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Recreational boating as a major vector of spread of nonindigenous species around the Mediterranean

Aylin Ulman

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Sorbonne Université
Università di Pavia

Ecole doctorale

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**Recreational boating as a major vector of spread of non-
indigenous species around the Mediterranean**

*La navigation de plaisance, vecteur majeur de la propagation d'espèces non-indigènes
autour des marinas Méditerranéenne*

Par Aylin Ulman

Thèse de doctorat de Philosophie

Dirigée par Agnese Marchini et Jean-Marc Guarini

Présentée et soutenue publiquement le 6 Avril, 2018

Devant un jury composé de :

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Katell Guizien (President, Sorbonne University)

Ana Costa (Reviewer, University of the Azores)

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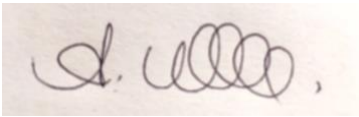
Agnese Marchini (Principal supervisor, University of Pavia)

Jean-Marc Guarini (Co-supervisor, Sorbonne University)

Christos Arvanitidis (Co-supervisor, Hellenic Centre of Marine Research, Greece)

CERTIFICATION

I, Aylin Ulman declare that this thesis, submitted in partial fulfilment of the requirements for the award Doctor of Philosophy, in the School of Earth and Environmental Sciences, University of Pavia, and for Sorbonne Université: Université de Pierre et Marie-Curie, is fully my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.



Aylin Ulman

March 7th 2018

STATEMENT OF CONTRIBUTION

My supervisors, Professors Agnese Marchini and Anna Occhipinti-Ambrogi conceptualized and focused the study topic of this thesis. I, Aylin Ulman, completed the study design, the marina sampling and interviews with boaters. I identified the majority of samples, with the contributions of the supervisor Agnese Marchini, the post-doc fellow Jasmine Ferrario, and the undergraduate students Gemma Martinez-Laiz, Giovanni Scribano, Guenda Merlo, Elisa Princisch, Ada Bandi at the Department of Ecology Laboratory at the University of Pavia. Some samples were sent or brought to the following experts for identification or verification: Christos Arvanitidis, Yanan Sun, Alfonso Ramos-Esplá, Cesare Bogi, Marco Bertolino, Cengiz Kocak and Giorgos Chatzigeorgiou. A few of these experts (Agnese Marchini, Jasmine Ferrario, Alfonso Ramos-Esplá, Marco Bertolino, Cengiz Kocak) further helped in shaping the paragraphs about the taxonomic characters and of the current known distributions for certain species.

The three data chapters (Chapters 2-4) presented in this thesis has been prepared as manuscripts in collaboration with my supervisors Agnese Marchini, Anna Occhipinti-Ambrogi and Christos Arvanitidis and are presented here in journal format.

The first data chapter has been published as the following journal article:

❏ **Chapter 2** – Ulman A, Ferrario J, Occhipinti-Ambrogi A, Arvanitidis C, Bandi A, Bertolino M, Bogi C, Chatzigeorgiou G, Çiçek BA, Deidun A, Ramos-Esplà A, Koçak Ç, Lorenti M, Martínez-Laiz G, Merlo G, Princisgh E, Scribano G and A Marchini (2017). **A massive update of non-indigenous species records in Mediterranean marinas.** *PeerJ*

The second data chapter (Chapter 3) has been submitted and is currently in review:

❏ **Chapter 3** – Ulman A, Ferrario J, Forcada A, Arvanitidis C, Occhipinti-Ambrogi A and A Marchini. **A hitchhiker's guide to non-indigenous species marina settlement.** *Diversity and Distributions*

The second data chapter (Chapter 3) assimilated the results learned from Chapter 2, and combined these results with additional data previously collected by the post-doc fellow Jasmine Ferrario from University of Pavia for a very wide-scale geographic univariate and multi-variate statistical analysis on NIS in Mediterranean marinas. Aitor Forcada offered assistance with the statistical analysis. Aylin Ulman analyzed the results and led the writing of the paper.

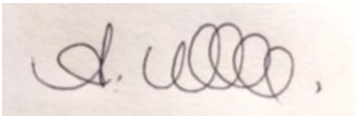
The third data chapter (Chapter 4) is in the final stages before submission:

❏ **Chapter 4** – Ulman A, Ferrario J, Seebens H, Arvanitidis C, Occhipinti-Ambrogi A and A Marchini.

Bowed down in a sea of troubles: How recreational boating is spreading alien species around the Mediterranean Target Journal: *Journal of Applied Ecology*

For the third data chapter (Chapter 4), Aylin Ulman analyzed the boater survey results along with the sampling results of the NIS found on the fouled boat-hulls. Hanno Seebens offered assistance with the statistics involving ‘Generalized Linear Models’ and all interpretations and writing of the paper were done entirely by Aylin Ulman.

As the primary supervisor, I, Professor Agnese Marchini, declare that the greater and most critical part of the work in each article listed is attributed to the candidate, Aylin Ulman. In each of the above manuscripts or chapters, Aylin led the study design and was primarily responsible for data collection, analysis and interpretation, with the exception of Chapter 3, where she had substantial assistance for the statistical analysis by Aitor Forcada, and Chapter 4, where she was provided some statistical assistance from Hanno Seebens. The manuscript presented in Chapter 2 was led by Aylin Ulman, with contributions from the expert taxonomists. Aylin was in charge of the manuscript review process with the journal. Manuscript and chapter were written by the candidate, who was then responsible for responding to comments made by her co-authors. The manuscript and Chapter presented in Chapter 3 was led by Aylin Ulman, as was Chapter 4.



Aylin Ulman
PhD Candidate

3rd March 2018



Dr. Agnese Marchini
Principal supervisor

3rd March 2018

LIST OF PUBLICATIONS, PRESENTATIONS and POSTERS

Publications in journals with impact factor

Ulman A, Ferrario J, Occhipinti-Ambrogi A, Arvanitidis C, Bandi A, Bertolino M, Bogi C, Chatzigeorgiou G, Çiçek BA, Deidun A, Ramos-Esplà A, Koçak Ç, Lorenti M, Martínez-Laiz G, Merlo G, Princisgh E, Scribano G and A Marchini · 2017. **A massive update of non-indigenous species records in Mediterranean marinas.** *PeerJ* 5: e3954.

Ulman A, Ferrario J, Forcada A, Arvanitidis C, Occhipinti-Ambrogi A, and A Marchini (In review). **A hitchhiker's guide to marina travel for alien species.** *Ecological Indicators*

Palero F, Torrado H, Perry O, Kupriyanova E, **Ulman A**, Ten Hove H & R Capaccioni-Azzati. (In prep.) Establishment of *Spirobranchus cf. tetraceros* (Annelida: Serpulidae) in the Western Mediterranean.

Ulman A, Ferrario J, Seebens H, Arvanitidis C, Occhipinti-Ambrogi and A Marchini (In prep.) Bowed down in a sea of troubles: **The role of recreational boats in the spread of alien species in the Mediterranean Sea.** Target journal: *Journal of Applied Ecology*

Other Publications

Ferrario J, **Ulman A**, Marchini A, Saracino F and A Occhipinti-Ambrogi. 2016. Non-indigenous fouling species in the marina of Rome. *Biologia Marina Mediterranea* 23(1): 224-225.

Martinez-Laiz G, **Ulman A**, Ros M and A Marchini. 2017. A combined biological and social approach to test the role of recreational boating as vector for exotics: the case of peracarids in the Mediterranean Sea. *Biodiversity Journal*, 8(2): 421-423.

Merlo G, **Ulman A**, Martinez-Laiz G, Scribano G and A Marchini. 2017. New records of alien Amphipoda in Mediterranean marinas. *Biodiversity Journal*, 8(2): 535-537.

Conference Presentations

Ulman A, Ferrario J, Forcada A, Arvanitidis C, Occhipinti-Ambrogi A and A Marchini. 9 March 2018. ICES Working Group on Introduction and Transfer of Marine Organisms (WGITMO), Madeira, Portugal. Talk presented on 'Abiotic factors affecting NIS richness and NIS assemblage compositions in Mediterranean marinas.'

Ulman A, Ferrario J Marchini, A, Arvanitidis A, Ramos-Espla- A, Guarini JM and A Occhipinti-Ambrogi. European Marine Biology Conference, Piran, Slovenia, 22-26 September 2017. Presented a talk on '*Recreational boating as a major vector of spread of marine non-indigenous species.*'

Martinez-Laiz G, **Ulman A**, Ros M and A Marchini. The 17th International Colloquium on Amphipoda, Trapani, Italy. 4-7 September 2017. Talk presented on '*A combined biological and social approach to test the role of recreational boating as vector for exotics: the case of peracarids in the Mediterranean Sea.*'

Conference Posters

Merlo G, **Ulman A**, Martinez-Laiz G, Scribano G, and A Marchini. 17th ICA International Colloquium on Amphipoda, Trapani, Sicily, 4-7 September 2017. Poster presented on '*New Records of Alien Amphipoda in Mediterranean Marinas.*'

Ferrario J, Lorenti M, Martínez-Laiz G, **Ulman A**, Marchini A and A Occhipinti-Ambrogi. 1° Congresso Nazionale Congiunto SItE – UZI – SIB. Milano, 30 Agosto -Settembre 2016. '*Anthurid invasion along the Mediterranean coasts: the spreading of *Mesanthura cf. romulea* and *Paranthura japonica*.*'

Ulman A, Marchini A, Arvanitidis C and A Occhipinti. Mares Conference in Marine Ecosystem Health, Olhao, Portugal, 2-4 February 2017. Presented a poster on '*Recreational Boating as a vector of spread of alien species around the Mediterranean.*'

Ulman A, Marchini A, Arvanitidis C and A Occhipinti. A. 9th International Conference on Marine Bioinvasions. Sydney, Australia, 19-21 January 2016. Presented a poster on '*Recreational Boating as a vector of spread of alien species around the Mediterranean.*'

Ramos-Esplá A, Berecibar E, Chainho AC, Castanheira AC, Dias F, Frias P, Henriques FF, Henriques M, Jesus DC, Moreira PM, Pilar-Fonseca T, Sá J, Tavares SC, and **A Ulman**. XIX Iberian Symposium on Marine Biology Studies. Porto 5 - 9 September 2016. Poster presented on '*Rapid Assessment Survey of two contrasting marinas near Lisbon: Ascidiacea (Chordata: Tunicata).*'

Ferrario J, **Ulman A**, Marchini A, Saracino F and A Occhipinti. Societa' Italiana Di Biologia Marina (SIMB), Torino, 24-28 July 2016. Poster presented on '*Non-indigenous species in the Port of Rome.*'

Ulman A and A Ramos-Esplá. COST Alien Ascidian Taxonomy and Identification Training Poster. 14 January 2017. University of Alicante, Spain. Submitted to COST Alien Challenge for Training Award Beneficiary. COST-STSM-TD1209-051116-080962.

OTHER PUBLICATIONS DURING PHD DURATION (not directly related to this PhD research subject)

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- Demirel N, **Ulman A** and Daskalov G (In prep.) Reassessing the role of bonito and bluefish as top predators in the Black Sea using the Catch-MSY method. Target journal: *Fish & Fisheries*
- Ulman A**, Gerami M, Giovos I and Z Jahromi (In prep.) The status of sharks of the Iranian Persian Gulf fisheries. Target journal: *Endangered Species Research*
- Ulman A**, Demirel N, Zengin M and D Pauly (In prep.) The fish of Turkey's past: A recent history of disappeared species and commercial fishery extinctions for the Black and Marmara Seas. Target journal: *PLoS ONE*
- Abudaya M, **Ulman A**, Selah J, Fernando, D, Wor C and Notarbartolo di Sciara G (2017). Speak of the devil ray (*Mobula mobular*) fishery in Gaza. *Reviews in Fish Biology and Fisheries*. DOI: 10.1007/s11160-017-9491-0
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- Ulman A**, Burke L, Hind E, Ramdeen R and Zeller D (2016) Conched out: Turks and Caicos reconstructed fisheries catches (1950-2012) demonstrate an unsustainable future. *Frontiers in Marine Science* 3(71): 9.
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- Ulman A**, Saad A, Zyllich K, Zeller D and Pauly D (2015) Reconstruction of Syria's fisheries catches from 1950-2010: Signs of overexploitation. *Acta Ichthyologica et Piscatoria*. 45(3): 259-272.
- Ulman A**, Shlyakhov V, Jatsenko S and Pauly D (2015) A reconstruction of the Ukraine's marine fisheries catches, 1950-2010. *Journal of the Black Sea/Mediterranean Environment*. 21(2): 103-124.
- Piroddi C, Gristina M, Zyllich K, Greer K, **Ulman A**, Zeller D and Pauly D (2015) Reconstruction of Italy's marine fisheries removals and fishing capacity, 1950-2010. *Fisheries Research*. 172: 317-327.

Edited book contributions

- Unal V and **A Ulman**. (In press) Economic viability of small-scale fisheries in Turkey. In: *Too Big To Ignore (TBTI) Economic viability of small-scale fisheries in Europe*.
- Ulman A**. 2016. Box 2.1. Catch reconstructions: the challenges for and of local authors. In: D. Pauly and D. Zeller (eds.) *Global Atlas of Marine Fisheries: Ecosystem Impacts and Analysis*. Island Press, Washington, D.C.
- Bultel E, Le Manach F, **Ulman A** and D Zeller. 2016. France (Mediterranean) In: D. Pauly and D. Zeller (eds.) *Global Atlas of Marine Fisheries: Ecosystem Impacts and Analysis*. Island Press, Washington, D.C.
- Ulman A**. 2015. Georgia. 2016. In: D. Pauly and D. Zeller (eds.) *Global Atlas of Marine Fisheries: Ecosystem Impacts and Analysis*. Island Press, Washington, D.C.
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- Funes M, Box S, Zeller D, Zylich K, **Ulman A** and D Pauly. 2016. Honduras (Pacific) In: D. Pauly and D. Zeller (eds.) *Global Atlas of Marine Fisheries: Ecosystem Impacts and Analysis*. Island Press, Washington, D.C.
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- Ulman A**, Zengin M, Knudsen S, Zeller D and D Pauly. 2016. Turkey (Black Sea) In: D. Pauly and D. Zeller (eds.) *Global Atlas of Marine Fisheries: Ecosystem Impacts and Analysis*. Island Press, Washington, D.C.
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- Ulman A**, Burke L, Hind E, Ramdeen R and D Zeller. 2016. Turks and Caicos Islands In: D. Pauly and D. Zeller (eds.) *Global Atlas of Marine Fisheries: Ecosystem Impacts and Analysis*. Island Press, Washington, D.C.
- Ulman A**, Shlyakhov, V, Jatsenko, S and Pauly D. 2016. Ukraine In: D. Pauly and D. Zeller (eds.) *Global Atlas of Marine Fisheries: Ecosystem Impacts and Analysis*. Island Press, Washington, D.C.

STATEMENT OF STYLE

This thesis has been prepared in journal article compilation style format. With the exception of Chapter 1 (General Introduction) and Chapter 5 (General Discussion), each chapter has been written with the aim of publication in a marine ecology or conservation scientific journal. As a result of this, there is some overlap between chapters, in particular in relation to the introduction themes, study descriptions and acknowledgements written in Chapters 2, 3 and 4.

ABSTRACT

Many stressors, such as climate change, overfishing, pollution and biological invasions, are currently devastating the marine domain. Despite species being passively transported to new marine ecosystems since the onset of boat travel, invasion biology is a relatively new field of science. Recreational boating as a major vector in the transport of non-indigenous species (NIS) has largely been overlooked until very recently, mostly because of the perceived effectiveness of antifouling coatings on boat-hulls. The role of recreational boating in facilitating marine bioinvasions urgently necessitated a proper evaluation, especially in the Mediterranean Sea which is the second most popular region for global charter boating traffic (after the U.S.A.) and is also *the* global hotspot for alien species. This study addresses this shortfall by completing the first-ever Mediterranean basin-wide study investigating the influence of recreational boats in the transfer of NIS from biofouling both in marinas and from boat-hulls.

First, a thorough investigation of NIS was conducted in 34 marinas across the Mediterranean (spanning from Spain to Turkey), targeting benthic macroinvertebrates. All marinas were found to host NIS, ranging from 2 to 27 per marina. This first output of this research provides a massive update of new NIS records and updated species distributions for the Mediterranean, and presents three new species in the Mediterranean basin, 51 new NIS country records and 20 new subregional records, which can now be fed into models and databases to gain a better comprehension of the composition and scale of NIS colonizing marina habitats.

Next, boat owners/captains were surveyed on their vessel characteristics including hull-cleaning and antifouling application history along with their recent travel itinerary. Subsequently, biological samples of the biofouling were collected from approximately 600 of the same boat-hulls whose captains had already completed the survey, in order to search for correlations between the findings of the two. After the biofouling samples were identified, it was realized that almost 80% of sampled fouled vessels were found to host at least 1 NIS, while 11 was the maximum NIS found on one boat-hull. It was also found that recreational vessels visiting new marinas sometimes carry NIS not yet present neither in that marina nor in the country in which they are visiting, thus providing ample evidence of recreational boating supplying new NIS to marinas. Some factors found here to influence higher non-indigenous species richness on vessels were time since last hull-cleaning, and the visual antifouling estimation of the fouling of the niche areas on the boat-hulls. Additionally marinas with high species richness correlated to boats in those marinas also having a higher species richness. Vessel specifics such as boat type, hull composition and average cruising speed did not influence NIS richness, nor did increased travel patterns or durations.

The results of this large-scale Mediterranean marina assessment were combined with other existing data on NIS in Italian marinas for a total sample size of 50 marinas, which were then used to feed both univariate and multivariate statistical tests aimed at identifying which abiotic factors mainly contribute to total species richness of NIS in marinas and also which factors contribute to similar NIS assemblages between marinas. The results revealed that a higher species richness of NIS in Mediterranean marinas was influenced by the following factors: water temperatures above 25°C, a higher number of berths, absence of floating pontoons, proximity to the Suez Canal and proximity to commercial harbours. Whereas the similarities between NIS assemblages amongst marinas were more influenced by environmental factors such as temperature, biogeographical region, climate type, primary productivity and again proximity to the Suez Canal. The significance of the Suez Canal as a prominent factor in both analyses coincides with the general trend of higher total NIS found in the Eastern Mediterranean strongly influencing NIS distributions.

The results presented within this thesis, adding to those marinas surveyed from around the world, form a robust case that recreational boating provides an extremely important pathway in facilitating primary NIS introduction events and their associated secondary spread to other coastal areas as 'stepping stone' habitats. These results indicate that the recreational boating vector requires urgent management to reduce the scale of future invasions and the further spreading of established NIS. The results presenting the NIS in 50 marinas (35 which were sampled from this study) across the Mediterranean also indicates which of these marinas present a high-risk of spreading owing to either their high NIS richness or their unique NIS assemblages demonstrating risk for future spreading, and should hence be routinely investigated, to improve our knowledge on the dynamics of NIS distributions, especially for their possible socio-economic consequences.

ACKNOWLEDGEMENTS

The past three years have certainly contributed greatly to my personal and professional development. Since the geographical range of the study area was so wide, I was able to gain a unique perspective of the scale and severity of marine bioinvasions across different regions. Also, my scientific network has considerably expanded, with many who either contributed their expert knowledge to this project, or taught me new skills, both, which helped me grow as a researcher. This was an enormous project that was only made possible by the knowledge, enthusiasm and collaborations of a few key people.

First and foremost, I would like to thank my super positive, dedicated, passionate and brilliant main supervisor, Agnese Marchini for sharing her knowledge, advice, enthusiasm, endless support, problem solving skills and especially her friendship during these last three years. She made it a joy to work at the Orto Botanico of the University in Pavia. Without her, this grand project would never have been initiated. I eternally thank her for having really raised the bar in showing how commitment, enthusiasm and a dedicated team are the ingredients for success.

I shall feel perpetually indebted to my exceptional secondary supervisory Anna Occhipinti-Ambrogi for the provision of your expert advice and knowledge throughout these years and especially for helping get me out of sticky situations, and most enjoyably, for her quirky sense of humour which kept me smiling amidst my heavy workload.

Thirdly, I would like to thank the MARES-EU Program for offering this remarkable opportunity and especially for your very thoughtful and helpful scientific advisory committee, who perpetually helped advise on improving my inspection of this large-scale issue, outlook and scope with their cumulative guidance, and ensured that I performed my best with the task at hand.

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For taxonomic expertise and welcoming me to their teams, laboratories and countries, I especially thank Christos Arvanitidis and Alfonso Ramos-Esplá. For help with taxonomic advice, I thank Michel Bariche, Fernando Boero, Şermin Açık Çınar, Melih Ertan Çınar, Victoria Fernandez-Gonzalez, Bella S. Galil, Jean-Georges Harmelin, Valiallah Khalaji- Pirbalouty, Traudl Krapp-Schickel, Elena Kupriyanova, Gretchen Lambert, Carlos Navarro-Barranco, Gary Poore, Rosanna Rocha, Jean-Claude Sorbe, Yanan Sun and Kevin J. Tilbrook. For helping to obtain marina permissions I thank GianPaolo Sacchi, Giulio Franzitta, Paola Gianguzza and Stefanos Kalogirou. For collection of samples, a big thank you to Alessandro Bolis, Gina Donnarumma, Ali Murat Elagoz, Andres Izquierdo-Muñoz, Alberto Orengo, Francesco Saracino, the late Arif Sipahi and Tevfik Yilmaz.

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Spain

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France

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La Grand-Motte: Marina Director- Eric Pallier

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Saint-Tropez: Marina Director- Jean-Francois Turret

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Saint-Maxime: Marina Manager- Mr. Ellul

Cannes Le Vieux Port: Marina Director- Francois Gillet

Antibes Port Vauban: Marina manager- Emilie Pluenet

Villefranche-sur-Mer: Chef d'Exploitation- Sarah Castanie

Italy

Porto Turistico di Roma/ Lido di Ostia: Marina Manager- Daniele Cossu

Ischia Marina di Sant'Angelo: Giulio Lauro; Porto d'Ischia: Mr. Di Maio

Sorrento Marina Piccola: Italian Coast Guard/ Sorrento Location

Marina Villa Igia Palermo: Marina Owner - Gioacchino Guccione

Porto La Cala Palermo: Marina Manager- Nicola Rossa

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Marina di Marzamemi: Marina Director- Salvatore Gurrieri

Marina di Ragusa: Marina Manager- Enza Di Raimondo

Marina di Cale del Sole (Licata): Marina Manager- Maria Sitibondo

Porto dell'Etna/ Marina di Riposto: Marina Manager- Emiliano Indelicato

Malta

Msida Yacht Marina: Marina Director- George Mercieca

Grand Harbour Marina Valletta: Marina Director- Gordon Vassolo

Greece

Old Venetian Harbour (Heraklion): Marina Director- Dr. Bras Ioannis

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1 INTRODUCTION

1.1 Importance of the issue

Marine non-indigenous species (NIS) are a major threat in the marine realm since they are practically impossible to remove once established, can outcompete native species for space and nutrients, and can also cause harm both to human health and the economy. The two major understood vectors of transfer of NIS (ballast water and transfers via aquaculture farming) have recently been internationally regulated. Biofouling was not considered a major vector of spread of NIS until very recently due to the perceived effectiveness of antifouling coatings on boat-hulls. However, the active applied compound in these coatings - tributyltin (TBT), was completely prohibited for use in antifouling coatings as of 2008 (Hyder Consulting 2006) due to chemical harm it released into the marine realm and subsequently up the food chain. Other popular antifouling coating types (mostly copper-based) commonly used today are not as effective in deterring biofouling growth (See 1.6.2), so the contribution of NIS spread by biofouling has subsequently been revived and now is the largest unregulated vector for the spread of NIS. Studies elsewhere have shown biofouling to be the major vector in the transfer of NIS (Acosta et al. 2009; Floerl et al. 2009; Clarke-Murray et al. 2011; Ashton et al. 2014), however, despite the Mediterranean being a hotspot both for NIS and for recreational boating traffic, no directed studies have been completed in the region to properly understand the role of recreational boats in the spread of NIS. Invasive species experts and managers in the Mediterranean are in the process of drafting new regulations to help control the biofouling vector, however, no large-scale regional studies have yet been completed on this topic to test its strength and to understand which stimuli influence NIS success in recreational marinas and on boats. This study sets out to fill in these major gaps in data and provide usable advice to help improve the management of the biofouling vector via recreational boating in the Mediterranean.

1.2 The Mediterranean Sea

The word 'Mediterranean' is derived from the Latin 'mediterraneus', meaning "in the center of the land". Up to about 5.3 million years ago, it was a dry valley, which was rapidly flooded by the Atlantic Ocean through a barrier which was eroded west of the Strait of Gibraltar, disconnecting it from the world's oceans (Garcia-Castellanos et al. 2009).

The Mediterranean basin is a semi-enclosed basin, with limited water exchanges occurring in the west (Strait of Gibraltar), the northeast (the Dardanelles and Bosphorus Straits exchanging with the Black Sea), and the southeast (the Suez Canal exchanging some water with the Red Sea). Overall, the basin loses a thickness of about 145 cm each year due to intense evaporation, 27% of which is replaced by rainfall, 6% by rivers, 6% by the Black Sea and 6% by the Atlantic via Gibraltar Strait (Hughes 2005), resulting still in a net loss of water. Water flows in through the Strait of Gibraltar as surface water, and water exits the basin counter-currently as denser deeper water. Due to these limited inputs and outputs caused by its almost total confinement, the Mediterranean basin can be considered as a unique marine laboratory useful for understanding larger global processes. The water in the basin varies a great deal geographically, with higher primary production in the western portion of the basin, whereas the eastern portion is ultra-oligotrophic with much higher salinity (Pauly et al. 2014).

1.2.1 The Strait of Gibraltar

The Strait of Gibraltar separates the southern Spanish from the northern Moroccan coast. It is the only place where the Atlantic Ocean mixes with the Mediterranean Sea, with a minimum width of about 13 km (Bergamasco & Malanotte-Rizzoli 2010) and a total length of about 60 km. The Strait includes a system of sills and narrows and its shallowest depth is just 290 m (Soto-Navarro et al. 2010). Hundreds of vessels pass through this strait each day making it an extremely busy shipping route¹.

1.2.2 The Suez Canal

The Suez Canal is an artificial waterway joining the Gulf of Suez (the northern branch of the Red Sea) to the Mediterranean. It formally opened in 1869, and was designed by the French engineer Ferdinand de Lesseps to reduce shipping voyage time. Preceding its opening, captains had to navigate around the Cape of Good Hope in Southern Africa in order to transfer goods from Asia to Europe and the Americas. Currently, the canal supports about 8% of global shipping traffic (www.marineinsight.com). The canal begins at Port Said on the Egyptian Mediterranean coast and extends approximately 160 km to the south, where it meets the Red Sea.

In 2015, the Egyptian government performed an enlargement project on the canal, adding 72 km to the channel, in addition to widening and deepening the canal to facilitate additional shipping traffic. This

¹ <https://www.livescience.com/29738-strait-of-gibraltar-where-atlantic-meets-mediterranean.html>

enlargement project decreased shipping passage time from 18 to 11 hours and is now able to accommodate nearly twice the amount of daily passages (from 50 to 97 ships); however, thus far, the annual toll revenue received by the Egyptian government has actually decreased, as many captains claimed the tolls are overinflated and rather prefer to journey the much lengthier route around the Cape of Good Hope (<http://freightplus.com/suez-canal-expansion-brings-more-options-to-sea-freight/>). However, this recent enlargement of the Suez was completed only for local economic benefit without any consideration to the scale of impacts from the accelerating marine biological invasions into the Mediterranean Sea as no 'Environmental Impact Assessment' was required (Galil et al. 2015). It has also been suggested through the fore-mentioned source that bioinvasions through the ballast water vector may increase, as ships will have to empty their ballast tanks before travelling through the Suez Canal to be lighter and higher to facilitate ease of travel.

