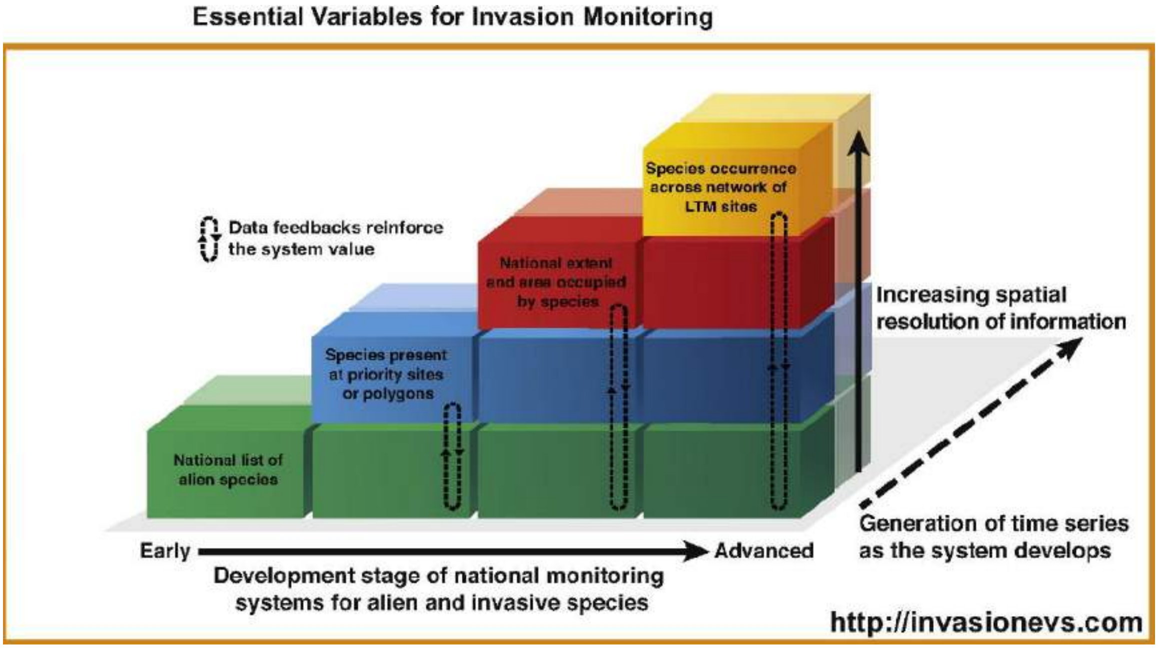

Figura 3. Variables esenciales para el seguimiento de las invasiones y enfoque modular para el Desarrollo de un Sistema nacional de observación y monitorización, construido a partir de la variable “presencia de especies exóticas”, la cual es una de las tres variables esenciales para el monitoreo de la invasión y también una Variable Esencial de la Biodiversidad (*Essential Biodiversity Variables – EBV*) según Pereira et al. (2013). LTM= Long term monitoring. Gráfico extraído de GEO BON (2015) y Latombe et al. (2017).

En El Plan de acción contra las especies invasoras en España se indica específicamente como acción a desarrollar: “conocer la ubicación precisa de las localidades donde son detectadas por primera vez las EEI” (Medida 4.2 del eje de acción investigación y conocimiento); así como mejorar la “red de alerta temprana” (Medida 6 en el eje de vigilancia y control). Para este fin, los puertos deportivos nos ofrecen enclaves estupendos donde levantar información y situar estaciones centinelas de prevención. Por una parte, son puntos calientes de introducciones y actúan como fuentes y reservorios de especies exóticas marinas (Glasby et al., 2007, Ferrario et al., 2017). Por otra, al ser ambientes muy accesibles para el ser humano, permiten labores de muestreo con una relación coste-beneficio muy asumible. Además, los protocolos de monitoreo pueden extraerse a múltiples escalas. De hecho, varios grupos de trabajo colaboran actualmente para poner a punto protocolos estandarizados para el monitoreo de las comunidades del *fouling* en puertos comerciales y deportivos; tanto para la fracción sésil (Tamburini et al., 2021), como para móvil (Ros et al., 2019).

Los muestreos y seguimientos frecuentes de las comunidades del *fouling*, ya sea como parte de investigaciones paralelas (recolecciones "*by catch*", Ruiz et al., 2000) o monitoreos específicos implementados, pueden contribuir en gran medida a mitigar estos retrasos en la detección y el reconocimiento de los invasores (Hayes et al., 2019). Gracias a ellos es posible mitigar problemas como bases de datos de distribución incompletas; incertidumbre taxonómica (*i.e*. especies mal identificadas, no identificadas, complejos de especies, especies crípticas etc.); o falta de estandarización de las bases de datos (Gatto et al., 2013; Ojaveer et al., 2014; Marchini and Cardeccia, 2017; Galil et al., 2018; Carlton and Fowler, 2018; Zenetos et al., 2019). Esto cobra especial relevancia en el caso de los llamados "*hidden-invaders*", incluyendo taxones unicelulares como foraminíferos bentónicos (Guastella et al., 2019) o invertebrados de pequeño tamaño, complejidad taxonómica, o con comportamiento críptico (Xavier et al., 2009; Carlton, 2011; Marchini et al., 2016). Por último, incrementar estos esfuerzos es imprescindible para contribuir a configurar bases de datos de especies introducidas a largo plazo que permitan investigar patrones a nivel global, algo que escasea en el medio acuático (Bailey et al., 2020).

1.2.3 La importancia de la dimensión social

Integrar las ciencias Sociales y Humanidades es ahora uno de los temas transversales imprescindibles del Programa Marco de Investigación e Innovación Horizonte Europea 2021-2024; sobre todo en materia de transición digital y ecológica (https://op.europa.eu/en/web/eu-law-and-publications/publication-detail/-/publication/3c6ffd74-8ac3-11eb-b85c-01aa75ed71a1, accedido el 3/12/21). En invasiones marinas, la dimensión social es también de suma importancia. Al margen de los aspectos ecológicos, los hábitos de los dueños de las embarcaciones van a jugar un papel importante en el riesgo de propagar especies marinas a través de la navegación de recreo. A saber: las prácticas de mantenimiento como tipo de limpieza (dentro del agua o fuera, en el embarcadero), frecuencia de limpieza, frecuencia de renovación de pintura antifouling, velocidad de crucero, rutas de viaje y variedad de puertos visitados (Floerl et al., 2005; Floerl et al., 2009; Acosta et al. 2010; Jurk, 2011; Zabin et al., 2014; Ulman et al., 2019). Como tales, deben ser tenidos en cuenta a la hora de evaluar el riesgo de invasión, y priorizar medidas de intervención (Ferrario et al., 2016; Floerl et al., 2016). Muchas de estas variables dependen también de las normativas de cada puerto deportivo; por tanto, la disposición y el apoyo de las autoridades portuarias va a ser clave a la hora de definir un enfoque preventivo de la gestión.

Cualquier hábito o comportamiento medioambiental por parte de los agentes implicados, desde sus actitudes hasta su implicación pública y el nivel de cumplimiento de una regulación, viene en parte determinado por percepciones tales como su nivel de concienciación, percepción de riesgo, o sus valores sobre la naturaleza, entre otros (Bremmer and Park, 2007; Hourston et al., 2015; Kemp et al., 2017; Colet et al., 2019). Mapear estas percepciones se torna, por tanto, urgente de cara a implementar una regulación justa y efectiva para prevenir la propagación de especies mediante la navegación de recreo (GloFouling Project, 2011). Concretamente, incorporar la percepción social en el estudio de invasiones marinas nos permite:

A) Identificar la falta de concienciación, conocimiento o percepción de riesgo de la población implicada (Gelcich et al., 2014) y diseñar campañas de educación ambientales eficaces para solventarlos. El nivel de concienciación pública rige, como uno de los ejes fundamentales, los escenarios de futuro predichos para la gestión de invasiones marinas (Roura-Pascual et al., 2021). Como tal, es clave para lograr una gestión preventiva: por una parte, es un indicador útil para la evaluación del riesgo de incursión de especies exóticas (Cliff & Campbell, 2012); por otra, una alta concienciación ambiental facilita la detección temprana y una respuesta rápida coordinada si dicha incursión ocurre (Piola and McDonald, 2012).

B) Evaluar la viabilidad de determinadas medidas de gestión. Se sabe que los valores, ideologías y factores emocionales de los ciudadanos modelan sus actitudes hacia ciertas estrategias de gestión de las especies invasoras (Carballo-Cárdenas, 2015). Es por ello que conocer la intención (*intended behaviour*) de los grupos implicados es una herramienta útil para informar sobre la oposición o cumplimiento potencial (Diedrich et al., 2013; Drake et al., 2015; Cerri et al., 2016). Del mismo modo, evaluar su cumplimiento a posteriori (*self-reported behaviour*) permite detectar las causas de no cumplimiento así como los puntos a mejorar (Scianni et al., 2021, Shannon et al., 2019; Wright, 2021)

C) Aunar esfuerzos para las campañas de actuación a través de iniciativas de ciencia ciudadana. Dichas campañas han demostrado ser de utilidad para un enfoque preventivo de la gestión de invasiones marinas mediante la detección temprana y los esfuerzos de vigilancia (Azzurro et al., 2013; Bodilis et al., 2014; Mannino and Balistreri, 2018; Kleitou et al., 2019; Mannino et al., 2021). Además, aportan múltiples beneficios como mejorar la confianza y el flujo de comunicación entre los diferentes grupos interesados, ejercer la propia ciudadanía ambiental (*marine citizenship*: los derechos y responsabilidades de un individuo hacia el medio marino) y, en última instancia, fomentar el cambio de actitud y comportamiento hacia prácticas más responsables (Kelly et al., 2019; Locritani et al., 2019).

1.3 Los crustáceos peracáridos como modelo de estudio

El Superorden Peracarida es un grupo de crustáceos malacostráceos que comprenden actualmente 12.000 especies. Entre ellos, los órdenes Amphipoda, Isopoda y Tanaidacea engloban multitud de especies de pequeño tamaño que viven como epibiontes sobre una gran variedad de sustratos como briozoos, hidrozoos, ascidias, esponjas, balanos, macroalgas y fanerógamas marinas, entre otros (Conradi et al. 2000; Galil 2008; Galil et al., 2011; Guerra-García et al., 2011; Cabezas et al., 2013; Ros et al., 2014; Bamber, 2012; Tait et al. 2021). Cabe destacar que el grupo de los anfípodos es el más numeroso, con más de 9000 especies de las 12.000 mencionadas, a pesar de ser de los crustáceos menos descritos (Coleman, 2015, Hughes and Ahyong, 2016; Arfianti et al., 2018). Además, representan el grupo de crustáceos más diverso en cuanto a estilo de vida, hábitat y tamaño (De Broyer and Jazdzewski, 1996, Hacker and Stenech 1990; Scipione, 2013; Florido et al., 2019), jugando un papel clave como mediadores de cadenas tróficas (Jeong et al., 2012; Vazquez-Luiz et al., 2013); además de tener aplicaciones como bioindicadores de contaminación ambiental (Aono and Takeuchi, 2008;

Guerra-García et al., 2005), y alimento en acuicultura y acuariofilia (Woods, 2009; Baeza-Rojano et al., 2014; Guerra-García et al., 2016; Jiménez-Prada et al., 2021).

Los anfípodos poseen gran capacidad para colonizar sustratos artificiales de puertos (pantalanes, boyas, cuerdas etc.), cascos de barcos, o granjas de acuicultura; alcanzando hasta densidades de 319.000 individuos por m^2 (Frey et al., 2009; Ashton et al., 2010; Ros et al., 2013; Frey et al., 2014; Fernández-González y Sánchez-Jerez, 2017; Martínez-Laiz et al., 2018; Martínez-Laiz et al., 2019; Tait et al., 2021). Incluso son colonizadores frecuentes de objetos flotantes transoceánicos como la basura marina (Carlton et al., 2017; Rech et al., 2021). Constituyen, por tanto, el componente principal de la fracción móvil de las comunidades incrustantes o *fouling.* Como tal pueden ser dispersados largas distancias a causa de la acción humana asociados a numerosos vectores, y en general, viajando como polizones (*stowaways*) de barcos comerciales y de recreo (Gollasch et al. 2002; Ros et al. 2013; Ashton et al. 2014; Marchini and Cardeccia 2017). Algunas familias, como Corophiidae o Caprellidae, están particularmente bien dotadas para el transporte en barco ya que, por ejemplo, construyen tubos sobre basibiontes del fouling o bien se anclan fuertemente mediante los pereiópodos a sustratos de porte arborescente que son dispersados pasivamente (Crawford, 1937; Takeuchi and Hirano, 1995). Así mismo, este grupo posee atributos que favorecen la invasión, como alta capacidad reproductiva, tolerancia medioambiental, agresividad y plasticidad trófica (Grabowski et al., 2007; Ros et al., 2015; Ros et al., 2021). Varias especies de anfípodos introducidos han demostrado ser invasores de éxito, expandiéndose rápidamente, a veces llegando a colonizar ambos hemisferios, y acarreando efectos negativos en las comunidades nativas, así como costes económicos en infraestructuras (Boos et al., 2011, Ros et al., 2015; Dick and Platvoet, 2000; Borza et al., 2018; Marchini and Cardeccia, 2017).

Por todo ello, los crustáceos peracáridos, y en especial los anfípodos, nos ofrecen un modelo adecuado para estudiar las invasiones marinas asociadas a la navegación de recreo. Concretamente, desde un punto de vista metodológico:

A) Nos permiten obtener muestras abundantes con excelente relación coste-beneficio (accesibles mediante rascado en pantalanes o recolectando a mano). En el caso de técnicas moleculares, por ejemplo, esto llega a ser un requisito. Resulta muy interesante para definir puntos calientes de contaminación biológica, o poner a punto metodologías estandarizadas para los programas de vigilancia y monitoreo.
B) Nos permiten establecer el vector asociado con mayor robustez: al ser un taxon con desarrollo directo (sin larva), podemos atribuir con mayor confianza la introducción en regiones geográficas distantes al vector antropogénico, y no a la dispersión natural. Esto aporta claridad a la hora de establecer rutas de introducción asociadas al tráfico marítimo; y permite entender mejor las fases de transición en el proceso de invasión, como el transporte en barco o la colonización vector - medio receptor.
C) Con ellos abordamos retos que suelen ser pasados por alto en el estudio de las invasiones marinas, como la fracción móvil del *fouling*, o el caso de los llamados *hidden-invaders*; esto es, taxones de difícil detección debido a su tamaño o difícil resolución taxonómica.

1.4 General objectives and thesis structure

This PhD projects aims to overcome some gaps of knowledge for preventive management of marine bioinvasions and a future regulation of the recreational vessels' hull-fouling vector in our region. The project keeps in mind the three aforementioned pilars of prevention: vector assessment to inform regulation, baseline surveys, and community participation and awareness; and makes an effort to integrate a socio-ecological approach.

- **CHAPTER 2. THE ROLE OF RECREATIONAL BOATING AS A VECTOR**

  This chapter addresses the role of recreational boating as vector for marine exotic species, with a focus on vessel hull-fouling, with the aim of increasing evidence that warrants regulation. First, it explores the probability of vessels carrying exotic fauna as stowaways in their hull, and their potential to connect fouling communities at regional scale in the Mediterranean Sea. This is approached from a socio-ecologial point of view; this is, evaluating which habits from boat owners favor this accidental exchange. Secondly, we focus on exploring what factors may drive the survivorship of fouling mobile epifauna during this vessel journey, in order to put the transport phase into context within the invasion process and draw light to pre-arrival processes (usually overlooked, yet fundamental for a preventive management approach). This chapter will consist on two parts:

  - PART 2.1 Is recreational boating a potential vector for non-indigenous peracarid crustaceans in the Mediterranean Sea? A combined biological and social approach

  - PART 2.2. The journey of hull-fouling mobile invaders: basibionts and boldness mediate dislodgement risk during transit

- **CHAPTER 3. BASELINE SURVEYS TO UNCOVER MISSING INFORMATION**

  This chapter aims to outline the importance of surveys and monitoring to serve a preventive approach by mitigating detection time-lags in the future. First it addresses the challenge of hidden invaders and uses several case studies to illustrate how they demand extra efforts in monitoring, record dissemination and taxonomical expertise to overcome missing data issues (record uncertainty, misidentifications, gaps in distribution range etc.). Secondly, it explores the application of molecular temporal data as a monitoring tool for invaders; concretely, by exploring the introduced population status (haplotype diversity, vulnerability periods) during the post-establishment phase. Here, the research is focused in marinas as optimal sites for monitoring and vigilance of marine exotic introductions. This chapter will consist on two parts:

  - PART 3.1 The challenge of hidden invaders

    3.1.1 Marine exotic isopods from the Iberian Peninsula and nearby waters

3.1.2 Scientific collaboration for early detection of invaders results in a significant update on estimated range: lessons from *Stenothoe georgiana* Bynum & Fox 1977

3.1.3 Hitchhiking northwards: on the presence of the invasive skeleton shrimp *Caprella scaura* in the UK

- PART 3.2 Using molecular data to monitor the post-establishment evolution of the invasive skeleton shrimp *Caprella scaura*

- **CHAPTER 4. COMMUNITY PARTICIPATION AND AWARENESS**

This chapter investigates the social-perception of Spanish stakeholders about marine bioinvasions associated to the recreational boating vector. Knowledge, awareness, risk perception, values and attitudes towards management are explored for the first time. The objectives are a) set baselines for a future fair and effective implementation of the biofouling regulation in our country; b) promote dialogue among the scientific community and policy makers around marine biosecurity; and c) provide preliminary recommendations to better orientate outreach for raising awareness on the issue. A comparison is carried out between the public views in Spain and those in the only two countries with regulations at national level: Australia and New Zealand. This chapter gathers some preliminary results of this ongoing research, and will consist on a single part:

- Social-perception of marine bioinvasions associated to recreational boating: first insights from Spanish stakeholders

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2

THE ROLE OF RECREATIONAL BOATING AS A VECTOR: PART 1

Is recreational boating a potential vector for non-indigenous peracarid crustaceans in the Mediterranean Sea?

A combined biological and social approach

Adapted from: Martínez-Laiz G, Ulman A, Ros M, Marchini A (2019). Is recreational boating a potential vector for non-indigenous peracarid crustaceans in the Mediterranean Sea? A combined biological and social approach. Marine Pollution Bulletin 140:403-15.

ABSTRACT. Shipping is understood to be a major vector for the introduction and spread of marine non-indigenous species (NIS). However, recreational boating is still unregulated and its influence as vector has not yet been assessed for the Mediterranean Sea, which is the second most popular recreational boating destination worldwide. This is the first large-scale study to examine this by a combined biological (analyzing hull and marina fouling) and social approach (boaters surveys on maintenance habits, travel patterns and awareness), focused on peracarid crustaceans. A surprisingly high number of NIS were found on vessels cruising Mediterranean waters, and species compositions suggest an exchange between marina and vessel assemblages. This means recreational boating presents a risk for NIS spread which should warrant regulation. Results also implied that regionally coordinated management should be supported by effective local-scale-based management in the Mediterranean, which could improve upon with targeted environmental education to solve lack of awareness.

1. INTRODUCTION

Globally, the Mediterranean Sea stands out as a marine biodiversity hotspot, hosting more than 17.000 described marine species and contributing around 7% of the world's marine biodiversity (Coll et al. 2010). Located between Europe, Africa and Asia, it serves as a major shipping route and is ringed by numerous ports and marinas (Seebens et al., 2013; Carreño and Lloret, 2021); and it is considered to be currently the most invaded Sea (Edelist et al., 2013; Bailey et al. 2020; Fig. 1). The history of introductions in the Mediterranean Sea is centuries-long, with many exotic species being overlooked as pseudo-indigenous *sensu* Carlton (2009), but with a late response from stakeholders and the scientific community until notorious negative impacts arose in the 80s. Indeed, Galil et al. (2018) indicates that perceptions of introduced species in the Mediterranean Sea evolved from ignorance and indifference to "biological pollution" impairing potentially exploitable marine resources and harming indigenous species and habitats. The native ranges of most NIS in the Mediterranean Sea stem from the Indo-Pacific region, and the Suez Canal pathway (corridor) is responsible for over 50% of introduced species, followed by shipping (commercial and recreational) and mariculture (Galil et al. 2018). The number of NIS introduction events in the Mediterranean has doubled since 1970 until now (Galil et al., 2018), probably related to multiple factors: the intensification in commercial shipping (Seebens et al., 2013), increased coastal urbanization and recreational populations (Airoldi and Beck, 2007; Tosun 2011), the warming of the sea (Bianchi et al., 2013), and the progressive enlargements of the aforementioned Suez Canal (Galil et al., 2017).

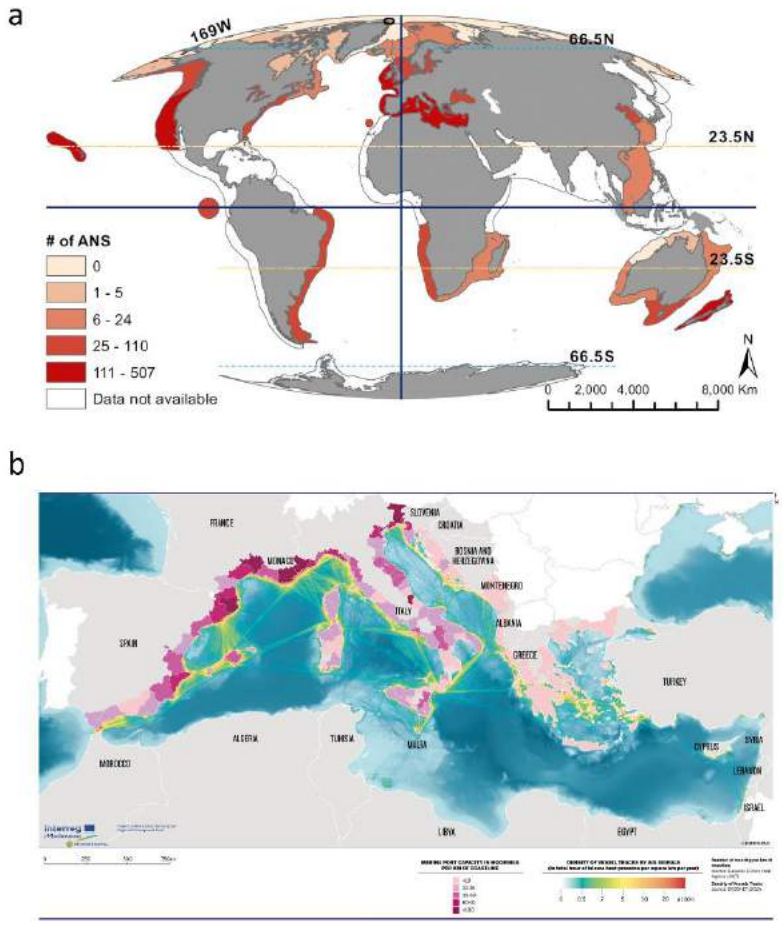

Figure 1. a) Retrieved from Bailey et al. (2020). Records of primary detections of aquatic non-indigenous species between 1965 and 2015 in each of 49 coastal marine, estuarine and freshwater ecosystems. b) Retrieved from Carreño and Lloret (2021); source: Interreg Pharos4MPAs, Alessandro Mulazzani - in Carreño et al. (2019). Marina port capacities in number number of moorings per km of coastline in EU countries (except Cyprus), and sailing and pleasure craft routes using Automatic Identification System (AIS) signals (crafts > 24m).

Despite the role of commercial shipping in the spread of NIS was discovered more than 30 years ago (Carlton, 1985), concern for recreational boating as a vector only later arose (Minchin et al., 2006; Clarke-Murray et al., 2011, 2014). In 2011 the Mediterranean represented the second most popular destination for nautical tourism worldwide (Cappato, 2011). In 2015, between 50 and 70% of the worlds superyacht charter contracts were in the Mediterranean (Superyacht Business Happening, 2015; Piante and Ody, 2015). Today, leisure boating is one of the pillars of the European Union Blue Economy (EC, 2018) and the Mediterranean Sea continues to be a top nautical tourism destination in the world (Carreño and Lloret, 2021), generating 59% of the economic output of the European Union's nautical tourism sector (EU, 2017). It bears around 400,000 berths, distributed in 940 marinas (Bille and Lowezanin, 2010; Cappato, 2011) with more than half of these concentrated in Italy (253), Spain (191), and France (124). However, the efficacy of recreational boating in transporting marine NIS still had not been tested yet for the Mediterranean Sea at regional scale (at the time this research took place).

This research aims to gain insight into the role of recreational boating as a vector for non-indigenous species across the Mediterranean Sea. This will be done by using a combined biological and social approach. For the biological analysis, the fouling assemblages of marinas and hulls of transient vessels will be examined to determine the distribution patterns of native versus non-indigenous species. This portion will focus on peracarid crustaceans as model taxon, mainly encompassing the orders Amphipoda, Isopoda and Tanaidacea. Despite being a key component of the fouling communities colonizing artificial substrates and really good models for exploring bioinvasions, studies dealing with boating as a vector for non-indigenous peracarids are scarce and mainly focus on either single localities or single species (*e.g.* Ros et al., 2013; Ros et al., 2014; Marchini et al., 2015, 2016; Ros et al., 2020). As for the social portion, the vessel maintenance habits, travel history and awareness of boat owners will be explored via behavioural questionnaires, which have proven a useful, rapid and cost-effective method in the assessment of fouling risk in studies performed in New Zealand, Scotland, Canada and USA (Floerl et al., 2005a; Ashton et al., 2006; Acosta et al., 2010; Clarke-Murray et al., 2013; Zabin et al., 2014). A few studies undertaken overseas have warned about how boater's maintenance habits affect the level of biofouling on boats (Brine et al., 2013; Clarke-Murray et al., 2013; Zabin et al., 2014), but knowledge on Mediterranean boater habits remains very scarce (Ferrario et al., 2016). This study presents a unique approach to determine the role of recreational boating in the spread of non-indigenous peracarids across the Mediterranean Sea, as only boat owners/captains who had completed an interview had their boat hulls sampled for fouling, so data from the two approaches could be directly compared and tested. The two aims of this research are to: (1) explore the presence of native versus non-indigenous peracarid species in artificial habitats across the Mediterranean and explore the linkage among boat hulls and marinas, thus determining if recreational boating is a potential vector in the transfer of non-indigenous peracarids; and (2) investigate NIS awareness of boat owners and determine which boating behaviors increase the risk for NIS occurrence in biofouling colonization on their boat hulls.

2. MATERIAL AND METHODS

2.1 Study area and sampling procedure

Ten marinas were selected across the northern rim of the Mediterranean Sea (Fig. 2). We first compiled a list of marinas that were highly representative of recreational boating traffic in three subregions of the Mediterranean (eastern, central, western), on the basis of data about non-residential traffic obtained from marinas websites and telephone interviews. Selection of the final shortlist was then dependent on gaining permissions to conduct the scientific study. As shown in Table S3 in Supplementary material, the marinas sampled ranged in sizes (number of berths), all of them being important resorts and major connecting hubs for recreational boating traffic, as well as being able to host pleasure crafts ranging from small boats to mega-yachts.

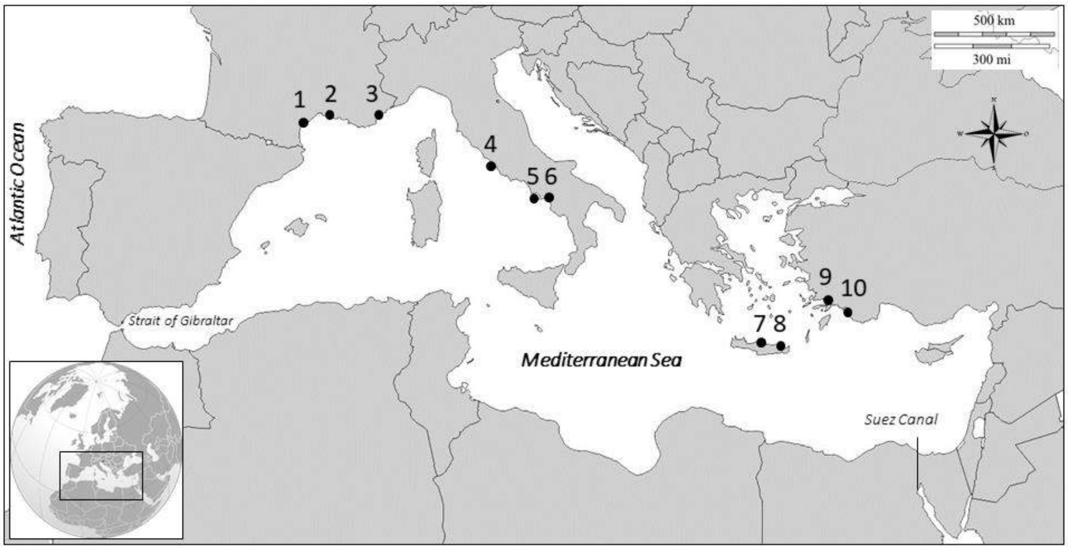

Figure 2. Location of sampled marinas across the Mediterranean Sea. 1) Port Camargue, 2) Cap d'Agde, 3) Antibes, 4) Rome, 5) Ischia, 6) Sorrento, 7) Heraklion, 8) Agios Nikolaos, 9) Marmaris, and 10) Fethiye.

Sampling was conducted in 2015 during the boating season, namely between April and October. In each marina locality, a minimum of 15 and maximum of 30 vessels were sampled depending on feasibility, boater presence in the marina and their willingness to cooperate. Firstly, a short behavioral survey was conducted with the vessel owners/crew to determine vessel characteristics, hull maintenance practices (frequency of cleaning and painting, costs, type of paint used and recent one year of travel history, etc.) [See behavioral questionnaire, Supplementary material S1]. Next, the same boats were sampled for fouling biota, which included (where applicable) the hull, keel, propeller shaft, propeller, stairs, bow thrusters and water vents. Sampling was performed underwater by snorkel or SCUBA dependent on the permission granted from the marina, except for the boats sampled in French marinas, most of which were sampled at the shipyard, immediately after they were removed from the water for cleaning/painting purposes. In all cases, samples of the fouling community were collected using a paint scraper (3.81 cm in blade diameter) and captured in aquarium nets (0.381 cm mesh size), and then quickly preserved in 70% ethanol. In addition to the boat sampling, the artificial structures of the marinas were also sampled. A rapid-assessment sampling approach was used for this purpose (see Cohen et al., 2005; Pederson et al., 2005; Ashton et al., 2006); which was adapted to approximately 8 h of sampling time for the artificial structures of the marina including docks, floating pontoons, tires, buoys, ladders and ropes. The biofouling was collected by scraping the substrate with a hand-held metal rigid net, with one edge sharpened to dislodge encrusting fouling biota (1mm net mesh size, and 25 cm×25 cm net size), extending to a depth of 1.5 m. The procedure was repeated around the marina, paying special attention to sample both exposed and enclosed areas of the marina, in order to obtain a comprehensive sample. Here, peracarids were carefully removed from the substrate they were clinging on, sorted in situ using a sieve (1mm mesh size) and immediately fixed in ethanol for further laboratory analysis.

2.2. Data analysis

2.2.1. Taxonomic identification and classification of species

In the laboratory, peracarid species were sorted from the boat samples and identified to species level whenever possible. Then, data from both boats and marinas were organized into a presence-absence matrix. Each species was classified into their status as ‘native’, ‘NIS’ or ‘cryptogenic’, following a literature analysis of their geographical distributions. Species with a restricted Mediterranean or Atlanto- Mediterranean distribution were classified as ‘native’, while the category ‘NIS’ was assigned to species introduced by human agency to an area outside their natural distribution (EEA, 2012). To assess a likeliness of human-mediated introduction, Chapman and Carlton (1991) criteria were followed. Species with unknown or uncertain native origin were classified as ‘cryptogenic’, sensu Carlton (1996). Additionally, we also created the category ‘potential invader’ to refer to species with a history of introduction in some region, either in the Mediterranean or elsewhere, plus cryptogenic species with a worldwide distribution. Hence, this category includes all NIS, plus cryptogenic and even some native species from our dataset, and distinguishes them from species restricted to the Mediterranean or Atlanto-Mediterranean region that have no history of invasion anywhere. The purpose of this second classification is to assess the influence of the recreational boating vector under a global perspective (i.e. considering the Mediterranean Sea not only as a ‘recipient’ region for NIS, but also as a ‘source’ of NIS for other regions potentially visited by recreational boats). Furthermore, the category ‘potential invaders’ allows for reducing the inherent uncertaintyposed by the high number of cryptogenic species found within this taxon (Marchini and Cardeccia, 2017). Remarks on the assigned potential invader status can be found in Suppl. material S2 in Supporting Information. In the case of Tanaidacea, identification was carried out to genus level, since the available taxonomic literature is limited and in need of revision. For example, contrasting descriptions of the same species occur in the family Tanaididae (e.g. Sieg, 1980 and Riggio, 1996). Tanaids were then excluded from the classification and the community analysis, in order to avoid errors in the status assignment (see Suppl. material S2).

2.2.2. Community exploration and NIS presence

For each boat and marina the number of native, cryptogenic and NIS were calculated, as well as the NIS ratio (number of NIS/total number of species), as recommended for the evaluation of marine environmental status (Olenin et al., 2010), and the potential invaders ratio (number of potential invaders/total number of species). Data from Marmaris marina were excluded from this latter analysis, since the collected material unfortunately was lost during transportation. Moreover, an exploratory one-way SIMPER analysis based on presence/absence data was conducted to explore similarities between the faunal compositions found on the boats vs. marinas. This analysis was conducted using PRIMER 6 software (Clarke and Gorley, 2006).

2.2.3. Effect of behavioral aspects on the occurrence of fouling peracarids

In order to get a broad perspective about the travellers profiles, three aspects were explored: cleaning habits, travel patterns and awareness. Regarding cleaning habits, we considered the frequency (categories: ≤1 time/year, 1 time/year,>1 time/year) and cleaning method (categories: in-water, out of water (dry); and professional cleaning, staff cleaning). Regarding travel patterns we considered the travel duration (categories: ≤1 month, 2–3 months, 3–6 months, 6–12 months) and the longest distance travelled (categories: local or > 200 nautical miles; regional or 200–600 nautical miles; Mediterranean Sea or 600–2000 nautical miles and International/outside the Mediterranean or > 2000 nautical miles). As for the awareness, we considered the categories: insufficient knowledge (unaware of what NIS are), fair (aware of the term 'NIS' but unable to give correct examples) and aware (named correct examples). For each aspect, percentage of boaters falling into each category was assessed. In addition, a chi-squared test was used to check whether awareness patterns varied significantly among countries. In order to investigate whether the variation of peracarid assemblages on boat hulls could be explained by behavioral aspects (data obtained from interviews to boat owners), the probability of finding NIS was modeled using Generalized Linear Models (GLM; McCullagh and Nelder, 1989). The response variable was coded in the format [successes, failures]; specifically: [number of NIS, number of natives] and modeled through a Binomial distribution (logit link). Explanatory variables considered for the analysis were: marina sampled (10 levels, one for each marina); last cleaning method performed (2 levels, in water versus dry); person who performed the cleaning (2 levels, professional versus self or staff); time the boat spent travelling (in number of days); time since the last dry professional cleaning and painting (number of months); boat length (in meters); boat average speed (in knots); and longest distance travelled (in miles). It needs to be emphasized that the contribution of data about behavioral aspects depended on boaters willingness to cooperate. Therefore, only boats for which all data was collected for each variable could be considered for analysis (n=135 observations). Prior to analysis, multi-collinearity was explored to ensure the absence of correlation among predictor variables, following the criterion variation inflation factor (vif)<10.

Model selection was carried out based on second order Akaike's information criterion (AIC) for small sample sizes (AICc, Burnham and Anderson, 2002), as a measure of relative quality, and the preferred model was identified by the minimum value of AICc. The Akaike weight (wi) of each model was calculated within the significant models, i.e. those with<2 AIC units of difference from the model with the smallest AIC. All significant models were used to calculate model-averaged parameter estimates and unconditional 95% confidence intervals to better represent model-selection uncertainty. The relative importance of each predictor was based on the combined Akaike weights (Σwi) for all significant models that contained a given predictor. None of the interaction terms were considered well-supported by our data and thus were removed to simplify subsequent analyses. Variability explained by the best models was computed using Phi coefficient for binary data (Jackson et al., 1989). The performance of the significant probability models was assessed using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The analysis was carried out using R studio version 3.4.0.

3. RESULTS

A total of 247 interviews were conducted, and the same number of boat hulls were inspected from 10 different Mediterranean marinas. There was a surprisingly high number of non-indigenous peracarids found: overall, 64% of the inspected boats had peracarids in their fouling assemblages, and the majority (56%) had NIS (increasing to 95% when cryptogenic species were also incorporated). When considering the amount of potential invaders, the results were even more striking: 97% of the boats showed values above 50%, and 79% of the boats reached 100%. In total, 50 taxa were identified, out of which 28 species belonged to 19 genera and 14 families in the order Amphipoda; 17 species from 10 genera and 5 families in the order Isopoda; and 5 genera belonging to 2 families in the order Tanaidacea. Out of all species identified, excluding tanaids, 12 were NIS, 14 were cryptogenic and 19 were native (see Table 1).

Marinas and boats assemblages from the same location showed, for most of the cases, relatively high similarities. In six out of the nine sampled locations, boats assemblages shared between 50 and 78% of their compositions with their respective marina assemblages (see Table 2). Exceptions were Cap d'Agde, whose marina fouling composition was mostly similar to that of vessel hull fouling in Rome (50% similarity); and boats and marina assemblages in Fethiye and Sorrento, which had rather unique compositions. Regarding the composition of marina fouling assemblages, the ratio of NIS/total number of species was highly variable even among marinas within the same country (Fig. 3); the highest values were found in Fethiye (Turkey) and Cap d'Agde (France), where 50% of the peracarid species were non-indigenous, followed by Heraklion (Greece) with 40%. Port Camargue (France) and the Italian marinas had intermediate values (20–33%), whereas Agios Nikolaos (Crete, Greece) had 17% of non-indigenous species. Only in Antibes (France), no NIS were detected. When considering the ratio potential invaders/total number of species, all marinas showed values above 67%, and reached 100% in Cap d'Agde, Rome and Fethiye. Noteworthy is the high percentage of cryptogenic peracarid species in marinas, which was above 30% for all marinas, and highest in Rome (67%).

Table 1. List of peracarid species identified from the boats (B) and marina (M) fouling assemblages in each locality. Statuses are as follows: NIS=non-indigenous species; C=Cryptogenic species; N=native species; PI=potential invader; NR=native species with a restricted distribution; US=unassigned status (only for Tanaidacea; see main text for justification.

				PORT CAMARGUE		CAP D'AGDE		ANTIBES		OSTIA-ROME		ISCHIA		SORRENTO		HERAKLION		AG. NIKOLAOS		FETHIYE		MARMARIS
STATUS	**ORDER**	**FAMILY**	**SPP**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**
NIS / PI	Amphipoda	Aoridae	*Aoroides longimerus* Ren & Zheng, 1996	X																		
NIS / PI	Amphipoda	Aoridae	*Bemlos leptocheirus* Walker, 1909														X		X			
NIS / PI	Amphipoda	Caprellidae	*Caprella scaura* Templeton, 1836	X	X	X	X	X		X	X	X	X	X	X	X	X	X				X
NIS / PI	Amphipoda	Ischyroceridae	*Ericthonius pugnax* Dana, 1852	X		X				X												
NIS / PI	Amphipoda	Stenothoidae	*Stenothoe georgiana* Bynum & Fox, 1977	X		X								X	X							
NIS / PI	Isopoda	Paranthuridae	*Paranthura japonica* Richardson, 1909	X	X	X	X	X		X	X		X			X	X					
NIS / PI	Isopoda	Anthuridae	*Mesanthura* cf. *romulea* Poore & Lew Ton, 1986												X							
NIS / PI	Isopoda	Sphaeromatidae	*Cymodoce fuscina* Schotte & Kensley, 2005													X	X					
NIS / PI	Isopoda	Sphaeromatidae	*Cymodoce* sp. A														X					
NIS / PI	Isopoda	Sphaeromatidae	*Paracerceis sculpta* Holmes, 1904										X			X	X	X		X		
NIS / PI	Isopoda	Sphaeromatidae	*Paradella dianae* Menzies, 1962													X					X	
NIS / PI	Isopoda	Sphaeromatidae	*Sphaeroma walkeri* Stebbing, 1905													X	X	X		X	X	
C / PI	Amphipoda	Caprellidae	*Caprella equilibra* Say, 1818	X	X	X		X				X		X		X		X				
C / PI	Amphipoda	Caprellidae	*Phtisica marina* Slabber, 1769	X																		
C / NR	Amphipoda	Colomastigidae	*Colomastix pusilla* Grube, 1861	X																X		
C / PI	Amphipoda	Corophiidae	*Apocorophium acutum* Chevreux, 1908	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C / PI	Amphipoda	Corophiidae	*Monocorophium acherusicum* Costa, 1853	X		X		X	X	X	X		X	X			X			X		
C / PI	Amphipoda	Corophiidae	*Monocorophium sextonae* Crawford,1937	X				X		X												
C / PI	Amphipoda	Ischyoceridae	*Ericthonius brasiliensis* Dana, 1853	X						X	X	X	X	X		X	X	X	X	X		
C / PI	Amphipoda	Ischyroceridae	*Jassa marmorata* Holmes, 1905	X	X	X	X	X	X	X		X		X	X	X	X			X		
C / PI	Amphipoda	Ischyroceridae	*Jassa slatteryi* Conlan, 1990	X	X	X		X	X	X		X		X			X					
C / PI	Amphipoda	Maeridae	*Elasmopus rapax* Costa, 1853	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X
C / NR	Amphipoda	Maeridae	*Quadrimaera inaequipes* Costa, 1857												X							
C / NR	Amphipoda	Podoceridae	*Podocerus variegatus* Leach, 1814													X		X				
C / PI	Amphipoda	Stenothoidae	*Stenothoe valida* Dana, 1852			X										X						

Table 1. (Continued).

C / PI	Isopoda	Sphaeromatidae	*Dynamene bidentata* Adams, 1800																	X
N / NR	Amphipoda	Amphithoidae	*Ampithoe riedlii* Krapp-Schickel, 1968											X						
N / NR	Amphipoda	Dexaminidae	*Dexamine spiniventris* Leach, 1814									X								
N / NR	Amphipoda	Hyalidae	*Hyale crassipes* Rathke, 1837		X															
N / PI	Amphipoda	Ischyroceridae	*Ericthonius punctatus* Bate, 1857			X				X		X			X		X		X	X
N / NR	Amphipoda	Ischyroceridae	*Ericthonius* sp. (females)	X						X	X		X		X		X	X		X
N / NR	Amphipoda	Leucothoidae	*Leucothoe denticulata* Costa, 1851													X				
N / NR	Amphipoda	Lysianassidae	*Lysianassa pilicornis* Heller, 1866			X										X				
N / NR	Amphipoda	Lysianassidae	*Orchomene humilis* Costa, 1853											X						
N / NR	Amphipoda	Photidae	*Gammaropsis maculata* Johnston, 1828	X																
N / NR	Amphipoda	Stenothoidae	*Stenothoe marina* Bate, 1856												X	X	X			
N / NR	Isopoda	Paranthuridae	*Paranthura costana* Spence Bate & Westwood, 1866	X				X	X			X					X			
N / NR	Isopoda	Gnathiidae	*Gnathiidae* sp. Leach, 1814			X		X												
N / NR	Isopoda	Janiridae	*Janiridae* ind. A Sars, 1897											X						
N / NR	Isopoda	Janiridae	*Janiridae* ind. B Sars, 1897														X			
N / NR	Isopoda	Janiridae	*Janiridae* ind. C Sars, 1897	X																
N / NR	Isopoda	Hemioniscidae	*Hemioniscidae* ind. Bonnier, 1900										X							
N / PI	Isopoda	Sphaeromatidae	*Sphaeroma serratum* Fabricius, 1787	X	X	X		X	X			X								
N / PI	Isopoda	Sphaeromatidae	*Dynamene edwardsi* Lucas, 1849	X	X	X		X	X		X	X			X	X	X	X		X
N / NR	Isopoda	Sphaeromatidae	*Dynamene bicolor* Rathke, 1837			X		X	X						X					X
US	Tanaidacea	Tanaididae	*Hexapleomera* sp. Dudich, 1968	X	X	X		X		X	X	X	X		X		X	X	X	X
US	Tanaidacea	Tanaididae	*Zeuxo* sp. Templeton, 1840	X	X	X		X	X	X	X	X	X		X		X		X	X
US	Tanaidacea	Tanaididae	*Tanais* sp. Latreille, 1831				X		X		X			X			X	X		X
US	Tanaidacea	Tanaididae	*Anatanais* sp. Nordenstam, 1930			X		X	X	X	X	X					X	X	X	X
US	Tanaidacea	Leptocheliidae	*Leptochelia* cf *savignyi* Krøyer, 1842	X		X	X	X		X	X	X	X	X			X			

Table 2. One-way SIMPER analysis based on presence/absence species data exploring similarities between the faunal compositions on boats vs. marinas. Similarities 0–50% are show in white and those ≥50% in blue. Cells showing similarities among pairs of boats and their marinas are in bold.

Marina *Boats*	FRANCE			ITALY			GREECE		TURKEY
	PC	CD	ANT	ROM	IS	SOR	HER	A.NIK	FET
PC	58.06	32.00	53.33	44.44	54.55	32.26	47.37	37.04	16.00
CD	66.67	38.10	61.54	52.17	55.17	37.06	58.82	34.78	19.05
ANT	75.00	44.44	78.26	50.00	61.54	33.33	51.61	30.00	22.22
ROM	54.55	50.00	47.62	66.67	50.00	36.36	55.17	44.44	25.00
IS	63.64	37.50	57.14	55.56	58.33	36.36	55.17	55.56	25.00
SOR	47.62	40.00	40.00	47.06	34.78	38.10	42.86	35.29	13.33
HER	48.28	34.78	35.71	40.00	51.61	27.59	66.67	48.00	34.78
A.NIK	41.67	22.22	34.78	40.00	53.85	25.00	51.61	50.00	33.33
FET	31.58	30.77	44.44	53.33	47.62	31.58	53.85	40.00	46.15
MAR	42.11	30.77	44.44	53.00	47.62	31.58	38.46	66.67	30.77

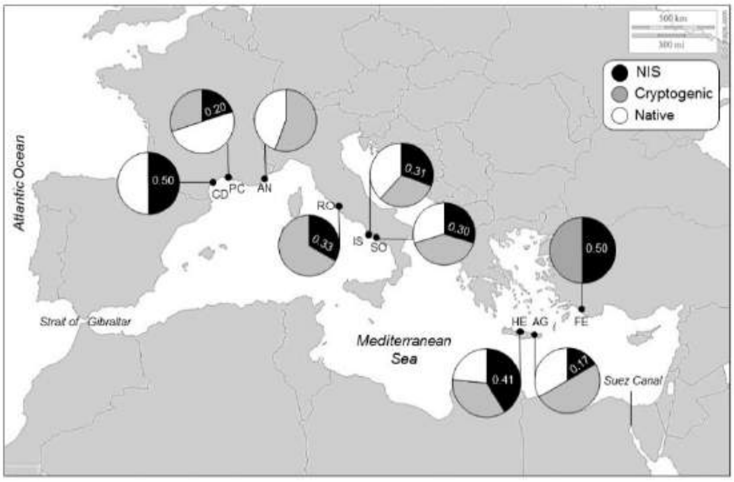

Figure 3. Relative contribution of NIS (black), cryptogenic (grey) and native (white) species to the total number of species found in each marina. The value of the ratio NIS/total species is given in each case.

Effect of boat characteristics, maintenance habits and travel history

From the boats surveyed, 43% had travelled for up to one month in the most recent 12 months leading up to the survey date, and another large percentage performed long journeys: 40% travelled for 2–3 months; 13% for 3–6 months; and 4% for half a year or a complete year (Fig. 4A). Half these journeys (49%) were performed locally and within the respective countries, or within one Mediterranean region (41% regional). Ten boats (8%) journeyed widely across the Mediterranean Sea (600–2000 nautical miles); and three boats (2%) travelled to the Mediterranean overseas; one from Australia and two from England (Fig. 4B). As for cleaning frequency (which included professional cleaning prior to antifouling application and additional in-water cleanings), 69% of the boaters cleaned their boat-hulls once a year; 21% cleaned their vessel more than once per year and 11% cleaned less than once per year. Half the boaters had their hulls professional cleaned (53%), while the other 47% had last cleaned their hulls themselves (usually in-water) within the last year. Finally, nearly half (47%)

of the interviewed boaters were unaware of the concept of NIS; 24% had heard of the term, but were not able to correctly name any NIS, and 29% were aware of the term and could name proper examples (Fig. 4C). Significant differences among countries were found to exist in level of NIS awareness, (Chi-squared = 37.60; df = 6; P < 0.00001). Boaters in Greece (56% named correct examples of NIS and only 16% were unaware of the term) had significantly higher NIS knowledge than those in France (64% were unaware of the term NIS) (P = 0.005) and Italy (37% were unaware) (P = 0.0008); and slightly higher than those in Turkey (44% were unaware) (P = 0.08).

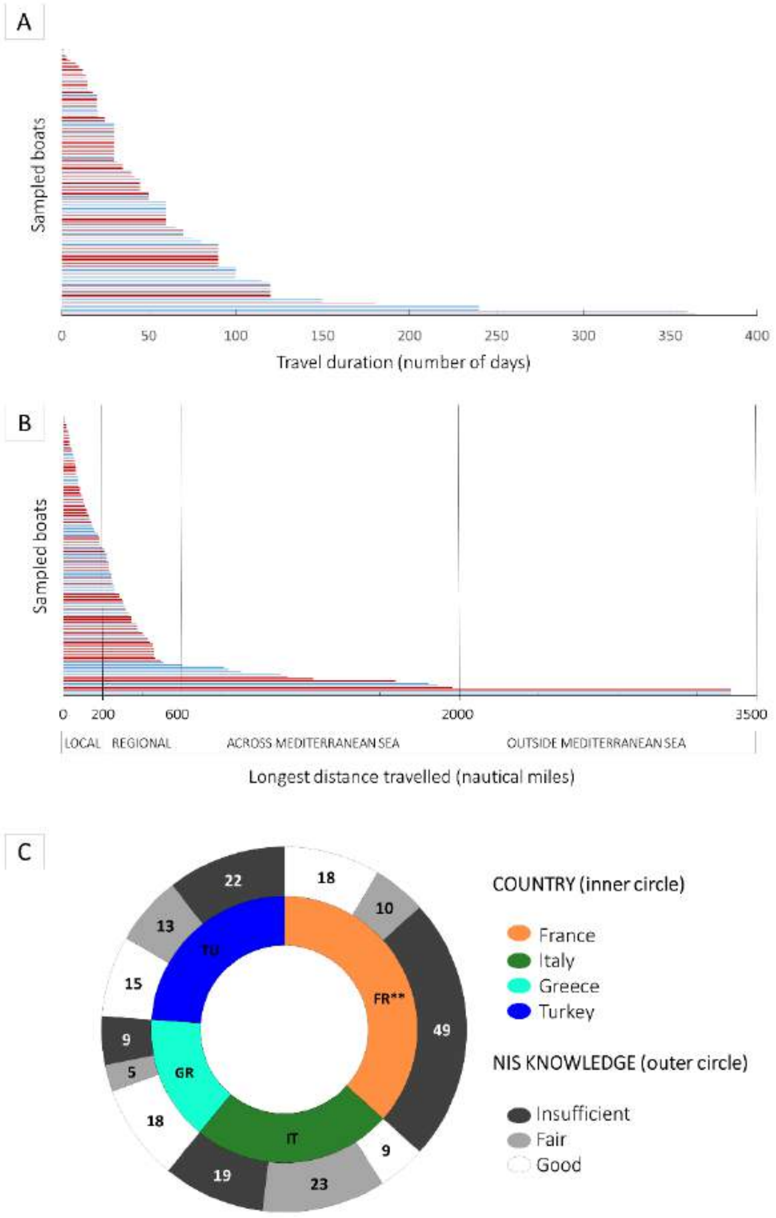

Figure 4. (A–C) Results from the interviews regarding travel patterns and awareness. Plots show (A) travel duration (number of days); (B) longest distance travelled by boats (in nautical miles); and (C) and awareness level of interviewed boaters plotted by country (C). Red lines in (A) and (B) indicate boats carrying NIS on their hulls. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Model selection for explaining probability of NIS occurrences generated five output models from a model set of 512, which were used to calculate model-averaged parameter estimates and unconditional 95% confidence intervals. The significant models ($\Delta i < 2$) included the following variables: marina, cleaning method (in water [IW] vs. dry), number of travel days, and vessel length (Table 3A). The models which were deemed significant ranged from explaining 61.82% to 64.13% variability (see Phi values in Table 3A) and explained 64% in the final model. Model averaged coefficient β showed that boats mooring in Cap d'Agde, Fethiye, Heraklion, Port Camargue, Rome and Sorrento had a higher probability of NIS occurrence than those in Antibes, Ischia and Marmaris (Table 3B; Fig. 5A). Probability of NIS occurrence was negatively related to the number of travel days (β = −0.001) and with vessel length [β = − 0.004] (Table 3B; Fig. 5B); and was greater when using the last time a vessel performed in-water cleaning as a proxy, rather than their last professional cleaning alone (Table 3B; Fig. 5C). The only significant relationship found was between probability of occurrence and marina (βCD = 1.20, PCD = 0.03; βHER = 1.25, PHER = 0.03; βPC = 1.09, PPC = 0.04), which was the most important variable for explaining the occurrence (∑wi = 1), followed by number of travel days (∑wi = 0.37); cleaning method (∑wi = 0.31) and vessel length (∑wi = 0.14) (Table 3A). The average performance of the chosen best model was very good (AUC =84.87).

Table 3. (A) Model selection for predicting probability of NIS occurrence on boat hulls. Akaike's information criterion corrected for small sample size (AICc), AICc weights (wi) and AICc difference between the AICc of each model and the AICc of the best fitted model (Δi) were used for comparison. Variables: 'Marina', marina where the boat was sampled; 'Days', travel duration (in number of days); 'CleaningMethod': in-water versus professional dry cleaning; and 'BoatLength', (in m). (B) Model averaged coefficients (β) of explanatory variables present in the significant models for probability of NIS occurrence. Adjusted standard error (SE) of model averaged coefficients, including z-values and P values.

A Best models	df	AICc	Δ_i	w_i	LogLik	Phi
response: Probability of NIS occurrence						
1. Marina	10	233.18	0.00	0.32	-105.65	61.82
2. Marina + Days	11	233.85	0.67	0.23	-104.79	63.07
3. Marina + CleaningMethod	11	234.45	1.27	0.17	-105.09	62.64
4. Marina + CleaningMethod + Days	12	234.79	1.61	0.14	-104.04	64.13
5. Marina + BoatLength	11	234.91	1.73	0.14	-105.32	62.31

B Predictor	*β*	Adj. SE	z	*P*
Intercept	-1.82	0.55	3.33	0.001***
MarinaANT	-1.42	0.88	1.62	0.11
MarinaCD	1.20	0.54	2.21	0.03*
MarinaFET	0.97	0.58	1.66	0.09
MarinaHER	1.25	0.56	2.21	0.03*
MarinaIS	-1.06	1.14	0.93	0.35
MarinaMAR	-0.40	0.90	0.45	0.65
MarinaPC	1.09	0.53	2.07	0.04*
MarinaROM	0.76	0.69	1.11	0.27
MarinaSOR	0.79	0.66	1.20	0.23
Days	-0.001	0.002	0.56	0.58
PlaceCleaning	0.10	0.21	0.46	0.64
Length	-0.004	0.02	0.23	0.82

*Significant coefficient value p<0.05

***Significant coefficient value p<0.001

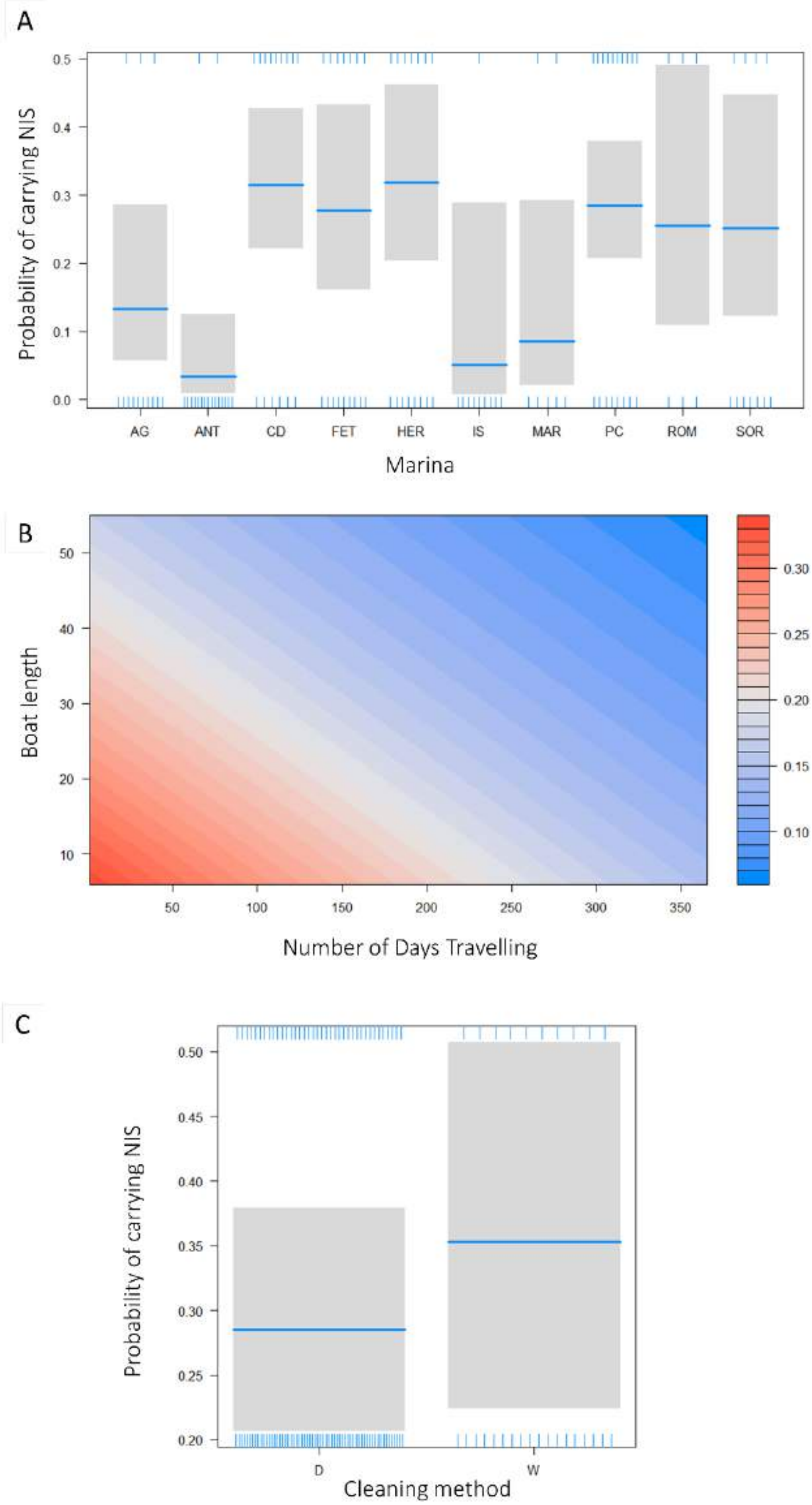

Figure 5. Binomial model plots showing the probability of NIS occurrence on boat hulls based on the predicted effect of marina* (A), days travelling and boat length combined (B), and cleaning method performed (C). Shading indicates 95% confidence limits for the fitted relationship.

4. DISCUSSION

This large-scale spatial study provides evidence that pleasure boats cruising Mediterranean waters carry rich and diverse peracarid communities on their hulls, including a notably high number of NIS and 'potential invaders' (as defined in Section 2.2.1): 56% of the boats carried NIS and 79% hosted exclusively potential invaders on their hull fouling. Nearly all marina assemblages were also dominated by these taxa: with the exception of Antibes (where no NIS were found), the assemblages in French, Italian, Greek and Turkish marinas exhibited a high percentage of potential invaders (67–100%). The predominance of these potential invaders affirms the strength of the recreational boating vector in the transport and relocation of these and other such species across Mediterranean marinas. Since peracarid crustaceans lack a larval stage, their spreading from marina to marina can mainly be attributed to transport by boats.

4.1 A NIS network connected by boats

These results indicate that the role of the marina is a significant predictor for the probability of NIS occurrence in hull fouling, since the longer a boat sits in a marina, it has a higher chance of becoming colonized by the same species as the marina itself. Boats moored in Port Camargue, Heraklion, Cad d'Agde and Fethiye had a higher probability of NIS occurrence on their hulls, and three of these four marinas had the highest contributions of NIS in their peracarid assemblages (Fig. 3). Also the three first mentioned marinas are in close proximity to other major vectors [aquaculture sites for Port Camargue and Cap d'Agde and a commercial shipping port for Heraklion (Ulman, 2018)], thereby providing reasoning for their higher incidence of NIS.

Furthermore, the exploratory SIMPER analysis revealed that in most cases, boats sampled in a marina hosted a highly similar species composition to the marina itself (see diagonal values in Table 2). This suggests an ongoing exchange between marina and moored boats and thus displays the propensity for boats to take on similar NIS as where they are moored, from where they can subsequently spread with boat travel. Additionally, the highest similarity values occurred within subregions of the Mediterranean Sea. This matches with the tendency of our sampled vessels to perform local or regional trips (90% of the cases), allowing for a higher flow or exchange between peracarid assemblages within these subregions. A tendency for similarities between species compositions is visible when looking at the bigger picture as well (frequent similarities ≥50% in Table 2), highlighting the strength of the biofouling vector for redistributing species across distant marinas in the Mediterranean. Recreational vessels performing long-distance trips, although representing a small percentage in our survey (10%), pose a risk as well, as they have the potential to connect faunal assemblages from opposite ends of the entire Mediterranean Sea, at distances that would normally inhibit the spreading of peracarids. Indeed, one third of these vessels were hosting non-indigenous peracarids on their hulls (Fig. 4B). The likely resulting scenario is an expansion of NIS that may eventually lead to biotic homogenization (Olden and Poff, 2004; Galil, 2007) from a lower beta diversity resulting from diminishing community distinctiveness among regions, similarly to urbanization above-land (McKinney, 2006; Olden et al., 2006).

The mentioned patterns raise concern regarding management of NIS in the Mediterranean Sea. Firstly, each marina bears different environmental and anthropogenic conditions that influence several stages of the invasion process (*i.e*., arrival, reproduction, settlement, establishment and population increase and range expansion, Lockwood et al., 2005). Concretely, whether an introduced NIS successfully reproduces and establishes is largely determined by a variety of local conditions as well as environmental factors (*e.g*., harbour design, temperature, salinity, proximity to nutrient inputs, etc.) (Floerl and Inglis, 2003; Foster et al., 2016), as well as biological factors relating to the local community present in the marina (*e.g.,* availability of suitable substrate) (Ros et al., 2012). Secondly, based on these results, certain marinas pose a higher risk for NIS occurrence in hull fouling (Fig. 4A). This means that each marina boats visit matters for calculating 'infection risk'; signalling that the management of the global issue of marine bioinvasions should take into account local factors, and that regionally coordinated management should be supported by effective local scale-based management. Certainly, these results support marinas as effective monitoring localities for the detection and the spreading process of NIS, as other studies have shown (Cohen et al., 2005; Glasby et al., 2007; Floerl et al., 2009; Lacoursiére-Roussel et al., 2012; Ros et al., 2013; Foster et al., 2016). This work supports the undertaking of a preventive approach against NIS, based on early detection of new invaders, and the development of measures for preventing new introductions needs to follow. The better predictive capability, the better implementation of eradication campaigns (Simberloff, 2009).

4.2 Role of boat owners: maintenance habits, travel patterns and awareness

The probability model demonstrated that maintenance habits and travel patterns are influencing variables for NIS occurrence on hull fouling. These factors have been reported as key when addressing the 'human dimension' of the issue (Mineur et al., 2008; Davidson et al., 2010; Jurk, 2011; Clarke-Murray et al., 2013; Brine et al., 2013; Ferrario et al., 2016); and listed by the IMO 'Guidance for Minimizing the Transfer of Invasive Aquatic Species as Biofouling for Recreational Craft' (IMO, 2012). The binomial model suggested that smaller and more stationary vessels (under 30 m in length, travelling up to 3 months maximum) were three times more likely to carry NIS than more active mega-yachts (Fig. 5B). The most common behavior of Mediterranean boaters observed from the 247 interviews corresponds to boaters owning a 6–15 m vessel, who perform local or regional trips during the summer season (lasting between 1 and 3 months) and haul-out the boat once a year for a professional dry cleaning and painting; usually combined with occasional in-water cleanings carried out by either staff or the owner themselves. This means that most of the Mediterranean boaters interviewed (70%) exhibited a higher risk profile for NIS occurrence; with a probability of carrying NIS in their vessel hulls of 30% (shown in Fig. 5B). This is specially concerning, as recreational boats between 2.5 and 24m constitute more than 90% of the Mediterranean fleet (Cappato, 2011). Additionally, in-water cleanings are a common practice among boaters to help reduce drag and hence gas consumption in between new antifouling coatings: nearly 1/4 of boaters performed their last cleaning in-water, and around one-third of boaters surveyed here exercised this method at least once a year. In water cleanings are also sometimes used as a strategy to prolong the need for expensive professional dry cleaning/antifouling treatment costs which were shown here to cost up to 4000 € for < 15 m long vessels and up to 10.000 € for 15-35 m long vessels. The model suggested that boats whose last cleaning method was in-water had higher probability of NIS occurrence, confirming what has already been shown in other studies: in-water cleaning does not guarantee the total removal of viable fouling organisms (Floerl et al. 2005b; Woods et al., 2007) and, when carried out in marinas, it may even induce or trigger spawning event for some organisms, which thus could present additional risks to biosecurity (Minchin and Gollasch, 2003; Hopkins and Forrest, 2008; Woods et al., 2012). IMO guidelines (2012) also state that 'in-water scrubbing of large biofouling (e.g. barnacles, tubeworms or fronds of algae) generates waste or debris that may create a pulse of biocide that could harm the local environment; as well as depleting some coating types which could then rapidly re-foul'. If used for removing light fouling, it is highly recommended to use 'appropriate technology that captures biological, chemical and physical debris so that it can be disposed of to an appropriate onshore facility', which is seldom the case, or to perform such cleanings outside the marina away from other vessels. Cleaning frequency is another maintenance practice to draw attention to. Most of the boaters (69%) performed one cleaning per year (minimum frequency recommended by the guidelines: IMO, 2012), which was found insufficient for preventing the colonization of their vessels' hulls by fouling peracarids in the Mediterranean Sea. A more frequent cleaning would be a more effective option (Ferrario et al., 2016).