The Suez Canal is logically considered the main vector for non-indigenous species (hereafter NIS) introductions in the Mediterranean Sea since about 2/3rds of the 750+ multicellular NIS introduced into the Mediterranean Sea are of Indo-Pacific origin (Galil et al. 2017), and the influence of NIS is much more pronounced in the eastern portion of the basin, now largely comprised of Indo-Pacific species (Çevik et al. 2008). The creation of the Suez Canal (Galil et al. 2017) added additional artificial habitat for species to travel through, but also decreases shipping times, consequently increasing the rate of transport survivorship of alien species.

1.3 Globalization and transport of NIS

Globalization, synonymous with "global economy" is an unstoppable growing force, and is the international solution to supporting constant economic growth around the world (Chase-Dunn 1999). While transportation for humans continually improves in terms of temporal and spatial reductions, the human-mediated transport of NIS to new areas is resultantly escalating and includes the shipping pathway (via ballast water and biofouling), recreational boating (biofouling), aquaculture and the live fish food and aquarium trades, and artificial canals (Carlton 1985; Clarke-Murray et al. 2011; Floerl & Inglis 2003; Naylor et al. 2001; Ojaveer et al. 2014; Semmens et al. 2004; Weigle et al. 2005). The spreading of marine NIS is redefining the biogeography of the oceans and seas (Occhipinti-Ambrogi 2007).

It was previously understood that the distribution of marine NIS was restricted due to geographic barriers, but empirical evidence has instead revealed that climate is the restricting factor (Capinha et al. 2015), as geographical barriers have been deconstructed due to globalization, thus as long as there is a means of transport, along with similar environmental conditions (i.e., temperature and salinity) that are within the

species' niche range to trigger reproductive events, NIS then have the chance to establish populations. The increase in global travel and commerce has provided increased opportunities for the transport of alien species across great distances (Kolar & Lodge, 2000; Padilla & Williams, 2004), and continued human population growth along with the ever-increasing speed and frequency of travel will likely intensify the number of new successful NIS establishments (Havel, Kovalenko, and Kats 2015).

These human-mediated transport events, unique both in space and time (Carlton 2003) have been occurring since the onset of marine navigation, primarily scientifically recorded by Charles Darwin (1854). One major impediment affecting bioinvasion science is not correctly being able to identify the native origins of species (Marchini & Cardeccia 2017), hence the term "cryptogenic" (of unknown origin) which is applied to such species and thus likely underestimates the scale of invasions (Carlton 2003) as it undervalues the true number of NIS. Due to a few key factors, such as climate change (Occhipinti-Ambrogi 2007), the increased frequency of transport (Seebens et al. 2013), the increased availability of source populations (i.e., from the subsequent widenings of the Suez Canal connecting the Red Sea to the Mediterranean; Galil et al. 2017; Galil BS et al. 2016), and lack of regulations to deter biofouling, there has been a gross escalation in the level of marine bioinvasions in recent decades (Seebens et al. 2017).

1.4 Pathways and vectors

The actual foundation of research on marine pathways and vectors of introduction is the paper by Carlton (1985) entitled *Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water*: "In the years subsequent to its publication, the field of marine vector ecology, and ballast research in particular, has grown dramatically" (Davidson & Simkanin 2012).

A new species introduction can exist as a single event to a particular locality, multiple events to a single locality, or several introduction events to the region, over time. Following an initial introduction event, the successful establishment of a species to the new area indicates that the environmental conditions between source and new location are compatible. A 'pathway' serves as a method of primary introduction to a region or country and the major pathways are due to shipping, aquaculture and artificial canals (Galil et al. 2017; Ruiz et al. 2000). Organisms are then transported following the initial introduction events as secondary transfers via 'vectors'. One pathway can have several vectors, for example, the shipping pathway can have the following associated vectors: ballast water, components in the sediment of ballast water tanks (Casas-Monroy et al. 2011; Hewitt et al. 2009), in sea chests [small underwater compartments used in the hull through which

seawater is ingested or expelled, i.e., for engine cooling for ballast water] (Coutts et al. 2003), and as part of the biofouling composition (Chapman et al. 2013; Gollasch 2002; Lacoursière-Roussel et al. 2016).

1.4.1 Global shipping (pathway)

The advent of container shipping in the 1950s propelled the world towards globalization as the world's biggest economies formed close trading ties with each other.² Shipping represents one of the most important global networks as the vast majority (90%) of global trade occurs by sea (Kaluza et al. 2010), including minerals, oils, and manufactured goods as it is the most cost-effective method to transport materials. Container shipping is largely responsible for the increase in global trade.

1.4.2 Recreational boating in the Mediterranean Sea

The Mediterranean Sea attracts over 2/3rds of global mega-yachting recreational boat traffic, and comes second to the USA in terms of number of boats (Cappato 2011). Mega-yachts generally only operate between the months of July and August, for durations lasting under nine days, and spend over half (55%) their time cruising the Western Mediterranean, 15% the Eastern Mediterranean, and 14% in the Caribbean. The total number of both passive and active recreational vessels in the Mediterranean remains unknown, however, approximately 1.5 million recreational boats were estimated using satellite images (Cappato 2011).

In 2010, the estimated number of operational marinas in the Mediterranean was 946 and were distributed as follows: 11 in Albania, 24 in Algeria, 3 in Cyprus, 81 in Croatia, 6 in Egypt, 191 in Spain, 124 in France, 3 in Gibraltar, 135 in Greece, 8 in Israel, 253 in Italy, 3 in Lebanon, 15 in Libya, 6 in Malta, 9 in Morocco, 2 in Montenegro, 3 in Slovenia, 3 in Syria, 29 in Tunisia and 37 in Turkey (Cappato 2011). However, these official data are likely an underestimation. For example, there are at least three operational marinas in the Republic of Northern Cyprus, which were overlooked in the previously mentioned assessment. According to www.pagineazzurre.com, the number of marinas in Italy exceeds 500. France has the highest abundance of boats (10,000 per 1000 km of coastline) compared to Italy's 8,000 boats per 1000 km of coastline. No such data or estimations were available for any other (southern) Mediterranean countries (Cappato, 2011).

²<https://worldview.stratfor.com/article/why-global-shipping-industry-will-be-tough-salvage>

1.5 Non-indigenous species (NIS)

If a species is endemic or indigenous, it is classified as native. By default, if there is no recorded introduction event, the species is deemed native (Carlton 1996). If a species crosses a barrier or enters a new sea, it is then an alien or non-indigenous species (NIS); however, the definition of NIS adopted here also deems that the species arrives via human mediation, albeit intentionally or unintentionally (European Commission 2014). Thus, new species to the Mediterranean Sea arriving naturally via natural range expansion either through the Gibraltar Strait or the Suez Canal are not considered here as NIS.

As taxa have been traveling around for millennia, if the origins or native ranges of species are unknown or questionable, they are labeled as cryptogenic species (crypt-, Greek, kryptos, secret; -genic, New Latin, genic, origin; Carlton 1996). This study focuses solely on NIS, thus native and cryptogenic species are excluded.

NIS are termed 'invasive' once they have a significant effect on the natural biodiversity, ecosystem services, human health or economic impact (i.e., loss to fisheries or aquaculture), but not all NIS are perceived negatively or pose a threat to the natural biodiversity. In Europe, of the roughly 12,000 alien species (both terrestrial and aquatic), 10-15% were estimated to be invasive (Barton 2015). Biological invasions play a pivotal role in restructuring communities, often displacing native species (Galil et al. 2015), especially in artificial environments (Occhipinti-Ambrogi 2007).

1.5.1 NIS in the Mediterranean Sea

The effects of bioinvasions are relentlessly increasing owing to various processes such as more efficient transportation technologies and routes, climate change, habitat alteration and also geopolitical events (Early et al. 2016). Regarding the effective regulation of alien species, prevention is the best chance for mitigation, since once a species is established in the marine realm, its eradication is often highly expensive and thus impractical with very low rates of success (Genovesi 2005).

1.6 Current Regulations

1.6.1 Ballast water

Ballast water is the local water ingested by ships to regulate their stability after offloading cargo shipments, and as this ingested water contains local marine microbes, phytoplankton, and zooplankton (including both adult and early life-cycle stages of marine organisms), these then can get transported and transferred to new localities as the ships expel this water before loading new cargo onboard. If this water is left untreated, it releases an array of organisms or propagules to the new locality, often far from the source. As the spread of NIS via the ballast water vector is well understood to pose a serious threat to Mediterranean biodiversity, the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention; www.imo.org) was adopted in 2004 to introduce global regulations to control the transfer of potentially invasive species. The treaty entered into force on September 8, 2017, requiring ballast water to be treated before its release into a new location, to ensure that any microorganisms or small marine species are first removed. However, this date was recently pushed forward now allowing existing vessels until 2024 to retrofit their ballast water treatment systems, so regulation will now be completed twenty years after the initiation of the Ballast Water Convention³. However, a recent investigation has shown that in comparison to commercial ships, a higher proportion of passenger vessels have already refitted their Ballast Water Treatment Systems (BWTS), showing that a global transition towards the commencement of a global transition of an effective ballast water management strategy is well underway (Davidson et al. 2017).

For the past three decades, ballast water was considered the primary vector for the introduction of marine species but recent studies suggest that introductions from this vector were probably overstated, and that the biofouling vector more likely accounts for over 2/3 of marine NIS introductions (in Australia, Hewitt et al. 2009).

1.6.2 Biofouling

Marine biofouling refers to the growth of undesirable marine organisms on immersed artificial structures such as boat-hulls, marina substrates and aquaculture cages. The major understood associated negative impact

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inflicted on boat owners from excess biofouling on their boat-hulls is increased gas consumption resulting in additional (unnecessary) expenses resultant from the additional drag. Due to this, preceding the boating season, boat owners generally apply a fresh antifouling coating to their hulls every year to hinder the attachment of fouling biota. Despite similar or possibly even greater risks that biofouling poses for the spread of NIS compared with ballast water, only a voluntary 'Code of Conduct' has recently been prepared in Europe titled "A European Code of Conduct on Recreational Boating and Invasive Alien Species" (Barton 2015), with the aim of compatibility with other international initiatives such as the International Maritime Organization (IMO) Guidance for minimizing the transfer of invasive aquatic species as biofouling on recreational boats (IMO 2012). One of the guidelines recommends that vessels apply new appropriate antifouling paint and that they also perform in-water cleanings if the boat has been in the water less than a year not frequently used, especially before enduring long-distance voyages; this was prepared on behalf of the Bern Convention, a legally binding international agreement focusing on the protection of natural habitats and endangered species, which entered force in 1982. This report stresses the need to manage pathways of alien species more effectively, but at this early stage, all measures are voluntary, as with the IMO Guidelines for the Control and Management of Ships' Biofouling (IMO 2011).

European management regulations have yet to be enacted to combat this vector. In a series of recent legislative documents (EC 2010, 2012, 2014a, 2014b), the European Union has repeatedly stated that boating, yachting and maritime tourism are amongst the main focus areas to be financially supported to foster sustainable growth and employment, and has thus recommended a series of actions to improve the sector and stimulate the development of sustainable tourism in coastal areas. Despite claims that 'A healthy environment is fundamental in supporting any sort of tourism in coastal areas and that every effort must therefore be made to protect it' (EC 2012), the previous documents listed above all fail to address the issue of the spread of marine bioinvasions via recreational boating. The reason this vector has been previously ignored until very recently was due to the perceived effectiveness of antifouling paints (Floerl & Inglis 2003), which have recently changed due to the banning of the harmful tributyltin (TBT) compound, which was an organic compound base for antifouling paints which was widely used in antifouling coatings from the 1960s. However, in the late 1970s, TBT was understood to have damaging effects on aquatic environments, in killing other sea-life unassociated with biofouling and was also found to enter the food chain, and was consequently banned by the International Maritime Organization for use in biofouling applications on ships in 2003, and a complete prohibition of its usage was enforced on January 1, 2008 (Hyder Consulting 2006). Popular antifouling coatings for vessels have reverted to using copper and other similar oxide-based paints, which were popular before the introduction of TBT, in addition to some biocide-free treatments, both which are less effective in the long-term. These biocide-free paint types are inefficient in their ability to constantly release biocides to repel biofouling. Resultantly, self-

polishing paints were created which wear-off layers of coatings with travel usage, to offer a constant release of biocides, but these are only effective for vessels that travel frequently as biota can easily colonize the substrate if the boat is inactive. Since these new anti-fouling types are much less effective at deterring biofouling organisms as the now prohibited TBT component, this has since renewed the risk and effects caused by biofouling growth on boats.

1.7 Current knowledge on recreational boating as a vector of spread of NIS

Recreational boating is receiving growing recognition for its contribution to the spread of NIS. In the United Kingdom, boating is thought to be responsible for over a third of NIS introductions (Gallardo & Aldridge 2013). Of 88 marinas sampled in the United Kingdom, 83 were found to contain at least 1 NIS (Foster et al. 2016).

Currently, the most thorough study of its kind from Western Canada has shown that recreational boats are likely the largest unregulated vector for the introduction and establishment of marine alien species (Clarke-Murray et al. 2011), as this study sampled over 600 boats and found ¼ of the vessels to contain NIS. Another study from Alaska found many NIS on newly arriving boats which were not yet present in the state (Ashton et al. 2014). A study from California found 80% of sampled vessels to be fouled, and 25% of those vessels to host NIS, with the most invaded vessel hosting 5 NIS (Zabin et al. 2014).

To date, other studies showing recreational boating as a major or prominent vector for the transfer of alien species have been from Hawaii (Davidson et al. 2010), the Great Lakes (Sylvester & MacIsaac 2010), the North Sea (Gollasch 2002), the UK (Brock-Morgan 2010), Norway (Ware et al. 2014), South Africa (Jurk 2011), New Zealand (Gordon & Mawatari 1992) Australia (Floerl & Inglis 2003), and globally (Minchin et al. 2006). For the Mediterranean the following countries have addressed the issue with small-scale studies conducted in Spain (Lopez-Legentil et al. 2015; Ros et al. 2013), Croatia (Marić et al.), Italy (Ferrario et al. 2017b) and Israel (Gewing & Shenkar 2017).

1.7.1 Current management of biofouling around the world

Australia is severely plagued by bioinvasions due to its isolation and blames the boating pathway, both from biofouling and ballast water vectors for the introduction of its marine invaders, and are now working towards

developing an adequate biosecurity regime to manage both the biofouling and ballast water vectors. However, the national monitoring program is severely under-resourced but they highly recommend conducting mandatory port marine pest assessments every five years (Australian Marine Conservation Society 2015). Thus far, Australia and New Zealand are the only countries in the world that have enforced any regulations pertaining to small vessels arriving from foreign ports which must present documentation on their previous antifouling application history, and contingent on those details, may have to undergo visual inspections of their boat-hulls (Zabin et al. 2014), often at the owners expense. Australia aims to develop their national regulatory system by first targeting visiting vessels and then secondly to include domestic vessels to combat biofouling as proposed in a recent bill on biosecurity (Australian Marine Conservation Society 2015).

1.8 This study

1.8.1 Main study aims

By addressing the following broad questions, the aim of this study is to separate the factors underlying NIS richness in marinas and biogeographic distribution of alien species in the Mediterranean, and the contribution of recreational boats in facilitating the spread of NIS to better understand the strength of the recreational boating vector in the transport of NIS. The important questions therefore are:

1. Are Mediterranean marinas hotspots for marine bioinvasions?
2. Which NIS are present on boat-hulls, and do these differ from the NIS found in the same marinas?
3. Do recreational boats in the Mediterranean carry a substantial amount of NIS?
4. Which abiotic factors (or combinations thereof) contribute to total NIS richness in certain marinas?
5. Which underlying factors shape similar NIS distribution patterns in marinas across the Mediterranean?
6. Which marinas or subregions present the greatest risk for the additional spreading of alien species to new localities?
7. Which factors influence boats to have higher species richness in their biofouling composition?
8. Are boaters cleaning and painting their boats often enough to prevent the growth of biofouling?
9. Does increased boat travel relate to higher NIS richness on boats?
10. What are boaters awareness levels of non-indigenous species?
11. What recommendations can this research give for future management of this vector?

1.8.2 The hypothesis

The null hypothesis is that recreational boating does not influence higher NIS richness in the Mediterranean.

1.8.3 Study tasks

To assess the contribution of recreational boating and marinas as a major vector of spread of non-indigenous species in the Mediterranean, the following tasks were carried out:

1. Marina selection across for each Mediterranean subregion (Western, Central, Eastern);
2. Obtaining relevant permissions from marinas for sampling;
3. Creation of a taxonomic field-guide identification booklet for applicable NIS target species;
4. Marina sampling for NIS;
5. Boater questionnaires;
6. Boat-hull biofouling sampling and visual fouling estimates;
7. Taxonomic identification;
8. Statistical analyses;
9. Manuscript writing; and
10. Publishing of results.

1.8.4 Novelty of the study

Marinas in many parts of the world have recently been shown to host many NIS, especially in comparison to their adjacent natural substrates (See section 1.8 for applicable references). These highly invaded marinas, built using artificial substrates mainly suggest biofouling on boat-hulls as a major vector for the spread and success of NIS, especially if no other major vectors lie within close proximity. Despite the Mediterranean being the most invaded region in the world and the second busiest region in terms of yachting traffic, no previous studies have been completed here strictly testing the strength of the recreational boating vector. This study is the largest and widest-ranging geographical study on the topic ever completed and the first of its kind in the Mediterranean which sampled 34 marinas spanning 7 countries, including marinas from each of the three subregions. The main aim was to better understand NIS presences and distributions in marinas and their

likelihood of species transfers via biofouling on recreational boats. The assumption was strong that the species transfer occurred by boating if the NIS were also found on boat-hulls in addition to their presence in the same marina and/or if the species lacked a larval stage. In general, NIS transfers are usually assigned a vector of spread due using expert knowledge mostly by assigning the closest major vector since first introductions are typically untraceable events. Additionally, after the taxonomic identification was completed, this study tested which abiotic factors influence marinas and boats to have more successful NIS establishment than others.

This is also the largest sample size collected on NIS from recreational boat hulls which had surveys first completed with captains or staff on both antifouling and cleaning history of the vessel, and travel history to improve on the testing quality. This type of surveying along with sampling allows for testing to determine what factors influences higher fouling, but is rarely undertaken since surveys can be extremely time consuming and in-water boat sampling in marinas can be dangerous and is more difficult to obtain permissions for.

1.8.5 Study Design

Marina selection

As this study aims at analyzing a Mediterranean-wide selection of marinas, the Northern Mediterranean region was chosen as the main study area owing to some pre-established cooperation and assistance initially offered from partner universities/institutions in Italy, France and Greece, and additionally due to feasibility of transport between these countries. The criteria used for marina selection initially included the sub-region to which they belong, the number of berths (marina size) and popularity as a tourist locality, and additionally, feasibility of conducting fieldwork and obtaining permissions.

The number of visiting vessels received for each marina per annum, staying at least one night, was meant to be used as a proxy for marina selection, however these data were only available for 20 marinas (Table 1.1).

Table 1.1 Number of visiting vessels received per annum to marinas. Marina # assigned from Table 2.1, locality and country, with number of visiting vessels per annum (from either 2014, 2015 or 2016).

#. Locality and country	#	#. Locality and country	#
1. Alicante, Spain	N/A	18. Siracusa, Italy	N/A
2. Barcelona, Spain	2122	19. Marzamemi, Italy	N/A
3. Cap d'Agde, France	1000	20. Ragusa, Italy	N/A

4. La Grande-Motte, France	N/A	21. Licata, Italy	500
5. Port Camargue, France	N/A	22. Msida, Malta	350
6. Saint-Tropez, France	700	23. Grand Harbour, Malta	459
7. Cogolin, France	N/A	24. Heraklion, Greece	N/A
8. Saint-Maxime, France	1221	25. Agios Nikolaos, Greece	N/A
9. Cannes, France	N/A	26. Rhodes, Greece	N/A
10. Antibes, France	1200	27. Istanbul, Turkey	N/A
11. Villefranche-sur-Mer	2000	28. Bodrum, Turkey	N/A
12. Rome, Italy	N/A	29. Datça, Turkey	450
13. Ischia, Italy	N/A	30. Marmaris, Turkey	2000
14. Sorrento, Italy	1200	31. Fethiye, Turkey	1500
15. Villa Igiea, Italy	476	32. Finike, Turkey	2500
16. La Cala, Italy	N/A	33. Karpaz, Cyprus	300
17. Riposto, Italy	800	34. Famagusta, Cyprus	50

Some smaller marinas were found to be extremely popular hubs for incoming vessels such as Villefranche-sur-Mer, France; Sorrento, Italy; and Finike, Turkey, which certainly increases opportunities for spreading NIS due to traffic intensity, but before these marinas can be found to pose a risk, they have to also host sufficient NIS.

Boater surveys

A preliminary screening was first completed with boat captains/owners to ensure that their vessel had travelled outside their home marina in the past 12 months for a minimum of one night duration, so that the vessel posed some risk of spreading NIS; if a boat had not ventured to another marina, it posed no risk of transferring NIS and was excluded. Interviews were conducted either with boat owners or the crew in the marinas, either when they were onboard their vessels, or when accompanying their vessel at the dry dock for hull cleaning/antifouling paint applications. As interviews were only conducted with boaters who were present during the five to seven days sampling period at each marina, there may be a slight bias towards surveying and sampling boats that travel more frequently over those that travel infrequently.

The surveys included questions on their boat specifics (size, hull-type, horsepower, average cruising speed), antifouling (type of paint used, price and date) and in-water cleaning history, recent travel history and awareness of NIS.

As the lead author either personally conducted or supervised all surveys, and speaks English, French and Turkish, native speaking assistants accompanied her for surveys in Italy and to some extent in Greece to ensure that surveys could be carried out in the native tongue, as necessary. The surveys were also translated to Spanish and Croatian (See Appendix 1 for English survey).

Boat-hull sampling

Next, after surveys were completed and after obtaining verbal permission from the boat owners/captains, fouling samples were collected from the boat-hulls. Further details on this type of sampling are presented in Chapter 2 and Chapter 4.

1.9 Description of subsequent chapters

After conducting this large-scale investigation on the role of recreational boating as a vector of spread in the Mediterranean, many new NIS records were realized, which are of great interest to the scientific community, and were first prepared for publication (**Chapter 2**), to quickly make these records available to scientists, managers and conservationists. This chapter clearly illustrates the prominence of the recreational boating vector, and shows many recreational marinas act as hot-spots for NIS, it also presents many NIS existing only on boats but not yet in the same marina or in many cases even in the country, therefore demonstrating the biofouling process in action as vessels bring new propagules to seed new marinas. This chapter also greatly increases the scientific knowledge on NIS distributions and expansions.

Next, data from this main study totaling 35 Mediterranean marinas were combined with additional data on NIS from 15 Italian marinas sampled in the framework of other projects (Ferrario et al. 2017; Ferrario unpublished data) for a total sample size of 50 marinas. The aim here is to determine which abiotic factors influence some marinas to host a higher NIS richness than others, and additionally which factors influence similarities between multivariate NIS communities across the Mediterranean (**Chapter 3**), including some factors which had never been tested before and are unique to the Mediterranean basin.

For **Chapter 4**, the survey results from the boaters were analyzed along with the sampling results from the found NIS from their boat-hulls to reveal the risks this sector poses in the spreading of non-indigenous species. Their maintenance routines, travel history details, a visual estimate of their level of fouling, and actual found NIS, and awareness of NIS are explored to determine their role in facilitating the spreading of NIS in the Mediterranean context. The last section presents answers to the research questions, explains the scientific contributions of this study, a general summary, management implications and ideas for future research objectives (**Chapter 5**).

Together, these three main chapters examining Mediterranean marinas and recreational boats for NIS will show the importance of recreational boating/biofouling as a vector of spread through showing marinas both as sources for primary introductions and as hubs secondary transfer of NIS from the new records (Chapter 2), then explores which factors make some marinas better suited hosts for NIS (Chapter 3), and which factors are related to increased NIS richness on vessels and also demonstrates how many of these boats pose a very high risk for the transfer NIS (Chapter 4).

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Cost of last hull cleaning? _____ (Euros)

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

Was the hull cleaning performed in water _____ or out of water _____?

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

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

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

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

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

Approximate number of **days** moored outside of your home marina in last 12 months?

2 A MASSIVE UPDATE OF NON-INDIGENOUS SPECIES RECORDS FROM MEDITERRANEAN MARINAS

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2.1 Abstract

The Mediterranean Sea is home to most of the world's charter mega yachting traffic, and second to the United States for overall recreational boating traffic. Studies elsewhere have shown marinas as important stepping-stones for the transport of non-indigenous species (NIS), many of which seem to prefer artificial substrates over natural ones. However, only a handful of studies have specifically addressed recreational boating and its role as a vector for the spread of NIS. This study addresses this vector on a large-scale across the Mediterranean Sea. From April 2015 to November 2016, 34 marinas were sampled in 7 countries spanning the Mediterranean Sea from Spain to Turkey, in order to investigate occurrence of NIS in hard substrate assemblages. In addition, fouling samples were collected from approximately 600 boat hulls from 25 of these marinas to determine if some boats were hosting further NIS not yet found in the marina. Here, we present data illustrating that Mediterranean marinas do act as hubs for the settlement of marine NIS, and we provide evidence that recreational boats act as powerful vectors of introduction and spread. We report three new NIS never observed before in the Mediterranean Sea (*Achelia sawayai sensu lato*, *Aorides longimerus*, *Cymodoce* aff. *fuscina*), and the re-appearance of NIS previously known but nowadays considered extinct in the Mediterranean (*Bemlos leptochirus*, *Saccostrea glomerata*). We also compellingly update the distributions of many NIS in the Mediterranean Sea showing some recent spreading. On top of the previously mentioned records, we present here ten new records for NIS at the sub-regional scale, in new parts of the Mediterranean, 51 new country records and finally 19 new NIS records which were found on boat hulls in marinas, but were not present neither in the marina nor in the perspective country. However, these new NIS records from boats cannot be classified as new country records, since the boats are mobile habitats, yet these records do provide an early warning signal that these species have the chance to settle in these new localities. For each record, their current distributions both globally and in the Mediterranean are provided. The species found in the marinas sampled should now be added to the relevant NIS databases compiled by several entities. Records of uncertain identity are also discussed, to assess the probability of valid non-indigenous status.

2.2 Introduction

The seas are being rapidly being tainted by many harmful stressors such as climate change, overfishing, pollution and non-indigenous species [hereafter NIS] (Occhipinti-Ambrogi 2007). The Mediterranean recreational boating fleet is estimated to contain approximately 1.5 million vessels and hosts over 70% of global mega-yachting traffic, and is only second to the United States in its sheer number of recreational boats (Cappato 2011). It is also the world's most invaded sea, hosting over 700 NIS (Galil et al. 2017a); over half of which are of Indo-Pacific origin and have probably arrived via the Suez Canal (Galil et al. 2017b). The human-mediated transport of species across boundaries is dramatically altering the natural distribution of marine biota, impacting biodiversity as well as human well-being (Carlton 1989; Occhipinti-Ambrogi 2007).