When interviewed, numerous boaters considered their boat hulls to be perfectly clean despite the presence of some biofouling containing several taxa. Moreover, most boaters were completely unaware that their boat could be carriers of NIS. We could observe from our interviews that only 29% of the boaters had heard about non-indigenous species and correctly named examples. They mainly mentioned 'Caulerpa' (Caulerpa taxifolia [M.Vahl] C.Agardh), 'pufferfish' (*Lagocephalus* sp. Swainson, 1839) and broad terms for taxa such as 'worms', 'barnacles', 'algae' and 'jellyfish'. Less often, they mentioned the Red-Sea 'moray eel' (*Gymnothorax reticularis* Bloch, 1795) and numerous cases of Indo-Pacific fish such as 'trumpetfish' (*Fistularia commersonii* Rüppell, 1838), 'squirrelfish' (*Sargocentron rubrum* [Forsskål, 1775]), the lessepsian 'parrotfish' (Scarus ghobban Forsskål, 1775) and 'rabbitfish' (*Siganus rivulatus* Forsskål & Niebuhr, 1775 and *Siganus luridus* [Rüppell, 1829]). Additionally, they were aware of some freshwater or terrestrial exotic species such as 'zebra mussel' (*Dreissena polymorpha* Pallas, 1771), 'grass carp' (*Ctenopharyngodon idella* Valenciennes, 1844), 'grey squirrel' (*Sciurus carolinensis* Gmelin, 1788), and 'Japanese knotweed' (*Fallopia japonica* (Houtt.) Ronse Decr.. It was evident that awareness varied among Mediterranean regions and were biased to those species appearing in the media; especially for *Caulerpa taxifolia*, well-known among French boaters (64% of those knowing NIS mentioned it); and *Lagocephalus sceleratus* among Greek (83%) and Turkish boaters (47%). Futhermore, more than half the boaters (59%) were either unaware of the NIS concept, or unable to indicate reasonable examples, naming native species such as 'dolphinfish' (*Coryphaena hippurus* [Linnaeus, 1758]), 'trança' (Turkish name for sea bream, *Sparus aurata* Linnaeus, 1758), moonfish (*Mola mola* [Linnaeus, 1758]) and the endangered Mediterranean monk seal (*Monachus monachus* [Hermann, 1779]). This means there is an urgent need of raising awareness among Mediterranean boat owners, and their general low knowledge of the issue should be taken into consideration when implementing environmental education strategies (Ghilardi-Lopes et al., 2015; Nunes et al., 2015). In this regard, it would be advisable to develop effective outreach programs (see

Campbell et al., 2017) as well as developing citizen science initiatives (see Crall et al., 2010; Jurk, 2011; Dickinson et al., 2010; Hourston et al., 2015).

It is acknowledged that further research is required to test other variables such as a combination of environmental and human-mediated factors and more comprehensive fouling assemblages (*i.e.* including sessile species) on larger data-sets. Unfortunately, these limitations come with the difficulty of obtaining such data, which highly depends on willingness of both boaters and marina to cooperate. In this sense, we highlight the need of mobilizing cooperation among the scientific community and port authorities in countries bordering the Mediterranean Sea.

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SUPPLEMENTARY MATERIAL

S1: Behavioural questionnaire for boat owners

Language________ Mediterranean boater survey Identification number: ___________
Date Survey completed: _ _ / _ _ / _ _ _ _ (day-month-year)

1. Current marina: _________________ / ___________________/______________________
   Marina name City Country
2. Home marina: ____________________ / ___________________/_________________

   Same as above (Y/N) Marina name City Country

3. Boat storage (one):
   In water 12 months: ____
   In water some of the year: ____ (# months in water) ________ (# months dry storage)
   Dry docked all year: ____

4. Type of vessel ( ):
   Sailboat __Powerboat ___ Other (Please specify) __________

5. Length of vessel: _____ meters or _____ feet

6. Motor size? _____ hp or ________ kW

7. Hull material ( ):
   Wood ___ Steel ___ Fibreglass ___

8. Average cruising speed?

9. Are you aware of any invasive species in the Mediterranean? If yes, please give name:
   ____

10. If yes to above, have you been affected by an Invasive species?
    Name of species_________________________

    Please explain impact _______________________________

Last 12 months only:

11. Last hull painting date? _____ / _____ / _____________ (Day-Month-Year)
12. Cost of last hull painting?

13. Type of paint used ( ): conventional ______ ablative ____ self-polishing _____?

14. Last hull cleaning date? ______ / ______ / ______________ (Day-Month-Year)

15. Cost of last hull cleaning? ______ (Euros)

16. Was the last hull cleaning completed professionally (by a company) ___ or personally (by yourself or your crew)? _____

17. Was the hull cleaning performed in water ________ or out of water ________?

18. How many times was the hull cleaned in the last 12 months?

19. Is price a consideration/factor for the number of times you clean your hull each year?

20. Are you willing to pay more for more frequent and more effective hull cleaning? If yes, what is the maximum (specify euro or $)?

21. Number of ports visited in last 12 months and number of days spent in each.

Name of Marina City # of days Month

	Name of Marina	City	# of days	Month
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

22. Approximate number of days moored outside of your home marina in last 12 months

S2: Remarks on the assignment of introduction status in the category 'potential invader' and on the 'unassigned status' for tanaidacea. Species are listed in alphabetical order

- ***Anatanais* spp.**: Unassigned status. Assignment to this genus is complicated due to an overlap in characters used to distinguish the generas *Anatanais*, *Zeuxo* and *Zeuxoides* (Edgar, 2008). According to the literature, *Anatanais* only differs from *Zeuxo* in the first article of antenna 1 being

2 times the length of second article versus 2.5, or sometimes 3 in *Zeuxo* (Sieg & Winn, 1981). This diagnostic character is, however, naturally highly dependent not only on the length and proportions of the first article but also the second (see Bird, 2008). Given this issue combined with a lack of updated taxonomic literature for this taxon, a precautionary approach was followed here to avoid mistaking the identification.

- ***Aoroides longimerus***: Non-indigenous species / Potential invader. The genus *Aoroides* was known to be exclusively distributed in the Pacific Ocean coastal regions until last year, when it was reported for the first time in European waters (Gouillieux et al., 2015). It was found from the French Atlantic coast, on floating pontons in the Bay of Brest (NW Brittany) and associated with an oyster reef in Arcachon Bay (SW Brittany), likely introduced via the aquaculture vector from Japanese oysters.
- ***Apocorophium acutum***: Cryptogenic / Potential invader. This is a tube-building species with wide distribution in warm temperate and tropical regions worldwide, very frequently found in harbours and fouling communities (Crawford, 1937). Several authors point to the North Atlantic Ocean (either western or eastern coasts, including the Mediterranean Sea) as the most likely native range, on account of the wide distribution of the species in these areas. Conversely, it is considered non-indigenous in the Gulf of Mexico, South Africa, Brunei, Australia and New Zealand (see Marchini & Cardeccia, 2017 for revision of all records), where its distribution is more patchy; however, records from the NW Pacific still need to be assessed, as well as the possibility of dealing with a complex of species. Given this uncertainty, we consider A. acutum as cryptogenic and potential invader.
- ***Bemlos leptocheirus***: Non-indigenous species / Potential invader. First described from Eastern Africa, *Bemlos leptocheirus* is an Indo-Pacific species which was only once previously recorded in the Mediterranean Sea (Bellan-Santini et al., 1998) in natural habitats on the Egyptian coast, and thus characterized as a likely lessepsian migrant. The present finding in marina habitats suggest its association to the hull fouling vector, which was previously not considered, and confirms it as a human-mediated introduction.
- ***Caprella equilibra***: Cryptogenic / Potential invader. *Caprella equilibra* is a cosmopolitan species, distributed from shallow water to the deep sea. The Mediterranean and East Atlantic coasts have traditionally been considered its native range given the established records in these areas (McCain & Steinber, 1970). However, its wide distribution (McCain, 1986) makes it difficult to determine its origin and it has also been classified as cryptogenic (Carlton, 1996). It has been associated with a wide range of substrate ranging from artificial constructions such as aquaculture facilities and ship fouling to marine turtles (see Marchini & Cardeccia, 2017 and references therein). The fact that it has also been found in plankton samples (Takeuchi & Sawamoto, 1998) suggests a possible natural dispersal capability, apart from the human-mediated transport. McCain in (1968) already suggested the hypothesis of a complex of several morphological forms; thus, its status remains as cryptogenic, until further studies will solve the multiple angles of uncertainty regarding this species.

- ***Caprella scaura***: Non-indigenous species / Potential invader. Caprella scaura was first described by Templeton in 1836, from individuals collected in Mauritius (south Indian Ocean), but the real origin of this species is actually unknown. After its first description, other records in Brazil, Caribbean Sea, Australia and Japan followed within two years; therefore, its native range could also include any of these regions (Ros et al., 2014; Ramalhosa & Canning-Clode, 2015). During the twentieth-century, it was introduced to several regions worldwide by human-mediated vectors such as fouling and/or ballast water. It was recorded for the first time in the Mediterranean Sea in 1994, associated with the fouling community of the Lagoon of Venice in the northern Adriatic Sea (Sconfietti & Danesi, 1996). Since then, it has been reported many times in several Mediterranean countries: Greece (Krapp et al., 2006), Spain (Martínez & Adaraga, 2008), Turkey (Bakir & Katagan, 2011) and Tunisia (Ben Souissi et al., 2010). There is some evidence of multiple introduction events having occurred through time in the Mediterranean Sea (Cabezas et al., 2014), followed by an intensive secondary spread likely mediated by boating (Ros et al., 2014).
- ***Cymodoce fuscina***: Non-indigenous species / Potential invader. *Cymodoce fuscina* was first described from seagrass beds in Saudi Arabia, Persian Gulf between Safaniya and Manifa (Schotte & Kensley, 2005), from material collected by McCain in 1982. Until now, this isopod has not been described outside its native range. Specimens of *C. fuscina* were found associated to hull-fouling and marinas structures, which suggest a human-mediated introduction.
- ***Dynamene edwardsi***: Native / Potential invader. *Dynamene edwardsi* (Lucas, 1849) widely occurs in the NE Atlantic Ocean and the Mediterranean and Black Seas, suggesting it to be native in these regions. Vieira et al. (2016) reviewed the distribution of the genus and extended the northern, eastern and western limits of *D. edwardsi*. It occurs from Galicia (north-western Spain) to Nouadhibou in Mauritania. It is widespread in the Macaronesian islands and in the eastern and western Mediterranean. Glynn (1972) also reported it from the Suez Canal, suggesting that it had migrated from the Mediterranean throughout the whole length of the Canal and Vieira et al. (2016) showed it has now reached the Gulf of Aqaba in the Red Sea. In 2011, Picker & Griffiths (2011) recorded it from South Africa and suggested it has been introduced as a fouling organism or in ballast water. Considering these records, and its frequent occurrence in our samples of boats and marinas, we classify it as a potential invader. The finding of a male clinging on fouling on a ship in Tangiers harbour (Morocco) (Supplementary material 1, Vieira et al., 2016) supports this hypothesis.
- ***Elasmopus rapax***: Cryptogenic / Potential invader. It has been long thought to be a native species in the Mediterranean Sea, on account of its first description from Italy. However, it has been reported worldwide and therefore considered to be cosmopolitan. *Elasmopus rapax* was later established as a complex species of about 20 species (Hughes & Lowry, 2010; Vader & Krapp-Schickel, 2012), whose center of biodiversity seems to be located in the Pacific Ocean (see Marchini & Cardeccia, 2017 and references therein). It is considered a non-indigenous species in Australia, where all records come from ports and populations are very likely conspecific with the Mediterranean ones (Hughes & Lowry, 2010). Given the wide occurrence in artificial habitats, the

introduced status in Australia and the taxonomic intricacy of this species, we consider it as cryptogenic and potential invader.

- ***Ericthonius brasiliensis***: Cryptogenic / Potential invader. It was first described by Dana in 1852 in Rio de Janeiro. There has been some confusion with this species since European workers usually allocated *E. puntatus* (Bate) to *E. brasiliensis* (Dana). It distribution includes Venezuela, Brasil, West Indies, New England and the Mediterranean Sea according to Myers & McGrath (1984). Later, it was reported as cryptogenic in North-East Pacific (Needles & Wendt, 2013) and alien in Hawaii (Carlton & Eldredge, 2009) and East/West coast of South Africa (Mead et al., 2011).
- ***Ericthonius pugnax***: Non-indigenous species / Potential invader. This species was first described by Dana in 1853 from the coast of Japan. It has a wide Indo-Pacific distribution including Australia, New Zealand, Papua New Guinea, Korea, Malaysia, India, Madagascar and Mauritius (Hirayama, 1985). Recently, it was also reported from South Africa, on mussel rafts adjacent to Port Elizabeth Harbour (Milne & Griffiths, 2013).
- ***Ericthonius punctatus***: native / potential invader. E. punctatus is native from the North Atlantic, distributed from Norway to tropical west Africa including the British Isles and the Mediterranean (Myers & McGrath et al., 1984). It has also been indicated as NIS in Argentina (Albano, 2012; Rumbold et al., 2016) and Mauritius (Appadoo & Myers, 2004).
- ***Hexapleomera* spp.**: Unassigned status. This genus had long been considered to be monotypic, comprising a cosmopolitan species predominantly commensal on turtles or manatees. However, Dr. John Bishop found a distinct species of *Hexapleomera* living on boat hulls in a marina in Plymouth (UK) in 2009 and 2010. Bamber (2012) re-assessed the literature of this taxon and re-examined material from eastern Mediterranean. He proposed five species within the *Hexapleomera robusta* species complex, from which several were found in artificial habitats and could be candidates for the potential invader category. *Hexapleomera* wombat was associated to boat hulls, located in Queen Anne's Battery Marina in Plymouth (UK) and several marinas in Brittany (France). *Hexapleomera moverleyi* (before *Pancoloides moverleyi* Edgar, 2008) was collected from pylon-scrapings in Port Hobart, in Tasmania. *Hexapleomera satella* (before H. robusta sensu Bamber et al., 2009) was found in Tripoli (Libya) and Beirut (Lebanon) harbours. On the other hand, *H. robusta* sensu Sieg (1980) is the Mediterranean *Hexapleomera* commensal with turtles, whose distribution also includes the Northwest Atlantic, possibly the Caribbean (Heard et al., 2004), and probably Brazil. Given the lack of information about the distribution on artificial substrates of these species out of the Atlanto-Mediterranean region, a precautionary approach was followed here to avoid making mistaking this classification. Further studies are needed to continue shedding light into this species complex.
- ***Jassa marmorata***: Cryptogenic / Potential invader. It has global distribution originally described from New England (USA). The putative native range is either the Atlantic coast of North America and Gulf of Mexico or the Mediterranean and Black Seas, since in both areas it occurs in natural habitats. There is controversy about its status in the North-East Atlantic and it has been considered an introduced species in numerous locations (see Marchini & Cardeccia, 2017). These authors point to the North Atlantic as the putative native region but explain that a native origin

in the South Atlantic also may be a possibility. The non-indigenous status in Europe, Africa, and South America (Atlantic coast) cannot be assured and therefore considered it as a cryptogenic species, with a clear invasive potential.

- ***Jassa slatteryi***: Cryptogenic / Potential invader. The type locality is California although there is some controversy surrounding this. It is distributed worldwide and considered an introduced species in numerous locations: South Africa, eastern and western Argentina coasts, Japan and New Zealand; repeatedly found associated with harbours, bays and ships (see Marchini & Cardeccia, 2017 and references therein). According to these authors, *J. slatteryi* is a cryptogenic species that has undergone several misidentifications and whose native versus introduced range is yet to be clarified. After conducting a molecular analysis, Pilgrim & Darling (2010) suggest the Pacific coast of North America as the native range for *J. slatteryi*, however, no eastern Atlantic or Mediterranean populations were included in that study for comparison. We hereby follow a precautionary approach and consider it cryptogenic species with a clear invasive potential.
- ***Leptochelia cf savignyi***: Unassigned status. Species of the genus *Leptochelia* represent some of the most challenging tanaidaceans to identify, especially the members of *Leptochelia dubia* complex. Leptochelia savignyi Krøyer, 1842 was originally described from Madeira, and has been subject of some confusion over the last 150 years, including erroneous synonymy with *L. dubia* Krøyer, 1842. *Leptochelia savignyi* was considered virtually cosmopolitan occurring in Macaronesia, Atlantic coast of Europe and Africa, the Mediterranean Sea, South Africa, eastern and western coasts of North America, Brazil, the Indo-West Pacific, Hawaii and Tuamotu Archipelago (Holdich & Jones, 1983). Bamber et al. (2010) pointed out the error in attributing this cosmopolitan tag to either *L. dubia* or *L. savignyi*. They defined *L. savignyi* sensu stricto to be distributed from the English and French coasts to the Isles of Scilly, northwestern Spain, the Azores and Madeira; and suggested that past Mediterranean samples belong to *L. savignyi* sensu stricto and not *L. dubia*. Since controversy still surrounds this issue, a precautionary approach was followed here, until further re-examination of Mediterranean material is undertaken, especially from artificial habitats. We stress the need of paying attention to this group with regards to marine introductions.
- ***Mesanthura cf. romulea***: Non-indigenous species / Potential invader. The genus Mesanthura comprises 45 species predominantly distributed in tropical to warm temperate seas, living mainly in shallow cryptic habitats. The first record of this species in the Mediterranean Sea, identified as 'Mesanthura sp.', was from Lake Burullus, a brackish lagoon situated in the coast of Egypt (Samaan et al., 1989). The second record occurred in the harbours of Salerno and Taranto (Italy) in 2000, where it was well-established (Lorenti et al., 2009). These authors suggested an exotic origin for this species, since there is a total lack of prior records of the genus *Mesanthura* on a basin scale and the mentioned records occurred in confined areas, lagoons and harbours, which are likely habitats for introductions. The samples from Italy are most similar to the species *Mesanthura romulea*, described by Poore & Lew Ton (1986) in New South Wales (Australia). In this work, we report a single specimen from Sorrento marina that we identified as Mesanthura cf. romulea.

- ***Monocorophium acherusicum***: Cryptogenic / Potential invader. Even though described from the Mediterranean Sea, this species has an incredible worldwide distribution and has been widely dispersed by shipping throughout history. This makes it particularly difficult to elucidate its true native versus introduced range. Records of this species include the Black Sea, Mediterranean Sea, Iberian Coast, South Africa, Canada, Mexico, Hawaii, Brazil, Mauritius, India, Thailand, Australia, New Zealand, Korea, China and Japan (see Marchini & Cardeccia, 2017); and its association with the fouling and ballast water vectors are clear (Crawford, 1937; Hurley, 1954; Barnard, 1971; Gollasch et al., 2000; Kitsos et al., 2005). Marchini & Cardeccia (2017) consider the North Atlantic as its putative native range, confirm its non-indigenous status in the Indo-Pacific region and in the Pacific American coast, and warn about the possibility that a complex of species is involved. Due to the huge uncertainty regarding this taxon, it was considered a cryptogenic species here with a clear invasive potential.
- ***Monocorophium sextonae***: Cryptogenic / Potential invader. Several authors consider it to be native to New Zealand and introduced to England, where it was first described by Crawford (1937), but was not present in previous studies there (Costello, 1993; Eno et al., 1997; Goulletquer et al., 2002; Nehring, 2002; Kerckhof et al., 2007; Zorita et al., 2013). Contrarily, Australian authors, consider it native from Europe and introduced to Australia and New Zealand (Hewitt et al., 2004; Ahyong & Wilkens, 2011). Commonly found in artificially made habitats such as harbours and lagoons (see Marchini & Cardeccia, 2017). These authors consider it could be either native to the southwest Pacific, alien in Europe and pseudo-indigenous in the Mediterranean, or native to Europe and introduced elsewhere. Given this uncertainty, it is classified here in the same category as the other corophiids: cryptogenic and potential invader.
- ***Paracerceis sculpta***: Non-indigenous species / Potential invader. *Paracercis sculpta* was first described by Holmes (1904) from San Clemente Island (California, USA). It is a widely distributed species, occurring along the North American Pacific coast from Mexico, California to Hawaii, and also Hong Kong, Australia and Brazil (Espinosa & Hendricks, 2002). It was first recorded in the Mediterranean in the Lake of Tunis (Rezig, 1978); and next in Italy from the Lagoon of Venice (Forniz & Sconfietti, 1983). It then spread to other Italian localities (Forniz & Maggiore, 1985; Savini et al., 2006) and the Strait of Gibraltar (Castelló & Carballo, 2001). The latest records are from the Thermaikos and Toroneos Gulfs in Greece (Katsanevakis et al., 2014), Grande-Motte in France (Marchini et al., 2015) and the Azores (Marchini et al., 2017). The authors, Katsanevakis et al., (2014) suggest that the small-scale distribution of P. sculpta may be due to the recreational boating vector, and predict further spread of the species.
- ***Paradella dianae***: Non-indigenous species / Potential invader. Paradella dianae is an intertidal species, first reported from Bahia de San Quintin, Baja California and Mexico (Menzies 1962). Its native range is the northeastern Pacific region and it is considered to have entered the Mediterranean Sea via hull fouling (Galil et al., 2008; Occhipinti-Ambrogi et al., 2011). It first Mediterranean record is from Civitavecchia, near Rome in the Italian coast (Forniz & Maggiore, 1985), and later from Alexandria (Egypt) (Atta 1991), Bay of Algeciras (Spain) in 1988- 1992 (Castelló & Carballlo 2001), Izmir Bay (Turkey) in 2004 (Çinar et al., 2008), Cyprus in 2003 (Kirkim

et al., 2010), Al Gazala Lagoon (Libya) in 2002 (Zgozi et al., 2002) and Fethiye Bay (Turkey) (Kirkim et al., 2015).

- ***Paranthura japonica***: Non-indigenous species / Potential invader. Paranthura japonica was first described by Richardson (1909) from Muroran (north Japan) and its native range only includes Japanese coast localities (Yamada et al., 2007), eastern Russia (Nunomura, 1975, 1977), and eastern China (Ong Che & Morton 1991; Li, 2003; Zhang et al., 2009; Wang et al., 2010). It was reported as a non-indigenous species in San Francisco Bay in 1993 (Cohen & Carlton, 1995) and Southern California in 2000 (Cohen et al., 2005). Between 2007 and 2010, it was first recorded in European waters, collected in several locations in Arcachon Bay (Bay of Biscay, France), where accidental introduction with oyster transfers is the most likely vector of introduction (Lavesque et al., 2013). Between 2010 and 2012 it was found in numerous localities from the Italian Peninsula: the Lagoon of Venice, La Spezia and Olbia harbours (Marchini et al., 2014) and Taranto (Lorenti et al, 2015). Next, it was reported in La Grande Motte marina (southern France near Montpellier) (Marchini et al., 2015) and Tunisia (Tempesti et al., 2016).
- ***Phtisica marina***: Cryptogenic / Potential invader. Its type locality is Zeeland (the Netherlands) and it is widely distributed. Records include the northeastern Atlantic, from Norway to North Africa (Eagle, 1973; Fincham, 1973; Marques & Bellan-Santini, 1991; Guerra-García, 2001; Guerra-García, 2002; Wernberg et al., 2004; Fredriksen et al., 2007; Guerra-García et al., 2012); Canary Islands (Riera et al., 2003), Azores (Guerra-García, 2004; Zeina et al., 2015), Madeira (Zeina et al., 2015); northwestern Atlantic, from North Carolina to the Gulf of Mexico (Sedberry, 1989; McCain, 1968; Foster et al., 2004); Caribbean and South America (Lalana et al., 2005; Serejo, 1998: McCain, 1968). Also widely distributed in the Mediterranean and Black Seas (Bellan-Santini et al., 1993), northwestern Pacific (Irie, 1958; Arimoto, 1980), and found in a port in California (Fairey et al., 2000). Zeina et al. (2015) calls for caution regarding P. marina since many specimens may have been quickly assigned to this species only on the basis of pereopod 3 and 4 fully developed, which is a very distinctive character. They advise a re-examination of the material from the Mediterranean and Atlantic, combined with molecular studies. Given its wide distribution and its potential for to misidentifications, as well as possibility dealing with a complex of species, here this taxon is considered cryptogenic.
- ***Sphaeroma serratum***: Native / Potential invader. According to Jacobs (1987) the species distribution range includes the eastern Atlantic, from the British Isles to Morocco, plus the Mediterranean, the Black Sea and the Suez Canal. It has also been found in South Africa (Kensley, 1969) and West Australia (Holdich & Harrison, 1983). Holdich & Tolba (1985) pointed out that the last records are probably a result of shipping. Due to its invasion history elsewhere, this species is treated here as a potential invader.
- ***Sphaeroma walkeri***: Non-indigenous species / Potential invader. *Sphaeroma walkeri* was first described by Stebbing (1905) in Ceylon (now Sri Lanka, Indian Ocean). It was first recorded in the Mediterranean Sea in 1924, collected from Port Said (Egypt) from ship hulls (Omer-Cooper 1927). Subsequently, it was reported from Toulon (France) in 1977 (Zibrowius, 1992) and Alicante (Spain) in 1981 (Jacobs 1987). Years later it was found again on boat hulls in Haifa Harbour (Israel) (Galil,

2008), and was confirmed to be completely established in Tunisia harbours and lagoons (Ben Amor et al., 2010). In 2010-2011 it was first recorded in Italian waters; in La Spezia, Olbia and Lampedusa harbours (Lodola et al., 2012). It is considered to be a Lessepsian migrant due to its presence on boat hulls in the Suez Canal.

- ***Stenothoe georgiana***: Non-indigenous species / Potential invader. Stenothoe georgiana was first described by Bynum & Fox (1977) from Chesapeake Bay (Virginia) and Florida. It is distributed from Chesapeake Bay to Brazil, including North Carolina, Georgia and Florida records (Bynum & Fox, 1977). In 2010 it was first found in the Mediterranean Sea, and associated with algae, cnidarians and mussels in fish farms in Alicante and Murcia (Spain) (Fernández-González & Sánchez-Jerez, 2017).
- ***Stenothoe valida*****:** Cryptogenic / Potential invader. It was first described from Rio de Janeiro. Krapp-Schickel (2015) hypothesized that the previously thought cosmopolitan "S. valida" was actually a complex of species. There are reports as alien or cryptogenic species from Hawaii, Spain, California, Gulf of Mexico, Australia and New Zealand (Cohen et al., 2005; Martìnez & Adarraga, 2006; Carlton & Eldredge, 2009; Ahyong & Wilkens, 2011; Paz-Ríos et al., 2013). Marchini & Cardeccia (2017) consider it a cryptogenic species. They point out that the wide distribution can be due to human transport or to the sum of multiple restricted distributions of distinct local species belonging to a complex.
- ***Tanais* spp.**: Unassigned status. The taxonomy of the Tanaidae family remains quite problematic, since new species have been discovered since the review of Sieg (1980) and also from idiosyncrasies found in the methods applied by Sieg (see Edgar, 2008). Some species may represent complexes of cryptic species, so matches with Sieg's illustrations are very susceptible to misidentifications. Moreover, some of the characters applied by Sieg vary with growth and also between populations. *Tanais dulongii* is one of the main cases. In Sieg (1980) it was reported from British waters, Azores (probably mistaken and belonging to *T. grimaldii* according to Bamber & Costa, 2009), the Faroes, Norway, Denmark, Holland, Belgium, France, Spain, the Mediterranean and Black Seas, east coast of North and South America and Southwest Australia. Bamber et al. (2012) also reported it from Capo Verde and Madeira. According to Edgar (2008) there may be cryptic species similar in appearance to *T. dulongii*. These authors hypothesize it is a non-indigenous species translocated to Australia by shipping and established in the Swan estuary since 1943; however, the records in Australia are still doubtful. Therefore, a precautionary approach was followed here to avoid identification mistakes, and stress the need to pay attention to the group with regards to marine introductions. A comprehensive review of the phylogeny of tanaids including genetic analysis and re-examination of type material is urgently needed.
- ***Zeuxo* spp.**: Unassigned status. Individuals belonging to this genus are very difficult or impossible to identify without dissection of mouthparts (Sieg, 1980), which requires specialistic taxonomic expertise. Moreover, Larsen (2014) and Larsen et al. (2014) determined the previously thought cosmopolitan *Zeuxo normani* (Richardson 1905) is actually a large complex including species from California, Japan, Korea, Australia, France and Spain, several of which are found in harbours. They also revealed that a number of currently considered diagnostic characters for species

identification, such as the number of uropod articles, are variable even within a single individual and thus unreliable for identification. *Zeuxo (Parazeuxo) coralensis* represents a similar case, known from warm and temperate waters from the Maldive Islands (type locality), Red Sea, eastern Mediterranean, Japan, Brazil, the Pacific coast of Panama (see Sieg, 1980) and Florida (Heard et al., 2004). In addition, if mature adults are unavailable, is very difficult to distinguish between other species reported in in the Mediterranean species such as *Z. fresii* (i.e., the first species to be recorded in this Sea) and *Z. turkensis* (Larsen et al., 2014b). Therefore, a precautionary approach was followed to avoid misidentification. We stress the need of paying attention to the group regarding marine introductions and of conducting a deep revision of this genus in the Mediterranean Sea

Table S3. List of sampled marinas divided according to national and administrative divisions. Coordinates in World Geodetic System 1984, number of berths, maximum vessel length it can host, and use (R=recreational, Fe=ferrylines, Fi=Fishing, H=hydrofoil) are given for each sampled marina. (References: ΔAEAN, 2017; Ece Marina, 2017; Explore Crete, 2017; Fédération français des Ports de Plaisance, 2017; Heraklion Port Authority, 2017; Marine Traffic, 2017; Netsel Marina, 2017; Pagine Azzurre, 2017; Port Camargue, 2017; Port du Cap d'Agde, 2'17; SeturMarinas, 2017).

Country	**Region**	**Region subdivision**	**City/Town**	**Marina**	**Coordinates**	**# berths**	**Vessel length (m)**	**Use**
FRANCE	Provence-Alpes-Côte d'Azur	Alpes-Maritimes Department	Antibes	Port Vauban	43°35'08''N 07°07'38'' E	1700	165	R
FRANCE	Languedoc-Roussillon-Midi Pyrénées	Gard Department	Port Camargue	Port Camargue	43°31'18''N 04°07'36''E	5000	60	R
FRANCE	Languedoc-Roussillon-Midi Pyrénées	Hérault Department	Cap D'Agde	Port principal	43°16'22''N 03°30'31''E	3100	30	R
ITALY	Latium	Roma Province	Ostia	Porto turistico di Roma	41°44',15''N 12°14',59'' E	796	60	R

ITALY	Campania	Naples Province	Sorrento	Marina Piccola di Sorrento	40°37'48''N 14°22'33'' E	280	-	R, Fe
ITALY	Campania	Naples Province	Ischia Island	Porto d'Ischia	40°44'41''N 13°56'33'' E	200	50	R, Fe, Fi
			All samples from these 5 marinas were pooled and analyzed as only one marina.	Casamicciola	40°44'56''N 13°54'24'' E	275	80	R
				Lacco Ameno	40°45'09''N 13°53'34'' E	12	90	R, Fe
				Forio	40°44'19''N 13°51'37'' E	350	40	R,H
				Sant'Angelo	40°41'48''N 13°57'38'' E	100	21	R, Fi
GREECE	Crete	Heraklion regional unit	Heraklion	Old Venetian harbour	35°20'36''N 25°08'09''E	200	20	R, Fi
GREECE	Crete	Lasithi regional unit	Agios Nikolaos	Agios Nikolaos marina	35°11'10''N 25°43'00''E	255	70	R
TURKEY	Aegean	Muğla Province	Fethiye	Ece marina	36°37'25''N 29°06'05''E	460	60	R, Fi
TURKEY	Aegean	Muğla Province	Marmaris	Netsel marina	36°51'02''N 28°16'38''E	720	90	R

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2

THE ROLE OF RECREATIONAL BOATING AS A VECTOR: PART 2

The journey of hull-fouling mobile invaders: basibionts and boldness mediated dislodgement risk during transit

Adapted from: Martínez-Laiz G, MacLeod C, Hesketh AV, Konecny CA, Ros M, Guerra-García JM, Harley CDG (2021). The journey of hull-fouling mobile invaders: basibionts and boldness mediate dislodgement risk during transit. Submitted to Biological Invasions.

ABSTRACT. Vessel hull-fouling is responsible for most biopollution events in the marine environment, yet it lacks regulation in most countries. Although experts advocate a preventative approach, research efforts on pre-arrival processes are limited. We evaluated the performance of mobile epifauna during vessel transport via laboratory simulations, using the well-known invasive Japanese skeleton shrimp (*Caprella mutica)*, and its native congener *Caprella laeviuscula* as case study. The invader did not possess any advantage in comparison to the native species in terms of inherent resistance to drag. Instead, its performance was conditioned by the complexity of secondary substrate. Dislodgement risk was significantly reduced as we progressively added sessile fouling basibionts; which provided refugia and boosted the probability of *Caprela mutica* remaining attached from 7% to 65% in laboratory flow exposure trials. Interestingly, the invader exhibited significantly higher exploratory tendency and motility than its congener at zero-flow conditions. Implications in terms of *en-route* survivorship, invasion success and macrofouling management are discussed.

1. INTRODUCTION

In the marine environment, species introductions have increased in frequency in recent decades, with no signs of leveling off (Seebens et al. 2018; Tingley et al. 2018). The human-mediated dispersal of marine introduced species is increasing and is facilitated by the trade of a massive quantity of goods and services (*i.e*. vectors) (see IMO 2020; Grosholz et al. 2015; Carlton et al. 2017; Passarelli and Pernet 2019; Valls et al., 2016). Trade moves species beyond their native ranges when they are intentionally or inadvertently included as cargo, entrained as part of the transport process (*e.g.*, in ballast water), or attached directly to transport vessels (*e.g.*, biofouling). In particular, the accumulation of organisms attached to or associated with man-made underwater or wetted surfaces is responsible for between 55.5% and 69.2% of the currently established non-indigenous species (NIS) in coastal waters worldwide (Hewitt and Campbell 2010). Its economic costs are acknowledged worldwide, and considered substantial to the shipping industry (ACT, 2003; Schultz et al. 2010), shellfish aquaculture (Carman et al. 2010; Fletcher, Forrest and Bell 2013; Davidson et al. 2014), and the marine renewable energy sector (see Vinagre et al. 2020), among others. At present, the International Maritime Organization (IMO) recognizes the biosecurity risks associated to transport hubs like commercial merchant vessels and recreational boating (see GloFouling IMO project 2019, https://www.glofouling.imo.org/?lang=es), despite regulation being non-existent, insufficient, or still in its infancy in most countries. In 2008, Floerl et al. revealed that up to 80% of fouled recreational vessels arriving to New Zealand were carrying non-native taxa (NIS+cryptogenic species). Simard et al. (2017) established an infestation probability of 60-100% for most recreational vessels cruising marine ecoregions on the Atlantic Coast of Canada. Additionally, Ulman et al. (2019) showed that 71% of recreational vessels travelling in the Mediterranean Sea also harbored fouling NIS as inadvertent stowaways.

Experts agree that biosecurity needs to operate with a precautionary approach (Rout et al. 2011; Lewis, 2016; Faulkner, Robertson, and Wilson 2020). This implies that, in order to provide efficient management, efforts should be placed first in targeting the early stages of the invasion process (*i.e.* introduction phase) rather than later ones (*i.e.* spreading phase). Indeed, accidental human-mediated dispersal causes serious eco-evolutionary consequences from the very beginning of the invasion pathway: departure; transfer; and, finally, settlement in recipient environment (see Bullock et al. 2018). Therefore, the study of the transport process and the performance of fouling stowaways becomes of critical importance, since transportation represents the very first node of the process invaders must overcome (see Simberloff 2009). Nevertheless, the majority of literature dealing with invasion success focuses on the establishment and secondary spread phases rather than the initial steps; as noted by Marsico et al. (2010); Clarke Murray, Therriault, and Martone (2012); and Schimanski et al. (2017).

The probability of fouling organisms surviving the vessel voyage to a new destination is dependent on the interplay of multiple factors. Firstly, hull-associated organisms must withstand the impact of hydrodynamic conditions (*i.e.* currents and water flow) and the shear forces generated near the substratum. Different areas of a vessel (hull *vs* niche areas) experience different water velocities (Coutts et al. 2007; Davidson et al. 2010; Clarke Murray et al. 2011) that ultimately modify hydrodynamic selective pressure. Indeed, sessile organisms have developed a variety of surface adhesion mechanisms to avoid dislodgement (Rittschof et al. 2008; Clarke Murray et al. 2012; Kamino 2013; Kauano et al. 2017), but little information is available regarding mobile taxa. Although several studies have evaluated the post-transport survivorship of an epifouling assemblage in terms of diversity (Davidson et al. 2008; Chan et al. 2016; Schimanski et al. 2016), they do not target the strategies of mobile epifauna to cope with hydrodynamic stress. In fact, these small taxa inhabiting secondary substratum are generally overlooked (Chapman et al. 2005; People 2006; Wilhelmsson & Malm 2008; Marzinelli et al. 2009).

Basibionts – secondary biotic substrata – and sometimes habitat complexity, also play a critical role for these invasive epifauna from the early stages of colonization (Ros et al. 2020) until the range expansion phase (Gribben et al. 2020). When water flows over a solid surface, the portion in direct contact with the substratum experiences friction and the water-flow layers above are hindered, creating what is called a “boundary layer” (Schlichting 1979, Jumars and Nowell 1984). This layer has a different flow regime that may act as a refuge against the prevailing flow, and the complexity of substratum is one key factor modifying the level of this protection (Koehl 1982, 1984). In this regard, numerous studies have evidenced the role of marine habitat-forming species such as corals (Sebens et al. 1997), oyster reefs (Whitman and Reidenbach 2012), and mussel beds (O’Donnell 2008) in reducing water flow speeds and providing attachment surfaces (Norling and Kautsky 2007). In freshwater environments, like the Great Plains rivers, zoobenthos also benefits from physical habitat complexity when seeking refugia from abrupt flow events (O’Neill and Thorp 2011). Taking all this into account, basibionts are expected to modify the flow regime experienced by epifauna during the vessel transfer phase; however, such a role has not yet been experimentally addressed.

Finally, a series of inherent factors, or biological traits, may come into play when determining the performance of invasive stowaways. To date, studies have focused on exploring the influence of life stages, reproduction strategy, metabolism, and body size (Waterkeyn et al. 2010; Lenda et al. 2014; Valls et al. 2016; Schimanski et al. 2017) as well as thermal tolerance (Lenz et al. 2018) and desiccation tolerance during overland transport (*i.e*. trailered vessels) (Hillock and Costello 2013; De Ventura et al. 2016; Bollen et al. 2017; Kauano et al. 2017). Less attention has been paid to purely behavioral traits such as boldness (*i.e*. the tendency to explore). The shyness-boldness axis is one major temperament trait category in animals (Réale et al. 2007), where boldness and exploratory activity form a common behavioral syndrome, that is heritable and determinant in the colonization of novel environments and dispersal (see Cote et al. 2010a; Mazué et al. 2015 and references therein). This trait has turned out to be an important predictor of the invasion pattern of the mosquitofish *Gambusia affinis* (Cote et al. 2010b) and a good proxy for the distance travelled by the killifish *Rivolus hartii* (Fraser et al. 2001). In addition, Ros et al. (2020) determined that the propensity for unaided local dispersal was indeed critical in the initial stage of even low-motility invaders; and one of the components shaping the invasion potential in mobile epifauna.

Amphipod crustaceans are an excellent example of small mobile species that are poorly understood in terms of the dynamics of transport and introduction, yet are commonly dispersed by vessel traffic.

One striking example of an invasive amphipod is the 'Japanese skeleton shrimp' *Caprella mutica* Schurin, 1935, which has proved to be an extremely successful invader worldwide. This crustacean, native to sub-boreal areas of northeast Asia (Schurin, 1935; Vassilenko 1967; Arimoto, 1976; Fedotov, 1991), spread across both hemispheres in just 30 years via commercial and recreational vessels, aquaculture transfers, and occasionally on drifting algae (Schückel et al. 2010; Boos et al. 2011 and references therein; Daneliya and Laakkonen 2014; Almón et al. 2014; Peters and Robinson 2017). Indeed, it is the most common aquatic non-indigenous species reported as primary detection across the world in the period 1965-2015 (Table 2 in Bailey et al. 2020). This caprellid amphipod exhibits multiple invasiveness traits (Ashton et al. 2007; Matthews 2008; Boos et al. 2011), successfully displaces ecologically similar native species (Shucksmith et al. 2009), and increases the economic cost of maintenance work in the aquaculture industry (Boos et al. 2011). Yet, to our knowledge, no studies have explored how *C. mutica* or any other non-indigenous mobile crustacean surmount the challenges faced during transportation to new habitats.

Based on the aforementioned gaps of knowledge, the goal of this study was to evaluate the response of mobile invasive epifauna to a simulated vessel voyage, in order to gain insight into the factors facilitating or hampering this phase of the invasion process. We used the invasive amphipod *Caprella mutica* and its native congener *Caprella laeviuscula* Mayer, 1903, as model systems. This native species occurs exclusively in its native range, with no invasive character reported so far. We evaluated specifically: 1) the resistance of both species to hydrodynamic stress, by measuring their dislodgement risk after a simulated trip; 2) whether the sessile component of the fouling (basibionts) enhances the chances of the invader overcoming the vessel trip. 3) The boldness behaviour of both species at zero-flow conditions. Lessons learnt from this research will help to understand the factors driving the successful arrival of new biota to the recipient environment, as well as to better predict their future spread.

2. MATERIAL AND METHODS

2.1 Sampling procedure

The study area was located in the Salish Sea in southwestern British Columbia (Canada). Here, *C. mutica* has been present from at least the 1990s (Frey et al. 2009), and represents one of the most widespread marine invaders (Gartner et al. 2016), usually coexisting with the native *C. laeviuscula* in coastal ports and marinas. Sampling was conducted in two marinas in Burrard Inlet: Royal Vancouver Yacht Club (49°17'42"N 123°07'41"W) and Reed Point Marina (49°17'29"N 122°53'10"W) (Fig. 1). Fauna was collected from artificial structures (encompassing floating docks, ropes and boat hulls) in October and November 2019. The material included: the invasive caprellid amphipod *C. mutica* Schurin, 1935; the native *C. laeviuscula* Mayer, 1903; the fine filamentous hydrozoan *Obelia dichotoma* (Linnaeus, 1758), which is a preferred caprellid substratum (Lim & Harley 2018); the mussel *Mytilus trossulus* Gould, 1850; and the encrusting bryozoans *Celleporella hyalina* (Linnaeus, 1767) and *Membranipora membranacea* (Linnaeus, 1767), associated with *O. dichotoma* and *M. trossulus*. Most of the sorting procedure for caprellids was completed *in situ* to make sure there were enough individuals for experiments. *O. dichotoma*, *M. trossulus*, and their associated encrusting bryozoans were not further manipulated. All material was transported to the lab in aerated and insulated seawater containers. Material used for the final experiments was collected exclusively from Royal Vancouver Yacht Club on the 12th and 19th of November 2019.

Figure. -- Sampling locations in the southwest coast of British Columbia

2.2 Laboratory set up and experimental design

While kept in the laboratory, animals were distributed in multiple small tanks (20 x 15 x 30cm) covered with a plexiglass lid, where they received a continuous aerated seawater flow at 11.4°C under a photoperiod of 9 hours light: 15 hours dark. Multiple preliminary tests were conducted in order to fix the necessary parameters for the experimental set up, with a focus on how to manipulate animals causing the minimum disturbance prior to the experiments. Around 200 caprellid individuals were required for preliminary tests; and a total of 340 individuals were used for the final assays; each caprellid was used only once. These tests were conducted in the following sequence: on day 1, field sampling was carried out and fresh material was taken to the laboratory and placed in the small tanks. On day 2, the necessary number of individuals from both species were sorted and placed in separate tanks with some natural substratum and artificial mesh for them to cling to. The rest of the material was kept in additional holding tanks to avoid unnecessary manipulation. We allowed acclimation for 48 hours, and experiments were conducted on day 4. This sequence was repeated during two consecutive weeks until completion of the following investigations:

1) Response of *C. laeviuscula* and *C. mutica* to water flow

In order to evaluate interspecific differences in the response to simulated boat travel, caprellids were placed on an experimental plate and exposed to a 30km h^{-1} water flow for 30 minutes. This chosen speed aims to represent common sailing/cruising conditions of recreational vessels on an average trip. The aquarium setup built for this purpose is shown in Figure 2. The experimental plate consisted of a 5 x 10cm piece of a 1mm-mesh integrated in a 14 x 14cm rigid structure, which was affixed with two bars inside a large tank (93 x 48 x 46cm) filled with seawater. The water flow was generated with two submersible pumps E160713 LISTED 8C99 MODEL 12B (300 L h^{-1} flow rates, https://wholesalepumps.com) and directed towards the platform in order to create a laminar flow parallel to the mesh. At the opposite end of the tank, a double layer of mesh was placed as a barrier to catch the dislodged caprellids, and to avoid having them redirected by the water flow back into the experimental plate area.

The experiment was conducted separately for the two species. At the beginning of each simulation, a total of 6 caprellids in the case of *C. mutica* and 12 in the case of *C. laeviuscula* were placed on the mesh with a paintbrush (Fig. 2C). These numbers were selected based on preliminary tests as well as their natural densities found in the field. Once the individuals had recovered from their transfer and attached to the mesh, the water flow was initiated and we quantified the individuals that remained attached after 2, 3 and 30 minutes. A total of 10 randomized trials (replicates) were conducted for each species, resulting in 180 individuals (120 of *C. laeviuscula* and 60 of *C. mutica*) being used in this experiment.

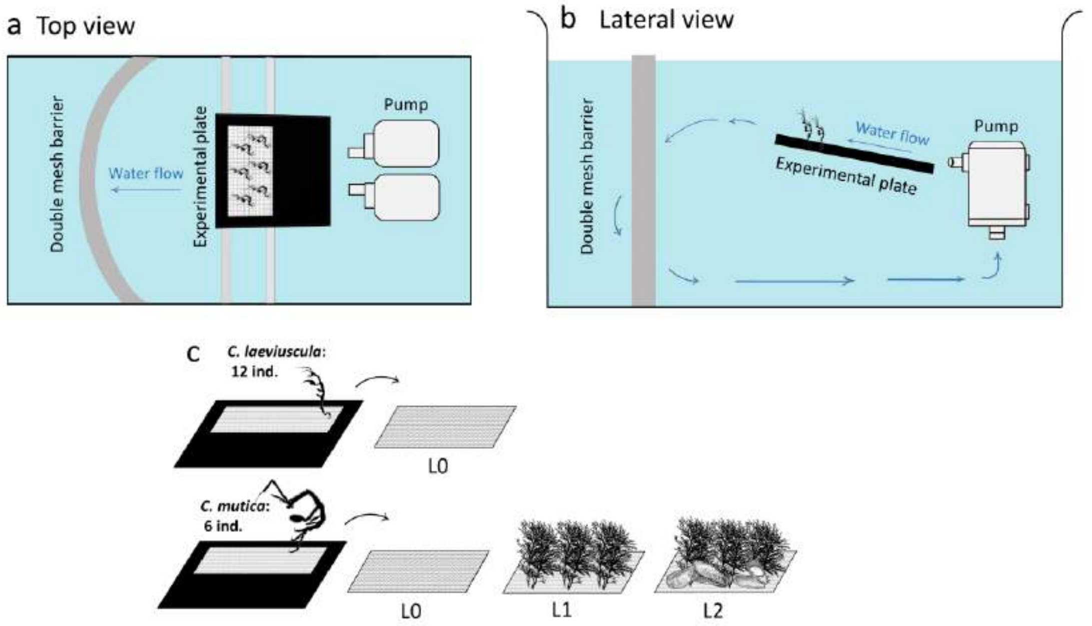

Fig. 2abc Aquarium setup for evaluating caprellids' response to water flow (experiments 1 and 2), and behaviour strategies (experiment 3). a) and b) Top and lateral views of the aquarium, respectively. Submersible pumps, experimental plate, location of the caprellids, wáter flow direction and barrier mesh for dislodged caprellids are indicated. The experimental plate was affixed to the tank walls with two bars (visible in a). c) number of individuals used for each trial in experiments 1 and 2; and levels of refugia treatments for each species

2) Response of *C. mutica* to water flow when offered different levels of refugia

To evaluate the response of the invasive species when refugia were available, the same assay was conducted with two additional levels of fouling complexity: level 1 (L1; mesh with pieces of *O. dichotoma* attached) and level 2 (L2; mesh with pieces of *O. dichotoma* plus mussels) (Fig. 2C). These are the natural fouling taxa to which our two caprellid species are associated in the field; and so they were selected for this experiment. The individuals of *C. mutica* remaining after a 30-min simulated vessel trip were recorded for each treatment, and compared with the previous experiment (L0; bare mesh). A total of 120 individuals of *C. mutica* (60 for each treatment group) were used for this experiment.

For experiments 1 and 2, the heart rate (hereafter HR) of *C. mutica* individuals that remained attached was quantified immediately at the end of the trip simulation. As HR varies from individual to individual, we would ideally have measured this parameter before and after the simulation. However, this would have notably stressed the specimens and may have biased our results. Therefore, measurements were taken exclusively after the simulation, and compared between the three different refugia treatments. HR was chosen as a proxy for performance against biomechanical stress, based on previous work quantifying the physiological tolerance of crustaceans to abiotic variables (DeFur and Mangum 1979; Lim and Harley 2018; McGaw et al. 2018). For this procedure, some individuals from those remaining attached were haphazardly selected after the simulation and transferred on a small piece of *O. dichotoma* into a Petri dish filled with treatment water, for filming under a dissecting microscope following the methods of Lim and Harley (2018). Previous tests indicated that water temperature in the Petri dish did not vary during the procedure. Just before starting the observation, we drained most of the water, leaving only enough to surround the caprellid, which minimized their movement. The heart beat visible in the second pereonite was filmed for approximately one minute to obtain, when possible, 2-3 sequences of at least 20 seconds. In total, we obtained HR data for one individual from treatment L0 (the only one remaining in the mesh), for 10 individuals from treatment L1, and for 15 individuals from treatment L2. For each individual, HR was calculated as the average number of heart beats counted in the 20-second recordings. The distance from the top of the head to the end of the 7th pereonite was measured to account for among-individual variation in body size.

3) Observation of exploratory behavior

In order to gain insight into the boldness of each species, we filmed caprellid behavior when placed alone on the mesh substratum and left without any further disturbance or manipulation. Recording was done over a two-minute period and repeated for a total of 10 adult males and 10 adult females for each species (in total, 40 individuals used). Adult males were differentiated by the presence of a fully developed gnatopod 2 (second forelimb on caprellids), and females by the presence of a fully developed brood pouch. Footage was analyzed using Windows Media Player and ImageJ, and the following variables were quantified: time spent resting (*i.e.* no shifting/displacement of pereopods); distance covered when moving, quantified as the longest distance (measured as a straight line) from the initial to the final position; and probability of actively departing from the experimental plate into the water column (*i.e.* swimming).

2.3 Statistical analysis

The probability of success (*i.e.* probability of remaining attached to the mesh) in experiments 1 and 2, as well as the probability of actively departing into the water column in experiment 3 were modeled through a binomial distribution (logit link) using Generalized Linear Models (GLM; McCullagh and Nelder, 1989). Explanatory variables considered were: 'Species' (2 levels: *C. mutica* and *C. laeviuscula*) and 'Time of exposure' (4 levels: 0, 2, 3 and 30 minutes) in experiment 1; 'Level of refugia' (3 levels: L0, L1 and L2) in experiment 2; and 'Species' and 'Sex' in experiment 3. Additionally, to analyze *C. mutica*'s HR, the response variable number of beats/minute was modeled through a gamma distribution (inverse link), also with GLM. The explanatory variables considered for HR models were level of refugia and body size (in mm).

GLM model selection was carried out based on second order Akaike's information criterion (AIC) for small sample sizes (AICc, Burnham & Anderson 2002), as a measure of relative quality, and the preferred model was identified by the minimum value of AICc. The Akaike weight (w_i) of each model was calculated within the significant models, *i.e.* those with < 2AIC units of difference from the model with the smallest AIC. All significant models were used to calculate model-averaged parameter estimates and unconditional 95% confidence intervals to better represent model-selection uncertainty. The relative importance of each predictor was based on the combined Akaike weights ($\sum w_i$) for all significant models that contained a given predictor. Variability explained by the best models was computed using Phi coefficient for binary data (Jackson et al. 1989) and using R^2 for the gamma model. The performance of the significant probability binomial models was assessed using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The goodness of fit of our HR data to a gamma distribution was assessed by using the Kolmogorov-Smirnov (KS) test.

In experiment 3, mean and standard error were calculated for the remaining variables: 'time spent resting' and 'distance covered when moving', and the results were analyzed with a two-way ANOVA. Here we considered the factors 'Sex' (two levels, fixed) and 'Species' (two levels, fixed). Homogeneity of variances was checked with Levene's test. All data analysis was performed using R studio version 4.0.2 (R Core Team, 2020)

3. RESULTS

3.1 Response to water flow

As soon as the experimental flow regime was initiated, caprellids lost their natural body position (*i.e.* arched body with sporadic swinging when resting), and aligned their body in a parallel position (Takeuchi and Hirano 1995; Guerra-García et al. 2002), vibrating due to the water flow and with their pereopods as the only grip point. The probability of caprellids remaining attached to the mesh at L0 significantly decreased through time ($p<0.0001$); and differed among the two species ($p<0.0001$) (AUC

= 0.91, phi coefficient = 0.94) (Table 1AB). The native *C. laeviuscula* exhibited a better performance than the invasive *C. mutica* (Fig. 3AB); for *C. laeviuscula*, only 16-33% of the individuals were detached after 30 minutes, whereas in the case of *C. mutica*, 67-100% of the individuals were lost after just the first two minutes of exposure to water flow.

Table 1. (A) Model selection for predicting probability of caprellids remaining attached to the mesh at level 0 (without refugia). Akaike's information criterion corrected for small sample size (AICc), AICc weights (wi), and phi coefficient are presented as well. Variables: 'Species' (*C. mutica* and *C. laeviuscula*); and 'Time of exposure to water flow' (in min). **(B)** Model averaged coefficients (β) of explanatory variables present in the significant model for probability of success. Adjusted standard error (SE) of model averaged coefficients, including z-values and P values

A Best model	AICc	wi	Phi
response: Probability of success			
1. Species + Time of exposure	133.85	0.10	94

B Predictor	β	Std. Error	z	P
Intercept	2.59	0.24	10.4	<2e-16***
Species *C. mutica*	-4.32	0.32	-13.28	<2e-16***
texposed	-0.06	0.01	-5.4	7e-08***

***Significant coefficient value p<0.001

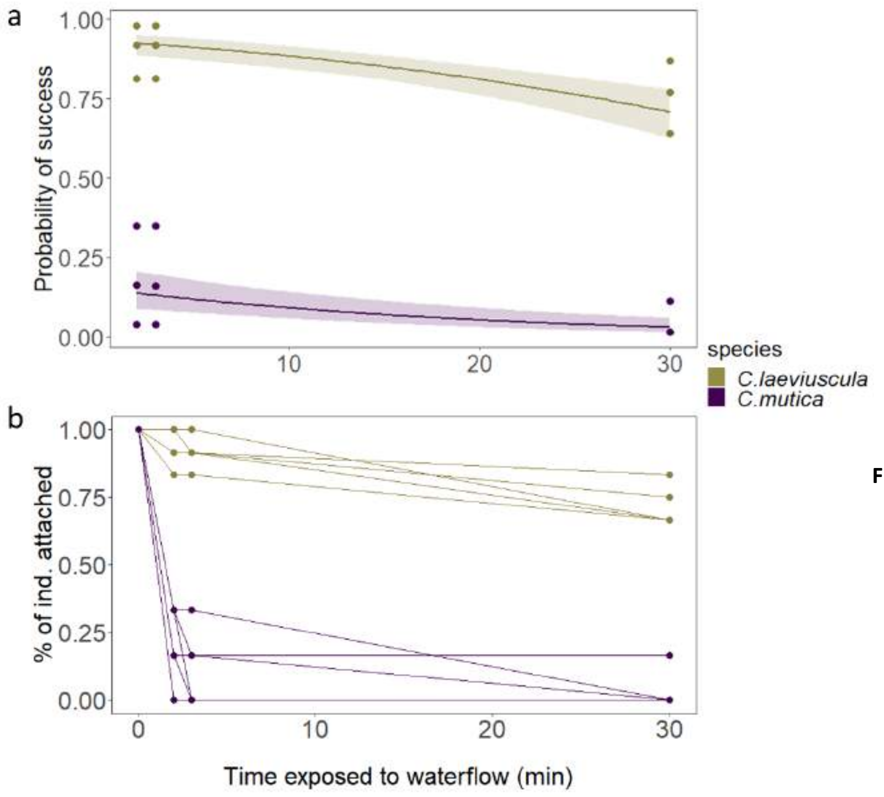

Fig. 3ab Response to waterflow of *C. laeviuscula* versus *C. mutica* as a function of time (experiment 1). a) binomial model output of predicted probability of success (*i.e.* probability of remaining attached to the mesh); and b) observed individuals remaining attached

Meanwhile, when offered different levels of substratum complexity (L1: mesh with hydroids attached; L2; hydroids plus mussels), *C. mutica* performed much better than without them (L0), becoming increasingly likely to remain attached as we increased the complexity (p=0.002 for factor L1, p<0.0001 for factor L2, AUC = 0.87, phi=0.97) (Table 2, Fig. 4).

Table 2. Model predicting probability of *C. mutica* remaining attached to the mesh when offered different levels of secondary substrate complexity. Variable: 'Refugia' (L0 - without secondary substrate, L1 - with hydrozoans, and L2 - with hydrozoans and mussels). Model averaged coefficients (β) of explanatory variables present in the significant model for probability of success. Standard error (SE), z-values and p values are included. Note that, for calculating the parameters of the final model, factor variable 'level L0' is taken as the baseline for the model.

Predictor	β	Std. Error	z	P
Intercept	-4.08	1.00	-4.04	5.27e-15***
Refugia L1	3.23	1.05	3.09	0.002**
Refugia L2	4.70	1.04	4.50	6.86e-06***

***Significant coefficient value p<0.001

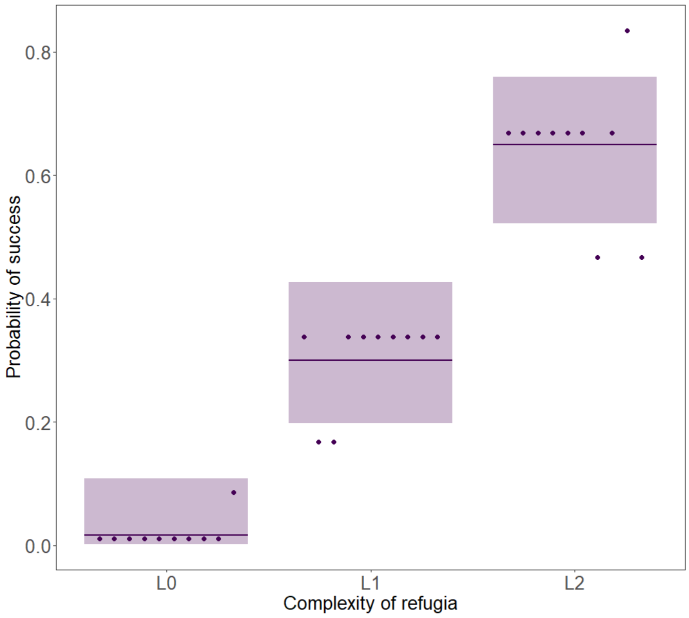

Fig. 4 Response of *C. mutica* to waterflow when offered different levels of basibiont complexity (experiment 2). Binomial model output predicting probability of remaining attached (success) at L0 (bare mesh), L1 (mesh with pieces of *O. dichotoma* attached) and L2 (mesh with pieces of *O. dichotoma* plus mussels). Violin plot shows data distribution for each group and and outliers (grey dots)

The invasive species exhibited a reduced heart rate when exposed to L1 and L2 refugia (Fig 5). Note that, due to the limited success of the species in the absence of refugia, only one individual could be recovered for analyzing the HR at the lowest level of substrate complexity (L0); therefore, this level was removed from the GLM model. When modelling the HR, only the factor body size was selected, but it was not significant (GLM gamma, p=0.44, p from KS test=0.79, R^2=0.11) (Table 3).

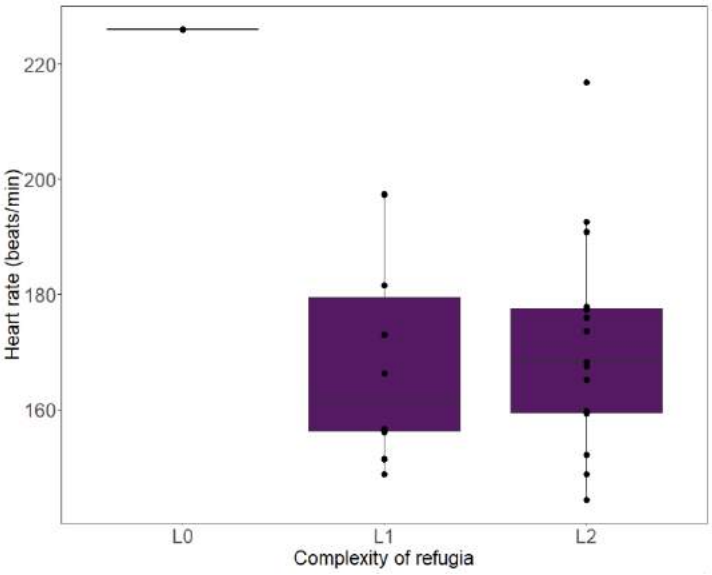

Fig. 5 Heart rate of *C. mutica* after exposure to waterflow with the three levels of refugia

Table 3. **(A)** Model selection for predicting heart rate (beats/min) of *C. mutica* when exposed to waterflow and offered different levels of secondary substrate complexity. Akaike's information criterion corrected for small sample size (AICc), AICc weights (wi) and AICc difference between the AICc of each model and the AICc of the best fitted model (Δi) were used for comparison. Variables: 'Refugia' (L1 and L2); and 'Body size' (in mm). **(B)** Model averaged coefficients (β) of the explanatory variable present in the significant model for probability of success. Adjusted standard error (SE), z-values and p values are included.

A Best models	df	AICc	Δi	wi	LogLik
response: Probability of success					
1. Size	3	218.28	0.00	0.53	-105.57
2. NULL	2	218.50	0.22	0.47	-106.98

B Predictor	β	Adj. SE	z	P
Intercept	5.18e-3	4.67e-4	11.51	<2.03e-16***
Size	2.27e-5	2.87e-5	0.77	0.44

***Significant coefficient value p<0.001

3.2 Behavioural observations in zero-flow conditions

Without hydrodynamic disturbance, caprellids always maintained their natural (upright, arched) body positions when attached to the mesh, but the two species differed in their movements. Firstly, *C. mutica* spent significantly less time resting than *C. laeviuscula* (ANOVA, p<0.001), with a significant interaction between species and sex (p=0.03) where male *C. mutica* tended to rest less than females, but male and female *C. laeviuscula* resting times were similar (Table 4A, Fig. 6A). Secondly, the maximum distance covered by *C. mutica* when crawling was, for both sexes, significantly higher than that of *C. laeviuscula*, which instead stayed rather close to the area of the mesh where they were initially placed (ANOVA, p=0.01) (Table 4B, Fig. 6B). Finally, the tendency for actively departing into the water column was significantly higher for *C. mutica*, particularly in males (expected probability= 90%; p=0.01, AUC=0.94, phi=0.76) in comparison to females (expected probability= 36%) and to the native species (expected probability= 0%), which never detached from the mesh (Table 4CD, Fig. 6C).

Table 4. Results for behavior observations of *C. mutica* and *C. laeviuscula*. ANOVA output for the variable 'time spent resting'(in sec) **(A)**; and for the variable 'maximum distance covered' (in mm) **(B).** Factor 'species' (2 levels) and 'sex' (2 levels) were fixed. N_A=41, N_B =40. **(C)** and **(D):** Model selection and model parameters for predicting the variable 'probability of voluntary detachment' from the mesh, for both sexes of the species *C. mutica* (Note: none of the individuals of *C. laeviuscula* actively detached).

A	dF	MS	F	P
Species	1	25351	26.78	8.8e-6***
Sex	1	2002	2.12	0.155
Species : Sex	1	4601	4.86	0.034*
RES	36	947		
Total				

*Significant coefficient value p<0.05

***Significant coefficient value p<0.001

B	dF	MS	F	P
Species	1	7190	26.36	9.3e-6***
Sex	1	28	0.103	0.75
Species : Sex	1	387	1.419	0.24
RES	37	273		
Total				

***Significant coefficient value p<0.001

C Best model	AICc	wi	Phi
response: voluntary detachment			
1. Species + Sex	27.57	0.91	76

D Predictor	β	Std. Error	z	P
Intercept	-21.49	3598.06	-0.01	0.99
Species *C. mutica*	20.69	3598.06	0.01	0.99
Sex male	1.63	0.65	2.5	0.01*

*Significant coefficient value p<0.05

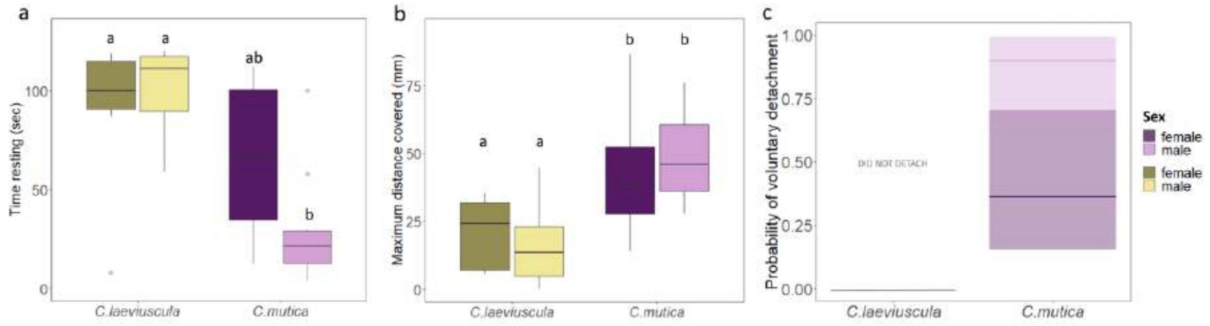

Fig. 6abc Behaviour observations of both caprellid species and sexes in no distress conditions (experiment 3). a) Time spent resting (no displacement of pereopods) in seconds. b) Maximum distance covered when crawling (in mm). c) Binomial model output predicting probability of actively departing the plate into the water column (*i.e.* swimming). The line shows the expected probability and the shading band the confidence interval (note: the native species did not jump to the water column). Significant differences computed by Tukey HSD test are indicated with lowercase letters

4. DISCUSSION

4.1 Strategies to overcome dislodgement

This study suggests that the invasive *C. mutica* does not bear an inherent advantage over its native congener in terms of resistance to hydrodynamic forces. The native species was the most successful in avoiding dislodgement during our simulated transport. In accordance, it exhibited limited exploratory tendency (lack of tendency to detach from the mesh into the water column, limited movement and long resting times) and a strong grip to the (artificial) substrate even in zero-flow conditions. These characteristics were also notable during manipulation in the laboratory and in natural field conditions: *C. laeviuscula* was extremely reluctant to leave its substratum, not even to escape manipulation (personal observation). In contrast, the invasive species struggled to resist the drag experienced in the absence of fouling. This performance may result from multiple non-exclusive factors. Firstly, the body morphology of a biofouling organism is a key factor determining how hydrodynamic forces will act upon it. In particular, the height of protrusion from the settlement plate will determine whether the organisms find themselves in the low or high-energy region of this boundary layer and, in turn, how much they will be affected by vessel translocation (Coutts et al. 2010). Specimens of *C. mutica* had a larger body size and height of protrusion than *C. laeviuscula* (mean length = 16mm vs. 13.5mm; maximum length= 27 mm vs. 17mm, for males). These two factors imply that, for a similar morphological body plasticity, *C. mutica* was exposed to a higher magnitude of drag, which could partly explain its higher tendency to dislodge under flow. Most importantly, our experiments empirically demonstrate that chances of *C. mutica* overcoming vessel transport highly depend on secondary substrate complexity. When offered a flat surface, the species struggled to avoid dislodgement in comparison to its native congener. Here, the different size of appendages 5-7 in the two species may have also played a role. In amphipods, the relationship of prehensile pereopod morphology and basibiont size can shape attachment strength (*e.g.* see the case of *C. andreae* and its adaptation for attachment to the small algae growing on the turtle carapace; (Aoki and Kikuchi 1995; Cabezas et al. 2013). The shorter propodus (pereopod's last segment) of *C. laeviuscula* may have conferred a better adjustment to the size of our particular bidimensional artificial substrate, and thus a better grip, than that of *C. mutica.* Instead, the presence of basibionts protected *C. mutica* from hydrodynamic disturbance, to the point of resisting water flow as well as the native *C. laeviuscula* in the zero-substrate treatment. When the substratum complexity increases, the number of crevices available for hiding, as well as the suitable attachment points for caprellids multiply. Hydroid basibionts offer thallus/stems with variable thickness, which would remarkably improve *C. mutica* attachment in comparison to the horizontal, uniform mesh. A recent study based on isopods from the genus *Idotea*, also establishes a significant relation among morphological properties of the substratum (specifically, breaking stress and thickness of thallus in seaweeds) and the attachment strength applied by the epifauna to avoid dislodgement from the marine intertidal (Starko et al. 2016). Besides, the hydroids and especially mussels themselves increase boundary layer thickness, reducing the hydrodynamic forces actually experienced. This confirms that the basibiont component acts as a refugium for mobile invasive epifauna during vessel voyage, with higher basibiont complexity conferring better protection. A similar idea was hypothesized by Chan et al. (2016), based on the findings of Lewis, Bergstrom, and Whinam (2006) and Davidson et al. (2009). Although we could only test one individual's HR at L0, our results invite further studies to confirm whether the stowaway epifauna actually experiences a reduced physiological stress when it can use a hydrodynamic refuge habitat. This highlights once again the tight association between basibionts and epifauna. Furthermore, invasive epifauna will benefit especially from the survivorship of erect, flexible, filamentous morphologies such as *Obelia dichotoma*, which tend to survive voyages even at high speeds (Coutts et al. 2010), and sometimes even better than rigid, encrusting forms (Ashton, Davidson, and Ruiz 2014).

Another non-exclusive factor influencing the performance of *C. mutica* could be its bold behavior. Our behavior experiments demonstrated that the native species assumes a conservative strategy regarding exploratory behaviour, whereas the invasive species embraces an opportunistic, more risky strategy (also visible in natural field conditions: *C. mutica* usually exhibited a quick inter-patch movement by swimming and crawling, in order to escape manipulation – personal observation). This implies that the invasive species may also choose an "escaping" strategy, or at least offer less resistance to release grip on the substratum when exposed to unfavorable conditions (in our case, hydrodynamic disturbance). Emigrating allows for escape from deteriorating conditions in a wide range of taxa (Ronce 2007 and references therein; Vinatier et al. 2011; McDougald et al. 2012), yet it's highly variable even at population level (Maggs et al. 2019): each individual integrates the information from different environmental cues and thereby reaches its decision of whether to leave or not, but it's context-dependent and can vary with phenotype (morphological, physiological or behavioural attributes (Clobert et al. 2009). Sex-based differences in emigration propensity have also been reported in numerous vertebrate and invertebrate taxa, both male-biased and female-biased (Trochet et al. 2016; Mishra et al. 2018; Li and Kokko 2019). In our case study, males of *C. mutica* also tend to have higher boldness than females. This finding is consistent with the observations of Parretti et al. (2021) for the invasive congener *Caprella scaura* and the "cruising male" pattern reported for multiple peracarid species (see Borowsky 1983). This author and Caine (1991) noted the active search of males for receptive females, who instead invest energy in maternal care (Aoki 1997).

It is worth mentioning the fact that individuals getting detached during vessel transport does not necessarily imply death. Several invaders are known to survive in the water column awaiting the opportunity to re-attach to a different structure (vector) in a different transport pathway (for example, by rafting on floating material); thus increasing their spreading potential (Thresher et al. 2003). In the case of *C. mutica*, individuals are capable of dispersing over distances of at least 1km naturally in the water column (Ashton 2006). The congener *C. equilibra*, considered as cosmopolitan, is known to tolerate long periods in the water column, dispersing as part of the plankton (Takeuchi and Sawamoto 1998). This phenomena is also observed in taxa frequently found as fouling basibionts, such as ascidians, sponges and bryozoans. In this case, unattached fragments are known to survive suspended in the water column and later re-attach when substrate becomes opportunistically available, diversifying the introduction pathways along routes (Morris and Carman 2012; Kauano et al., 2017).".

4.2 Implications for invasion success and fouling management

Our results suggest that the transport phase can considerably hamper the invasion of *C. mutica* if faced with unfavourable substrate on transport vectors. At the same time, a complex fouling basibiont assemblage may protect the species and increase survivorship during the vessel journey; especially if the species is transported in protected "niche areas". Hydrodynamically sheltered areas, including those associated with fouling organisms, may help to offset the lower attachment probablilities of *C. mutica* relative to its congener *C. laeviuscula*. Our results emphasize that invasion success cannot be predicted solely on the basis of single traits (e.g., attachment ability) or on one phase of the invasion process. Despite being more vulnerable than its congener to hydrodynamic drag, the invader *C. mutica* still possesses several traits consistent with the dispersal syndrome *sensu* Clobert et al. (2009) that may confer an advantage at the post-arrival phases of the invasion process (*i.e.* colonization, establishment, and spread). These would be a higher tendency to explore, higher motility, larger body size and, according to literature, a higher fecundity than *C. laeviuscula* (maximum eggs/female 363 versus 150) (Caine 1991; Ashton 2006). As a note, the trait aggressiveness is common to *C. mutica*, the native *C. laeviuscula* and one invasive congener, *C. scaura* (Ashton, 2006). The tendency to explore is a particularly important factor setting apart dispersers from residents (Bowler & Benton 2005; Rehage et al. 2016). At the beginning of the invasion process, it increases the likelihood of attaching to a vector in the first place, and the odds of finding a favourable microhabitat on that vector. Only 1-2 days are needed for species inhabiting a marina to colonize a new vessel in transit (Schimanski et al. 2017), and the initial colonists will be those species characterized by a higher propensity for local dispersal (Ros et al. 2020). It also favors detaching and transitioning to the recipient environment; potentially colonizing a new location. Several authors state that boldness is likely to complement the ability of invaders to colonize novel environments, also compensating for the great energy expenditure experienced during transit (Cote et al. 2010a; Sih et al. 2012; Bensky & Bell 2021). Finally, it is also expected that resident fouling basibionts will act as facilitators (*i.e.* habitat/protection providers) for this invader upon arrival, thus favoring its establishment. All this implies that, if hydrodynamic refuges for *C. mutica* are sufficient during transit, it may pose a serious invasion risk in the recipient environment.

In the case of non-indigenous species with several invasiveness traits that may rely on fouling refugia to overcome translocation, the most effective management strategy is removal of biofouling prior to departure to a new destination. This stresses the need for implementing proper hull-cleaning regulations for small craft from the early stages of biofouling growth. Here, the use of proactive in-water cleaning (PIC) as a preventative measure to control the growth of microfouling (slime) can help to minimize biosecurity risks (reviewed in Scianni and Georgiades 2019). If patches of macrofouling organisms (potential basibionts) have already grown on the structure, the desirable reactive approach is through dry-docking and re-application of anti-fouling substances, including careful inspection of niche areas, which have been found to host considerably higher amounts of fouling (Coutts et al. 2013; Coutts and Taylor 2004; Davidson et al. 2010; Clarke Murray et al. 2011; Frey et al. 2014). In-water cleaning via encapsulating systems would also be an alternative for macrofouling removal, although these techniques are still under evaluation for feasibility and environmental safety (Hopkins and Forrest 2008; Roche et al. 2015; Keanly and Robinson 2020). Regardless, frequent hull-cleaning is key in order to avoid a rank 3 Level of Fouling (LoF *sensu* Floerl et al. 2005) that would most probably harbor and protect non-indigenous mobile epifauna in transit, preventing natural loss of propagules during the transport phase.

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3

BASELINE SURVEYS TO UNCOVER MISSING INFORMATION: PART 1

The challenge of hidden invaders

3.1.1 Marine exotic isopods from the Iberian Peninsula and nearby waters

Adapted from: Martinez-Laiz G, Ros M, Guerra-Garcia JM (2018). Marine exotic isopods from the Iberian Peninsula and nearby waters. PeerJ, 27;6:e4408.

ABSTRACT. Effective management of marine bioinvasions starts with prevention, communication among the scientific community and comprehensive updated data on the distribution ranges of exotic species. Despite being a hotspot for introduction due to numerous shipping routes converging at the Strait of Gibraltar, knowledge of marine exotics in the Iberian Peninsula is scarce, especially of abundant but small-sized and taxonomically challenging taxa such as the Order Isopoda. To fill this gap, we conducted several sampling surveys in 44 marinas and provide the first comprehensive study of marine exotic isopods from the Iberian Peninsula, the southern side of the Strait of Gibraltar (northern Africa) and the Balearic Islands. Exotic species included *Ianiropsis serricaudis* (first record for the Iberian Peninsula and Lusitanian marine province), *Paracerceis sculpta* (first record for the Alboran Sea ecoregion), *Paradella dianae*, *Paranthura japonica* (earliest record for the Iberian Peninsula) and *Sphaeroma walkeri*. Photographs with morphological details for identification for non-taxonomic experts are provided, their worldwide distribution is updated and patterns of invasion are discussed. We report an expansion in the distribution range of all species, especially at the Strait of Gibraltar and nearby areas. *Ianiropsis serricaudis* and *Paranthura japonica* are polyvectic, with shellfish trade and recreational boating being most probable vectors for their introduction and secondary spread. The subsequent finding of the studied species in additional marinas over the years points at recreational boating as a vector and indicates a future spread. We call for attention to reduce lags in the detection and reporting of small-size exotics, which usually remain overlooked or underestimated until the invasion process is at an advanced stage.

1. INTRODUCTION

The Order Isopoda includes marine, brackish, freshwater and terrestrial species, occupying areas from the desert to the deep sea. It comprises 379 genera in 37 families of marine isopods inhabiting all marine habitats including temperate realms, tropical regions and polar seas (Espinosa-Pérez and Hendrickx, 2006; Poore and Bruce, 2012). They show a variety of feeding modes including detritus feeders, carnivores, parasites, filter feeders and browsers. They also have been attributed a certain economic impact, being either diet for fish or their ectoparasites and thus potentially affecting commercial stocks, as well as causing damage of wharf and timber structures (see Poore and Bruce, 2012). Indeed, they are also great invaders around the world (Galil et al. 2011; Chapman and Carlton, 1991; Orensanz et al., 2002), and are potentially transportable by a number of vectors such as vessels, aquaculture, live seafood, contaminated gear and footwear, marsh restoration and floating plastic debris, among others (Carlton, 2011). For example, the invasive burrowing isopod Sphaeroma quoyanum has caused several impacts in California saltmarshes by reducing sediment stability and increasing erosion, ultimately converting this habitat to mudflats (Talley et al. 2001). Nevertheless, this group poses limitations for a correct assessment of exotics, mainly because they are small and taxonomically challenging; thus, they can thus remain detected for many years even if they pose a threat to surrounding species (Carlton, 2011). Reports of updated distribution of exotics and arrivals in new areas are vital to overcome these obstacles. For example, in the Iberian Peninsula, Baleares and northern coast of Africa, studies dealing with Isopoda include the catalogs published by Castelló (1986), Castelló and Carballo (2001), Castellanos et al. (2003) and Junoy and Castello (2003); however, no further revisions or checklists about exotic isopods are available at present. This is an urgent issue to solve, since the Iberian Peninsula is highly threatened by exotic species introduction due to its biogeographical position; it bears intense maritime traffic all around, with numerous shipping routes converging at the Strait of Gibraltar (see Seebens et al. 2013). Approximately 60,000 vessels transit the Strait each year; and it serves as gateway connecting areas like the Mediterranean Sea, West Africa, the Caribbean, northern Europe and Australia (Gibraltar Port Authority, 2017; Gibraltar Port marina staff, pers. comm., 2017), thus being a high-risk pathway for exotic species (see Drake and Lodge, 2004).

Several sampling surveys along the marinas of the Iberian Peninsula, the Baleares Islands and the northern coast of Africa were carried out from 2011 to 2017, exploring a wide range of fouling substrates, in order to provide the first comprehensive study of marine exotic isopods in the Iberian Peninsula and adjacent waters, and discuss potential pathways and vectors of introduction

2. MATERIAL AND METHODS

Examined material was collected during several sampling surveys carried out from 2011 to 2017, in order to study the fouling epifauna in 44 marinas around the Iberian Peninsula, the Southern side of the Strait of Gibraltar (northern Africa) and Baleares. Marina choice was based on its vessel traffic and popularity as tourist locality (see Table 1 including number of berths and population density). Data for number of berths was obtained from the FEAPDT (Federación Española de Puertos Deportivos y

Turísticos: http://www.feapdt.es) and from the IPTM (Instituto Portuário e dos Transportes Marítimos: http://www.atlanticstrategy.eu/en/partners/iptm-instituto-portu%C3%A1rio-e-dos-transportes-mar%C3%ADtimos-ip, accessed 16/11/21). Census data for the locality to which each marina belongs was obtained from the National Statistical Systems of Spain (http://www.ine.es, accessed 16/11/21), Portugal (http://www.ine.pt, accessed 16/11/21) and Morocco (http://www.hcp.ma, accessed 16/11/21) (Ros et al. 2015). In 2011, the abundant bryozoans *Bugula neritina* and *Amathia verticillata*, together with its associated epifauna, were collected from marinas around the Peninsula and the Strait of Gibraltar (Ros et al. 2015). Additionally, two monitoring programmes were carried out along the year 2012 in Puerto de Palma marina (Palma de Mallorca, Balearic Islands) and Puerto América marina (Cádiz), in which the substrates *Amathia verticillata* and *Eudendrium* sp. were sampled. Finally, a sampling survey was carried out during 2017 along the southern coast of the Iberian Peninsula to cover the main marinas of Andalusian coast. This area was selected as convergence zone between the Mediterranean Sea and the Atlantic Ocean, bearing a big gateway for marine introductions as it is the Strait of Gibraltar. Fouling organisms growing on artificial hard substrate including pontoons, ropes, wheels, buoys and ship hulls were sampled. These included red and green algae, hydroids, bryozoans, ascidians and mollusks plus their associated mobile epifauna. Samples were hand-collected, fixed in 90% ethanol and taken to the laboratory. Isopods were sorted, counted and identified to species level following updated literature on the group. Valid alien status was assigned following the European Environmental Agency criteria EEA, 2012, and valid human-mediated introduction was assessed based on Chapman and Carlton (1991). Photographs of full specimens and morphological parts of interest were taken using the camera Sony DSC-WX50. Worldwide distribution maps were developed using QGIS 1.8.0 Lisboa (QGIS, 2015), and shapefiles of marine ecoregions were obtained from http://maps.tnc.org/gis_data.html (accessed 20/08/2017). Voucher material of each species was deposited in the Museo Nacional de Ciencias Naturales (MNCN,CSIC), Madrid, Spain. The rest of the material was kept in the Laboratorio de Biología Marina, University of Seville, Spain.

Table 1. Data of sampled marinas and presence of exotic isopods. List of sampling localities (stations), coordinates, number of marina berths, population density (mean number of people per km2) and sampling year of each sampled marina. Exotic isopod species present in each marina are indicated; Is, Ianiropsis serricaudis; Ps, Paracerceis sculpta; Pj, Paranthura japonica; Pd, Paradella dianae and Sw, Sphaeroma walkeri; ``_'', no exotic isopods or no isopods at all present; ``blank'', no sampled. In grey, the cases in which an increased in exotic isopod species was found in 2017.

Station (St)	Coordinates	No. of marina berths	Population density	Exotic isopods 2011/2012	Exotic isopods 2017
1. Santander	43.45° N, 3.82° W	900	5176	-	
2. Gijón	43.54° N, 5.67° W	779	1527	-	
3. Ferrol	43.48° N, 8.26° W	250	883	Is	
4. A Coruña	43.37° N, 8.40° W	700	6503	-	
5. Nazaré	39.59° N, 9.07° W	52	180	-	
6. Cascais	38.69° N, 9.42° W	650	1832	-	
7. Sines	37.95° N, 8.87° W	230	67	-	
8. Albufeira	37.08° N, 8.27° W	475	251	-	
9. Faro	37.01° N, 7.94° W	300	289	Ps	Ps
10. Isla Cristina	37.19° N, 7.34° W	231	448	-	Ps
11. El Rompido	37.22° N, 7.13° W	387	85	-	-
12. Chipiona	36.74° N, 6.43° W	447	573	-	Ps,Pj
13. Rota	36.62° N, 6.35° W	209	347	Ps	Ps,Pj,Pd

14.1 Cádiz, Puerto América	36.54° N, 6.38° W	319	10154	Ps	Ps,Pd,Sw,Pj
14.2 Cádiz, V. de Levante	36.52° N, 6.30° W	270	10154		Ps, Pj
15. Sancti Petri	36.40° N, 6.21° W	94	389	-	-
16. Conil	36.29° N, 6.14° W	97	245	Ps	
17. Barbate	36.19° N, 5.93° W	314	160	-	Ps, Pd
18. La Línea	36.16° N, 5.36° W	624	3370	Ps	-
19. Fuengirola	36.54° N, 4.62° W	275	7145	-	Ps
20. Benalmádena	36.60° N, 4.51° W	1140	2373	-	
21. Málaga	36.72° N, 4.41° W	107	1437	-	-
22. Caleta Vélez	36.75° N, 4.07° W	277	488	Pd	Pd
23. Motril	36.72° N, 3.53° W	193	555	-	Pd
24. Almerimar	36.70° N, 2.79° W	1100	371	-	
25. Roquetas	36.76° N, 2.61° W	237	1506	-	
26. Almería	36.83° N, 2.46° W	277	643	-	-
27. Carbonera	36.99° N, 1.90° W	48	86	-	
28. Torrevieja	37.97° N, 0.68° W	570	1430	Ps	
29. Alicante	38.34° N, 0.49° W	400	1661	Ps	
30. Dénia	38.85° N, 0.11° W	300	676	Ps	
31. Valencia	39.43° N, 0.33° W	206	5928	Ps	
32. Borriana	39.86° N, 0.07° W	713	126	-	
33. Oropesa Mar	40.08° N, 0.13° W	668	126	-	
34. Benicarló	40.42° N, 0.43° W	293	126	Ps,Pj	
35. Tarragona	41.11° N, 1.25° W	441	2436	-	
36. Vilanova Geltrú	41.21° N, 1.73° W	812	1976	-	
37. Barcelona	41.38° N, 2.18° W	200	16449	Pj	
38. L'Estartit	42.05° N, 3.21° W	738	172	-	
39. Tánger	35.79° N, 5.81° W	500	229	-	
40. Ceuta	35.89° N, 5.32° W	325	4229	Ps	
41. MSmir	35.75° N, 5.34° W	450	283	Ps	
42. M'Diq	35.68° N, 5.31° W	120	283	Ps	
43. Puerto de Palma	39.34° N, 2.38° E	996	1931	Pj	

3. RESULTS

Five exotic marine isopods were found on fouling communities associated to marinas: *Ianiropsis serricaudis*, *Paracerceis sculpta*, *Paradella dianae*, *Paranthura japonica* and *Sphaeroma walkeri* (Table 1). From the sampled marinas, 53% hosted exotic isopods, with marinas around the Strait of Gibraltar being the most invaded ones (*e.g.,* Cádiz Bay hosting four of the five species) and *Paracerceis sculpta* the most widespread species. Out of the 14 marinas that were sampled in 2011/2012 and again in 2017, seven (50%) had increased the number of exotic species, sometimes by 200% or more (see Table 1). We provide the first record of *Ianiropsis serricaudis* for the Iberian Peninsula and the Lusitanian marine province, the first record of *Paracerceis sculpta* for the Alboran Sea ecoregion, and the earliest (2011) record of *Paranthura japonica* from the Iberian Peninsula. We report an extension in the distribution range for all species along the coasts of the Iberian Peninsula and adjacent waters.

Suborder Asellota Latreille, 1802
Family Janiridae G.O. Sars, 1897
Genus *Ianiropsis* G.O. Sars, 1897a
***Ianiropsis serricaudis* (Gurjanova, 1936)**
(Figs. 1A_1F)

Figure 1. Useful morphological details for identification of marine exotic isopods on fouling communities associated to marinas (Families Janiridae and Paranthuridae). Families Janiridae (A_F) and Paranthuridae (G_K). Ianiropsis serricaudis from La Graña marina (Ferrol, Spain) (St 3); male dorsal view (A), Pereopod 1(B), maxilliped (C), pleotelsonic dentation (D), two claws on pereopod 1(E), three claws on pereopod 7(F). Paranthura japonica from Puerto America marina (Cádiz, Spain) (St 14.1); male lateral view (G), female (H), female pointed mouthparts (I), semi-segmented pleon (J), antenna 1 (K). Bar 1 mm: A,G,H,J. Bar 0.2 mm: B,C,D,I,K. Bar 0.05 mm: E,F. Arrows show specific morphological details described in the text.

Janiropsis serricaudis Gurjanova, 1936, pg. 251_252, Fig. 1
Ianiropsis notoensis Nunomura, 1985, pg. 130_132, Figs. 7_8
Ianiropsis serricaudis Kussakin, 1962, pg. 49_50, Fig. 25; Kwon and Heon, 1990, pg. 195, Fig. 2B; Shimomura and Kajihara, 2001, pg. 48; Yokoyama and Ishihi, 2007, pg. 132; Doti and Wilson, 2010, pg. 16; Heiman and Micheli, 2010, Table 1; McIntyre et al., 2013, pg. 30; Wells et al., 2014, pg. 6 and 19; Hobbs et al., 2015, pg. 179_182, Figs. 1_ 5; Marchini et al. 2016a; Marchini et al. 2016b, pg. 333, Figs. 2_3; Ferrario et al., 2017, pg. 4_6; Ulman et al., 2017, pg. 9, Table 2, pg. 13, Table 5, pg. 26.
Ianiropsis sp. Pederson et al., 2005, pg. 12.
Ianiropsis sp. Faasse, 2007, pg. 126, Fig. 2.

Material examined (total: 139 specimens): St3: 2 males (MNCN 20.04/11439), 18 males and 119 females clinging on bryozoan Bugula neritina, floating pontoons, 07/05/2011. Taxonomical remarks: The genus *Ianiropsis* is similar to *Janira* and *Carpias*: three claws on walking legs, coxae visible in dorsal view and usually can only be definitely identified from the males. *Ianiropsis* can be distinguished from the other two by bearing an elongated carpus of male pereopod I (Fig. 1B), instead of enlarged or swollen propodus and carpus (*Carpias*) or not elongated propodus and carpus at all (*Janira*) (Wilson & Wägele, 1994). Our specimens showed the features pointed out by Doti & Wilson (2010), Hobbs et al. (2015), Marchini et al. (2016a) and Marchini et al. (2016b) for *I. serricaudis*: (i) antennal peduncle segments 6 and 7 particularly elongated relative to the overall length of the antennae (Fig. 1A); (ii) head anterior margin in dorsal view concave; (iii) distinctive maxilliped palp of adult male, projecting substantially, enough to be visible on head in dorsal view (Figs. 1A, 1C) (Doti and Wilson, 2010); (iv) dactylus of pereopod 1 bearing two claws while that of pereopod 7 bearing three (Figs. 1E, 1F respectively); (v) four marginal denticles on pleotelson (Fig. 1D).

Ecological remarks: The species presents a cosmopolitan distribution according to Doti and Wilson (2010), inhabiting mostly temperate to cold temperate coastal waters. In its native range it is distributed under rocks, on sponges, ascidians, coralline and brown algae, and rhizoids of kelp Laminaria, in water temperatures from 1.8 _C to 24 _C (Gurjanova, 1936; Kussakin, 1962, Kussakin, 1988).

Suborder Cymothoida Wägele, 1989
Family Paranthuridae Menzies and Glynn, 1968
Genus *Paranthura* Spence Bate and Westwood, 1866
***Paranthura japonica* Richardson, 1909**
(Figs. 1G_1K)

Paranthura japonica Richardson, 1909, pg. 77_78, Figs. 4_5; Kussakin, 1975, pg. 53, 67; Nunomura, 1975, pg. 28_31, Figs. 10_12; Nunomura, 1977, pg. 86_87, Fig. 12; Che and Morton, 1991, pg. 205, Table 4; Moshchenko and Zvyagintsev, 2004, pg. 8, 13, table 2, Fig. 2; Li, 2003, pg. 139, table 1, pg. 156, table 3; Cohen et al., 2005, pg. 1001, Appendix A table; Yamada et al., 2007, pg. 346_348, 352, table 2; Zhang et al., 2009, pg. 306, table 2, 308; Wang, Ren and Xu, 2010, pg. 610, 612, table 3; Frutos et al. 2011, pg. 17; Lavesque et al., 2013, pg. 215_218, Fig. 2; Marchini et al., 2014, pg. 545_551, Figs. 2_5; Marchini, Ferrario and Minchin, 2015, pg. 358, Fig. 4; Lorenti et al., 2016, pg. 12792_12794, Figs. 2_4; Tempesti et al., 2016, Fig. 1; Ferrario et al., 2016b, pg. 224, 225, table 1; Dailianis et al., 2016, pg. 609, table 1, pg. 615, Fig. 9; Ferrario et al., 2017, pg. 4_5,7; Ulman et al., 2017, pg. 9, Table 2, pg. 13, Table 5, pg. 26, 27, 36.
Paranthura sp. (Cohen & Carlton, 1995), pg. 84, 146, Table 1, pg. A4-2, Table 1.

Material examined (total: 139): St12: Two females and two juveniles from fouling community on floating structures (pontoons, ropes and buoys), 01/07/2017. St13: Six males, 13 females and 24 juveniles from Corallinaceae algae and green algae, 13/05/2017. St14.1: Four females and one juvenile on B. neritina, one male and one juvenile on *Eudendrium* sp., and one male and two female on Coralline algae, floating pontoons, 14/05/2017; four males, 12 females and 33 juveniles from fouling community on floating pontoons, 14/05/2017; one male and two females (MNCN 20.04/11443), three males six females and 16 juveniles collected from fouling community on floating structures, 02/07/2017. St14.2 One female and one juvenile from fouling substrates, floating structures, 02/07/2017. St34: One juvenile on *A. verticillata*, floating pontoons, 27/06/2011. St37: One female and one juvenile on *A. verticillata*, floating pontoons, 26/06/2011. St43: One female on *Eudendrium* sp., floating pontoons, 09/2012.

Taxonomical remarks: the specimens match the descriptions by Richardson (1909), redescriptions by Nunomura (1975), Lavesque et al. (2013) and Lorenti et al. (2016). They display stinging mouthparts, typical of the family Paranthuridae (Fig. 1I), and a particular combination of characters that distinguish it from other known Japanese Paranthura species. These are: eyes well developed composed of less than 17 dark ommatidia; anterolateral angles of cephalon exceeding rostral projection; antenna 1 with 8 distinct articles (Fig. 1K); pereonite 6 shorter than pereonite 5; short pleotelson barely exceeding the tip of uropods; and particularly, semi-segmented pleon, with pleonites fused in the middle of their dorsal region but distinct at their sides, which allow to clearly identify P. japonica (Fig. 1J) (Lavesque et al., 2013; Lorenti et al., 2016).

Ecological remarks: *Paranthura japonica* is reported from coastal transitional ecosystems, such as lagoons, estuaries, and mangroves (Lorenti et al., 2016). It adapts to a wide range of habitats including sandy bottoms in seagrass beds (Zostera), among algae (Sargassum) and in mussel beds and oyster reefs (Golovan and Malyutina, 2010; Lavesque et al., 2013). It is a successful colonizer of boat wreck and pontoons fouling, inhabiting crevices and free spaces between colonial animals as well as burrows made by other organisms (Cadien and Brusca, 1993; Kussakin 1982; Lorenti et al., 2016).

Suborder Sphaeromatidea Wägele, 1989
Family Sphaeromatidae Latreille, 1825
Genus *Paracerceis* Hansen, 1905
***Paracerceis sculpta* (Holmes, 1904)**
(Figs. 2A_2E)

Dynamene sculpta Holmes, 1904, pg. 300_302, pl. XXXIV, Figs. 1_7.
Cilicaea sculpta Richardson, 1905, pg. 318_319, Fig. 349.
Paracerceis sculpta Menzies, 1962, pg. 340_341, Fig. 2; Miller, 1968, pg. 14, Fig. 3; Schultz, 1969, pg 120, Fig. 167; Rezig, 1978, pg. 175; Brusca, 1980, pg, 226, Fig. 12.5_12.6; Pires, 1981, pg. 219_220; Harrison and Holdich, 1982, pg. 440_441, Fig. 10; Pires, 1982, pg. 45,53, Fig. 26_27; Forniz and Sconfietti, 1983, pg. 197_203, Figs. 1_2; Forniz and Maggiore, 1985, pg. 780; Shuster, 1987, pg. 321_323, Figs. 1, 3; 1990, pg. 390, Fig. 1; 1992, pg. 232_234, Fig. 1; Rodríguez et al., 1992, pg. 95_96, Figs. 2A, 2B; Loyola and Silva, 1999, pg. 109_123, Figs. 1_18; Yasmeen and Javed, 2001, pg. 43_48, Figs. 1_3; Yu and Li, 2001, pg. 48_49; Hewitt and Campbell, 2001, pg. 925_934; Espinosa-Pérez and Hendrickx, 2002, pg. 1172_1176, Fig. 2C; Ariyama and Otani, 2004, pg. 54_55, Figs. 2A_2E; Yasmeen and Yousuf, 2006, pg. 116_118, Fig. 3; Brusca et al. 2007, pg. 518_19, 537_538, pl. 243A; Dailianis et al., 2016, pg. 609, Table 1, pg. 614, 615, Fig. 9; Marchini et al., 2017, pg. 3, Fig. 2; Ferrario et al., 2017, pg 5; Ulman et al., 2017, pg. 9, Table 2, pg. 11, Table 3, pg. 13, Table 5, pg. 28, 37; Ramalhosa et al., 2017, pgs. 1747_1749, pg. 1751_1752, Fig. 2, pg. 1755_1759.

Sergiella angra Pires, 1980a, pg. 212_218, Figs. 1_24; Pires, 1981, pg. 219_220.
Paracerceis japonica Nunomura, 1988, pg. 4_7, Figs. 3_4.

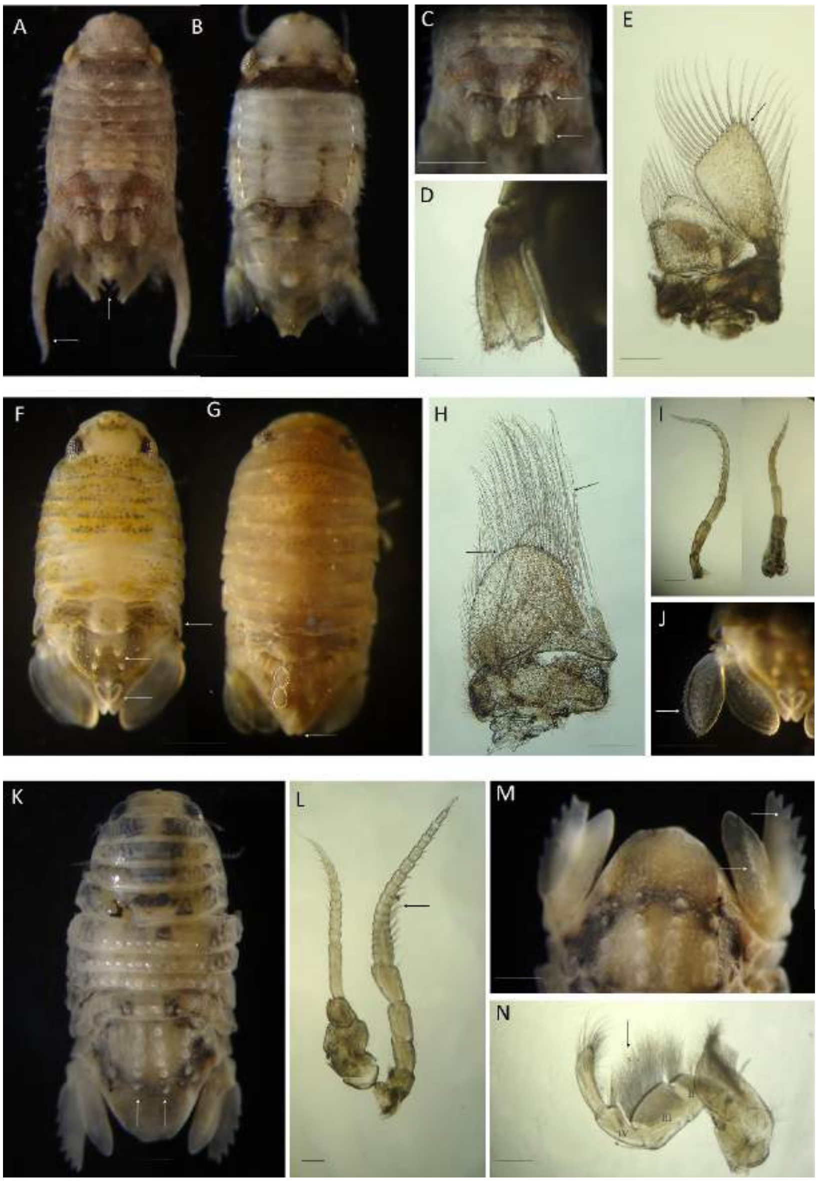

Figure 2. Useful morphological details for identification of marine exotic isopods on fouling communities associated to marinas (Family Sphaeromatidae). Family Sphaeromatidae. Paracerceis sculpta from Barbate marina (Cádiz, Spain) (St 17) (A_E); male dorsal view (A), female (B), male pleotelson (C), female uropods (D), male pleopod 2 (E). Paradella dianae male from Barbate marina (Cádiz, Spain) (St 17) and female from Caleta-Vélez marina (Málaga, Spain) (St 22) (F_J); male dorsal view (F), female (G), male pleopod 2 (H), male antenna (left) and antennule (right) (I), male uropods (J). Sphaeroma walkeri from Puerto America marina (Cádiz, Spain) (St 14.1) (K_N); female dorsal view (K), female antennule (left) and antenna (right) (L), female pleotelson and uropods (M), female maxilliped (N). Bar 1 mm: A, B, E, F, G, K, M. Bar 0.2 mm: C, D, I, J, L, N. Arrows and dashed circles show specific morphological details described in the text.

Material examined (total: 1,188): St9: Three females and five juveniles on Bugula neritina, three females on *A. verticillata*, floating pontoons, 11/05/2011; two males and two females (MNCN 20.04/11440), 14 males, 224 females and 192 juveniles on fouling substrates, floating structures (pontoons, ropes and buoys), 26/06/2017. St10: one female and four juveniles on fouling substrates, floating structures, 26/06/2017. St12: one female on fouling substrates, floating structures, 01/07/2017. St13: Three juveniles on *B. neritina*, one female and 10 juveniles on *A. verticillata*, floating pontoons, 17/05/2011; six juveniles on Coralline algae and green algae, floating pontoons, 13/05/2017. St14.1: One male, nine females, 19 juveniles on B. neritina, one male, 29 females, 23 juveniles on *A. verticillata*, floating pontoons, 17/05/2011; one female and six juveniles on *A.*

verticillata, 12/2011; one juvenile on *A. verticillata*, one male and one female on hydrozoan *Eudendrium* sp., 05/2012; one juvenile on *A. verticillata*, 06/2012; one juvenile on A. verticillata, 07/2012; one female and 23 juveniles on *A. verticillata*, 08/2012; 15 females and 39 juveniles on *A. verticillata*, 09/2012; one female and five juveniles on *A. verticillata*, 10/2012; two females and nine juveniles on *A. verticillata* 11/2012; 8 females and 155 juveniles on fouling community, floating pontoons, 14/05/2017. St14.2: One male, six females and six juveniles on fouling substrates, floating structures, 01/07/2017. St16: One juvenile on *B. neritina*, floating pontoons, 17/05/2011. 18 females and 139 juveniles on fouling substrates, floating pontoons, 06/2017. St17: One male, 18 females and nine juveniles on fouling substrates, floating structures, 01/07/2017. St18: One juvenile on *B. neritina*, floating pontoons, 15/05/2011. St19: Two males, 18 females and 26 juveniles on fouling substrates, floating structures, 29/06/2017. St28: three females and seven juveniles on B. neritina, floating pontoons, 29/06/2011. St29: 8 females and 10 juveniles on B. neritina, floating pontoons, 29/06/2011. St30: Two juveniles on *A. verticillata*, floating pontoons, 28/06/2011. St31: One female and three juveniles on *B. neritina*, three females and seven juveniles on *A. verticillata*, floating pontoons, 28/06/2011. St34: five juveniles on *B. neritina*, six females and 54 juveniles on *A. verticillata*, floating pontoons, 27/06/2011. St40: Two juveniles on *B. neritina*, floating pontoons, 29/95/2011. St41: Seven juveniles on *B. neritina*, floating pontoons, 30/05/2011. St42: Two females and four juveniles on *B. neritina*, one juvenile on *A. verticillata*, floating pontoons, 30/05/2011.

Taxonomical remarks: Our specimens match the description and illustrations given by Menzies (1962), Rodríguez et al. (1992), Brusca et al. (2007) and Marchini et al. (2017). The genus *Paracerceis*, together with other *Cerceis*-like genera, can be distinguished by bearing pronounced marginal teeth on exopods of pleopods 1_3, especially obvious on pleopod 2 (Fig. 2E), in contrast to the crenulate margin or toothless margin on genera like *Dynamene*, *Sphaeroma* or *Paradella* (Fig. 2H) (Harrison and Ellis, 1991). Male specimens of *P. sculpta* collected in our survey presented a granulated pleon, with three tubercles on the anterior and posterior margins (Fig. 2C). The most peculiar feature are the greatly elongated cylindrical uropod exopods, which largely exceed margin of pleotelson, and a cleft posterior margin of pleotelson with three pairs of notches, indicative of P. sculpta. (Fig. 2A). Some variation was reported though regarding some minute characters of the pleotelson, for example the variation in setation of pleotelsonic and pleon tubercles (see Marchini et al., 2017). Our specimens bear dorsal tufts of setae on the pereonite, pleon and pleotelson tubercles (Fig. 2C), like populations from California (Brusca et al. 2007), Azores (Marchini et al., 2017) and Mediterranean Sea; and unlike other specimens with rather poor or absent setation from the Iberian Atlantic coast (Rodríguez et al., 1992), Brazil (Loyola et al., 1999) and Japan (Ariyama and Otani, 2004). Futhermore, the apex of male endopods are markedly pointed (Fig. 2A), similarly to the aforementioned specimens from Azores and Brazil. According to Shuster (1987), *P. sculpta* exhibits three distinct sexually mature male morphs in its native range, corresponding to different strategies for reproduction. The `` α-males'' are the largest, they bear distinct morphological characteristics of *Paracerceis* and defend a harem. The `` β-males'' are smaller; they resemble females and mimic their courtship behavior. The ``γ-males'' are the smallest; they resemble juveniles and attempt to sneak into _-male harems. Our populations were also examined in search of all morphs but only alpha males (6.55 _ 0.72 mm in length according to Shuster, 1992) were present. Ecological remarks: The species inhabits coasts and lagoons of subtropical to temperate regions. It has been found in association with a range of substrates such as shallow water calcareous sponges (Richardson, 1905; Holmes, 1904; Brusca, 1980), Sargassum C. Agardh, 1820 and Galaxaura Lamouroux, 1816 in Brazil (Pires, 1981), barnacles (Loyola and Silva, 1999), oyster reefs (Munguia and Shuster, 2013) and bryozoans (Marchini et al., 2015; Marchini et al., 2017). As a stenohaline species (thus low tolerance to freshwater conditions) it would have crossed the Panama channel via ballast water of ships (Espinosa-Pérez and Hendrickx, 2002).

Family Sphaeromatidae Latreille, 1825
Genus Paradella Harrison & Holdich, 1982
***Paradella dianae* (Menzies, 1962)**
(Figs. 2F_2J)

Dynamenopsis dianae Menzies, 1962, pg 342, Fig. 3; Glynn, 1968, pg 573; Schultz, 1969, pg 123
Dynamenella dianae Menzies and Glynn, 1968, pg 63, 113, Fig. 3; Glynn, 1970, pg 24, Figs. 9_10; Iverson, 1974, pg 166; Pires, 1980b, pg 134, Figs. 1_7
Paradella dianae Harrison and Holdich, 1982, pg 104, Fig. 6; Pires, 1982, pg. 45, 51_53, Figs. 21_23; Fox and Ruppert, 1985, pg. 317; Javed and Ahmed, 1987, pg. 216, Fig. 1; Kensley and Schotte, 1989, pg. 224_225, Figs. 98A_98C, pg. 266, 268, Table 6; Atta, 1991, pg. 213_217, Figs. 2,3; Rodríguez et al., 1992, pg. 96, Fig. 2; Nelson and Demetriades, 1992, pg. 648_649, Figs. 1_2, pg. 650, 652; Kensley et al., 1995, pg. 137, table 1, pg. 138, table 2; Kensley and Schotte, 1999, pg. 702_705, Figs. 4_5; Hass and Knott, 2000; pg. 461, table1; Castelló and Carballo, 2001, pg. 230; García-Guerrero and Hendrickx, 2004, pg. 1159; Wetzer and Bruce, 2007, pg. 39, 40, 42, 46 and 48; Çinar et al., 2008, pg. 1, 6_7, Table 2, pg. 12, 14; Knott and De Victor 2010, pg. 2_6, Figs. 1_3; Kirkim et al., 2010, pg. 102; Galil, 2011, pg. 231, Appendix 1, 236, Appendix 2, 242, Appendix 3, 384, table 1, 463, table 2; Ates et al., 2013, pg. 23; Do§an et al., 2015, pg. 857, 860_864, table 2; Kirkim et al., 2015, pg. 323_325, Fig. 2; Ferrario et al., 2017, pg. 4_5; Ulman et al., 2017, pg. 11, Table 3, pg. 28, 37.
Paradella quadripunctata Van Dolah et al., 1984, pg.52

Material examined (total: 49): St13: One male and two females (MNCN 20.04/11441), five females and 36 juveniles collected from Corallinaceae algae and green algae, floating pontoons, 13/05/2017. St14.1: Two juveniles collected from fouling community, floating pontoons, 14/05/2017. St17: One male collected from fouling community of floating structures (pontoons, buoys, ropes) 01/07/2017. St23: One female collected from fouling substrates, floating structures, 28/06/2017. St22: One female from fouling substrates, floating structures, 28/06/2017 and one female on *B. neritina*, floating pontoons, 03/07/11.

Taxonomical remarks: The specimens coincide with the characters explained by Menzies and Glynn (1968), Pires (1980b) (on *Dynamenella dianae*), Harrison and Holdich (1982), Wetzer and Bruce (2007) and Rodríguez et al., (1992). The genus *Paradella* can best be identified by males having a distinct dorsally-directed, Y-shaped and posteriorly closed pleotelson foramen; long, tapering and basally fused penial processes, and a long and basally narrow appendix masculina that usually extends beyond the distal margin of the endopod (Fig. 2H) (Wetzel and Bruce, 2007). *Paradella dianae* males can be distinguished by the aforementioned Y-shaped or heart-shaped and posteriorly closed pleotelson foramen; by paired sub-median nodules on the pleon and two pairs of longitudinal carinae centrally arranged on the dorsal surface of the granulose pleotelson; and by large or expanded pereonite 7 coxae (Fig. 2F). *Paradella dianae* has ovate uropods, subequal in length, and with exopod and endopod of mature male large, with heavy, decidedly crenulate margins, with an evenly convex lateral margin on the uropodal exopod, characters that allow for its distinction from the similar congener P. garsonorum (Fig. 2J) (from Wetzer and Bruce, 2007; Harrison and Holdich, 1982). Uropoda of female are smaller than in male and apex of pleotelson has a slight reduced depression (Fig. 2G). Antennula flagellum has 11 artciles and antenna flagellum with 16 (Fig. 1I), similarly to the Arabian Sea and Cádiz specimens (Javed and Ahmed, 1987; Rodríguez, Drake and Arias, 1992) and unlike the Australian ones, which bear 12 and 13 articles respectively (Harrison and Holdich, 1982). Female submedian pair of tubercles are not completely fused (dashed circles in Fig. 1G), as indicated by Atta (1991) for Mediterrranean specimens. Size was consistent with populations previously reported from Cádiz Bay (Spain) (Rodríguez et al., 1992).

Ecological remarks: This isopod is commonly found amongst barnacles tests, intertidal green algae, bryozoans, empty polychaete tubes and rock oysters on rocks and man-made structures from upper to lower shore, in exposed and sheltered shores (Harrison and Holdich, 1982). It is known to survive at temperatures as low as 14 _C (Nelson and Demetriades, 1992), tolerant to some salinity variations, 31_38 pt. (García-Guerrero and Hendrickx, 2004) and also known to withstand heavy pollution (Pires, 1980b). It is protogynous hermaphrodite (Kensley and Schotte, 1999) and females can bear a peak of egg production during June (García-Guerrero and Hendrickx, 2004) or more than one peak in the introduced population (Nelson and Demetriades, 1992).

Family Sphaeromatidae Latreille, 1825
Genus *Sphaeroma* Bosc, 1801
***Sphaeroma walkeri* Stebbing, 1905**
(Figs. 2K_2N)

Sphaeroma walkeri Stebbing, 1905, pg. 31_33, pl. VII; 1910, pg. 220; 1917, pg. 444; Barnard, 1920, pg. 360; 1936, pg. 178; 1940, pg. 405; Omer-Cooper, 1927, pg. 240; Baker, 1928, pg. 49; Nierstrasz, 1931, pg. 192; Monod, 1931, pg. 36; Monod, 1933, pg. 198; Larwood, 1940, pg. 28; Pillai, 1955, pg. 132, pl. VI; Loyola e Silva, 1960, pg. 41, Figs. 6_7; Joshi & Bal, 1959, pg. 61_62; Menzies and Glynn, 1968, pg. 56, Fig. 23; Miller, 1968, pg. 8_11, Fig. 3; Glynn, 1972, pg. 286, Fig. 5; Carlton and Iverson, 1981: 31_46; Estevez and Simon, 1976, pg. 288; Harrison and Holdich 1984, pg. 279_282, Fig. 1; Jacobs, 1987, pg. 22_24, Fig. 6; Mak, Huang and Morton, 1985, pg. 75; Morton, 1987, pg. 504, Fig. 1; Kensley and Schotte, 1989, pg. 235, Fig. 101; Kussakin and Malyutina, 1993, pg. 117; Bruce, 1993, pg. 156, Fig. 1; Loyola e Silva, 1998, pg. 629; Ghani and Qadeer, 2001, pg. 871_872; Ramadan et al., 2006, pg. 22, table 1; Galil, 2008, pg. 443, Fig. 1; Ben Amor et al., 2010, pg. 615, Fig. 1; Khalaji-Pirbalouty and Wägele, 2010, pg. 10_16, Figs. 6_10, 11D; Ben Amor et al., 2015, pg. 37, Fig. 2; Ulman et al., 2017, pg. 9, Table 2, pg. 11, Table 3, pg. 13, Table 5, pg. 29.

Material examined (total: two females): St14.1: One female from fouling community, floating pontoons, 14/05/2017; one female (MNCN 20.04/11442) collected from fouling community, floating structures (pontoons, ropes, buoys), 02/07/2017. Taxonomical remarks: The specimens coincide with the descriptions by Jacobs (1987), Khalaji-Pirbalouty and Wägele (2010) and Ben Amor et al., (2015). Sphaeroma can be distinguished from related genera like *Exosphaeroma* and *Lekanesphaera* by bearing a robust maxilliped, particularly the palp, articles II_IV without lobes and a fringe of robust, plumose setae on internal border of endite (Fig. 2N). The uropodal rami of Sphaeroma are subequal, usually reaching beyond the posterior margin of pleotelson and the external margin of exopod is pronouncedly serrated (Fig. 2M). The assignment to the species *S. walkeri* was based on the presence of two longitudinal rows of five prominent tubercles flanked on either side by a shorter longitudinal row of three prominent tubercles on the dorsal surface of pleotelson, two on either side of midline (Fig. 1K). This character is also reported from the Persian Gulf specimens (Khalaji-Pirbalouty and Wägele, 2010), Tunisian ones (Ben Amor et al., 2015) and Africa ones (Jacobs, 1987). The pleotelson is long and tapers to a rounded point that is slightly upturned; margin of telson crenated. Endopod of uropod has dorsally prominent, median tubercles, and exopod with five to six large, triangular, external teeth plus an acute apex of the exopod (Fig. 1M), as other authors pointed out (Pillai, 1955; Harrison and Holdich, 1982; Ben Amor et al., 2015). The number of teeth varies also within the same individual. The number of articles in the antenna flagellum varies, depending on size, and bears a fringe of smooth setae at the distal interior angle, in female reaching only as far as end of next segment (Fig. 2L).

Ecological remarks: This species is a shallow, warm-water, fully marine isopod common in crevices and in fouling. Occasionally, it has been recorded as a wood-boring species (Khalaji-Pirbalouty and Wägele, 2010); however, it is to be noticed that traces of wood have not been found in the stomach contents of this species and its mouthpart morphology is not that of a true wood-boring sphaeromatid (see Carlton and Iverson, 1981). Instead, these authors suggest a thigmotactic response. This means *S. walkeri* has a predilection for holes and crevices, which explains its occasional observations in wood, benthic algae, stones, dead sea squirts, mangrove roots, empty barnacle shells like those of *Balanus amphitrite* Darwin, 1854, oscula of sponges and dead ascidians including *Ciona intestinalis* (Ben Amor et al., 2010; Ben Amor et al., 2015). It is a thermophilic isopod, with high densities during spring and summer. Its reproductive biology was positively correlated with salinity, transparency of water and temperature, and it breads continuously throughout the year in some introduced populations (see Ben Amor et al., 2015).

4. DISCUSSION

At present, 12 marine exotic isopod species are known to be present in European waters. Ten of them are free-living species, most of them considered to be established, and two are parasites and considered to be casual (Streftaris, Zenetos & Papathanassiou, 2005; Zenetos et al., 2010; Galil, 2011; Noël, 2011; Lavesque et al., 2013; Chainho et al., 2015; Lorenti et al., 2016; Marchini, Ferrario & Occhipinti-Ambrogi, 2016a; Ulman et al., 2017) (see Table S1). The Iberian Peninsula alone hosts 50% of these ten free-living species, proving to be an important monitoring point for spread as well as future arrivals of exotics. Moreover, 50% of the marinas sampled in 2017 had increased their number of exotic isopods within the timeframe of only six years (Table 1). The case of the marinas in Cádiz Bay (Strait of Gibraltar) is to be noticed. Only *Paracerceis sculpta* was found in 2011, but they hosted *P. sculpta*, *Paradella dianae*, *Sphaeroma walkeri* and *Paranthura japonic*a in 2017 (see the case of St. 12, 13 and 14.1 in Table 1). It is to be noticed that, despite more habitat-forming species were analyzed in 2017 in comparison with 2011, the increase in NIS was verified for the same species. In fact, a previous study conducted by Ros et al. (2013) demonstrates that about 50% of the dominant sessile species present throughout the year in Puerto América marina (St. 14.1) are introduced. Several factors may be favouring the introduction and establishment of exotic species in this area. Some of these factors may be due to particular environmental conditions of each marina; but others are most likely human-related, like the proximity of these marinas to a major international port in southern Spain (Cádiz Port), together with the high maritime traffic occurring across the Strait of Gibraltar. History of introduction, pathways, vectors and potential spread of each species are discussed below.

Histories of introduction and worldwide distribution

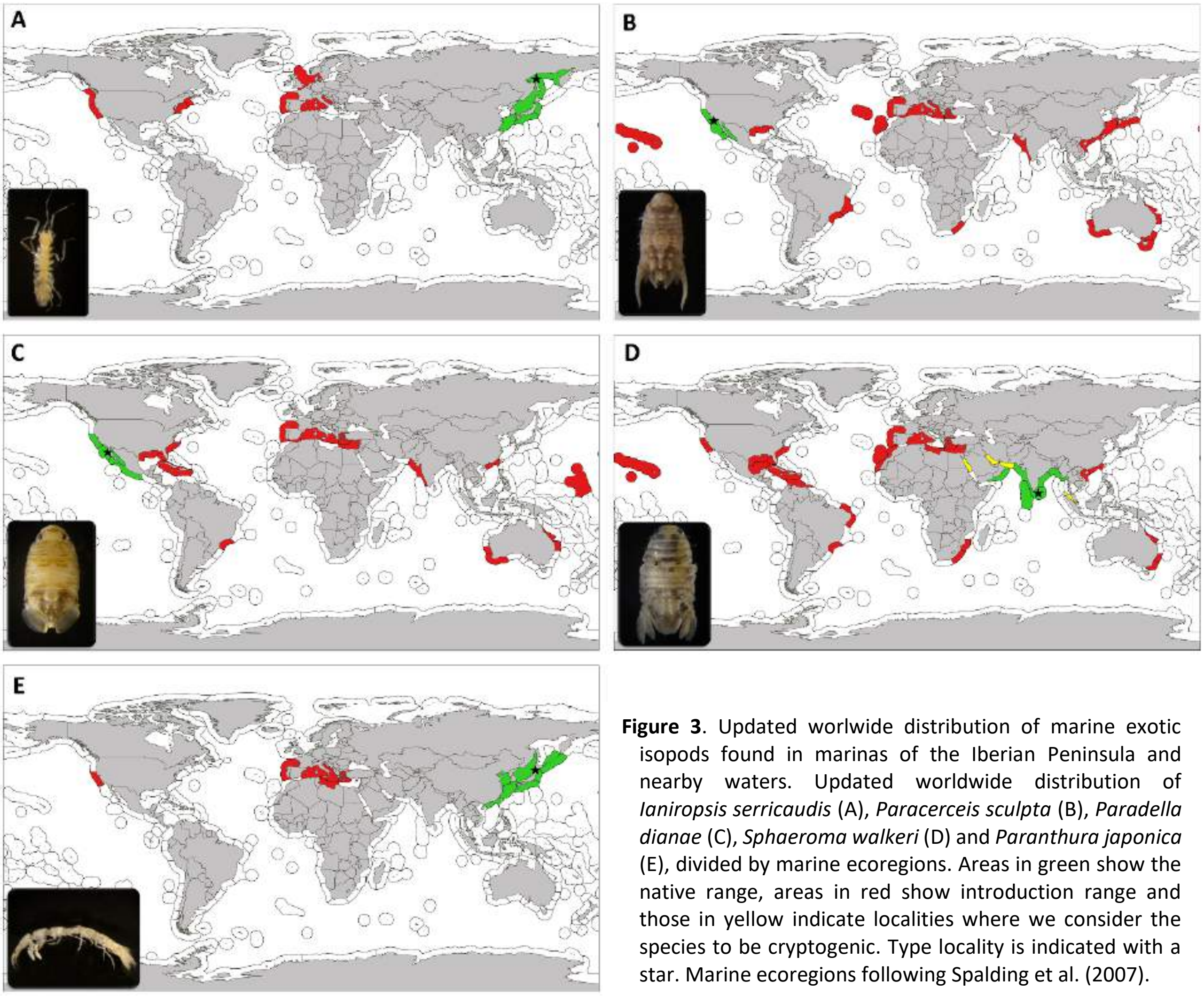

Figure 3. Updated worlwide distribution of marine exotic isopods found in marinas of the Iberian Peninsula and nearby waters. Updated worldwide distribution of *Ianiropsis serricaudis* (A), *Paracerceis sculpta* (B), *Paradella dianae* (C), *Sphaeroma walkeri* (D) and *Paranthura japonica* (E), divided by marine ecoregions. Areas in green show the native range, areas in red show introduction range and those in yellow indicate localities where we consider the species to be cryptogenic. Type locality is indicated with a star. Marine ecoregions following Spalding et al. (2007).

Ianiropsis serricaudis is native to the western Pacific, from the Sea of Okhotsk to the Sea of Japan, including Russia, Japan and Korea (Kussakin, 1962; Jang and Kwon, 1990; Shimomura et al., 2001; Yokoyama and Ishihi, 2007) (Fig. 3A). It was reported as NIS in San Francisco Bay, California (Carlton, 1979) in association with the introduced ascidians *Ciona intestinalis* Linnaeus, 1767 and *Styela clava* Herdman, 1881, possibly transported in shipping associated with the Vietnam War (Carlton, 1979). In the following years, reports of unknown *Ianiropsis* or erroneously identified specimens started to appear in the East and West coast of the United States and in 2004 it was already present in Europe, associated with the introduced ascidian *Syela clava* in Southampton (England) (see Hobbs et al., 2015 and references herein). In the Netherlands it was first observed in 2000 (Faasse, 2007) in an estuary used for shellfish aquaculture, and near the port of Rotterdam, among other locations. In 2010 and 2011, Hobbs and collaborators realized that all the mentioned reports involved the same species, potentially globally distributed by ships. From 2010 to 2013 it was widely reported from Maine to New Jersey (United States, western Atlantic), in association with both native and introduced algae, bryozoans and ascidians from fouling communities on floating dock sites and pilings (Pederson et al., 2005; McIntyre et al., 2013; Janiak and Whitlatch, 2012; Johnson, Winston and Woolacott, 2012; Wells et al., 2014; Hobbs et al., 2015). Also in California and Washington (United States, eastern Pacific), in mudflats near reefs of the introduced Australian serpulid polychaete *Ficopomatus enigmaticus* (Fauvel, 1923) (Heiman and Micheli, 2010) or in association to the non-native tunicate D. vexillum colonizing mussel aquaculture facilities (Cordell et al., 2013).

The first evidence of its occurrence in the Mediterranean Sea took place in 2012, when it was found to be abundant in the Lagoon of Venice (Adriatic Sea, Italy) (Marchini et al., 2016a; Marchini et al., 2016b). The Lagoon of Venice is a big center for recreational and commercial harbour as well as flourishing mariculture that hosts a high number of introduced species (Occhipinti-Ambrogi, 2000; Marchini et al., 2015). A couple of years later it was present in Olbia (Ferrario et al., 2017), again a major site for mussel farming which, in fact, imports stocks from Adriatic lagoons (Marchini et al., 2016b); and subsequently in French marinas (Ulman et al., 2017). Our results fill a gap in its distribution, providing the first record for the Iberian Peninsula and the Lusitanian province. We now have evidence that it was already present in 2011 in the North of Spain, in La Graña marina (Ferrol, Galicia). Ferrol city has been a major naval shipbuilding centre for most of its history, and today, aquaculture and fishing stand as its primary industries.

Interestingly, the specimens found in Ferrol bear four marginal denticles on pleotelson (Fig. 1D). There are some minor discrepancies regarding this character; Gurjanova (1936) described it as possessing four or five, Kussakin (1962) established a range of four to seven, Jang and Kwon (1990) showed four on the material from Korea et al., (2010) established ``five denticles or more'' but not ``up to four denticles'' and Marchini et al, (2016a); Marchini et al., (2016b) reported three or four from the specimens collected from the Mediterranean Sea. In any case, Hobbs et al. (2015) considered this to be a variable character and they relied on additional characteristics instead. They suggested a founder effect from the narrower range of denticle counts in introduced populations (three to four) versus the reported from native regions (up to seven). Moreover, our specimens were considerably large (males up to 5 mm and ovigerous females up to 3 mm) in comparison to those reported from Russia (2.9 mm for males and 2.7 for females) (Kussakin, 1962, Kussakin, 1988) from the East coast of the United States (largest male being 3.2mmand female 2.4 mm) (Hobbs et al., 2015) and from the Mediterranean Sea (around 3 mm) (Marchini et al, 2016a; Marchini, Ferrario and Occhipinti-Ambrogi, 2016b). Whether these morphological changes imply changes in the ecological performance of the species in the new range and whether these are the result of changes at the genetic or only phenotypic level are uncertain. The biological, social and economic impact *I. serricaudis* may have in the introduced areas cannot be estimated until further ecological studies are carried out, since there is a severe lack of information for this species, even in its native range (Hobbs et al., 2015).

In the Iberian Peninsula, the arrival of *I. serricaudis* is probably linked to accidental introduction with shellfish transfers. This is a likely associated vector (see Marchini et al., 2016a; Marchini et al., 2016b), judging by the occurrence of the species in European mussel aquaculture facilities and hotspots for mariculture and shellfish trade. In fact, Galicia, together with Cataluña, bear the highest oysters, clams and mussel production of Spain, including production of nonnative species such as the Pacific oyster (*Crassostrea gigas*) and the Japanese clam (*Ruditapes philippinarum*), and export to other countries of Europe (Instituto Galego de Estatistica, 2017; Ministerio de Agricultura y pesca, 2017). This vector has been attributed to several species with similar routes of introduction (see Gruet et al., 1976), including the isopod *Paranthura japonica* (see Figs. 3A, 3E) (Lavesque et al., 2013). Nevertheless, shipping transport is an associated vector of this species as well, given its presence in ports and its nature as fouling species of hard substrates such as docks, as well as its adaptability to different substrates (Hobbs et al., 2015). Our finding in a marina adds recreational boating as a vector, at least, for secondary transport. This means *I. serricaudis* has the potential to spread to further Mediterranean marinas as well as along the Iberian Peninsula coast. This would be not surprising since this species bears broad temperature tolerance and is expected to spread through Europe as was well as temperate waters of the southern hemisphere (see Hobbs et al., 2015). As a small-size organism, it is likely to be overlooked though; therefore, we call for prevention for the detection of this species in the mentioned areas.

Paracerceis sculpta is the most widespread species within the genus and a successful species colonizing new areas. Its type locality is San Clemente Island, California (USA) (Menzies, 1962) and its putative native range includes the northeastern Pacific region, including California (Richardson, 1905; Wallerstein, 1980; Austin, 1985; Reed and Hovel, 2006), San Quintin Bay, southern Baja California (Menzies, 1962); Puerto Peñasco, Sonora (Ohmart, 1964) and northern and central Gulf of California (Mexico) (Brusca, 1980). It has been present in Hawaii at least since 1943, probably introduced by naval shipping from southern California (Miller, 1968; McCain, 1975); and at least since 1978 in Brazil (Pires, 1980a; Pires, 1981; Loyola e Silva, Masunari and Dubiaski-Silva, 1999). It was only detected from the Gulf of Mexico in 2009 (Munguia and Shuster, 2013). From the 1990s onwards, it was reported from distant locations; from China (Yu and Li, 2001), Hong Kong (Bruce, 1990; Yu and Li, 2003), Taiwan (Yu and Li, 2003) and Japan (Ariyama and Otani, 2004), to Australia (Hass and Knott, 2000; Hewitt & Campbell, 2001) and northwest Indian Ocean, in Pakistan (Yasmeen and Yousuf, 2006). It is also considered introduced in South Africa, being ship fouling and/or ballast water its associated vector (Barnard, 1940; Griffiths et al., 2009; Mead et al., 2011). In the Mediterranean Sea, it is known from the central region since the 1970s (Rezig, 1978; Forniz and Sconfietti, 1983; Forniz and Maggiore, 1985;

Lombardo, 1985; Savini et al., 2006; Cosentino et al., 2009; Vincenzi et al., 2013), and decades after it was reported from the eastern (Katsanevakis et al., 2014) and western Mediterranean as well (Marchini et al., 2015). In the eastern Atlantic Ocean it was found for the first time in 1988_1989 in Cádiz bay (Spain) (Rodríguez et al., 1992). In the Macaronesia biogeographical region (northwestern Africa) it was detected only in 2014 (Marchini et al., 2017), collected from Ponta Delgada marina (Azores archipelago) and in 2015 (Ramalhosa et al., 2017), collected from Funchal marina (Madeira archipelago).