Biological invasions are not only important to understand due to their associated ecological and economic impacts; but they also provide an opportunity to understand other important biogeographic processes such as long-distance dispersal, rapid adaptation and range-expansion processes (Viard et al. 2016). To properly assess the bioinvasion process and understand the scale of the associated threats, it is first necessary to have the most up-to-date information regarding species distributions, which are used to feed the many databases such as the European Alien Species Information Network (Katsanevakis et al. 2015), the World Register of Introduced Alien Species- WRIMS (Pagad et al. 2017) and AquaNIS- Information system on aquatic NIS and cryptogenic species (Olenin et al. 2014). These databases are highly utilized by scientists and legislators wishing to assess the breadth of the ecological and socio-economic consequences of biological invasions by understanding species' distributions, measuring trends, and generating ecological models.

Most records of NIS in the Mediterranean Sea originate from occasional or casual findings, while only a few monitoring programs thus far have specifically targeted Mediterranean marine NIS, mainly addressing Marine Protected Areas (MPAs, e.g. Mannino et al. 2017), commercial harbours (López-Legentil et al. 2015; Ferrario et al. 2017), or aquaculture sites (Verlaque 2001). Recreational marinas have not yet been systematically surveyed in the Mediterranean, despite the recent international literature indicating they are important hubs for new species introduction and secondary spreading events (Acosta et al. 2009; Floerl et al. 2009; Clarke-Murray et al. 2011; Ashton et al. 2014). Furthermore, several recent records of marine NIS in the Mediterranean come from marina habitats (Ros et al. 2013; Marchini et al. 2015a; Marić et al. 2016; Ferrario et al. 2017; Steen et al. 2017), suggesting that marinas are part of the stepping-stone invasion process.

The definition of NIS adopted here is: "An organism introduced outside its natural past or present distribution range by direct or indirect human activity (European Environment Agency 2012). This definition implies an anthropogenic-assisted transport via various pathways, albeit intentional or unintentional. The route that a new species is transported through to a recipient region is treated as a "pathway". In the Mediterranean Sea, in addition to shipping and aquaculture (together considered the principal pathways of global NIS introductions), the Suez Canal is frequently referenced as another relevant pathway for the migration of Indo-Pacific species (Galil et al. 2017a, and references therein). Each of these "pathways" can have several "vectors" attributed to them, which is the means by which they were transported (Minchin et al. 2009; Olenin et al. 2014). For example, the "shipping" pathway can have the following associated transport vectors: hull-fouling, ballast water, and sea chests. There is a high level of uncertainty associated with many of these pathways and vectors since it is rather impossible to prove how a species had been transported, although inferential reasoning on the locality, and proximity to known hubs for NIS introductions such as major ports, aquaculture

farms or the Suez Canal make it possible to put forth scientifically sound hypotheses. For this reason, a NIS is often defined as “polyvetic species” *sensu* Carlton et al. (Box 1, 2005), because it could have been introduced by a certain combination of pathways or vectors.

This contribution presents new records from the first large-scale survey of Mediterranean marinas for NIS. From April 2015 to November 2016, 34 marinas were sampled for NIS across the Mediterranean spanning from Spain, France, Italy, Malta, Greece, Turkey and Cyprus. Additionally, when permitted, boat-hulls were also inspected for NIS and their captains interviewed about the boats recent travel history since its last hull-cleaning to investigate if recreational boats indeed do seed new NIS propagules to marinas they are visiting, i.e., to verify the role that recreational boating plays as a vector of spread of NIS. Here, we present new NIS records either for the Mediterranean basin, sub-region, country or locality. The new records are presented by taxa, with information on the native origin of the species, their global and Mediterranean distributions, and details of the present record. Here, new records are provided for 32 macroinvertebrate species in Mediterranean marinas and an additional six species found on boat-hulls but not in the marina.

2.3 Materials and Methods

A total of 34 marinas were sampled in 7 countries, along with a subset of recreational boat-hulls from 25 of the marinas (Table 2.1; Figure 2.1). Table 1 provides an assigned number for each sampled marina, along with their coordinates and sampling dates.

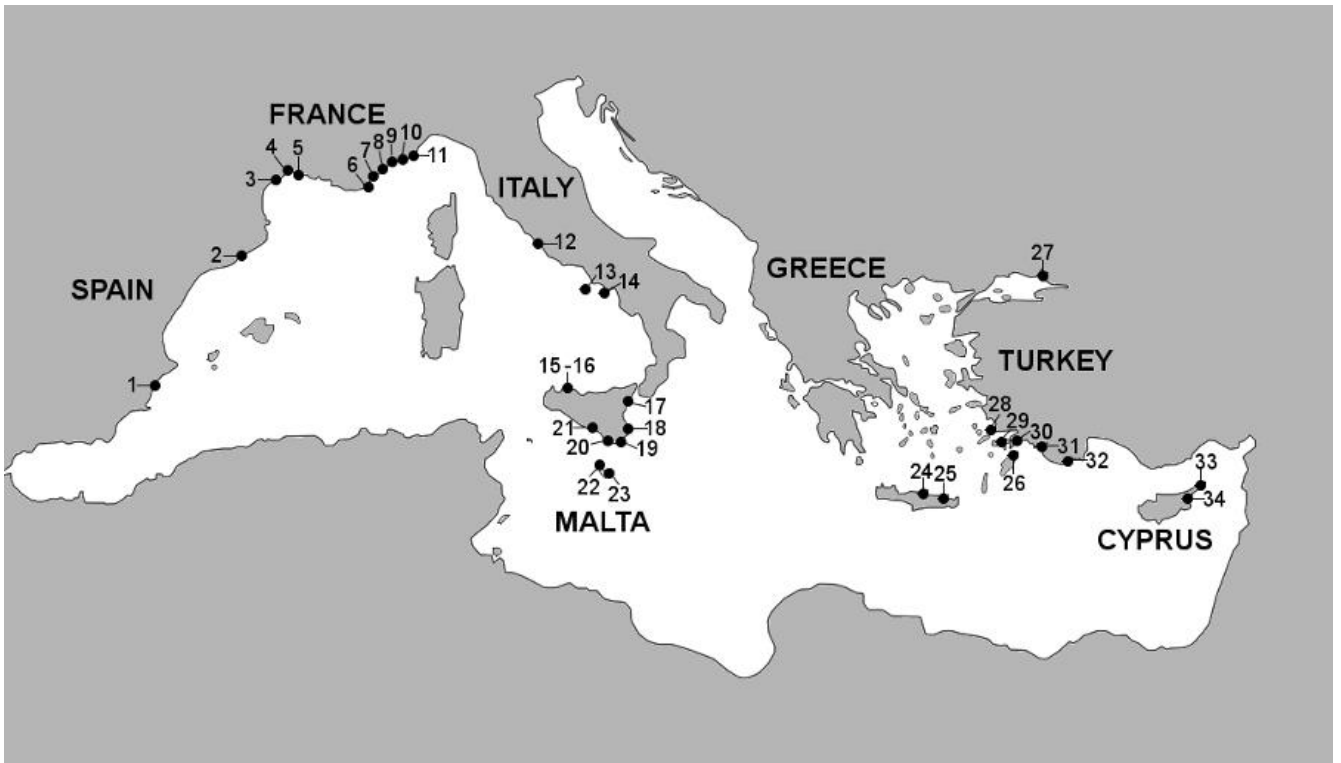


Figure 2.1 Marinas sampled in this study; numbers correspond to marinas provided in Table 2.1.

When reporting new sub-regional records for the Mediterranean, each sub-region includes the following countries: Western Mediterranean (Spain and France); Central Mediterranean (Italy and Malta); and Eastern Mediterranean (Greece, Turkey, and Cyprus).

Table 2.1 List of marinas sampled for this study, with sampling date, geographical coordinates and a category if boats-hull sampling was also performed.

Country	Number assigned	Locality name	Marina name	Geographical coordinates	Sampling dates	Boats sampled (Y/N)	
Spain	1	Alicante	Marina de Alicante	38.339 N; 0.4799 W	14, November 2016	N	
	2	Barcelona	One Ocean Port Vell Barcelona	41.376 N; 2.187 E	22, November 2016	N	
France	3	Agde	Port Cap d'Agde	43.281 N; 3.501 E	5-18, June 2015	Y	
	4	La Grande-Motte	Port de la Grande-Motte	43.557 N; 4.082 E	2, November 2016	N	
	5	Le Grau-du-Roi	Port Camargue	43.515 N; 4.132 E	16-28, May 2015	Y	
	6	Saint-Tropez	Port de Saint-Tropez	43.278 N; 6.637 E	1-30, April 2016	Y	
	7	Cogolin	Marines de Cogolin	43.065 N; 6.586 E	1-30, April 2016	Y	
	8	Sainte-Maxime	Port Privé de Sainte-Maxime	43.307 N; 6.638 E	1-30, April 2016	Y	
	9	Cannes	Cannes Le Vieux Port	43.540 N; 7.032 E	19-28, April 2015	Y	
	10	Antibes	Port Vauban	43.585 N; 7.127 E	1-12, May 2015	Y	
	11	Villefranche-sur-Mer	Port de Villefranche	43.698 N; 7.307 E	22-30, September 2016	N	
	Italy	12	Lido di Ostia	Porto Turistico di Roma	41.737 N; 12.250 E	12-19, July 2015	Y
		13	Ischia Island	Marina di Casamicciola; Marina di Lacco Ameno; Marina del Raggio Verde;	40.748 N; 13.906 E – 40.752 N; 13.891 E – 40.738 N; 13.860 E	30-8, July-August 2015	Y
14		Sorrento	Marina Piccola Sorrento	40.629 N; 14.375 E	22-29, July 2015	Y	
15		Palermo	Marina Villa Igiea	38.142 N; 13.370 E	26-29, July 2016	Y	
16		Palermo	Porto La Cala	38.120 N; 13.368 E	2-3, August 2016	N	
17		Riposto	Porto dell'Etna	37.732 N; 15.208 E	17-28, September 2016	Y	
18		Siracusa	Porto Grande (Marina Yachting)	37.063 N; 15.284 E	15-16, August 2016	N	
19		Marzamemi	Marina di Marzamemi	36.733 N; 15.119 E	8, September 2016	N	
20		Marina di Ragusa	Porto Turistico Marina di Ragusa	36.781 N; 14.546 E	29-7, August-September 2016	Y	
21		Licata	Marina di Cala del Sole	37.097 N; 13.943 E	5-10, August 2016	Y	
Malta		22	Msida	Msida Yacht Marina	35.896 N; 14.493 E	1-8, July 2016	Y
	23	Valletta	Grand Harbour Marina	35.890 N; 14.523 E	11-18, July 2016	Y	
Greece	24	Heraklion	Old Venetian Harbour	35.343 N; 25.136 E	13-14, October 2015	Y	

	25	Agios Nikolaos	Agios Nikolaos Marina	35.187 N; 25.136 E	4-12, November 2015	Y
	26	Rhodes	Mandraki Port	36.449 N; 28.226 E	2-11, June 2016	Y
Turkey	27	Istanbul	Setur Kalamış Marinas	40.976 N; 29.039 E	28, August 2015	Y
	28	Bodrum	Milta Bodrum Marina	37.034 N; 27.425 E	9-11, September 2015	Y
	29	Datça	Datça Marina	26.722 N; 27.689 E	13, May 2016; 5, April 2017	N
	30	Marmaris	Setur Marmaris Netsel Marina	36.852 N; 28.276 E	14-18, September 2015	Y
	31	Fethiye	Eçe Marina	36.623 N; 29.101 E	19-24, September 2015	Y
	32	Finike	Setur Finike Marina	36.294 N; 30.149 E	18-27, May 2016	Y
Cyprus	33	Karpaz	Karpaz Gate Marina	35.558 N; 34.232 E	21-27, June 2016	Y
	34	Famagusta	Famagusta Port	35.123 N; 33.952 E	13-19, June 2016	Y

2.3.1 Taxonomic identification

This study focused on fouling invertebrates; plants and algae were not examined. All macronivertebrate taxa were collected for identification. Samples requiring expert identification were sent to appropriate specialists. The preserved specimens were examined under a dissecting microscope and, as necessary, taxonomic slides were prepared and analyzed under an optical microscope. Photographs of magnified specimens or morphological parts were taken directly from the microscope using the Olympus TG-4 camera (i.e., for serpulids and crustaceans), or with the Tescan FESEM (Field Emission Scanning Electron Microscope) series Mira 3XMU for SEM pictures, with increasing magnification, at 6-19 mm working distance, using an accelerating voltage of 10 kV, with graphite metallization and detection by secondary electrons (i.e., for bryozoans). Bryozoan specimens used for SEM pictures were cleaned beforehand using a combination of bleach and hydrogen peroxide to remove organic residues. Ascidians were stained with Masson's haemalum for dissection.

Some of our records refer to species completely new to the Mediterranean Sea, whose taxonomic identity has been verified morphologically, but may still be requiring further genetic confirmation, since they pertain to taxonomically challenging taxa which have often revealed complexes of cryptic species. Moreover, a couple of our findings include species not yet properly described scientifically; thus it is not possible to assign a certain identification until formal descriptions are completed. These records are discussed in detail to verify the

likelihood of representing introduced populations of NIS. To assign a NIS status for such species, the Chapman & Carlton (1991) criteria were followed taking into account factors such as: “appearance in local regions where not found previously”; “association with human mechanisms of dispersal”; “prevalence or restrictions to artificial environments”; “insufficient active or passive dispersal capability” and “exotic evolutionary origin”. Records of species found only on boat-hulls but not in marinas should only be considered as new NIS country records if certain that the boat did not leave that country’s waters, since boats represent mobile habitats and are hence affected by an “uncertain occurrence” (see Marchini et al. 2015b).

NIS status is dependent on their establishment success in a new locality, and can be defined as either: not established (a single specimen reported in one or two localities, rare, uncommon), or established (evidence of a reproducing population in one or more localities, common or abundant). Additionally, a couple of cases are presented here for “pseudoindigenous species” (NIS perceived to be native species).

2.4 Results

Within the framework of this study, a total of 74 NIS were collectively identified from 34 marinas from the seven countries, however, only new country records and interesting new locality records are presented here. First, we present the number of new NIS found in this study per country and by taxa (Figure 2.2).

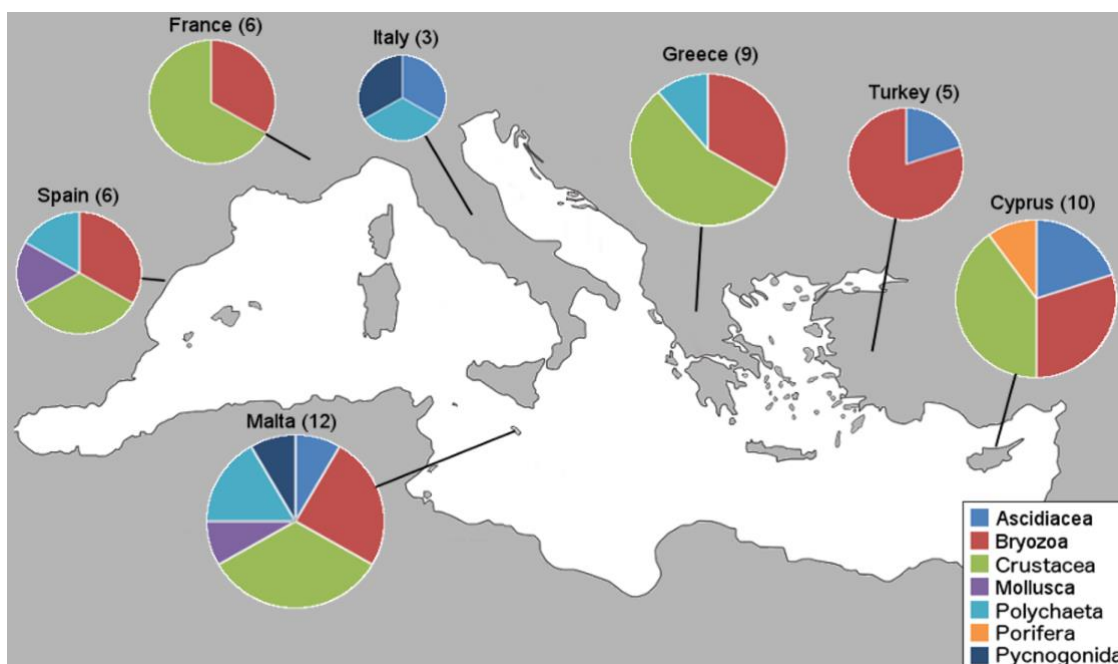


Figure 2.2 Number of NIS records found in this study represented by taxa in pie charts.

This study revealed three species new to the Mediterranean basin (*Achelia sawayai sensu lato*, *Aoroides longimerus*, and *Cymodoce aff. fuscina*), 11 new subregional records (*Watersipora arcuata*, *Hydroides brachyacantha sensu lato* and *Saccostrea glomerata* now present in the Western Mediterranean; *Symplegma brakenhielmi*, *Stenothoe georgiana*, *Spirobranchus tertaceros sensu lato*, *Dendostrea folium sensu lato* and *Parasmittina egyptiaca* now present in the Central Mediterranean, and *Watersipora arcuata*, *Bemlos leptochirus* and *Dyspanopeus sayi* in the Eastern Mediterranean), for an overall number of 51 new country records and a few new locality records exhibiting distribution expansions. These new Mediterranean basin and country records are presented (Table 2.2) with the corresponding marina numbers in which they were found from Table 2.1. Additionally, NIS found on boat-hulls but not in the respective marina, locality or country, are presented as a warning signal for future monitoring (Table 2.3). The numbers of new NIS found per marina are shown (Table 2.4), and also the new NIS records are presented by country, specifically twelve for Malta, ten for Cyprus, nine for Greece, six for Spain and France, five for Turkey and three for Italy (Table 2.5). Subsequently, all new NIS records are discussed by species (first ordered by class and family, and then alphabetically by species, see ‘New NIS records: notes on individual species’ below, accompanied with specimen photos). The key taxonomic characters used to identify these species are also presented along with specimen photos from this study. Comprehensive reviews of global and Mediterranean distributions for all NIS listed in Tables 2.2 and 2.3 are presented below, along with details on the new record type and if they were found in the marina, on a boat-hull or both.

Table 2.2 New NIS record table by species, with numbers in locality corresponding to marina details from Table 1. Record Type: * New country record, ** New Mediterranean record; Letters indicate a new subregional record (WM=Western Med.; CM=Central Med.; EM=Eastern Med.).

Family	Species	Country and Marina	Record
Asciacea	<i>Clavelina oblonga</i>	Cyprus (#34)	*
	<i>Clavelina oblonga</i>	Turkey (#29)	*
	<i>Phallusia nigra</i>	Cyprus (#33, #34)	*
	<i>Styela plicata</i>	Malta (#22, #23)	*
	<i>Symplegma brakenhielmi</i>	Italy (#15)	*, CM
Bryozoa	<i>Amathia verticillata</i>	Malta (#22, #23)	*
	<i>Amathia verticillata</i>	Cyprus (#34)	*
	<i>Amathia verticillata</i>	Turkey (#28, #30)	*
	<i>Celleporaria brunnea</i>	Spain (#1)	*
	<i>Celleporaria brunnea</i>	France (#4, #5, #6,	*
	<i>Celleporaria brunnea</i>	Malta (#22, #23)	*
	<i>Celleporaria brunnea</i>	Greece (#24)	*
	<i>Celleporaria vermiformis</i>	Greece (#24, #25,	*

	<i>Celleporaria vermiformis</i>	Cyprus (#33, #34)	*
	<i>Hippopodina</i> aff. <i>feegeensis</i>	Turkey (#32)	*
	<i>Parasmittina egyptiaca</i>	Turkey (#32)	*
	<i>Parasmittina egyptiaca</i>	Cyprus (#33)	*
	<i>Tricellaria inopinata</i>	France (#3, #5)	*
	<i>Tricellaria inopinata</i>	Greece (#24)	*
	<i>Watersipora arcuata</i>	Spain (#1, #2)	*
	<i>Watersipora arcuata</i>	Malta (#22)	*, CM
	<i>Watersipora arcuata</i>	Turkey (#28, #32)	*, EM
Crustacea	<i>Ampithoe bizseli</i>	Cyprus (#33, #34)	*
	<i>Aorides longimerus</i>	France (#5)	**
	<i>Bemlos leptocheirus</i>	Greece (#24, #25)	*, EM
	<i>Charybdis (Gonioinfradens)</i>	Cyprus (#34)	*
	<i>Cymodoce</i> cf. <i>fuscina</i>	Greece (#24)	**
	<i>Dyspanopeus sayi</i>	Greece (#24)	*, EM
	<i>Erichthonius</i> cf. <i>pugnax</i>	France (#5)	*
	<i>Ianiropsis serricaudis</i>	France (#3, #5)	*
	<i>Mesanthura</i> cf. <i>romulea</i>	Spain (#1)	*
	<i>Mesanthura</i> cf. <i>romulea</i>	Malta (#22)	*
	<i>Mesanthura</i> cf. <i>romulea</i>	Greece (#26)	*
	<i>Mesanthura</i> cf. <i>romulea</i>	Cyprus (#33, #34)	*
	<i>Paracerceis sculpta</i>	Malta (#22, #23)	*
	<i>Paracerceis sculpta</i>	Cyprus (#34)	*
	<i>Paranthura japonica</i>	Spain (#1, #2)	*
	<i>Paranthura japonica</i>	Malta (#23)	*
	<i>Sphaeroma walkeri</i>	Greece (#24)	*
	<i>Stenothoe georgiana</i>	France (#5)	*
	<i>Stenothoe georgiana</i>	Malta (#23)	*, CM
Mollusca	<i>Arcuatula senhousia</i>	Spain (#2)	*
	<i>Dendostrea folium</i> s.l.	Malta (#22, #23)	*, CM
Polychaeta	<i>Hydroides brachyacantha</i> s.l.	Spain (#2)	*, WM
	<i>Hydroides brachyacantha</i> s.l.	Greece (#24)	*
	<i>Hydroides dirampha</i>	Malta (#22, #23)	*
	<i>Hydroides elegans</i>	Malta (#22)	*
	<i>Spirobranchus tetraceros</i> s.l.	Italy (#18)	*, CM
Porifera	<i>Paraleucilla magna</i>	Cyprus (#34)	*
	<i>Achelia sawayai</i> s.l.	Malta (#23)	**
Pycnogonida	<i>Achelia sawayai</i> s.l.	Italy (#17, #18)	**

Table 2.3 NIS found on boat-hulls but not found in the marina or country: Λ= Not previously known from the locality, *=Not previously known from the country. Letters indicate a new subregional record (WM=Western Med.; CM=Central Med.; EM=Eastern Med.).

Family	Species	Country and Marina #	Record type
Asciacea	<i>Clavelina oblonga</i>	Cyprus (#33)	Λ
Bryozoa	<i>Amathia verticillata</i>	Turkey (#31)	Λ
	<i>Celleporaria brunnea</i>	France (#3, #7, #9, #10)	Λ
	<i>Tricellaria inopinata</i>	Turkey (#27)	*
	<i>Parasmittina egyptiaca</i>	Italy (#21)	*, CM
	<i>Parasmittina egyptiaca</i>	Greece (#25)	*
	<i>Watersipora arcuata</i>	France (#7)	*
	Crustacea	<i>Amphibalanus improvisus</i>	France (#5)
<i>Balanus trigonus</i>		Cyprus (#33)	*
<i>Cymodoce aff. fuscina</i>		Greece (#25)	Λ
<i>Erichthonius cf. pugnax</i>		France (#3)	Λ
<i>Paracerceis sculpta</i>		Turkey (#31)	*
<i>Paradella diana</i>		Italy (#20)	Λ
<i>Paradella diana</i>		Greece (#24)	*
<i>Sphaeroma walkeri</i>		Greece (#25)	Λ
<i>Stenothoe georgiana</i>		France (#3, #10)	Λ
Mollusca		<i>Dendostrea folium s.l.</i>	Italy (#17)
	<i>Saccostrea glomerata</i>	France (#10)	*, WM
Polychaeta	<i>Hydroides homoceros</i>	Cyprus (#33)	*

Table 2.4 Number of NIS per marina, using marina numbers given in

#. Marina locality	# NIS	#. Marina locality	#
1. Alicante, Spain	10	18. Siracusa, Italy	16
2. Barcelona, Spain	11	19. Marzememi, Italy	11
3. Cap d'Agde, France	8	20. Ragusa, Italy	14
4. La Grand-Motte, France	7	21. Licata, Italy	11
5. Port Camargue, France	17	22. Msida, Malta	14
6. Saint-Tropez, France	4	23. Grand Harbour, Malta	13
7. Cogolin, France	6	24. Heraklion, Greece	27
8. Saint-Maxime, France	3	25. Agios Nikolaos, Greece	12
9. Cannes, France	5	26. Rhodes, Greece	16
10. Antibes, France	5	27. Istanbul, Turkey	4
11. Villefranche-sur-Mer,	2	28. Bodrum, Turkey	12
12. Rome, Italy	9	29. Datça, Turkey	9
13. Ischia, Italy	5	30. Marmaris, Turkey	6
14. Sorrento, Italy	8	31. Fethiye, Turkey	10
15. Villa Igiea, Italy	20	32. Finike, Turkey	14
16. La Cala, Italy	16	33. Karpaz, Cyprus	17
17. Riposto, Italy	13	34. Famagusta, Cyprus	18

Table 2.5 New NIS country records from marinas.

Country	Species	Country	
Spain	<i>Celleporaria brunnea</i>	Greece	<i>Celleporaria brunnea</i>
Spain	<i>Watersipora arcuata</i>	Greece	<i>Celleporaria vermiformis</i>
Spain	<i>Mesanthura cf. romulea</i>	Greece	<i>Tricellaria inopinata</i>
Spain	<i>Paranthura japonica</i>	Greece	<i>Bemlos leptocheirus</i>
Spain	<i>Arcuatula senhousia</i>	Greece	<i>Cymodoce cf. fuscina</i>
Spain	<i>Hydroides brachyacantha s.l.</i>	Greece	<i>Dyspanopeus sayi</i>
France	<i>Celleporaria brunnea</i>	Greece	<i>Mesanthura cf. romulea</i>
France	<i>Tricellaria inopinata</i>	Greece	<i>Sphaeroma walkeri</i>
France	<i>Aorides longimerus</i>	Greece	<i>Hydroides brachyacantha s.l.</i>
France	<i>Erichthonius cf. pugnax</i>	Turkey	<i>Clavelina oblonga</i>
France	<i>Ianiropsis serricaudis</i>	Turkey	<i>Amathia verticillata</i>
France	<i>Stenothoe georgiana</i>	Turkey	<i>Hippopodina aff. feegeensis</i>
Italy	<i>Symplegma brakenhielmi</i>	Turkey	<i>Parasmittina egyptiaca</i>
Italy	<i>Spirobranchus tetraceros s.l.</i>	Turkey	<i>Watersipora arcuata</i>
Italy	<i>Achelia sawayai s.l.</i>	Cyprus	<i>Clavelina oblonga</i>
Malta	<i>Styela plicata</i>	Cyprus	<i>Phallusia nigra</i>
Malta	<i>Amathia verticillata</i>	Cyprus	<i>Amathia verticillata</i>
Malta	<i>Celleporaria brunnea</i>	Cyprus	<i>Celleporaria vermiformis</i>
Malta	<i>Watersipora arcuata</i>	Cyprus	<i>Parasmittina egyptiaca</i>
Malta	<i>Mesanthura cf. romulea</i>	Cyprus	<i>Mesanthura cf. romulea</i>
Malta	<i>Paracerceis sculpta</i>	Cyprus	<i>Ampithoe bizseli</i>
Malta	<i>Paranthura japonica</i>	Cyprus	<i>Charybdis (Gonioinfradens) paucidentatus</i>
Malta	<i>Stenothoe georgiana</i>	Cyprus	<i>Paracerceis sculpta</i>
Malta	<i>Dendostrea folium s.l.</i>	Cyprus	<i>Paraleucilla magna</i>
Malta	<i>Hydroides dirampha</i>		
Malta	<i>Hydroides elegans</i>		
Malta	<i>Achelia sawayai s.l.</i>		

2.5 New NIS records: notes on individual species

Please note that the numbers used in describing the locality of the new records correspond to the marinas listed in Table 2.1.