There is evidence for attributing shipping, including recreational boating, as vector to *Paracerceis sculpta* (Hewitt et al., 2004; Katsanevakis et al., 2014; Mead et al., 2011; Marchini et al., 2017). It is commonly found in locations of intense vessel traffic; in marinas, bays or coastal lagoons near major harbor facilities (Rezig, 1978; Forniz and Sconfietti, 1983; Rodríguez et al., 1992; Castelló and Carballo, 2001; Espinosa-Pérez and Hendrickx, 2002; Marchini et al., 2017). In the 1990s it was already present in the Mediterranean Sea and the Strait of Gibraltar. From there, it has been subsequently found in additional marinas along the southern and eastern sides of the Iberian Peninsula coast from 2011 to 2017 (Table 1); and it currently occurs from southern Portugal to eastern Spain. We report it for the first time for Alboran sea ecoregion, where all the males found belonged to the alpha morph *sensu* Shuster (1992). This supports the idea that only the alpha morph has made it into the introduced populations, consistent with the lack of beta and gamma male records in other non-native locations (Pires, 1981; Forniz and Maggiore, 1985; Rodríguez et al., 1992; Loyola and Silva, 1999; Hewitt and Campbell, 2001; Yu and Li, 2001; Ariyama and Otani, 2004; Munguia and Shuster, 2013; Marchini et al., 2017). In fact, Shuster and Wade (1991) hypothesized that the shorter lifespan of beta and gamma males is a handicap for surviving long trips and colonizing new regions.

In the Iberian Peninsula, *Paracerceis sculpta* is mainly associated to the introduced/cryptogenic bryozoan Bugula neritina and the introduced *A. verticillata*, which may have facilitated the transport and establishment of this exotic isopod (Marchini et al., 2015; Marchini et al., 2017; Gavira-O Neill et al., 2016). Additionally, we have observed a non-overlapping presence of *P. sculpta* and the native isopod *Dynamene edwardsii* in most of the stations. A further study investigating the interspecific interaction of these two species is scheduled, in order to determine the potential biological impact of *Paracerceis sculpta*. Similar to *Paracerceis sculpta*, *Paradella dianae* was first reported from Bahia de San Quintin, Baja California and Mexico (Menzies, 1962). Its native range is supposed to be Northeast Pacific, from Ventura County (California, USA) to Michoacán (Mexico), including the Gulf of California (Iverson, 1974; García-Guerrero and Hendrickx, 2004) (Fig. 3C). Before the 1980s it was reported in the western Atlantic in Puerto Rico (Menzies and Glynn, 1968) and Brazil (Pires, 1980b). First record outside of its native range was in Marshall Islands in 1967 (Glynn, 1970). From the 1980s onwards, it was found in distant areas of the world. In western Pacific, in Hong Kong in 1986 (Bruce, 1990); in Australia (Harrison and Holdich, 1982; Furlani, 1996; Hass and Knott, 2000), collected from small boats jetties; and at the other side of Indian Ocean in Pakistan (Arabian Sea) in 1984 (Javed and Ahmed, 1987). At the same time, *Paradella dianae* arrived to the southeastern coast of USA (western Atlantic) (Clark and Robertson, 1982; Van Dolah, Knott and Calder, 1984; Fox and Ruppert, 1985; Kensley and

Schotte, 1989; Nelson and Demetriades, 1992), being ship fouling the most likely vector (Knott and De Victor 2010).

It is unknown whether *P. dianae* arrived to the Iberian Peninsula and the Mediterranean Sea from the Indian Ocean, from the Atlantic Ocean, or from both through multiple introductions. It was reported from the Italian coast in 1980 (Forniz and Maggiore, 1985) and the coast of Alexandria (Egipt) (Atta, 1991); but at the same time reported across the Strait of Gibraltar, in Cádiz Bay (Atlantic side of the Strait) in 1988_1989 (Rodríguez et al., 1992) and Algeciras Bay (Mediterranean side of the Strait) in 1992 (Castelló and Carballo, 2001). From 2000 onwards it was collected and reported from additional locations in Central Mediterranean Sea (Bey et al., 2001; Ferrario et al., 2017; Ulman et al., 2017); and Eastern Mediterranean Sea (Zgozi et al., 2002, Kirkim et al., 2010; Çinar et al., 2008, Do§an et al., 2015, Kirkim et al., 2015; Ulman et al., 2017).

As well as *P. sculpta*, it was probably introduced to new locations by hitchhiking on the hulls or other surfaces of ships (Rodríguez et al., 1992; Galil, 2011). Hass and Knott (2000) also point to recreational boating as a likely vector, at least for its introduction to Australia. Our study supports this hypothesis, since it was found again in marinas located in Cádiz Bay (Strait of Gibraltar's vicinity) plus others along the Alboran Sea coast. Marinas of southern Iberian Peninsula coasts are well connected by frequent local traffic; 90% of visiting boats in the sampled marinas are Spanish, plus a percentage of foreign boats usually coming from Europe (UK, France, Holland) and other parts of the world (America, Australia, Arabic countries) (marina staff, personal communication). In fact, our data shows an ongoing expansion of *Paradella dianae* into additional marinas, potentially colonizing the eastern side of the Iberian Peninsula into the western Mediterranean Sea. Even having the same native range and potentially bearing a similar pattern of introduction than *P. sculpta*, *P. dianae* does not seem to be as successful, bearing lower densities than *P. sculpta* and a smaller introduction range (Figs. 3B, 3C).

Sphaeroma walkeri is the most widespread of these species, reaching numerous ports worldwide (see Carlton and Iverson, 1981). Stebbing (1905) first described it from in Ceylon (now Sri Lanka, Indian Ocean), with the northern Indian Ocean being its native range, including India, Arabian Sea and Bay of Bengal (Carlton and Iverson, 1981). It was known from the Persian Gulf some years later and the introduction status in this locality is doubtful, thus considered cryptogenic (Fofonoff et al., 2017) (Fig. 3D). Carlton and Iverson (1981) propose an episodic dispersal for this species. An initial local transport (pre-1870 period) would have occurred around the Indian Ocean plus South Africa (Stebbing, 1917), where it was found in fouling on pilings, Mozambique (Barnard, 1955) and Australia (Baker, 1928; McNeill, 1932; Iredale, Johnson and McNeill, 1932). A second period would be related to the opening of the Suez Canal in 1869. The record of this species in Port of Suez already in 1904_1905 (Stebbing, 1910) is doubtful; therefore, we agree with Fofonoff et al. (2017) and consider *S. walkeri* cryptogenic from this locality as well (Fig. 3D). From there, it would have travelled through the Suez Canal into the Mediterranean Sea (Omer-Cooper, 1927; Larwood, 1940). A post 1940 period would have been coincident with World War II. *Sphaeroma walkeri* would have been transported to the American continent associated to the intense shipping traffic since that time. It was found in Brazil (Loyola e Silva, 1960), Puerto Rico (Menzies and Glynn, 1968), Florida (Miller, 1968; Camp et al., 1977; Nelson and Demetriades, 1992) and Hawaii (Miller, 1968). From those areas, it continued to increase its distribution to different parts of the world. To the western Pacific in Hong Kong in 1972 (Vrijmoed, 1975; Morton, 1987), Hainan (southern China) from pier fouling samples (Kussakin and Malyutina, 1993) and other locations in Australia (National Museum of Natural History (Smithsonian Institution) collections (NMNH), 1967; Montelli and Lewis, 2008). To the eastern Pacific in San Diego Bay (California), it was first detected in 1973 in fouling on pilings, floats and small boats at yacht harbours (Carlton and Iverson, 1981). Along the western Atlantic coast it was found in other locations of the Gulf of Mexico (Clark and Robertson, 1982; Cházaro-Olvera et al., 2002), Cuba in 1994 (USNM 280039, US National Museum of Natural History 2007) and Isla Margarita (Venezuela) in 2004 (Gutiérrez, 2012). Along the Northwest coast of Africa, it was also associated with harbours (Jacobs, 1987). On the Indian Ocean it was reported from Malaysia only in the 1990s (Rai-Singh and Sasekumar, 1996) and from Iran in 2006_2010 (Khalaji-Pirbalouty and Wägele, 2010). Across the Mediterranean Sea, it continued spreading to further eastern locations until the present year (Glynn, 1972; Kocata_s, 1978; Galil, 2008; Ulman et al., 2017). It was recorded in the Italian Peninsula (Lodola, 2013) and found to be completely established with successful populations in Tunisia harbours and lagoons (Ben Souissi et al., 2004; Ben Amor et al., 2010). In was also reported in the western Mediterranean (Zibrowius, 1992), being reported from Spain for the first time in 1981 (Jacobs, 1987). In 2017, we report *Sphaeroma walkeri* from the southern Iberian Peninsula, in Cádiz Bay.

The route of introduction to southern Spain and the Strait of Gibraltar is unknown and several are possible. Initially, specimens may have arrived to the Mediterranean Sea from faraway ports in Indian Ocean or Australia; or from the long-established population in Suez Canal, and subsequently spread towards the western Mediterranean Sea, arriving to France and eastern Spain. It may also have arrived from western Atlantic populations from America or northwestern Africa and entered through the Strait of Gibraltar (Spanier and Galil, 1991; Galil, 2008); or from both Indian and Atlantic populations through multiple introduction events. In any case, its presence in Puerto América marina also indicates a transport via shipping, including recreational boating as vector. This supports the findings of Ulman et al. (2017), who collected individuals of *S. walkeri* directly from hull fouling of recreational vessels in Mediterranean marinas. Interestingly, *S. walkeri* was first reported from the Macaronesia biogeographical region only two years ago; at Funchal marina, presumably introduced by means of recreational boating from populations in the Canary Islands (Spain) or the Madeira island system itself (see Ramalhosa et al., 2017). Considering that *S. walkeri* was already present in Marocco and Mauritania (northwestern Africa) since the early 1980s (Jacobs, 1987), it could have introduced to marinas across Madeira, Canary Islands and the Strait of Gibraltar years ago, even though it was detected only now. An interspecific competition pressure among *S. walkeri* and its congener *S. serratum* has been suggested for the Lagoon of Tunis (Ben Amor et al., 2015), but further studies are necessary to evaluate its biological impact in the Iberian Peninsula.

Finally, Richardson (1909) first described *Paranthura japonica* from material collected from Muroran (North Japan). Its native range only includes localities from Japanaese coasts (Nunomura, 1977; Yamada et al., 2007), eastern Russia (Sea of Japan) (Nunomura, 1975; Moshchenko and Zvyagintsev, 2004), Kurile Islands (Kussakin, 1975) and eastern China (Che and Morton, 1991; Li, 2003; Zhang et al., 2009; Wang et al, 2010) (Fig. 3E). It was reported as alien for San Francisco Bay in 1993 (Cohen and Carlton, 1995) and found to be widespread in southern California harbours in 2000 (Cohen et al., 2005). Between 2007 and 2010 it was first found in European waters; in Arcachon Bay (Bay of Biscay, France), probably introduced with oyster transfers. This Bay is one of the major French oyster farming sites (Verlaque et al., 2008), and during the 1970s, the exotic Pacific cupped oyster Crassostrea gigas (Thunberg 1793) from the Senday Bay (Japan) was massively introduced (Mineur et al., 2014), in order to sustain the local industry after a viral disease of *Crassostrea angulata* (Lamarck 1819). *Paranthura japonica* probably remained unnoticed or misidentified since then (see Lavesque et al., 2013). It was found in the Mediterranean for the first time in the Lagoon of Venice, probably in 2000 (Marchini et al., 2014). It is thought to have arrived as shellfish import directly from Arcachon Bay, associated with the clam *Ruditapes philippinarum* (Adams and Reeve 1850) during the 1970s; and secondary spread to further Mediterranean marinas (see Marchini et al., 2014; Marchini et al., 2015; Lorenti et al., 2016; Ferrario et al., 2016b; Dailianis et al., 2016; Tempesti et al., 2016; Ferrario et al., 2017; Ulman et al., 2017).

It was reported only recently from the Iberian Peninsula, from samples collected from fouling assemblages in marinas of the eastern coast in 2016 (Ulman et al., 2017). Nevertheless, our study proves that *P. japonica* has been present in Barcelona and Valencia (eastern Iberian Peninsula) at least since 2011. Ulman et al. (2017) suggest this species to be `polyvectic' (meaning it has been transported by multiple mechanisms, according to Cohen (1977), Carlton and Ruiz (2005)), and points at recreational boating as vector for its secondary spread across the Mediterranean Sea. Our data supports this hypothesis, since *P. japonica* was found in Barcelona, Benicarló and Mallorca (Balearic Islands), which are popular destinations for vessels cruising the western Mediterranean in between Barcelona to the West and northwestern Italy to the East (Ulman, personal communication). In 2014, two individuals of *P. japonica* were found within the Strait of Gibraltar's vicinity, in Chipiona rocky shores (Cádiz) (Cabezas, pers.comm); and three years later, it was abundant in marinas located in Cádiz Bay. Cádiz is a great hotspot for both international commercial shipping and pleasure craft, as well as a center for aquaculture production, including the Japanese clam *Ruditapes philippinarum* (Junta de Andalucía, 2014). Just as in Italy, this clam was intentionally introduced for commercial use in Spain in the 1970s. Despite having conducted several samplings in Cádiz marinas before 2014, this species was never found to be present before that date. On one hand, it is possible that P. japonica has arrived to Cádiz bay due to shellfish transfers since the 1970s, but have remained unnoticed and located only in aquaculture facilities instead of spreading to nearby marinas, thus undetected during sampling campaigns. On the other hand, it seems more likely that it spread via recreational boating from the Italian Peninsula to the eastern Iberian Peninsula (present in 2011), and later on to Cádiz marinas (present in 2017). It is to be noticed that *P. japonica* was not present in the bryozoan B. neritina in Puerto América marina in 2011; but it was found associated to the same host in 2017. This fact supports this record as a new arrival of NIS into a particular region, and thus represents a Marine Strategy Framework Directive indicator to establish Cádiz Bay as a hotspot for marine introductions, following Olenin et al. (2016).

In the light of the results, we outline that building comprehensive data on the presence and distribution range of exotic species, especially on new arrivals is the first step in order to be ready for preventing invasions (see Bishop and Hutchings, 2011; Groom et al., 2015; Olenin et al., 2016). The next step is to evaluate their potential biological, social and economical impact, however, there are gaps of knowledge that hamper this task. Baseline studies delving into the ecology of all these species (i.e. role as prey-predator in the trophic chain, habitat selection, role in their ecosystem functioning) are of great need in here (see Table 1 Blackburn et al., 2014). Although none of the NIS found in the present study were found in the extensive survey of natural coastal habitats by Guerra-García et al. (2012), future surveys including natural areas would be necessary to detect a potential secondary spread into these habitats. Lags in detection of small-size and scarcely studied organisms need to be mitigated; as small does not mean ''unimportant'' (Carlton, 2011) and, since biological invasion processes are ''irritatingly idiosyncratic'' (Richardson et al., 2000), exotics can exist in relatively low numbers before exploding. This means we risk underestimating the potential impact of taxa like the Order Isopoda that, as shown in the present study, can subsequently spread across additional marinas within a short timeframe. We consider this account serves as documentation and update about the marine exotic isopods dwelling in the Iberian Peninsula; as well as to draw attention to these overlooked taxa and the risk of recreational boating as a vector.

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SUPPLEMENTARY MATERIAL

Table S1. List of introduced isopod species in European waters, updated with the findings of the present study. Name of the species, parasite/free-living status, origin, distribution in European waters, introduction status remarks and likely vectors of introduction are provided. MED, Mediterranean Sea; WMED, Western Mediterranean; CMED, Central Mediterranean; EMED, Eastern Mediterranean; ATL, Atlantic; NOR, North Sea; C, casual; E, established; NE, non-established; nd, no data available. Species with asterisk are those found to be present in the Iberian Peninsula.

Species	Origin	Distribution (European waters)	Introduction status remarks	Likely vectors of introduction
Parasites				
Anilocra pilchardi Bariche & Trilles, 2006	Indo-Pacific	WMED, CMED, EMED	C (Zenetos et al., 2010)	Canals (Galil, 2011)
Cymothoa indica Schioedte et Meinert, 1884	Indo-Pacific	EMED	C (Zenetos et al., 2010)	Canals (Galil, 2011)
Free - living				
Apanthura sandalensis Stebbing, 1900	South Africa	CMED, EMED	E (Zenetos et al., 2010)	nd
Cymodoce fuscina Shotte & Kensley, 2005	Persian Gulf	EMED	nd	Vessel fouling (Ulman et al. 2017)
** Ianiropsis serricaudis* Gurjanova, 1936	NW Pacific	CMED, WMED, ATL (Spain)	E in CMED (Marchini et al. 2016a) and Spain (present study)	Vessels (Ulman et al. 2017; present study)
Limnoria quadripunctata Holthuis, 1949	S Indo-Pacific	ATL (British Isles to Spain)	NE in Portugal (Chainho et al. 2015)	Vessel fouling (Noël, 2011; Chaino et al. 2015)
Limnoria tripunctata Menzies, 1951	S Indo-Pacific	ATL (British Isles), NOR	nd	Vessel fouling (Noël, 2011)
** Paradella dianae* (Menzies, 1962)	NE Pacific	WMED,CMED,EMED, ATL (Spain)	E in CMED,EMED (Zenetos et al. 2010)	Vessel (Galil, 2011); vessel fouling (Ulman et al. 2017; present study)
** Paracerceis sculpta* (Holmes, 1904)	NE Pacific	WMED, CMED,EMED, ATL (Spain)	E in MED (Zenetos et al. 2010) and Spain (present study)	Vessels (Galil, 2011); vessel fouling (Ulman et al. 2017; present study)

** Paranthura japonica* Richardson 1909	NW Pacific	WMED,CMED,EMED, ATL (France, Spain)	E in CMED (Ulman et al. 2017)	Vessel fouling and shellfish trade (Lavesque et al. 2013; Marchini et al. 2014; Lorenti et al.2016; Ulman et al. 2017; present study)
** Sphaeroma walkeri* Stebbing, 1905	Indian Ocean	WMED,CMED,EMED, ATL (Spain)	E (Zenetos et al. 2010)	Vessels (Galil, 2011); vessel fouling (Ulman et al. 2017; present study)
Synidotea laticauda Benedict, 1897	Japan	ATL (France)	nd	Vessel fouling and mariculture (Noël, 2011)

3

BASELINE SURVEYS TO UNCOVER MISSING INFORMATION: PART 1

The challenge of hidden invaders

3.1.2. Scientific collaboration for early detection of invader results in a significant update on estimated range:

lessons from *Stenothoe georgiana* Bynum & Fox 1977s

Adapted from: Martinez-Laiz G, Ros M, Guerra-García JM, Marchini A, Fernández-González V, Vázquez-Luís M, Lionello M, Scribano G, Sconfietti R, Ferrario J, Ulman A, Costa AC, Micael J, Poore A, Cabezas MP, Navarro-Barranco C (2020). Scientific collaboration for early detection of invaders results in a significant update on estimated range: lessons from Stenothoe georgiana Bynum & Fox 1977. Mediterranean Marine Science 21(2):464-481

ABSTRACT. Detection of new non-indigenous species is often delayed when taxa are taxonomically challenging, such as small-sized marine organisms. The present study highlights the relevance of scientific cooperation in the early detection of the invader amphipod Stenothoe georgiana. Originally described from North Carolina (USA), the species was recently found in Chile and the Western Mediterranean. Here, we provide the first record of the species in Macaronesia, Atlantic coasts of continental Europe, North Africa and Australia, and extend its known distribution along the Mediterranean coast. Just like other small crustaceans, shipping (both ballast water and recreational boating) and aquaculture are probably the main vectors of introduction and secondary spread for this amphipod species. This case of S. georgiana sheds light on the importance of promoting taxonomical knowledge, and building multidisciplinary networks of experts that ensure an effective diessemination of alien species information. We also encourage the implementation of standardized monitoring methodologies to facilitate early detection of small mobile invaders.

1. INTRODUCTION

As commented in previous chapters, management of non-indigenous species (NIS) in the marine environment becomes particularly challenging due to the magnitude of goods and services traded, their emerging pathways and the inherent complications of accessing and sampling this biome (Tingley et al. 2018; Townsend et al. 2018). In order to ensure early detection of introductions, rigorous baseline surveys are required, along with surveillance and monitoring programs (Lehtiniemi et al., 2015). Unfortunately, such missions are hampered by issues such as incomplete and inaccurate data availability on species distributions; scattered information across different unstandardized datasets; and cumulative time-lags in detection, acknowledgment and reporting of invaders (Gatto et al., 2013; Ojaveer et al., 2015; Marchini & Cardeccia, 2017; Galil et al., 2018; Zenetos et al., 2019). As commented in previous sections, this is especially relevant for the so-called “hidden invaders” like small-size invertebrates. For example, when conducting monitoring campaigns in harbours and marinas, rapid assessment surveys may suffice for larger-sized taxa or sessile invertebrates. Contrarily, *in situ* detection of numerous epifaunal species is often impossible; usually due to their small sizes and crypsis within their arborescent substrate. In these cases, it is crucial to also sample the fouling substrate inhabiting the surface of artificial structures, which have a tendency to harbor non-native species (Dafforn *et al.*, 2012; Airoldi *et al.*, 2015; Foster *et al.*, 2016). Despite their small size, small invertebrates have a crucial role in marine food webs, nutrient cycling, habitat structuring and ecosystem functioning (*e.g*. Ieno *et al.*, 2006; Mermillod-Blondin and Rosenberg, 2006). In many cases, managing newly detected alien species is neither efficient nor affordable, often due to time-lags in the timing of records and uncertainty about potential impacts. Consequently, there is a great need of taxonomic expertise focusing on smaller organisms, a better understanding of their functional role in ecosystems, and sustained cooperation among the scientific community through open communication and knowledge dissemination. For example, while there are some well documented invasions of amphipods, the number of introduced species is almost certainly underestimated due to the presence of cryptogenic species, unresolved taxonomy and overlooked introductions (see Marchini and Cardeccia, 2017). To tackle this difficult group, a strong and active cooperation between senior, experienced taxonomists, early-career taxonomists or parataxonomists, and those involved in processing large numbers of samples from monitoring surveys, are key factors that need to be encouraged.

The amphipod species *Stenothoe georgiana* Bynum & Fox, 1977 stands out as a primary example of how the combination of taxonomical expertise and scientific cooperation can result in the early detection of small marine invaders. Indeed, the genus *Stenothoe* stands out as one of the most taxonomically challenging amphipod genera, due to smaller than average sizes and minute differences among species in the relevant characters for identification (Krapp-Schickel, 2015). *Stenothoe georgiana* recently emerged as a new non-indigenous species (NIS) in the Mediterranean Sea (Fernandez-González & Sanchez-Jerez, 2017; Ulman *et al.*, 2017; Servello *et al.* 2019) and Southeastern Pacific (Pérez-Schultheiss & Ibarra, 2017). It was first described from North Carolina (USA) by Bynum & Fox (1977) and all of its records in the subsequent decades were limited to the Atlantic coast of USA (*e.g.* Fox and Ruppert, 1985; Nelson and Demetriades, 1992). In 2017, one of the co-authors of the present study, Victoria Fernández-González (thereafter VF-G), found the species across the Atlantic in 2010 from the Spanish Mediterranean coast. An early and rapid exchange of information amongst several amphipod experts aided other researchers working with NIS in fouling communities to detect the new invader in other Mediterranean regions (Ferrario *et al.*, 2017, 2018; Ulman *et al.*, 2017, 2019; Servello *et al.,* 2019). As a further advancement of the above-mentioned collaboration, we present here an updated worldwide distribution of the species, including new records, along with a discussion about invasion dynamics and *S. georgiana's* new status as a neo-cosmopolitan species (*sensu* Darling & Carlton, 2018). Using this case study, we highlight the need for scientific cooperation to properly address early detections and better manage invasive species.

2. MATERIAL AND METHODS

2.1 Collection of samples

Samples for this study were collected during several sampling surveys between 2010 to 2017 in Portugal, Spain, Morocco, Italy, Tunisia, Malta, Croatia, Greece, Turkey and Australia (Fig. 1).

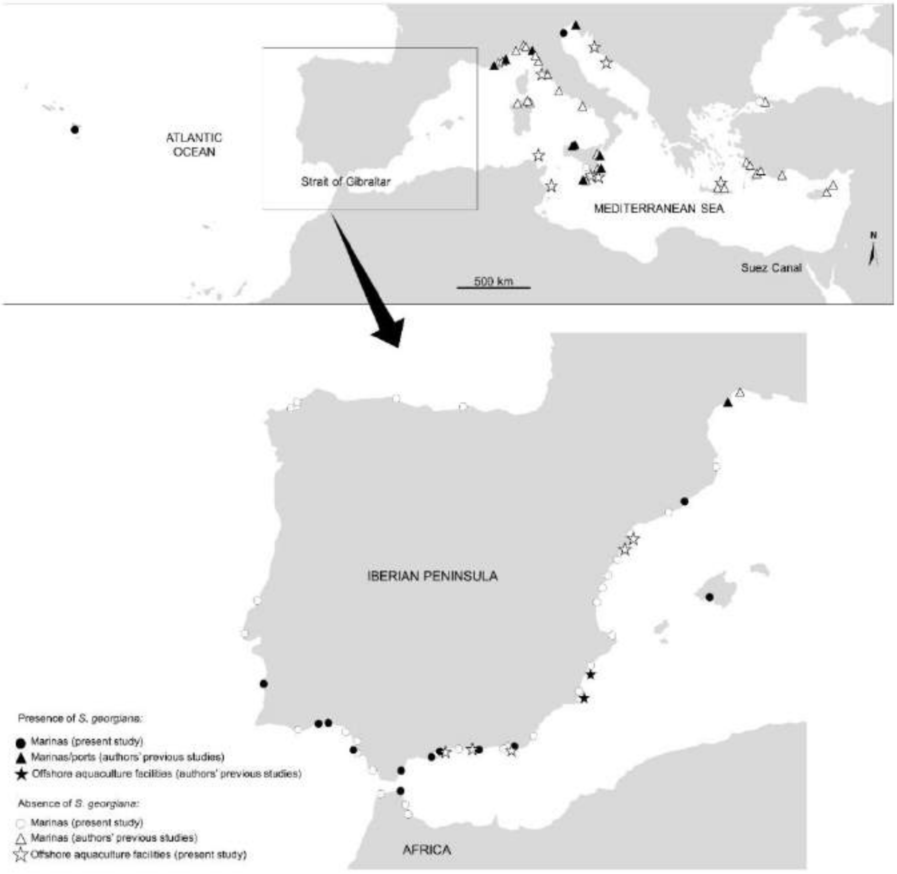

Figure 1. Presence/absence of *Stenothoe georgiana* in localities studied by the authors. Previous records are taken from Fernández-González & Sánchez-Jerez, 2017; Ferrario *et al.* 2017, 2018; Ulman *et al.,* 2017, 2019; Lo Brutto *et al.*, 2018. See also Table 2 for presence records and Supplementary material for absence records.

2.1.1 Iberian Peninsula, Balearic Islands and North Africa

A total of 42 marinas were surveyed along the whole Iberian Peninsula and North Africa during the late spring-summer of 2011 (see Ros *et al.*, 2014 for details). In each marina, three colonies of the bryozoan *Bugula neritina* (Linnaeus 1758) were hand-collected from the submerged portion of pontoons close to the surface (see Ros *et al.*, 2015 for details). Out of these, twelve marinas of the Andalusian coasts (Iberian Peninsula) were sampled a second time in 2017 using the same methodology. Additionally, extra samples from fouling communities growing on artificial hard substrate including pontoons, ropes, wheels, buoys and ship hulls were inspected in these marinas. These included red and green algae, hydroids, bryozoans, ascidians and molluscs and their associated mobile epifauna. Finally, the Marina of Ceuta (North Africa) was sampled in 2015 and 2016 as part of an experimental field study (Ros *et al.*, 2020). In this case, a survey of the floating pontoons was carried out by scraping the artificial substrate. To explore seasonal fluctuations of *S. georgiana*, we also analysed data from two monitoring programs in 2012, aimed to assess the peracarid community associated with fouling substrates. In Puerto América marina (Cádiz), three replicates of the hydroid *Eudendrium racemosum* (Cavolini, 1785) and the bryozoan *Amathia verticillata* (delle Chiaje, 1822) were sampled monthly from floating pontoons. In Palma Marina (Balearic Islands), three replicates of the hydroid *E. racemosum* were collected monthly for the same purpose.

Between 2010 and 2013, a total of 10 offshore aquaculture facilities were sampled around the Spanish border of the western Mediterranean coast and Tunisia (see Fernandez- Gonzalez & Sanchez-Jerez, 2017 for details). In each facility, samples were collected from mooring ropes by scraping all fouling organisms from 20 cm of rope. Between 2010 and 2011, two of these fish farms (located in Alicante) were additionally sampled to study seasonal fluctuations in different fouling substrates (i.e. mussels, hydroids and algae) using an air-lift device to ensure a quantitative sampling method. In 2014, as part of an integrated multi-trophic aquaculture study, artificial collectors were deployed around two fish farms in Málaga and Almería (Andalusian coast).

In all surveys, samples were preserved (in ethanol 70%, formalin or frozen until examined) and checked for the presence of *S. georgiana* (see Ros *et al.*, 2015; Fernandez- Gonzalez *et al.*, 2018 for details).

2.1.2 Italian Peninsula, Croatia, Malta, Greece and Turkey

Between 2010 and 2013, as part of the offshore aquaculture study, seven facilities were sampled in the coasts of Italy, Croatia, Malta and Greece, using the same methods described above. In all surveys, the biota collected off the substrates (both natural and artificial) were preserved in ethanol or formalin and sorted later at the laboratory to check for the presence of *S. georgiana*. Similarly, from April 2014 until November 2015, marinas across the northern rim of the Mediterranean Sea were sampled for their biofouling compositions focusing on alien species in Malta, Greece and Turkey. A hand-held rigid net with one sharpened edge was used to scrape the submerged portion of pontoons; and a paint scraper was used to sample buoys and ladders. The samples were preserved in 96% ethanol, sorted and identified to species level. In the Lagoon of Venice (Italy, North Adriatic Sea), a sampling survey was conducted in July 2017 along the estuarine gradient of the River Dese, within the northern part of the lagoon; using a hand-held net, eight stations were sampled focusing on the subtidal portion of wooden piles, which mark the navigable canals. Temperature and salinity were measured during both low and high tidal conditions. Additionally, samples were obtained from an offshore platform located in the North Adriatic Sea, at 7.8 nautical miles from the coast. Located just in front of the Lagoon of Venice, this plaftorm is a stop-over site for cruise and commercial ships, while they are waiting for access permission into the Lagoon, and is one of the sites where the Regional Agency for Environmental Protection and Prevention of the Veneto (ARPAV) regularly conducts monitoring surveys. In March and September 2019, the fouling community was collected by scuba divers by scraping the surface of 33 x 33 cm quadrats from a zinc-coated iron pillar, at both 6 m and 14 m depths.

2.1.3 Azores (Macaronesia)

In spring of 2013, 2014 and 2015, surveys of the fouling communities associated with boat hulls and pontoons were conducted in Ponta Delgada (Azores), as part of a general study aimed at identifying alien species in the area. The marina in Ponta Delgada had been expanded in 2008 to allow the simultaneous mooring of 600 recreational boats plus a transatlantic cruise pontoon. Fouling organisms (mainly bryozoans) growing on artificial hard substrata were hand-collected by snorkeling and immediately placed into zip-lock plastic bags. Samples were subsequently cleaned in seawater and Amphipoda were sorted, preserved in 96% ethanol, and identified to species level.

2.1.4 New South Wales (Australia)

In 2017, artificial collectors made of several small branches of frayed polypropylene rope were deployed in Chowder Bay (Sydney, Australia) during one month, as part of an experimental recolonization study for peracarid crustaceans (Navarro-Barranco *et al.*, unpublished data). Samples were preserved in 70% ethanol, and the associated fauna was sorted and examined for the presence of *S. georgiana.*

2.2 Dissemination of records information

In 2012, VF-G observed an unusual species of the genus *Stenothoe* in samples collected in 2010 from the Spanish Mediterranean coast. The identity of the specimens remained unknown until a key publication was published which included a taxonomical revision of the *Stenothoe* genus by a senior taxonomist (Krapp-Schickel, 2015), providing a comprehensive key to global species. The same year, VF-G performed a thorough taxonomic analysis, and personally communicated with Traudl Krapp-Schickel which confirmed the identity as *S. georgiana*. When it was clear that the finding represented a new record for the Mediterranean Sea, prior to the publication of the record, the author personally communicated her finding with some previous Spanish collaborators and co-authors of the present work. In parallel work, Italian co-authors found additional specimens of “unknown” *Stenothoe* sp. in fouling samples from multiple Mediterranean marinas in 2016, and reported these findings to the same amphipod team to help determine the identity.

This early exchange of information enabled researchers working with NIS to detect *S. georgiana* in further Mediterranean regions. It is worth mentioning that these and multiple other researcher's scientific networks were born from participating in the International Colloquium of Amphipoda (ICA), a biannual specialistic international conference on this group taxa. All teams raised the flag on the presence of a new invader among the Amphipod network's experts working with fouling communities; and engaged researchers from 12 institutions from different countries to coordinate the present work.

2.3 Identification remarks

Detailed morphological descriptions of *S. georgiana* are provided by Bynum & Fox (1977) and Pérez-Schultheiss & Ibarra (2017). The species is distinguishable by the following characters for males (Fig. 2): palmar corner of gnathopod 2 defined by a semicircular rounded spinose hump; large coxa, rounded anteroventrally and straight posteriorly; gnathopod 1 article 6 with posterior border convave, palm defining angle bearing 4 spines and dactyl fitting palm; U1 peduncle slightly longer than rami, with small distal tooth; U2 peduncle and rami subequal, rami with longitudinal rows of fine serrations; U3 peduncle shorter than entire ramus, article 2 of ramus shorter than article 1; telson apex acute, lateral margins bearing two spines. These and other authors (*e.g.* Krapp-Schickel, 2006) highlighted the morphological similarities between *S. georgiana* and *S. estacola* J.L. Barnard 1962, the latter described from the Pacific coast of USA (Barnard, 1962). *Stenothoe georgiana* and S. *estacola* were also grouped with the Hawaiian species *Stenothoe haleloke* J.L. Barnard 1970, within the key to all *Stenothoe* species provided by Krapp-Schickel (2006). Males of *S. haleloke* were not available at that time but the later synonymization between *S. haleloke* and *Stenothoe qingtaoensis* Ren, 1992 contributed to the establishment of distinguishable characters (*e.g.* unlike *S. estacola* and *S. georgiana*, gnathopod 2 propodus in males and females of *S. haleloke* lack a clear palmar corner; Krapp-Schickel *et al.*, 2015, Krapp-Schickel, 2015).

Males of both *S. georgiana* and *S. estacola* are characterized by a defining palmar hump on the propodus of gnathopod 2. However, only *S. georgiana* bears robust spines on the hump. Other noticeable differences are: a narrow palmar carpal lobe of *S. estacola* gnathopod 2 (broader in *S. georgiana*), the presence of several additional small palmar blunt cusps (absent in *S. georgiana*), a clearly longer antenna 2 in comparison with antenna 1 in *S. estacola* (antenna 1 is a slightly longer in *S. georgiana*), and the number of dorsal setae on peduncle of the uropod 3 (1 in *S. georgiana* vs 3 in *S. estacola*) (Bynum & Fox, 1977; Pérez-Schultheiss & Ibarra, 2017). However, after examination of additional material (also collected in the California coast and presumably attributed to *S. estacola*) and type series specimens of *S. estacola,* Barnard (1969) reported contrasting characters with those described in the holotype which included the presence of spines on the surface of the palmar hump, a less noticeable cusp, a broader carpal lobe, a slightly longer antenna 1 and differing number of spines on uropod 3. At that time, Barnard (1969) suggested that the differences could be attributable to ontogenetic changes, but this hypothesis was not later confirmed. Likewise, the specimens examined by Barnard (1969) more closely resemble *S. georgiana* (that would be described eight years later) than the *S. estacola* holotype described by Barnard (1962). Unfortunately, Bynum & Fox (1977) did not mention the particularity of this material on the original description of *S. georgiana.* Under this scenario, further molecular and morphologic phylogenetic studies would be helpful to help clarify the relationship between *S. estacola* and *S. georgiana*.

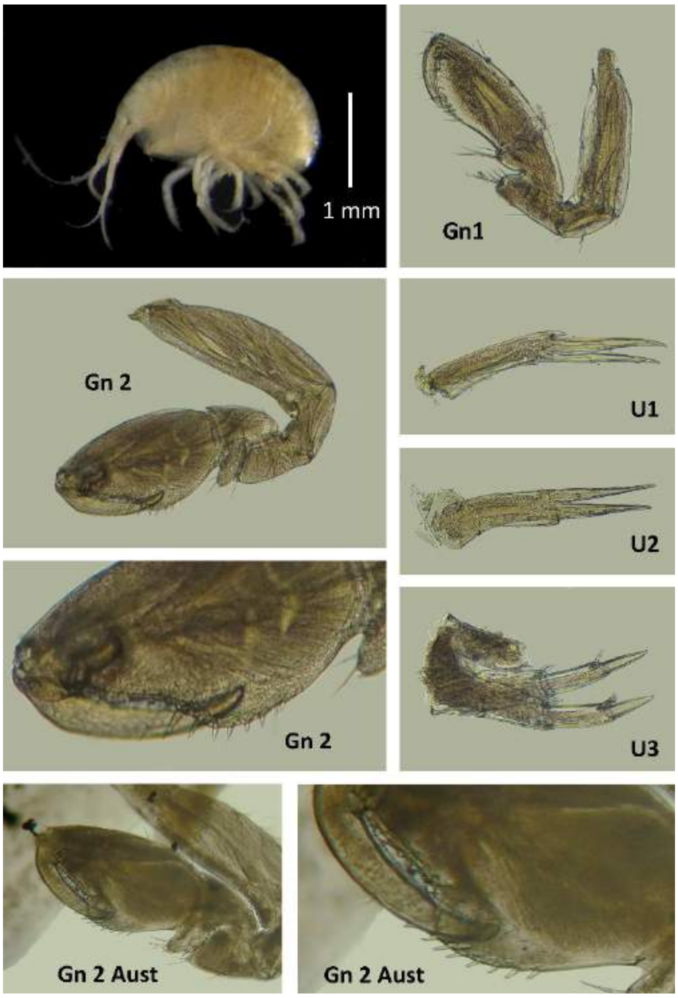

Fig. 2: *Stenothoe georgiana* Bynum & Fox, 1977. Lateral view, gnathopod 1 (Gn1), gnathopod 2 (Gn2) with propodus detail, uropods 1, 2 and 3 (U1, U2, U3) of males from Southern Iberian Peninsula. Gnathopod 2 with propodus detail (Gn2 Aust) of male from Sydney, Australia.

3. RESULTS

3.1 Previous records and habitat use of *S. georgiana*

The known distribution records of *S. georgiana* prior to the present study (from 1935 to 2017 in both putative native and introduced ranges) are listed in Table 1. Fifty-four percent of those locations represented records of its putative native range. Of those, 31% of records correspond to natural habitats (offshore hard-bottom areas, nearshore habitats including mud, sand, patches of sponges, and soft corals), 46% to artificial habitats (piling and seawalls, rock jetties, artificial reefs, and piers) and 23% to anthropized sounds and estuaries. In contrast, all records from the putative introduced range are from artificial habitats (75% in harbours and marinas; and 25% in aquaculture facilities).

New records and spreading of S. georgiana

Table 1. Previously known Stenothoe georgiana records worldwide. Records from its putative introduced range are shaded. Likely vectors: 'SF'= ship fouling; 'SF (rb) = recreational boating; 'BW' = ballast water; 'AQ' = aquaculture. * in vectors represents assumptions made by the authors. '-' = data not available.

Date	Country	Localities	Collected from	Vector	Author of record
1935	USA	Sapelo Island (Georgia)	Offshore, 11 miles off-coast	-	Identified by Mr. Clarence R. Shoemaker GBIF.org (2019) [1]
-	USA	Chesapeake Bay and Norfolk (Virginia); New River, Shackelford Bank and Beaufort (North Carolina); St. Catherine Sound (Georgia); Sarasota Bay, Tampa Bay and Loggerhead Key (Florida)	-	-	Mr. Clarence R. Shoemaker, unpublished data in Bynum & Fox (1977)
-	Brazil	Rio de Janeiro and Sacco São	-	-	Mr. Clarence R. Shoemaker, unpublished data in Bynum & Fox (1977)
1957-1966	USA	Institute of Marine Science pier station, Drum Inlet station, Lockwoods Folly Inlet station (North Carolina)	Nocturnal surface macroplankton in estuaries	-	Williams and Bynum (1972) in Bynum & Fox (1977) (as '*Stenothoe* sp.' and '*S. minuta'*
1971	USA	North Carolina, Chesapeake Bay (Virginia), Tybee Inlet and Sebastian Inlet (Florida) *	Fouling community on a pier	-	Bynum & Fox, (1977)
1975	USA	North Carolina estuaries	Fouling community and shelly bottom	-	Fox and Bynum (1975)
1980-1981	USA	Charleston (South Carolina), Sapelo Island (Georgia), Jacksonville (Florida)	Stomach contents of *Archosargus probatocephalus* at offshore reef habitats	-	Sedberry (1987)
1981-1985	USA	South Carolina, shallow waters	Pilings and Seawalls, creeks and sounds	-	Fox and Ruppert (1985)

1982	USA	St.Catherine’s Island (Georgia)	Sponges and corals at hard-bottom area	-	Wendt et al. (1985)
April 1984-March 1985	USA	Sebastian Inlet (Florida)	Sabellariid worm *Phragmatopoma lapidosa* at rock jetties	-	Nelson and Demetriades (1992)
1985-1987	USA	South Carolina	Stomach contents of *Chaetodipterus faber* in shallow nearshore habitats (sponges, soft corals); estuarine habitats; and artificial reefs and jetties	-	Hayse (1990)
2004	USA	Tampa Bay (Florida)	Artificial reefs dominated by mussels and oysters	-	Dix et al. (2005)
2010	Spain	Alicante and Murcia, off-coast sea bass-sea bream aquaculture facilities	Fouling dominated by algae, hydroids and mussels	AQ	Fernandez-Gonzalez and Sanchez-Jerez (2017)
2011	Spain	Alicante, off-coast sea bass-sea bream and oyster aquaculture facilities	Fouling dominated by algae, mussels and anemones	AQ	Fernandez-Gonzalez and Sanchez-Jerez (2017)
2012	Chile	Los Lagos and Atacama	Salmonid farms	AQ*	Pérez-Schultheiss and Ibarra (2017)
2013	Italy	Lerici marina	Fouling communities on dock walls and floating pontoons	SF (rb)	Ferrario et al. (2017)
2014	Italy	Porto Torres harbour	Fouling communities on the subtidal level of dock walls	SF	Ferrario et al. (2017)
2015	Mexico	Puerto Progreso (Yucatan)	Macroalgae associated to buoys, chains, pilings and seawalls	SF*	Winfield et al. (2015)
2015	France	Port du plaisance du Port Camargue (Le Grau-du-Roi), Port Principal du Cad d’Agde (Agde) and Port Vauban (Antibes)	Marina fouling at Port Camargue; recreational vessel hull-fouling at Cap d’Adge and Port Vauban	SF (rb)	Ulman et al. (2017)

2015	Italy	Porto Turistico Marina Piccola di Sorrento (Sorrento, Campania)	Marina fouling and vessel hull-fouling	SF(rb)	Ulman et al. (2017)
2016	USA	Tampa Bay (Florida)	Artificial reefs dominated by barnacles, sponges ascidians and bryozoans		Karlen et al (2017)
2016	Italy	Marina Villa Igiea (Palermo), Porto Grande (Siracusa), Porto dell'Etna (Riposto), Marina di Cala del Sole (Licata) (Sicily)	Marina fouling and vessel hull-fouling at Palermo and Licata; marina fouling at Siracusa; hull-fouling at Riposto	SF(rb)	Ulman et al. (2017)
2016	France	St. Tropez marina (Cote d'Azur)	Sailing boat hull-fouling	SF(rb)	Ulman et al. (2017)
2016	Malta	Valletta	Marina fouling at Grand Harbor Marina	SF(rb)	Ulman et al. (2017)
2017	Slovenia	Harbour of Piran	Dock fouling	SF	Ferrario et al. (2018)
2017	Italy	Porto di Palermo (Palermo, Sicily)	Rope fouling	SF	Lo Brutto et al. (2018)

[1] The earliest record of the species are some specimens collected by M.C.R. Shoemaker from Sapelo Island (Georgia) and deposited in 1935 in the National Museum of Natural History, Smithsonian Institution.

A - New regions

The present study provides new records of S. georgiana in Portugal, North Africa (Ceuta) and Australia, as well as new locality records in countries where the species had already been detected: Spain and Italy (Table 2, Figs. 1, 2).

In the marinas of the Iberian Peninsula, the species is mainly distributed in the southern region, from Sines to Almería, but is also present in Barcelona (North – Western Mediterranean Sea) and Palma (Balearic Islands). In Venice Lagoon (Northeastern Italy, Adriatic Sea), where salinity varies with tidal conditions, S. georgiana was found in the higher salinity portion of the estuarine gradient, up to a station where salinity dropped to about 25 PSU under low tide conditions. Two years later (2019), it was also observed outside the lagoon (at an offshore platform) in rather high abundances (up to 2420 ind. m^{-2}).

In Sines (Portugal, Iberian Peninsula), three specimens were collected in 2011; and in Azores (Portugal, Macaronesian region) another three specimens were found in Ponta Delgada marina in 2013, 2014 and 2015. These findings represent the first record of the presence of S. *georgiana* in the Central-East Atlantic coast. In North Africa coast, *S. georgiana* (ca. 10 specimens) was found for the first time in September 2015 (late summer); and later in January 2016 (winter); in association with fouling substrata attached to floating pontoons, including *Eudendrium* sp., *Ellisolandia elongata* (J.Ellis & Solander) K.R. Hind & G.W. Saunders, *Mytilus* sp., *B. neritina*, *Dictyota dichotoma* (Hudson) J.V. Lamouroux, and plumularid hydroids. Finally, *S. georgiana* was recorded for the first time in Australian waters (ca. 15 specimens), on piers from Chowder Bay (Sydney). Localities sampled by the authors where *S. georgiana* was not detected are included in the supplementary material (Table 1. Supplementary).

Table 2. New records for *Stenothoe georgiana*. Monthly presence/absence of the species in Cádiz (2012) and Palma (2012) is also included. PT= Portugal, ES= Spain, IT=Italy, AU= Australia.

Country	Locality	Date	Coordinates	Substrates
PT	Sines marina, Alentejo	9 May 2011	37.95º N; 8.87º W	Pontoons (*Bugula neritina*)
	Ponta Delgada marina, São Miguel, Azores	22 May 2013, 21 April 2014, 10 March 2015	37.73º N; 25.65º E	Boat hulls, pontoons
ES	Isla Canela marina, Huelva	26 June 2017 (Absent in 2011)	37.19º N; 7.34º W	Pontoons (*Bugula neritina*)
	El Rompido marina, Huelva	26 June 2017 (Absent in 2011)	37.22º N; 7.13º' W	Pontoons (fouling communities)
	Puerto América marina, Cádiz	17 May 2011, 2 July 2017	36.54º N; 6.28º W	Pontoons (fouling communities)
	La Línea marina, Cádiz	15 May 2011, 30 June 2017	36.16º N; 5.36º W	Pontoons (fouling communities)
	Fuengirola marina, Málaga	29 June 2017 (Absent in 2011)	36.54º N; 4.62º W	Pontoons (fouling communities)
	Málaga marina, Málaga	3 July 2011, 29 June 2017	36.72º N; 4.41º W	Pontoons (*Bugula neritina*)
	Motril marina, Granada	2 July 2011, 28 June 2017	36.72º N; 3.53º W	Pontoons (*Bugula neritina*)
	Almería marina, Almería	27 June 2017 (Absent in 2011)	36.83º N; 2.46º W	Pontoons (fouling communities)
	Barcelona marina, Barcelona	26 June 2011	41.38º N; 2.18º E	Pontoons (*Bugula neritina*)
	Palma marina, Baleares	18 December 2011	39.57º N; 2.63º E	Pontoons (*Eudendrium racemosum*)
	Ceuta, North Africa	September 2015, January 2016 (Absent in 2011)	35.89º N; 5.31º W	Pontoons (see Results)
IT	Venice lagoon	12 June 2017	45.47º N; 12.41º E	Wooden piles
	Adriatic Sea (off the Venice Lagoon)	12 March 2019; 16 September 2019	45.31° N; 12.51° E	Offshore platform pillar
AU	Chowder Bay, Sydney	3 December 2017	33.84º S; 151.25º E	Piers (Artificial collectors)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Locality: Puerto América marina, Cádiz, SPAIN (36º 32' 29.2" N; 6º 17' 02.4'' W) ***Substrates***: *E. racemosum*, *A. verticillata*	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓
Locality: Palma marina, Baleares, SPAIN (39º 34' 2'' N; 2º 37' 56'' E)) ***Substrates***: *E. racemosum*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

B - New locations (within regions previously sampled)

In the Southern Iberian Peninsula, *Stenothoe georgiana* was recorded in four marinas in 2011 [Puerto América (Atlantic Ocean), La Línea, Málaga and Motril (Mediterranean Ocean)]; and six years later, it was found in eight marinas from the same region: the previous ones plus Isla Canela and El Rompido in the Atlantic Ocean, and Fuengirola and Almería in the Mediterranean Sea. It should be noted that in the last three marinas, the species was found in samples collected using a slightly different methodology than in 2011. Finally, *S. georgiana* was not present in Ceuta in 2011, but was found there in 2015 and 2016 (Table 2).

C - Evidence for seasonal patterns (establishment at new locations)

The presence of *S. georgiana* in all months sampled confirm the existence of established populations in the new localities in Spain and Italy. The monitoring studies also reveal that *S. georgiana* populations are present throughout the entire year. In Palma Marina, a total of ca. 100 specimens were collected from *E. racemosum*, being present in all sampled months, with higher densities found in February and December. In Puerto América, Cádiz, the species was also found most of the year associated to the substrates *E. racemosum* (ca. 90 specimens collected) and the alien bryozoan *A. verticillata* (ca. 110 specimens collected) (Table 2).

3.2 Taxonomical remarks

Although Pérez-Schulthesiss & Ibarra (2017) reported some minute morphological differences in the propodus of gnathopod 2 of introduced Chilean populations of *S. georgiana* and the original description by Bynum and Fox (1977) (see Pérez-Schultheiss and Ibarra, 2017), we did not find constant differences within the rest of introduced populations [Mediterranean, East Atlantic coast, Chile, Australia] (Fernandez-Gonzalez and Sanchez-Jerez, 2017, Ulman et al., 2017, Pérez-Schultheiss and Ibarra, 2017 and present study). Furthermore, preliminary molecular analysis indicates that populations from the Iberian Peninsula and Australia belong to the same species (Cabezas et al. unpublished data). Further molecular studies are advised to confirm *S. georgiana* as a neo-cosmopolitan species an introduced species having achieved a widespread distribution through anthropogenic dispersal (Darling and Carlton, 2018).

The voucher materials have been deposited in Museo Nacional de Ciencias Naturales de Madrid (MNCN, Madrid, Spain), Museo di Storia Naturale di Venezia (MSNVE, Venice, Italy), and Museo di Storia Naturale dell'Università di Pavia (MSNPV, Pavia, Italy). In MNCN: 7 males collected from Puerto América Marina, Cádiz, Spain, 36.54º N; 6.28º W, associated to *E. racemosum* colonizing floating pontoons, August 15, 2012 (MNCN 20.04/12009) were deposited; 9 males collected from Palma Marina, Spain 39.57º N; 2.63º E, associated to *E. racemosum* colonizing floating pontoons, February 18, 2012 (MNCN 20.04/12010); 5 males collected from Barcelona Marina, Barcelona, Spain, 41.38º N; 2.18º E; associated to *B. neritina* colonizing floating pontoons, June 16, 2011 (MNCN 20.04/12011); 2 males collected from Chowder Bay, Sydney, Australia, 33.84º S; 151.25º E, associated to artificial collectors deployed in piers, December 3, 2017 (MNCN 20.04/12012). In MSNVE: 25 specimens (10 males, 8 females, 7 juveniles) collected from an offshore platform located in front of the Venice Lagoon (45.31° N; 12.51° E); associated to mussels colonizing a zinc-coated iron pillar (MSNVE 25100). In MSNPV: 7 specimens collected from the Lagoon of Venice, Italy (45.47º N; 12.41º E); associated with a rich macrofouling community composed by mussels, sponges, tunicates and bryozoans colonising wooden piles (MSNPV-2019/8).

4. DISCUSSION

Stenothoe georgiana has successfully extended its introduced range, now currently present in both hemispheres, both the Atlantic and the Pacific Ocean, and the Mediterranean Sea. The present case is that of a very successful colonizer on artificial structures, though easily overlooked due to its small-size and tricky identification (hidden invader). In this scenario, early detection is especially challenging; and in this particular case, it was only possible due to effective communication amongst expert groups and thorough taxonomic works (see section 4.3 below). The research conducted here becomes then of particular relevance; and it serves to point out certain challenges, and to provide suggestions to facilitate detection of hidden invaders.

4.1 On the invasion dynamics of *S. georgiana*: biogeographical patterns and vectors of introduction

Stenothoe georgiana was described from individuals collected from fouling communities on a pier at the University of North Carolina (type locality) by Bynum & Fox (1977). They reported *S. georgiana* as abundant in sounds and estuaries of that region. Several years before the species was described, another researcher (M.C.R. Shoemaker) was working on the description of what he called *'S. georgiana'* at the time of his death. Therefore, Bynum & Fox (1977) examined his unpublished manuscript and included part of his data in their description. Shoemaker's manuscript comprised material from Virginia, North Carolina, Georgia and Florida from the east Atlantic coast of USA, as well as Rio de Janeiro and Sacco São from the southeastern coast of Brazil. The earliest, but unpublished record of the species actually dates back to 1935, and belongs to specimens collected by Shoemaker from Sapelo Island, Georgia which were deposited in the National Museum of Natural History, Smithsonian Institution (*Stenothoe georgiana* Shoemaker in GBIF Secretariat, 2017; Orrell, 2019). It would be of interest to know the habitats of all the found materials, and especially to separate records from natural *versus* artificial habitats, in order to have hints on the possible native range of the species (when species associated to fouling communities are involved, records from an artificial habitat should be considered with caution, see Chapman & Carlton, 1991). However, such information was not provided with Shoemaker's material, hence preventing us from knowing if the specimens from its putative native region were associated with artificial habitats or not. Analysing the global distribution records of the species (Table 1), we found that no additional records of *S. georgiana* have been reported from Brazil, which may be attributable to a lack of sampling effort or misidentifications. Instead, most records belong to the western Atlantic coast of USA and the Gulf of Mexico (included in its putative native range). Taking these facts into account, we think the record from Brazil should be considered as questionable (Fig. 3a) and we suggest further sampling in this geographical region in order to validate this.

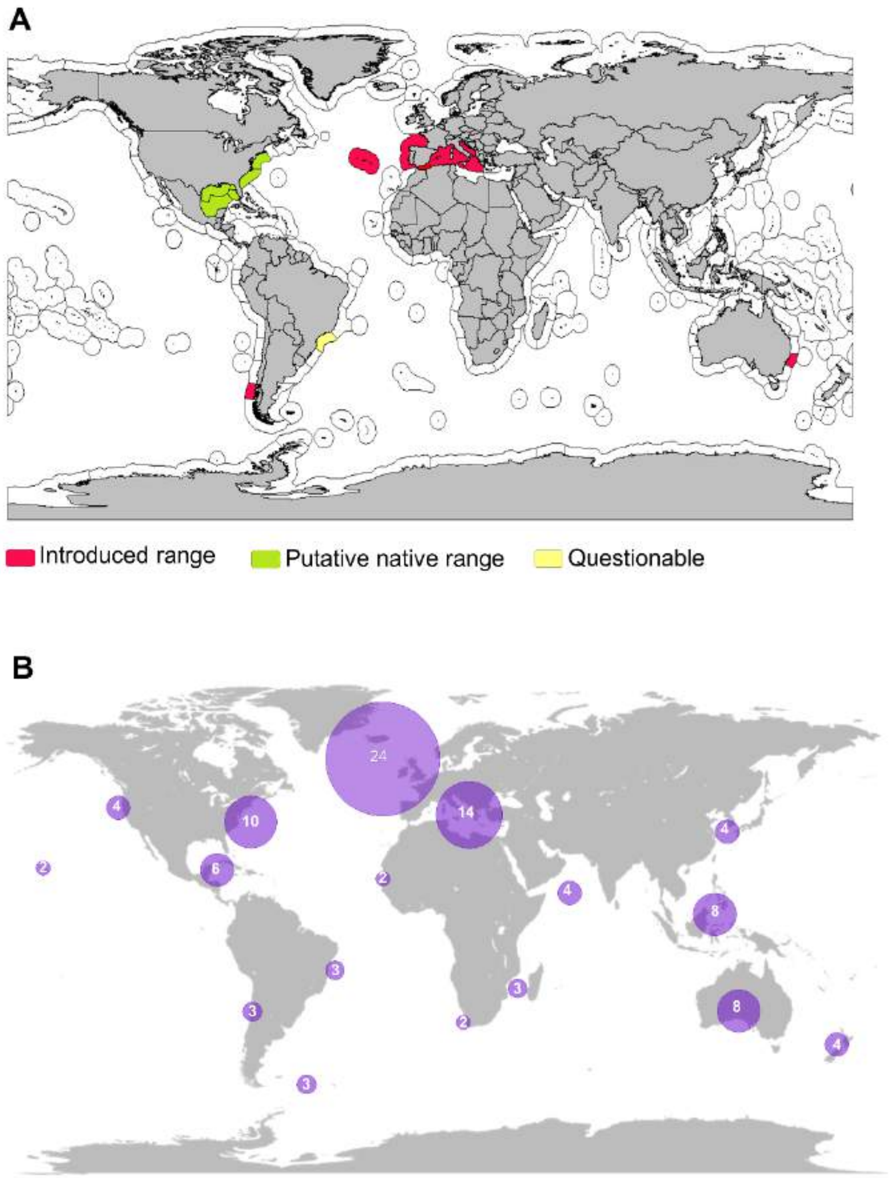

Fig. 3: a) Updated worldwide distribution of *Stenothoe georgiana* including its introduced range (in red) and its putative native range (in green). Records from Brazil are considered questionable (in yellow). Information based on data showed in Tables 1 and 2. b) Number of *Stenothoe* spp. recorded in each region. Information based on Krapp-Schickel (2015), GBIF.org (26 June 2019) and WORMS (Horton *et al.* 2019).

Since its original description in 1977, and until 2017, the distribution of the species was assumed to be restricted to the Western Atlantic region. However, in 2010 and 2011, established populations of *S. georgiana* were found by Fernandez-Gonzalez & Sanchez-Jerez (2017) associated with offshore sea bass and sea bream aquaculture facilities in two Mediterranean localities. After raising the flag on the presence of *S. georgiana* amongst scientific colleagues, the species was found in additional locations in the Mediterranean Sea (published records from Italy, France, Malta and Slovenia, Table 1), and confirmed here with new Mediterranean records. This study also confirms the presence of *S. georgiana* in Macaronesia, North Africa and Australia, significantly enlarging its distributional range. Simultaneously, records of the species have also appeared from the other side of the world, Pérez-Schultheiss & Ibarra (2017) also found the species in 2012 in salmonid farms in Chile. Currently, populations of *S. georgiana* are known from both the Northern and Southern Hemispheres, specifically from: the Northwestern Atlantic coast of USA, the Gulf of Mexico, the Southwestern coast of Brazil, the Eastern Atlantic coast of Europe (including Macaronesia), the Mediterranean Sea, and the Southeastern and Southwestern Pacific coasts of Chile and Australia, respectively (Fig. 3a).

Records of *Stenothoe georgiana* from its putative native range occur in both natural and artificial substrata. Meanwhile, records from its introduced range were exclusively associated with anthropogenically altered environments and artificial substrata (harbours, recreational marinas, aquaculture facilities). This leads to two assumptions about its native range and vectors of introduction. Firstly, although the species was described from individuals associated with artificial substrata, its presence in natural habitats along the western Atlantic coasts could support the assignment of this area as its putative native range. The Northern Atlantic Ocean represents the region with the highest diversity of *Stenothoe* species (Fig. 3b), hosting twenty-four species in the Eastern side, ten in the Western side, and six in the Gulf of Mexico and the Caribbean Sea. Although the higher number of *Stenothoe* spp. in the North-East Atlantic region could hint at the evolutionary origin of this genus in this area (Chapman & Carlton, 1991), the absence of *S. georgiana* in natural habitats, together with the lack of early records in important and comprehensive taxonomic works on Amphipoda for the area (*e.g*. Lincoln, 1979; Ruffo, 1993; 1998), suggests a recent introduction of the species in European waters. Molecular analysis would be necessary to confirm this assumption. Furthermore, considering the numerous records of S. georgiana in port habitats that are connected to offshore aquaculture facilities and other ports/marinas by boating activity (Sarà et al., 2007), the likely vectors of introduction of this amphipod are recreational and commercial boating. Indeed, Ulman et al. (2017) provides direct evidence of the presence of *S. georgiana* found on recreational boat hulls moored in France and Italy. Although no evidence has yet been found on the spreading of *S. georgiana* specimens through rafting on floating substrates, it is worth noting that other species of the same genus were recently reported associated with floating debris originating from the Japanese tsunami (Carlton et al., 2017).

In its putative native range *S. georgiana* was also found in nocturnal surface macroplankton of estuaries (Bynum and Fox, 1977). This could also imply a high ability for short-distance natural dispersal compared to other benthic amphipod species (traditionally considered poor dispersers). A high natural dispersal tendency may increase the probability of being transported by a human-mediated vector (Ros et al., 2020). This, combined with its strong affinity for artificial environments in its introduced range and its high plasticity for colonizing different biogenic substrata, may help to explain its rapid spread in European waters. Unfortunately, not enough data exists on the ecology of *S. georgiana* to enable a comprehensive understanding of its invasion potential. Therefore, we highlight the need for subsequent *S. georgiana* studies which address, among other aspects: population dynamics, trophic ecology, habitat use, behavior or tolerance to environmental stress from different areas of its distribution. In any case, this study confirms the recent presence of established populations of S. *georgiana* over the years (*e.g.* in Andalusia and Azores) as well as throughout the year (in southern Iberian Peninsula, Balearic Islands and Sicily). This may imply its rapid establishment success after its initial introduction to new region. The presence of the species in very distant biogeographical areas, including tropical, subtropical and temperate regions, suggests its high adaptation capabilities to different environmental conditions. Furthermore, its occurrence in intermediate salinity conditions (25 PSU) in the Venice Lagoon suggests high salinity tolerance, a trait commonly exhibited by successful invaders (*e.g*. see Lejeusne *et al.*, 2014; Hobbs *et al.*, 2015).

Stenothoe georgiana is not the only *Stenothoe* species with invasion potential. For example, *Stenothoe valida* Dana, 1852 presents a cosmopolitan distribution and is considered as an introduced or cryptogenic species in the Eastern Pacific Coast of North America, Hawaii, Gulf of Mexico, New Zealand, Australia and Spain (Fofonoff *et al.*, 2009 –NEMESIS). Another example is the case of the *Stenothoe gallensis* species complex, with some species of the complex frequently associated with fouling communities in port habitats, like *Stenothoe crenulate* Chevreux, 1908 (see Carlton and Eldredge, 2009; Carlton *et al.*, 2017). Although morphological evidence supports the conspecificity of *S. georgiana* populations (see taxonomical remarks section), molecular evidence is needed to confirm this species as a neo-cosmopolitan species. Similarly, future molecular studies are necessary to understand the invasion pathway of *S. georgiana* in Europe and other introduced regions. For example, such studies could confirm whether *S. georgiana* entered the Mediterranean through the Strait of Gibraltar, as suggested by the current distribution of the species (*i.e.* from its absence in the Red Sea and the Eastern Mediterranean Sea).

4.2 Lesson from *S. georgiana*: taxonomical expertise, scientific cooperation and efficient monitoring programs

In marine bioinvasion science, the continuous improvement and updating of the taxonomic knowledge provide the foundation for early detection of invaders, in order to develop effective management strategies and limit their further spreading. Contrarily, insufficient taxonomical expertise and poor communication between taxonomists and researchers or professionals involved in marine alien species monitoring can lead to mistakes in species' identification, with introduced species often being either overlooked or mistakenly identified as common native species in the area (Wotton and Hewitt, 2004; Carlton, 2009). For example, the invasive Northern Pacific seastar, *Asterias amurensis*, was misidentified in Tasmania as a native species for nearly 10 years, and by the time it was correctly assessed as introduced it had reached pest proportions (Goggin 1998). These issues cause under-reporting of introduced in technical reports, and in national inventories and databases, which are essential tools for bioinvasion management providing the basis for decision-making.

Unfortunately, invertebrate taxonomy is currently experiencing a dramatic decline (see Kim and Byrne, 2006; Coleman et al., 2015; Bik, 2017). In most parts of the world, museums, research centers and academia are all suffering a loss of professional taxonomists, a high percentage of whom are nearing retirement, and are unlikely to be replaced due to low recruitment of emerging scientists into this discipline. Taking into account the long period necessary for training taxonomists, this decline of professional experts poses a threat for present and future marine bioinvasions research and management; as both conventional and molecular biological surveys are dependent on it (Costello et al., 2010). An example is the fact that the magnitude of organisms transfer is inversely proportional to body size, yet relatively few new detections are recognized for fungi, protists, parasitic and free-living flatworms, viruses and microbes, which reflects the high uncertainty in taxonomic identification and geographic origin (Lohan et al., 2020; pointed out in Bailey et al., 2020). Also, experts themselves sometimes fail in promoting their research to both colleagues and the general public (Hutchings, 2017); which is accentuated by the underestimation of valuable taxonomic contributions (usually relegated to lower impact journals). In fact, critical updated taxonomy advancements are sometimes ignored or not properly incorporated into alien taxa inventories (see discussion in Marchini and Cardeccia, 2017). Additionally, routine monitoring from local environmental agencies is often constrained by very tight deadlines, with little opportunity for in depth examination of the more taxonomically challenging taxa constituting a high-risk of overlooking new alien species arrivals.

Consequently, we advocate the importance of taxonomy, and communication between taxonomists and those monitoring marine environments for improved alien species management. In order to ensure that alien species information is effectively disseminated at all levels, the following tools should be promoted:

A. increase opportunities for exchanges of knowledge between senior taxonomists and early-career taxonomists or para-taxonomists, for example by the provision of visiting fellowships/grants to universities, museums, and summer/winter schools on taxonomy;
B. establishment of local/national taxonomy networks, such as ‘SCAMIT’ in the United States (https://www.scamit.org/), and ‘MOTax’ in Italy (https://www.szn.it/index.php/it/ricerca/infrastrutture-di-ricerca-per-le-risorse-biologiche-marine/piattaforme-tecnologiche/tassonomia-degli-organismi-marini-motax); these networks ensure support in cases of difficult species identifications;
C. support the participation of national experts to international events, *e.g.* specialistic workshops/ conferences on taxonomy (such as the ICA – International Colloquium of Amphipoda), and on bioinvasions, in order to establish connections and facilitate information exchanges with international colleagues;
D. increased opportunities for data /information exchanges between research scientists and environmental management agencies through workshops and stakeholders meetings, in order to cross-check advancements on knowledge of alien species;
E. more frequent updating of standardized, global alien species databases such as WRiMS (http://www.marinespecies.org/introduced/), AquaNIS (http://www.corpi.ku.lt/databases/index.php/aquanis/) and EASIN (https://easin.jrc.ec.europa.eu/easin). Ideally, new species records being accepted for publication should immediately be forwarded to database managers, in order to ensure an early update of open- access databases.

In addition, we call attention to the number of confusing genera awaiting revision in the Amphipoda group (Navarro-Barranco, 2015; Bonifazi et al., 2018). Certainly, findings from the present study would not have been possible without the foundational work of Krapp-Schickel (2015), which provided a comprehensive key to worldwide species of *Stenothoe*. Owing to that work, specimens were successfully identified as a *Stenothoe* species previously unrecorded in the Macaronesian, Atlanto-Mediterranean and Australian coastal areas rather than being erroneously attributed to congeneric species or remaining unidentified.

Finally, the time-lag between new arrivals and documentation of an invader is far too long to serve management (see Crooks *et al.*, 2005, 2011). This temporal window could be reduced by enhanced cooperation and communication amongst the scientific community. Sometimes, academic publishing requirements and hyper- competition push researchers to avoid disseminating their new alien records prior to publication. Instead, we highly recommend participation with integrative and collective studies encompassing global records, rather than more local, individual publications. In the present case of *S. georgiana*, several factors notably contributed to the cooperative early detection of a hidden invader. Firstly, a key taxonomic revision drew light to the correct identities of doubtful specimens. Secondly, the willingness of exchanging opinions and sharing knowledge among colleagues raised the flag on the presence of this invader in several distinct regions. And lastly, open communication within the amphipodologist group allowed the acceleration of its identification and revealed its introduced distribution range as soon as possible. If the information had not been circulated quickly and effectively, this NIS may have gone overlooked, and several publications would have probably been submitted without acknowledging its presence. This would then have had consequences on studies that were analysing and modelling the richness of NIS (*e.g.* Ferrario *et al.*, 2017; Ulman *et al.*, 2019). Yet, a time lag of 7 years passed between the first collection of samples in which *S. georgiana* was present (in 2010), and the first published record in the introduced range (Fernandez-Gonzalez & Sanchez-Jerez, 2017), not surprising considering the small size and taxonomic difficulty of this taxon. In order to minimize the detection time-lag of small mobile epifauna, it is urgent to provide the scientific community with a standardized and quantitative monitoring methodology for fouling communities. This methodology should: i) facilitate sampling processing and identification at both the morphological and molecular levels, ii) allow comparisons across spatio-temporal scales and different habitats (*e.g.* artificial and natural), and iii) reduce sampling bias when absence records are provided. We thus strongly encourage scientific colleagues to include absence data of invaders in their research. This is important for properly addressing the propagation rate and range of introduced species, as well as their invasion potential.

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SUPPLEMENTARY MATERIAL

Table S1. List of localities sampled by the authors where (to date) presence of *Stenothoe georgiana* Bynum & Fox, 1977 have not been detected

Country	**Locality**	**Date**	**Coordinates**	**Source**
PORTUGAL	Nazaré, Marina, Eastern Atlantic	9 May 2011	39.59º N; 9.07º W	Present study
	Cascais Marina, Eastern Atlantic	9 May 2011	38.69º N; 9.42º W	Present study
	Faro Marina, Gulf of Cádiz	11 May 2011; 26 Jun 2017	37.01º N; 7.94º' W	Present study
MOROCCO	Tanger, Eastern Atlantic	31 May 2011	35.79º N; 5.81º W	Present study
	Marina Smir, Alboran Sea	30 May 2011	35.75º N; 5.34º' W	Present study
	M-Diq, Alboran Sea	30 May 2011	35.68º N; 5.31º W	Present study
SPAIN	Santander Marina, Bay of Biscay	2 May 2011	43.45º N; 3.82º W	Present study
	Gijón Marina, Bay of Biscay	3 May 2011	43.54º N; 5.67º W	Present study
	A Graña Marina, Eastern Atlantic	7 May 2011	43.48º N; 8.26º W	Present study
	A Coruña Marina, Eastern Atlantic	4 May 2011	43.37º N; 8.39º W	Present study
	Chipiona Marina, Gulf of Cádiz	17 May 2011; 1 Jun 2017	36.74º N; 6.43º W	Present study
	Rota Marina, Gulf of Cádiz	17 May 2011	36.62º N; 6.35º W	Present study
	Sancti Petri Marina, Gulf of Cádiz	17 May 2011	36.40º N; 6.21º W	Present study
	Barbate harbour, Gulf of Cádiz	17 May 2011; 1 Jun 2017	36.19º N; 5.93º W	Present study
	Benalmádena Marina, Alboran Sea	15 May 2011	36.43º N; 4.51º W	Present study
	Málaga, Alboran Sea (aquaculture facilities)	May 2010; Nov 2012; Sep 2014	36.71º N; 4.36º W	Present study
	La Caleta Marina, Alboran Sea	3 Jul 2011; 28 Jun 2017	36.75º N; 4.07º W	Present study
	Salobreña, Alboran Sea (aquaculture facilities)	May 2010	36.74º N; 3.61º W	Present study
	Almerimar Marina, Alboran Sea	1 Jul 2011	36.70º N; 2.79º W	Present study
	Roquetas de Mar Marina, Alboran Sea	1 Jul 2011	36.76º N; 2.61º W	Present study
	Almería, Alboran Sea (aquaculture facilities)	Sep 2014	36.83º N; 2.46º W	Present study
	Carboneras harbour, Western Mediterranean	30 Jun 2011	36.99º N; 1.90º W	Present study
	Marina Salinas, Torrevieja, Western Mediterranean	29 Jun 2011	37.97º N; 0.68º W	Present study
	Alicante Marina, Western Mediterranean	29 Jun 2011; 14 Nov 2016	38.34º N; 0.49 W	Present study
	Dénia Marina, Western Mediterranean	28 Jun 2011	38.85º N; 0.11º W	Present study
	Valencia Marina, Western Mediterranean	28 Jun 2011	39.43º N; 0.33º' W	Present study

	Burriana Marina, Western Mediterranean	28 Jun 2011	39.86º N; 0.07º W	Present study
	Oropesa Marina, Western Mediterranean	28 Jun 2011	40.08º N; 0.13º E	Present study
	Benicarló Marina, Castellón Western Mediterranean	27 Jun 2011	40.42º N; 0.43º E	Present study
	Tarragona Marina, North Western Mediterranean	27 Jun 2011	41.1º N; 1.25º E	Present study
	Tarragona A, North Western Mediterranean (aquaculture facilities)	Oct 2010	40.88º N; 0.81º E	Present study
	Tarragona B, North Western Mediterranean (aquaculture facilities)	Oct 2010	40.53º N; 0.59º E	Present study
	L’Estartit Marina, North Western Mediterranean	25 Jun 2011	42.05º N; 3.21º E	Present study
FRANCE	Port de la Grande-Motte, North Western Mediterranean	2 Nov 2016	43º 33' N; 4º 5' E	Ulman et al., 2017
	Cogolin Marina, North Western Mediterranean	1 Apr 2016	43.55º N; 4.08º E	Ulman et al., 2017
	Sainte-Maxime Marina, North Western Mediterranean	1 Apr 2016	43.3º N; 6.63º E	Ulman et al., 2017
	Cannes Le Vieux Port, North Western Mediterranean	19 Apr 2015	43.53º N; 7.03º E	Ulman et al., 2017
	Port de Villefranche, North Western Mediterranean	22 Nov 2016	43.7º N; 7.3º E	Ulman et al., 2017
ITALY	Alassio Marina, North-Western Mediterranean	27 May 2016	44.02º N; 8.18º E	Ulman et al., 2019
	Genoa harbour, North-Western Mediterranean	29 Jul 2013	44.4º N; 8.93º E	Ferrario et al., 2017
	Santa Margherita Ligure Marina, North-Western Mediterranean	6 Jul 2013	44.33º N; 9.22º E	Ferrario et al., 2017
	La Spezia harbour, Ionian Sea	11 Jul 2013	44.02º N; 9.83º E	Ferrario et al., 2017
	Leghorn harbour, Ionian Sea	28 Jun 2013	43.55º N; 10.3º E	Ferrario et al., 2017
	Viareggio harbour, Ionian Sea	27 Jun 2013	43.87º N; 10.25º E	Ferrario et al., 2017
	Follonica, Ionian Sea (aquaculture facilities)	Apr 2011	42.91º N; 10.64º E	Present study
	Terre Rosse Marina, Ionian Sea	26 Jul 2016	42.95º N; 10.55º E	Ulman et al., 2019
	Scarlino Marina, Ionian Sea	27 Jul 2016	42.88º N; 10.78º E	Ulman et al., 2019
	Punta Ala Marina, Ionian Sea	27 Jul 2016	42.80º N; 10.73º E	Ulman et al., 2019
	Olbia harbour, Ionian Sea	10 Jun 2014	40.92º N; 9.52º E	Ferrario et al., 2017
	Castelsardo harbour, North-Western Mediterranean	11 Jun 2014	40.92º N; 8.7º E	Ferrario et al., 2017
	Porto Rotondo Marina, Ionian Sea	9 Jun 2014	41.03º N; 9.55º E	Ferrario et al., 2017
	Porto Turistico di Roma, Ionian Sea	12 Jul 2015	41.73º N; 12.25º E	Ulman et al., 2017
	Casamicciola Marina, Ionian Sea	1 Aug 2015	37.75º N; 15.9º E	Ulman et al., 2017

	Porto dell’Etna, Riposto, Central Mediterranean	18 Sep 2016	37.75º N; 15.2º E	Ulman et al., 2017
	Marzamemi Marina, Central Mediterranean	8 Oct 2016	36.73º N; 15.12º E	Ulman et al., 2017
	Marina di Ragusa, Sicily Channel, Central Mediterranean	15 Dec 2017	36.73° N ;14.53° E	Present study
MALTA	Qwara (aquaculture facilities)	Oct 2010	35.62º N; 14.70º E	Present study
	Il-Hofriet (aquaculture facilities)	Feb 2011	35.40º N; 14.94º E	Present study
	Msida Yatch Marina, Central Mediterranean	1 Jul 2016	35.9º N; 14.5º E	Ulman et al., 2017
TUNISIA	Ghar el Melh (aquaculture facilities)	May 2013	37.32º N; 10.28º E	Present study
	Mahdia (aquaculture facilities)	May 2013	35.46º N; 11.09º E	Present study
CROATIA	Brac (aquaculture facilities)	Oct 2010	43.30º N; 16.46º E	Present study
	Uglan (aquaculture facilities)	Oct 2010	44.03º N; 15.22º E	Present study
GREECE	Crete (aquaculture facilities)	Sep 2010	35.58º N; 25.25º E	Present study
	Old Venetian harbour, Heraklion, Aegean Sea	1 Nov 2015	35.35º N; 25.13º E	Ulman et al., 2017
	Agios Nikolaos Marina, Aegean Sea	18 Nov 2015	35.18º N; 25.13º E	Ulman et al., 2017
	Mandraki Port, Rhodes, Aegean Sea	2 Jun 2016	36.45º N; 28.23º E	Ulman et al., 2017
TURKEY	Ataköy Marina, Istanbul Marmara Sea	20 Aug 2015	41.00º N; 29.52º E	Present study
	Setur Kalamış Marina, Istanbul, Marmara Sea	28 Aug 2015	40.98º N; 29.03º E	Ulman et al., 2017
	Milta Bodrum Marina, Aegean Sea	9 Sep 2015	37.03º N; 27.43º E	Ulman et al., 2017
	Datça Marina, Aegean Sea	10 Oct 2015; 13 May 2016	26.72º N; 27.68º E	Ulman et al., 2017
	Setur Marmaris Netsel Marina, Aegean Sea	14 Sep 2015	36.85º N; 28.28º E	Ulman et al., 2017
	Eçe Marina, Fethiye, Eastern Mediterranean	19 Sep 2015	36.62º N; 29.1º E	Ulman et al., 2017
	Setur Finike Marina, Eastern Mediterranean	18 May 2016	36.30º N; 30.15º E	Ulman et al., 2017
CYPRUS	Karpaz Gate Marina, Eastern Mediterranean	21 Jun 2016	35.55º N; 34.23º E	Ulman et al., 2017
	Famagusta harbor, Eastern Mediterranean	13 Jun 2016	35.12º N; 33.95º E	Ulman et al., 2017

3

BASELINE SURVEYS TO UNCOVER MISSING INFORMATION: PART **1**

The challenge of hidden invaders

3.1.3. Hitchhiking northwards: on the presence of the invasive skeleton shrimp *Caprella scaura* in the UK

Adapted from: Martínez-Laiz G, Guerra-García JM, Ros M, Fenwick D, Bishop JD, Horton T, Faasse MA, Cabezas MP. Hitchhiking northwards: on the presence of the invasive skeleton shrimp *Caprella scaura* in the UK. Marine Biodiversity, 51(5):1-8.

ABSTRACT. Early detection of invasive species becomes particularly challenging in the case of small-sized, neglected or taxonomically challenging taxa. The invasive skeleton shrimp, *Caprella scaura* Templeton, 1836, stands out as a primary example of this and is a successful widespread invader in Europe. This study presents its first record in the UK, confirming that the species is now expanding towards higher latitudes by means of commercial shipping and recreational boating. Molecular analysis indicates that the UK population bears the most widespread haplotype across the northern hemisphere and suggests a potential introduction pathway shared with populations from the Iberian Peninsula, the Mediterranean Sea, Sea of Japan and Eastern US. We propose an Indo-Pacific origin, via the Suez Canal, with Mediterranean and Iberian populations acting as stepping stones. Considering the continuous development of European coastlines, the high molecular connectivity of *C. scaura* with transoceanic donors and its wide environmental tolerance, a further spread is expected in this continent. Lessons learned from its invasion in Southern Europe suggest the potential for competitive exclusion of native taxa. We outline the importance of promoting taxonomic expertise and effective dissemination of records, in order to reduce misidentifications, unresolved cases and lags in the detection of alien species.

1. INTRODUCTION

The amphipod *Caprella scaura* Templeton 1836 stands out as another example of “hidden-invader”; this time being one of the most widespread exotic species in the last 10 years (Galil et al. 2014), currently recorded in all oceans except for the Arctic (reviewed and updated in Fig. 1). It has high fecundity, reproducing all year around with a high number of eggs (Guerra-García et al., 2011, Ros et al., 2013a,b). It shows aggressive intra and interspecific behavior; bears trophic plasticity, responding to food availability (Ros et al., 2014b); and in the Iberian Peninsula it has rapidly taken over its congener species *Caprella equilibra* Say, 1818 (Ros et al., 2015). The case of *C. scaura* is also a primary example illustrating the huge importance, however neglected, of taxonomic expertise. Applied studies and lessons learned from the history of this invader have been possible only after years of thorough taxonomic studies to resolve the challenging complex *Caprella scaura* sensu lato. *Caprella scaura* was originally described from Mauritius (the type locality) by Templeton in 1836. Mayer (1890, 1903) described six forms: *C. scaura* f. *typica* Mayer, 1890, from Brazil and Australia; *C. s.* f. *diceros* Mayer, 1890, from Japan; *C. s*. f. *cornuta* Mayer, 1890, from Brazil; *C. s*. f. *californica* Mayer, 1890, from California; *C. s.* f. *scauroides* Mayer, 1903, from Hong Kong, China and Japan; and *C. s*. f. *spinirostris* Mayer, 1890, from Chile. Utinomi (1947) added a seventh form, *C. s.* f. *hamata* from Japan. The nominal species from Mauritius is represented as *C. scaura scaura* (see Krapp et al. 2006) and, according to the morphological and molecular studies of Ros et al. (2014) and Cabezas et al. (2014), *C. scaura scaura* and *C. scaura typica* are conspecific, representing *Caprella scaura* sensu stricto. Moreover, *C. scaura californica* and *C. scaura scauroides* were raised to species rank and following a period in synonymy with *C. californica* Stimpson, 1857, are now considered to belong to two separate species (see Dougherty and Steinberg 1953; Takeuchi and Oyamada 2013). Cabezas et al. (2014) provided molecular evidence to consider the subspecies *C. s. spinirostris* and *C. s. diceros* as another two valid species, although complete morphological redescriptions are lacking for the moment. The taxonomic status of the subspecies *C. s. hamata* and *C. s. cornuta* remains unclear. Meanwhile, we cannot rule out the possibility of dealing with several cryptic species within the *C. scaura* complex (see discussion regarding *C. scauroides* in Peart et al. 2019).

Caprella scaura sensu stricto is the only species within the complex with confirmed invasive character so far; there is sound evidence of its introduction via hull fouling and ballast water into several locations of the northern hemisphere (see Ros et al. 2014; Cabezas et al. 2014; Martínez-Laiz et al. 2021). According to these works, most European populations have an Indo-Pacific origin and have resulted from east to west stepping-stone events in the Mediterranean Sea by means of recreational boating (aspects regarding the history of introduction are given in more depth in chapter 3.2 of this thesis). Eventually, the species colonized the Iberian Peninsula, and most recently, it has reached the Netherlands (Faasse 2017). Ecophysiological experiments on *C. scaura* populations introduced in southern Europe indicate a potential to expand to higher European latitudes (Ros et al. 2021). In this study, we report on the first confirmed records of this species in the UK and discuss the biogeographical implications.

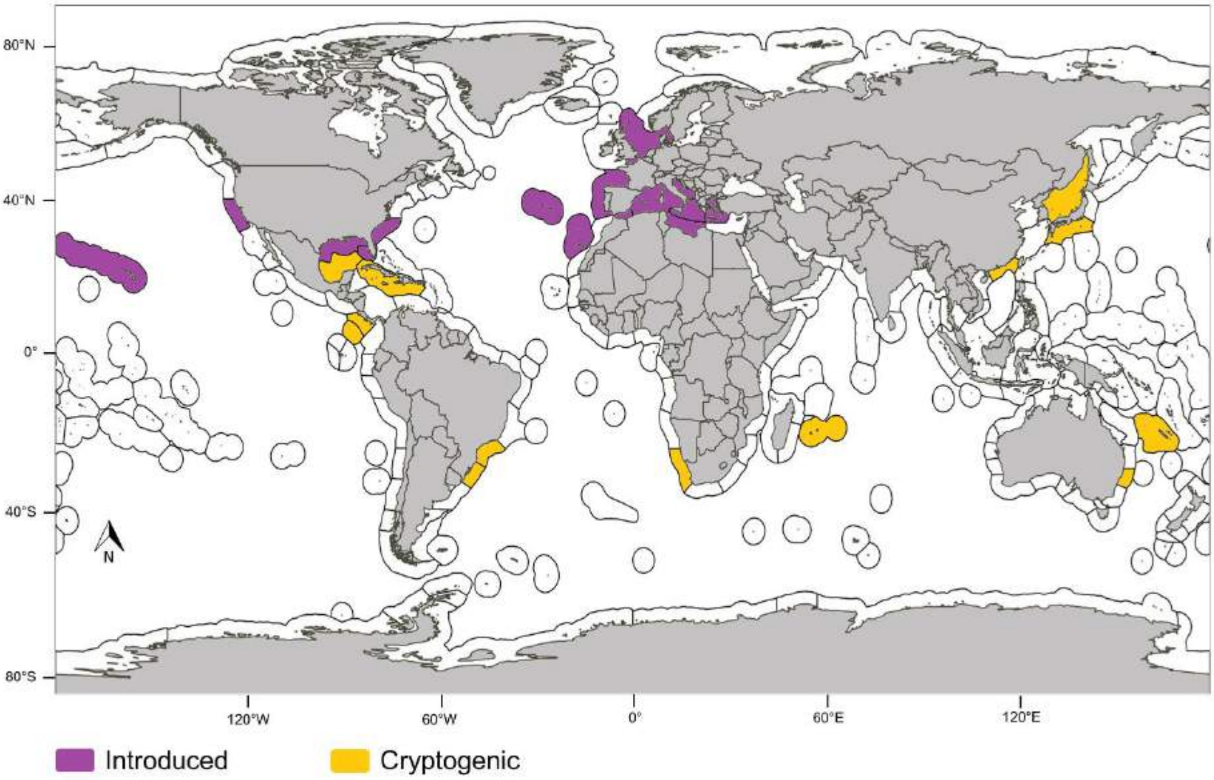

Figure 1. Global distribution of *Caprella scaura* sensu stricto (including records of *C. scaura scaura* and *C. scaura typica)*, indicating its introduced range and those areas where the species is considered cryptogenic. Questionable records (i.e. where it was not possible to verify the identity of *Caprella scaura* sensu stricto against other 'varieties') have been excluded. Records from other subspecies have not been included. Years of the first record for each ecoregion are indicated. Information is based on Rodríguez-Aimaraz and Ortega-Vidales (2013), Ros et al. (2014) and references therein, Cabezas et al. (2014), Ramalhosa and Canning-Clode (2015), Marchini et al. (2015), Faasse (2017), Gillon et al. (2017), Ferrario et al. (2018), Chebaane et al. (2018), Kamcha et al. (2020), and Martínez-Laiz et al. (2021). Marine ecoregions follow Spalding et al. (2007)

2. MATERIAL AND METHODS

2.1 Sampling and dissemination of records

Individuals of *C. scaura* were found inhabiting fouling communities of Sovereign Harbour Marina (50°47′28.4″ N, 0°19′38.8″ E), Eastbourne (UK). The Marine Biological Association of the UK conducted surveys of sessile fauna in the marina in October 2009 and August 2014. In 2009, 5 mature females and 37 juvenile caprellids were collected and, in 2014, 1 mature female and 1 immature male. The *C. scaura* individuals were by-catch in small samples of erect bryozoans and hydroids, including the non-indigenous bryozoans *Bugula neritina* (Linnaeus, 1758) (abundant) plus *Bugulina stolonifera* (Ryland, 1960) and *Bugulina simplex* (Hincks, 1886) (both occasional), that fouled the floating pontoons at shallow depth. The identity of the caprellids was not investigated at that time, but they were preserved for future examination. In August–September 2020, around 40 individuals were independently detected inhabiting the fouling community of the same marina. Morphological identification of the aforementioned material as *C. scaura sensu stricto* was possible thanks to the dissemination of records among the co-authors in October 2020. Immediately, additional sampling was carried out. The fouling community of the marina pontoon (up to 1-m depth) was collected using a hand-held net with a scraper, and specimens were fixed in situ in 90% ethanol. A total of 11 individuals (comprising mature males and females) were selected for barcoding, in order to confirm the invasive status (*i.e.* whether the UK specimens belonged to *C. scaura s.s.*) and to investigate the potential introduction history of the population.

2.2 Molecular analysis

Genomic DNA extraction was conducted as in Martinez- Laiz et al. (2021). Approximately 658 bp of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified by PCR using jgLCO1490 and jgHCO2198 (Geller et al. 2013) primers and reaction conditions following Cabezas et al. (2014; 2.5 mM MgCl2). PCR product purification and bidirectional sequencing were provided by a commercial company (GENEWIZ, Leipzig, Germany). The resulting sequences were checked and edited using Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and verified as Caprellidea DNA using GenBank's BLASTn search (Altschul et al. 1990). They were thereafter deposited in GenBank (accession numbers: MZ573397-MZ573407). To confirm the morphological identification of UK specimens, all available COI sequences from *C. scaura* in GenBank and BOLD (see Table 2 in Martínez-Laiz et al. 2021) were included in the final dataset. In addition, eight sequences of the closely related species *Caprella cf. diceros* Mayer, 1890 (Cabezas et al. 2014; accession numbers: KF743329, KF743331–KF743337), and 14 sequences of *C. simia* Mayer, 1903 (KF743434–KF743447), were also included. All sequences were aligned using MUSCLE (Edgar 2004) as implemented in MEGA X (Kumar et al. 2018) and trimmed to a length of 657 bp for the final alignment. They were also translated into amino acids to search for stop codons that would indicate the presence of pseudogenes.

Phylogenetic relationships were estimated using Bayesian inference (BI) and maximum likelihood (ML) methods of phylogenetic inference. The protocols, parameters and software used are detailed in Sect. 2.3.2 in Martínez-Laiz et al. (2021). In this case, only one individual (or sequence) per haplotype was included, and the species *C. simia* was selected as the outgroup. When examining the best-fit model of sequence evolution for the dataset, the best one was GTR + I + G (1st–3rd partition), according to the corrected Akaike information criterion (AIC, Akaike 1974). The consensus tree inferred for each phylogenetic approach was visualized and rooted using FigTree v1.4.4 (Rambaut 2018) and later edited in Inkscape v1.0.1. Haplotypes were coded as in Table 4 and Fig. 1 in Martínez-Laiz et al. (2021). Relationships among haplotypes were also examined via a haplotype network using the statistical parsimony method in TCS v1.21 (Clement et al. 2000) with a 95% connection limit and plotted with tcsBU (Santos et al. 2016).

3. RESULTS AND DISCUSSION

Morphological analysis assigned all individuals from the UK to *C. scaura* sensu stricto. This was also supported by the barcoding results: only one haplotype was identified in the 11 individuals sequenced, which matched 100% with sequences of *C. scaura* from Spain, Florida, Amursky Bay (Sea of Japan) and the Mediterranean Sea. The morphological analysis confirms that the invader has been present in the UK since at least 2009 and appears to be established in the single known location, judging by the species' persistence there for 11 years and the presence of mature males, brooding females and juveniles (Fig. 2).

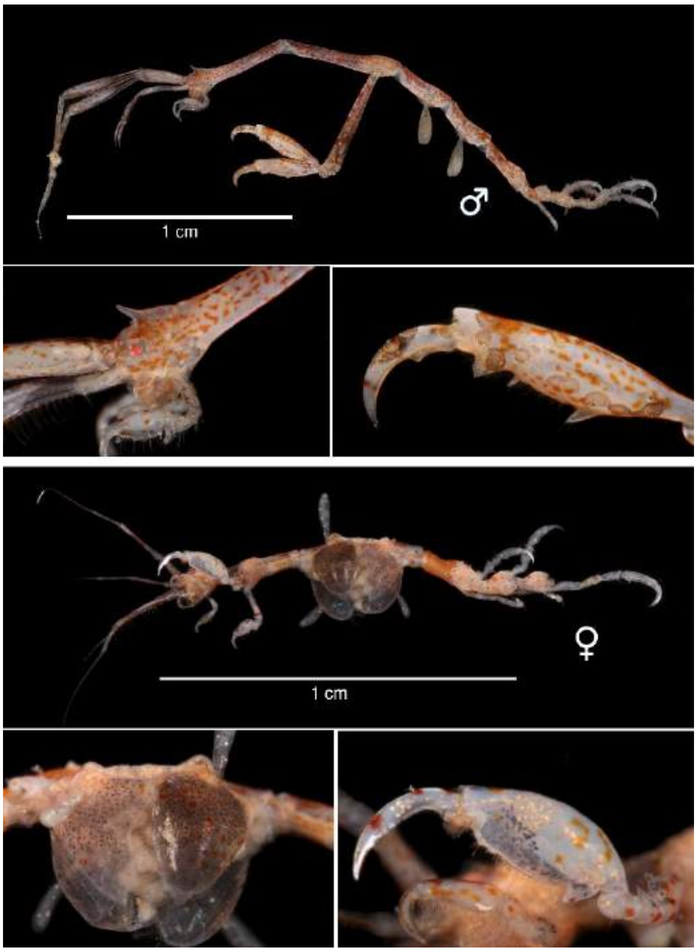

Figure 2 *Caprella scaura* from Sovereign Harbour Marina, Eastbourne, UK. **a** Lateral view of the male, with details of head and gnathopod 2 represented in **b** and **c**, respectively; **d** lateral view of the female, with brood pouch and gnathopod 2 represented in **e** and **f**, respectively

Phylogenetic analyses supported the species-level assignment, with sequences from *C. scaura* sensu stricto being monophyletic and grouped into six well-supported clades, designated as Cs1-6, separated by 5–15 nucleotide changes (Fig. 3). The last group, Cs6, included the sequenced individuals from the UK and *C. scaura* sequences from Spain, France, Italy, Greece, Charleston, Florida, Sea of Japan and the potentially native population of Mauritius (Fig. 3). The haplotype present in the UK corresponds to Hap 2, the most widespread haplotype across the northern hemisphere and the most common one found in the Iberian Peninsula and the Mediterranean Sea (see Table 4 and Fig. 4 in Martínez-Laiz et al. 2021). This suggests that the same pathway or source population may have been responsible for the introduction of *C. scaura* at these localities. Considering the invasion pattern of this species in the European region proposed by Cabezas et al. (2014), our results suggest that UK populations might have an Indo-Pacific origin, via the Suez Canal, with Mediterranean and Iberian populations acting as stepping stones along the same introduction pathway. On the other hand, despite their proximity, the Netherlands and UK populations did not share any haplotypes (Fig. 3b), confirming that more than one introduction pathway is operating in the European region (see Cabezas et al. 2014). This aligns with the conclusions from Martínez-Laiz et al. (2021) who suggested that, despite experiencing periods of vulnerability because of reduced genetic diversity in populations close to the Strait of Gibraltar (a key gateway for introductions in the Mediterranean Sea), European *C. scaura* populations benefitfrom multiple transoceanic donors that can refuel propagule pressure. The marina habitat of the new records in the UK supports the idea of this caprellid hitchhiking by means of recreational boating, which in turn creates new sources for exchange. Indeed, the two northwestern Europe populations may eventually mix, increasing within-population genetic diversity in this region and thus reinforcing the prevalence of this invader in the European continent (Fig. 1).

In addition, coastal areas continue to be the top tourism destination in Europe. Judging by the growing tendency of the European 'blue' economy, the continuous 'building up the coast and protecting the built environment' will provide abundant man-made structures to act as stepping stones for this and other marine NIS (EEA 2017). These habitats usually offer fluctuating environmental conditions that can favour the local establishment of NIS. Sovereign Harbour Marina is a shallow, enclosed and lock-gated basin likely to be warmed above ambient open-coast temperatures in the summer (21.7 °C was recorded at both the surface and 2-m depth during the survey in early August 2014). This somewhat mirrors the Netherlands occurrence of *C. scaura*, which was in the year-round outflow of cooling water from a power station in the Westerschelde, in a region that already experiences generally elevated summer sea temperatures (Faasse 2017). Sovereign marina also has an abundant population of the non-indigenous bryozoan *Bugula neritina* that seems to be the preferred substrate for *C. scaura* in the Iberian Peninsula and elsewhere (Guerra-García et al. 2011; Cabezas et al. 2014; Ros et al. 2013, 2014). Taking all factors into account, in addition to the wide environmental tolerance of the species (Ros et al. 2021), further spread of *C. scaura* can be expected. This spread would have implications first at the ecological level in terms of competitive exclusion of native communities (Ros et al. 2015; Parretti et al. 2021). Besides, the introduction may also have economic impacts as observed for the invader congener *Caprella mutica* Schurin, 1935, also present in the UK. This species overpopulates fouling with densities around 50,000 ind/m2 during the summer months, peaking up to 200,000 (Ashton 2006; Boos 2009) in northwestern European harbour environments; and it increases the costs of maintenance work in the aquaculture industry by blocking pumps for the feeding system of caged finfish sites or heavily fouling cage nets during summer months (Boos et al. 2011). The invader *Caprella scaura* is known to reach similar densities (over 100,000 ind/L) in marinas of the Southern Iberian Peninsula (Ros et al. 2015). Further studies are required to assess the potential impact of *C. scaura* on the northwestern European epifaunal community.

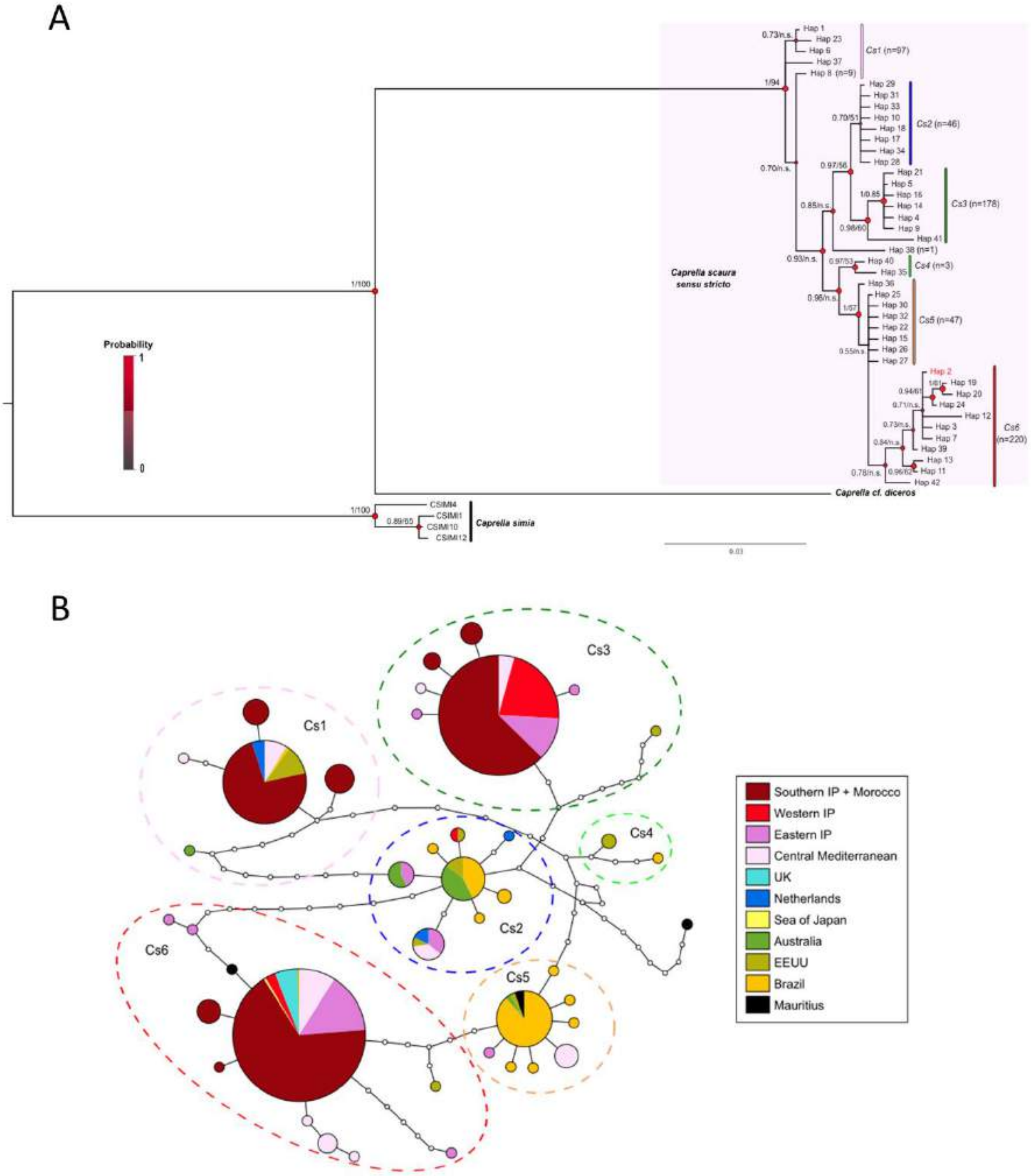

Figure 3. a Bayesian consensus tree obtained for the mitocondrial COI gene. Node values correspond to Bayesian posterior probabilities, also represented by colour and node size, and bootstrap support given by the maximum likelihood analysis (n.s. indicates less than 50% support). Haplotypes are coded as in Martínez-Laiz et al. (2021) (see Figs. 4 and 5 for locations) and the haplotype present in the UK population (Hap 2) is highlighted in red. The number of the analysed *Caprella scaura* sequences is provided in parentheses following clade designation (Cs1–Cs6) if any. The tree was rooted with *Caprella simia* (sequences available in GenBank: KF743434–KF743447); **b** COI haplotype network (95% parsimony connection limit) for *C. scaura*. Sampling areas are coded by filling patterns (see legend) and circle sizes are proportional to their frequency among all haplotypes found. Non-observed haplotypes are represented by small white circles, and each line connecting haplotypes represents a single mutational change. Distinct clades (Cs1–Cs6) are depicted as dashed-line ovals

Finally, we would like to outline the importance of (a) promoting synergy among taxonomists, molecular scientists and invasion ecologists; and (b) enforcing regular monitoring with a standardized and quantitative methodology for fouling communities (see Ros et al. 2019; Tamburini et al. 2021). This cooperation is needed to fill the gaps in knowledge that currently hamper trustworthy identifications of taxonomically challenging taxa with invasive potential. Indeed, the lack of detailed studies dealing with British caprellids is concerning. They are limited to the brief synopses by Harrison (1944), Smaldon (1990, in the volumes of the Marine Fauna of the British Isles and Northwest Europe) and Howson and Picton (1997); the later redescriptions by Guerra-García (2002); the guide of Guerra-García (2014); and the reports of Willis et al. (2004) and the present study calling attention to the presence of the aliens *C. mutica* and *C. scaura*, respectively. Scientific collaborations also mitigate, as much as possible, time lags in detection of new arrivals by favouring sharing of new records and effective transfer of knowledge (see discussion in Martínez- Laiz et al. 2020). This would favour cost-effective management and set precedents for responding to future incursions of hidden invaders.

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3

BASELINE SURVEYS TO UNCOVER MISSING INFORMATION: PART 2

Using molecular data to monitor the post-establishment evolution of the invasive skeleton shrimp *Caprella scaura*

Adapted from: Martínez-Laiz G, Ros M, Guerra-García JM, Faasse M, Santos AM, Cabezas MP (2021). Using molecular data to monitor the post-establishment evolution of the invasive skeleton shrimp *Caprella scaura.* Marine Environmental Research, 166:105266.

ABSTRACT. The study aims to monitor the post-establishment success of the invasive skeleton shrimp *Caprella scaura* in the Atlantic-Mediterranean transition zone and understand its connectivity with other world areas, providing new information on the status of the introduced population and its global distribution. By using mitochondrial markers (16S and COI) we examined the temporal variation of populations in Cadiz Bay, Spain (hotspot for introductions in Europe) in between 2010 and 2017; as well as their linkage with foreign populations in its native and introduced distribution ranges. Cadiz Bay populations exhibited a connection with several European introduced populations (Iberian Peninsula, Canary Islands, Mediterranean Sea and The Netherlands), eastern USA, Sea of Japan and Australia. We found no evidence to support a Brazilian origin (one potential native area) of the Iberian Peninsula populations. We identified a progressive decrease in haplotype diversity and a low connectivity at the end of the monitoring period in one of the stations. Human-mediated changes in propagule pressure, and unfavorable environmental fluctuations are probably responsible for this. Meanwhile, populations in Cadiz Bay count on numerous foreign donors that could easily refuel the propagule input by exchanging gene flow. This implies that a vector regulation strategy has the potential of compromising the success of established non-native populations, which usually undergo vulnerability periods due to the challenging conditions of marinas. The use of molecular tools in a time series approach is then useful to identify the ideal time window to put in action management measures so that they are cost-effective.

1. INTRODUCTION

The increasing magnitude of introduction events in the marine environment is resulting in cooperative efforts to develop prevention management actions, as well as eradication or at least contingency programs to avoid future spread (Simberloff et al. 2013, 2018; Crowley et al., 2017). Still, establishment, long-term success, and spreading of invaders depend not only on extrinsic factors, but also on their intrinsic genetic composition and population diversity; which determines their plasticity and adaptability to the environment (Allendorf et al., 2013). In this regard, barcoding has been recognized as a powerful and useful tool for drawing light into the mechanisms underlying the invasion process. It has been widely used to determine source populations, unravel the introduction history, and understand the processes of colonization, as well as the genetic structure of introduced taxa populations (Holland 2000; Geller et al., 2010; Rius et al., 2015). This, however, comes with several difficulties. Resolving the past dynamics of an introduced species is especially challenging in the case of cryptogenic taxa (*i.e*. taxa whose native range is unknown, usually due to ancient introductions) (Carlton 1996); and taxa that experience multiple geographically distance source populations and/or repeated introductions, thus mixing native and introduced haplotypes. Furthermore, little effort has been placed on studying how population genetic patterns change temporally across the invasion history and expansion phase of introduced populations. In this regard, genetic monitoring can provide information about the temporal stability of the invader's genetic composition (Moule et al., 2015; Chiesa et al., 2014; Díez-del-Molino et al., 2016); as well as about their vulnerability, ecological success, or expansion potential (Roman and Darling 2007; Fonseca et al., 2010; Forsstr¨om et al., 2017). This becomes of great importance when predicting their risk of spreading to new areas (Guardiola et al., 2012; Lehnert et al., 2018), as well as when evaluating the efficiency of control programs (Zalewski et al., 2016). From a management point of view, monitoring is especially relevant because, even if prevention and early detection strategies are the most cost-effective when dealing with bioinvasions, big detection lags happen most of the time. By the time a new invader is acknowledged by the scientific community and decision makers, it may already be established in the recipient environment (Crooks 2005, 2011; Azzurro et al., 2016). At this stage of the management process, monitoring surveys and time-series quality data are essential for developing contingency and/or eradication programs, as well as for preventing future spreading (IUCN 2009; see Fig. 1 in Simberloff et al., 2013).

Colonizers of highly fluctuating habitats such as ports and marinas are especially challenging, often showing a huge potential for quick spread and rapid adaptation. Indeed, these anthropogenic habitats are characterized by stressful factors, drastic changes in environmental conditions, and variable propagule pressure (Bax et al., 2003; Arenas et al., 2006; Hedge et al., 2012; Hedge and Johnston 2012). In these circumstances, introduced taxa can suffer huge changes in recruitment along the years (alternating latency periods with density peaks), and yet continue reproducing successfully (Crooks and Soul´e 1999; Cabezas et al., 2019). As explained in previous sections, amphipod crustaceans are good examples of this type of colonizers; and the species *Caprella scaura* one primary example of successful worldwide hidden-invader with, however, a complicated history of introduction.

The native range of *C. scaura* is still under discussion. The most ancient records of the species correspond to Templeton (1836), who described it based on specimens collected from Mauritius Island; and to Dana (1853), who collected individuals from Rio the Janeiro (Brazil) two years later. A short time later, the species was reported as well from the Caribbean Sea, Australia and Japan (see Table 3 and discussion in Ros et al., 2014a). Therefore, its native range may be situated in a) the Central and southwestern Atlantic Ocean, b) the IndoPacific region and Australia, or c) the temperate northwestern Pacific Ocean; but *C. scaura* is, to date, considered as cryptogenic in these areas. In any case, there is sound evidence that the species *Caprella scaura sensu stricto* (including the forms *Caprella scaura scaura* and *Caprella scaura typica*) has been introduced by human transport into several locations of the Northern Hemisphere (see Ros et al., 2014a; Cabezas et al., 2014). In the present study, we will refer to the nominal form *C. scaura* (*Caprella scaura sensu stricto*) as '*C. scaura*'. During the 20th century, it was introduced to both coasts of USA via hull-fouling and ballast water (Marelli 1981; Foster et al., 2004). In Europe, it was reported for the first time from the Venice Lagoon (Italy) in the Mediterranean Sea (Sconfietti and Danesi 1996). In the following years, it quickly spread to further Mediterranean countries by means of recreational boating (Krapp et al., 2006; Martinez and Adarraga 2008; Bakir and Katagan 2011; Ben Souissi et al., 2010; Fernandez-Gonzalez et al., 2011; Ros et al., 2014a; Ulman et al., 2017); as well as westwards to the Atlantic Ocean, including the Canary Islands (Faasse 2017; Guerra-García et al., 2011). Cabezas et al. (2014) reported multiple source populations in this invasion process: an Indo-Pacific origin via the Suez Canal, most likely with Mediterranean populations acting as stepping-stone events; plus a Pacific Australian origin. Last records of the species are from The Netherlands (Northern Europe) (Faasse 2017) and UK (Martínez-Laiz et al., 2021); which proves that it continues to expand its introduced distribution range towards higher latitudes. Although efforts were made to unravel the introduction history of *C. scaura,* the use of molecular markers to monitor changes in its introduced populations has not been carried out so far.

The main objective of the present study is to monitor the post-establishment success of *C. scaura* in southern Spain by using mitochondrial markers. To address this aim we analysed the temporal genetic variation of *C. scaura* populations in the Southern Iberian Peninsula in between 2010 and 2017, starting shortly after it was first detected in that area. Additionally, we aim to understand the influence of multiple introduction events in this process by exploring the genetic connectivity among Cadiz Bay populations and other world areas (including both its native and introduced distribution range). Therefore, this study also provides new information regarding the world spatial distribution of *C. scaura*; examining populations from a potential native range area *versus* recently introduced ones. This knowledge is critical to better understand the worldwide invasion routes of this species; as well as for informing the most effective spatio-temporal scale of management actions.

2. MATERIAL AND METHODS

2.1 Sample collection

A total of 322 individuals were collected from marinas in Cadiz Bay (see Table 1), as the result of a series of sampling programs developed from 2010 to 2012, 2016 and 2017 (Ros et al., 2015a,b plus other campaigns). This area was selected due to its strategic location at the Strait of Gibraltar, which serves as gateway connecting areas like the Mediterranean Sea, West Africa, The Caribbean, northern Europe and Australia; with approximately 60,000 vessels transiting each year (Gibraltar Port Authority 2017; Gibraltar Port marina staff, pers. comm. 2017). Samples from fouling communities growing on artificial hard substrate including pontoons, ropes, wheels, buoys and ship hulls were hand-collected. These included mainly the bryozoan *Bugula neritina* (Linnaeus 1758), plus other arborescent substrates such as red and green algae, hydroids, other bryozoans, ascidians, mollusks and their associated mobile epifauna. Unfortunately, there was a gap for the period 2013–2015 due to financial and logistics constraints, which impeded carrying out the sampling of this geographic area at that time. In addition, a total of 111 specimens of *C. scaura* were collected in 4 countries across its introduced geographic range (Spain – Balearic Islands, Italy, France and The Netherlands); plus three states across the southeastern coast of Brazil, included in its potential native range (see Table 1). Fouling communities growing on artificial substrate (*i.e.* ports, marinas and a power station) and natural habitats (*i.e* rocky shores protected/exposed to wave action in beaches/protected areas) were sampled following a similar methodology (see Material and methods section in Ros et al. (2013a) and Ros et al. (2016) for more details). Samples were fixed *in situ* in 96% ethanol and taken to the laboratory, where individuals of *C. scaura* were sorted, identified and kept aside for molecular analysis.

Table 1. Sample information including population code, site location, source country, geographical coordinates, site description and substratum, date of collection, number of sequences obtained per site, reference and GenBank accession numbers. Note that for samples from Cadiz Bay, "Number of sequences" refers to concatenated COI+16S sequences. For remaining samples and those from Cabezas et al. (2014), Leray and Knowlton (2015), and MGOTX150-19 N (BOLD), it refers to COI sequences. GenBank codes are included, respectively.

Population Code	Locality	Country	Coordinates	Site description, Substratum	Date of collection	Number of sequences	Reference	GenBank codes (COI; 16S)
PAM10	Puerto America (Cadiz Bay)	Spain	36°32'36.9"N 6°16'47.8"W	Marina, *Bugula neritina*	Jun-Dec 2010	7	This study	MW550737- MW550743; MW539401- MW539407
PAM11	Pto. America (Cadiz Bay)	Spain	36°32'36.9"N 6°16'47.8"W	Marina, *B. neritina*	17 May-14 Dec 2011	53	This study	MW550744- MW550796; MW539408- MW539460
PAM12	Pto. America (Cadiz Bay)	Spain	36°32'36.9"N 6°16'47.8"W	Marina, *B. neritina*	19 Jan-23 Dec 2012	119	This study	MW550797- MW550915; MW539461- MW539579
PAM16	Pto. America (Cadiz Bay)	Spain	36°32'36.9"N 6°16'47.8"W	Marina, *B. neritina*	6 Mar-10 Sep 2016	16	This study	MW550916- MW550931; MW539580- MW539595
PAM17	Pto. America (Cadiz Bay)	Spain	36°32'36.9"N 6°16'47.8"W	Marina, fouling substrate	12 Mar 2017	13	This study	MW550932- MW550944; MW539596- MW539608
VLE16	Viento de Levante (Cadiz Bay)	Spain	36°31'13.8"N 6°16'26.6"W	Marina, *B. neritina*	6 Mar, 10 Sep 2016	28	This study	MW550945- MW550962; MW539609- MW539626
VLE17	Viento de Levante (Cadiz Bay)	Spain	36°31'13.8"N 6°16'26.6"W	Marina, fouling substrate	12 Mar, 2 Jun 2017	18	This study	MW550963- MW550990; MW539627- MW539654
ROT11	Rota (Cadiz Bay)	Spain	36°36'59.4"N 6°21'12.4"W	Marina, *B. neritina*	17 May 2011	2	This study	MW550991- MW550992; MW539655- MW539656
ROT16	Rota (Cadiz Bay)	Spain	36°36'59.4"N 6°21'12.4"W	Marina, *B. neritina*	6Mar, 10 Sep 2016	17	This study	MW550993- MW551009; MW539657- MW539673
CSICA	Isla Canela	Spain	37°11'11.4"N, 7°20'24.0"W	Marina, *B. neritina*	16 May 2011	9	Cabezas et al. (2014)	KF743182–KF743190
CSCHP	Chipiona	Spain	36°44'41.5"N, 6°25'49.2"W	Marina, *Zoobotryon verticillatum*	12 Dec 2009	9	Cabezas et al. (2014)	KF743191–KF743199

CSCON	Conil	Spain	36°17'41.8"N, 6°08'10.8"W	Marina, *B. neritina*	17 May 2011	5	Cabezas et al. (2014)	KF743220–KF743224
CSBAB	Barbate	Spain	36°11'09.4"N, 5°56'01.9"W	Marina, *B. neritina*	31 Jan 2010	2	Cabezas et al. (2014)	KF743225–KF743226
CSTEN	La Laguna (Canary Islands)	Spain	28°30'29.83" N, 16°11'3.78"W	Aquaculture facilities next to fishing dock	20 May 2009	6	Cabezas et al. (2014)	KF743300–KF743305
CSALM	Almería	Spain	36°49'53.4"N, 2°27'42"W	Marina, *B. neritina*	1 July 2011	15	Cabezas et al. (2014)	KF743227–KF743241
CSTOR	Torrevieja	Spain	37°58'29.0"N, 0°40'59.6"W	Marina, *B. neritina*	29 Jun 2011	5	Cabezas et al. (2014)	KF743242–KF743246
CSALI	Alicante	Spain	38°20'22.7"N, 0°29'10.7"W	Marina, *B. neritina*	29 Jun 2011	10	Cabezas et al. (2014)	KF743247–KF743256
CSVAL	Valencia	Spain	39°25'41.2"N, 0°19'56.6"W	Marina, *B. neritina*	28 Jun 2011	10	Cabezas et al. (2014)	KF743257–KF743266
CSBAR	Barcelona	Spain	41°22'38.1"N, 2°10'58.5"E	Marina, *Z. verticillatum*	26 Jun 2011	15	Cabezas et al. (2014)	KF743267–KF743281
CSLET	L'Estartit	Spain	42°03'14.4"N, 3°12'21.7"E	Marina, *Z. verticillatum*	25 Jun 2011	3	Cabezas et al. (2014)	KF743282–KF743284
CSBAL	Cala Rajada (Balearic Islands)	Spain	39°33'4.68"N, 2°37'52.03"E	Marina, *B. neritina, Ceramium* sp.	5 Nov 2011	10	Cabezas et al. (2014)	KF743375–KF743384
MAH	Mahón (Balearic Islands)	Spain	39°53'28.5"N 4°15'55.0"E	Marina, fouling substrate	20 July 2012	9	This study	MW551010- MW551018
CSCAS	Cascais	Portugal	38°41'27.4"N, 9°25'06.8"W	Marina, *B. neritina*	9 May 2011	1	Cabezas et al. (2014)	KF743146
CSSIN	Sines	Portugal	37°57'01.9"N, 8°51'54.4"W	Marina, *B. neritina*	10 May 2011	15	Cabezas et al. (2014)	KF743147–KF743161
CSALB	Albufeira	Portugal	37°05'05.54" N, 8°15'58.2"W	Marina, *B. neritina*	10 May 2011	5	Cabezas et al. (2014)	KF743162–KF743166
CSFAR	Faro	Portugal	37°00'53.9"N, 7°56'12.1"W	Marina, *B. neritina*	11 May 2011	15	Cabezas et al. (2014)	KF743167–KF743181
CSMAR	Marina Smir	Morocco	35°45'16.3"N, 5°20'31.3"W	Marina, *B. neritina*	30 May 2011	15	Cabezas et al. (2014)	KF743285–KF743299
COR	Ajaccio (Corsica)	France	41°55'06.3"N 8°44'35.9"E	Marina, *B. neritina, Eudendrium* sp.	31 May 2012	15	This study	MW551019- MW551033;
CIV	Civitavecchia	Italy	42°05'33.7"N 11°47'00.1"E	Marina, *B. neritina*	29 May 2012	13	This study	MW551034- MW551046
CSPTO	Porto Torres (Sardinia)	Italy	40°50'4.20"N, 8°24'35.70"E	Natural habitat, *Dilophus* sp.	10 Dec 2010	1	Cabezas et al. (2014)	KF743306
CSPAL	Palermo (Sicily)	Italy	38°06'28.50" N, 13°22'5.78"E	Marina, *Bugula neritina*	30 Sep 2011	15	Cabezas et al. (2014)	KF743353–KF743367
CSGRE	Gulf of Amvrakikos (Koronissia)	Greece	38°44'N, 20°55' E	Natural habitat, small seagrass	16 Aug 2002	7	Cabezas et al. (2014)	KF743307–KF743313
NL	Borssele	The Netherlands	51°25'49.41'' N, 3°42'34.24''E	Power station, bushy seaweeds on boulders	8 Sep 2017	6	This study	MW551047- MW551052
SEB	São Sebastião	Brazil	23°45'16.2"S 45°24'37.1"W	Yacht club, *B. neritina* and *Z. verticillatum*	4 Oct 2012	14	This study	MW551053- MW551066
ARA	Praia do Araçá (Santa Catarina) (protected area)	Brazil	27°08'06.2"S 48°31'50.7"W	Natural hábitat, *Z. verticillatum* and *B. neritina*	2 Oct 2012	14	This study	MW551067- MW551080
ARC	Arraial do Cabo	Brazil	22°58'13.5"S 42°01'03.6"W	Fishing port, fouling substrate	18 Oct 2012	15	This study	MW551081- MW551095

NIT	Niteroi (Rio de Janeiro)	Brazil	22°56'05.59"S 43°06'26.08" O	Harbor, fouling substrate	21 Oct 2012	8	This study	MW551096- MW551103
CSBRA	Ilha Redonda, Cagarras	Brazil	23°5'44.68"S, 43°12'11.29" W	Oyster farm	7 Oct 2006	1	Cabezas et al. (2014)	KF743385
CSMAU	Pointe aux sables	Mauritius	20°10'19.36"S , 57°26'53.54"E	Natural habitat, *Gracilaria* sp.	24 Jul 2002	4	Cabezas et al. (2014)	KF743368–KF743371
CSCHA	Ft Jonson (Charleston)	EEUU	32°45.12'N, 79°53.93' W	Floating dock in SCDNR boatship	10 Dec 2006	3	Cabezas et al. (2014)	KF743372–KF743374
FLO	Indian River Lagoon, Florida	EEUU	28°03'37.6"N, 80°34'35.4"W	Autonomous reef monitoring structure (ARMS) plates	26 May 2013	1	Leray and Knowlton (2015)	KP254447
TEX	Baffin Bay, Texas	EEUU	27°15'58''N, 97°25'18.8''W	-	30 May 2019	1	-	MGOTX150-19 (BOLD)
CSHON	Honolulu (Hawaii)	EEUU	21°16'52.10" N, 157°50'33.75" W	Marina, *Eudendrium* sp.	6 Jun 2011	15	Cabezas et al. (2014)	KF743338–KF743352
CSJAP	Amursky Bay	Russia	42°36'34.42" N, 131°47'36.42" E	Natural habitat, intertidal algae	20 Jul 2010	9	Cabezas et al. (2014)	KF743329–KF743337
CSAUS	Scotland Islands	Australia	33°38'38.91"S , 151°17'40.82" E	Marina, *Sargassum* sp. and *Padina* sp.	14 Jan 2011	15	Cabezas et al. (2014)	KF743314–KF743328

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from gnathopods, pereopods, antennae and gills; along one side of the body of each specimen. We used the commercial kit PureLink Genomic DNA Mini Kit (Invitrogen, UK) according to the manufacturer’s protocol. The DNA was eluted in 120 µl of elution buffer and stored at □ 20 ◦C. Fragments of two mitochondrial (COI and 16S rRNA) genes were amplified by polymerase chain reaction (PCR), the latter gene only for individuals of Cadiz Bay populations (Table 1). PCR amplifications consisted of 25 µl reaction volumes containing 3 µl of template DNA, 10 × MgCl2-free buffer (Invitrogen, UK), 3 mM (for COI gene)/2.5 mM (for 16S gene) MgCl2, 0.2 mM dNTPs, 1 µM of each primer, 0.1 µg µl□ 1 Bovine Serum Albumin (BSA, Promega, Madison, WI), 0.3 U Platinum Taq DNA polymerase (Invitrogen, UK), and double-distilled H2O to volume. Primers for amplification and PCR conditions are listed in Table 2. PCR product purification and bidirectional Sanger sequencing were provided by a commercial company (GENEWIZ, Leipzig, Germany). The DNA amplification was successful in 85% of the sampled material; this is for 273 individuals from Cadiz Bay and for 94 individuals from other localities.

2.3. Sequence analysis

The resulting sequences were checked and edited using SEQUENCHER version 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and Codon Code Aligner version 8.0.1 (Codon Code Corporation, MA, USA). Mitochondrial COI sequences were translated into amino acids to search for stop codons that are indicative of the presence of pseudogenes. All sequences were deposited in GenBank (Table 1), all sequences of COI and 16S were aligned using MUSCLE (Edgar 2004) as implemented in MEGA version 7 (Kumar et al., 2016). Sequences of both mitochondrial genes from Cadiz Bay samples were subsequently concatenated using the APE package (Paradis et al., 2004) in RStudio (RStudio Team 2016). In addition to the sequenced data produced in this study, COI sequences from other nine countries across *C. scaura* worldwide distribution range were taken from Cabezas et al. (2014), Leray and Knowlton (2015) and MGOTX150-19 (BOLD systems). These data were pooled together for the spatial analysis and the corresponding estimates of genetic diversity.

Table 2. Primers used for amplification and PCR conditions used in the present study

Primer	Sequence (5’-3’)	Source	PCR conditions
COI			
jgLCO1490	TITCIACIAAYCAYAARGAYATTGG	Geller et al. (2013)	94 °C (4'); [x40] 94 °C (45''), 45 °C (50"), 72 °C (1'); 72 °C (10')
jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA		
16S rRNA			
16STf	GGTAWHYTRACYGTGCTAAG	Macdonald et al. (2005)	94 °C (150"); [x36] 94 °C (40''), 54 °C (40"), 65 °C (80"); 65 °C (8')
16SBr	CCGGTTTGAACTCAGATCATGT	Palumbi et al. (1991)	

2.3.1. Estimates of genetic diversity and population structure at spatio-temporal scale

Based on mitochondrial data, number of haplotypes, haplotype diversity (*Hd*, Nei 1987) and nucleotide diversity (π, Nei 1987), were estimated for *C. scaura* populations in all sampled locations, using DnaSP version 6 (Rozas et al., 2017). Frequencies of haplotypes over time and location were calculated with this program as well. The genetic differentiation among populations was determined by means of the statistics FST (Weir and Cockerham 1984) with Arlequin version 3.5.1.2 (Excoffier and Lischer 2010), using the pairwise differences distance method and assessing statistical significance through 10,000 permutations. These parameters were obtained analyzing the mithocondrial COI region. Besides, for Cadiz Bay populations, we focused on the concatenated COI+16S region for calculating the aforementioned parameters as well as the four analyses presented below. For Cadiz Bay populations, a multidimensional scaling (MDS) analysis was performed on the matrix of FST values, for a graphical depiction of the spatio-temporal structure of concatenated COI+16S. The software TIBCO STATISTICA version 13 (TIBCO Software Inc., CA, USA) was used for this purpose. Additionally, a hierarchical analysis of molecular variance (AMOVA, Excoffier et al., 1992) was conducted in Arlequin version 3.5.1.2 (Excoffier and Lischer 2010) to study the distribution of genetic variability (concatenated COI+16S) within and between the three sampled marinas in Cadiz Bay. Three groups were used for the AMOVA tests, corresponding to the three marinas: Puerto America, Viento de Levante, and Rota. Statistical significance of variance components was tested with 16,000 permutations. To test if the selection of demographic events (population expansion or contraction) affected the genetic structure of Cadiz Bay populations, neutrality tests (Tajima’s D, Fu’s FS and Ramos-Onsis and Rozas’ R2) (Fu 1997; Tajima 1989; Rozas and Ramos-Onsins 2002) and mismatch distribution were performed for the mitochondrial concatenated dataset. Neutrality tests provide trends with respect to equilibrium and non-equilibrium conditions and indicate recent population expansion when the null hypothesis of neutrality is rejected due to significant negative values. They were assessed for each population with the statistical significance obtained by 10,000 coalescent simulations. To test the goodness-of-fit between the observed and the expected distributions under the sudden expansion model, the sum of squared deviations (SSD) (Schneider and Excoffier 1999) and Harpending's raggedness index (Rg) (Harpending 1994) were also computed using 10,000 bootstrap replicates. DnaSP version 6 (Rozas et al., 2017) was used to calculate R2 statistic, and the remaining estimates and respective significance tests were obtained with Arlequin version 3.5.1.2 (Excoffier and Lischer 2010). Finally, as part of the long monitoring in Puerto America (PAM) population, a linear regression analysis was performed to test whether variation in *Hd* of COI+16S was linearly related to time (in months), using the ggplot2 package (Wickham, 2016) in RStudio (RStudio Team 2016).

2.3.2. Phylogenetic reconstruction

At local scale, relationships among the mitochondrial haplotypes (concatenated dataset) from Cadiz Bay across the years were examined via a haplotype network using statistical parsimony method (Templeton et al., 1992) in TCS version 1.21 (Clement et al., 2000) with a 95% connection limit. The network was plotted with tcsBU (Santos et al., 2016).

At global scale, phylogenetic relationships based on the COI dataset were estimated using two model-based methods of phylogenetic inference: Bayesian inference (BI) in MRBAYES version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) and maximum likelihood (ML) in Garli version 2.0.1 (Zwickl, 2006). Analyses were conducted using data partitions by codon (1+2+3) for the mitochondrial COI gene, to minimize saturation effects of codon positions on phylogenetic reconstructions (Salemi 2009) and to account for different rates of evolution of each one (Pond et al., 2009). The species *Caprella simia* Mayer, 1903 and *Caprella linearis* (Linnaeus, 1767) (sequences available in GenBank: COI, KF743434–KF743447 and FJ581572 respectively) were used as outgroups. The best-fit model of sequence evolution was estimated using PartitionFinder version 2.1.1 (Lanfear et al., 2016). According to the corrected Akaike Information Criterion (AICc) (Akaike 1974), the best models was GTR + I (1st - 3rd partition). For BI analyses, two independent runs, of four chains each, were conducted for 2 × 107 generations (runs converged with average standard deviation of the split frequencies below 0.01). Trees and parameters were sampled every 1,000 generations, with the heating parameter set to 0.25. The consensus (majority-rule) tree was estimated combining results from duplicated analyses, after discarding 25% of total samples as burn-in, determined from plotting log-likelihood values against generation time in Tracer version 1.7.1 (Rambaut et al., 2018). ML analysis was performed using 10 independent searches and 1,000 bootstrap replicates. The convergence between tree topologies was confirmed by examining log likelihood values across searches. The SumTrees command from the package DendroPy (Sukumaran and Holder, 2010) was used to summarize non-parametric bootstrap support values for the best tree after generating a majority-rule consensus tree. Consensus tree inferred for each molecular dataset was visualized and rooted using FigTree version 1.4.3 (Rambaut 2017).

3. RESULTS

3.1 Haplotype variation in Cadiz Bay

The mitochondrial markers COI and 16S rRNA were successfully amplified for 367 individuals of C. scaura. A total of 14 haplotypes were observed for Cadiz Bay populations (Fig. 1AB, Table 3). The complete alignment of the COI dataset had a total length of 657 bp; and the one of the 16S rRNA 404bp; resulting in a concatenated alignment of 1061 bp. No insertions or deletions were detected in any of the COI sequences, and when they were translated into proteins, no stop codons were found. For the 16S rRNA alignment, we observed an insertion of a thymine (T) at position 722 for seven individuals from Puerto America (PAM) marina, corresponding to the private haplotype 47.