2.5.1 Class: Ascidiacea

Some ascidians whose likely origin is the Northeast Atlantic (i.e., *Clavelina lepadiformis*, *Ciona intestinalis*, *Ascidella aspersa* and *Botryllus schlosseri*) have been excluded from this study which focuses exclusively on NIS.

Genetic studies have shown that these species include different clades in the Mediterranean, some which can be considered non-native, and in some cases native (Turón *et al.* 2003; Pérez-Portela *et al.*, 2013; Bouchemousse *et al.*, 2016; Nydam *et al.* 2017). These cryptogenic species (Carlton, 1996), their origins and status require additional genetic analyses, which exceeds the breadth of the present study, which is based on morphological characters.

Family: Ascidiidae



Figure 2.3.0 Ascidians: (A) *Phallusia nigra* in #34; (B) *Clavelina oblonga* in #34; (C) *Diplosoma listerianum* in #23; (D) *Microcosmus squamiger* in #20; (E) *Styela plicata* in #14; (F) *Symplegma cf. brakenhielmi* in #32. Photo credits (A-F): Aylin Ulman.

Phallusia nigra Savigny, 1816

Potential native origin: Uncertain, could be from the Red Sea, Indo-Pacific, or Western Atlantic Ocean.

Distribution: First recorded and described from the Red Sea (Savigny 1816), then in the Gulf of Guinea and Angola (Millar 1965), the Arabian Gulf (Monniot et al. 1997), the Pacific Ocean (Lambert 2003), Indian Ocean (Abdul Jaffar Ali et al. 2009), and the Western Atlantic and Caribbean (Van Name 1945; Bonnet et al. 2011; Vandepas et al. 2015).

In the Mediterranean, it has only been reported in the Eastern Mediterranean from Israel, Lebanon and the Turkish Levantine coast (Çinar et al. 2006; Shenkar 2008; Izquierdo-Muñoz et al. 2009), and most recently from Greece, specifically from Chalkidiki and Rhodes (Kondilatos et al. 2010; Thessalou-Legaki et al. 2012).

New records: This finding represents the first country record for Cyprus (#33 and #34: Supplementary Data [hereafter S.D.]; Fig. 2.3.0 A).

Boat-hull records: Found on one boat-hull moored in Cyprus (#34).

Notes: Although its native origin is uncertain, it is considered a NIS in the Mediterranean (Çinar et al. 2006; Shenkar 2008). Vandepas et al. (2015) highlighted some uncertainty regarding some *Phallusia nigra* Mediterranean records due to resemblances to the also dark, native congeneric tunicate *Phallusia fumigata* (Gruber, 1864), and confirmed the presence of the introduced *P. nigra* in the Eastern Mediterranean basin. For this reason, the morphology of the *Phallusia* specimens collected from Cyprus were carefully compared to specimens of the native *Phallusia fumigata* (found in our own samples from Port Vell, Barcelona).

Key taxonomic characters: Solitary ascidian with a very smooth, jet or ink-black tunic (can also be dark brown) devoid of epibionts, however juvenile specimens appear greyish. Long, curved oral siphon, bringing it near short atrial siphon, up to 10 cm in length. After alcohol preservation, the tunic turns a dark blue hue. Removal of tunic exposes a dense network of longitudinal and transverse muscular bands on right side.

Family: Clavelinidae

Clavelina oblonga Herdman, 1880

Native origin: Western Atlantic US coast and Caribbean Sea.

Distribution: Its initial record (Herdman, 1880) is from Bermuda (Van Name 1945). It is hypothesized to be an introduced species to Brazil, first sighted there in 1925 (Rocha et al. 2012). In the Eastern Atlantic, it has been reported as NIS in Cape Verde (Hartmeyer R 1912), Senegal (Pérès 1951), and the Azores (Monniot C et al. 1994). It was described in the Mediterranean half a century after its initial record as *Clavelina phlegraea* from southern Italy and Corsica (Salfi 1929). It was also found in natural habitats on the Iberian Coast, about 100 km west of Gibraltar (Ordóñez et al. 2016).

New records: This finding represents a first country record for Turkey (Marina #29) and Cyprus (#34: Fig. 2.3.0B), and two new locality records for mainland France (#5, #7).

Boat-hull records: Found on boat-hulls moored in Cyprus (#33 and #34).

Notes: The species identified earlier as *C. phlegraea* (Salfi, 1929) in the Mediterranean was thought to be a native species, but recent genetic analysis confirmed it as the introduced *C. oblonga* (Ordóñez et al. 2016). In France, it had only previously been reported in Corsica, so these new records from the French mainland indicate its possible expansion along the coast.

Key taxonomic characters: Colonial ascidian, joined at base to others by short stolons. Soft tunic, mostly transparent with white speckled dots. On thick basal tunic, numerous fine stolons ending in white pigmented budding chambers. Zooids 25 mm in length, with some white pigment in branchial sac and stomach, but can also be blue or pink. 20 simple tentacles of various orders, vertical oval neural gland aperture and about 20 rows of stigmata in the branchial sac (with 50-60 stigmata per half row). Digestive system contains a descending esophagus, a subterminal squared stomach with marked ridges, followed by a mid-intestine and an ascending rectum. The gonads left of intestinal loop, and stomach dorsally containing numerous ovoid and small male follicles, with a mass of oocytes in middle of testes.

Family: Didemnidae

Diplosoma listerianum (Milne-Edwards 1841)

Native origin: North Sea.

Distribution: This species was first described from England but is well known from marinas and harbours worldwide including the Pacific Northwest, Panama, Chile, Japan, Tahiti, Guam, South Africa and Australia (Rocha RM et al. 2005; Perez-Portela et al. 2013). In the Mediterranean, its first record was from Italy in 1975 (Lafargue, 1975), and is now widespread throughout European and Mediterranean waters (Millar, 1969; Ramos-Esplá, 1988; Koukouras et al. 1995; Çinar 2014).

New records: This study presents a new locality record for the Turkish Levantine Sea/Mediterranean coast (#31, #32). During this study, it was also found in France (#5), Malta (#23: Fig. 2.3.0C), Turkey (#28) and Greece (#24 and #26).

Key taxonomic characters: Colonial ascidian with immersed zooids. Forms thin, flat, jelly-like transparent or milky-white sheets less than 2 mm thick. Common tunic without spicules, it may contain grey or brown pigmented flecks. Zooids with fixator's appendix. Esophagus and intestine crossed. Gonads to left of gut, ovary with more than two oocytes.

Family: Pyuridae

Microcosmus exasperatus Heller 1878

Potential native origin: Unknown.

This species has a broad global distribution from all continental waters, including remote localities such as Hawaii and the Mariana Islands, but does not occur in Antarctica (Nagar et al. 2016).

In the Mediterranean, it was first reported from south-eastern Tunisia in 1998 (Meliane 2002; Ramos-Esplá et al. 2013), then from Lebanon (Bitar et al. 2007), Israel (Shenkar 2008), around the Lebanese coast in 2009 (Ramos-Esplá et al. 2013), the Aegean Sea of Turkey (Ramos-Esplá et al. 2013), and North-Western Cyprus (Gewing et al. 2016).

New records: This study presents a new locality record for Turkey (#29) as the southernmost record for Turkey, and a new locality for Cyprus (#33), illustrating its ongoing expansion.

Notes: *Microcosmus exasperatus* and *Microcosmus squamiger* are both present in the Mediterranean, however, they do not overlap in distributions: *M. squamiger* is present in the Western and Central Mediterranean whereas *M. exasperatus* is only present in the Eastern Mediterranean (Ramos-Esplá et al. 2013). Thus, it has been hypothesized these two species invaded via different entrances to the basin: *M. squamiger* via the Strait of Gibraltar and *M. exasperatus* via the Suez Canal (Turón et al. 2007, Ramos-Esplá et al. 2013). Noteworthy is that *M. exasperatus* was not found in late 2014 in Karpaz Marina, Cyprus (#33) by Gewing et al. (2016) when specifically looking for this species, however, we found it present there in 2016.

Microcosmus squamiger Michaelsen, 1927

Potential native origin: Australia.

Distribution: Globally, this species is found in the waters of California, South Africa, Hawaii, and the Western Indian Ocean (Mastrototaro et al. 2005).

In the Mediterranean, it was first reported from Tunisia in 1967 (Monniot 1981), and is now found throughout the Western Mediterranean (Monniot 1981; Ramos-Esplá 1988; Mastrototaro et al. 2005; Turón et al. 2007) and from the Central Mediterranean: Taranto, Italy and Grand Harbour, Malta (Izquierdo-Muñoz et al. 2009).

New records: This finding represents a new locality for Italy around Sicily (#15, #17, #19, #20: Fig. 2.3D). From this study, it was also found in Spain (#2).

Key taxonomic characters: Solitary ascidian with hard tunic. Globular and irregular shape with prominent siphons. Brown in colour sometimes with small epibionts. Up to 4 cm in length. Found in small clusters, not dense aggregations. Two species impossible to distinguish without dissection (See remarks below).

Remarks: *Microcosmus exasperatus* is differentiated taxonomically from *M. squamiger* by the shape of the siphonal spines from the inhalant siphon, which are much longer and pointy (and curved like a shark fin, Kott 1985) in *M. exasperatus* and shorter and rounded at the top ('roma' shaped) in *M. squamiger*.

Family: Styelidae

Styela plicata (Lesueur, 1823)

Potential native origin: Unknown, cosmopolitan species.

Distribution: This species has been reported worldwide (Harant et al. 1933; Van Name 1945; Pérès 1951; Tokioka 1963; Ramos-Esplá 1988). It is considered a NIS in California (Lambert et al. 2003), Gulf of Mexico (Lambert 2005), Brazil (Rocha et al. 2005) and the Mediterranean Sea (Maltagliati et al. 2016).

New records: This finding represents a new country record for Malta (#22 and #23). From this study, *S. plicata* is extremely widespread and was found in all sampled marinas aside from #6, #9, #11, #29 and #33.

Boat-hull records: Found on boat-hulls moored in the following marinas: France (#3, #5, #7, #10), Italy (#12, #14: Fig. 2.3E, #15, #21), Malta (#22), Greece (#24, #26), and Turkey (#31, #32).

Notes: This is a well-known cosmopolitan hull-fouling species found from many localities across the Atlantic Ocean from Philadelphia, USA (Van Name 1945) to Senegal (Pérès 1951). Recent genetic analysis suggests that its wide geographic distribution is attributed to many introductions stemming from human-mediated hull fouling, triggering multiple introduction events (Barros et al. 2009). Additionally, most records are from artificial substrates or harbours, also supporting the hypothesis of an ongoing invasion (Barros et al. 2009).

Key taxonomic characters: Solitary, unstalked ascidian. Externally, can be whitish to tan or greyish in color, often containing epibionts; tunic quite lumpy, although juveniles are less lumpy in appearance but have small pleats. Internally, from 4 to 8 gonads on left and 2 on right (Ramos-Esplá 1988).

Symplegma brakenhielmi (Michaelson, 1904)

Potential native origin: Unknown

Distribution: It has been found in Australian waters (Kott 2004), the Pacific Panamanian coast (Carman et al. 2011), and from the Atlantic in French Guianese waters (Monniot 2016). In the Mediterranean, it was reported from Israel in the 1950s (as *Symplegma viride* Herdman, 1886), then from Lebanon (Bitar et al. 2001; Bitar et al. 2007), Israel (Shenkar 2008) and Turkey (Çinar et al. 2006).

New records: This study presents a new country record for Italy (#15), and a new Central Mediterranean subregional record. During this study, it was also found in Turkey (#31 and #32: Fig. 2.3.0F).

Boat-hull records: Found on one boat-hull moored in Turkey (#31).

Notes: It is likely that, Pérès (1958) is referring to this species under the name *S. viride*. Antoniadou et al. (2016), in their recent update of ascidians found in Greek waters warned of a high-likelihood of a Greek invasion due to its proximity to the Turkish Levantine coast. This study confirms its spread to the Central Mediterranean. Soon after this finding in Cyprus from June 2016, it was also reported from Cyprus in Larnaca Bay in November 2016 by Gerovasileiou et al. (2017).

Key taxonomic characters: Encrusting colonial ascidian, zooids joined by common base. Thin transparent tunic with many vascular processes. Branchial sac with 4 longitudinal lines. Long and curved caecum connected to main intestinal tract with two bands of tissues; 4-5 longitudinal stomach plications. Testes with lobes.

2.5.2 Bryozoa

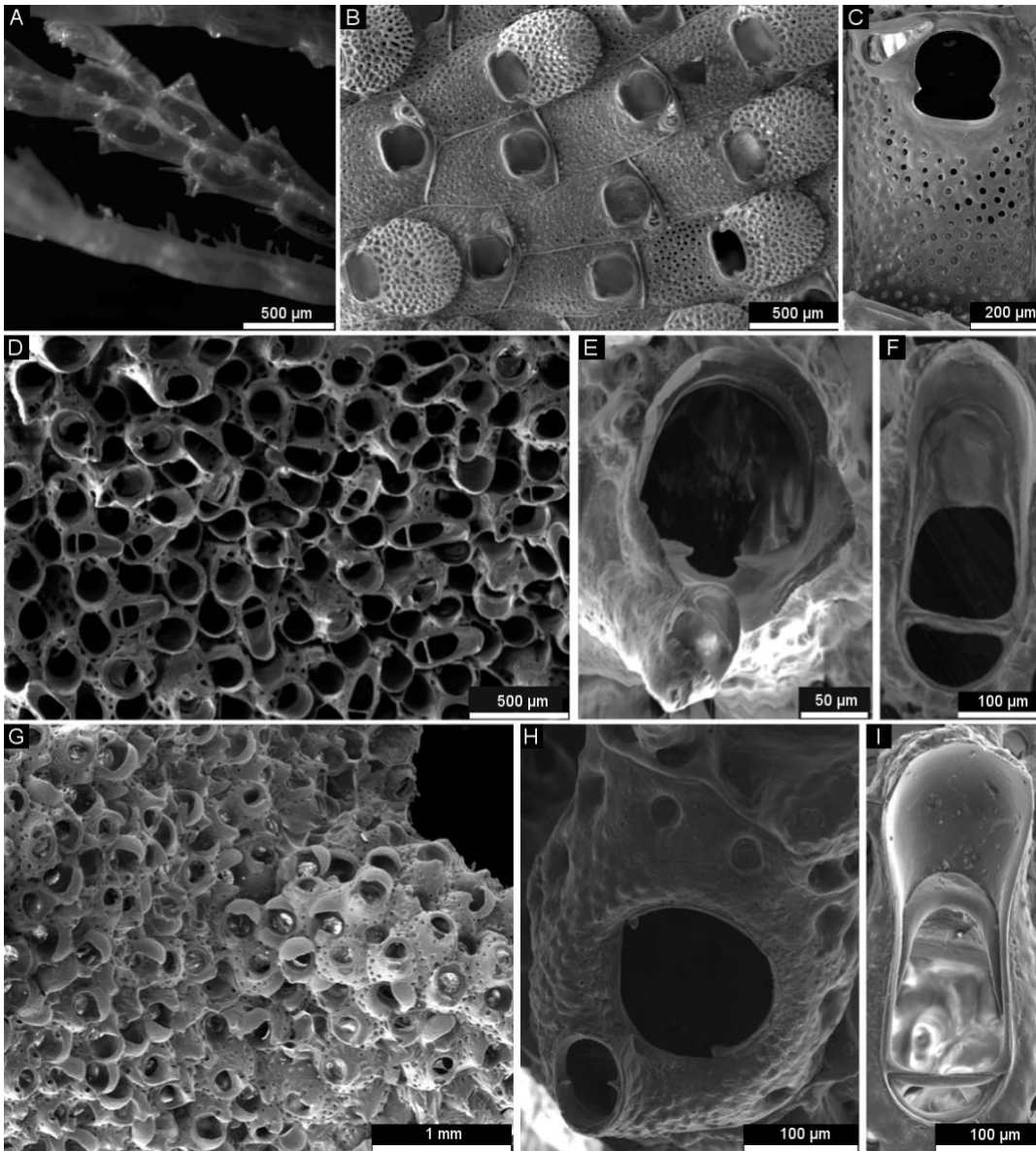


Figure 2.3.1 Bryozoans specimen photos, part 1: (A) *Tricellaria inopinata* in #5; (B-C) *Hippopodina* sp. A: (B) colony with ovicelled autozooids in #26, (C) close-up of the autozooid with avicularia in #32; (D-F) *Celleporaria brunnea* in #5: (D) colony, (E) close-up of the orifice and the sub-oral adventitious avicularium, (F) close-up of the interzooidal avicularium; (G-I) *Celleporaria vermiformis* from #33: (G) colony with ovicelled zooids; (H) close-up of the orifice and the sub-oral adventitious avicularium; (I) gigantic vicarious avicularium. Photo credits: Maria Pia Riccardi and Ilenia Tredici (CISRIC-Arvedi Laboratory) at the University of Pavia assisted Jasmine Ferrario with the use of the Scanning Electronic Microscope (SEM).

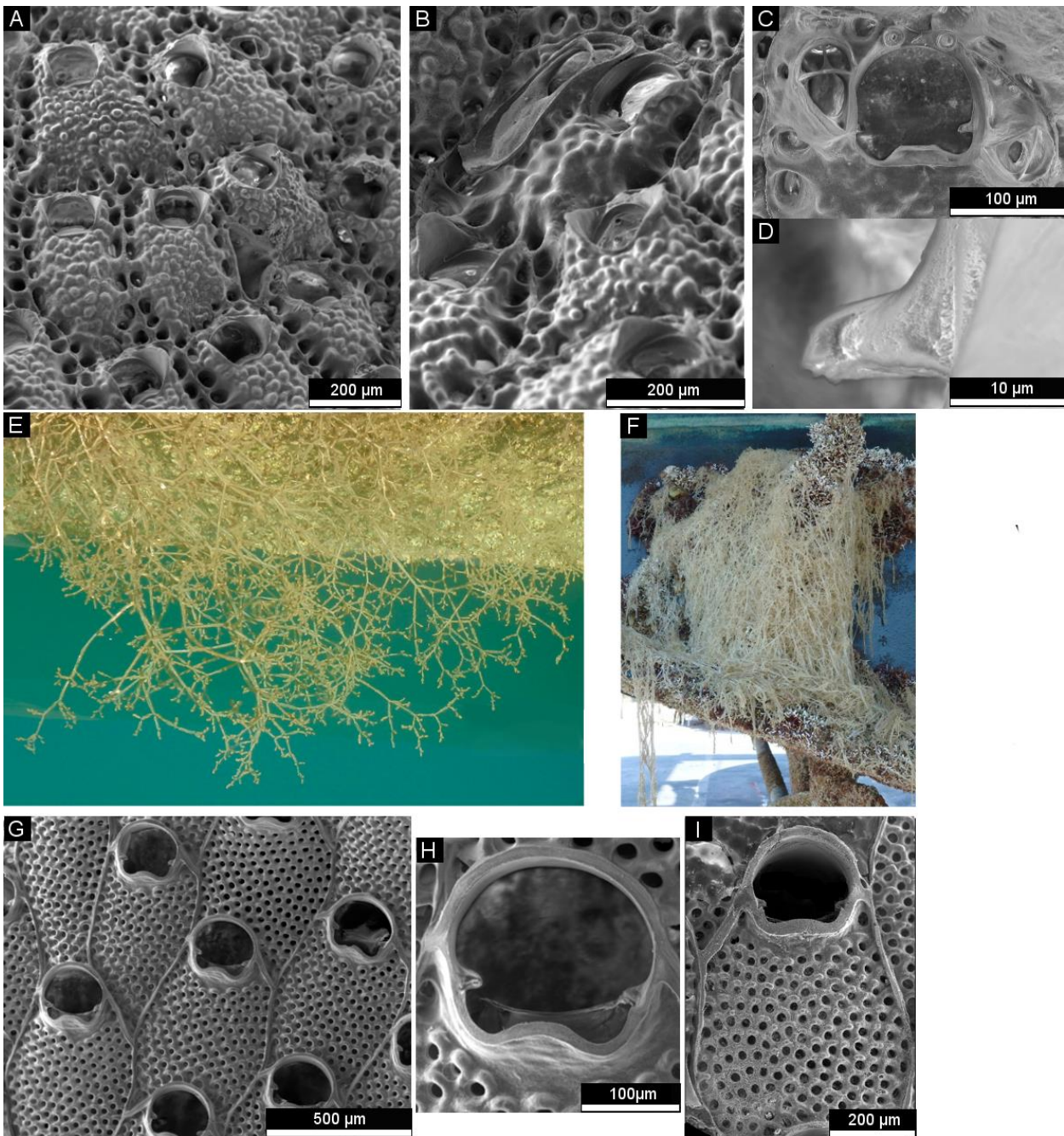


Figure 2.3.2 Bryozoans specimen photos, part 2: (A-B) *Parasmittina egyptiaca* on boat hull in #25: (A) colony, (B) autozoid with a gigantic spatulate avicularium with triangular flaps; (C-D) *Parasmittina egyptiaca* in #33: (C) close-up of the orifice with two small avicularia, (D) condyle; (E-F) *Amathia verticillata*: (E) colony in #30, (F) colony on boat hull in #12; (G-H) *Watersipora arcuata* in #18: (G) colony, (H) close-up of the orifice; (I) *Watersipora arcuata* in #22: autozoid. Photo credits: (A-C, G-I) Maria Pia Riccardi and Ilenia Tredici (CISRIC-Arvedi Laboratory) at the University of Pavia assisted Jasmine Ferrario with the use of the Scanning Electronic Microscope (SEM); (E, F) Aylin Ulman.

Family: Candidae

Tricellaria inopinata d'Hondt & Occhipinti Ambrogi, 1985

Potential native origin: Indo-Pacific Ocean.

Distribution: It is considered a NIS in New Zealand and cryptogenic elsewhere in the Pacific, from Japan to Taiwan, Australia and the Northeast Pacific (Dyrynda et al. 2000). It was also reported from the Northeast Atlantic coasts of Great Britain, Ireland, Belgium, France, the Netherlands, Spain, Portugal and Germany (Dyrynda et al. 2000; Arenas et al. 2006; Cook et al. 2013). This species has also been transported via aquaculture and in association with marine debris stemming from the 2011 Japanese tsunami which landed in Oregon (Calder et al. 2014).

In the Mediterranean, *Tricellaria inopinata* was first described in the Lagoon of Venice in 1982 (d'Hondt & Occhipinti-Ambrogi 1985) and is considered a NIS in the Mediterranean Sea because the genus *Tricellaria*, typical of the Indo-Pacific Ocean, was previously absent from the basin. After its initial Venetian record, it was reported from Tunisia (Ben Souissi et al. 2006), and from several other Italian localities (Lodola et al. 2012b; Ferrario et al. 2017).

New records: This finding represents new country records for France (#3 and #5: Fig. 2.3.1 A) and Greece (#24).

Boat-hull records: Found on boat-hulls moored in Italy (#14), France (#3 and #5), and Turkey (#27).

Notes: In Europe, it was found on various types of artificial substrates, e.g., boat-hulls, ropes, docks and also natural substrates (Dyrynda et al. 2000; De Blauwe et al. 2001). Generally, *T. inopinata* is known to establish successfully in marinas lacking strong freshwater inputs (Occhipinti-Ambrogi 1991; Johnson et al. 2012; Cook et al. 2013). If it establishes from boat to marina in Turkey, it would then present a new country record.

Key taxonomic characters: Dichotomously branched whitish arborescent colonies. Well-marked joints at the bases of the rami. Autozooids disposed in alternating series, bearing avicularia with jaw-like mandible. Autozooids with spines, generally three in the outer and two in the inner side. Scuta usually small, variable in shape and size within the colony.

Family: Hippopodidae

Hippopodina sp. A

Potential native origin: Indo-Pacific Ocean.

Distribution: The species *Hippopodina feegeensis* (Busk, 1884) from the Indo-Pacific and the Red Sea, was reported as NIS in the Eastern Mediterranean Sea (Powell 1969; Morri et al. 1999; Corsini-Foka et al. 2015). However, Tilbrook (1999) had observed strong morphological variations within *H. feegeensis* colonies from different geographical regions, and some species were later designated to be new species (Tilbrook 2006). Particularly, Tilbrook (2006) recognised that the true *H. feegeensis* is restricted to the Philippines Islands, South China Sea and Australia, while two other *Hippopodina* spp. were left undescribed (named as *H. "feegeensis"*, Holothuria Bank and *H. "feegeensis"*, Ethiopia (*sic*) in Tilbrook, 2006). The material presented here is most likely conspecific with the still undescribed *Hippopodina* sp. collected by Tilbrook (2006) from Massawa Harbour, Erythraea (Kevin J. Tilbrook, pers. comm., 2017), and is indicated here as *Hippopodina* sp. A.

New records: This study presents a new country record for Turkey (#32). It was also found in Rhodes, Greece (#26: Fig. 2.3.1B). Recently, Corsini-Foka et al. (2015) recorded *H. feegeensis* from Mandraki Harbour in Rhodes, in the same locality where it was also collected during this study (at the Three Windmills wall), and those specimens will likely be re-assigned to *Hippopodina* sp. A, after a more comprehensive and detailed taxonomic comparison is undertaken.

Boat-hull records: Found on two boat-hulls moored in Turkey (#32: Fig. 2.3.1 C).

Notes: This species is morphologically similar to *H. feegeensis*, with only a few varying characters (see Supplementary Data). Further morphological and genetic comparisons are necessitated to compare the Mediterranean specimens thus far identified as *H. feegeensis* (Powell 1969; Morri et al. 1999; Corsini-Foka et al. 2015) with samples from the Red Sea, which will then lead to a proper taxonomic description for these *Hippopodina* samples.

Key taxonomic characters: Unilaminar encrusting colonies, autozooids rectangular in shape with the frontal wall perforated by numerous small pores. Primary orifice bell-shaped, with proximal border shallowly concave (which is generally straight in *H. feegeensis*); orifice with medium-sized lateral indentation, poster 90% width of anter (80% in *H. feegeensis*). Single or paired medium-sized adventitious avicularia, elongated-triangular in shape and medially directed.

Family: Lepraliellidae

Celleporaria brunnea (Hincks, 1884)

Native origin: Northeast Pacific Ocean.

Distribution: It is widely distributed in the Pacific Ocean (British Columbia, Ecuador, Gulf of California, Hawaiian Islands, Korea and Panama Canal: see Soule et al. 1995; Seo et al. 2009). Recorded as a NIS along the North-eastern Atlantic (Portugal and France: Canning-Clode et al. 2013; Harmelin 2014) and Mediterranean Sea (from Croatia, Italy, Lebanon, Turkey: Koçak 2007; Harmelin et al. 2009; Harmelin 2014; Lezzi et al. 2015; Lodola et al. 2015; Ferrario et al. 2016; Marić et al. 2016).