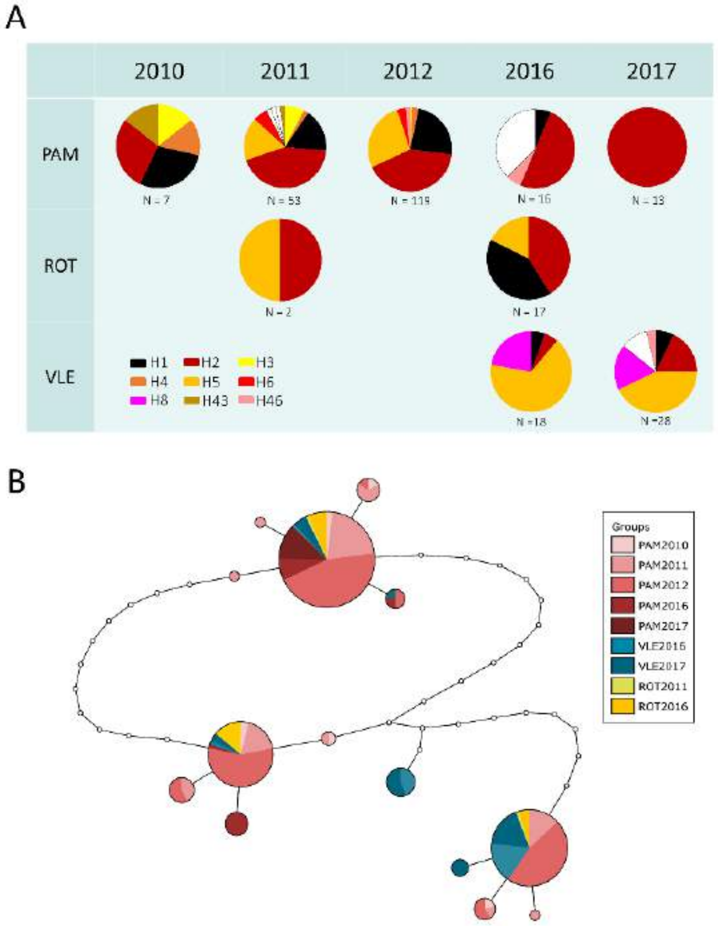

Fig. 1. Temporal genetic variation of concatenated COI+16S in *C. scaura* populations from Puerto America (PAM), Rota (ROT), and Viento de Levante (VLE) (Cadiz Bay) in 2010–2012, 2016 and 2017. A - Each year's population is represented by a pie chart showing population composition and relative haplotype frequency. Number of analysed individuals per population appears below. White-shaded areas are the proportion of private haplotypes per location (H7, H44, H45 and H47 from PAM and H9 from VLE). Rota was sampled only in 2011 and 2016, and Viento de Levante in 2016 and 2017. B - Haplotype network (95% parsimony connection limit) representing the relationships between the 322 individuals sampled from Cadiz Bay. Localities and years are coded by filling patterns (see legend). Circle sizes are proportional to haplotype frequency. Non-observed haplotypes are represented by small white circles. Each line connecting haplotypes represents a single mutational change.

3.2. Temporal variation in between 2010 and 2017 in Cadiz Bay

Twelve of the 14 haplotypes found in Cadiz Bay were present in Puerto América (H1–H7, H43–H47) (Fig. 1A). In this location, the haplotype H2 was the only prevalent across all monitoring years, increasing its frequency over time. Haplotype H1 was the second most common haplotype, but it was absent in 2017. Haplotype 5, the third most abundant haplotype, was detected in high frequency in

2011 and 2012, but disappeared afterwards. The remaining diversity corresponded mainly to private haplotypes restricted to that marina (H3, H4, H6, H7, H43, H44, H45, H47); which decreased their frequency over time. Overall, PAM population showed a progressive linear decrease in haplotype diversity over time (p = 0.002; Adjusted R2 = 0.46; Table 3, Fig. 2), with maximal value occurring in 2010 (*Hd* = 0.905) and lowest value occurring in 2017 (*Hd* = 0), where all *C. scaura* individuals exhibited exclusively the haplotype H2. Nucleotide diversity dropped from π = 0.009 to π = 0 (Table 3) across monitoring years. In accordance, neutrality tests (Tajima's D, Fu's FS and Ramos-Onsis and Rozas' R2), were positive and not statistically significant across all years (Table 1a Supplementary material). This indicates that Puerto America population is not under a recent expansion event.

Table 3. Estimates of genetic diversity for concatenated COI+16S sequences of *C. scaura* for each sampling site in Cadiz Bay (Spain) and across the 7-year period. N, number of individuals per location; Haplotype codes (private haplotypes indicated in bold); H, number of haplotypes; Hp, number of private haplotypes; *Hd*, haplotype diversity; π, nucleotide diversity. Note that N, H and Hp are indicated first for each sampling site and time, and secondly for the geographical area as a whole.

Geographical location	Population	N	Haplotype codes	H	Hp	*Hd*	π
Puerto America	PAM10	7	H1, H2, H3, H4, H43	5	0	0.905	0.009
	PAM11	53	H1, H2, H3, H4, H5, H6, **H7**, H43, **H44**, **H45**	10	3	0.758	0.008
	PAM12	119	H1, H2, H3, H4, H5, H6, H46	7	0	0.707	0.008
	PAM16	16	H1, H2, H46, **H47**	4	1	0.642	0.007
	PAM17	13	H2	1	0	0.000	0.000
Viento de Levante	VLE16	18	H1, H2, H5, H8	4	0	0.529	0.005
	VLE17	28	H3, H4, H5, H8, **H9**, H46	6	1	0.762	0.008
Rota	ROT11	2	H2, H5	2	0	1	0.010
	ROT16	17	H1, H2, H5	3	0	0.669	0.008
	Cadiz Bay	*273*	*H1-H9, H43-H47*	*14*	*5*		

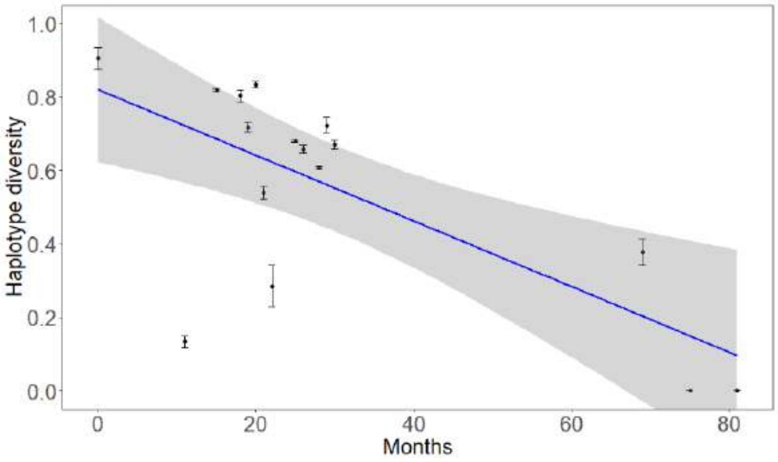

Fig. 2. Temporal linear regression of genetic diversity (haplotype diversity) in Puerto America populations from 2010 to 2017. Horizontal axis represents time (in months) passed since the beginning of the monitoring.

Populations from Viento de Levante (VLE) marina showed 6 out of the 14 haplotypes found in Cadiz Bay, including two private haplotypes (H8 and H9) (Fig. 1A). Haplotype 5 was the dominant one in the two sampled years; and haplotypes H1 and H2 increased their frequency from one year to another. In Rota populations, haplotypes H1, H2 and H5 were also present. Haplotype H1 was only detected in 2016, but in higher frequency when comparing with PAM population in the same year. Overall, haplotype and nucleotide diversities were high in both VLE and ROT populations (Table 3). Neutrality tests did not show any negative and significant values, which suggest that neither these populations are under expansion (Table 1b Suppl. material).

Estimates of pairwise FST values showed mostly low and intermediate levels of divergence between populations of Cadiz Bay, with significant values ranging from 0.091 (VLE17-PAM12; $p < 0.01$) to 0.755 (VLE16-PAM17; $p < 0.001$) (Table 2 Suppl. material). Results revealed that populations from VLE were genetically more differentiated from the rest of populations inhabiting Cadiz Bay. However, no significant differentiation was found between ROT and PAM populations during most of the monitoring period (Table 2 Suppl. Material; Fig. 1B). Only the year 2017 in PAM population was genetically differentiated from the rest. These patterns are clearly reflected in the MDS plot (Fig. 3). Hierarchical AMOVA tests revealed significant genetic differences between populations of different marinas and within populations of the same marina (Table 3 Suppl. material). Intrapopulation variance explained most (over 82%) of the genetic variation found in *C. scaura*. Finally, neutrality tests were positive but not significant for all 3 marina populations (Table 1b Suppl. material). Regarding the sum of the square deviations (SSD), statistically significant differences were observed in PAM (SSD = 0.13, $p < 0.01$), VLE (SSD = 0.13, $p < 0.05$) and ROT populations (SSD = 0.18, $p < 0.001$). These results agree with the significant values of Harpending's raggedness index (Rg) found; thus suggesting the rejection of the null hypothesis of recent population expansion.

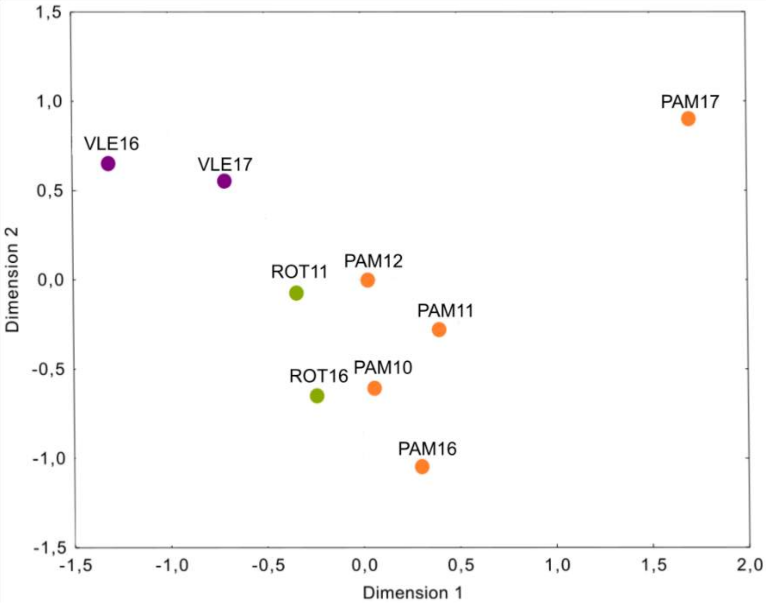

Fig. 3. Multidimensional scaling plot (MDS) based on FST values for *C. scaura* in Cadiz Bay. Populations are coloured according to the marina they belong, and points correspond to samples per year.

3.3. Worldwide connectivity and phylogenetic pattern

The worldwide geographical distribution of mtDNA COI haplotypes of *C. scaura* is shown in Fig. 4. At a global scale, a total of 42 COI haplotypes were observed. Most of them (34) were private and only 8 were shared between different geographical locations. A total of 12 haplotypes were identified in the Brazilian populations sequenced in the present study. However, only two of them (H25 and H29) were shared with other foreign populations: H25 with Mauricio (CSMAU) and Charleston (CSCHA); H29 with Honolulu (CSHON); and both haplotypes with Australia (CSAUS). These Brazilian populations, included in the potential native range of *C. scaura*, accounted for a higher percentage of private haplotypes (83% - 10 private haplotypes), in comparison with the 71% (12 haplotypes) and 67% (6 haplotypes) observed in the introduced Mediterranean and Cadiz Bay populations, respectively. Among populations from the introduced regions, a total of 15 haplotypes were identified, and only two of them were shared with potential native populations: H1, present in Ilha Redonda, Brazil (CSBRA, artificial habitat); and H2, present in a Russian population in the Sea of Japan (CSJAP, natural habitat) (Fig. 4). Moreover, populations in Puerto Am´erica showed significantly low differentiation with the potential native populations of Niteroi (FST = 0.23; $p < 0.05$) and Mauritius (FST = 0.21–0.24; $p < 0.001$) (Table 4 Suppl. material). Corsica (COR) and Mahon (MAH) populations were characterized by the two main haplotypes found in Spain populations (H2 and H5 in Fig. 4; H1 and H2 in Cabezas et al., 2014), and that were also present in other Mediterranean populations (PAL and CSGRE); but also in Florida (FLO) and the Sea of Japan (CSJAP) (Fig. 4). Moreover, Mahon also shared haplotype H10 with Australia (CSAUS) and Alicante (CSALI) populations. The Netherlands (NL) population of *C. scaura* shared one haplotype (H18) with Balearic Islands (CSBAL), Greece (CSGRE) and Texas (TEX) populations; and the other one (H1) with populations of Civitavecchia (CIV), Ilha Redonda (CSBRA), Honolulu (CSHON), and Cadiz Bay. Finally, as expected, Cadiz Bay populations were mainly characterized by the dominant haplotypes present in Iberian and Mediterranean populations (Fig. 4). The estimates of pairwise FST values showed mostly low to intermediate levels of differentiation between Cadiz Bay and the other worldwide populations, except for Puerto America in the year 2017 (PAM17) (Table 4 Suppl. material). The phylogenetic tree suggested that Cadiz Bay populations are the result of multiple introductions, with a clear linkage with Australian populations plus three geographical areas in the Northern hemisphere: North Atlantic Ocean (both EEUU and Europe), Mediterranean Sea and Northwestern Pacific Ocean (Sea of Japan) (Fig. 5).

Overall, populations showed similar values of haplotype diversity (Table 4). The highest values of haplotype diversity was found in populations from Puerto America in 2010 (PAM10) (*Hd* = 0.905) and Niteroi (NIT) (*Hd* = 0.857); whereas lowest values were obtained for the population from Puerto America in 2017 (PAM17) (*Hd* = 0.00) and that of the yacht club in S˜ao Sebasti˜ao (SEB) (*Hd* = 0.396). Nucleotide diversity values ranged from 0 in PAM17 to 0.011 in NIT and CIV.

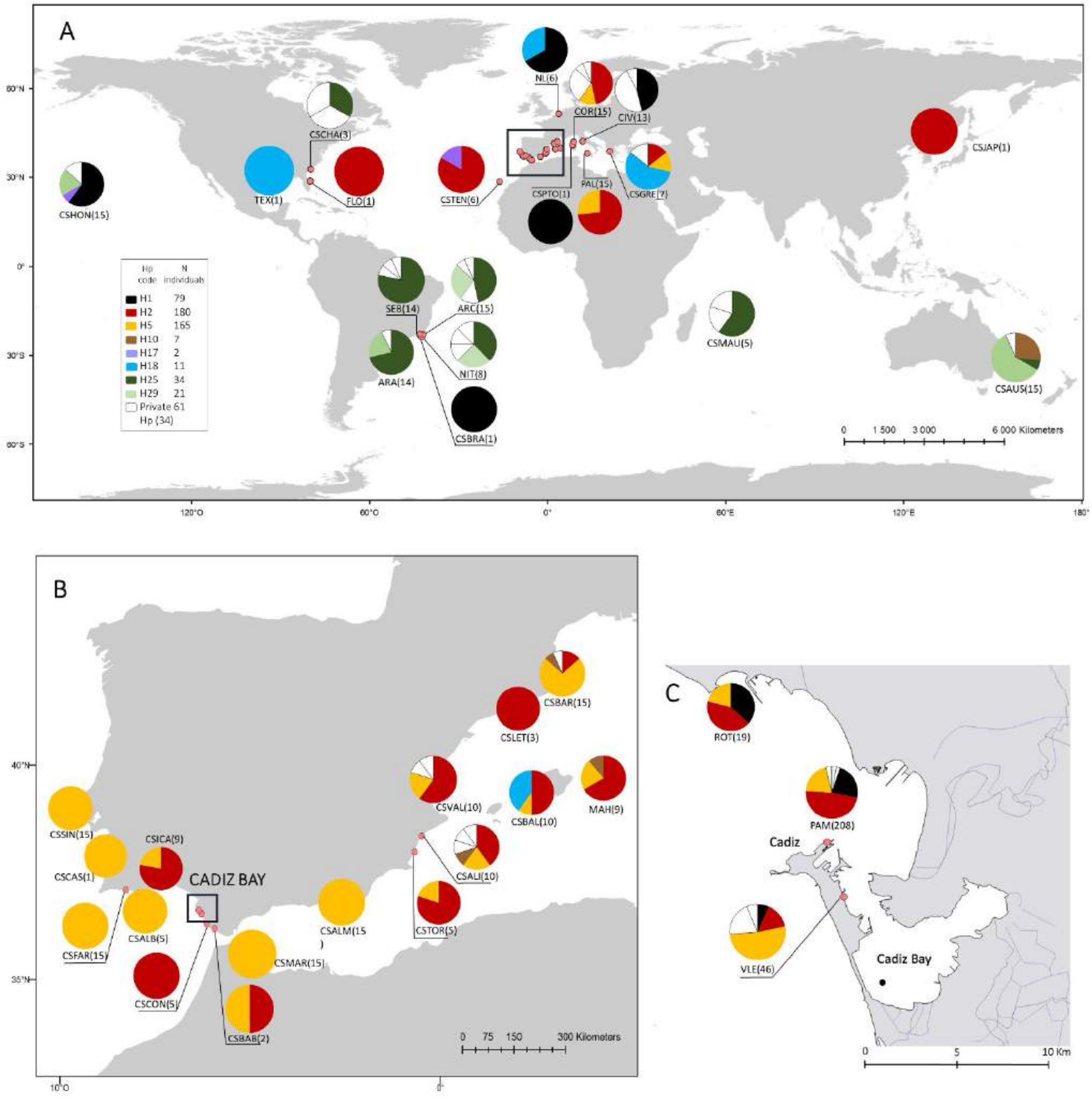

Fig. 4. Geographical distribution of mtDNA COI haplotypes of *C. scaura* at global scale (except for those in Cadiz Bay) (A). The view in B represents a closer look to the Iberian Peninsula and Balearic Islands. The view in C represents Cadiz Bay sampling sites. Each site is represented by a pie chart showing population composition and relative haplotype frequency. White areas represent private haplotypes per location, and number of individuals sequenced are shown in brackets. The map includes results from Cabezas et al. (2014) (coded as 'CS + localion'), Leray and Knowlton. (2015) (population coded as FLO) and MGOTX150-19 (BOLD) (population coded as TEX). Sites are coded as in Table 1. The legend gives information about the existing haplotypes across all locations and the number of individuals carrying each haplotype.

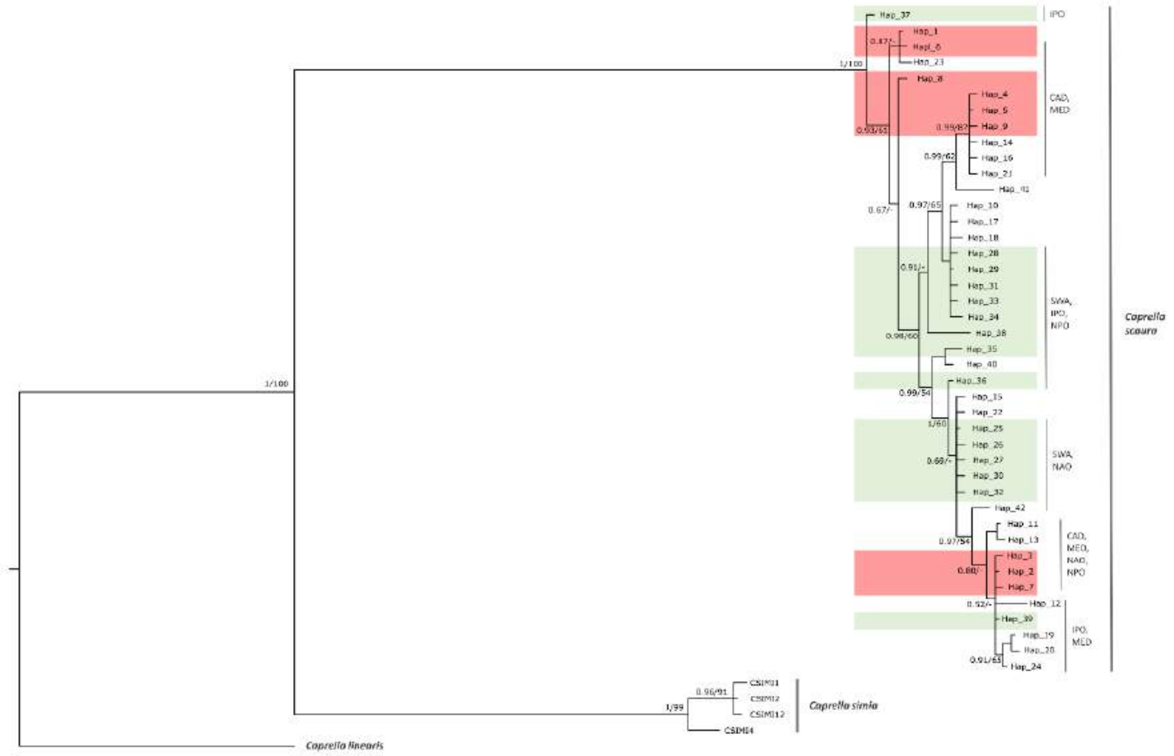

Fig. 5. Bayesian consensus tree obtained for the mtDNA COI gene for all sequenced data. The tree was rooted with *Caprella linearis* (sequence available in GenBank: FJ581572). Node values correspond to Bayesian posterior probabilities and bootstrap support given by the maximum likelihood analysis (both above branches). '-' indicates <50% support; CAD Cadiz Bay; MED Mediterranean; NPO North-Pacific Ocean, IPO Indo-Pacific Ocean, NAO North-Atlantic Ocean, SWA South-west Atlantic. Haplotypes present in Cadiz Bay are highlighted in red; and those present in the potential native range are highlighted in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4 Estimates of genetic diversity for COI sequences of *C. scaura* for each sampling site. N, number of individuals per location; Haplotype codes (private haplotypes indicated in bold); H, number of haplotypes; Hp, number of private haplotypes; *Hd*, haplotype diversity; *π*, nucleotide diversity. Note that N, H and Hp are also indicated for the geographical areas of Cadiz Bay and Southeastern Brazil as a whole. Private haplotypes are considered taking into account the worldwide variation of COI (see Fig. 4).

Geographical location	Population	N	Haplotype codes	H	Hp	*Hd*	*π*
SPAIN	*Cadiz Bay*	*273*	*H1-H9*	*9*	*6*	0.715	0.012
	MAH	9	H2, H5, H10	3	0	0.556	0.010
BRAZIL	*SE Brazil*	*51*	*H25-H36*	*12*	*10*	0.606	0.007
	SEB	14	H25, **H26**, **H27**, **H28**	4	3	0.396	0.003
	ARA	14	H25, H29, **H30**	3	1	0.473	0.006
	ARC	15	H25, **H33**, H29, **H31**, **H32**	5	3	0.733	0.009
	NIT	8	H25, H29, **H34**, **H35**, **H36**	5	3	0.857	0.011
FRANCE (Corsica)	COR	15	H2, H5, **H19**, **H20**, **H21**	5	3	0.733	0.008
ITALY (Civitavecchia)	CIV	13	H1, **H22**, **H23**	3	2	0.615	0.011
THE NETHERLANDS (Borssele)	NL	6	H1, H18	2	0	0.533	0.010

4. DISCUSSION

4.1. Post-establishment evolution of C. scaura at spatio-temporal scale

Average values of haplotype diversity in Cadiz Bay were similar to those in potential native range populations. This is not surprising, since the case of *C. scaura* in Europe is that of a very successful invasion, where the species benefited from multiple pathways and introduction events (Cabezas et al., 2014). European marinas are big hubs for maritime traffic (see Fig. 2 in Seebens et al., 2013), hence receiving a high propagule pressure that sustains their populations. This can provide a genetic diversity that can match or even exceed that of native sources (Roman and Darling 2007; Rius et al., 2015; Viard et al., 2016), which is the case for some other marine non-indigenous amphipods such as *Caprella mutica* (Ashton et al., 2008), *Ampithoe valida (*Pilgrim and Darling 2010), *Jassa marmorata* and *J. slatteryi* (Beermann et al., 2020).

Propagule pressure plays, with no doubt, a major role in determining the fate of an introduced population (see section 3.2 in Simberloff 2009). It has been identified as a key determinant in explaining invasion patterns, according to the “human activity” hypothesis [human activities facilitate the establishment of non-native species by disturbing natural landscapes and by increasing propagule pressure] (Chown et al., 1998; Taylor and Irwin 2004; Meyerson and Mooney 2007; Hedge et al., 2012; Zimmermann et al., 2014). In marine bioinvasions, non-indigenous populations are nourished by human-assisted introductions and the propagule pressure they receive is determined by their vectors' patterns: fouling risk, marine traffic, vessel routes, etc. (Seebens et al., 2013; Murray et al., 2014; Ferrario et al., 2016). At spatial scale, this implies that a marine exotic species with limited autonomous dispersal capability (*i.e.* lacking a larval stage), like *C. scaura*, may not spread following a ‘natural’ geographical pattern in their introduced range. Instead, it usually results in disjunct introduced populations that may or may not be further connected, depending on the propagule pressure they receive (magnitude, frequency, and temporal and spatial pattern of introduction events). This explains two aspects of the spatial evolution observed in Cadiz Bay populations. 1) Their genetic structure at very small scale (10 km) is more differentiated than those of other marine exotic species (see Fig. 3 in Chiesa et al., 2014; FST values within Finnish populations in Table 1, Forsstr¨om et al., 2017; Calazans et al., 2017). And 2) Puerto America (except for PAM17) and Rota (ROT) bear the highest connectivity despite being 10 km far away; whereas Viento de Levante (VLE) is the most differentiated population despite being less than 4 km away from Puerto America. We hypothesize that Rota population could be the result of propagules from Puerto America, while Viento de Levante population, that exhibits two private haplotypes, may be recipient for a different source that provides new genetic variants (Fig. 1B).

At temporal scale, propagule pressure significantly alters and explains the establishment success of invasive inoculants in ports and harbors environments (Hedge and Johnston 2012; Hedge et al., 2012); as it influences the available gene pool for facing foreign challenging conditions (Kolar and Lodge 2001; Lockwood et al., 2005; Suarez and Tsutsui 2008; Rius and Darling 2014). In this regard, numerous studies on marine introduced species have reported low levels of genetic diversity at some point of the invasion phase (Golani et al., 2007; Tepolt et al., 2009; Rius and Shenkar 2012). Concretely, a recent study on the invasive tropical caprellid *Paracaprella pusilla* identified a temporal instability at the westernmost limit of its geographical range in Europe; compromising its long-term success in the area (Cabezas et al., 2019). Dramatic changes in its population density were also detected, and the diversity status of the species seems to be the result of the fluctuating human-mediated propagule pressure it receives from adjacent areas. Likewise, the invader *Caprella scaura* showed a progressive decrease of haplotype diversity in Puerto America marina over time, which is the closest one to the international commercial harbor in Cadiz province. It is to be noticed that the resulting population after those 7 years is also the one with the lowest connectivity with adjacent marinas. Besides, populations of introduced caprellids in marinas are subjected to big density fluctuations across seasons and/or years on a regular basis (Ros et al., 2013a); driven by the highly fluctuating conditions of the marina environment [(*e.g*. pollutants, changes in salinity/temperature, maintenance works, marine traffic, oil and diesel spills, etc. (Mack and D'Itri 1973; Burgin and Hardiman 2011; Murray et al., 2014)]. In recent years, density fluctuations in *C. scaura* populations became more noticeable, and annual average density data from *C. scaura* in Puerto America started to show a decreasing trend: from 60,000–75,000ind/1,000 ml of substrate in 2011–2012, to half of it (around 30,000ind/1,000 ml) in 2016 and 2017 (data pooling from Ros et al., 2013, 2015; Molina et al., 2017; and unpublished data). Taking everything into account, it seems that *C. scaura* population in Southern Spain is decreasing and remaining isolated, left with small diversity and few new supplements. In these conditions, a population is susceptible to experience genetic drift over time (Guardiola et al., 2012; P´erez-Portela et al., 2012; Pineda et al., 2016; Stepien et al., 2018), which in turn makes it more vulnerable to buffer the impact of unfavorable environmental conditions. Concretely, in 2017, Cadiz experienced the highest temperature and rain fluctuations recorded in the last 10 years (AEMET database), which could have led to mortality and contributed to the lower diversity and higher genetic differentiation observed in 2017.

4.2. International connectivity pattern of C. scaura populations

Meanwhile, when looking at the international connectivity pattern, we observe that *C. scaura* populations in Southern Spain may as well be exchanging gene flow with a number of foreign sources, which pose a risk for propagule input. Firstly, the introduced populations in Cadiz Bay are highly connected with the rest of the Iberian Peninsula, Canary Islands, Balearic Islands, Northern Europe, Italy and Greece. These regions (mostly archipelagos and peninsulas) serve as frequent layovers for European maritime routes, and it is well-known that Mediterranean vessels are effective vectors connecting exotic communities both at regional and basin wide scale (Martinez-Laiz et al., 2019; Ulman et al., 2019). Moreover, Cadiz Bay populations are potentially enriched by very distant transoceanic donors such as Australia, Sea of Japan, EEUU, Honolulu and Brazil. The population of Sea of Japan bears the most prevalent haplotype in Cadiz Bay and the most widespread one across the Atlanto-Mediterranean populations. These two geographical regions are frequently connected due to the shellfish trade in between the Northwestern Pacific and Europe, which has already facilitated the introduction and spread of other non-indigenous peracarid crustaceans along the coast of the Iberian Peninsula (Martínez-Laiz et al., 2018). Indeed, Southern Spain, Galicia and Catalonia regions are hotspots for aquaculture production, leading the oyster, clam and mussel production in Spain. This involves, for instance, growing the non-native Pacific oyster (*Crassostrea gigas*) and the Manila clam (*Ruditapes philippinarum*), as well as exporting to other countries of Europe (Ministerio de Agricultura y Pesca 2018). Similarly, introduced populations of *C. scaura* at the opposite side of the Atlantic Ocean (EEUU and Gulf of Mexico) seems to be another frequent donor population to Northern Europe and the Mediterranean Sea. The incursions via shipping and/or aquaculture of introduced peracarids such as *Ianiropsis serricaudis* or *Stenothoe georgiana* are further evidence of the exchange between fouling communities in eastern EEUU, Europe, and Japan (see (Hobbs et al., 2015); (Martínez-Laiz et al., 2020)). Finally, Cadiz Bay populations also showed connectivity with populations as far as Brazil and Honolulu. Their common haplotype (H1) shows a very scattered pattern in Europe, contrary to the quasi-natural northwards propagation of H2 and H5 (Fig. 4), and still is one of the most widespread haplotypes worldwide. This points out to punctual independent introductions and reveals once again a huge potential for multiple transoceanic exchange routes and/or donors.

Regarding the history of introduction, our results support the pattern proposed by Cabezas et al. (2014): an IndoPacific origin and East to West stepping stone events in the Mediterranean Sea. As for Brazilian populations, they accounted for a higher number of private haplotypes (83%) than that found for introduced regions, such as the Mediterranean Sea or Cadiz Bay, in spite of a smaller sample size. This suggests that: 1) Brazilian populations are long-term residents, far exceeding the time-frame of human introductions. This would point to the Central and Southwest Atlantic Ocean as the native range of the species; or 2) there is a lack of sampling in the truly native region, which may be preventing us from finding shared haplotypes with other geographical locations. Unfortunately, the alternative native area, the Indo Pacific, remains largely under-sampled. Until this missing data is available, we cannot draw conclusions regarding the geographical origin of the species. Furthermore, the hypothesis of Brazilian populations being the origin of the ones in the Southern Iberian Peninsula (pointing to Central and southwestern Atlantic as native range) is not fully supported by our data. Despite the intensive sampling in both regions and the high diversity of Brazilian samples, no strong linkage was found except for the mentioned haplotype H1. However, this similarity is based only on a single individual (CSBRA) collected from an artificial habitat (oyster farm) and not present in other adjacent Brazilian regions (both natural and artificial habitats) (Fig. 4A). According to Chapman and Carlton (1991), when establishing the native range of species associated to fouling communities, records from an artificial habitat should be considered with caution. All populations exhibiting H1, except for Sardinia (CSPTO), were collected from artificial habitats and have a wide geographically disjunct distribution. This case is one more evidence of the difficulty of identifying biogeographic patterns of ancient introductions in such a hyperconnected world. The invasion process in a region and its subsequent secondary spread involves a complicated interplay of processes: single or multiple introduction events through time, into single or multiple sites, via single or multiple vectors (Murray et al., 2014). It is rare for the native region to remain isolated and to prevent mixing of introduced and native haplotypes.

In cases like that of *C. scaura* (with multiple potential donors, however vulnerable at a key area of their introduced range), the most cost-effective managing strategy is to target the vector of introduction itself. The prevention of future hull-fouling transfers would circumvent propagule pressure and alleviate gene flow that could ‘refuel’ further spread (Stepien et al., 2018). Since *C. scaura* has not yet colonized natural adjacent areas, one would expect that, with insufficient propagule pressure plus a diminishing population size and genetic diversity, these populations could progressively disappear, as their adaptability to face the drastic fluctuations in the marina environment would be weakened. By managing the vector, the success of *C. scaura* could be compromised at one of the main hotspots and maritime gateways of introduction in Europe. This reiterates the urgent need of implementing regulations for hull-fouling transfers (see http://www.imo.org/en/MediaCentre/Press Briefings/Pages/20-biofouling.aspx), addressing not only commercial shipping but also small private craft. Besides, these conclusions are not exclusive for introduced caprellids, but can be applied to numerous fouling exotic species that recently colonized European waters and succeeded in the establishment phase. At present, the number of animal taxa introduced by anthropogenic vectors and established in European Seas has risen up to 800 (including Lessepsian and Inland canal migrants) (EASIN 2019; Tsiamis et al., 2019). However, there is a time lag between this phase and widespread invasion, which responds to the needed propagule pressure to transition from established to expanding (see 3.4 section in Simberloff 2009), and during which management actions can still be cost-effective. Together with this, monitoring molecular studies are crucial not only to detect vulnerable introduced populations or predict future spreading, but also to evaluate, in the coming future, the effectiveness of this vector regulation.

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SUPPLEMENTARY MATERIAL

Table S1a. Neutrality tests for mitochondrial sequences of Puerto America (PAM), Viento de Levante (VLE) and Rota (ROT) marinas populations over time. R2 = Ramos-Onsis and Rozas test; SSD = sum of squared deviations between observed and expected distributions; Rg = Harpending's raggedness index; *p < 0.05, **p < 0.01, ***p < 0.001.

PUERTO AMÉRICA (PAM)

	2010	2011	2012	2016	2017
Tajima's D	0.936	2.260	3.693	2.517	0.000
Fu's FS	1.567	5.339	15.498	6.870	0.000
R_2	0.222	0.187	0.216	0.244	NA
SSD	0.081*	0.104**	0.140***	0.222	NA
R_g	0.091	0.079**	0.179***	0.324	NA

VIENTO DE LEVANTE (VLE)

	2016	2017
Tajima's D	- 0.273	1.487
Fu's FS	5.878	6.861
R_2	0.140	0.182
SSD	0.397***	0.098**
R_g	0.403	0.138**

ROTA (ROT)

	2011	2016
Tajima's D	0.000	2.050
Fu's FS	2.639	10.676
R_2	0.500	0.225
SSD	NA	0.199***
R_g	NA	0.353**

NA: The variance of the mismatch distribution is too small, no demographic parameters could be estimated

Table S1b. Neutrality test and mismatch distribution analysis for mitochondrial sequences of C. scaura for each marina. R2 = Ramos-Onsis and Rozas test; SSD = sum of squared deviations between observed and expected distributions; Rg = Harpending's raggedness index: *p < 0.05, **p < 0.01, ***p < 0.001.

	Puerto América (PAM)	Viento de Levante (VLE)	Rota (ROT)
Tajima's D	3.307	1.374	2.284
Fu's FS	10.785	8.658	11.570
R_2	0.188	0.162	0.228
SSD	0.130**	0.133*	0.183***
R_g	0.125***	0.188**	0.319**

Table S2. Pairwise FST values between populations of *C. scaura* from Cadiz Bay, based on mtDNA COI+16S sequences. Significant values: $p < 0.05$(*); $p < 0.01$ (**); $p < 0.001$ (***). Values in light grey represent medium levels of differentiation between populations and values in dark grey represent high levels of differentiation.

	PUERTO AMERICA MARINA					**VIENTO DE LEVANTE**		**ROTA**	
	PAM10	**PAM11**	**PAM12**	**PAM16**	**PAM17**	**VLE16**	**VLE17**	**ROT11**	**ROT16**
PAM10	0.000								
PAM11	-0.048	0.000							
PAM12	-0.044	0.005	0.000						
PAM16	-0.043	0.031	0.067*	0.000					
PAM17	0.508**	0.220**	0.284***	0.362**	0.000				
VLE16	0.297**	0.306***	0.205***	0.443***	0.755***	0.000			
VLE17	0.126	0.168***	0.091**	0.283***	0.554***	0.000	0.000		
ROT11	-0.182	-0.187	-0.219	0.044	0.748	0.075	-0.172	0.000	
ROT16	-0.097	-0.001	-0.011	0.001	0.416**	0.278***	0.135*	-0.118	0.000

Table S3. AMOVA tests. Results of the AMOVA tests comparing variation in mitochondrial sequences of *C. scaura* from Cadiz Bay, grouped by sample marina population. Significance at $p < 0.05$(*) and $p < 0.001$ (***). Statistical probabilities derived from 16,000 permutations.

Group	**Source of variation**	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Locations (marinas)	Among groups	2	83.713	0.591	12.378 ($F_{CT} = 0.124$***)
	Among populations within groups	6	60.806	0.249	5.205 ($F_{SC} = 0.059$***)
	Within populations	264	1039.070	3.936	82.417 ($F_{ST} = 0.179$***)
	Total	272	1183.590	4.776	

Table S4. Pairwise FST values between populations of Caprella scaura worldwide, based on mtDNA COI sequences. Bold numbers indicate statistical significant ($p < 0.05$). Values highlighted in grey represent small levels of differentation (<0.25) between Cadiz Bay and the others worlwide populations.

	NORTHEASTERN ATLANTIC SEA																		MEDITERRANEAN SEA														NORTH SEA	WESTERN ATLANTIC SEA								PACIFIC OCEAN			INDIAN OCEAN
	Cadiz Bay									Portugal and Southern Spain								Canary Islands	Eastern Spain and Marroco							Balearic Islands		Central Mediterranean Sea				Eastern Med.		South America - Brazil					North America - EEUU			Sea of Japan	Australia	Hawaii	
	PAM10	**PAM11**	**PAM12**	**PAM16**	**PAM17**	**VLE16**	**VLE17**	**ROT11**	**ROT16**	**CSCAS**	**CSSIN**	**CSALB**	**CSFAR**	**CSICA**	**CSCHP**	**CSCON**	**CSBAB**	**CSTEN**	**CSALM**	**CSTOR**	**CSALI**	**CSVAL**	**CSBAR**	**CSLET**	**CSMAR**	**CSBAL**	**MAH**	**PAL**	**CSPTO**	**COR**	**CIV**	**CSGRE**	**NL**	**CSBRA**	**SEB**	**ARA**	**ARC**	**NIT**	**CSCHA**	**FLO**	**TEX**	**CSJAP**	**CSAUS**	**CSHON**	**CSMAU**
PAM10	0.000
PAM11	-0.045	0.000
PAM12	-0.044	0.005	0.000
PAM16	-0.059	0.013	0.051	0.000
PAM17	**0.524**	**0.230**	**0.296**	**0.370**	0.000
VLE16	**0.265**	**0.292**	**0.191**	**0.423**	**0.751**	0.000
VLE17	0.097	**0.152**	**0.076**	**0.252**	**0.551**	0.000	0.000
ROT11	-0.193	-0.200	-0.228	0.023	0.748	0.072	-0.173	0.000
ROT16	-0.098	-0.001	-0.013	-0.012	**0.428**	**0.246**	**0.103**	-0.139	0.000
CSCAS	0.098	0.207	0.048	0.440	1.000	-0.556	-0.406	-1.000	0.148	0.000
CSSIN	**0.684**	**0.483**	**0.368**	**0.713**	**1.000**	**0.200**	**0.229**	0.776	**0.557**	0.000	0.000
CSALB	**0.490**	**0.419**	**0.319**	**0.606**	**1.000**	0.076	0.127	0.474	**0.428**	0.000	0.000	0.000
CSFAR	**0.684**	**0.483**	**0.368**	**0.713**	**1.000**	**0.200**	**0.229**	0.776	**0.557**	0.000	0.000	0.000	0.000
CSICA	0.129	0.030	0.095	0.130	0.183	**0.490**	**0.287**	-0.158	0.147	0.500	**0.807**	**0.685**	**0.807**	0.000
CSCHP	**0.253**	0.103	**0.181**	0.195	0.043	**0.607**	**0.403**	0.156	**0.247**	0.750	**0.906**	**0.838**	**0.906**	-0.076	0.000
CSCON	**0.333**	0.162	**0.248**	0.248	0.000	**0.678**	**0.474**	0.474	**0.312**	1.000	**1.000**	**1.000**	**1.000**	0.034	-0.078	0.000
CSBAB	-0.193	-0.200	-0.228	0.023	0.748	0.072	-0.173	-1.000	-0.139	-1.000	0.776	0.474	0.776	-0.158	0.156	0.474	0.000
CSTEN	0.169	0.070	**0.150**	0.143	0.139	**0.561**	**0.356**	0.003	0.191	0.634	**0.897**	**0.799**	**0.897**	-0.092	-0.105	-0.034	0.003	0.000
CSALM	0.684	**0.483**	**0.368**	**0.713**	**1.000**	**0.200**	**0.229**	0.776	**0.557**	0.000	0.000	0.000	0.000	**0.807**	**0.906**	**1.000**	0.776	**0.897**	0.000
CSTOR	0.085	-0.004	0.071	0.093	0.207	**0.493**	**0.275**	-0.215	0.117	0.500	**0.876**	**0.750**	**0.876**	-0.182	-0.146	0.000	-0.215	-0.160	**0.876**	0.000
CSALI	0.072	0.033	0.073	0.120	**0.270**	**0.378**	**0.205**	-0.254	0.106	0.211	**0.667**	**0.508**	**0.667**	-0.040	0.044	0.118	-0.254	-0.045	**0.667**	-0.087	0.000
CSVAL	0.059	0.005	0.043	0.110	**0.270**	**0.368**	**0.185**	-0.314	0.088	0.219	**0.671**	**0.512**	**0.671**	-0.078	0.020	0.118	-0.314	-0.028	**0.671**	-0.123	-0.060	0.000
CSBAR	**0.381**	**0.328**	**0.232**	**0.509**	**0.810**	0.050	0.067	0.064	**0.342**	-0.836	0.082	-0.034	0.082	**0.520**	**0.654**	**0.742**	0.064	**0.608**	0.082	**0.536**	**0.391**	0.381	0.000
CSLET	0.230	0.104	0.204	0.177	0.000	**0.646**	**0.435**	0.250	0.247	1.000	**1.000**	**1.000**	**1.000**	-0.068	-0.180	0.000	0.250	-0.154	**1.000**	-0.132	0.023	0.023	**0.712**	0.000
CSMAR	**0.684**	**0.483**	**0.368**	**0.713**	**1.000**	**0.200**	**0.229**	0.776	**0.557**	0.000	0.000	0.000	0.000	**0.807**	**0.906**	**1.000**	0.776	**0.897**	0.000	**0.876**	**0.667**	**0.671**	0.082	**1.000**	0.000
CSBAL	0.112	**0.102**	**0.109**	**0.198**	**0.446**	**0.329**	**0.178**	-0.208	**0.142**	0.109	**0.620**	**0.451**	**0.620**	0.099	0.212	0.287	-0.208	0.090	**0.620**	0.060	-0.008	0.033	**0.336**	0.200	**0.620**	0.000
MAH	0.064	0.003	0.046	0.109	0.275	**0.388**	**0.197**	-0.304	0.092	0.268	**0.710**	**0.551**	**0.710**	-0.092	0.006	0.114	-0.304	-0.062	**0.710**	-0.138	-0.091	-0.100	**0.403**	0.015	**0.710**	0.003	0.000
PAL	0.130	0.035	**0.087**	**0.142**	0.196	**0.453**	**0.264**	-0.197	**0.145**	0.429	**0.714**	**0.603**	**0.714**	-0.092	-0.020	0.078	-0.197	-0.041	**0.714**	-0.140	-0.027	-0.069	**0.467**	-0.006	**0.714**	0.099	-0.080	0.000
CSPTO	-0.305	0.125	0.023	0.067	1.000	0.269	0.069	-0.238	-0.182	1.000	1.000	1.000	1.000	0.554	0.752	1.000	-0.238	0.634	1.000	0.544	0.318	0.357	0.579	1.000	1.000	0.292	0.391	0.514	0.000
COR	0.188	**0.090**	**0.148**	**0.183**	**0.169**	**0.507**	**0.329**	-0.073	**0.203**	0.491	**0.745**	**0.642**	**0.745**	-0.048	-0.009	0.054	-0.073	-0.019	**0.745**	-0.100	0.028	-0.008	**0.523**	-0.032	**0.745**	**0.163**	-0.016	-0.018	0.546	0.000
CIV	0.093	**0.213**	**0.192**	**0.175**	**0.629**	**0.384**	**0.267**	0.188	**0.127**	0.362	**0.699**	**0.572**	**0.699**	**0.383**	**0.467**	**0.510**	0.188	**0.394**	**0.699**	**0.358**	**0.294**	**0.289**	**0.505**	**0.453**	**0.699**	**0.277**	**0.318**	**0.383**	-0.188	0.418	0.000
CSGRE	0.127	**0.183**	**0.141**	**0.271**	**0.642**	**0.205**	0.108	-0.133	**0.156**	-0.147	**0.547**	**0.342**	**0.547**	**0.281**	**0.414**	**0.483**	-0.133	**0.292**	**0.547**	0.247	0.126	0.171	**0.241**	0.400	**0.547**	-0.041	0.155	**0.272**	0.118	**0.328**	**0.240**	0.000
NL	0.116	**0.271**	**0.200**	**0.286**	**0.838**	**0.256**	**0.177**	0.217	0.139	0.232	**0.765**	**0.585**	**0.765**	**0.519**	**0.643**	**0.724**	0.217	**0.551**	**0.765**	**0.505**	**0.367**	**0.400**	**0.455**	**0.660**	**0.765**	**0.280**	**0.408**	**0.497**	-0.600	**0.537**	0.135	0.117	0.000
CSBRA	-0.305	0.125	0.023	0.067	1.000	0.269	0.069	-0.238	-0.182	1.000	1.000	1.000	1.000	0.554	0.752	1.000	-0.238	0.634	1.000	0.544	0.318	0.357	0.579	1.000	1.000	0.292	0.391	0.514	0.000	0.546	-0.188	0.118	-0.600	0.000
SEB	**0.556**	**0.416**	**0.407**	**0.537**	**0.855**	**0.683**	**0.530**	**0.643**	**0.501**	0.853	**0.929**	**0.891**	**0.929**	**0.599**	**0.688**	**0.797**	**0.643**	**0.629**	**0.929**	**0.632**	**0.465**	**0.469**	**0.747**	**0.772**	**0.929**	**0.484**	**0.526**	**0.552**	0.846	**0.568**	**0.400**	**0.552**	**0.719**	0.846	0.000
ARA	**0.426**	**0.351**	**0.343**	**0.450**	**0.736**	**0.579**	**0.434**	0.411	**0.405**	0.668	**0.839**	**0.762**	**0.839**	**0.475**	**0.565**	**0.644**	0.411	**0.478**	**0.839**	**0.479**	**0.337**	**0.348**	**0.636**	**0.602**	**0.839**	**0.335**	**0.392**	**0.451**	0.676	**0.479**	**0.303**	**0.387**	**0.576**	0.676	0.008	0.000
ARC	**0.323**	**0.306**	**0.285**	**0.396**	**0.660**	**0.429**	**0.311**	0.195	**0.325**	0.387	**0.693**	**0.577**	**0.693**	**0.400**	**0.495**	**0.558**	0.195	**0.390**	**0.693**	**0.386**	**0.241**	**0.278**	**0.471**	**0.509**	**0.693**	**0.181**	**0.294**	**0.386**	0.484	**0.429**	**0.271**	**0.174**	**0.400**	0.484	**0.253**	0.064	0.000
NIT	**0.234**	**0.253**	**0.239**	**0.331**	**0.677**	**0.398**	**0.267**	0.072	**0.257**	0.279	**0.733**	**0.570**	**0.733**	**0.339**	**0.455**	**0.526**	0.072	**0.326**	**0.733**	**0.310**	**0.173**	**0.206**	**0.454**	**0.448**	**0.733**	0.127	**0.225**	**0.331**	0.359	**0.376**	**0.200**	0.125	**0.338**	0.359	**0.238**	0.038	-0.066	0.000
CSCHA	0.086	0.147	0.168	0.207	**0.637**	**0.422**	**0.253**	-0.253	0.162	-0.100	**0.815**	**0.594**	**0.815**	0.155	0.290	**0.365**	-0.253	0.131	**0.815**	0.074	0.037	0.038	**0.479**	0.185	**0.815**	0.063	0.064	0.175	-0.048	0.218	0.170	0.122	0.321	-0.048	**0.322**	0.147	0.102	0.005	0.000
FLO	-0.203	-0.296	-0.093	-0.200	0.000	0.562	0.270	-1.000	-0.067	1.000	1.000	1.000	1.000	-0.750	-1.000	0.000	-1.000	-1.000	1.000	-1.000	-0.547	-0.546	0.644	0.000	1.000	-0.201	-0.577	-0.571	1.000	-0.627	0.258	0.118	0.495	1	0.720	0.488	0.355	0.198	-0.630	0.000
TEX	0.295	0.386	0.322	0.520	1.000	0.374	0.227	-0.238	0.353	1.000	1.000	1.000	1.000	0.594	0.782	1.000	-0.238	0.644	1.000	0.587	0.282	0.389	0.478	1.000	1.000	-0.076	0.391	0.551	1.000	0.587	0.375	-0.506	0.200	1	0.839	0.620	0.231	0.151	-0.073	1.000	0.000
CSJAP	-0.203	-0.295	-0.093	-0.200	0.000	0.562	0.270	-1.000	-0.067	1.000	1.000	1.000	1.000	-0.750	-1.000	0.000	-1.000	-1.000	1.000	-1.000	-0.547	-0.546	0.644	0.000	1.000	-0.201	-0.577	-0.571	1.000	-0.627	0.258	0.118	0.495	1.000	0.720	0.488	0.355	0.198	-0.630	0.000	1.000	0.000
CSAUS	**0.529**	**0.448**	**0.382**	**0.591**	**0.875**	**0.444**	**0.353**	**0.509**	**0.478**	0.549	**0.774**	**0.679**	**0.774**	**0.656**	**0.752**	**0.827**	**0.509**	**0.686**	**0.774**	**0.679**	**0.467**	**0.541**	**0.497**	**0.807**	**0.774**	**0.347**	**0.536**	**0.608**	0.721	**0.644**	**0.533**	**0.201**	**0.482**	0.721	**0.752**	**0.597**	**0.305**	**0.338**	**0.532**	0.767	0.199	0.767	0.000
CSHON	0.132	**0.264**	**0.197**	**0.270**	**0.737**	**0.278**	**0.200**	0.269	**0.143**	0.311	**0.655**	**0.531**	**0.655**	**0.499**	**0.593**	**0.650**	0.269	**0.523**	**0.655**	**0.492**	**0.378**	**0.403**	**0.447**	**0.611**	**0.655**	**0.333**	**0.409**	**0.480**	-0.472	**0.521**	**0.157**	**0.217**	-0.063	-0.472	**0.642**	**0.528**	**0.384**	**0.330**	**0.374**	0.504	0.375	0.504	**0.447**	0.000
CSMAU	0.186	**0.215**	**0.237**	**0.267**	**0.696**	**0.496**	**0.331**	0.010	0.236	0.333	**0.861**	**0.706**	**0.861**	**0.261**	**0.383**	**0.479**	0.010	0.239	**0.861**	0.209	0.127	0.136	**0.566**	0.345	**0.861**	0.161	0.175	**0.270**	0.307	**0.300**	0.171	**0.223**	**0.412**	0.307	0.144	0.022	0.084	-0.004	-0.122	-0.156	0.307	-0.156	**0.587**	**0.423**	0.000

4

COMMUNITY PARTICIPATION AND AWARENESS

Social-perception of marine bioinvasions associated to recreational boating: first insights from Spanish stakeholders

Adapted from: Martínez-Laiz G, Leonard K, Hewitt C, Guerra-García JM, Navarro-Barranco C, García-Garrido S, Campbell M. Social-perception of marine invasions associated to recreational boating: first insights from Spanish stakeholders. In preparation

ABSTRACT. Perceptions of stakeholders are one pilar of environmental policy-making, involving aspects like awareness, risk perception and, ultimately, engagement and compliance of citizens. Spain represents a well-known hotspot for marine exotic species introductions in Europe, and there is scientific evidence proving the role of recreational boating as a vector in the Mediterranean Sea. The International Maritime Organization is already taking action for a future regulation of the hull-fouling vector; however, perception of stakeholders in our country have not been explored so far. This is the first research to approach the issue in Spain. Surveys were undertaken to identify public opinions, knowledge and beliefs around exotic marine species and management of the recreational boating vector. A comparison with consistent methodology was carried out with the only two countries that so far have implemented measures at National level: Australia and New Zealand. Risk perception of marine exotic species and their vectors in Spain was significantly lower than in the other two countries; and was significantly associated to the perceived responsibility of good vessel maintenance practices. Baseline knowledge, connection feeling with the environment, and risk perception were the main factors influencing attitudes towards NIS management, and were interconnected among themselves in the three countries. In the light of our initial results, we provide some preliminary recommendations to better orientate outreach for raising awareness; and discuss the next steps to follow in order to better map our stakeholders perception on this environmental problem. With this study we also hope to promote dialogue among the scientific community and policy makers around marine biosecurity decision-making; and start creating valuable information for a future fair and effective implementation of the biofouling vector in our country

1. INTRODUCTION

As explained before in the introduction and chapter 2 - part 1, the human dimension of marine bioinvasions is of crucial importance. Vessel maintenance practices, travel itineraries, marina guidelines, as well as public awareness can shape the risk of spreading marine NIS via recreational boating (Floerl et al., 2005a,b; Floerl et al., 2009; Acosta et al., 2010; Clarke Murray et al., 2013; Jurk, 2011; Zabin et al., 2014; Ferrario et al., 2016; Martínez-Laiz et al. 2019; Ulman et al. 2019). Nevertheless, marine biosecurity regulations for the biofouling vector are still in its infancy in most countries and institutions except for two nationwide enforcement examples: New Zealand and Australia. Globally, these countries stand out as early-adopter of mandatory biofouling regulations for both commercial and recreational vessels. Their National Standards are in continuous revision and improvement (https://www.agriculture.gov.au/biosecurity/avm/vessels/marine-pest-biosecurity/biofouling/anti-fouling-and-inwater-cleaning-guidelines/in-water-cleaning-standard ; Scianni and Georgiades, 2019), and they represent a blue-print for other international jurisdictions (Georgiades et al. 2018).

Institutions who took a step forward in biofouling regulation agree on certain essential principles to serve marine biosecurity, and public awareness and stakeholders' involvement is one of them (Seaward et al., 2015; https://www.mpi.govt.nz/biosecurity/how-to-find-report-and-prevent-pests-and-diseases/tips-for-finding-pests/ ; McAllister et al., 2020; https://www.qld.gov.au/environment/coasts-waterways/marine-pests; GloFouling, 2021). It is also considered one main axis clustering potential future scenarios regarding biological invasions (Roura-pascual et al., 2021); and is key to bridging the gap among scientists and policy makers around marine biosecurity. Arroz et al. (2016) explain that this does not mean "forcing the former to value science according to the same criteria as experts. The aim, instead, is that by combining different agendas and interests, personal and significant reasons will emerge and allow people to understand and value the positive role of science in dealing with everyday life situations". Indeed, insufficient appreciation of socio-ecological context and values system of the different actors, non-existent or limited community engagement, and unidirectional communications can notably foster destructive conflicts (*i.e.* undesirable environmental, social and economic outcomes) to arise from invasive species management (see cases in Estévez et al., 2015 and Crowley et al., 2017). Moreover, emotional factors of stakeholders can facilitate public engagement in protection of native species (Arroz et al. 2016); but also hamper eradication strategies of invasive species (Carballo-Cárdenas et al., 2015). Perception and level of concern are also important tools to inform biosecurity risks assessments of future NIS incursions (Cliff and Campbell, 2012), as well as predicting citizen behavioural intentions in these situations; for example, informing policy makers on the potential opposition or compliance of stakeholders (Diedrich et al., 2013; Drake et al., 2015; Nunes et al., 2015; Cerri et al., 2016). Investigating the inner perceptions of stakeholders as well as their interests not only favour the uptake of management actions; but is vital to build effective environmental education strategies. This starts by improving the flow of information between scientists and the local community via, for instance, outreach campaigns and citizen science initiatives that, in turn, benefice the community by "informing, consulting, involving, collaborating and empowering" (IAP2, 2007; Crall et al. 2010; Dickinson et al. 2010, Piola and McDonald 2012). It needs to be noticed though, that the last one requires considerable training effort; therefore, discrepancy exists on to what extent devolved responsibility to biosecure citizens is feasible (Campbel et al. 2017). In any case, a good level of public awareness and environmental literacy of stakeholders represents a valuable resource for conservation (Vasconcelos et al. 2009, Rowe and Frewer, 2005); as it translates into better support and engagement of management actions (Bremmer and Park 2007; Hourston et al. 2015; Cole et al., 2016).

As stated before, the Mediterranean Sea stands out as the most invaded sea worldwide (Edelist et al., 2013). Here, the Strait of Gibraltar (Andalucia region, Southern Spain) represents one of the two main high-risk pathways for exotic species and introduction events; connecting areas like Europe, West Africa, the Caribbean and Australia, with more than 35 millions of passengers/year (www. Puertos.es, accessed 25 August 2021; marine operators, personal communication). So far, the human-dimension of this problem in the Mediterranean Sea has only been addressed in the studies by Ferrario et al. (2016), Martínez-Laiz et al. (2019) and Ulman et al. (2019). Their research focused on the relation between boat owners' behaviour (*i.e.* hull cleaning and painting schedule, frequency of travel, destination history, etc.) and the probability/risk of carrying and/or spreading NIS via hull-fouling. Boaters' awareness in terms of self-declared knowledge was briefly targeted here as well. Ferrario et al., (2016) conducted 208 interviews and indicated that 66% of Italian respondents had heard about marine NIS; but another 24% had misconceptions. When testing similar questions in France, Italy, Greece and Turkey, Martínez-laiz et al. (2019) indicated a smaller self-declared awareness (53%); and outlined that only 29% of the total boaters interviewed were able to name correct examples (N=247). Both authors also noted that numerous boaters were unaware of the role recreational boating plays as a vector, and rather think that the fouling organisms underneath their craft are "something natural" and/or "harmless" (especially for smaller taxa). Some of the respondents even considered their hulls to be perfectly clean, despite the presence of some biofouling. Apart from this material, we do not count yet with a comprehensive social-perception investigation that precisely reflects stakeholder's views regarding the transfer of marine NIS in a Mediterranean country; and no information whatsoever is available for Spain.

This is the first study exploring social perception of Spanish stakeholders' about marine bioinvasions associated to hull-fouling; and the first to conduct a comparison with consistent methodology with the two countries leading marine biosecurity research and management efforts worldwide: Australia and New Zealand. Our objectives are 1) identify, start to understand and highlight trends in social perception of Spanish stakeholders regarding marine NIS and their regulation; 2) evaluate if boater's perceptions vary between the countries with implemented marine biosecurity at National Level and ours; and 3) provide preliminary practical recommendations on how to increase stakeholder's awareness in our country. By highlighting these perceptions and their influences we hope to better orientate public awareness and outreach regarding the issue of marine NIS; as well as to contribute to set baselines for a future fair and effective implementation of biofouling management for the recreational boating vector.

2. MATERIAL AND METHODS

2.1 Study areas and selection of sampling size

Three regions (one in each country) were selected to represent our target population based on the following criteria: representing a relevant maritime hotspot for recreational boating at national level, in terms of number of marinas and density of international maritime routes; being geographically located in the same climate zone (Temperate – Mediterranean and Oceanic *sensu* Köppen climate classification); and bearing demographic profiles as similar as possible within our target population. The selected regions were Western Australia, the North Island, and Andalucía, respectively. In Australia, the Western region shares climate zone with the North Island and Andalucía; represents one of the main hubs for recreational boating in the country and a very suitable area to study trends of local boaters; plus their community notably performs trips at national scale, covering a high diversity of destinations all over Australia (http://data.daff.gov.au/data/warehouse/9aai/2018/RecBoatSelfMgtBiofouling/RecBoatOperators_SelfMgtBiofouling_v1.0.0.pdf). In New Zealand, the North Island concentrates the highest number of marinas and the highest density of international shipping routes. Tauranga and Auckland are the two main hubs concentrating local boaters in this Island, and we find here the largest marina in the Southern Hemisphere in terms of surface and berths (Westhaven marina, Auckland). In Spain, Andalucía represents a key region at national level for the recreational boating sector, in terms of economical profit and % of employment; plus it bears the highest number of marinas and second highest number of berths/marina of the Spanish Iberian Peninsula (Anen, 2017). The sampled size required to represent each country region was calculated with Survey Software (https://www.surveysystem.com/sscalc.htm), according to the total population inhabiting each region, to assure a robust sample with a confidence level of 95%. This resulted in a minimum of 384 surveys needed in each country region. Once the target numbers are decided, these need to be obtained from a stratified sampling that ensures a suitable representation of our target group. Following Munro et al. (2017), stakeholders in marine and coastal environments are those individuals, groups, or organization interested, involved or affected by a given project or action towards resource use. In our case, we targeted three population groups when studying perception of marine bioinvasions associated to recreational boating: boat owners, marina managers, and general public that make a recreational use of the marine environment (beach goers). As we are interested in gathering the perceptions of local stakeholders, the sampling frame targeted residents only. Accordingly, we selected sampling areas inside each region that met these criteria and, across these areas, we selected high-occupancy locations, frequented by our stakeholders, as sampling points (Figure 1; Table 1). These included marinas, beaches and beach surroundings like green strands or quays.

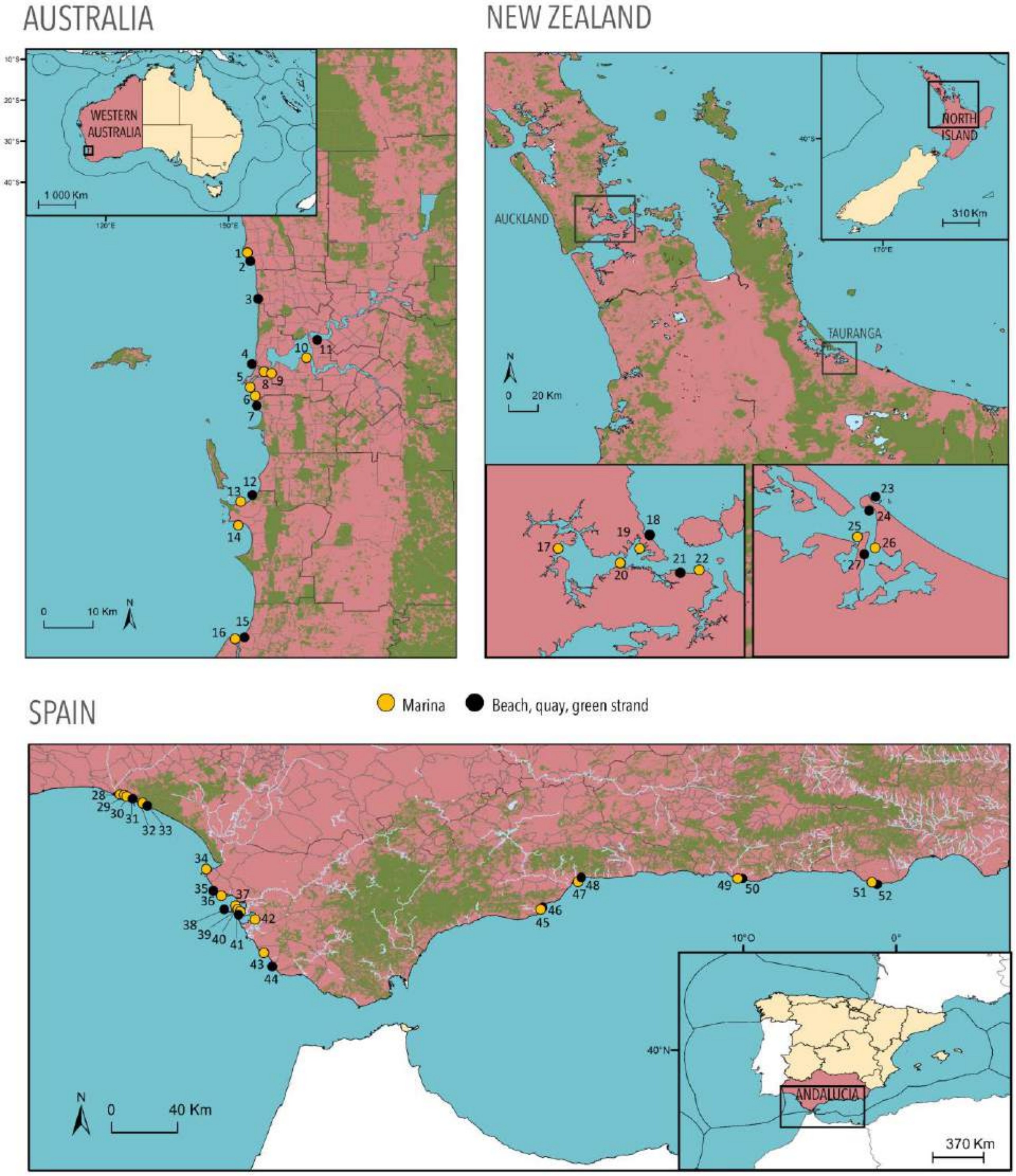


Figure 1. Locations or sampling points for the three regions (in red) selected for each country (light yellow). **Western Australia:** Hillarys yacht club (1), Sorrento Quay (2) and Scarborough Beach (3) in Hillarys area; Fremantle Leighton Beach (4), Royal Perth Yacht Club (5), Fremantle Sailing club (6) and South Beach (7) in Fremantle area; Swan yacht Club (8), East Fremantle Sailing Club (6), South of Perth Yacht Club (10) and South Perth Foreshore (11) in Swan River - Perth; Rockingham Beach (12), The cruising yacht club of Western Australia (13) and Safety Yacht Club (14) in Rockingham; and Mandurah Offshore Sailing Club (15) and Mandurah Beach (16) in Mandurah. **North Island:** Hobsonville marina (17), Takapuna beach (18), Bayswater marina (19), Westhaven marina (20), Mission Bay (21) and Saint Heliers Beach (12) in Auckland; and Mount Manganoui Beach (23), Pilot Bay (24), Tauranga marina (25), Tauranga Bridge marina (26) and The city center Strand (27) in Tauranga. **Andalucía**: El Rompido Puerto Marina (28), Club Naútico Río Piedras (29), Marina Nuevo Portil (30), El Portil Beach (31), Club naútico Punta Umbría (32) and Punta Umbría Beach (33) in Huelva; Puerto Deportivo Chipiona (34), Puerto de Rota (35), Rota Beach (36), Puerto América (37), La Caleta Beach (38), Viento de Levante (39), Puerto de ElCano (40), La Victoria Beach (41), Puente de Hierro-San Fernando (42), Club naútico Sancti Petri (43) and Conil Beach (44) in Cádiz; Puerto Deportivo Fuengirola (45), Fuengirola Beach (46), Real Club Naútico Mediterráneo (47) and La Malagueta Beach (48) in Málaga; Real Club Naútico Motril (49) and Motril Beach (50) in Granada; and Puerto Deportivo Almerimar (51) and Almerimar Beach (52) in Almeria. More details are indicated in Table S1.