New records: These findings represent first country records for Spain (#1), France (#4, #5: Fig.2.3.1 D-F, #6, #8), Malta (#22 and #23), and Greece (#24). In Turkey, *C. brunnea* was previously found in Izmir Bay by Koçak (2007), and during this study, three additional localities are added to its previously known Turkish distribution (#28, #30 and #31), illustrating its wider expansion along the Turkish south-western and southern coasts. During this study, it was also present all around Sicily (#15, #16, #17, #19, #20, #21).

Boat-hull records: Found on boat-hulls moored in France (#3, #7, #9, #10) and Greece (#25, #26), but was not found from the artificial substrates of those same marinas.

Notes: Many species of the genus *Celleporaria* are tolerant and opportunistic, and may exhibit invasive attributes (Dunstan et al. 2004). *Celleporaria brunnea* was reported as a fouling organism from different substrates, both natural and artificial (i.e., Koçak 2007; Canning-Clode et al. 2013; Lezzi et al. 2015). Furthermore, it can be easily spread via hull-fouling, but its introduction via the aquaculture trade cannot be ruled out, as some of the Mediterranean findings refer to sites in close proximity to shellfish farms (i.e., Lezzi et al. 2015; Lodola et al. 2015).

Key taxonomic characters: Unilaminar or multilaminar encrusting irregular colonies. Opercula, sclerites of avicularia mandibles, base of spines and the lophophore tentacles dark-brown in colour. Orifice proximal border with midline notch and small horizontal condyles. Peristome usually with black joined spines. Suboral and large interzooidal avicularia present.

Celleporaria vermiformis (Waters, 1909)

Native origin: Red Sea.

Distribution: Apart from the Red Sea, its distribution is not well known (Vine 1986; Ostrovsky et al. 2011). However, it has recently been found in the Gulf of Oman (Dobretsov 2015). Its first and only Mediterranean record (prior to our new records listed below) is from Tripoli, Lebanon (Harmelin 2014).

New records: This study presents new country records for both Greece (#24, #25, #26) and Cyprus (#33: Fig. 2.3.1 G-I, and #34).

Boat-hull records: Found on boat-hulls moored in Greece (#25 and #26), and Cyprus (#33 and #34).

Notes: Since *Celleporaria vermiformis* was previously recorded from only a single record from a single site in Lebanon, it was not previously considered as an established species (Harmelin 2014). However, these five new locality records presented here now qualify it as an established NIS in the Mediterranean, and signifies its likely spreading in the Eastern portion of the basin.

Key taxonomic characters: Multilaminar encrusting colonies black in colour. Autozooids with large marginal pseudopores, subcircular primary orifice with condyles. Orificial spines lacking. Both small adventitious and gigantic vicarious avicularia present.

Remarks: *Celleporaria vermiformis* specimens analyzed in this study differ from specimen described in having an orifice with condyles, less concave proximal edge, shorter suboral umbo, lower and narrower ovicell and presence of gigantic vicarious avicularia. Specimens described here are more similar to those of *C. vermiformis* from Safaga N Bay (Red Sea) while the Lebanese specimen described by Harmelin (2014), under the name *C. vermiformis*, seems to be more similar to *Celleporaria melanodermorpha* Liu, 2001 (J.-G. Harmelin personal communication).

Family: Smittinidae

Parasmittina egyptiaca (Waters, 1909)

Native origin: Red Sea and Indo-Pacific Ocean.

Distribution: *Parasmittina egyptiaca* was reported along the Suez Canal (Hastings 1927, Harmelin et al. 2009), in the Red Sea (Ostrovsky et al. 2011), and from the Indo-Pacific region (Menon 1972). In the Mediterranean Sea, it has only been reported from Lebanon (Harmelin et al. 2009) and Israel (Sokolover et al. 2016).

New records: This finding represents two new country records for Turkey (#32) and Cyprus (#33).

Boat-hull records: Found on boat-hulls moored in Greece (#25: Fig. 2.3.2 A-B), and Italy (#21). The Italian finding presents a new Central Mediterranean record for this species.

Notes: In our samples, *P. egyptiaca* was mostly found growing on *Amphibalanus amphitrite* (Darwin 1854) specimens and oysters. The captain of the boat hosting this species in Italy explained that his home marina was Finike, Turkey (#32), and he had just recently travelled from there, through Greece to Sicily. Interestingly, one could expect many similar examples of new country records for Greece as several dozens of liveaboard recreational sailboats that used to winter in the Finike marina in Turkey, explained to the first author that since 2014, many had collectively relocated their vessels to now winter in Agios Nikolaos, Crete (#25). Despite thorough sampling procedures in Agios Nikolaos, this species was not found present in the marina.

Key taxonomic characters: Unilaminar encrusting colonies, generally small. Autozooids generally arranged in regular rows, with large marginal pores. Primary orifice rounded with 2-3 spines. Lyrula broad, with distal edge straight and the sides at 45°. Two condyles digitate with denticulate tips. Peristome interrupted distally. Polymorphic adventitious avicularia variable in number. Less frequently a single gigantic spatulate avicularium is present, facing the lateral side of the autozooids with triangular flaps.

Family: Vesiculariidae

Amathia verticillata (delle Chiaje, 1822)

Native origin: Caribbean Sea.

Distribution: It has a cosmopolitan distribution from tropical to subtropical regions in the Atlantic and Indo-Pacific Oceans, the Mediterranean Sea and Macaronesia (Amat et al. 2009; Wirtz et al. 2009; Minchin 2012; Ferrario et al. 2014; Marchini et al. 2015a).

In the Mediterranean, it was first recorded in the Gulf of Naples (Delle Chiaje 1822) and is well-known from the following countries: Algeria, Croatia, Egypt, France, Greece, Israel, Spain, Syria and Tunisia (Marchini et al. 2015a).

New records: This finding represents new country records for Malta (#22, #23), Turkey (#28 and #30: Fig. 2.3.2 E) and Cyprus (#34). During this study, it was also found in Spain (#2), France (#4, #5, #6, #11), the Tyrrhenian coast of Italy (#12 and #14), the Ionian Sea (around Sicily, #15-21), and Greece (#24 and #26).

Boat-hull records: Found on boat-hulls moored in France (#10), Italy (#12: Fig. 2.3.2F, #13, #14, #15, #17, #20-21), Malta (#22, #23), Greece (#24-#26), Turkey (#28, #30, #31), and Cyprus (#34).

Notes: It was recently confirmed to originate from the Caribbean (see Galil et al. 2014). Due to its rapid growth rate, it can pose ecological and economic impacts by forming extensive and resistant colonies on many types of artificial substrates (Ferrario et al. 2016), and can also facilitate introductions of additional fouling species (Marchini et al. 2015a), such as *Caprella scaura*, which was found to be intertwined with it in large abundances in La Grand-Motte, France when we sampled there.

Key taxonomic characters: Stoloniferous fouling bryozoan with bushy or more elongated colonies; irregularly-branching arrangement can exceed one meter in length. Colony translucent, zooids oval in shape and, in young branches, arranged on two regular parallel rows, while irregularly arranged on the basal branches.

Family: Watersiporidae

Watersipora arcuata Banta, 1969

Potential native origin: Tropical Eastern Pacific.

Distribution: It is a widespread species distributed from the tropical Pacific such as the Mexican Pacific coast, California and Hawaii, extending down to Australasia (Wisley 1958; Skerman TM. 1960; Banta 1969; Coles et al. 1999). In the Mediterranean, it had only been reported from Porto Santa Margherita Ligure in NW Italy and Porto Rotondo Marina in Sardinia (Ferrario et al. 2015; Ferrario et al. 2017).

New records: This finding represents new country records for Spain (#1 and #2), Malta (#22: Fig. 2.3.2 I) and Turkey (#28 and #32). This also represents an additional Italian locality record for Sicily (#18: S.D. Fig. 3G-H, and #20). Therefore, this study shows this species is now present in all regions of the Mediterranean, presenting here two new subregional records for the Western and Eastern Mediterranean.

Boat-hull records: Found on a boat-hull moored in France (#7), but not found from the marina substrate.

Notes: In this study, *W. arcuata* was especially abundant in Siracusa, Sicily. The captain of the boat in Cogolin, France (#7) hosting this species had recently travelled from Barcelona, where it was also found in the marina from this study. If it does establish in France, it would then present a new country record.

Key taxonomic characters: Unilaminar or multilaminar encrusting colonies, transparent to reddish-brown/black in colour. Operculum and orifice semicircular, with the orifice proximal margin curved inward. Cardelles (projections of the border of the orifice) present at about one-third (or more proximally) of the orifice length. Pair of transparent opercular lucidae present and frontal wall perforated by pseudopores. *Watersipora* genus characterized by the absence of spines, avicularia and ovicells.

2.53 Crustacea

Cirripedia

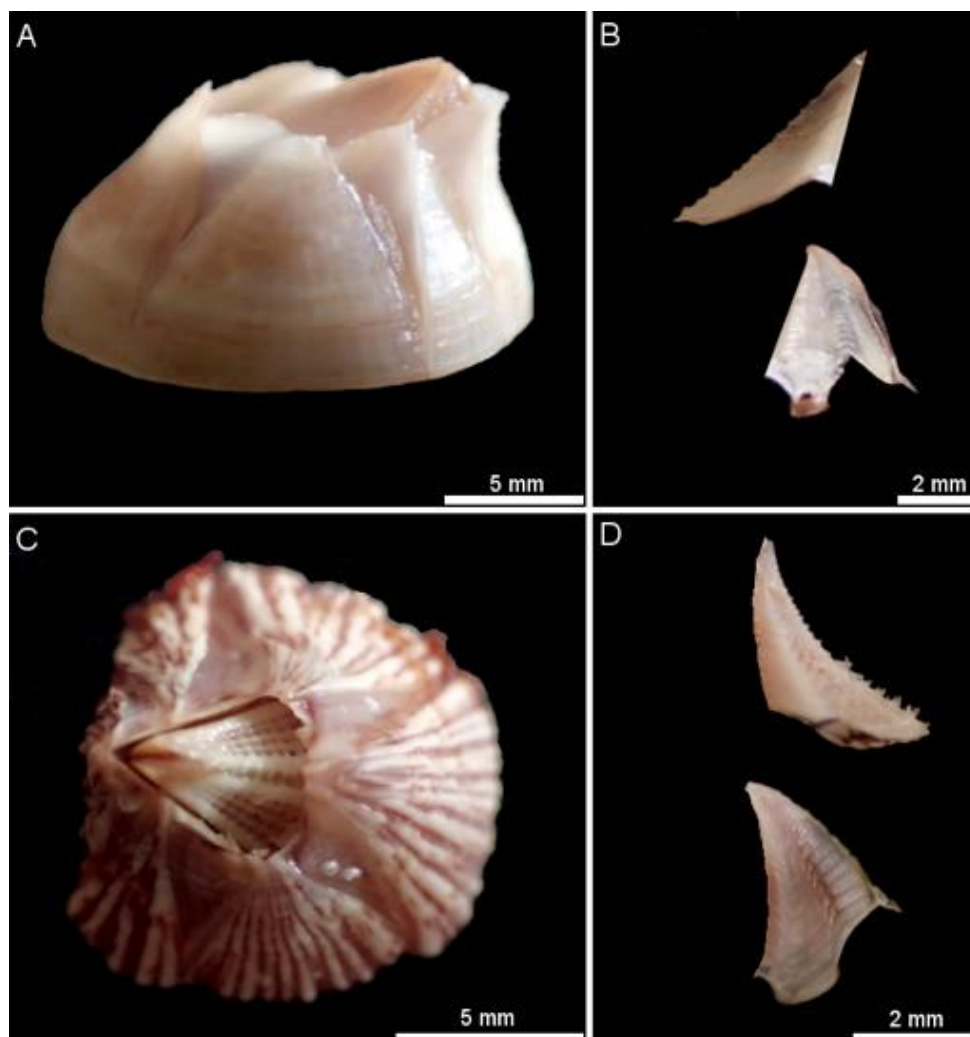


Figure 2.3.3 Cirriped/Barnacle specimens: (A-D). (A-B) *Amphibalanus improvisus* on boat-hull in Marina #5: (A) complete specimen, (B) scutum and tergum; (C-D) *Balanus trigonus* on boat-hull in Marina #33: (C) complete specimen, (D) scutum and tergum. Photo credits: Aylin Ulman.

Family: Balanidae

Amphibalanus improvisus (Darwin 1854)

Potential native origin: Western Atlantic Ocean.

Distribution: It is considered NIS in the Pacific Northwest, and is also present in the Sea of Japan, New Zealand and northern Europe (Foster et al. 1979; Zullo 1979; Furman 1989; Iwasaki 2006).

In the Mediterranean region, it was first reported from the Black Sea in 1844 (Gomoiu et al. 2002). Next, it was found in the Bosphorus Strait, Turkey (Neu, 1935), which connects the Black Sea to the Aegean Sea. By the late 1940s it was also reported in Barcelona (Spain), Catania (Italy), and Alexandria and Abukir (Egypt; Kolosvary 1949).

Marina records: This finding represents a new locality record for Italy (#12). It was also found in Turkey (#27).

Boat-hull records: Found on boat-hulls moored in France (#5: Fig. 2.3.3 A-B) and Turkey (#28).

Notes: If *A. improvisus* happens to establish in Port Camargue marina, this would then present a new country record for France. The captain of a boat hosting *A. improvisus* in Port Camargue (France #5) had recently travelled from Barcelona (where it was recorded long ago), as well as the Balearic Islands. Another captain from Port Camargue also hosting this species had recently only travelled to the Balearic Islands, so it is likely that *A. improvisus* is present there. The captain hosting this species from Bodrum, Turkey (#28) had recently travelled to Istanbul, where this species has long been present, in addition to travelling through Italy and Greece.

Key taxonomic characters: Maximum 17 mm diameter. Plates white and smooth with slightly toothed orifice. Plates have white longitudinal radii narrowing as they reach the top. Scutum with well-developed adductor ridge on interior face, while tergum has blunt apex and spur long and narrow. Spur length about 1/3 length of basal margin, width about 1/5 of basal margin.

Balanus trigonus Darwin 1854

Native origin: Indo-Pacific.

Distribution: It was first described from the Pacific Ocean (Darwin 1854), and has a wide Indo-Pacific distribution extending to the Red Sea. It is considered NIS in the Atlantic Ocean and Mediterranean Sea, its first Atlantic record coming from Brazil in the 1860s (Zullo 1992). It was introduced to the Atlantic coast of North America around the 1950s to the 1960s (Moore et al. 1963; Gittings 1985), and has also been reported from the eastern Atlantic from the Azores to South Africa.

Its first Mediterranean record was from the Gulf of Catania, Italy in 1927 (Patane 1927). It was abundant in the Italian Tyrrhenian, Ionian and Adriatic Seas in the 1960s (Relini 1968). It is also reported from Egypt (Ghobashy 1976), Lebanon (Bitar et al. 2001), Turkey (Kocak et al. 1999), Greece (Koukouras et al. 1998), Croatia (Igić 2007) and Slovenia (Mavrič et al. 2010).

Boat-hull records: Found on boat-hulls moored in Italy (#14 and #15), Turkey (#32), Greece (#24 and #26), and Cyprus (#33: Fig. 2.3.3 C-D).

Notes: Although reported on boat-hulls in north-western Europe, it has not established in that region (Hayward et al. 2017). Relini (1968) questioned a lack of other Mediterranean records for this species despite its earlier abundance in the Italian Ionian, Tyrrhenian and Adriatic Seas. In Cyprus, *B. trigonus* has not yet been reported for the country, and the boat captain in Cyprus hosting this species explained that he had just travelled along Turkey's Mediterranean coast and also through Rhodes, Greece since his last hull-cleaning. If this species establishes itself in Karpaz Marina, Cyprus, where it was found on boats, it would then present a new country record for Cyprus. This species can also be transported via both the aquaculture or 'Live Fish Food Trade' (LFFT) pathways due to its custom of gluing itself to other marine species such as shellfish and crabs (Zullo 1992).

Key taxonomic characters: Maximum 25 mm diameter, triangular aperture, six external plates, purplish to pink with white striations. Distinguishable by scutum, with 1 to 6 longitudinal rows of pits, while tergum is broad, smooth and flat.

Decapoda

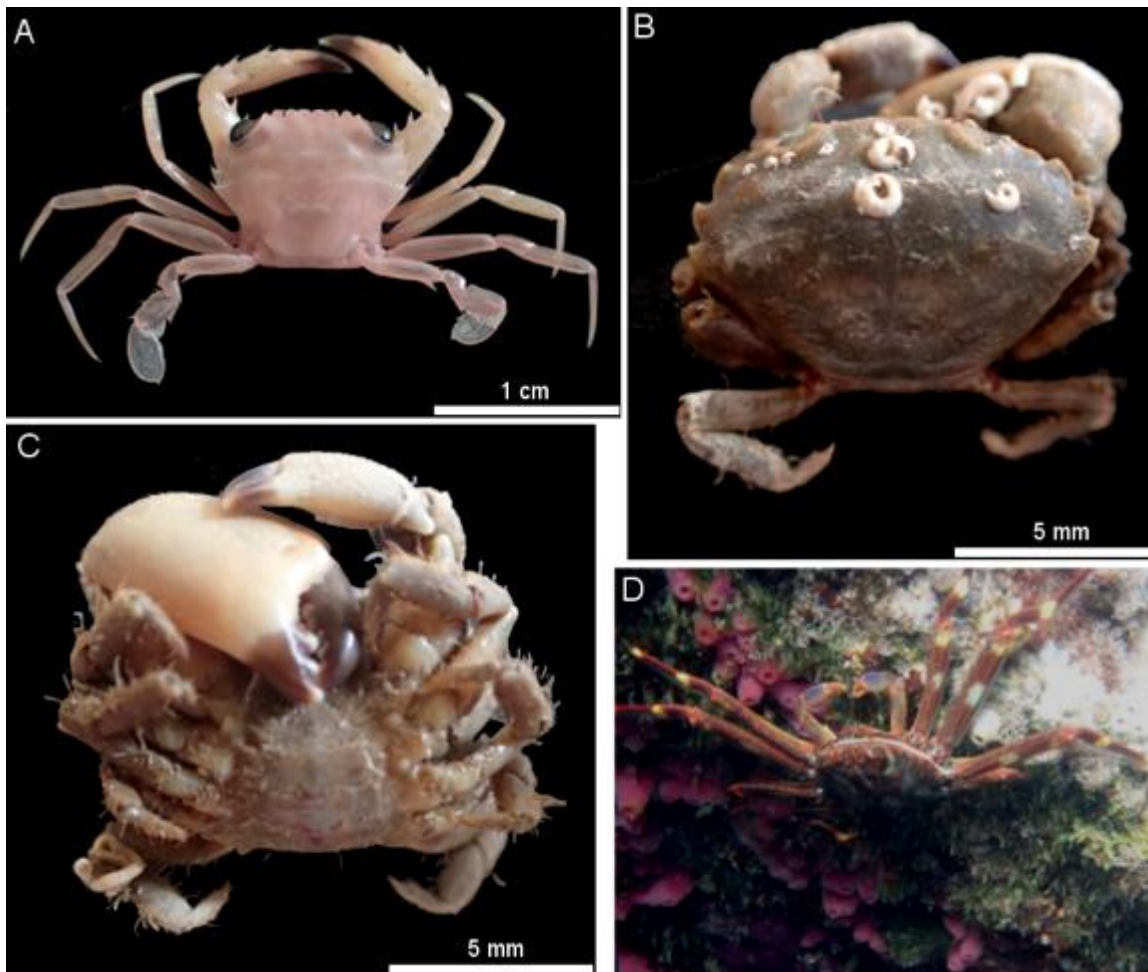


Figure 2.3.4 Decapods (A-D). (A) *Charybdis (Gonioinfradens) paucidentatus* in Marina #34; (B-C) *Dyspanopeus sayi* dorsal and ventral view in Marina #24; (D) *Percnon gibbesi* in Marina #24. Photo credits: Aylin Ulman.

Family: Portunidae

Charybdis (Gonioinfradens) paucidentatus (A. Milne-Edwards, 1861)

Native origin: Indo-Pacific.

Distribution: This species has a wide Indo-Pacific distribution, including the Red Sea, eastern Africa, Australia, New Caledonia, Japan (Poupin 1994; Poupin 1996; Apel et al. 1998; Apel 2001), Madagascar (Crosnier 1962), the Persian Gulf (Naderloo et al. 2007) and Hawaii (Davie 1998).

Its first Mediterranean record was in Turkey in 2009 from the Kaş-Kekova Specially Protected Area (SPA) from the Turkish Levantine coast (Karhan et al. 2012). A 2010 record from Rhodes, Greece provided the second Mediterranean record (Corsini-Foka et al. 2010), which is only about 140 km from Kaş, Turkey.

New records: This finding represents a new country record for Cyprus (#34: Fig. 2.3.4 A).

Notes: It may have been introduced to the Eastern Mediterranean via ballast water (Corsini-Foka et al. 2010).

Key taxonomic characters: Maximum length of 52.5 mm with a hexagonal, smooth carapace. Six teeth on front, with median and submedian teeth truncate, lateral teeth triangulate and rounded at tip, separated by deeper groove from previous ones. With four large anterolateral teeth, first is more rounded and last spiniform. Two accessory denticles at base of external border of first and second teeth, second very small. Chelipeds have three strong spines on anterior border, carpus with strong interior spine and 3 smaller spines on outer face, chela has two large spines on superior surface and two other marginal spines near movable finger.

Remarks: The presence of only four large anterolateral teeth allows *Gonioinfradens* to be easily distinguished from all the other subgenera retained in *Charybdis*.

Dyspanopeus sayi (Smith 1969)

Native origin: Western Atlantic, from Canada to Florida.

Distribution: It spread from the Western Atlantic to the Northeast Atlantic and also to the North Sea: Great Britain, France and Netherlands (Ingle 1980; Clark 1986). Its first Mediterranean record was from the Lagoon of Venice in 1991 (Frogliani et al. 1993), then next a little south in the Adriatic Sea in the Po River Delta (Turolla 1999). In 2009, it was found in a Romanian harbour in the Black Sea (Micu et al. 2010), and in 2010 from the Ebro Delta of the Iberian Peninsula, providing the first Western Mediterranean record (Schubart et al. 2012). In 2011, it was collected from the central-southern Adriatic Sea lagoon of Varano (Ungaro et al. 2012), and in 2011 it was reported in Mar Piccolo, Gulf of Taranto (Ionian Sea, Kapisris et al. 2014) another known hotspot for NIS, and then in Lago Fusaro (a brackish lagoon north of Naples), where it was the most abundant crab (Thessalou-Legaki et al. 2012).

New records: This finding represents a first subregional record for the Eastern Mediterranean and additionally a new country record for Greece (#24: Fig. 2.3.4 B-C). It was also found in Sicily (#18) from this study.

Notes: Its first Mediterranean record from Venice is hypothesized to have arrived either via the ballast water or aquaculture vector (Frogliani et al. 1993).

Key taxonomic characters: Maximum carapace width of 30 mm. Live specimens greenish to brown with reddish dots on dorsal side, creamy ventral side. Oval, arcuate carapace, small median notch on front, minutely granular. Five teeth on each anterolateral margin, first two coalescent and near ocular lobe margin, last three prominent but variable in shape. Walking pereopods have long and slender dactylus; pereopods 2 to 5 are shorter than pereopod 1. First male pleopod has a low mesial lobe which is broadly rounded, differing it from *D. taxana* which has an elongate and narrow mesial lobe. Chelipeds unequal in size in male only, chelipeds with small tubercles. Fingers of chelae variable in colour, from ivory to dark brown to black.

Peracarida – Amphipoda

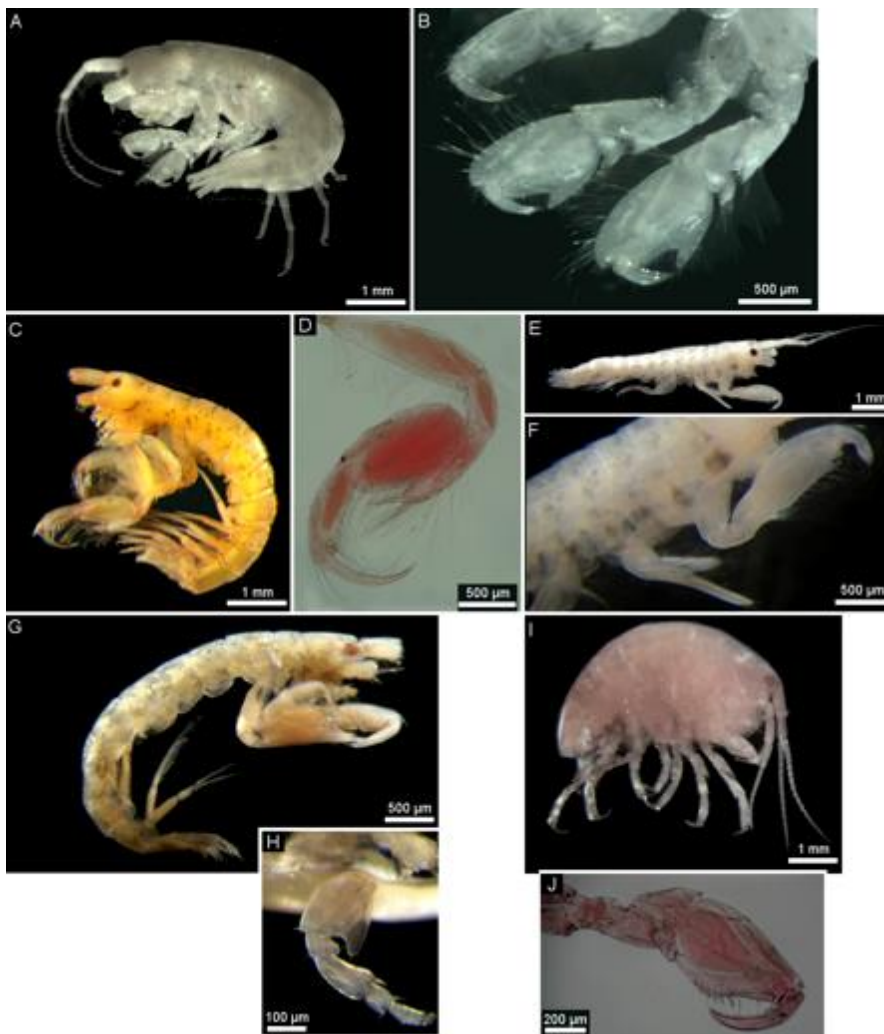


Figure 2.3.5 Amphipods. (A-J). (A-B) *Ampithoe bizseli* in Marina #34: (A) male specimen, (B) right and left gnathopod 2; (C-D) *Aoroides longimerus* in Marina #5: (C) male specimen, (D) merochelate gnathopod 1; (E-F) *Bemlos leptochirus* in Marina #24: (E) male specimen, (F) gnathopods 1 and 2; (G-H) *Ericthonius* cf. *pugnax* in

Marina #5: (G) male specimen, (H) pereopod 5; (I-J) *Stenothoe georgiana* in Marina #14: (I) male specimen, (J) gnathopod 2 with conspicuous lobe on the propodus palm. Photo credits: (A-B) Agnese Marchini; (C-J): Gemma Martinez-Laiz.

Family: Ampithoidae

Ampithoe bizseli Özaydinli & Coleman, 2012

Potential native origin: Red Sea and Indian Ocean.

Distribution: To date, the species has only been reported from Tanzania and Turkey (Izmir Bay, Özaydinli & Coleman 2012). Its distribution may be much wider than currently known, but this species could have been misidentified as *Ampithoe ramondi* Audouin, 1826, following Schellenberg's (1928) record of "*A. ramondi*" (see notes below).

New records: This finding represents a new country record for Cyprus (#33 and #34: Fig. 2.3.5 A-B).

Boat hull records: Found on boat-hulls moored in Cyprus (#33 and #34).

Notes: According to Özaydinli & Coleman (2012), specimens from Tanzania identified as *Ampithoe ramondi* by Schellenberg (1928) display ischium lobes identical to *A. bizseli*. For this reason, the native origin of *A. bizseli* is hypothesized to be the Indian Ocean, from where it could have been transferred to the Mediterranean via hull-fouling. Its current presence in two marinas and also on boat-hulls in those marinas provides evidence of biofouling as a vector for its wider spread.

Key taxonomic characters: Gnathopods (Gn) 1 and 2 with rounded lobes on basis anterodistal corner and ischium anterior margin (small in females, large in males). Particularly, male Gn2 lobe very large and reaches beyond ischium. Male Gn2 propodus bearing a prominent anterodistal lobe, with long setae; palm excavate, defined by posterodistal tooth.

Family: Aoridae

Aoroides longimerus Ren & Zheng 1996

Native origin: Northwest Pacific Ocean.

Distribution: It has been reported from Daya Bay, China (Ren & Zheng 1996) and from Osaka and Wakayama, Japan (Ariyama 2004). It has also been recorded from the Northeastern French Atlantic coast where it is considered NIS (Gouillieux et al. 2016).

New records: This finding represents a new Mediterranean record (#5: Fig. 2.3.5 C-D), and a new regional record for the French Mediterranean.

Boat-hull records: Found on boat-hulls in France (#5).

Notes: Port Camargue, France, is situated in close proximity to Thau lagoon, the most important Mediterranean locality for aquaculture farming of Japanese oysters (Boudouresque et al. 2011). This information and our new record from boat-hulls suggests that both aquaculture and shipping are possible vectors of introduction, similarly to what has been indicated for the French Atlantic record (Gouillieux et al. 2016).

Key taxonomic characters (males only): Gnathopod (Gn)1 long, merochelate, densely setose, much larger than Gn2. Coxa bearing a few plumose setae and spine. Basis elongated, bearing dense plumose setae along the anterior margin and some setae in lateral margin. Maerus bearing long plumose setae, prolonged into a long distal tooth extending way beyond the carpus; tip abruptly narrowed. Carpus large, ventral and medial surface with long plumose setae. Propodus bearing simple setae at anterodistal corner and along posterior margin. Dactylus robust, long, curved, bearing several simple setae along posterior margin. Gn2, basis with long simple setae and a plumose seta in anterior margin, a few simple setae in posterior margin. Carpus and propodus with simple setae along posterior margin. Propodus with transverse palm, posterior margin with a spine. Uropod 3 biramous.

Bemlos leptochirus (Walker, 1909)

Native origin: Red Sea, Indian Ocean.

Distribution: Aside from early records from its native region: Kenya, Tanzania, South Africa, Suez Canal (Walker 1909; Schellenberg 1928; Sivaprakasam 1968; Myers 1975), its first and only Mediterranean record was from Egyptian coast from Port Said, Alexandria, and Abu Kir in the early 20th century (Schellenberg 1928; Bellan-Santini et al. 1998). However, it was considered to be absent from the Mediterranean, as it had not been reported since (Zenetos et al. 2017).

New records: This finding represents a new country record for Greece (#24: Fig. 2.3.5 E-F, and #25), and confirms its presence and reappearance in the Eastern Mediterranean.

Notes: Previous findings of *B. leptochirus* in the Suez Canal and from the Egyptian Mediterranean coast, near the canals entrance, suggest it to have a “Lessepsian migrant” vector status (Bellan-Santini et al. 1998), especially since it was also recorded from buoys and boats (Schellenberg 1928). Our findings support that it should rather be assigned to the “biofouling or hull-fouling” vector.

Key taxonomic characters: Antenna 1 with 5-articulated accessory flagellum. Gnathopod (Gn) 1 bigger than Gn2; Gn1 carpus shorter than half the length of propodus, propodus subchelate, with long setae and (in males) a triangular tooth at posterodistal margin. Gn2 merus, carpus and propodus bringing long plumose setae; carpus and propodus elongated and slender. Third uropod uniramous.

Family: Ischyroceridae

Ericthonius cf. pugnax (Dana 1852)

Potential native origin: Indonesia.

Distribution: *Ericthonius pugnax* has a wide Indo-Pacific distribution including Australia (Great Barrier Reef, New Caledonia and New South Wales), Papua New Guinea, Singapore, Japan, Korea, Malaysia, India, Sri Lanka, Madagascar and Mauritius (Marchini et al. 2017 and references therein). It was reported from New Zealand as a NIS (Ahyong et al. 2011). Records of *E. pugnax* from South Africa on mussel rafts (Milne et al. 2013) may also represent an introduction event. In the Mediterranean Sea, a record of *Ericthonius dydimos* from the Adriatic Sea (Krapp-Schickel 2013) may refer to this species.

New records: This finding represents a new subregional record for the Western Mediterranean and a new country record for France (#5: Fig. 2.3.5 G-H).

Boat-hull records: Found on boat-hulls moored in France (#3 and #5).

Notes: An *Ericthonius* species strikingly similar to *E. pugnax* was described by Krapp-Schickel (2013) from the Lagoon of Venice (and to date, has not been reported from other localities): *E. didymus*. The latter presents a strongly posteriorly lobate pereopod 5 basis, identical to *E. pugnax*. Krapp-Schickel (2013) justifies the establishment of the new species *E. didymus* on the basis of differences in pereopod 5 postero-distal lobe (in *E. didymus* only visible in adult males; in *E. pugnax*, visible in both adult and juvenile males), in shape of gnathopod 2 carpus (bearing two teeth in *E. didymus*, versus only one tooth in *E. pugnax*), shape of pereopods 3 and 4 basis, as well as body size. However, a re-examination of *Ericthonius* material collected in 2012 from the Lagoon of Venice (A. Marchini, private collection), together with a cross-comparison of descriptions and drawings of both *E. didymus*, provided by Krapp-Schickel (2013), and *E. pugnax*, provided by Moore (1988), Just (2009) and Azman & Othman (2013), shows that the differences pointed out by Krapp-Schickel (2013) may not support the separation between the two species. With regards to gnathopod 2 carpus, Azman et al. (2013) showed that the number of teeth in male *E. pugnax* varies with maturity. Furthermore, we observed some males from Venice having a single-toothed gnathopod 2 carpus, consistent with the description of *E. pugnax* hyperadult males of Moore (1988). The basis of pereopods 3, 4 is bottle-shaped, and distally expanded in both species. Furthermore, body length is largely variable (*E. didymus* described from Venice is 4.5 mm; *E. pugnax* described from Australia by Moore (1988) and Just (2009) is 3.0 - 3.7 mm, from Malaysia by Azman & Othman (2013) is 3.8 mm, from Japan by Nagata (1965) is up to 7.5 mm).

Therefore, we hereby suggest that the "endemic" *E. didymus* in Venice may be an introduced population of the Indo-Pacific *E. pugnax*, and therefore may be a pseudoindigenous species. However, it is also possible that the global populations of *Ericthonius* with a posteriorly lobated pereopod 5 basis represent a complex of cryptic species. We consider that in this case the hypothesis of the valid introduced status is supported by the following facts:

- 1) *Ericthonius pugnax* has a notably wide distribution in the Indo-Pacific region, which supports a human-mediated dispersal hypothesis, and is already known as a NIS from New Zealand (and possibly, South Africa);
- 2) In the Lagoon of Venice, it has developed populations with high densities (A. Marchini, personal observation), which is consistent with "invasive" behaviour; and
- 3) The Lagoon of Venice is a well-known hotspot of introductions, where over 70 NIS have already been recorded, many with Pacific/Indo-Pacific origins, which were introduced to Venice *via* oyster imports (Marchini et al. 2015b). The present records from Cap d'Agde and Port Camargue are both nearby another popular

hotspot for oyster introductions, the Thau lagoon (Boudouresque et al. 2011). This further supports the hypothesis of introduction from the Indo-Pacific region, with aquaculture being the main pathway of primary introduction.

Key taxonomic characters (males only): Gnathopod (Gn) 2 very large and carpocheled; coxa with stridulating ridges, carpus with two posterodistal teeth, the outer being longer (while hyper-adult males exhibit a single prominent tooth); propodus shorter than carpus; dactylus slender, with apical tuft of long setae. Pereopods 3-4 basis flask-shaped; pereopod 5 basis with a distinctive lobe on the posterodistal margin. Posterior margin of epimeral plate 3 minutely serrated.

Family: Stenothoidae

Stenothoe georgiana Bynum & Fox 1977

Native origin: Western Atlantic.

Distribution: Its first record outside its native range was reported just recently in 2010, in association with fouling communities of offshore fish farms (about 10 km from shore) in Alicante and Murcia, Spain (Fernández-González et al. 2017). Its subsequent Mediterranean records were from the Ligurian Sea and from Sardinia, Italy (Ferrario et al. 2017).

New records: These findings represent new country records for France (#5) and Malta (#23). This study increases its known Italian distribution by incorporating Sicily (#14: Fig. 2.3.5 I-J, #15, #18, #21). The Maltese and Sicilian findings from this study represent a new Central Mediterranean subregional record.

Boat-hull records: Found on boat-hulls moored in France (#3, and #10), and Italy (#14, #15, #17, and #21).

Notes: Since this species has only very recently been reported in the Mediterranean, we hypothesize that it may have gone previously overlooked, since it is already present in at least 4 countries. It may soon establish in Cap d'Agde Marina or Port Vauban, Antibes, and this should serve as a warning for future monitoring of those marinas. This study demonstrates that this species is likely polyvectic (i.e., has been transferred by more than one vector): in addition to its likely transfer via aquaculture (Fernández-González et al. 2017), recreational boating is also facilitating its spread.

Key taxonomic characters (males only): Gnathopod (Gn) 1 carpus triangular, propodus with weakly convex palm, finely denticulate, bringing a few spines. Gn2 propodus palm defined by a characteristic spinose hump

(weak in females, prominent in males); dactylus reaching palmar hump. Telson with two rows of longitudinal spines.

Peracarida – Isopoda

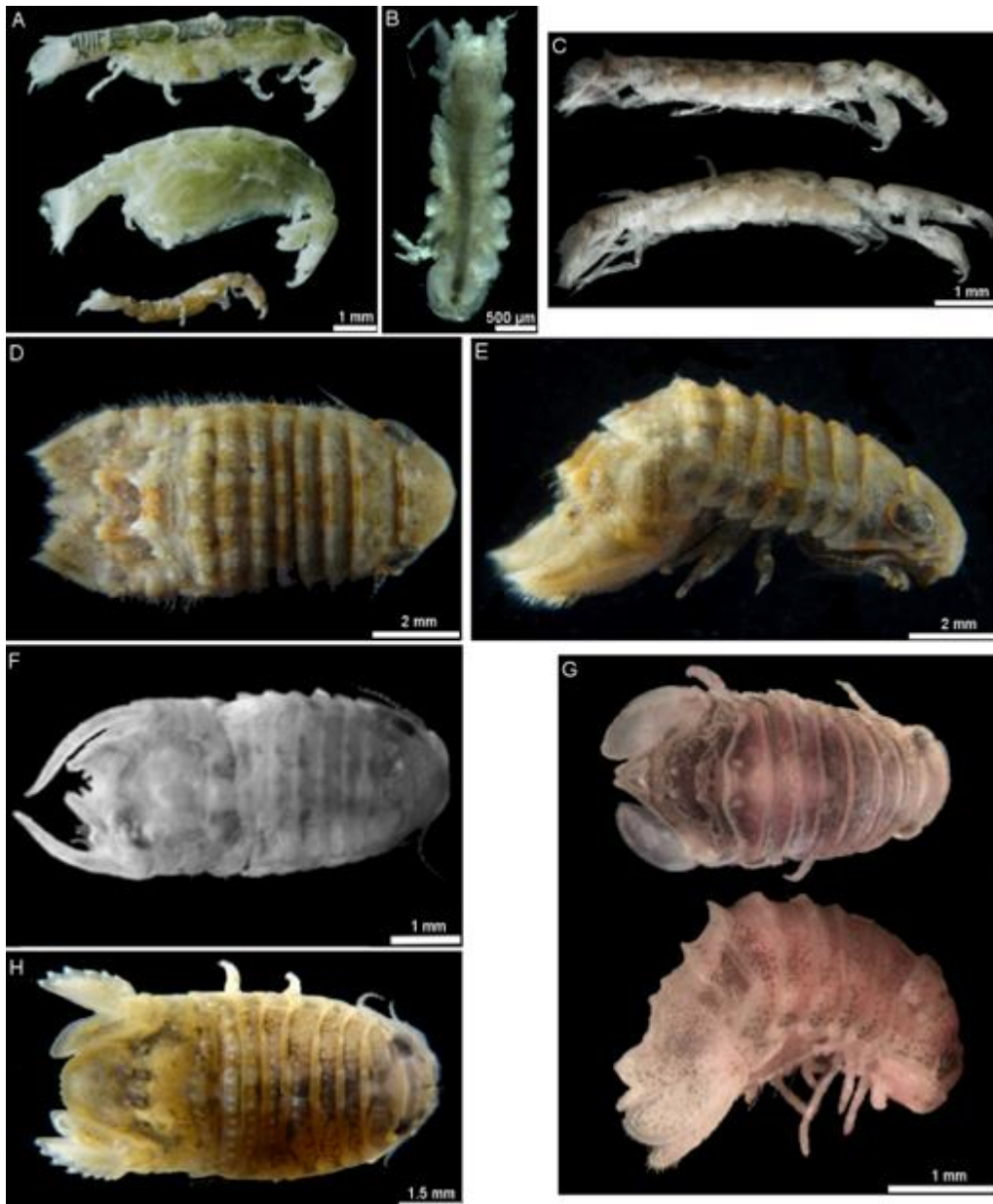


Fig. 2.3.6 (A-H). Isopods: (A) *Mesanthura* cf. *romulea* in (from top to bottom) Marinas #1, #16 and #22: female specimens; (B) *Ianiropsis serricaudis* in Marina #5; (C) *Paranthura japonica* specimens in (from top to bottom) Marinas #1 and #21: female specimens; (D-E) *Cymodoce* aff. *fuscina* in Marina #24: (D) frontal and (E) lateral view of a male specimen, (F) *Paracerceis sculpta* in Marina #22: male specimen; (G) *Paradella diana* in Marina #15: male specimen; (H) *Sphaeroma walkeri* in Marina #24: male specimen. Photo credits: (A-E) Agnese Marchini; (F-H) Gemma Martinez-Laiz.

Family: Anthuridae

Mesanthura cf. romulea Poore & Lew Ton, 1986

Potential native origin: Tropical to sub-tropical southern seas.

Distribution: *Mesanthura* specimens belonging to the same species and sharing major diagnostic characters with *M. romulea* described from Australia (Poore & Lew Ton 1986) were subsequently (2000) collected from the harbours of Salerno and Taranto (Italy), where they were well established (Lorenti et al. 2009), and also from Ischia Island (Kroeker et al. 2011). More recently, this species has been reported by Ferrario et al. (2017) from marinas in Northern Italy (Liguria).

New records: This finding represents new country records for Spain (#1: Fig. 2.3.6 A), Malta (#22: Fig. 2.3.6.A), Greece (#26) and Cyprus (#33 and #34), the latter two records also confirming the presence of *Mesanthura cf. romulea* in the Eastern Mediterranean. From this study, we additionally report specimens from Italy (#14, #15, #16: Fig. 2.3.6 A, and #18).

Notes: The earliest mention of the presence of the genus *Mesanthura* in the Mediterranean region was from Lake Burullus, Egypt (Samaan et al. 1989); however, the record was not supported with taxonomic details and needs confirmation. Castellò (2017) recently described a new *Mesanthura* species from both the Lebanese coast and Cyprus (*Mesanthura pacoi*, Castellò, 2017), whose females vary from those of the present species in the dorsal colour pattern and in other subtle morphological features. As mentioned above, the species found by Lorenti et al. (2009) and reported here is comparable and most probably conspecific (Poore, G., pers. comm., 2017) with *M. romulea* described by Poore & Lew Ton (1986), which is based only on two specimens collected from Sydney Harbour and Port Stephens, New South Wales, Australia. No other records of this species have been published.

The fact that the extant description of the Australian *M. romulea* lacks a number of taxonomic characters and is based on only two specimens prevents from determining if features observed in all Mediterranean specimens lie within the natural range of morphological variation of the species, or allow for the determination of a different species.

As long as these cases of taxonomic identity are unsolved, and no new material of *M. romulea* is found from its putative native range, the origin of populations occurring in the Mediterranean remains obscure. However, the Mediterranean finding of the present species of *Mesanthura* shows strong indications of a human-mediated introduction. Following Chapman & Carlton's (1991) criteria, the lack of previous records of the genus *Mesanthura* on a basin scale (except for the recent discovery of *M. pacoi* from the Levantine Sea), the mentioned occurrences from confined areas such as lagoons and harbours, the notably poor capabilities of

active or passive spreading by natural means of the genus, and its likely exotic evolutionary origin, cumulatively support the hypothesis of a human-mediated introduction.

Key taxonomic characters: This new species is awaiting proper description. *Mesanthura* genus only one amongst anthuridean isopods, along with *Chelanthura*, exhibiting species-specific pigmented patterns (Poore, 2001). The species reported here present a characteristic pigmented dorsal pattern arranged in composed patches on head and pereonites, with interruptions of blank areas. Pleon contains five stripes, delimited by two semi-circles, a narrower anterior one and a wider distal one; patches of pigment also cover telson and uropods. Morphological features of diagnostic relevance include, the presence of 6-7 spines on the distal mandibular palp article, the palm of pereopod 1 with a step, the broadly notched uropod exopod.

Remarks: Our material is obviously conspecific with the species recorded by from two Italian harbors (Maurizio Lorenti, personal obs.) and compared to the Australian species *M. romulea* primarily based on similar identity of cephalic and pereional decoration.

Family: Janiridae

Ianiropsis serricaudis Gurjanova, 1936

Native origin: Sea of Okhotsk to the Sea of Japan.

Distribution: In addition to its native range, it has been reported from the Northeastern Pacific (from Puget Sound to Monterey Bay), the Northwestern Atlantic (from Maine to New Jersey) and the Eastern Atlantic and North Sea (England and the Netherlands) (Hobbs et al. 2015).

Its first Mediterranean record was in 2012 from the Lagoon of Venice (Marchini et al. 2016a), and soon after from Olbia, Sardinia in 2014 (Marchini et al. 2016b).

Key taxonomic characters: Elongate segments 6 and 7 of antennal peduncle. Maxilliped palps elongated and visible in dorsal view. Pereopod 1 with two claws on dactylus, and three claws on pereopod 7. Pleotelson lateral margin with three or four denticles along the posterior half.

New records: This finding represents a new country record for France (#3 and #5: Fig. 2.3.6 B).

Notes: In North America, this species is now known as a common fouling species. It was hypothesized that this species is likely more established along North America and the European coasts than what is known, but may go undetected due to its minuscule size (< 3 mm) and the taxonomic complexity of the genus (Hobbs et al.

2015). All the Mediterranean findings (Venice, Olbia, Port Camargue) refer to sites in close proximity to aquaculture sites.

Family: Paranthuridae

Paranthura japonica Richardson 1909

Native origin: Northwest Pacific Ocean.

Distribution: It was first reported from Muroran, northern Japan and from eastern Russia (Nunomura 1977). It was reported as a NIS for San Francisco Bay in 1993, then from southern California in 2000 (Cohen et al. 1995; Cohen 2005). Between 2007 and 2010 it was first recorded in European waters from the Bay of Biscay, France, most likely via the aquaculture vector (Lavesque et al. 2013).

Its first Mediterranean records occurred only recently; between 2010 and 2012 it was found in numerous localities around Italy: the Lagoon of Venice, La Spezia and Olbia harbours (Marchini et al. 2014), and Taranto (Lorenti et al. 2015). Next, it was found in La Grande-Motte, France (Marchini et al. 2015a) and then in Tunisia and Greece (Tempesti et al. 2016).

New records: These findings represent new country records for Spain (#1: Fig. 2.3.6 C, and #2) and Malta (#23). Furthermore, *P. japonica* was found in countries where it was already reported from, extending its known distribution to new localities in France (#3, #4, and #9), Italy (#12, #13, #16-21: #21 Fig. 2.3.6 C), and Greece (#24 and #26). These new Sicilian records (#16-21), and Maltese record (#23) show it is already well-established in the Central Mediterranean.

Boat-hull records: Found on boat-hulls moored in France (#3, #5, #9 and #10), Italy (#12, #17, #20 and #21) and Greece (#24).

Notes: The current findings dramatically increase the known distribution of *P. japonica*, revealing it as one of the most widespread NIS in the Mediterranean Sea. While the initial findings of *P. japonica* suggested an association with aquaculture transfers, these new records show that it most likely is a polyvetric species, which complicates the possibility of reconstructing its invasion trajectory.

Key taxonomic characters: Elongated body, covered with scattered pigmentation. Cephalon with anterolateral lobes extending beyond rostrum; mouth appendages produced in an acute piercing/sucking apparatus. Pereon segment 5 slightly longer than 6 and 7. Pleon segments fused dorsally but not laterally.

Family: Sphaeromatidae

Cymodoce aff. fuscina Schotte & Kensley 2005

Native origin: Persian Gulf.

Distribution: *Cymodoce fuscina* was first described in 2005 from seagrass beds in Saudi Arabia, the Persian Gulf by Schotte et al. (2005). Until now, this isopod had not been reported outside the Persian Gulf.

New records: This finding represents a new record for the Mediterranean basin, and a new country record for Greece (#24: Fig. 2.3.6 D-E).

Boat-hull records: Found on boat-hulls moored in Greece (#24 and #25).

Notes: Our specimens show very strong affinity to *C. fuscina* from the Persian Gulf (Valiollah Khalaji- Pirbalouty, pers. comm., 2015), and they certainly differ from all other known *Cymodoce* species reported in the Mediterranean Sea in several characters of the pleotelsonic region, while also being similar to other species described from the Western Indian Ocean (Khalaji-Pirbalouty et al. 2014). Its association with marina structures and hull-fouling further supports the hypothesis of a human-mediated introduction, possibly from boats travelling from the Red Sea through the Suez Canal. However, slight differences between our material and the original description of *C. fuscina* by Schotte et al. (2005) should be noted, for example the pleotelsonic apex of *C. fuscina* has the three apical lobes subequal in length and rounded apically, while in our material the central lobe is slightly longer than the lateral ones, and ends in a tiny bifid spike. We stress the fact that not all Indo-Pacific species within this genus may be known (many new species have been described in the recent decade), and a complex of species is also a possibility. Therefore, we recommend that genetic analyses should be undertaken to compare the Mediterranean material with specimens from the native range, to confirm the identity of these samples from Heraklion, Greece.

Key taxonomic characters (males only): Pereon weakly setose laterally and with tufts of dorsal setae, bearing dorsal tubercles on the three distal segments. Pleotelson densely setose and rugose, ending in a deeply notched tridentate apex. Proximal part of pleotelson with two prominent longitudinal ridges, flanked by bifid distal tubercles. Uropod rami extending beyond the pleotelsonic medial lobe; endopod with two clearly visible dorsal tubercles. Appendix masculina straight.

Paracerceis sculpta (Holmes 1904)

Native origin: California.

Distribution: This is a widely distributed species naturally found along the North American Pacific coast from California to Mexico, and has also been reported from Hawaii, Hong Kong, Australia, Brazil and the Azores (Marchini et al. in press and references therein).

In the Mediterranean Sea, it was first reported from the Lake of Tunis, Tunisia (Rezig 1978); and next from several Italian localities (Forniz et al. 1983; Forniz et al. 1985; Savini et al. 2006; Ferrario et al. 2017), and the Strait of Gibraltar (Castelló et al. 2001). Most recently, it was reported in Thermaikos and Toroneos Gulf in Greece (Katsanevakis et al. 2014) and La Grande-Motte in France (Marchini et al. 2015a).

New records: This finding represents new country records for both Malta (#22: Fig. 2.3.6 F, and #23) and Cyprus (#34). It was also found in France (#4), Greece (#24 and #26) and Italy (#13, #15-#21).

Boat-hull records: Found on boat-hulls from Sicily (#17, #20 and #21), Greece (#24), and Turkey (#31).

Notes: This species has often been reported from marinas, indicating that recreational boating plays an important role in the spread of this global invader. In Fethiye (#31), it was found on a boat-hull but not in the marina and so far was unknown from Turkey; When interviewed, the boat captain hosting this species explained he had just travelled from Rhodes (#26), where it was found in the marina. Attention should be paid to see if it spreads to the marina in Fethiye, Turkey, where it would then constitute a new country record.

Key taxonomic characters (males only): Easily distinguished from other sphaeromatid isopods by shape of pleotelsonic region. Pleon large, granulated, bearing three tubercles in distal margin. Pleotelson large, also with three tubercles, granulated in anterior part and setose in distal margin. Pleotelsonic apex cleft, with six notches, middle ones deeper than lateral ones. Uropod endopods flattened and short; exopods markedly elongated, cylindrical, with acute apex.

Paradella diana (Menzies, 1962)

Native origin: Eastern Pacific Ocean.

Distribution: The first description of this species was from the Bay of San Quintin, Baja California (Menzies 1962).

Its first Mediterranean record was from Civitavecchia, Italy (Forniz et al. 1985), followed by a series of findings in Egypt (Atta 1991), Spain (Castelló et al. 2001), Turkey (Çinar et al. 2006), Cyprus (Kırkım et al. 2010), Libya (Zgozi et al. 2002) and Sardinia, Italy (Ferrario et al. 2017).

New records: This finding represents a new locality record for Sicily, Italy (#15: Fig. 2.3.6 G), and an additional record for Turkey from the same locality (Fethiye) it had previously been reported in (#31).

Boat-hull records: Found on boat-hulls moored in Greece (#24), and Italy (#20).

Notes: This species has not yet been reported in Greece, so this finding on a boat-hull in Heraklion, Crete, which had only travelled through Greek islands since its last cleaning alludes to its presence in Greek waters. Interestingly, the boat-hull it was found on in Sicily had just travelled from Fethiye, Turkey, where it is known from. It is assumed that this sphaeromatid isopod arrived to the Mediterranean via hull-fouling on vessels from the Northeast Pacific, its alleged original native range (Galil et al. 2008).

Key taxonomic characters: Males: segments 5-7, distal margin dorsally protruding (visible in lateral view). Pleotelson granulated, with two pairs of prominent tubercles, Pleotelson ending with a characteristic heart-shaped indentation. Uropods enlarged, finely crenulated and surrounded by short setae. Females lack the prominent distal ridges in segments 5-7 and the heart-shaped indentation of the pleotelson, reduced to a weak depression.

Sphaeroma walkeri Stebbing 1905

Native origin: Indian Ocean.

Distribution: This species is commonly found in intertidal fouling communities and has been widely reported from ports in warm and warm-temperate waters worldwide, including the Pacific coast of North America (Carlton et al. 1981).