2.2 Questionnaire survey

Each face-to-face survey took approximately 7 minutes to complete and consisted on 22 questions divided into five main sections: 1. About you (use of the marine environment and connection feeling); 2. Attitudes towards exotic marine species (knowledge, risk perception, awareness of local NIS and perception of impacts); 3. Attitudes towards regulations of marine NIS (vessel maintenance practices and opinions towards management); 4. Communicating about marine NIS (preferred sources of information); and 5. Demographics (place of residence, age, gender, level of education and job sector). The surveys were designed following the validity and reliability guidelines by Thayer-Hart et al. (2010). A previous field study was conducted to test and improve questions; and all surveys were submitted for Human Ethics evaluation and approval at the correspondent institutions. Surveys for each population group and country are compiled in Supplementary material S2-S4; and further details about the survey process in the field, including control for biases, are described in S5. In order to control for data quality, data screening and cleaning were carried out after completion of sampling. During these processes, we deleted surveys from respondents who a) did not meet our target criteria (usually residency or minimum age); b) respondents who either showed clear signs of being under the effect of alcohol, or a clear careless/dishonest attitude during the interview process (speeding throughout the survey, giving inconsistent responses repetitively etc.); and c) respondents who only answered 50% or less of the questions. Answers given in the “other” category were examined and, when possible, pooled into one of the categories. In section 4, two extra categories, “government” and “education” were created *a posteriori* to encompass respondent’s views. In section 3, trailer boats were not taken into account for gathering information about maintenance practices.

2.3 Statistical methods

Variables considered in the present study are compiled in Table 2. Descriptive statistics were used to explore the demographic profiles of our target groups; their use of the marine environment in terms of entertaining activities, and the most popular maintenance vessel practices among boaters. We used likert scales to explore respondent’s connection with the marine environment; to have a look at their perceptions towards regulations in the three countries; and to examine how they perceive multiple impacts caused by marine NIS. These included loss of biodiversity and natural habitats, local extinction of native species, economic loss of fisheries and marine farming/aquaculture, interference with recreational activities, food poisoning by ingesting toxic species, human exposure to injury (biotoxins, stings, bites etc.), loss of aesthetic value of coastal landscapes, and loss of historical/cultural value of an area. Factor analysis was further used to evaluate underlying trends in these impacts’ perceptions. Chi-square tests of independence and Fisher’s Exact analysis were used to explore differences in perceptions of the three countries and population groups; to examine whether demographics influence respondent’s perceptions; and whether there is a relation between knowledge of marine NIS, connection feeling, risk perception, and opinions towards regulations. When exploring regulation views of Australia and New Zealand, their data were pooled together whenever there were not significant differences between the two countries. Besides, 5-level likert scale data was converted into 3-level likert scale data whenever was convenient to meet the minimum count of N=5 in each cell for

Chi-square test; or in order to explore trends at different resolution scales. When this conversion was carried out, categories were grouped together as follows: "strongly agree"/"very high" and "agree"/"high" were grouped together; "neutral" or "fair" reminded untouched; and "strongly disagree"/"very low" and "disagree"/"low" were grouped together. Open-ended questions were filtered and/or thematically coded and visualized through cloud words. Statistical analyses were carried out using R studio version 295 4.0.2 (R Core Team, 2020) and IBM SPSS Statistics 26.

Table 2. List of variables obtained from the interviews and used for the present study (note that only data from certain questions was used, as this study is still in preparation). The columns "type of variable" and "levels or items" indicate how these variables were coded for statistical analysis, when convenient. ME= marine environment; VMP= vessel maintenance practices. NOM= Nominal; ORD= Ordinal ; DIC= Dichotomous ; INT= Interval ; Y/N= Yes/No question, LIKERT= Likert scale; MULT= Multiple choice; OPEN= Open-ended question

Variables	Question (survey section)	Type of question	Type of variable	Levels or items
Use of the ME	Which of the following marine (*i.e.* ocean or beach) activities do you participate in? (1)	MULT	NOM	Items in q1
Connection with the ME	"I feel a connection to the areas where I do marine activities" (1)	LIKERT	ORD	1="TOTALLY DISAGREE" 2="DISAGREE" 3="NEUTRAL" 4="AGREE" 5="TOTALLY AGREE"
Knowledge about marine NIS	Do you know what an exotic marine species is? Can you name any examples of exotic marine species? (2)	Y/N and OPEN	ORD	-NONE: answered "no" to q4 -SELF-ADMITTED BUT INACCURATE: answered "yes" to q4, but gave incorrect examples in q6 -ACCURATE: answered "yes" in q4 and gave correct examples in q6
Risk perception of marine NIS	Do you consider marine exotic species to be a relevant problem? (2)	Y/N	DIC	-Yes -No
Risk perception of their vectors	How do you think exotic marine species can spread? Click the one(s) you consider (2)	MULT	ORD	-NONE: no vector acknowledged -LOW: one vector acknowledged -MODERATE: 2 vectors acknowledged -HIGH: 3 vectors acknowledged -VERY HIGH: all vectors acknowledged
Perceived relevance of impacts	Please rate the following impacts of exotic marine species according to how relevant you consider them (2)	LIKERT	ORD	1="NOT RELEVANT" 2="A BIT RELEVANT" 3="UNSURE" 4="SOMEWHAT RELEVANT" 5="VERY RELEVANT"
Perceptions about management necessity	"Regulations for management of exotic marine species are necessary" (3)	LIKERT	ORD	1="TOTALLY DISAGREE" 2="DISAGREE"

				3="NEUTRAL" 4="AGREE" 5="TOTALLY AGREE"
Satisfaction with NIS management	"Exotic marine species in Australia/New Zealand are well managed" (3)	LIKERT	ORD	Same as above
Satisfaction with recreational boating guidelines	"Biofouling guidelines from the Australian /New Zealand government for recreational vessels are fair" (3)	LIKERT	ORD	Same as above
Awareness of responsibility	"Good boat maintenance helps to stop the spread of exotic marine species" (3)	LIKERT	ORD	Same as above
Position on a potential biofouling regulation in Spain	"A biofouling regulation for the recreational boating vector is necessary to reduce NIS spread" (3)	LIKERT	ORD	Same as above
Intention of getting more involved	"I intend to get more involved in preventing exotic marine species spread *(e.g.* by documenting myself, participating in campaigns, spreading the word, etc.) (3)	LIKERT	ORD	Same as above
Preference for concrete sources of risk communication	"Which source of information do you consider the most useful to get informed about exotic marine species?" (4)	MULT		-
VMP – frequency of dry-cleaning + antifouling	How frequently do you haul your craft out of the water to clean it /redo your anti-fouling coating?" (3)	LIKERT	ORD	-Less than once a y ear -Once a year -More than once a year
VMP – iw- cleaning use	How frequently do you undertake in-water cleaning of your hull? Do you conduct in-water cleaning of the hull? (3)	LIKERT and MULT	DIC	-Yes -No
VMP – iw-cleaning purpose	Do you conduct in-water cleaning of the hull? (3)	MULT	NOM	-Preventive -Reactive -Other/didn't specify
VMP – iw-cleaning site	Where do you conduct the in-water cleaning? (3)	MULT	NOM	-In the marina -In the Gulf/Bay -Offshore -Other
Country, region and location	-	-	NOM	Items indicated in Table 1
Age	What is your age? (5)	MULT	INT	-16-25 - 26-35 -36-45 -46-55 -56-65 -65 and over
Gender	What is your gender? (5)	MULT	DIC	-Male -Female
Education level	What is your highest level of education? (5)	MULT	ORD	-Primary -Secondary -Tertiary -Postgraduate

3. RESULTS

3.1 Demographic data

A total of 1306 surveys were obtained. In Australia, a total of 409 valid surveys were recovered (97%); in New Zealand a total of 408 (95%) and in Spain, 431 (95%). Response ratios were 83.5%, 88.5% and 89.3%, respectively. Respondents were not evenly distributed across locations (Figure 2) nor amongst the different categories of the demographic variables (Figure 3A). Sixty-five percent of respondents were male; in particular, the marine operators population group was exclusively composed by males except for one female in Andalucía (Spain). The majority of boat owners and marine operators respondents were in between 56-65 years old (30%); whereas the majority of general public respondents were in between 26-35 (20%), with the rest being more evenly distributed. The most prevalent education level was tertiary (49%) (Figure 3B). Respondents showed a very similar recreational use of the marine environment for all 3 countries, with swimming/sunbathing (67-83% of respondents), recreational boating (54-73%) and fishing (41-61%) being the most popular activities (Figure 4).

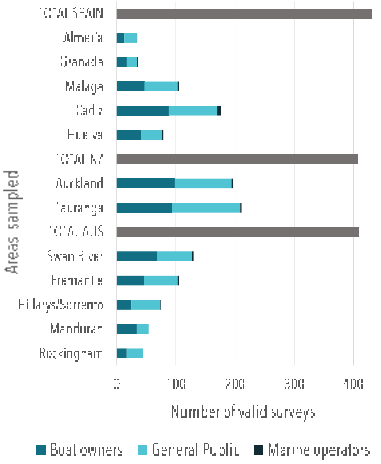
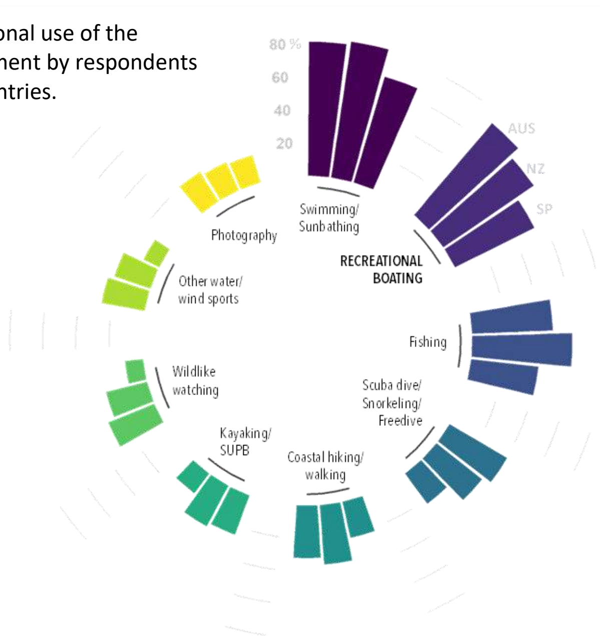


Figure 2. Distribution of valid surveys across the three countries and regions

Figure 3. (A) Sampled population pyramids for each group and country. BO+MO= boat owners plus marine operators; GP= general public. Total population in Spain in 2019 is included for reference; obtained from Instituto Nacional de Estadística (2019), https://www.ine.es/covid/piramides.htm . (B) Education level of respondents in the three countries

Figure 4. Recreational use of the marine environment by respondents in the three countries.



3.2 Awareness, risk perception, and perceived relevance of impacts

3.2.1 Knowledge on marine exotic species

In Spain, 84.2% of respondents declared self-admitted knowledge (*i.e.* they said they knew what marine NIS are). From these, only 27.17% were able to name at least one correct example of marine NIS (increasing to 36.42% if freshwater and terrestrial correct examples were also considered). There were significant differences among the three countries (χ^2=59.07, df=4; p<0.001) and population groups (χ^2=123.54, df=2; p<0.001; N=1182). Boaters and marine operators in New Zealand were the only ones showing significantly more accurate knowledge than all the other groups (p=0.000; stress=8.07). In Australia and New Zealand, general public showed a significant lack of knowledge (p<0.001, $stres_{AUS}$= 6.89, $stres_{NZ}$= 7.67); representing 35% and 32% of their population, respectively. In the case of Spain, general public was significantly overconfident (p<0.000; stress=6.26), but unable to give correct examples (p=0.004; stress= -3.67). The connection feeling with the marine environment was significantly related with the knowledge pattern (Fisher exact test; p= 0.005): people who strongly agreed with the statement “I feel a connection with the areas where I do marine activities” significantly tended to show accurate knowledge, in comparison to those who went neutral, who showed a lack of knowledge (Figure 6). The most frequent answers given in Australia for NIS examples were “Crown of thorns” (*Acanthaster planci* (Linnaeus, 1758)) (29% of respondents), followed by “Asian crab” (10%). In New Zealand, 41% of respondents named the “Fan worm” (*Sabella spallanzanii* (Gmelin, 1791)), followed by “Didymo” (*Didymosphenia geminate* (Lyngbye) M.Schmidt, 1899)) (12%). In Spain, 71% of respondents answered “Algae”; followed by “Portuguese man o’war” (*Physalia physalis* (Linnaeus, 1758)) (11%). Further details are shown in Figure 7. In Spain, the factor age turned out significant for marine NIS knowledge (χ^2=20.50; df=10; p=0.03; N=406): 31-35% of respondents aged between 46-65 years old showed accurate knowledge, while only 10% of the young respondents did. The factor education also reflected a significant association: respondents with primary education level usually did not know what marine NIS are (=0.001; stress=4.00).

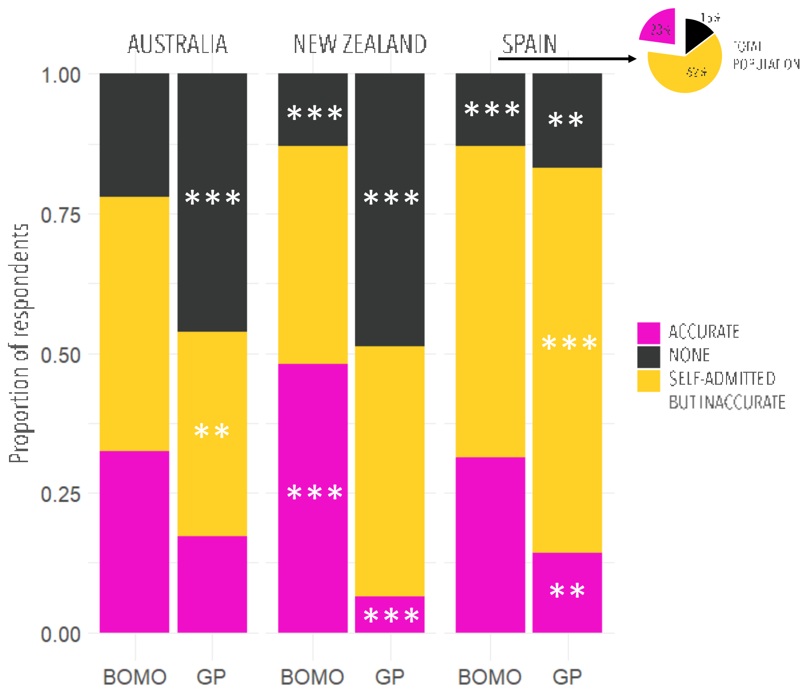


Figure 5. Knowledge about exotic marine species for each country and population group. BOMO = Boat owners and marine operators; GP = general public. Significance levels are indicated as *(p<0.05), **(p<0.01) and *** (p<0.001).

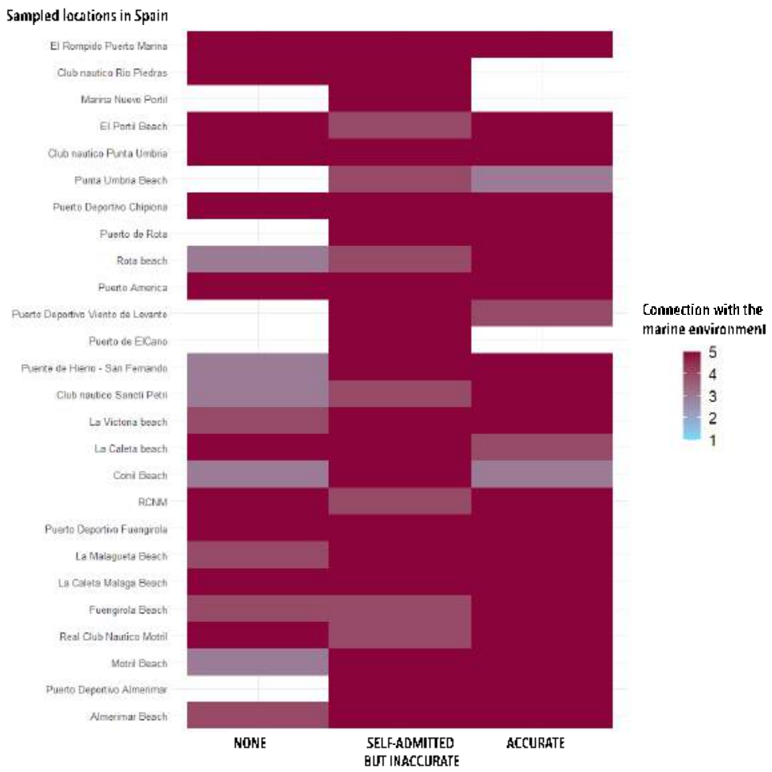


Figure 6. Tilemap showing knowledge level about marine exotic species of Spanish stakeholders, indicated per location sampled. Their declared connection with the marine environment is shown ranked from a 5-level likert scale, obtained from q3: "I feel a connection to the areas where I do marine activities. Note: tiles are of equal shape and do not inform on the % of population falling into each category

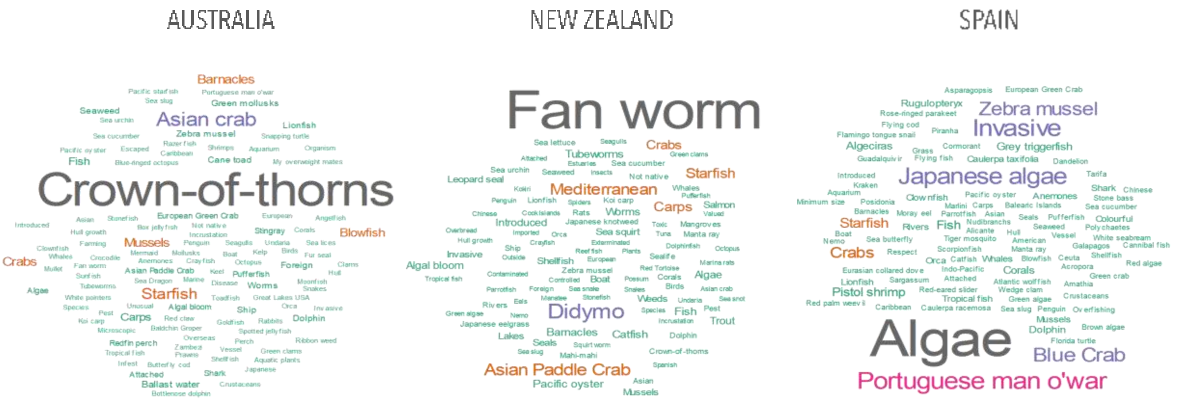


Figure 7. Word clouds representing respondent's answer to NIS examples. Font size indicates frequency of each answer; and similar frequencies appear with the same colour.

3.2.1 Risk perception of marine exotic species and their vectors

There were significant differences among countries when it comes to consider marine exotic species as a relevant problem or not (χ^2= 101.94; df=4; p<0.001; N=1200), mainly due to discrepancies between population groups (Figure 8). For boaters and marine operators, marine exotic species were a relevant problem (AUS: p=0.04, stress=3.04; NZ: p=0.0000; strest=7.58; SP: p=0.0001, strest=4.02); with the perception of new Zealanders being the clearest and strongest one (they rarely answered "no"; p=0.01, strest= -4.14; or "unsure"; p=0.000, strest=-5.26). Meanwhile, general public significantly tended to answer "unsure" (AUS: p=0.000, strest= 8.37; NZ: p=0.000, stress=4.7; SP: p=0.000; stress= 8.21). As a note, almost one third of Spanish general public think they're not a relevant problem; a significantly higher proportion than in the other two countries(p=0.000, strest=-4.4)

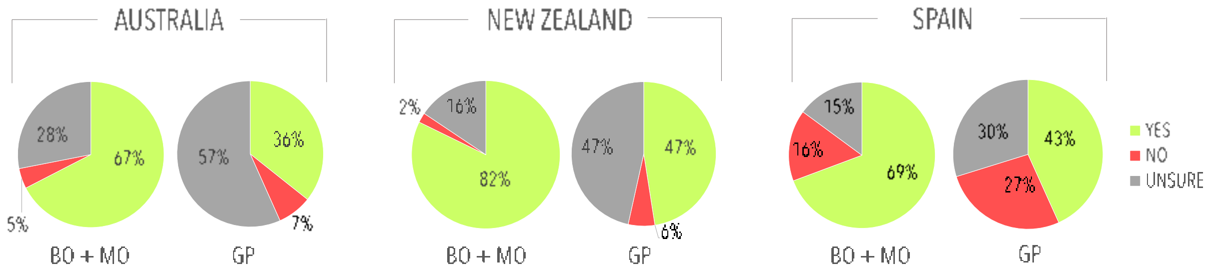


Figure 8. General risk perception of marine exotic species. Answers divided by country and population group to the question " Do you think exotic marine species are a relevant problem?". BO+MO= boat owners and marine operators; GP= general public

Regarding total risk perception of vectors, respondents showed a similar trend of thought, with clear differences between countries (χ^2=220,644, df=8; p<0.001; N=1243) and population groups (χ^2=40.599, df=4; p<0.001; N=1243) (Figure 9A). Australian and New Zealand stakeholders exhibited a very high risk perception of vectors (pAUS=0.000, stress= 4.36; Pnz=P=0.000, stress= 4.53); whereas Spanish respondents had a lower risk perception (p=0.000; stress=12); and rarely acknowledged more than one vector (p=0.000, stress: -7.25). In all three countries, boat owners and marine operators were rather associated to high risk perception (p=0.000, stress=3.90), and rarely showed low perception (p=0.000; stress=5); whereas general public exhibited the exact opposite pattern.

Furthermore, risk perception about vectors was significantly associated to knowledge level about marine NIS. In Australia, respondents who showed accurate knowledge also guaranteed a high to very high risk perception of vectors; whereas those who showed no knowledge or only self-admitted but inaccurate ranged from low to very high perception indistinguishably (Fishers' exact test, p=0.003; N=390). In New Zealand the gradation was even more clear (χ^2=56,53, df=8; p<0.0001; N=386). Respondents with accurate knowledge showed high risk perception (p=0.003, stress=3.719); and those with no knowledge showed either no risk perception (p=0.002, stress=3.80) or a fair risk perception (p=0.027, stress=3.11). Respondents with no knowledge of what marine NIS are, also were significantly incapable of reaching a very high risk perception (p<0.0001; stress=-4.54). In Spain, these variables were less related, but we still identified a significant trend when looking at only 3 levels of risk perception (low, fair and high) (χ^2=15,91, df=4; p<0.03; N=406): a low level of risk perception of vectors was negatively associated to accurate knowledge about NIS (p=0.003; stress=-3.61)

Figure 9. (A) Risk perception of vectors in each country and population group. The area surface of each group represents the % of risk perception attributed to that group. BO = boat owners; MO=marine operators; GP= general public. (B) Risk perception for each vector, indicated by country. BF= biofouling; BW= ballast water.

In Spain, female respondents showed a lack of risk perception for vectors (p=0.01; strest=3.27) in comparison to males (χ^2=43.671; df=4; p<0.001; N=431). Finally, there was no significant influence of age (χ^2= 13.782; df=8; p= 0.08) or of the education level of respondents (Fisher's exact test, p=0.31; N=419). The most acknowledged vector was, in all three countries, hull-fouling of commercial vessels (68 - 85% of awareness); followed by hull-fouling of recreational vessels in Australia and New Zealand (72.52% and 78.43%, respectively), and by ballast water in Spain (44%). Concretely, only 28.07% of Spanish stakeholders acknowledged recreational boating as a vector; in comparison with the other two countries (χ^2=264.92, df=2; p<0.001; N=1243). Aquaculture was notably the less acknowledged vector (21.81 - 40.09%) (Figure 9B).

3.2.1 Perceived relevance of impacts caused by marine exotic species

When asked to rate the relevance of multiple impacts of exotic marine species with a 5-level likert scale, Spanish respondents rated as the highest “Local extinction of native species” (4.44 ± 0.10; N=438), followed by Loss of biodiversity and natural habitats (4.22 ± 0.05) and Economic loss of fisheries and marine farming/aquaculture (4.01 ± 0.05) (Table 1?). For them, the less relevant impact was Interference with recreational activities, which was rated as neutral (3.17 ± 0.06).

The Kaiser-Mayer-Olkin (KMO) measure of sampling adequacy for factor analysis returned a value of 0.77, with values above 0.5 or 0.6 at the most conservative being considered acceptable (Field,2005). The scree plot test identified two eigenvalues greater than one, suggesting that two components could be extracted and rotated. These two components accounted for 98.18% of the total variance within the data. The first component was identified as food poisoning, exposure to injuries, and loss of historical/cultural value of an area. (Fig 10; Table 2). Impacts like local extinction of native species and loss of biodiversity and natural habitats loaded exclusively in component 2; and the underlying factor of this component also scored the highest (4.29 ± 0.04). Finally, economic impact on fisheries/aquaculture, interference with recreational activities; and loss of aesthetic value of the coasts loaded in both components. In NZ, 97.11% was represented; and respondents perceived all items as more relevant than in the other two countries. Component 1 included food poisoning and exposure to injuries (3.95 ± 0.04; N=408); component 2 was identical to Spain (4.4 ± 0.04; N=409); and the rest of items were spread across both components (3.07± 0.04; N=409). Finally, in Australia, the two components accounted for 97.36% of the total variance. This time, component 1 included loss of biodiversity and local extinction of native species (underlying factor scored 4.27 ± 0.04; N=412, and component 2 encompassed food poisoning, exposure to injuries and recreational activities (3.84 ± 0.04; N=413). Perceptions on economic profit and historical/cultural and aesthetic value grouped in both components (3.78 ± 0.04; N=413).

Table 1: Variables (impacts) loading on each component for a Principal Component analysis, based on answers from Spanish stakeholders

Impacts of marine exotic species	Component	
	1	2
Loss of historical/cultural value of an area	0.733	
Human exposure to injury	0.711	
Interference with recreational activities	0.702	0.302
Food poisoning by ingesting toxic species	0.701	
Loss of aesthetic value of coastal landscapes	0.671	0.354
Economic loss of fisheries and marine farming/aquaculture	0.645	0.315
Local extinction of native species		0.888
Loss of biodiversity and natural habitats		0.861

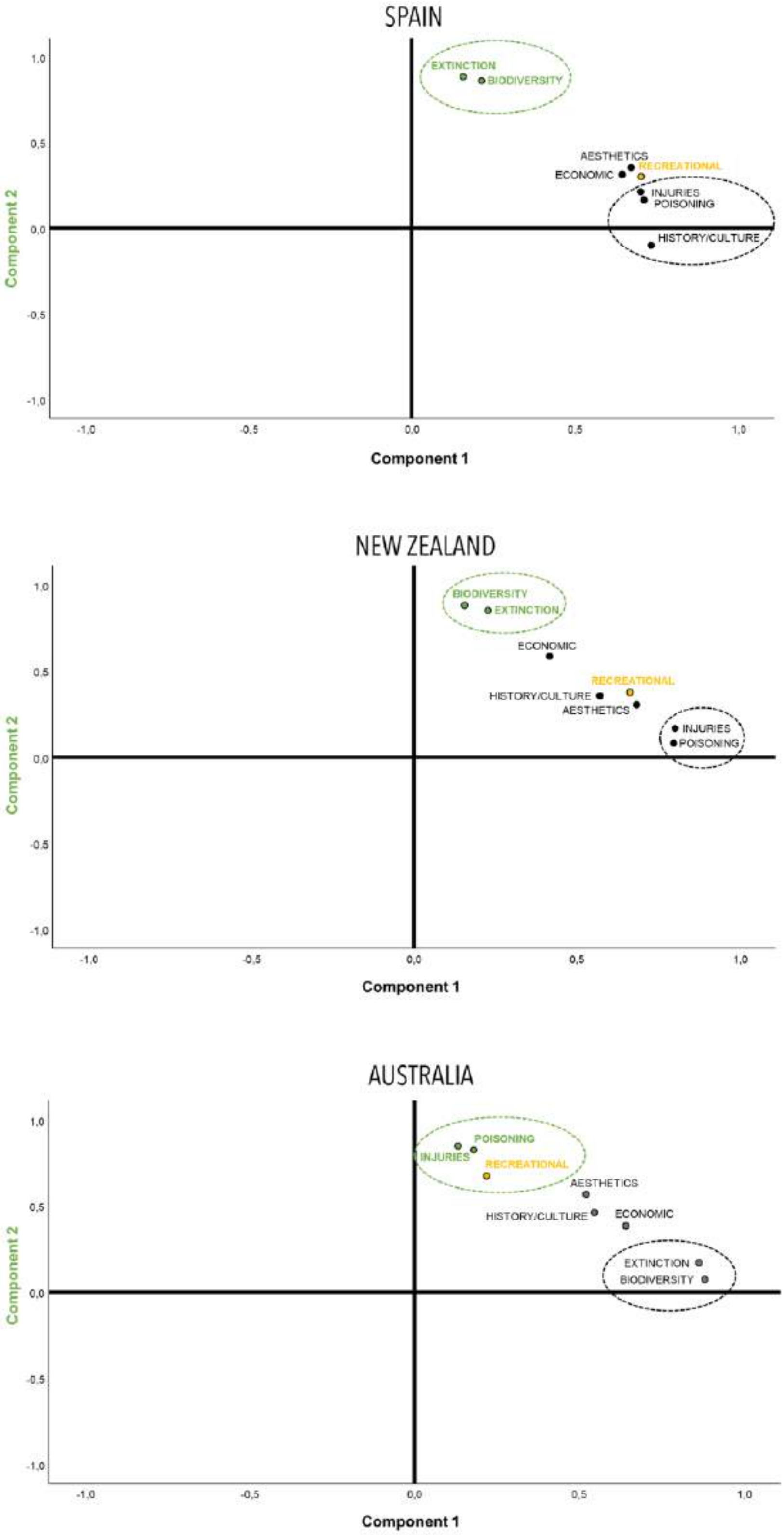


Figure 10. Factor analisis plot for the perception of impacts caused by marine exotic species, for the three countries explored.

3.2 Perception of NZ and Australian stakeholders towards their current regulations.

Boat owners and marine operators in New Zealand and Australia showed a high level of uncertainty and/or neutrality (49-51%) when asked on whether exotic species were well-managed in their countries (Figure 11). Only 25-36% answered certainly positive. Stakeholders in New Zealand were less satisfied and here, the variable knowledge about NIS and risk perception influenced their views (Fishers'exact test, p=0.005 and p=0.03, respectively; N=182). Respondents who had an accurate knowledge and a high risk perception were the ones expressing dissatisfaction rather than agreeing or remaining neutral; whereas those with self-admitted knowledge only, or a fair risk perception, were significantly more tendent to agree with the general management of NIS in New Zealand. In addition, respondents who declared a high or very high connection with the marine environment were also the ones giving a more diverse feedback regarding the general management of NIS; from strongly agreeing to strongly disagreeing (Fishers' exact test; p=0.03; N=182). Meanwhile, respondents answered generally positively regarding biofouling guidelines for recreational boating: 64-66% of them considered these regulations to be fair; and only 5-6% of respondents disagreed. No significant variation across regions was found for satisfaction with regulations.

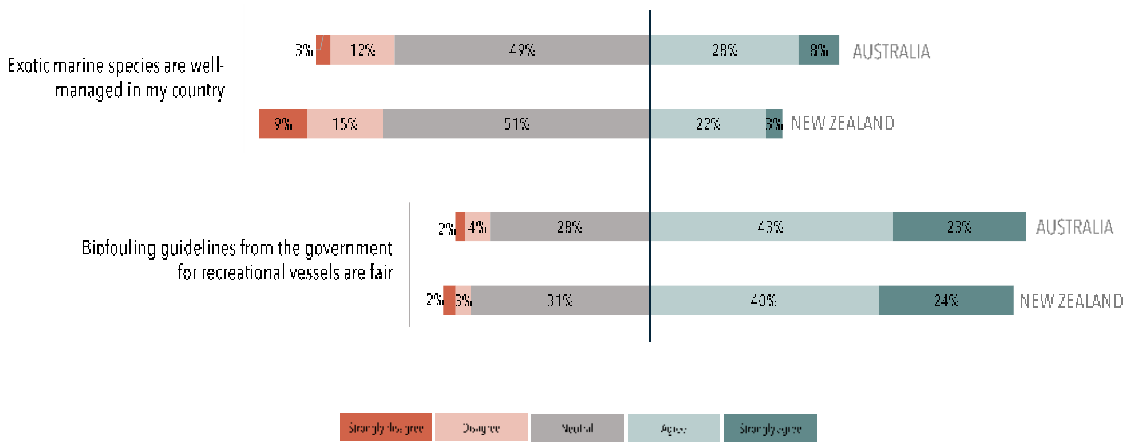


Figure 11. Perception of Australian and New Zealand boat owners and marine operators towards management of marine exotic species in their countries, based on q15C and B.

When considering other attitudes like interest in getting more involved or acknowledging the responsibility of good maintenance practices to prevent marine NIS, the variable connection was significantly associated to certain views. Respondents who declared a high connection with the marine environment were significantly more likely to agree with the responsibility of good maintenance practices than the ones who went neutral or denied this connection; who rather disagreed (Fishers'Exact test; p=0.0005; N=175 and p=0.001; N=182, respectively) (Figure 12A). Besides, respondents who felt neutral with their connection with the marine environment also went indecisive/showed a mitigated intention of getting more involved (Fisher's exact test; p=0.02; N= 366) (Figure 12B). Finally, the variable risk perception of vectors was related to perceptions about maintenance practices in New Zealand (Fisher's Exact test, p=0.03; N=141). Respondents who did not acknowledged any vector disagreed, almost exclusively, on the link between maintenance practices and the prevention of NIS spread.

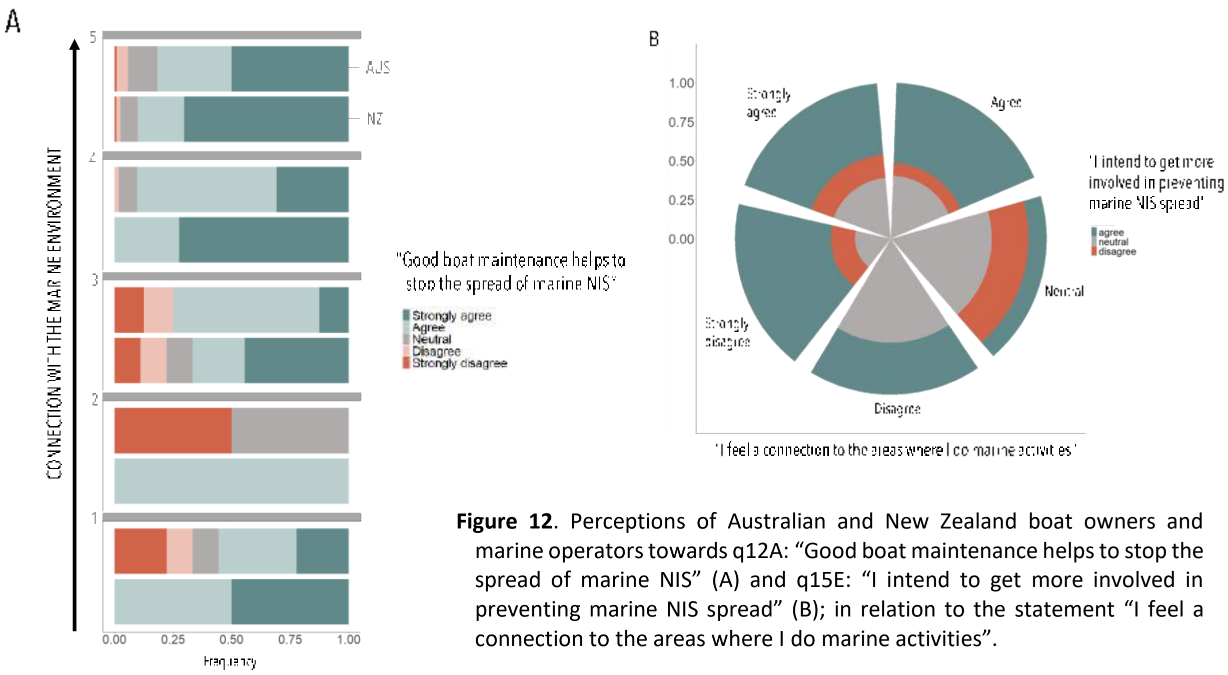


Figure 12. Perceptions of Australian and New Zealand boat owners and marine operators towards q12A: "Good boat maintenance helps to stop the spread of marine NIS" (A) and q15E: "I intend to get more involved in preventing marine NIS spread" (B); in relation to the statement "I feel a connection to the areas where I do marine activities".

3.3 Perception of Spanish stakeholders towards regulations and vessel maintenance practices

3.4.1 On the necessity of regulating the recreational boating vector

All countries agreed on the need to regulate marine exotic species (q15A), and on the fact that a good boat maintenance helps to stop their spread (q12A); with New Zealand respondents feeling the strongest about the last statement (Figure 13). When asked whether they think a biofouling regulation for recreational vessels is necessary to prevent NIS spread (q15F), Spanish boat owners and marine operators showed divided opinions; with 58.74% of them answering positively; 22% of them answering they disagree or strongly disagree; and 18.9% going neutral. Although there was no significantly different trends among regions (q15A:Fisher's exact test, p=0.22; q12A: p=0.06; q15F: p=0.776; N=206), nor among marinas (p=0.66, p=0.4758, and p=0.6592, respectively; N=204); opinions in this regard varied at spatial scale (Figure 13B). In particular, boat owners and the marine operators in El Rompido marina (Huelva) and Puente del Hierro – San Fernando (Cadiz) were the best supporters (73% and 77%, respectively). Finally, the interest to get more involved in preventing this environmental problem varied across regions (Fisher's exact test; p=0.013; N= 427; Figure 14), and marina as well (Fisher's exact test; p=0.03; N=140). Granada was the region showing the greatest intention; with a 69% positive responses and only 6% negatives (N=103). The boat owners and the marine operator of its sampled marina, Club Nautico de Motril, also scored the highest interest (83%; N=18). In Almeria, 66% of respondents showed interest and 11% rejected getting more involved; followed by Malaga

(50% versus 9%), which in turn had the highest frequency of “neutral” response (41%). Stakeholders in Cadiz and Huelva showed the most divided opinions, with 45-46% respondents willing to get more involved, and 23-24% who did not wish to. Boat owners and the marine operator in Puente de Hierro – San Fernando marina were the exception, with a 79% of respondents showing interest (N=14).

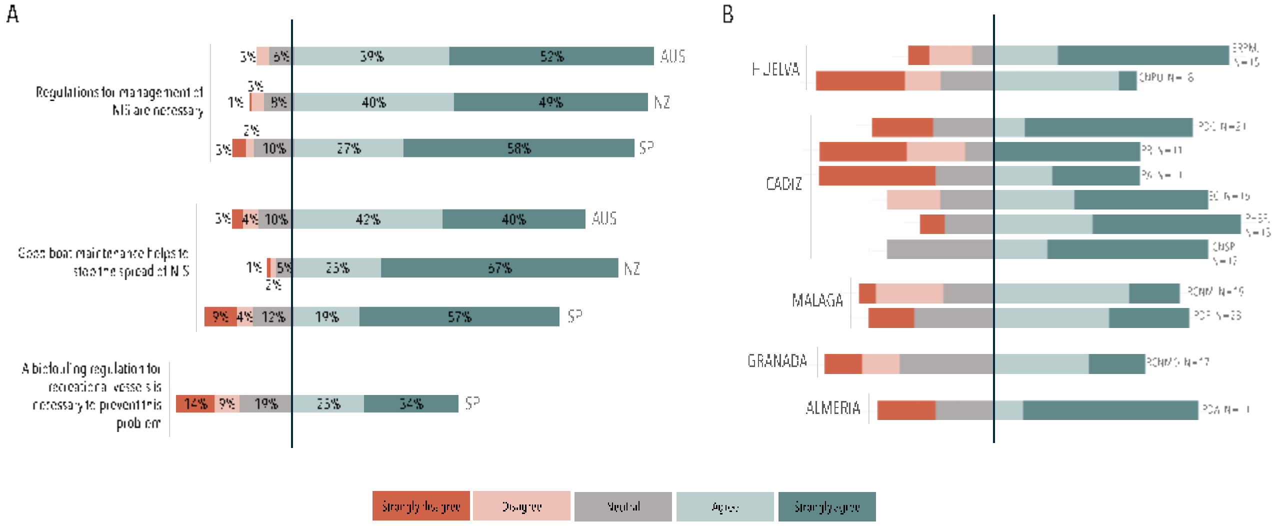


Figure 13. (A) Perception of boat owners and marine operators towards management of marine exotic species in the three countries. Question 15F “A biofouling regulation for the recreational boating vector is necessary to reduce NIS spread” only applies to Spain. Likert-scale: 1=”totally disagree”, 2=”disagree”, 3=”neutral”, 4=”agree”, 5= “totally agree”. (B Opinions of boat owners and marine operators in Spain on whether “ A biofouling regulation for recreational vessels is needed to prevent NIS spread” (q15F). Data is shown per marina and region sampled. N indicates sample size; only locations with a N>10 are included in the graph. ERPM = El Rompido Puerto Marina; CNPU= Club Naútico Punta Umbría; PDC= Puerto Deportivo Chipiona; PR= Puerto de Rota, PA= Puerto América; EC= Puerto de ElCano, PHSF= Puente de Hierro – San Fernando; CNSP=Club naútico Sancti Petri; RCNM= Real Club Naútico Mediterráneo; PDF= Puerto Deportivo Fuengirola; RCNMO= Real Club Naútico Motril, PDA= Puerto Deportivo Almerimar

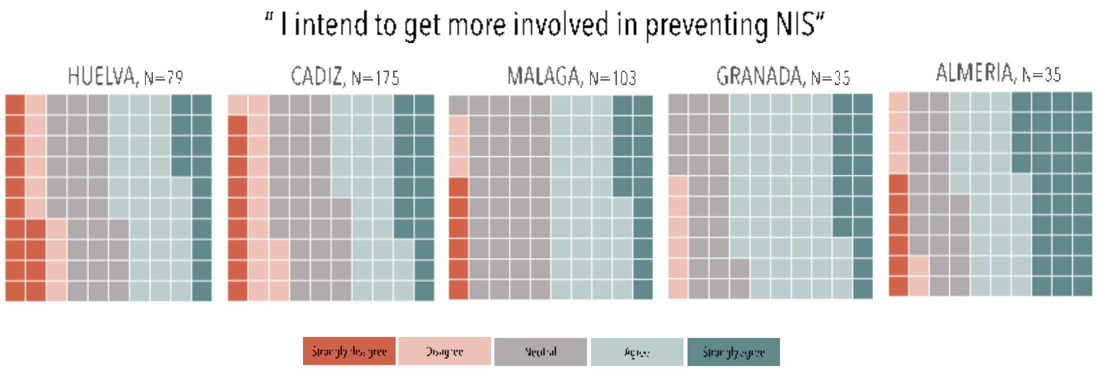


Figure 14. Intention of Spanish stakeholders (all population groups) to get more involved (q15E), indicated per region.

In Spain, there was a significant association between the risk perception of vectors in Spain and the agreement with the belief “good boat maintenance helps to stop the spread of marine NIS” (Fisher’s Exact test, p=0.03; N=136; for 3-level likert scales: agree/neutral/disagree). 94% of respondents with a very high risk perception supported this belief, acknowledging the responsibility of good maintenance practices. On the contrary, respondents with no risk perception of vectors whatsoever showed more mitigated opinions: 57% agreed and 33% disagreed with the statement (Figure 15 shows these data in more detail, with a 5-level likert scale for descriptive purposes). The factors knowledge about marine NIS and connection feeling were not significantly associated to specific views regarding management, though.

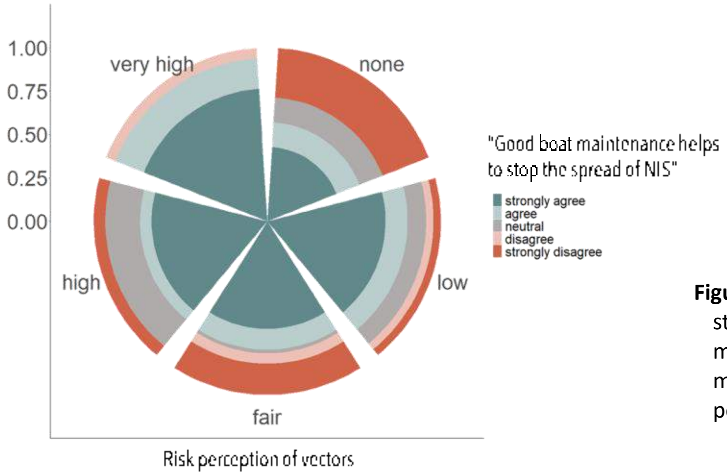


Figure 15. Perceptions of Spanish stakeholders towards q12A: “Good boat maintenance helps to stop the spread of marine NIS” depending on their risk perception of vectors.

3.4.2 Most common vessel maintenance habits by Spanish boaters

In Spain, most boat owners (68%) haul out their vessel for dock-cleaning (dry-cleaning) and redo the antifouling paint once a year. Twenty-one % do it less than once a year, and 11% more than once a year. As for in-water cleaning practice, seven out of 11 marina operators interviewed declared that either they didn’t allow in-water cleaning at all, or specified that only preventive cleaning (the slime) using a soft cloth was allowed. Two marinas indicated that it was allowed but only performed by professional divers. One answered that it was the full responsibility and decision was on the boat owners on how to perform it; and the last one said it was allowed both soft cleaning and scrapping. Surveys reveal that 39% of Spanish boaters do it regularly; with cases in all sampled marinas, although slightly less frequent in those with stricter regulations. From those, 34% use this practice to remove macrofouling from the hull (reactive in water cleaning); 31% only use it to remove microfouling; usually the green slime in the waterline (preventive in-water cleaning), and the rest 32% did not specify what use they gave. Half of these boaters did in-water cleaning in the marina; 26% offshore, 9% in the bay or gulf. The rest 15% did not specify the location of the practice. As a note, several respondents who engaged in a conversation during face-to face interviewers declared that they do not use scraping as “it would damage the antifouling paint” (usually self-polishing type), “causing more [fouling] growth underneath”.

3.4 Risk communication for Spanish stakeholders

Spanish stakeholders declared they had received information or environmental education mainly via TV (70% of respondents); Internet (58%) and personal contacts like family, friends or neighbours (43%); followed by magazines or newsletter articles (32%) and, with the same percentage, personal contacts associated to the marine recreational activities sector, like members of the boating club, bait shop vendor, marina staff etc. We found a significant positive correlation between the number of sources of information they had been exposed to and they knowledge they proved, which was especially clear in the marine operators' population group; although it only explained 16% of the variance (Kendall's rank correlation; Z=3.7831; p<0.001, tau coefficient=0.16, N=404; Figure 15). Overall, when asked about their preference, Spanish stakeholders pointed in broad lines to the media (93%), and less frequently to face-to face events (18%) (Figure 12). Only a small percentage selected personal contacts as their preferred source of risk communication (5%). Finally, 3% of the population considered that the best way of getting informed was via government institutions/organizations and/or via education. Concretely, the preferred sources of information for Spanish stakeholders were TV (45%); Internet (34%); and social networks (24%) (Figure 16).

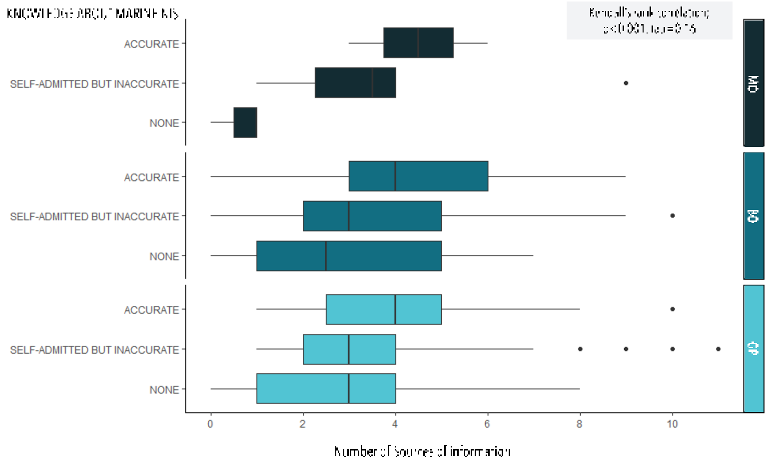


Figure 15. Correlation between knowledge level about marine NIS of Spanish stakeholders and the number of risk communication sources they have been exposed to.

MO= Marine operators;
BO= Boat owners;
GP= General public.

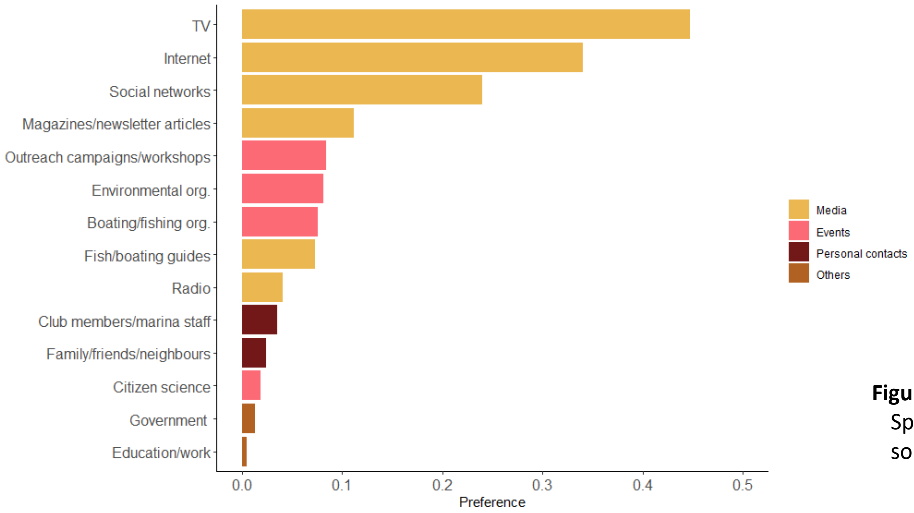


Figure 16. Preference (0-1) of Spanish stakeholders for each source of information

There was no significant different trends among population groups; except for “events organized by boating/fishing organizations” (χ^2=7,55; df=1; N= 367); which was positively associated to boaters and marine operators, while general public rarely chose it. When looking at different age groups (stacked in 3 categories), we found some significantly opposed trends in between the youngest and the oldest respondents (Figure 17). There was a strong tendence of respondents in the 16-35 category to choose social media as preferred source of communication; in comparison with respondents in 56-over 65 category, who rarely selected it (χ^2=13.436; df=2; p=0.001; N=367; stress = 2.78, p=0.03 for young respondents, stress = -3.33, p=0.01 for elderly ones). Young respondents were also significantly more inclined than others to choose events by environmental organizations (χ^2=8.81, df=2; p=0.01; N=367; stress= 2.67; p=0.046); as well as citizen science initiatives (Fishers’ exact test; p=0.016; N=367).

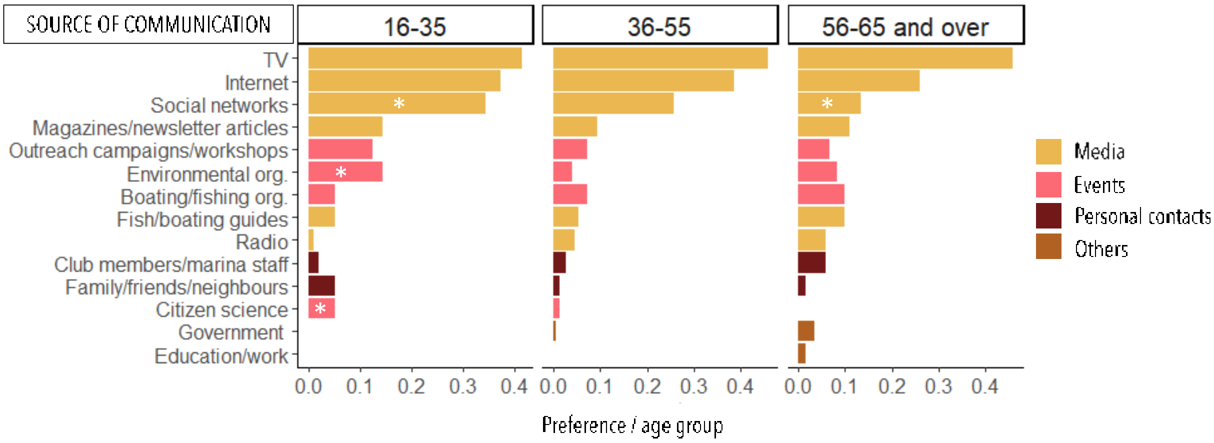


Figure 17. Preference (0-1) of Spanish stakeholders for each source of information, indicated per age groups. Significance levels are indicated as *(p<0.05)

4 DISCUSSION

4.1 Where do Spanish stakeholders stand in terms of awareness and risk perception, in comparison with countries with biofouling regulations?

Stakeholders in Spain are not clear on the concept of marine NIS and are far more overconfident in their knowledge than the two countries with regulations. This inaccuracy or ambiguity represents a hazard if a precautionary approach is to be assumed for management actions: respondents that believe they are aware of NIS but cannot actually distinguish among native and introduced species creates a false sense of a biosecure plebiscite and in turn results in overconfidence in managers (Campbell et al., 2017). A similar study in Madeira (Portugal) also detected a sound discrepancy between having heard about the issue and being unable to cite correct examples of marine NIS (Parretti et al., 2020). As a side note, Spanish stakeholders did have a considerable good (accurate) knowledge when also taking into account introduced species in the terrestrial and freshwater biomes; being especially aware of the Zebra mussel *Dreissena polymorpha* (Pallas, 1771), the blue crab *Callinectes sapidus* Rathbun, 1896 and urban birds like the Rose-ringed parakeet *Psittacula krameri* (Scopoli, 1769). They also proved to be really aware of the marine introduced species causing trouble in their local area; for example, boat owners in San Fernando marina were highly aware of the pistol shrimp (64% of boaters recognized it, and several elaborated on their answer in face-to-face conversation) and how this species affects the macrofauna by altering the substrate and causing less quality and deformed wedge clams (*Donax trunculus* Linnaeus, 1758) (boaters and marine operator in San Fernando, personal communication). The influence of the media on people's examples of marine NIS was also clear. In Spain, the Japanese algae *Rugulopteryx okamurae* (E.Y. Dawson) I.K.Hwang, W.J.Lee & H.S. Kim, 2009 was the most well-known example; probably thanks to its frequent appearance in the news and forums since 2019. A similar trend could be seen in Australia with the Crown of thorns. Interestingly, this species hasn't been introduced in their waters, but is highly mediatic due to the impact it causes to the Great Barrier Reef, among others; and we could observe how it was perceived as highly "invasive" and "dangerous", sometimes being referred to as the "coral reef killer". As for the Fan Worm in New Zealand, boaters outlined that they were aware thanks not only to the media but also to their own marinas and clubs, who raised the flag and provided frequent reports on the case.

Our study suggests that the highest gap of awareness in our country occurs in terms of risk perception; referring to both marine NIS as a relevant environmental problem and to which human vectors are responsible for their introduction and spread. There seems to be a weaker relationship between knowing the existence of marine introduced species and knowing the causes of the problem; whereas knowledge and risk perception are tightly associated in our sampled countries with implemented regulations. Although the enforcement of a clean hull regulation is key; it needs to be noted that, in Australia and New Zealand, this was also accompanied by a great involvement of the marina itself in tackling this problem via environmental education (personal observation and marine operators and boaters communication). Marinas usually offered multiple resources like slogans/posters alerting on new arrivals on nearby regions; visual guides on how to recognize the most common invasive species; clear information on how to act if ever spotting something "unusual" (phone number/website/way to contact the Marine Primary Industries – New Zealand government department - for example). In a similar vein, they used to organize campaigns in collaboration with either the local government or even universities for raising awareness among boaters on this issue; and they

Figure 18. Slogans and posters found in marinas in New Zealand, including an advert of a workshop to raise awareness on marine pests (A), a guide with the regulations applying to visiting boats in Bay of Plenty (North Island) (B), and a call for involvement or boat owners in preventing marine NIS via specific actions (C)

carried out regular inspections of vessels whenever somebody alerted on the presence of an introduced species in their surroundings (see examples for New Zealand in Figure 18). Cole et al. (2016) outlines the importance of this active involvement in outreach: as demonstrated for the recreational boater community in Illinois, people who had great access to outreach from their organization (recognition of prevention slogans) and who could recognize the highest number of local freshwater NIS, were the ones performing consistent prevention behaviour on the vectors (vessel and fishing equipment).

4.2 What can we expect from Spanish stakeholders regarding a future regulation?

Our preliminary results indicate a good disposition from Spanish stakeholders towards regulation of introduced species as an environmental problem in general. However, we cannot draw conclusions yet regarding their potential support for a regulation that applies to recreational vessels, due to highly mitigated opinions. Data is positive in terms of support of the IMO recommended guidelines regarding antifouling maintenance, dry-cleaning and in-water cleaning practices (around 60% support); however, this must be taken with caution, as one third of the respondents did not specify the details of, for example, their in-water cleaning practices. Further data needs to be collected regarding this; especially since several issues they were concerned about arose in the face-to face interviews: the need to regulate commercial vessels before/instead of recreational ones; the debate on whether the new biodegradable antifouling paints are effective; the importance of environmental education and personal education before implementing any law; the need of the local government to get involved in informing and educating on this issue; and the lack of effort from some marinas in offering waste disposal facilities.

Furthermore, in order to properly understand attitudes towards regulations for marine NIS introduced via hull-fouling, factors coming out as significant like “connection with the environment”, “previous knowledge on the subject”, and “risk perception” should warrant further investigation. According to literature and from a theoretical point of view, values and knowledge are at the base, influencing risk perception and, in turn, attitudes towards a specific environmental problem which ultimately shapes behaviour (see information revised in Figure 19 and references therein for more details). The weight of these interactions is context-dependent and highly variable depending on which environmental hazard we are dealing with. For example, when evaluating perceptions towards climate change, domain-specific knowledge has proven to be key when predicting public’s concern (Tobler et al. 2012; Aksit et al. 2017). On the contrary, Robinson et al. (2017) found the availability heuristic (“ease with which instances or occurrences come to mind”) and demographics to be far more significant when mapping risk perception towards the invasive Japanese knotweed *Fallopia japonica* in domestic gardens. Interestingly, values and beliefs like community reciprocity and knowing that other people also comply with an environmental regulation can be determining in multiple contexts; from engaging resident actions against the invasive tree albizia (*Falcataria moluccana* (Miq.) Barneby and J.W. Grimes) across private lands in Hawai’i (Niemiec et al. 2016), to recreational boaters complying with the Right Whale Regulations in Florida (Montes et al., 2018). In the freshwater and marine context, engagement with Aquatic Invasive Species can also be largely driven by the recreational use of the environment (*e.g*. transience level, number of lakes visited) (Wittzling et al., 2016; Nanayakkara et al., 2018); as well as the human-nature relationships stakeholders align with (Kemp et al. 2017). Finally, marine operators place a high importance on perceived effectiveness (through visible effects) when considering supporting or not certain biosecurity interventions (Vye et al., 2020). Our results indicated that some of these associations are country-specific (even with a similar demographic profile and recreational use of the marine environment); and therefore, need to be addressed separately. First, the perception of impacts varied considerably; which may indicate different value systems shaping the concern. In general, responses revealed an underlying perception of the marine environment *versus* human/social issues as two separate identities; with perceptions regarding ecosystem services (usually economic profits plus others, depending on the country) in the middle. This differentiation was more acute in Spain and more gradual in New Zealand and Australia. While there was a consensus regarding what applies to the environment itself; their perception on the second group differed. Australian stakeholders understood impacts on their human well-being (*i.e.* health and entertainment) as separate from the rest; for New Zealand, the issue of human health set aside when perceiving impacts; and for Spain this happened with impacts on society as a group (*i.e.* health and cultural history). Notably, the item "interference with recreational activities" was perceived differently in all three countries. A possible interpretation, based on these results plus face-to face interviews, is that Australia is regarded rather as a sport/entertainment; in New Zealand as something the marine environment provides; and in Spain as an income from the tourism sector. Based on these considerations, it would be interesting to investigate human-relationship views of our stakeholders (Van der Born, 2007). Secondly, factors like domain-specific knowledge and connection feeling were related to management perceptions and interest in getting involved only in the countries with regulations. On the contrary, views of Spanish stakeholders and capacity of acknowledging the responsibility of boat owners on the subject was rather related to individual risk perception.

In order to properly disentangle the attitudes towards management of Spanish stakeholders, further statistical analysis needs to be performed; which would involve weighing each factor through model building (in preparation). This analysis would be enriched with examination of value systems, which would complete the information raised by our perception of impacts' analysis. In here, we would test our stakeholders on a variety of human values towards nature to define which nature's contributions (positive or negative) are most relevant and thus affect their perception regarding introduced species (Verbrugge et al., 2013; Díaz et al. 2018; Rodríguez-Salinas, 2018). This is important because, for example, boaters with an anthropocentric orientation can be more likely to prevent the spread of marine NIS only if they impact on useful resources in an apparent way (Pradhananga et al., 2015); whereas those rather biocentric-oriented believe nature can be valued for its inherent qualities, and so preventive actions can be stimulated as long as awareness is high (van Riper and Kyle 2014). Evidently, environmental education campaigns need to be tailored accordingly. Once this mapping is completed, we would be able to provide some good quality information on how to target risk communication in this area; increasing the chances for public involvement, as well as readiness and uptake of measurements (see value-belief-norm application in Liobikiene and Juknys, 2016). Finally, after completion of environmental education campaigns, the next step would be to figure out what are the chances of those attitudes translating into specific actions (intended behaviour and self-reported actual behaviour) (see section 5.2 in Discussion and Future directions chapter).

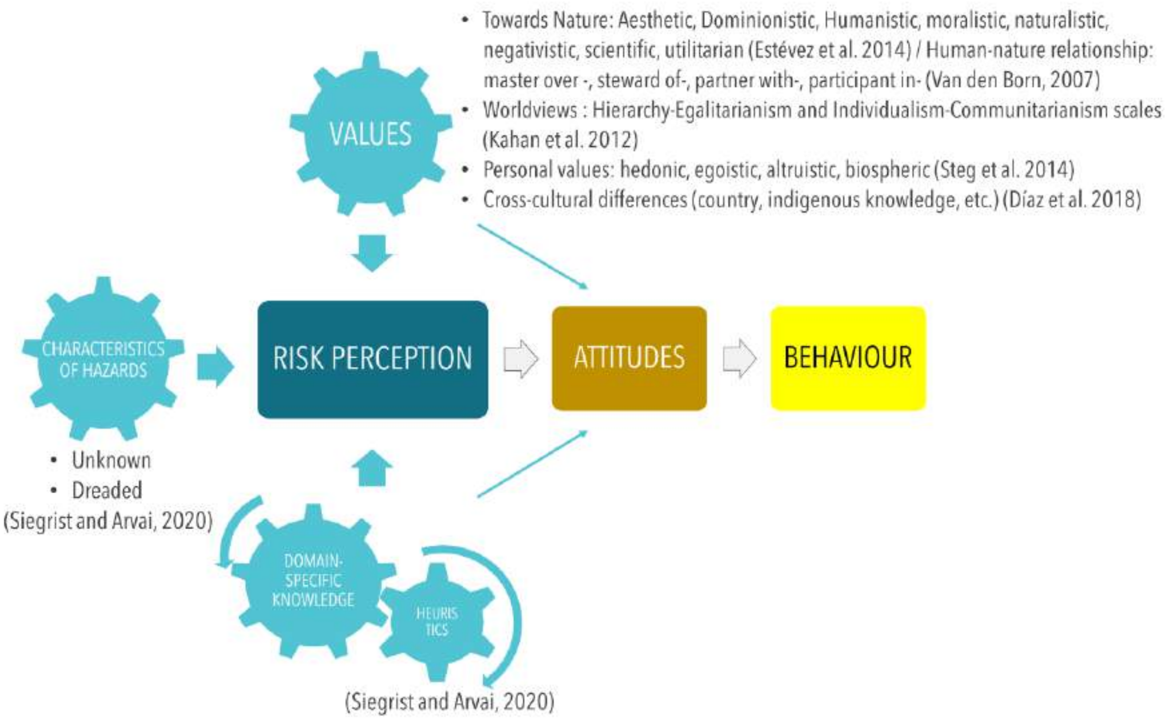


Figure 19. Factors influencing risk perception of stakeholders towards an environmental problem and, ultimately, behaviour. Information gathered from Van der Born (2008); Estévez et al. (2015); Kahan et al. (2012); Aksit et al. (2017), Steg et al. (2014) and Siegrist and Arvai (2020).

4.2 Preliminary recommendations for increasing stakeholders' awareness in Spain

In the light of the information and trends registered in our study, we can start proposing some preliminary recommendations for raising awareness among stakeholders' awareness in Spain.

A. The concept of what is an what is not an introduced species needs clarification, especially when addressing campaigns to the general public. This terminology issue was also raised by Verbrugge et al., (2021) in their evaluation of multiple international invasive alien species education projects; and they recommend establishing a clear context and reference frame (e.g. country or regional perspective) to solve this.

B. The main objective is increasing risk perception (*i.e.* knowledge on how these species are transported and how this can be impeded); which will increase the sense of responsibility of boaters on the issue. In here, the misconception that only commercial vessels are responsible needs to be addressed. This recommendation aligns with the ideas commented in Parretti et al. (2020) for stakeholders in Madeira Island (Portugal, Atlantic Ocean). They also advocate for increasing awareness on spreading vectors, based on the identified deficient risk perception towards recreational boating vector; and a significant association among this factor and the willingness to get involved in marine NIS management. In terms of language, Verbrugge et al., (2021) recommends using a combination of different type of messages: solution and behaviour-oriented messages, informative messages, and understanding impacts and processes messages; these last ones focusing on damages to the ecosystem and to the economic sector, according to our results from the factor analysis.

C. The most effective channel for risk communication would be the media, via news, documentaries and social networks. This also aligns with conclusions driven for other countries in the Mediterranean Sea, where the best known examples by boaters where the ones frequently appearing in tv news or internet news (*i.e.* "killer algae" *Caulerpa* sp and "pufferfish" *Lagocephalus* sp.) (Ferrario et al. 2016; Martínez-Laiz et al. 2019). Still, diversity of risk communication sources has proven to positively influence knowledge, at least for marine operators; and it is known that hands-on/local fieldwork activities work better than talks alone when increasing environmental awareness (Verbbruge et al., 2021). Therefore, we also suggest using slogans, workshops (exhibitions), and citizen science initiatives (*e.g.* reporting the presence of unknown fauna in their marinas, or volunteering to help in monitoring of sentinel stations – see section 5.3 in Discussion and Future directions chapter) addressed to the younger local stakeholders, advertised and broadcasted through social media, to increase concern and promote prevention.

D. In terms of maintenance practices, environmental education on the risks of reactive in-water cleaning needs to be conducted; promoting preventive in-water cleaning instead. For the marinas who declare a firm no in-water cleaning policy, compliance needs to be improved.

E. We recommend carrying out some pilot environmental education campaigns first on the issue, followed by and evaluation of their efficiency. Marinas like Club Naútico Motril in Granada, El Rompido in Huelva o Puente de Hierro-San Fernando in Cadiz could be good points for these tests, as they showed the highest intended engagement behavior. If campaigns are successful, they could be spread to other marinas in the Andalusian coast. Here we would like to specify that, according to marine operators interviewed in the study of Vye et al. (2020), leadership by individual marinas is important when driving behavioural change in the sector; nevertheless, when the time to implement biosecurity interventions arrive, this should occur universally across the sector.

F. If an implementation of a hull-fouling regulation is to occur in our country, it will definitely demand the collaboration of multiple actors; and this involves the local authorities to offer, for example, open-access, long-lasting and easy-to-use online resources for both boaters and general public on the subject. These initiatives were carried out in our country (especially in the years 2016-2017) to increase awareness on introduced species and especially to prevent pet release and/or trade (https://www.miteco.gob.es/es/biodiversidad/temas/ conservacion-de-especies/especies-exoticas-invasoras/ceeeicampanaeei.aspx; https://www.juntadeandalucia.es/medioambiente/portal/web/guest/landing-page-documento/-/assetpublisher/jXKpcWryrKar/content/folletos-de-especies-ex-c3-b3ticas-invasoras-1/20151?categoryVal=); all accessed 23 September 2021). Unfortunately, these resources are limited for the marine realm and, as they are addressed to the general public, few campaigns target information on specific vectors and what good prevention practices are desirable at individual level (https://www.aragon.es/documents/20127/674325/MANUAL_BUENAS_PRACTICAS_EVITAR_PROPAGACION_ESPECIES_EXOTICAS_INVASORAS.pdf/c1b45abf-09ef-fffb-1560-ff1242e9dfb; http://www.ceida.org/sites/default/files/adxuntos-publicaciones/guiaespeciesweb.pdf). Besides, only punctual ones directly call for public involvement and offer guidelines on what one can do to prevent their spread (see example for Zebra mussel: https://www.juntadeandalucia.es/medioambiente/portal/documents/20151/4058914/folletostopmejilloncebra.pdf/ddea5c3d-dea7-3144-22b9-b7efd0a99e20?t=1597395762690; gardening plants: https://www.juntadeandalucia.es/medioambiente/portal/documents/20151/469274/recomendacionesjardineriafolleto.pdf/0e843305-e7f9-8a53-43cf-8e320dc91a09?t=1410422211000; and the tiger mosquito: https://www.juntadeandalucia.es/medioambiente/portal/documents/20151/4058914/tripticomosquitotigre.pdf/2d822a44-1ebb-3dcb-435d-9f4dd7fd6ac 8?t=1597395981962). To our knowledge, there are no such initiatives addressed to boaters and how to prevent transporting and spreading marine exotic species in their hull-fouling.

G. Finally, it is worth mentioning that any environmental education campaign will benefit from targeting elementary schools to deliver messages, not only on the risks of marine exotic species but also on the importance of biodiversity conservation of our recipient environments. Kids are in general more receptive than teenagers and adults and they'll take the lessons learnt through to adulthood, shaping future communities; as well as into their homes, supporting intergenerational learning (Duvall and Zint 2007; Lawson et al. 2019; Verbbruge et al. 2021)

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SUPPLEMENTARY MATERIAL

Table S1. List of sampling locations in each selected area, to represent our target population in Western Australia (Australia), North Island (New Zealand), and Andalucía (Spain).

COUNTRY AND REGION	AREA	SAMPLED LOCATIONS	COORDINATES
AUSTRALIA – WESTERN AUSTRALIA	Hillarys	Hillarys yacht club (1)	31°49'17.8"S 115°44'10.2"E
		Sorrento Quay (2)	31°49'28.6"S 115°44'28.5"E
		Scarborough Beach (3)	31°53'36.8"S 115°45'17.4"E
	Fremantle	Fremantle Leighton Beach (4)	32°01'28.3"S 115°45'01.3"E
		Royal Perth Yacht Club (5)	32°03'34.6"S 115°44'30.5"E
		Fremantle Sailing Club (6)	32°04'06.8"S 115°45'00.7"E
		South Beach (7)	32°04'22.8"S 115°45'02.8"E
	Swan River	Swan yacht Club (8)	32°01'37.0"S 115°45'47.9"E
		East Fremantle Sailing Club (9)	32°01'44.8"S 115°46'32.0"E
		South of perth Yacht Club (10)	32°00'07.7"S 115°50'46.1"E
		South Perth Foreshore (11)	31°58'29.7"S 115°51'45.6"E
	Rockingham	Rockingham Beach (12)	32°16'03.0"S 115°44'24.4"E
		The cruising yacht club of Western Australia (13)	32°16'34.0"S 115°43'39.3"E
		Safety yacht Club (14)	38°18'25.8"S 144°59'39.7"E
	Mandurah	Mandurah Offshore Sailing Club (15)	32°31'19.1"S 115°42'47.1"E
		Mandurah Beach (16)	32°31'09.1"S 115°43'29.6"E
NEW ZEALAND - NORTH ISLAND	Auckland	Hobsonville marina (17)	36°48'35.3"S 174°38'44.6"
		Takapuna Beach (18)	36°47'16.1"S 174°46'32.5"
		Bayswater marina (19)	36°49'08.2"S 174°46'05.3"
		Westhaven marina (20)	36°50'09.0"S 174°44'34.9"
		Mission Bay (21)	36°50'50.0"S 174°49'54.4"
		Saint Heliers Beach (22)	36°51'00.9"S 174°51'22.5"
	Tauranga	Mount manganui Beach (23)	37°37'51.9"S 176°10'44.5"E
		Pilot Bay (24)	37°38'03.9"S 176°10'37.8"E
		Tauranga marina (25)	37°39'35.7"S 176°10'07.5"E
		Tauranga Bridge Marina (26)	37°40'15.1"S 176°10'45.9"E
		The strand (27)	37°41'00.4"S 176°10'14.2"E
SPAIN	Huelva	El Rompido Puerto Marina (28)	37°13'04.7"N 7°07'38.2"W
		Club Naútico Río Piedras (29)	37°12'57.1"N 7°06'55.2"W
		Marina Nuevo Portil (30)	37°12'52.7"N 7°04'51.3"W
		El Portil Beach (31)	37°12'31.3"N 7°03'00.2"W
		Club naútico Punta Umbría (32)	37°11'31.5"N 6°58'03.0"W
		Punta Umbría Beach (33)	37°10'26.0"N 6°57'38.5"W
	Cadiz	Puerto Deportivo Chipiona (34)	36°44'44.9"N 6°25'43.5"W
		Puerto de Rota (35)	36°36'55.6"N 6°21'10.6"W
		Rota beach (36)	36°37'12.2"N 6°21'54.2"W
		Puerto America (37)	36°32'33.8"N 6°16'56.5"W
		La Caleta Beach (38)	36°31'53.0"N 6°18'21.4"W
		Viento de Levante (39)	36°31'12.3"N 6°16'26.2"W
		Puerto de ElCano (40)	36°30'01.4"N 6°15'22.4"W
		La Victoria Beach (41)	36°29'55.8"N 6°16'29.0"W
		Puente de Hierro-San Fernando (42)	36°29'01.7"N 6°10'44.7"W
		Club naútico Sancti Petri (43)	36°23'44.5"N 6°12'27.9"W

- ANDALUCIA		Conil Beach (44)	36°17'14.9"N 6°06'22.7"W
	Malaga	Puerto Deportivo Fuengirola (45)	36°32'27.7"N 4°37'07.5"W
		Fuengirola Beach (46)	36°32'39.4"N 4°37'03.3"W
		Real Club Naútico Mediterráneo (47)	36°42'27.6"N 4°24'48.3"W
		La Malagueta Beach (48)	36°43'09.5"N 4°24'09.0"W
	Granada	Real Club Naútico Motril (49)	36°43'28.1"N 3°31'42.0"W
		Motril Beach (50)	36°43'05.1"N 3°32'37.1"W
	Almeria	Puerto Deportivo Almerimar (51)	36°41'46.7"N 2°47'39.0"W
		Almerimar Beach (52)	36°41'29.6"N 2°46'58.7"W

S2. QUESTIONNAIRES FOR AUSTRALIA

Social-perception of marine biological invasions associated to recreational boating

- SURVEY FOR MARINA OPERATORS -

Section 1. About you

1. Which of the following marine (*i.e.* ocean or beach) activities you participate in?
   - ☐ Boating
   - ☐ Swimming
   - ☐ Fishing
   - ☐ Scuba diving / Snorkeling
   - ☐ Photography
   - ☐ Kayaking
   - ☐ Surfing / Waterskiing /Jet ski
   - ☐ Marine wildlife watching/interaction
   - ☐ Coastal hiking
   - ☐ Other ____

2. Think of the areas where you do those activities. Why do you choose them?
   - ☐ They are close and accessible
   - ☐ There are no/few alternatives
   - ☐ They are my favourites

3. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I feel a connection to the areas where I do marine activities	1	2	3	4	5
I care about the environmental health of these areas	1	2	3	4	5

Section 2. Your attitudes towards exotic marine species

4. Do you know what an exotic marine species is? Yes / No
5. Do you know what an invasive species/pest is? Yes / No
6. Can you name any examples of exotic marine species?

7. How do you think exotic marine species can spread? Click the one/ones you consider
   - ☐ Attached to the hull of recreational vessels
   - ☐ Attached to the hull of commercial vessels
   - ☐ Via ballast water
   - ☐ Via aquaculture

8. Can exotic marine species live on artificial structures in the marinas (ropes, buoys, wheels, pontoons etc.)? Yes / No

9. Are you familiar with the following exotic marine species of the area?

Yes / No

Yes / No

Yes / No

10. Do you consider marine exotic species to be a relevant problem? Yes / No / Unsure

11. Please rate the following impacts of exotic marine species according to how relevant you consider them:

	Norelevant	A bit relevant	Unsure	Somewhat relevant	Very relevant
Loss of biodiversity and natural habitats	1	2	3	4	5
Local extinction of native species	1	2	3	4	5
Economic loss of fisheries and marine farming/aquaculture	1	2	3	4	5
Interference with recreational activities	1	2	3	4	5
Food poisoning by ingesting toxic species	1	2	3	4	5
Human exposure to injury (biotoxins, stings, bites etc.)	1	2	3	4	5

Loss of aesthetic value of coastal landscapes	1	2	3	4	5
Loss of historical/cultural value of an area	1	2	3	4	5

Section 3. Your attitudes towards regulations of exotic marine species

12. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Good boat maintenance helps to stop the spread of exotic marine species	1	2	3	4	5
Regulations for management of exotic marine species are necessary	1	2	3	4	5
I am familiar with the biofouling guidelines from the Australian Government for recreational vessels	1	2	3	4	5
Biofouling guidelines from the Australian Government for recreational vessels are created fairly	1	2	3	4	5
Exotic marine species in Australia are well managed	1	2	3	4	5
I intend to get more involved in preventing exotic marine species spread (e.g. by documenting myself, participating in campaigns, spreading the word, etc.)	1	2	3	4	5

13. Do you allow in-water cleaning in your marina? Yes / No

14. Do you have cleaning / antifouling requirements for visitor boats arriving to your marina? Yes / No. If yes, please specify____

15. Do you have cleaning / antifouling requirements for residents returning to your marina? Yes / No. If yes, please specify____

Section 3. Communicating about exotic marine species

16. Through which source have you heard information or received education about exotic marine species? Circle the one/ones you consider

Media
- ☐ magazines or newsletter articles
- ☐ info from fishing/recreational boating guides or brochures
- ☐ TV (news, documentaries etc)
- ☐ Internet websites
- ☐ Social networks (facebook, Instagram, twitter, youtube etc.)
- ☐ Radio

Events

- ☐ organized by recreational boating or fishing organization
- ☐ organized by environmental organizations
- ☐ Outreach campaigns or workshops
- ☐ Citizen science initiatives (*e.g.* collaborating reporting exotic marine species I encounter)
- ☐ Participating in surveys

Personal contacts

- ☐ family members, friends or neighbours
- ☐ members of a boating club, bait shop vendor, marina staff, etc.

- ☐ Other____
- ☐ None

17. Did these sources improve your understanding on the risks of exotic marine species? Yes / No

18. Which source from above do you consider the most useful to get informed about exotic marine species? Add a star next to the source(s)

19. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I trust the accuracy of information I see on social media	1	2	3	4	5
I trust the accuracy of information found on scientific articles	1	2	3	4	5
I trust the accuracy of information given by environmental organizations	1	2	3	4	5
I trust the accuracy of information I receive from personal contacts (family, friends, marina staff, etc.)	1	2	3	4	5

Section 4. Demographics

20. What is your place of residence (city and country)?

21. What is your age?

- ☐ 16-25
- ☐ 26-35
- ☐ 36-45
- ☐ 46-55
- ☐ 56-65
- ☐ 65 and over
- ☐ Choose not to say

22. What is your gender?

- ☐ Female
- ☐ Male
- ☐ Other
- ☐ Choose not to say

23. What is your highest level of education?
    - ☐ Primary
    - ☐ Secondary
    - ☐ Tertiary
    - ☐ Postgraduate (Masters, doctoral etc.)
    - ☐ Choose not to say

24. What is (or has usually been) your job sector?
    - ☐ Accountancy and Taxation
    - ☐ Advertising, Marketing and Public relations
    - ☐ Animals and Veterinary Science
    - ☐ Architecture, construction and Property
    - ☐ Art and Design
    - ☐ Banking and Financial services
    - ☐ Biological, chemical and Pharmaceutical science
    - ☐ Biomedical Technologies
    - ☐ Business management and Human resources
    - ☐ Charity and Voluntary work
    - ☐ Clerical and Administration
    - ☐ Computers and ICT
    - ☐ Earth and Environment
    - ☐ Education and Teaching
    - ☐ Engineering and Manufacturing
    - ☐ Farming, Horticulture and Foresty
    - ☐ Fashion and Beauty
    - ☐ Food and Beverages
    - ☐ Government, politics and law
    - ☐ History, Culture and Languages
    - ☐ Insurance
    - ☐ Leisure, Sport and Fitness
    - ☐ Marine and Maritime
    - ☐ Media and Publishing
    - ☐ Music and Performing Arts
    - ☐ Medical and Healthcare
    - ☐ Psychology and social care
    - ☐ Physics, Mathematics and Space Science
    - ☐ Sales and Retail
    - ☐ Security and Defense
    - ☐ Tourism and Hospitality
    - ☐ Transport and Logistics
    - ☐ Other_______________

Social-perception of marine biological invasions associated to recreational boating

- SURVEY FOR BOAT OWNERS -

********************* SAME STRUCTURE AND QUESTIONS EXCEPT FOR ******************

Section 3. Your attitudes towards regulations of exotic marine species

1. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Good boat maintenance helps to stop the spread of exotic marine species	1	2	3	4	5
Accidentally releasing exotic marine species during in-water cleaning or anti-fouling maintenance can contaminate waters	1	2	3	4	5
I am familiar with and understand the biofouling guidelines from the Australian Government for recreational vessels	1	2	3	4	5

2. How frequently do you…

	Less than once a year	Once a year	More than once a year	Monthly
conduct visual inspections of boats and equipment (including propeller, hull, anchor, fenders, cordate, tenders etc.)	1	2	3	4
redo your anti-fouling coating	1	2	3	4
haul your craft out of the water to clean it	1	2	3	4
undertake in-water cleaning of your hull	1	2	3	4
spray / rinse boat and equipment with high-pressure and / or hot water	1	2	3	4

3. Do you…

Conduct in-water cleaning in the marina? Yes / No

When performing in-water cleaning, capture biological, chemical and physical debris so that it can be disposed of to an onshore facility? Yes / No

Keep your biofouling maintenance information logged? Yes / No

Dry boat and all gear for at least 5 days before going to another waterbody? Yes / No

4. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Regulations for management of exotic marine species are necessary	1	2	3	4	5
Biofouling guidelines from the Australian Government for recreational vessels are fair	1	2	3	4	5
Exotic marine species in Australia are well managed	1	2	3	4	5
Intend to follow biofouling prevention guidelines	1	2	3	4	5
Intend to get more involved in preventing exotic marine species spread (*e.g.* by documenting myself, participating in campaigns, spreading the word, etc.)	1	2	3	4	5

Section 4. Demographics

5. What is your home marina?____
6. How many days in a year is your boat outside of its home marina? ___
7. What was the furthest destination you journeyed to with your boat in the last 12 months?_____
8. What type of trips do you do?
    - ☐ Local trips (within WA)
    - ☐ Regional trips (to other regions of Australia)
    - ☐ Overseas
9. What type of boat do you have? (motorboat, sailboat…)____________________________

Social-perception of marine biological invasions associated to recreational boating

- SURVEY FOR THE GENERAL PUBLIC-

******************** SAME STRUCTURE AND QUESTIONS EXCEPT FOR ********************

Section 3. Your attitudes towards regulations of exotic marine species

1. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Regulations for management of exotic marine species are necessary					
Exotic marine species are well managed in Australia					
I intend to get more involved in preventing exotic marine species spread (e.g. by documenting myself, participating in campaigns, spreading the word, etc.)					

S3. QUESTIONNAIRES FOR NEW ZEALAND

Social-perception of marine biological invasions associated with recreational boating

- SURVEY FOR MARINA OPERATORS -

Section 1. About you

10. Which of the following marine (*i.e.* ocean or beach) activities do you participate in?
    - ☐ Boating
    - ☐ Swimming
    - ☐ Fishing
    - ☐ Scuba diving / Snorkeling
    - ☐ Photography
    - ☐ Kayaking
    - ☐ Surfing / Waterskiing /Jet ski
    - ☐ Marine wildlife watching/interaction
    - ☐ Coastal hiking
    - ☐ Other ________________

11. Think of the areas where you do those activities. Why do you choose them?
    - ☐ They are close and convenient
    - ☐ There are no/few alternatives
    - ☐ They are my favourites

12. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I feel a connection to the areas where I do marine activities	1	2	3	4	5
I care about the environmental health of these areas	1	2	3	4	5

Section 2. Your attitudes towards exotic marine species

13. Do you know what an exotic marine species is? Yes / No
14. Do you know what an invasive species/pest is? Yes / No
15. Can you name any examples of exotic marine species?

16. How do you think exotic marine species can spread? Click the one(s) you consider

☐ Attached to the hull of recreational vessels
☐ Attached to the hull of commercial vessels
☐ Via ballast water
☐ Via aquaculture

17. Can exotic marine species live on artificial structures in the marinas (ropes, buoys, wheels, pontoons etc.)? Yes / No / Unsure

18. Are you familiar with the following invasive marine species of this area?

Yes / No

Yes / No

Yes / No

19. Do you consider marine exotic species to be a relevant problem? Yes / No / Unsure

20. Please rate the following impacts of exotic marine species according to how relevant you consider them

	Not relevant	A bit relevant	Unsure	Somewhat relevant	Very relevant
Loss of biodiversity and natural habitats	1	2	3	4	5
Local extinction of native species	1	2	3	4	5
Economic loss of fisheries and marine farming/aquaculture	1	2	3	4	5
Interference with recreational activities	1	2	3	4	5
Food poisoning by ingesting toxic species	1	2	3	4	5
Human exposure to injury (biotoxins, stings, bites etc.)	1	2	3	4	5
Loss of aesthetic value of coastal landscapes	1	2	3	4	5
Loss of historical/cultural value of an area	1	2	3	4	5

Section 3. Your attitudes towards regulations of exotic marine species

21. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Good boat maintenance helps to stop the spread of exotic marine species	1	2	3	4	5
Regulations for management of exotic marine species are necessary	1	2	3	4	5
I am familiar with the biofouling guidelines from the New Zealand Government for recreational vessels	1	2	3	4	5
Biofouling guidelines from the NZ Government for recreational vessels are created fairly	1	2	3	4	5
Exotic marine species in New Zealand are well managed	1	2	3	4	5
I intend to get more involved in preventing exotic marine species spread (*e.g.* by documenting myself, participating in campaigns, spreading the word, etc.)	1	2	3	4	5

22. What type of in-water cleaning of the hull do you allow in the marina?

☐ Only using a soft cloth for rubbing the hull to remove the slime (*e.g.* as some people do before a regatta)
☐ Rubbing plus scrapping in the case of hard substrate such as barnacles
☐ Only done by professional scuba divers
☐ It is up to the boat owner how to perform it
☐ We don’t allow in-water cleaning in the marina
☐ Other

23. What is the procedure if someone performs in-water cleaning in the marina in an inappropriate way (ignoring the marina regulations)?

24. Do you have cleaning / antifouling requirements for <u>visiting boats</u> arriving to the marina?

☐ Yes (please specify) ____
☐ No

25. Do you have cleaning / antifouling requirements for <u>resident boats</u> returning to the marina?

☐ Yes (please specify) ____
☐ No

Section 4. Communicating about exotic marine species

26. Through which source(s) have you heard information or received education about exotic marine species? Click the one(s) you consider

Media

☐ magazines or newsletter articles
☐ info from fishing/recreational boating guides or brochures
☐ TV (news, documentaries etc.)
☐ Internet websites
☐ Social networks (Facebook, Instagram, Twitter, Youtube etc.)
☐ Radio

Events

☐ organized by recreational boating or fishing organization
☐ organized by environmental organizations
☐ Outreach campaigns or workshops
☐ Citizen science initiatives (*e.g.* collaborating reporting exotic marine species I encounter)
☐ Participating in surveys

Personal contacts

☐ family members, friends or neighbours
☐ members of a boating club, bait shop vendor, marina staff, etc.

☐ Other____
☐ None

27. Did these sources improve your understanding on the risks of exotic marine species? Yes / No

28. Which source from question 17 do you consider the most useful to get informed about exotic marine species? Please add an asterisk next to the source(s)

29. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I trust the accuracy of information I see on social media	1	2	3	4	5
I trust the accuracy of information found on scientific articles	1	2	3	4	5
I trust the accuracy of information given by environmental organizations	1	2	3	4	5
I trust the accuracy of information I receive from personal contacts (family, friends, marina staff, etc.)	1	2	3	4	5

Section 5. Demographics

30. What is your place of residence (city and country)? ____________________

31. What is your age?

☐ 16-25
☐ 26-35
☐ 36-45
☐ 46-55
☐ 56-65
☐ 65 and over
☐ Choose not to say

32. What is your gender?

☐ Female
☐ Male
☐ Other
☐ Choose not to say

33. What is your highest level of education?

☐ Primary
☐ Secondary
☐ Tertiary
☐ Postgraduate (Masters, doctoral etc.)
☐ Choose not to say

34. What is (or has usually been) your job sector?

☐ Accountancy and Taxation
☐ Advertising, Marketing and public relations
☐ Animals and Veterinary science
☐ Architecture, construction and Property
☐ Art and Design
☐ Banking and Financial services
☐ Biological, chemical and Pharmaceutical science
☐ Biomedical Technologies
☐ Business management and human resources
☐ Charity and Voluntary work
☐ Clerical and Administration
☐ Computers and ICT
☐ Earth and Environment
☐ Education and Teaching
☐ Engineering and Manufacturing
☐ Farming, Horticulture and Forestry
☐ Fashion and Beauty
☐ Food and Beverages
☐ Government, politics and law
☐ History, Culture and Languages
☐ Homemaker
☐ Insurance
☐ Leisure, Sport and Fitness
☐ Marine and Maritime
☐ Media and Publishing
☐ Music and Performing Arts
☐ Medical and Healthcare
☐ Psychology and social care
☐ Physics, Mathematics and Space Science
☐ Sales and Retail
☐ Security and Defense
☐ Tourism and Hospitality
☐ Transport and Logistics
☐ Other __________

Thank you kindly for your participation

Social-perception of marine biological invasions associated to recreational boating

- SURVEY FOR BOAT OWNERS -

**************** SAME STRUCTURE AND QUESTIONS EXCEPT FOR *******************

Section 3. Your attitudes towards regulations of exotic marine species

35. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Good boat maintenance helps to stop the spread of exotic marine species	1	2	3	4	5
I am familiar with and understand the biofouling guidelines from the New Zealand Government for recreational vessels	1	2	3	4	5

36. How frequently do you…

	Less than once a year	Once a year	More than once a year	Monthly	NA
conduct visual inspections of boats and equipment (including propeller, hull, anchor, fenders, cordate, tenders etc.)	1	2	3	4	
redo your anti-fouling coating	1	2	3	4	
haul your craft out of the water to clean it	1	2	3	4	
undertake in-water cleaning of your hull	1	2	3	4	

37. Do you conduct in-water cleaning of the hull?

- ☐ Yes, using a soft cloth to remove the slime
- ☐ Yes, using a soft cloth to remove the slime and a scrapper/brush for organisms such as algae, barnacles, bivalves, tubeworms etc.
- ☐ Yes, in other way (please specify)____
- ☐ No

(if yes), where do you conduct the in-water cleaning?

- ☐ Within the marina
- ☐ Within the harbour / gulf
- ☐ Offshore
- ☐ Other ___________________

38. Do you keep your antifouling / cleaning maintenance information logged? Yes / No / NA

39. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Regulations for management of exotic marine species are necessary	1	2	3	4	5
Biofouling guidelines from the New Zealand Government for recreational vessels are fair	1	2	3	4	5
Exotic marine species in New Zealand are well managed	1	2	3	4	5
I intend to follow biofouling prevention guidelines	1	2	3	4	5
I intend to get more involved in preventing exotic marine species spread (*e.g.* by documenting myself, participating in campaigns, spreading the word, etc.)	1	2	3	4	5

Section 5. Demographics

40. What is your home marina?____
41. How many days in a year is your boat outside of its home marina? ___
42. What was the furthest destination you journeyed to with your boat in the last 12 months?_____
43. What type of trips do you do?
    - ☐ Local trips (within North Island)
    - ☐ Regional trips (to other regions of New Zealand)
    - ☐ International
44. What type of boat do you have? (motorboat, dinghy, sailboat, houseboat…) _______

Social-perception of marine biological invasions associated with recreational boating

- SURVEY FOR THE GENERAL PUBLIC -

**************** SAME STRUCTURE AND QUESTIONS EXCEPT FOR ****************

Section 3. Your attitudes towards regulations of exotic marine species

1. Please rate each of the following statements:

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Regulations for management of exotic marine species are necessary	1	2	3	4	5
Exotic marine species are well managed in New Zealand	1	2	3	4	5
I intend to get more involved in preventing exotic marine species spread (*e.g.* by documenting myself, participating in campaigns, spreading the word, etc.)	1	2	3	4	5

S4. QUESTIONNAIRES FOR SPAIN

Percepción social de las invasiones marinas asociadas a embarcaciones de recreo

- CUESTIONARIO PARA LA DIRECCIÓN DEL PUERTO -

Sección 1. Sobre usted

45. ¿Qué actividades practica en el mar?

☐ Naútica (yate, vela, barca…)
☐ Nadar
☐ Pescar
☐ Buceo / Snorkel
☐ Fotografía
☐ Kayak / Moto acuática
☐ Surf / Windsurf / Kitesurf / Esquí acuático
☐ Avistamiento / Interacción con fauna marina
☐ Senderismo por la costa
☐ Otra (especificar)________________________________

46. Piense en los lugares donde practica estas actividades. ¿Por qué los elige?

☐ Están cerca y son accesibles / convenientes
☐ Hay pocas o ninguna alternativa
☐ Son mis favoritos

47. Para las siguientes afirmaciones, marque la opción que mejor defina su opinión:

	Totalmente en desacuerdo	En desacuerdo	Neutral	De acuerdo	Totalmente de acuerdo
Siento una conexión con los lugares donde practico actividades en el mar	1	2	3	4	5
Me importa la salud ambiental de estos lugares	1	2	3	4	5

Sección 2. Su opinión sobre las especies marinas exóticas

48. ¿Sabe lo que es una especie exótica? Sí / No
49. ¿Sabe lo que es una especie invasora? Sí / No
50. ¿Podría nombrar algún ejemplo de especie marina exótica?

51. ¿Cómo piensa que se propagan las especies marinas exóticas? Marque las opciones que considere.

- ☐ Pegadas a los cascos de los barcos de recreo
- ☐ Pegadas a los cascos de buques mercantiles
- ☐ A través del agua de lastre
- ☐ A través de la acuicultura

52. ¿Cree que las especies marinas exóticas pueden vivir en las estructuras de los puertos deportivos (cuerdas, boyas, ruedas, pantalanes etc.) Sí / No / No lo sé

53. ¿Está familiarizado con las siguientes especies marinas invasoras de nuestras costas?

Sí / No

Sí / No

Sí / No

54. ¿Considera que las especies marinas exóticas son un problema? Sí / No / No lo sé

55. Estos son diferentes impactos causados por especies marinas exóticas. Clasifíquelos de acuerdo a la relevancia que tengan para usted.

	No relevante	Poco relevante	No estoy seguro/a	Relevante	Muy relevante
Pérdida de biodiversidad y hábitats naturales	1	2	3	4	5
Extinción local de especies nativas	1	2	3	4	5
Pérdidas económicas en pesquerías y acuicultura	1	2	3	4	5
Interferencia con actividades de ocio	1	2	3	4	5
Envenenamiento por ingestión de especies tóxicas	1	2	3	4	5
Exposición a heridas (biotoxinas, picaduras, mordeduras etc.)	1	2	3	4	5
Pérdida de valor estético/paisajístico de las costas	1	2	3	4	5
Pérdida de valor histórico/cultural de un área	1	2	3	4	5

Sección 3. Su opinión sobre el control de especies marinas exóticas

56. Marque la opción que mejor defina su opinión:

	Totalmente en desacuerdo	En desacuerdo	Neutral	De acuerdo	Totalmente de acuerdo
Un buen mantenimiento y limpieza de los barcos ayuda a evitar la propagación de especies exóticas	1	2	3	4	5
Conozco las medidas de la OMI para controlar la propagación de especies exóticas asociadas a la navegación de recreo	1	2	3	4	5
Es necesaria una regulación para el control de especies marinas exóticas	1	2	3	4	5
Es necesaria una regulación del mantenimiento de barcos recreativos para el control de especies marinas exóticas	1	2	3	4	5
Me interesaría involucrarme más en la prevención de especies marinas exóticas (ej. informándome, participando en talleres científicos, comentando el tema entre mis conocidos)	1	2	3	4	5

57. ¿Qué métodos de limpieza con el barco dentro del agua están permitidos en su puerto?

☐ Técnicas suaves (ej. esponja) para quitar la capa de verdina
☐ Técnicas suaves + raspado (ej. espátula o cepillo) para quitar incrustaciones más duras (ej. moluscos)

☐ Sólo los realizados por buzos profesionales
☐ Los propietarios pueden elegir cómo hacer la limpieza
☐ No permitimos limpiar el barco dentro del agua en este puerto
☐ Otro (especificar) ____

58. ¿Qué protocolo sigue la dirección del puerto si alguien realiza un método de limpieza no permitido?

59. ¿Hay algún requerimiento de limpieza / antifouling para barcos visitantes a su llegada al puerto?

☐ Sí (especificar) ____
☐ No

60. ¿Y para los barcos residentes a su vuelta al puerto?

☐ Sí (especificar) ____
☐ No

Sección 4. Divulgación sobre especies marinas exóticas

61. ¿A través de qué medio(s) ha recibido educación ambiental u oído hablar sobre especies marinas exóticas? Marque el / los que considere

Medios de comunicación

☐ revistas o artículos divulgativos / científicos
☐ información a través de guías / folletos de náutica o pesca
☐ televisión (noticias, documentales etc.)
☐ internet
☐ redes sociales (Facebook, Instagram, Twitter, Youtube etc.)
☐ radio
☐

Eventos

☐ organizados por asociaciones/grupos/clubs de pesca o puertos deportivos
☐ organizados por asociaciones medioambientales
☐ campañas de divulgación o talleres
☐ iniciativas de ciencia ciudadana (ej. informando a las autoridades sobre especies exóticas marinas que encuentro, haciendo seguimientos etc.)
☐ participando en cuestionarios

Contactos personales

☐ familia, amigos, vecinos
☐ miembros del club náutico, trabajadores de puertos, trabajadores de pesca deportiva etc.

☐ Otro (especificar)____
☐ Ninguno

62.¿Y aumentaron su conocimiento sobre los riesgos de dichas especies? Sí / No

63.¿Qué medios, de los mencionados en la pregunta 17, considera más útiles para informarse sobre especies exóticas marinas? Por favor, marque un asterisco a su lado

64.Marque la opción que mejor defina su opinión:

	Totalmente en desacuerdo	En desacuerdo	Neutral	De acuerdo	Totalmente de acuerdo
Confío en la información de las redes sociales	1	2	3	4	5
Confío en la información de los artículos científicos	1	2	3	4	5
Confío en la información que dan organizaciones medioambientales	1	2	3	4	5
Confío en la información que recibo de contactos personales (familia, amigos, trabajadores de puertos etc.)	1	2	3	4	5

Sección 5. Perfil demográfico

65. Lugar de residencia (ciudad y país) ____________________________

66. Edad

☐ 16-25
☐ 26-35
☐ 36-45
☐ 46-55
☐ 56-65
☐ Mayor de 65
☐ No contestar

67. Género

☐ Hombre
☐ Mujer
☐ Otro
☐ No contestar

68. Nivel educativo

☐ Primaria
☐ Secundaria
☐ Universidad
☐ Posgrado (Master, doctorado etc.)
☐ No contestar

69. Sector laboral

☐ Administración de empresas, recursos humanos
☐ Administrativos y secretariado
☐ Asesoría fiscal / auditoría
☐ Agricultura, ganadería, forestal
☐ Alimentación
☐ Arquitectura, construcción, inmobiliaria
☐ Arte, diseño
☐ Banca y seguros
☐ Ciencias biológicas, químicas y farmacéuticas
☐ Comercial, ventas
☐ Deporte, fitness y ocio
☐ Educación
☐ Energía y medio ambiente
☐ Física, matemáticas y astronomía
☐ Historia, cultura, idiomas
☐ Hostelería, turismo
☐ Ingeniería y producción
☐ Logística y Transporte
☐ Marítimo
☐ Medios de comunicación, editorial
☐ Minería
☐ Moda, textil, belleza
☐ Música y artes escénicas
☐ Pesca, acuicultura y silvicultura
☐ Política y derecho
☐ Publicidad, marketing, relaciones públicas
☐ Sanidad, salud y servicios sociales
☐ Seguridad y defensa
☐ Tecnología e Informática
☐ Telecomunicaciones
☐ Voluntariado
☐ Otro. Especificar____________

Percepción social de las invasiones marinas asociadas a embarcaciones de recreo

- CUESTIONARIO PARA PROPIETARIOS DE BARCOS -

**************** SAME STRUCTURE AND QUESTIONS EXCEPT FOR ****************

Sección 3. Su opinión sobre el control de especies marinas exóticas

70. Marque la opción que mejor defina su opinión:

	Totalmente en desacuerdo	En desacuerdo	Neutral	De acuerdo	Totalmente de acuerdo
Un buen mantenimiento y limpieza de los barcos ayuda a evitar la propagación de especies exóticas	1	2	3	4	5
Conozco las medidas de la OMI para controlar la propagación de especies exóticas asociadas a la navegación de recreo	1	2	3	4	5

71. ¿Con qué frecuencia realiza las siguientes tareas?

	<1 vez al año	Una vez al año	> 1 vez al año	Una vez al mes	No aplica
Cambiar la pintura antifouling	1	2	3	4	
Sacar el barco fuera del agua para limpiarlo	1	2	3	4	
Limpiar con el barco en el agua	1	2	3	4	

72. ¿Lleva a cabo la limpieza del casco con el barco dentro del agua?

☐ Sí, usando técnicas suaves (ej. esponja) para quitar la verdina
☐ Sí, usando técnicas suaves + una espátula o cepillo para organismos más duros como moluscos, gusanos tubo etc.
☐ Sí, de otro modo (especificar)___________________________________
☐ No

(en caso afirmativo), ¿dónde suele llevar a cabo la limpieza?

☐ En el puerto deportivo
☐ En el golfo / bahía
☐ Lejos de la costa
☐ Otro ________________

73. Marque la opción que mejor defina su opinión:

	Totalmente en desacuerdo	En desacuerdo	Neutral	De acuerdo	Totalmente de acuerdo
Es necesaria una regulación para el control de especies marinas exóticas	1	2	3	4	5
Es necesaria una regulación del mantenimiento de barcos recreativos para el control de especies marinas exóticas	1	2	3	4	5

Me interesaría involucrarme más en la prevención de especies marinas exóticas (ej. informándome, participando en talleres científicos, comentando el tema entre mis conocidos)	1	2	3	4	5

Sección 5. Perfil demográfico

74. Días al año que su barco pasa fuera de su puerto habitual: ___
75. Lugar más lejano al que viajó con su embarcación en los últimos 12 meses:___________
76. ¿Qué tipo de viajes realiza en su barco?
    - ☐ Locales (costa andaluza)
    - ☐ Regionales (a otras comunidades españolas)
    - ☐ Internacionales
77. Tipo de barco (yate, velero, lancha a motor, barco casa…)

Percepción social de las invasiones marinas asociadas a embarcaciones de recreo

- CUESTIONARIO GENERAL –

****************** SAME STRUCTURE AND QUESTIONS EXCEPT FOR ******************

Sección 3. Su opinión sobre el control de especies marinas exóticas

1. Marque la opción que mejor defina su opinión:

	Totalmente en desacuerdo	En desacuerdo	Neutral	De acuerdo	Totalmente de acuerdo
Es necesaria una regulación para el control de especies exóticas marinas	1	2	3	4	5
Me interesaría involucrarme más en la prevención de especies exóticas marinas (ej. informándome, participando en talleres científicos, comentando el tema entre mis conocidos)	1	2	3	4	5

S5. SURVEY CONSIDERATIONS AND CONTROL FOR BIASES

1. All different interviewers were previously trained to standard protocols to ensure replicability (Stewardt and Cash, 2008). Quality control and quality assurance of interview techniques was done by Gemma Martínez-Laiz, with the supervision of Dr. Marnie Campbell.
2. A stratified sampling was carried out with every third person asked to participate, with snowballing occurring if others ask to participate. Exceptions were people who showed clear signs of stress or being busy. Some examples were: people talking on the phone, parents telling off kids, people walking in a rush, or sailing competitors just after arrival to the marina while they clean up.
3. Only people in their free time were invited to fill in the survey, no one was bothered during working hours
4. We did not include people under age or with mental disability in the survey.
5. If a person declined to participate, we did not insist.
6. Sampling was randomized across days, locations and times to increase the selection of people in the survey frame.
7. The same formula was always used when approaching a potential respondent, and before carrying out the survey, we checked (when possible) if the respondent was a resident in the area.
8. When a person accepted to participate in the survey, we offered two ways of completing it: they could fill it themselves privately, or we could read the questions out loud to them. They chose freely the option that felt more comfortable and, in any case, we assured that the survey remained anonymous.
9. We controlled for halo effect as much as possible. When approaching a group, all surveys were delivered to individuals willing to participate at the same time, to avoid one person's feedback to influence in the views or willingness to participate of others.

5

DISCUSIÓN Y DIRECCIONES FUTURAS

De cara al futuro, lo primero a destacar es que estamos ante un problema ambiental que se espera siga aumentando de forma exponencial. Según el último estudio del ICES (2019), el riesgo de introducciones va a estar determinado por dos factores: en primer plano por los cambios en los vectores, rutas y vías de propagación; y en segundo plano por el grado de alteración antropogénica, incluyendo modificación costera y cambio climático. Un análisis reciente socioeconómico ha estimado que el tráfico marítimo internacional seguirá aumentando exponencialmente entre el 240 y el 1209% en los próximos 30 años; tanto en tonelaje y número de barcos como en diversificación de rutas; acarreando consigo un aumento del riesgo de invasión global de 3 a 20 veces el actual (UNCTAD 2017; Sardain et al. 2019). Por otra parte, el cambio climático está jugando un papel fundamental; siendo especialmente preocupante la situación en los polos, donde se esperan aumentos dramáticos de las tasas de establecimiento (Poloczanska and Butler 2010; Gold-smit et al., 2018). No sólo zonas de altas latitudes se convertirán en nuevos nodos de paso para especies marinas exóticas (Ware et al. 2014); sino que ya se habla de la creación de puertos árticos para facilitar el tráfico transártico uniendo Asia, Europa y Norteamérica, el cual se estima económicamente factible hacia mitad de siglo si la tasa de deshielo Ártico no se ralentiza (Hansen et al. 2016). De entrada hay evidencias de que el *biofouling* puede sobrevivir el tránsito polar en condiciones concretas (Chan et al., 2016); y recientemente se ha registrado el establecimiento del mejillón exótico *Mytilus cf. platensis* en la Antártica, procedente de un clado endémico de Patagonia del sur según evidencia genética, y donde se apunta al *biofouling* como vector más probable de introducción de propágulos (Cardenas et al. .2020).

Este escenario se puede modificar mediante la introducción de directivas, nuevas herramientas tecnológicas y mejores prácticas de gestión dirigidas al vector (Sardain et al., 2019); es decir, aliviando la presión de propágulos en las fases tempranas de invasión. Como se ha demostrado en los anteriores capítulos, la navegación de recreo es una vía de propagación secundaria muy efectiva, capaz de aumentar rápidamente los rangos de distribución de los invasores y de introducir poblaciones exóticas tanto a escala local, como entre ecoregiones, como a nivel de toda la cuenca mediterránea. Carreño et al. (2021) revisa de forma holística los impactos ecológicos del sector naútico en dicho mar, destacando el crecimiento que se espera en términos de número de barcos y puertos deportivos. De hecho, desde 2008 la producción de embarcaciones recreativas en países Mediterráneos Europeos ha crecido a razón de un 10% anual. Teniendo esto en cuenta, el papel de la navegación de recreo como vía de propagación deberá, sin falta, ser considerado dentro de los planes de gestión de invasiones marinas en los próximos años. Esta gestión a nivel internacional implicará enormes esfuerzos al tratarse de un mar rodeado de múltiples jurisdicciones, ya que la capacidad nacional para responder a las invasiones difiere de país en país (Early et al., 2016); por lo que, para la comunidad científica, se tornan especialmente importante aspectos como los esfuerzos colaborativos a nivel internacional ejemplificados en el capítulo 3, o mayor investigación del componente de percepción social que facilite el diálogo, como se ha explicado en el capítulo 4.

Para lograr una gestión a nivel internacional o global es imprescindible construir primero aportaciones a escala nacional (Latombe et al., 2017; Pyseck et al. 2020). Como se comentó anteriormente, España es el segundo país con más puertos deportivos del Mar Mediterráneo; y en su plan de actuación de 2021 se observa que hace referencia al vector *biofouling* y/o a la navegación de recreo en algunas ocasiones:

- Se reconoce que la dispersión de EEI se "acentúa por la navegación fluvial y marina", señalando tanto al tráfico comercial como recreativo (Medida 5.1, en el eje de acción coordinación y governancia).
- Se señala como agentes implicados a "Organismos públicos con competencia en EEI a nivel europeo, estatal, autonómico y local", "Propietarios y usuarios de embarcaciones en medio marino y terrestre" y " Operadores de buques mercantes y usuarios de embarcaciones de recreo, autoridades portuarias y concesionarios de puertos deportivos (marinas) e instalaciones de mantenimiento y reparación de naves (astilleros y varaderos) (ME 5.1)
- Se reconoce la necesidad de difundir "medidas de control para incrustaciones y aguas de lastre en aguas navegables tanto marinas como continentales" (ME 1.7, en el eje de acción Comunicación y Sensibilización), donde específicamente indica "contemplar como objetivo a la navegación recreativa" y "Controlar y registrar los eventos náuticos".

Aunque las medidas propuestas para estos puntos dan prioridad al agua de lastre, sí se indica para el *biofouling* "Intensificar la limpieza de cascos" y, específicamente para los barcos de recreo: "propiciar que pasen el invierno en dique seco y divulgar la guía de buenas prácticas elaborada por la Organización Marítima Internacional sobre la gestión del *biofouling*", así como "supeditar la autorización de actividades ligadas a las vías acuáticas en el ámbito competencial de la administración al cumplimiento de condiciones con el fin de prevenir y/o limitar la entrada y expansión de especies invasoras". Se concluye, por tanto, que contamos con el precedente del recientemente implementado Convenio para el agua de lastre y además sí hay una disposición en el marco legislativo español a reconocer este vector atajando también las embarcaciones privadas. Con la iniciativa GloFouling de la OMI en el horizonte, es cuestión de tiempo que se requiera incluir el vector *biofouling* en el Plan de Acción, así como medidas de gestión que apliquen también a la navegación de recreo como vía de propagación. De cara a ello y en base a la investigación desarrollada en esta tesis, se destacan las siguientes direcciones de investigación futuras:

5. 1 Mapeo del riesgo de invasión y monitorización a largo plazo en puertos deportivos españoles

En primer lugar, indicar la importancia de implementar un protocolo estandarizado unificado de muestreo para *biofouling* en puertos; de forma que las investigaciones destinadas a mapear o monitorizar que se proponen a continuación puedan producir datos comparativos a nivel internacional. Las metodologías de "SERC method" y "ANINEM protocol (Assessment of Non-Indigenous Mobile Epibenthic Macrofauna)" ofrecen soluciones cuantitativas de bajo coste y fáciles de aplicar, y se encuentran actualmente en ensayo (Tamburini et al., 2021; Ros et al., 2019). En concreto, se sugiere la puesta en marcha de las siguientes actividades:

- Listado de especies marinas introducidas en el territorio español. Como se explicó en la introducción y en el capítulo 3 – parte 1.1, es el primer paso hacia el desarrollo de un sistema estandarizado a nivel nacional de vigilancia y seguimiento a largo plazo (*long-term monitoring*).

- Mapeo del índice de biocontaminación (BCI) en puertos deportivos españoles. Los resultados descritos en el capítulo 1 - parte 1 ponen de manifiesto la relevancia del factor "identidad del puerto deportivo", mostrando cómo diferentes puertos suponen un mayor o menor riesgo de infección para las embarcaciones que paran en ellos. Recientemente Guerra-García et al. (2021) propusieron una metodología integradora para evaluar la salud ambiental de los puertos deportivos, ensayada en la costa andaluza y portuguesa, que incluye el índice de biocontaminación como un indicador clave. Es deseable el mapeo de todo el territorio español, identificando áreas o polígonos de prioridad de cara a 1) proporcionar información de calidad que nutran las bases de datos a nivel local, regional, nacional y europeo; 2) dar prioridad de recursos de vigilancia y urgencia de actuación de medidas de contingencia o erradicación; 3) identificar estaciones centinelas donde basar programas de vigilancia y seguimiento a largo plazo (*long-term monitoring*); y 4) poder adaptar las medidas de gestión a áreas especialmente vulnerables o protegidas; por ejemplo, exigiendo medidas de control extra a las embarcaciones que entran en ellas (ver ejemplo de gestión en las regiones de Northland https://www.nrc.govt.nz/maritime/marine-pollution-and-boat-cleaning/visiting-a-northland-marina-this-summer/ y Fiordland https://www.es.govt.nz/environment/biosecurity-and-biodiversity/marine-biosecurity/fiordland-marine-pathway-plan en Nueva Zelanda); o a aquellas embarcaciones que provengan de áreas con un BCI alto o donde se ha registrado una nueva invasión (https://marlboroughmarinas.co.nz/uncategorized/identifying-and-protecting-against-mediterranean-fanworm/; accedido el 3/02/21)
- Análisis de riesgo de invasión en puertos deportivos españoles y de los factores que lo determinan. Para ello es necesario un enfoque socio-ecológico, como se ha indicado en el capítulo 1 - parte 1 y en el capítulo 4, que permita considerar:
    - La carga de especies exóticas que portan las embarcaciones. Para esto es necesario cuantificar el BCI directamente de los barcos recreativos que llegan a puertos deportivos españoles, idealmente durante más de un año (ver ejemplo Inglis et al. 2010).
    - Dónde se enmarcan los dueños de barcos españoles en una escala de comportamientos de riesgo (ver ejemplo de Ferrario et al. 2016). Para ello se seguirá analizando los datos obtenidos en el capítulo 4 sobre principalmente las prácticas de limpieza y tipo de barco; a lo que sería necesario añadir otros como rutas de navegación, perfil operacional, diseño de barco y tiempo de residencia en puerto (Ulman et al., 2019); ya que se sabe que la forma óptima de gestionar el *biofouling* variará en función de ellas (ICES, 2019, Bell et al., 2011).
    - La percepción social de los dueños de los barcos, incluyendo en este modelo las variables que resulten determinantes al completar el análisis de datos del capítulo 4.
- Establecimiento de un sistema estandarizado a nivel nacional de vigilancia y seguimiento a largo plazo (*long-term monitoring*) de especies exóticas (*National alien species surveillance program*) (ver ejemplo en Latombe et al., 2017 y guía para países de GEO BON 2015 http://invasionevs.com/wp-content/uploads/2015/08/MonitoringBiologicalInvasions.TechnicalReport.pdf). Como se ha indicado en el capítulo 3 - parte 2, las técnicas moleculares son de gran ayuda a la hora de recabar información sobre la dinámica de post-establecimiento de las especies. Concretamente, hoy en día las técnicas de *high-throughput sequencing* ofrecen herramientas potentes ya sea para la detección temprana como para el seguimiento a largo plazo (Pochon et al., 2013; Mahon et al.,

2014; Rius et al., 2015; Xiong et al., 2016); siempre teniendo en cuenta que es necesaria una sinergia entre los datos genómicos y los esfuerzos "tradicionales" de muestreo a grandes escalas geográficas y análisis taxonómicos (Viard et al., 2016).

5.2 Viabilidad de medidas potenciales de gestión

Estudios previos y la experiencia de gestión de otros países coinciden en varios puntos en común sobre las pautas para unas buenas prácticas de limpieza que prevengan el crecimiento del *biofouling* y el transporte de especies exóticas de un puerto a otro (Georgiades et al. 2020 y referencias en tabla 3). Se insiste en la importancia de no usar la pintura antifouling más allá de su vida útil, de que se renueve al menos una vez al año (a veces se recomiendan 6 meses), que se lleve a cabo una limpieza al menos dos veces al año y antes de viajar a otras regiones; y de que un nivel de biofouling más allá de una capa de verdina (*slime, biofilm*) (a veces se aceptan balanos que no superen el 5% del total de la superficie del casco y áreas nicho) ya no es seguro desde el punto de vista de la bioseguridad. Los resultados del capítulo 1 - parte 2 apoyan esta idea, mostrando que hasta una especie que en principio tiene poca resistencia al hidrodinamismo puede sobrevivir con facilidad al transporte si encuentra matas de macrofouling adecuado, lo cuál de por sí es frecuente en las áreas nicho de las embarcaciones que escapan del mantenimiento más que otras zonas (Coutts et al., 2003; Coutts and Taylor 2004; Davidson et al. 2010; Clarke Murray et al. 2011; Frey et al., 2014). Una apuesta de gestión similar en España, en este caso en agua dulce, ha sido el endurecimiento de las normas de limpieza, navegación y cuarentena en la Cuenca del Ebro con el fin de frenar la propagación del mejillón cebra en los embalses (http://www.chebro.es/contenido.visualizar.do?id Contenido=64743; accedido el 2/12/21).

En el medio marino, sin embargo, existe debate sobre cómo regular la práctica extendida de limpieza dentro del agua (*vessel in-water cleaning and treatment – VICT*); ya que un punto clave sigue siendo cómo capturar el 100% de los residuos. Aunque está considerada por numerosas legislaciones como una herramienta importante para mantener las embarcaciones libres de *biofouling* (especialmente las de gran tamaño), las mismas también advierten de los riesgos de bioseguridad que acarrean de no ser debidamente reguladas (IMO 2011; Department of the Environment [DOE] and New Zealand Ministry for Primary Industries [MPI], 2015; Scianni et al., 2017; Georgiades et al., 2018). Esta práctica también es muy frecuente entre las embarcaciones de recreo para alargar la vida del antifouling y minimizar costes, no sólo con fines de mantenimiento sino también deportivos (*e.g.* contratando buzos para limpiar el casco el día de antes de una regata - observación personal -); siendo practicada por alrededor del 40% de propietarios en las costas españolas y del Mar Mediterráneo (Martínez-Laiz et al., 2019; capítulo 4). Es por tanto necesario buscar técnicas preventivas alternativas que ofrezcan una opción factible económicamente a los propietarios a la vez que minimicen la liberación de propágulos al medio receptor. Para ello se está evaluando el riesgo, viabilidad y eficacia de algunas iniciativas (Bell et al. 2011; Scianni and Georgiades, 2019; https://www.awe.gov.au/biosecurity-trade/aircraft-vessels-military/vessels/marine-pest-biosecurity/biofouling/anti-fouling-and-inwater-cleaning-guidelines/in-water-cleaning-standard; accedido el 2/12/21); sin embargo, todavía se necesitan esfuerzos de investigación antes de proceder a la implementación de una normativa (ver revisión de Scianni and Georgiades, 2019 al respecto).

Por otra parte, a fin de poder predecir la acogida que dichas medidas tendrán entre la comunidad de interés, será necesario recoger más datos sobre las intenciones y preferencias/aceptación potencial de dichas medidas (*intended behaviour*). Un ejemplo es llevando a cabo estudios de percepción social, mediante cuestionarios o mesas redondas, que evalúen con qué formas de regulación estarían más de acuerdo los gestores de puertos deportivos, así como qué reticencias tendrían en adoptarlas para su jurisdicción (Sharp et al., 2016, Vye et al., 2020). Las propuestas a debate podrían empezar por acciones individuales, como inspeccionar uno mismo su embarcación en busca de especies exóticas de interés, o portar consigo un libro de registro sobre el control del *biofouling* (*biofouling record book)* a modo de carnet de identidad que mostrar a la llegada a puerto. Y seguir con mayores regulaciones como el establecimiento de un nivel límite permitido de *fouling* a la llegada a puerto o de edad de la pintura; prohibición de prácticas específicas de limpieza; uso de métodos de encapsulamiento o zonas de embarcadero para la puesta en cuarentena; o instauración de un protocolo de acción - por ejemplo, inspecciones regulares por buzos profesionales - si sucede una incursión en áreas vecinas. También podría explorarse el interés ante ciertos incentivos para la población, como una bandera azul frente a especies invasoras a modo de sello de calidad para los puertos deportivos que decidan liderar estas acciones. En todo caso, se aconseja que la implantación de nuevas medidas también vaya acompañada de evaluaciones del nivel de eficacia. Esto puede hacerse con antelación; por ejemplo, mediante simulación (modelización) de campañas que nos ayuden a ajustar parámetros como en qué momento concreto exigir la renovación de la pintura antifouling, o qué nivel de cumplimiento es necesario para que la gestión sea eficaz (Floerl et al., 2016); o *a posteriori,* con observaciones más a largo plazo del cumplimiento (*self-reported behaviour*). Por ejemplo, Scianni et al. (2021) examinó las curvas de aprendizaje de la industria naval respecto a la nueva regulación para prevenir las comunidades incrustantes en Nueva Zelanda y California (2017-2018). Esto permitió identificar, además de los puntos a corregir de la nueva legislación, las causas del no cumplimiento: en su mayoría, una falta de entendimiento de la norma (a veces por falta de claridad del lenguaje genérico de gestión), que pudo solventarse con mayor instrucción y siendo flexible en conceder periodos de gracia para corregir las infracciones. Otro ejemplo fue el estudio de la respuesta ciudadana ante la campaña “Check Clean Dry” en el reino Unido; la cual manifestó una falta de infraestructura y de redes (*e.g*. puestos de limpieza – *wash down stations* - con sistemas de agua caliente para el material de pequeño tamaño que promovieran esa norma social) para pasar de intención a acción (Shannon et al., 2019). La observación de estas fases de aprendizaje basado en la experiencia también han sido propuestas por la Organización Marítima Internacional para asistir a los dueños de barcos y operarios en la implementación de la nueva directiva sobre el agua de lastre; la cual levanta incertidumbre en varios países sobre la implementación no uniforme entre jurisdicciones, la actuación y eficacia prolongada en el tiempo de los sistemas de tratamiento de nueva instalación (*Ballas Water Treatment Systems, BWTS*), o las barreras políticas que están dificultando la gestión (Wan et al., 2018; Gerhard et al., 2019; Wright, 2021).

5.4 Concienciación pública (*public awareness*) e iniciativas de ciencia ciudadana (*citizen science initiatives*)

Como se ha explicado anteriormente, construir un diálogo entre la comunidad científica y los agentes implicados es un tema urgente a resolver. Por un lado, porque una educación ambiental insuficiente de alguno de estos grupos puede desembocar en estrategias de gestión insatisfactorias, trayendo consigo un problema recurrente: falta de implicación política y aceptación de las medidas de bioseguridad que la comunidad científica lleva años sugiriendo (Davies 2016; Hulme et al. 2018; Pyšek et al., 2020). De ahí la relevancia de apostar por campañas de educación ambiental especialmente dirigidas a los dueños de barcos y gestores de puertos deportivos, como se indicó en el capítulo 4. Y por otro, porque a la vez nos brinda una de las cuatro herramientas principales que tenemos para luchar contra las especies exóticas, junto a la legislación, los programas de bioseguridad nacional y los avances tecnológicos para la gestión (Pyšek et al., 2020).

El desarrollo de campañas de ciencia ciudadana para la gestión de especies invasoras es una opción que permite abordar una gran escala a largo plazo y con bajo coste (Roy et al., 2015; Pocock et al., 2018; Groom et al., 2019). En Europa, la más longeva corresponde a la estación de monitoreo de macroflora exótica de los puertos de Le Havre y Antifer (costa francesa del Canal de la Mancha), donde el equipo de científicos ciudadanos lleva muestreando regularmente 50 años (Verlaque and Breton, 2019). El Plan de Acción español de 2021 para el control de las especies introducidas también recomienda la promoción de dichas iniciativas (medida MT5 del eje Vigilancia y control) y hace referencia a portales donde la población puede informarse y reportar la presencia de especies exóticas; como la app puesta a punto en las Islas Canarias para avistamiento de la Culebra real de California *Lampropeltis californiae* (Blainville, 1835) (https://play.google.com/store/apps/details?id=com.inventiaplus.lampropeltis&hl=es 419; accedido 1/1/21); el portal EXOS (https://www.biodiversidadcanarias.es/ exos/informacion; accedido 1/1/21); u otras no exclusivas de fauna introducida como la Red de Observadores del medio marino (https://redpromar.org/; accedido 1/1/21) o Ornitho Euskadi (https:// www.ornitho.eus/; accedido 1/1/21). Los proyectos de esfuerzo colaborativo involucrando a ciudadanos, ONGs y universidades han demostrado ser de utilidad en múltiples fases del proceso de invasión. Algunos ejemplos son los protocolos de detección temprana, alertando de nuevas incursiones gracias a esfuerzos de vigilancia (Azzurro et al., 2013; Bodilis et al., 2014; Mannino and Balistreri, 2018; Kleitou et al., 2019); las campañas de mitigación o contención (caso del pez león en el Océano Atlantico occidental y el Caribe - Green et al., 2014, Malpica-Cruz et al., 2016 ; Usseglio et al., 2017 ; https://www.reef.org/lionfish-derbies , accedido el 11/10/21); campañas de eradicación finalizadas con éxitos (caso de *Caulerpa taxifolia* en California - Anderson et al., 2005; Muñoz, 2016) las cuales, además, son escasas en el medio marino (Simberloff, 2021); alerta de nuevos records de especies ya establecidas (Delaney et al., 2008, Giovos et al., 2019); o el monitoreo en temas de interés emergentes también asociados a las invasiones, como la distribución e impactos ecológicos de la basura marina (revisado en Hidalgo-Ruiz and Thiel, 2015). Dichas iniciativas no están exentas de limitaciones, sobre todo en cuanto a la fiabilidad de la información recolectada; lo cual implica siempre incluir protocolos de control laboriosos (validación, supervisión in situ de expertos) y abre debate sobre hasta qué punto se puede delegar responsabilidad en los llamados *biosecure citizens* (Hidalgo Ruz and Thiel, 2015, Campbell et al., 2017). Sin embargo, hay varias medidas que contribuyen a paliar estas desventajas, como la preparación de protocolos directos y sencillos, adaptando objetivo si fuera necesario (Hidalgo-Ruz and Thiel, 2013); identificar los posibles sesgos y retos que los voluntarios puedan tener y que requieren un entrenamiento en mayor profundidad, como falsa confianza en la capacidad de reconocimiento de especies (Campbell, et al, 2017), o menor familiaridad con organismos de pequeño tamaño difíciles de fotografiar (Giovos et al., 2019); y ofrecer portales de información e intercambio online abiertas y fáciles de usar que a la vez sigan promoviendo la participación (Seaward et al., 2015; https://www.qld.gov.au/environment/coasts-waterways/marine-pests; https://www.mpi.govt.nz/biosecurity/how-to-find-report-and-prevent-pests-and-diseases/report-a-pest-or-disease/ ; https://www.waikatoregion.govt.nz/services/plant-and-animal-pests/mediterranean-fanworm/ ; (accedidos el 3/1/21); ver ejemplos en Tabla 1 de Johnson et al., 2020)

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6. CONCLUSIONS

CHAPTER 2 - PART 1

- Recreational boats cruising the Mediterranean Sea do host numerous non-indigenous species (NIS) of peracarids, exchanging fauna with marina's assemblages and transporting them to new localities.
- Vessel maintenance habits and travel patterns are influencing variables for the occurrence of NIS in hull-fouling.
- It is urgent to acknowledge recreational boating as a high-risk vector for NIS introduction in the Mediterranean Sea, and to address this by developing mandatory measures to be integrated in regulations for recreational boating. The introduction of environmental education strategies would contribute positively to the cause and would allow for a better collaboration among marina personnel and the scientific community.

CHAPTER 2 - PART 2

- *Caprella mutica* does not bear an inherent advantage over its native congener in terms of resistance to drag during vessel transport. Instead, its chances of surviving highly depend on secondary substrate complexity. This confirms that basibionts act as refugia for invasive mobile taxa during the early phase of the invasion process.
- The invasive species exhibited a higher boldness behaviour than the native one. On one side, this potentially incurs a considerable risk during translocation; on the other, it confers a higher dispersal potential in terms of vector microhabitat use and subsequent colonization of the recipient environment. When dealing with similar species of biosecurity concern, management of macrofouling would be more efficient if carried out prior to the translocation phase.
- Baseline information about behavioral type-dependent dispersal of NIS, as well as their *en-route* survivorship are interesting tools to map and predict the colonization process in advance.

CHAPTER 3 - PART 1

- The survey efforts for the study of hidden-invaders resulted in significant updates of their introduced range at local, regional and global scale. The first catalog of marine exotic isopods for the Iberian Peninsula, the Southern side of the Strait of Gibraltar and the Balearic Islands is now provided. The silent invader *Stenothoe georgiana* is now reported from both hemispheres, both the Atlantic and the Pacific Ocean, and numerous locations in the Mediterranean Sea. And the very successful invader *Caprella scaura* is now present in the UK, confirming its expansion towards higher latitudes by means of commercial shipping and recreational boating.
- Detection of the last two species was only possible due to thorough taxonomic works as baseline, followed by a notably collaborative effort: an effective communication amongst expert groups and willingness to disseminate new records to raise the alarm.

- Lesson-learnt from these cases are: 1) the need of counting with a baseline list of marine exotic species present in our coasts, as starting point for reducing lags in detection; 2) promoting the relevance of taxonomy on bioinvasion research; 3) building strong expert networks that ensure communication of records and transfer of knowledge among taxonomists, molecular scientists and invasion ecologists; and 4) the urgence of implementing an efficient standardized monitoring methodology to serve a preventive management approach.

CHAPTER 3 - PART 2

- Molecular monitoring during the post-establishment phase is useful to analyze the status of the introduced populations; to detect source populations that pose a risk for propagule input; and to detect vulnerability periods of the introduced population.
- This supports the idea that the most cost-effective managing strategy is to target the vector of introduction itself. A diminishing propagule pressure is the determinant factor to weaken a highly fluctuating population that naturally undergoes vulnerable periods (low-diversity or small population size).

CHAPTER 4

- Stakeholders in Spain are far more overconfident than those in countries with nationwide regulations. Their risk perception of marine NIS and their vectors is significantly lower and tightly associated to the perceived responsibility of good vessel maintenance practices. This warrants the implementation of environmental education strategies on the issue.
- Baseline knowledge, connection feeling with the environment, and risk perception are factors worth digging into when understanding stakeholders’ attitudes towards NIS management. There is a potential support of the IMO recommended guidelines for biofouling control in recreational vessels, but boaters provided rather ambiguous data when asked about cleaning practices.
- It is vital to continue this research in order to create valuable information for a future fair and effective implementation of the biofouling vector in our country.