Its first Mediterranean record is from Port Said, Egypt in 1924, where it was found on boat-hulls (Omer-Cooper 1927). Half a century later (in 1977), it was reported from Toulon, France (Zibrowius 1992), then from Turkey (Kocatas 1978), and Alicante, Spain in 1981 (Jacobs 1987). Decades later it was found once again on boat-hulls in Haifa Harbour, Israel (Galil et al. 2008), and also found to be well-established in Tunisian harbours and lagoons (Ounifi Ben Amor et al. 2010). In 2010 it was first spotted in Italy in the harbour of La Spezia (Lodola et al. 2012a).

New records: This finding represents a new country record for Greece (#24: Fig. 2.3.6 H). It was also found in Turkey (#31).

Boat-hull records: Found in Greece (#24 and #25), and Turkey (#31). This presents a new locality record for Greece (#25) in addition to the new country record presented above.

Key taxonomic characters: Pereon and pleotelson dorsally granulose, with rows of tubercles along posterior margins of pereonites, and four parallel rows of tubercles on pleotelson, directed longitudinally. Uropod endopod also with two or three prominent tubercles; exopod outer margin deeply serrated. Telson with denticulate posterior margin.

2.54 Mollusca

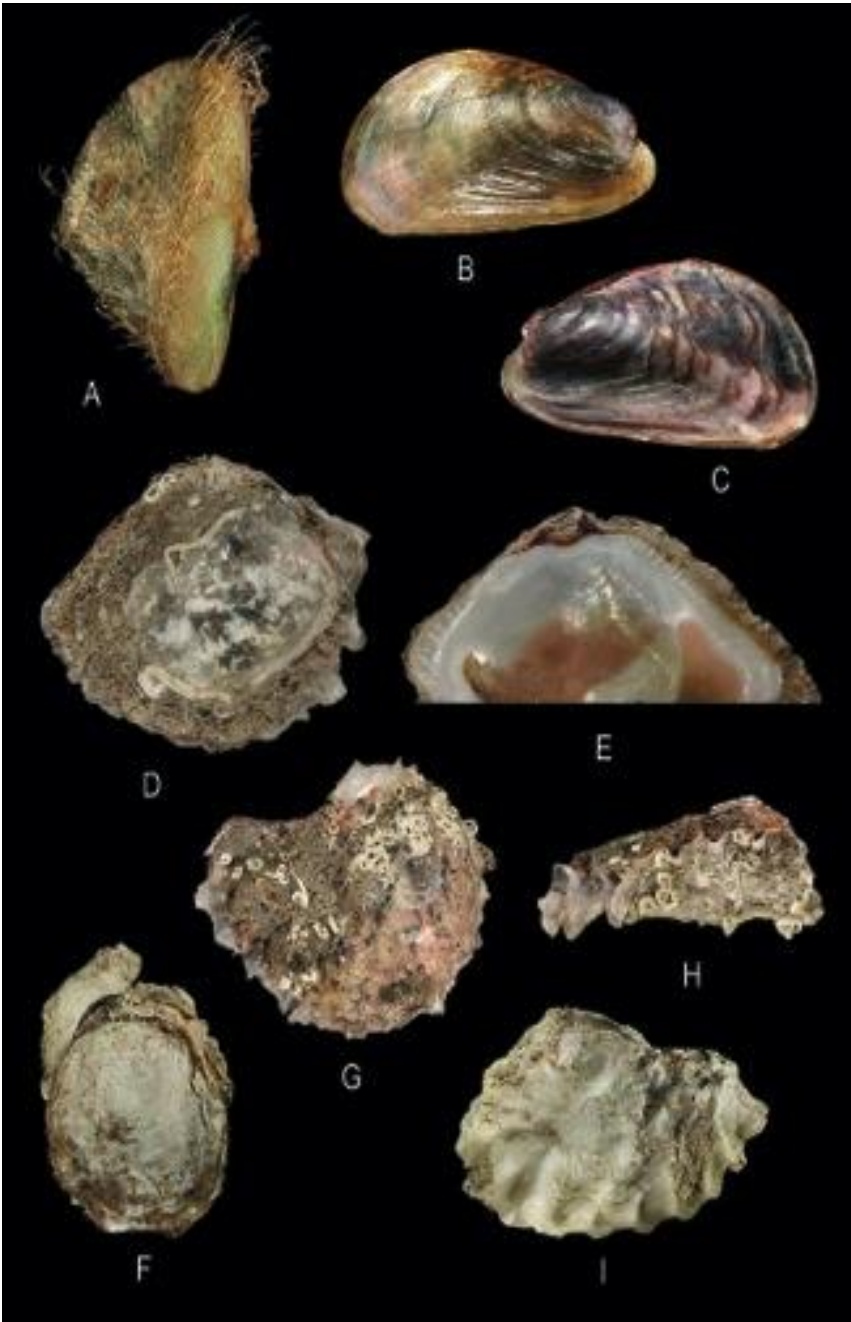


Figure 2.3.7 Molluscs. (A-I). (A) *Septifer cumingii* in Marina #25, L= 8,5 mm; (B-C) *Arcuatula senhousia* in Marina #2, L=19 mm; (D-E) *Saccostrea glomerata* in Marina #10, L= 40 mm; (F) *Pseudochama* cf. *corbierii* in Marina #20, L= 21 mm; (G-H) *Saccostrea* cf. *cucullata* in Marina #24, L= 25 mm; (I) *Dendostrea folium sensu lato* in Marina #33, L= 25mm. Photo credit: Cesare Boci.

Family: Chamidae

Pseudochama cf. corbieri (Jonas 1846)

Native origin: Red Sea, Gulf of Aqaba and Suez Canal.

Distribution: It is considered endemic to the Red Sea and Suez Canal (Barash et al. 1972). Its first Mediterranean record is from Greece (Ralli-Tzelepi 1946), and it has also been reported from Turkey (Cachia et al. 2017). The latest record from Malta represents its first Central Mediterranean record (Cachia et al. 2017).

New records: One juvenile specimen was found in Italy (#20: Fig. 2.3.7 F).

Notes: This species was formerly known as *Chama corbieri*, while *Pseudochama cornucopia* (Reeve, 1846) and *Pseudochama ruppelli* (Reeve, 1847) are both considered common synonyms. An additional record from Israel (Barash et al. 1972) as *Chama cornucopiae* Reeve, 1846 was excluded since the record was based on an empty shell. The present finding in Ragusa, Sicily (Italy) of a single young specimen remains dubious about its exact determination. Hence, we classify this finding as uncertain since the defining characters for this species were not yet fully developed in our juvenile specimen and suggest that the occurrence of *P. corbieri* awaits further confirmation before considering the species introduced to Italy.

Key taxonomic characters: Shell extremely inequivalve, lower valve (rv) deeply concave and upper valve quite flat with large area to attach to substrate. Irregular outline, exterior structure often eroded, can be subcircular. Very similar to *Pseudochama gryphina* but *P. corbieri* has a distinctly thicker shell. In addition, the exterior sculpture consists of fine to slightly roughened concentric, close-set chords. The interior colouration especially in upper valve suffused with purple, and crenulated margins, which are key identification characters. Lower point of shell's initial coiling in lower valve reaches to about 42% of total shell length, which is only about 25% in *P. gryphina*.

Remarks: Lower point of the shell's initial coiling in the lower valve reaches about 25% in the comparable *P. gryphina*, and exterior sculpture is more scale-like.

Family: Mytilidae

Arcuatula senhousia (Benson 1842)

Native origin: Siberian Peninsula to Indo-Pacific.

Distribution: It has been reported from Great Bitter Lake, the Suez Canal, the Red Sea, Mauritius, Zanzibar, and several Indo-Pacific and Indian Ocean countries including Thailand, Malaysia and New Caledonia (Barash et al. 1972).

Its first Mediterranean record was from Israel in 1960, and then from Lake Bardawil on the Egyptian Sinai Peninsula in 1982 (Barash et al. 1971). It was also found in Thau Lagoon, France in 1982, a popular oyster aquaculture locality (Hoenselaar et al. 1989) and then spread to the surrounding area including the Leucate Lagoon. Next, it was recorded in Ravenna, the Italian Adriatic coast in 1986 (Lazzari et al. 1994). In this century, it was found in the Gulf of Olbia, Tyrrhenian Sea (Savarino & Turolla 2000), then in 2001 it was established in the Gulf of Taranto, the Ionian Sea, from an area involving both mussel aquaculture and intense shipping (Mastrototaro et al. 2003). Next, it was reported again along the Adriatic Italian coast (Solustri et al. 2003), and the following year it had dense populations inside the dams of the Port of Leghorn (Livorno, Italy) (Campani et al. 2004). It was also found in Tunisia (Ben Souissi et al. 2005), then, between 2006 and 2009, in Siracusa's Porto Grande Marina, Sicily (Brancato et al. 2009). In 2010, it was found in the Eastern Adriatic from the Neretva River Delta growing on serpulid tubes of the polychaete *Ficopomatus enigmaticus* (Fauvel, 1923) (Despalatović et al. 2013). In Spain, it was reported from the Ebro River Delta in 2014 (Soriano et al. 2014), however, that record was based on four empty shells, therefore, its presence in Spain still awaits confirmation from live specimens.

New records: This finding represents the first confirmed country record for Spain (#2: S.D. Fig. 2.3.7 B-C). It was also found in France (#5 and #9), and Sicily (#15, #16 and #21).

Key taxonomic characters: Shell reaches a maximum 30 mm length, shell colour ranges from yellow-brown to dark-brown and is covered by a greenish periostracum. Has to 6 to 8 clearly visible ribs anterior to the umbone and is also accompanied by light-coloured radiating lines. Ventral margin a little bit concave, widened posteriorly. Anterior margin crenulated.

Family: Ostreidae

Dendostrea folium sensu lato (Linnaeus 1758)

Native origin: Indo-Pacific.

Distribution: Its first Mediterranean record is from Iskenderun Bay, Turkey in 1998 (Çeviker 2001), then from Cyprus (Zenetos et al. 2009), and next from the Greek islands of Astypalaia, Rhodes and Kastellerizo (Karachle et al. 2016). It has also recently been reported from Panama (Lohan et al. 2015).

New records: This finding represents a new subregional record for the Central Mediterranean, and a new country record for Malta (#22 and #23). It was also found in Greece (#24 and #26), Turkey (#29, #30 and #32) and Cyprus (#33: Fig. 2.3.7 I), where it was previously known.

Boat-hull records: Found on boat-hulls moored in Italy (#17), Greece (#26), Turkey (#31 and #32), and Cyprus (#33).

Notes: If it establishes in the marina in Italy, where it was found on a boat-hull, it would then present a new country record; the boat which was hosting *D. folium* in Italy had just returned from a long trip back from southern Turkey and the Greek Islands. *Dendostrea frons* (Linnaeus, 1758) and *D. folium* are very similar species. Huber (2010) rejects the possible presence of *D. frons* in the Mediterranean Sea, despite many reports of this species there. Based on genetic results, Crocetta et al. (2015) demonstrated that the Greek and Turkish material belongs to a single, morphologically highly variable species: *D. folium*, most likely representing a complex of species in need of revision (Marco Oliverio, pers. comm., 2017).

Key taxonomic characters: Highly variable morphology and colouration, often assuming nature of substrate. Foliate oyster, up to 60 mm in length, variable in colour, including brownish, whitish, reddish, pinkish. Thin, elongate oval; margin irregularly folded. Both valves concave having dichotomous radial ribs from umbo, top of ribs rounded. The submedian ridge not always present and number of plications is highly variable. Both valves with many fine and imbricate growth squamae, sometimes eroded dorsally. Adductor muscle scar kidney shaped, few chomata. Commissural shelf narrow. Umbonal cavity shallow.

Saccostrea cf. *cucullata* (Born 1778)

Native origin: Indo-Pacific.

Distribution: It is found from the Red Sea, East Africa down to South Africa including Madagascar, and West Africa up to Angola (Branch et al. 2002).

Its first Mediterranean record is from south-eastern Turkey in 1998-1999 from Erdemli, and later from Yumurtalik and Tasuçu (slightly west and east of Erdemli, respectively), where it is well-established with large populations (Çevik et al. 2001), followed by a record from El-Faham, Egypt (Gofas et al. 2003). An additional record from Tunisia remains questionable (Ounifi Ben Amor et al. 2016).

New records: This finding represents a possible new country record for Greece (#24). From this study, it was also found in Turkey (#31), presenting the most south-western record for the country.

Notes: The only specimen collected in Heraklion (Greece) was a juvenile (20 mm) and the crenulations along the margin (a key identification character) were only partially visible (Fig. 2.3.7 G-H), but were not well developed as in matured specimens. Therefore, we regard this finding as uncertain and suggest the occurrence of *S. cucullata* needs further confirmation before officially presenting this as a new country record in Greece.

Key taxonomic characters: Up to 60 mm length in Mediterranean. Shell inequivalve with lower valve (left valve, lv) larger, and can be deeply cupped, while right valve almost flat with plicated margin fitting margins of lower valve. Interior shell margin of right valve (rv) with prominent denticles fitting pits of lv margin. Sculpture highly variable from smooth, to strong radial ribs, and even spines. Shell also variable in outline, from nearly circular to oblong or roughly oval. Hinge untoothed. No sculpture at umbo.

Remarks: *Saccostrea* sp. are crenulated for entire perimeter whereas *Ostrea* sp. only have anterior margin near hinge crenulated. Indo-Pacific specimens reach larger dimensions (130 mm).

Saccostrea glomerata (Gould 1850)

Native origin: Australasia.

Distribution: Its native distribution extends from eastern Australia to New Zealand.

In the Mediterranean, it was intentionally introduced to the Adriatic Sea in 1984 for aquaculture (Cesari et al. 1985), but has not been found there since 1990 (Mizzan 1998), and is thus currently considered as locally extinct. In 1998 it was reported in Turkey, which was the first Eastern Mediterranean record (Çevik et al. 2001), but this record is considered a case of misidentification with either *S. cucullata* (according to Gofas 2011) or *Dendostrea frons* (according to Albayrak 2011), so this record remains questionable.

Boat-hull records: This species was found on one boat-hull moored in France (#10: Fig. 2.3.7 D-E), which had only travelled locally around the French Riviera (from Nice to Golfe-Juan) for the past 1.5 years since its last hull-painting.

New Mediterranean records: This finding confirms its presence in French waters and also presents a new subregional record for the Western Mediterranean.

Notes: This species was formerly known as *Saccostrea commercialis* (Iredale & Roughley, 1933), and is distinct from *S. cucullata* in terms of DNA 16S sequences (Lam et al. 2006; Salvi et al. 2014).

Key taxonomic characters: From 70-100 mm in length. Lower valve deep and cupped with weakly crenulated margin, flattened upper valve and folds towards lip to fit crenulations from lower valve. Presence of small denticles along edge near hinge which are closer together on lower valve compared to upper valve. Upper valve often has nodular ribs separated by large grooves.

2.55 Polychaeta

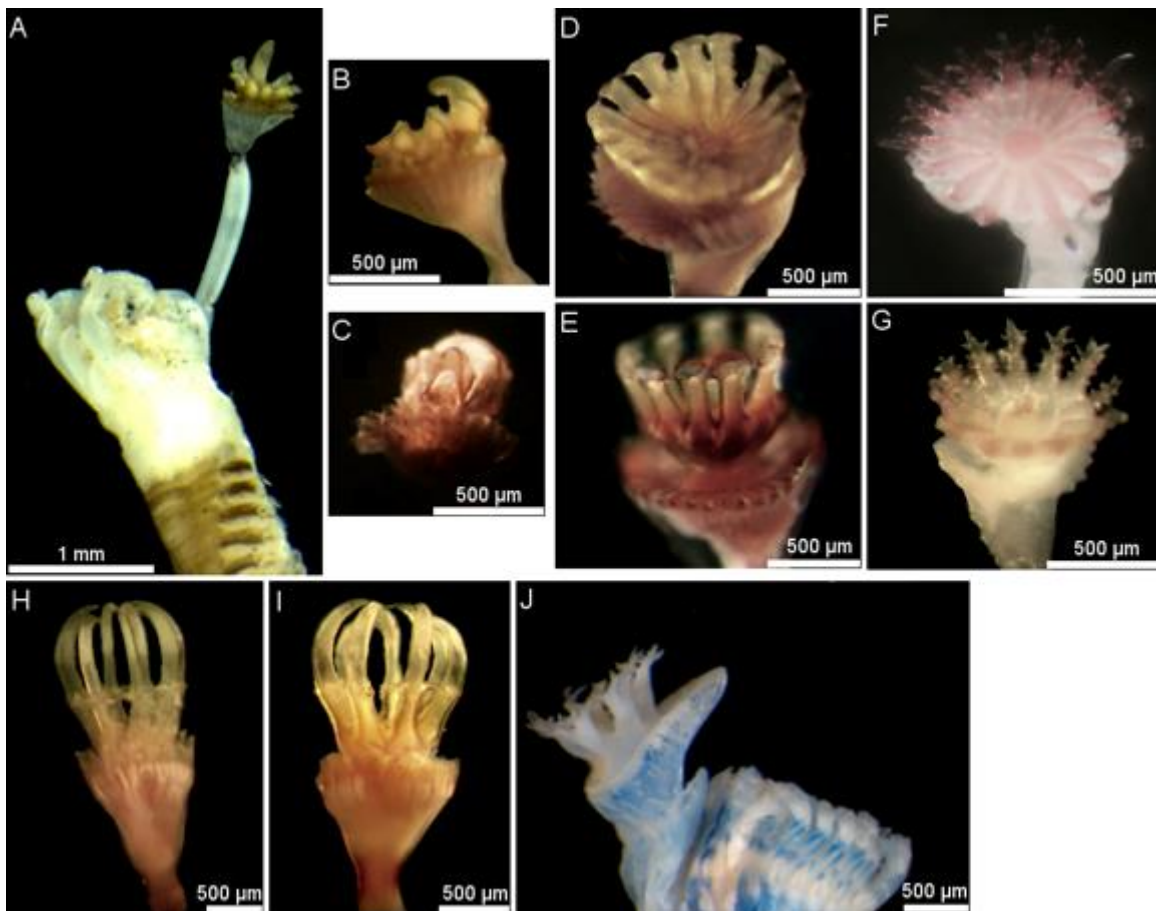


Figure 2.3.8 Serpulids. (A-J). Serpulids- Close-ups of the serpulid's opercula: (A-C) *Hydroides brachyacantha sensu lato* in Marina #2; (D-E) *Hydroides dirampha* in Marina #23; (F-G) *Hydroides elegans* in Marina #18; (H-I) *Hydroides homoceros* in Marina #33; (J) *Spirobranchus tetraceros sensu lato* in Marina #18. Photo credits: (A, J) Giorgos Chatzigeorgiou; (B-I) Aylin Ulman.

Family: Serpulidae

Hydroides brachyacantha sensu lato Rioja 1941

Potential native origin: Mexican Pacific.

Distribution: Since its initial Mexican record, it has been reported globally, from Hawaii (Straughan 1969), Brazil (Zibrowius 1970), Micronesia (Imajima 1982), Japan (Imajima 1987), Venezuela (Díaz Díaz et al. 2001), California (Bastida-Zavala et al. 2003) and India (Pati et al. 2015).

Its first Mediterranean record was from Israel in 1933 (Ben-Eliahu 1991), and its second from Turkey (Çinar 2006).

New Mediterranean records: This finding represents new country records for both Greece (#24) and Spain (#2: Fig. 2.3.8 A-C), the latter also presenting a new subregional record for the Western Mediterranean.

Boat-hull records: Found on boat-hulls moored in Greece (#24).

Notes: The recent paper by Sun et al. (2016) re-described *H. brachyacantha* as a complex of species, which renders the identity of the Mediterranean populations as unknown, until genetic analyses are performed and the status of the species within the complex is clarified. Consequently, the native origin of the Mediterranean populations is also unknown, and this serpulid should therefore be classified as "cryptogenic".

However, the possibility that *H. brachyacantha* is a native Mediterranean species having long escaped detection is not fully supported; it first appeared in the Mediterranean as early as in 1933 and so far has only from two records in the Levantine Sea (Israel and Turkey). According to Chapman & Carlton's (1991) criteria, these characteristics, combined with the fact that the species of *H. brachyacantha* complex are more widely distributed elsewhere (Sun et al., 2016), support a likely introduced status for the *H. brachyacantha* complex in the Mediterranean Sea.

The new records of this complex of species presented from this study in Greece and Spain demonstrate its ongoing spread, and additionally provide an important reference for future genetic analyses. Due to the uncertainty surrounding the real identity of any Mediterranean *H. brachyacantha* material, we here use the open nomenclature qualifier "*sensu lato*".

Key taxonomic characters: White calcaereous tubeworm. Opercular verticil possessing approximately 8 dark-brown strongly curved inward spines, dorsal hook longer and wider than others, spines covering the central disc, other spines similar to each other in size and shape (hooks near dorsal hook slightly larger than others). Spines have one short internal basal spinule.

Hydroides dirampha Mörch, 1863

Potential native origin: Tropical Western Atlantic.

Distribution: Circumtropical (Bastida-Zavala et al. 2003), originally described from the US Virgin Islands (Zibrowius 1971). It was reported in the Red Sea (Zibrowius 1971), the Western Atlantic (Bastida-Zavala et al. 2002), the Eastern Pacific (Bastida-Zavala et al. 2003), Australia (Hayes et al. 2003; Sun et al. 2015), and Hawaii (Bastida-Zavala 2008).

In the Mediterranean, it was first reported in Italy in 1870 as *Eupomatus lunifer* (Claparède 1870). It has since spread all over the basin, being next reported in Spain in 1923, Egypt in 1924 (for both records: Zibrowius 1973), Israel in 1937 (Ben-Eliahu and ten Hove 1992), Tunisia in 1969 (Zibrowius 1978), Lebanon in 1978 (Zibrowius 1981), Turkey in 2005 (Çinar 2006) and Greece in 2014 (Corsini-Foka et al. 2015).

New records: This finding represents a new country record for Malta (#22 and #23: Fig. 2.3.8 D-E).

Boat-hull records: Found on boat-hulls moored in marinas in France (#7), Italy (#12, #15, #17, #20, #21), Malta (#22 and #23), Greece (#24 and #25), Turkey (#31 and #32), and Cyprus (#33).

Notes: It is a NIS in the Mediterranean believed to be arrived by the shipping pathway from the tropical Western Atlantic (Zibrowius 1992).

Key taxonomic characters: White calcaereous tubeworm up to 36 mm in length (16 mm on average). Opercular verticil possessing 11 to 15 spines, similar in size and shape, with a distinct T or arrowhead flattened shape at the tips, and with one basal internal spinule. Without central tooth.

Hydroides elegans (Haswell, 1883)

Native origin: Australasia and Indian Ocean.

Distribution: Circumtropical: Pacific Ocean, Caribbean, Atlantic and Northern Europe. In the Mediterranean Sea, it has been reported since the 19th century (Claparède 1870), and has since spread to most countries in the basin (Galil et al. 2014).

New records: This finding represents a new country record for Malta (#22). This species was found in all marinas, except for #8, #13, #14, #20, #23, #29-31, #33, #34, #35. Fig. 2.3.8 F-G are from #18.

Boat-hull records: Found on boat-hulls from all marinas which had boats sampled.

Notes: It is considered the main fouling organism in the Mediterranean Sea (Kocak et al. 1999); our study confirms that it is the most widespread fouling species found here in terms of distribution.

Key taxonomic characters: White calcaereous tubeworm, sub-trapezoidal in cross-section, maximum tube length of 80 mm, with two longitudinal ridges; maximum body length of 20 mm. Opercular verticil with short central tooth possessing 14-17 radiating spines, each having 2-4 lateral processes and a medial row with 1-4 short internal spinules. Collar chaetae: bayonet, with 2-4 short teeth and a rasp behind them.

Hydroides homoceros Pixell, 1913

Potential native origin: Indo-Pacific.

Distribution: It was originally described from the Cape Verde Islands, in the Eastern Atlantic (Pixell 1913). Also reported from the Red Sea, Suez Canal, Arabian Gulf, Zanzibar and Maldives (Ben-Eliahu et al. 2011).

Its first Mediterranean record was from Israel in 1955 (Ben-Eliahu 1991), then in late 1970s from an aircraft carrier moored in Toulon, France (Ben-Eliahu et al. 2011). Next it was reported from the south-eastern Turkey (Çinar 2006).

Boat-hull records: Found on boat-hulls moored in Cyprus (#33: Fig. 2.3.8 H-I), but was not found in the same marina. The captain of one boat hosting this species in Cyprus had recently travelled to the Turkish Levantine coast, where it is known from. If it does establish in Cyprus, it would then present a new country record.

Key taxonomic characters: White calcaereous tube with three longitudinal ridges, less than 10 mm in body length. Opercular verticil chaeta possessing 6 spines, bayonet shaped with twinned spines in the middle of the chaetal length. Funnel with 18 radii, each with a pair of lateral spines pointed downwards, tips of radii T-shaped; Verticil with 6 spines of similar length, curved inwards with twinned lateral spinules at mid-length.

Spirobranchus tetraceros sensu lato (Schmarda 1961)

Native origin: Indo-Pacific.

Distribution: First described from Australia, it has a circumtropical distribution that includes the Suez Canal, Indian Ocean, South Africa, Australia, Malaysia, Japan, China and the Caribbean (Ben-Eliahu et al. 1992; Fiege et al. 1999).

Its first Mediterranean record was from Lebanon in 1965 (Laubier 1966) as *Spirobranchus giganteus coutierei* Gravier, 1908 (which is now understood as a sub-species of *S. tetraceros*, E. Kupriyanova, pers. comm., 2017.), followed by Rhodes, Greece (Dumont et al. 1989), Abu Kir Bay, the Egyptian Mediterranean (Selim et al. 2005), and the Turkish Levantine Sea (Çinar et al. 2006).

New records: This finding presents a new subregional record for the Central Mediterranean and a new country record for Italy (#18: Fig. 2.38 J). It was also found in Greece (#24).

Notes: *Spirobranchus tetraceros* has been treated as a complex of species since 1994 (Fiege et al. 1999; Ben-Eliahu et al. 2011) in need of taxonomic revision, hence, here it is referred to as *S. tetraceros sensu lato*.

Key taxonomic characters: Calcaereous tubeworm, pale purple in colour with three high longitudinal ridges and many transversal ridges. Broad peduncle with lateral wings crenulated on their inner distal margins; operculum flat with three long branching antler-like spines. Collar chaetae including 5 bayonet, 10 limbate, striate chaetae covered with minute denticles.

2.56 Porifera

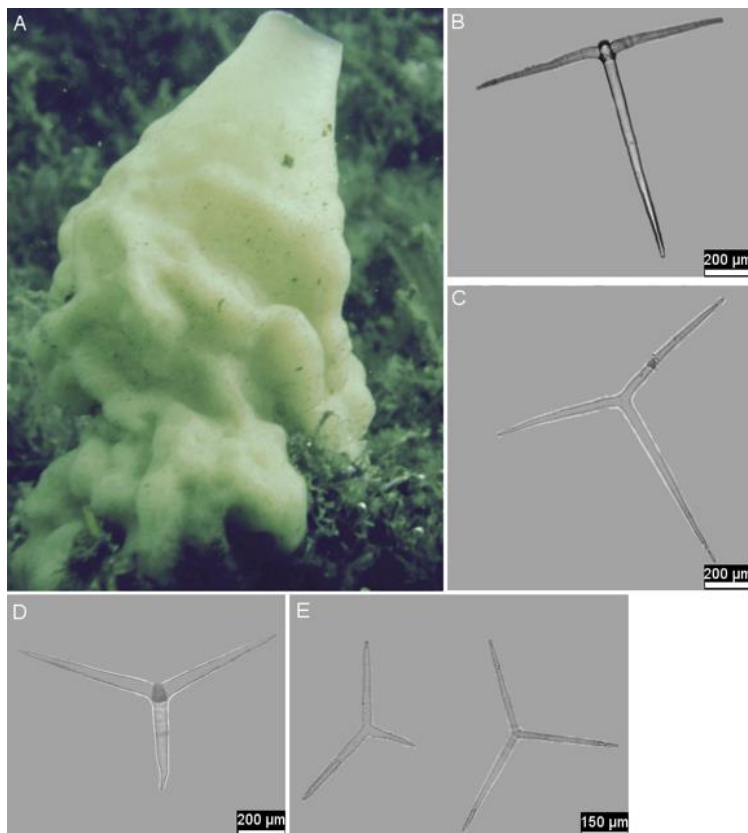


Figure 2.3.9 Porifera (A-E). *Paraleucilla magna* (A) Live colony in Marina #24. (B) cortical tetractine; (C) subatrial triactine; (D) subatrial tetractine; (E) atrial triactine (left) and cortical triactine (right). Photo credits: (A) Aylin Ulman; (B-E) Marco Bertolino.

Family: Amphoriscidae

Paraleucilla magna Klautau, Monteiro & Borojevic, 2004

Potential native origin: Indo-Pacific and Australia.

Distribution: First described from the Western Atlantic in Rio de Janeiro, Brazil, and was also found from the Azores, Madeira and Portugal (Bertolino et al. 2014; Guardiola et al. 2016).

Its first Mediterranean records were from multiple Italian localities, first in the Ionian then in the Tyrrhenian and Adriatic Seas, followed by the Ligurian Sea and Sicily (Longo et al. 2004, Longo et al. 2007, Bertolino et al. 2014, Marra et al. 2016). It was also reported from multiple localities in the Costa Brava region in Spain (Guardiola et al. 2012, Guardiola et al. 2016), as well as Malta (Zammit et al. 2009), and Croatia (Cvitković et al. 2013). Its first Eastern Mediterranean record is from the Gulf of Thessaloniki, Greece, where it was first

observed in 2014 in a mussel farm (Gerovasileiou et al. 2017). It has also emerged in the Sea of Marmara, Turkey in 2012 (Topaloğlu et al. 2016).

New records: This finding represents a new country record for Cyprus (#34), and new locality records for both Greece (#24: Fig. 2.3.9 A, and #26) and Sicily. Specifically, it was present at all seven sampled Sicilian marinas (#15-21), and was also found in Malta (#23).

Boat-hull records: Found on boat-hulls in France (#5), Malta (#22), Greece (#26) and Cyprus (#34).

Notes: Prior to the recent 2004 description of *P. magna*, this genus was only known from the Indo-Pacific region and Red Sea. As it was initially described from Rio de Janeiro in 2004, where it is considered cryptogenic (Cavalcanti et al. 2013), it was likely already present in several Mediterranean locations. For instance, the species had already been recorded (preceding its formal description) in 2001 from Mar Piccolo of Taranto (Longo et al. 2004), and according to local mussel farmers was present there as much as 20–30 years earlier (Longo et al. 2007). The opportunistic behavior of *P. magna*, with proliferation only close to either aquaculture facilities or harbours, may be the reason behind its late detection in the Mediterranean (Guardiola et al. 2012). Moreover, as several introductions probably occurred in a short period of time, the phylogeographic signal could be weak or even lost, making the determination of the introduction pathway a challenge (Pineda et al. 2011). Aquaculture and shipping are the most probable vectors for its recent expansion along the Western Mediterranean coast (Longo et al. 2007). This study shows that *P. magna* is now both a common and established species around Sicily and Malta. Noteworthy is the record on boat-hulls reported here from France (but not in the marina), which may represent the first step of subsequent spreading in the Western Mediterranean and recreational boating as another vector of its spread.

Key taxonomic characters: Calcareous sponge varying in shape from tubular to massive or irregular. Oscula located at ends of the tubes. Compressible consistency but sponge is friable, with smooth surface. Live specimens creamy-white coloured, not changing after alcohol preservation. Triactine and tetractine spicules.

2.57 Pycnogonida

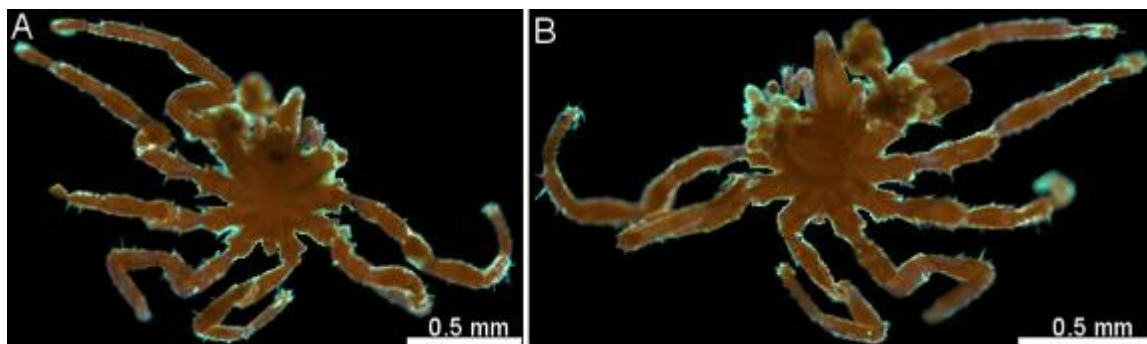


Figure 2.4.0 Pycnogonida. (A-B). *Achelia sawayai sensu lato* Marcus, 1940, ♂ (ovigerous) from Malta in Marina #23, (A) dorsal view; (B) ventral view. Photo credits: Cengiz Koçak.

Family: Ammotheidae

Achelia sawayai sensu lato Marcus, 1940

Native origin: Western Atlantic.

Distribution: Extremely common in the tropical shallow waters of the Western Atlantic. It is distributed from Georgia to the Gulf of Mexico and throughout the Caribbean Sea to Brazil. It has also been reported in Western Africa, Madagascar and in the southern Pacific in French Polynesia, Indonesia, Fiji and Papua New Guinea, although some of these records are still awaiting confirmation (Child 1992; Child 2004).

New records: This finding represents a new record for the Mediterranean Sea, and new country records for both Malta (#23: Fig. 2.4.0 A-B) and Italy (#17, #18).

Notes: Recent molecular studies suggest that the Atlantic and Pacific populations may belong to different entities within a complex of species (Sabroux et al. 2017). Therefore, it is referred to here as *A. sawayai sensu lato*. Since the origin of the Mediterranean material is unknown, further molecular studies are necessary to understand the invasion route taken by this pycnogonid. The local reproductive success of this species exhibiting paternal care was demonstrated by the finding of two ovigerous male specimens.

Key taxonomic characters: Trunk segments fused, outline circular; lateral processes touching or slightly separated, without major tubercles. Ocular tubercle height similar to width; large eyes, distinctly pigmented. Cheliferous scapes one segmented; chelae vestigial, globular. 8 segmented palps; 4 terminal short segments, very setose; 2nd and 4th segments longest. 10 segmented ovigers, with weak strigilis bearing denticulate spines; 4th

and 5th segments longest. Legs moderately slender; coxae 1 with 3 and coxae 2 with 2 laterodistal tubercles, terminating in one short seta; cement gland tube cone shaped at dorso-distal of femur, ending in very short tube-shaped duct; propodus large, slightly curved, without heel.

Notes: This species and *Achelia besnardi* Sawaya, 1951, known from the western Atlantic, are very similar except from the lateral processes of *A. besnardi* have conspicuous tubercles and the leg segments are relatively longer and more slender. Both the male and female of *A. besnardi* have fairly long dorso-distal tubercles on the femorae, almost as long as the femur diameter. The trunk of *A. besnardi* is not quite circular in outline, and the lateral processes do not always touch (Child, 1992). *Achelia sawayai* is also similar to *A. gracilis* Verrill, 1900, which is known from western Atlantic, and can be confused with this species. The dorso-distal tubercles on the lateral processes in the males of *A. gracilis* are stronger and coxa 1 of legs 1–2 show 4 tubercles as opposed to 3 with *A. sawayai*. A clear identification character in both sexes is the number and shape of palp articles of both species (7 in *A. gracilis*: 8 in *A. sawayai*) (Müller & Krapp, 2009). This study shows that this sea spider is likely establishing itself around Malta and Sicily, as one male specimen was ovigerous.

2.6 Discussion

This wide-scale study spanning the Mediterranean Sea provides a massive update of new NIS records, and in many cases their regional or local expansions, providing a warning for subsequent spreading. The 51 new country records presented in this study clearly indicate how inadequate our knowledge on Mediterranean marine NIS still is. There was a prevalence of new findings for bryozoans and crustaceans in almost all countries (Figure 2) because these are both poorly studied taxa in the Mediterranean owing to a lack of taxonomic expertise/focused studies. Additionally, typical rapid assessment surveys or citizen science initiatives searching for NIS usually target larger and eye-catching taxa (e.g. Zenetos et al. 2013, Mannino et al., 2017), so these minuscule or less charismatic components of fouling biota may have gone previously overlooked or not have had the applicable expertise available.

It is not uncommon for marine NIS to go overlooked for long periods of time (Carlton 2009), as in the case of *Paraleucilla magna* in the Mediterranean (Longo et al. 2007). Actually, for most ‘first country records’ documented in this study, the year of first introduction may have been much earlier than the first year of discovery presented here, but may have gone unnoticed due to a lack of taxonomic expertise or lack of focused study. Some probable examples of this include: *Paranthura japonica*, *Watersipora arcuata* and *Celleporaria brunnea*, whose current widespread Mediterranean distributions indicate they have likely been hitching rides

around the basin for quite some time. Another example of this is the sea spider *Achelia sawayai sensu lato*, first reported here for the Mediterranean basin, and specimens were already found in three marinas: two in Sicily and one in Malta. An exception to this is our finding of *Microcosmus exasperatus* in Karpaz Gate Marina, Cyprus, as this species was specifically sought two years prior to our sampling of the same marina, but was not found then (Gewing et al. 2016). Also, *Celleporaria brunnea* was not found to be present in Grand-Motte, France in 2014 (Marchini et al. 2015a), but was present there when we sampled in 2016.

In addition to the records presented here, *Percon gibbesi* (H. Milne Edwards, 1853) was sighted in Port Vauban, France, presenting a new country record for this species of western Atlantic origin, and already known from several other Mediterranean countries as a very successful invader (Katsanevakis et al. 2010). The non-indigenous status of *P. gibbesi* in the Mediterranean Sea is uncertain, because its long-lived planktonic larvae could have entered the Gibraltar Strait facilitated by natural means, i.e. the Atlantic Current, rather than human vectors, such as ballast water (Mannino et al. 2017 and references therein). Due to its questionable status regarding its mode of introduction, we have cautiously separated this species from the other NIS records. However, it is noteworthy that *P. gibbesi* was sighted feasting on a fouling community on a boat-hull in Greece (#25), suggesting hull-fouling as another possible vector for its ongoing spread.

This study focusing exclusively on marina habitats indicates that recreational boating represents the most plausible vector of introductions for the NIS we found, aside from the few marinas situated in very close proximity to either aquaculture facilities or shipping ports, as in Port Camargue, France and Heraklion, Greece. Hence, most of these new records suggest the pivotal role of recreational boating in facilitating both first introduction events to a given country and as a means of secondary spread.

Furthermore, some species reported here are likely polyvectic, but it is clear that recreational boating plays a determinant role in accelerating/facilitating the spread of many such species, especially those having only a very short and lecithotrophic larval stage. The presence of such species lacking the ability for natural long-distance dispersal found on boat-hulls and in marinas can confirm that the hull-fouling vector is instrumental in expediting primary introductions as well as facilitating secondary transfer for many ascidians, bryozoans and peracarids such as *Ampithoe bizseli*, *Bemlos leptocheirus*, *Celleporaria brunnea*, *Clavelina oblonga*, *Paraleucilla magna*, *Paracerceis sculpta* and *Paranthura japonica*, *Phallusia nigra*, *Styela plicata* and *Tricellaria inopinata*. The ongoing nature of the invasion process is further demonstrated by the observation of the same set of NIS

on boat-hulls and in the same marinas, clearly showing the exchange of organisms from marina to mobile habitats and *vice versa*.

The species which are not yet present in a country, but found only on boats obviously cannot formally be recorded as new country records, unless we are certain that the boat has not left that country since its last hull-painting/cleaning, e.g., as in our finding of *Paradella diana* on boat-hulls in Greece. Some other interesting cases of NIS found on boats but not yet in the country are (see Table 3 for details) the barnacle *Amphibalanus improvisus* and the bryozoan *Watersipora arcuata* both found on hulls in France, yet the boats which they were found on had only travelled to the Balearic Islands, alluding to the assumption that those NIS are likely present in the Balearic Islands. Also noteworthy is the finding of the oyster *Saccostrea glomerata* from a boat-hull in France, representing a new record for the Western Mediterranean, and of the bryozoan *Parasmittina egyptiaca* from a boat-hull in Italy, representing the first Central Mediterranean record for this species. Overall, the 20 records presented in this study of NIS attached to boats but not yet recorded in the respective marina illustrates the potential of the biofouling vector in seeding a new area with propagules.

In synthesis, a pool of NIS is circulating among Mediterranean marinas, linked by a dense network of boat voyages ensuring their dissemination by a steady multiplication of the number of occasions. It is also of interest to point out that nearly all marinas have a rule prohibiting the in-water cleaning of vessels, but this rule is genuinely not enforced, and in-water cleaning was commonly witnessed within marinas during this study, likely facilitating the 'stepping stone' invasion process by dislodging and exacerbating the resettlement of NIS propagules.

Recently, Ferrario et al. (2017) showed that marinas can host as many NIS as larger commercial harbours. This massive contribution of new NIS records confirms their result and reveals that Mediterranean marinas so far have been inadequately explored for NIS, despite the Mediterranean Sea being both a global hotspot for boating traffic, and for level of NIS invasions. We strongly recommend that major attention should soon be dedicated to recreational marinas as hotspots of introduction, and to pleasure boats as a vector of introduction and spreading. Management actions to combat NIS in the Mediterranean Sea need to also incorporate the recreational boating vector.

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3 A HITCHHIKER'S GUIDE TO MEDITERRANEAN MARINA TRAVEL FOR ALIEN SPECIES

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3.1 Abstract

The Mediterranean Sea is both a global hotspot for marine bioinvasions and for recreational charter boating traffic, the latter representing a vector for non-indigenous species (NIS) introductions and their spreading via biofouling. Here, a large-scale analysis was completed on NIS across Mediterranean recreational marinas to examine the drivers for NIS success and similarities between marinas. In total, 50 Mediterranean marinas spanning 7 countries from Spain to Turkey were investigated for NIS macroinvertebrate fauna. Then, total NIS richness of each marina was tested against several abiotic factors using multivariate statistics to determine which factors are significant in contributing to both higher NIS success and similar NIS assemblages between marinas. The marinas with the highest NIS richness were Heraklion, Crete, Greece (27), Villa Igiea, Sicily, Italy (20) and Port Camargue, France (18). The following factors were significant in shaping NIS richness in marinas: sea surface temperature, number of berths, proximity to Suez Canal, proximity to aquaculture sites, proximity to commercial harbours, absence of pontoons, biogeographic sector and climate type. However, the factors found to shape similarities of NIS assemblages across marinas contrasted from the previous results, owing almost entirely to environmental factors rather than proximity to known vectors of introduction; here a combination of temperature, primary productivity, biogeographic region, climate type and additionally proximity to the Suez Canal were found to be significant influences. These results can help prioritize monitoring and management efforts for controlling the introduction and spread of marine NIS in the Mediterranean Sea.

Key words: abiotic factors, alien species, biofouling, bioinvasions, Mediterranean, pathways, non-indigenous species (NIS), recreational boating, Suez Canal, vectors

3.2 Introduction

The seas are currently inundated with many stressors such as overfishing, pollution, climate change and invasive species, which combined are negatively affecting both ecosystem structure and function (Jackson, 2008; Worm et al., 2006). While many stressors, namely overfishing, have left much of the Mediterranean Sea barren (Guidetti et al., 2014), new species are constantly finding their way into the basin, and some of their preferred habitats are teeming with this 'foreign' life. While few of these new migrants have favourable effects on the economy, most are perceived negatively and are often considered a form of biological pollution (Olenin et al., 2007).

The Mediterranean is the second most prevalent place in the world both for recreational boating (Cappato et al., 2011), and the global hotspot for marine bioinvasions, hosting between 700 to 1000 marine non-indigenous species (hereafter referred to as NIS; Galil, 2009; Galil et al., 2015; Katsanevakis et al., 2014). This work aims to better understand the known distribution of NIS in the Mediterranean, particularly pertaining to recreational marinas and the boats which they host, and then explores which underlying abiotic factors influence certain marinas to be bioinvasion 'magnets'. The definition of NIS used here is (EEA, 2012): "An organism introduced outside its natural past or present distribution range by human agency, either directly or indirectly". Thus, these new arrivals must be assisted by anthropogenically-enabled facilitation.

Marinas, especially in the Mediterranean, have generally gone overlooked as source localities for NIS, due to (erroneous) perceptions about the effectiveness of antifouling paints (Minchin & Gollasch 2003), despite recent global research demonstrating marinas as important hubs both for primary introduction and for secondary stepping-stone invasion events (Acosta & Forrest, 2009; Ashton et al., 2014; Clarke-Murray et al., 2011; Floerl & Coutts 2009; Marchini et al., 2015; Ferrario et al., 2017).

'Pathways' facilitating transfers of species, such as shipping, aquaculture, and artificial canals are generally responsible for primary introduction events of NIS, and one pathway may have several associated 'vectors' for secondary transfers. For example, the principal vectors associated with the shipping pathway can be 'ballast water', 'ballast tank' (Casas-Monroy et al., 2011), or part of the 'biofouling'. Biofouling is the colonization of algae, plants and/or animals of submerged artificial surfaces, such as piers and boats. If biofouling biota adheres onto other live organisms, the process is called "epibiosis", but is still considered part of the biofouling assemblage.

Currently, only two pathways or vectors are under(going) regulation in Europe: direct transfers via aquaculture as of 2007 (#708/2007; EUROPA 2007), and ballast water as of September 2017 (Ballast Water Management

Convention; www.imo.org). Thus, the transfer of NIS via biofouling is now considered *the* largest unregulated vector for NIS introductions (Clarke-Murray et al., 2011; Gollasch, 2002; Zabin et al., 2014). Another major vector of concern in the Mediterranean is the man-made Suez Canal, discussed in detail both in Galil et al. (2017), and subsequently in this study, which likely helps expedite several other vectors of secondary spread such as ballast water and biofouling due to reduced shipping travel times thus improving survival opportunities for non-indigenous biota.

Boats of any type, size or class can have biofouling attached to their hulls (Carlton 1985, 2003). Thus, recreational marinas are an obvious place to conduct a large-scale study on NIS. These new migrants have been arriving more frequently especially to the Eastern Mediterranean in recent decades⁴; and from the Levantine basin they can hitch a ride wherever the wind or gas takes their host. After a NIS' initial arrival, settlement is first dependent on surviving in the new environment, and then on their reproductive success (Galil et al., 2017). Once a new marine species establishes in a locality, eradication is often unfeasible as it is nearly impossible, thus prevention is universally considered the best management option for NIS.

Bioinvasions are now a common component of global change, and many invaders flourish in artificial rather than natural habitats. Artificial structures can be fashioned from either man-made or natural materials but are specifically designed for human purposes (Mineur et al., 2012), thus all aspects of marinas are considered artificial structures. Fouling communities on artificial pontoons have been shown to host NIS different from their natural counterparts (Connell, 2000; Bacchiocchi & Airoidi, 2003; Megina et al., 2016). This is likely because these structures are usually located in sheltered habitats, with modified water circulation (Floerl & Inglis, 2003; Bulleri & Chapman, 2010), and home to intensive human traffic and vessel movement (Callier et al. 2009), which can sometimes host complex fouling communities (Glasby et al., 2007; Tyrell & Byers, 2007). In fact, in addition to NIS being shown to favour artificial substrates, increased habitat complexity resultant of biofouling has been shown to further exacerbate the establishment of additional fouling species (Simkanin et al., 2017) as it can provide additional habitat, food supplies and protected niche areas.

The successful establishment of NIS are thought to be reliant on combinations of both biotic and abiotic factors (Early & Sax 2014), but aspects of these factors fluctuate both spatially and temporally, thus each habitat has different underlying factors affecting settlement success. Firstly, there is the supply of new propagules to a marina from visiting vessels each hosting different fouling assemblages, i.e., propagule pressure (Bulleri & Airoidi, 2005). Secondly, the biological traits of each NIS, such as dispersal characteristics, nutrient accessibility and spatial requirements, are also contributing factors (Cardeccia et al., 2018). And thirdly, there are the

⁴<https://www.eea.europa.eu/data-and-maps/indicators/trends-in-marine-alien-species-mas-2/assessment>

complex interactions between local environmental conditions (Colautti et al., 2006, Wonham et al., 2013) and species characteristics (Simberloff & Von Holle 1999). All factors combined lead to a very complex matrix of possibilities for invasion success, and obviously, all probable factors cannot conceivably be concurrently tested.

Understanding some key underlying factors behind spatial distribution patterns of NIS communities in marinas can help clarify which contribute to settlement success (Clarke-Murray et al., 2014). Here, we perform the first large-scale study of NIS across marina habitats spanning the Northern Mediterranean region to test which abiotic factors of the marinas (or combinations thereof) are found to influence total NIS richness in marinas and additionally, which factors affect the multivariate structure of NIS assemblages between marinas or groups of marinas. These results will help influence effective management strategies to help deter marine bioinvasions via the biofouling vector in the future.

3.3 Materials and methods

3.3.1 Study area

The results from previous (published and unpublished) studies assessing NIS in Mediterranean marinas were combined to perform an extensive analysis of 50 Mediterranean marinas spanning seven countries along the northern rim of the Mediterranean Sea (Fig. 3.1). The marina names, along with their localities, geographic coordinates and sampling dates are presented in Table 3.1.

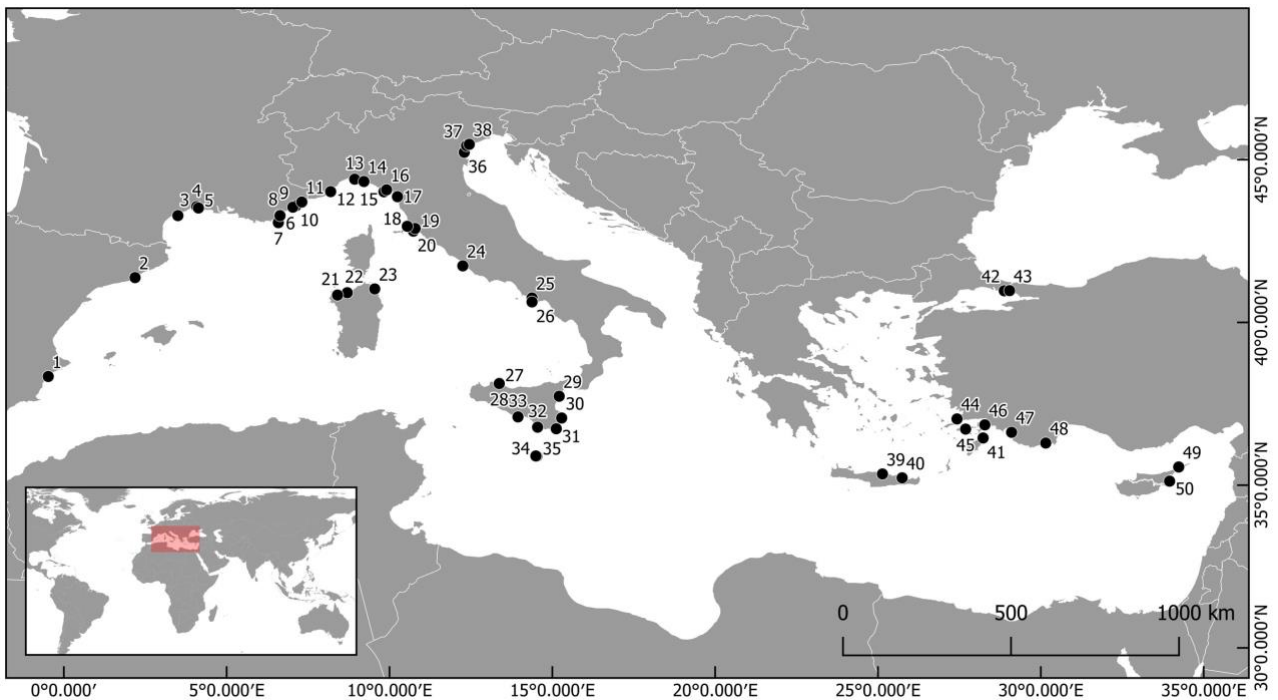


Figure 3.1 Map of the Mediterranean Sea showing marina localities sampled for this study, with their corresponding assigned number from Table 3.1.

3.3.2 Marina sampling strategy

This study sampled all collected macroinvertebrate taxa for identification. The samples were taken from submerged artificial substrates (docks and pontoons) using a hand-held rigid net (1 mm mesh size, surface area of 25 x 20 cm), having one sharpened edge capable of dislodging well-cemented taxa such as barnacles and oysters from the substrate. This net was used to scrape the substrate over an area of approximately 0.23 m² to collect one sample. For the marinas investigated by Ferrario et al. (2017), as well as the new material presented here (see “unpublished” records in Table 3.1), nine samples of biofouling were collected from each marina from the main docks or floating pontoons, covering all regions of the marina. Samples were preserved in 4% formalin solution and taxonomic identification was then completed in the University of Pavia laboratory.

Table 3.1 List of marinas sampled, with corresponding number, geographical coordinates, sampling dates and references.

Country	#	Locality name	Marina name	Lat. & Long.	Sampling dates	References
WESTERN MEDITERRANEAN SEA						
Spain	1	Alicante	Marina de Alicante	38.339°N; 0.480°W	14/11/2016	Ulman et al. 2017
	2	Barcelona	One Ocean Port Vell	41.376°N; 2.187°E	22/11/2016	Ulman et al. 2017
France	3	Agde	Port Principal du Cap d'Agde	43.281°N; 3.501°E	5-18/06/2015	Ulman et al. 2017
	4	La Grande-Motte	Port de la Grande-Motte	43.557°N; 4.082°E	02/11/2016	Ulman et al. 2017
	5	Le Grau-du-Roi	Port Camargue	43.515°N; 4.132°E	16-28/05/2015	Ulman et al. 2017
	6	Saint-Tropez	Port de Saint-Tropez	43.278°N; 6.637°E	1-30/04/2016	Ulman et al. 2017
	7	Cogolin	Marines de Cogolin	43.065°N; 6.586°E	1-30/04/2016	Ulman et al. 2017
	8	Saint-Maxime	Port Privé de Sainte-Maxime	43.307°N; 6.638°E	1-30/04/2016	Ulman et al. 2017
	9	Cannes	Cannes Le Vieux Port	43.540°N; 7.032°E	19-28/04/2015	Ulman et al. 2017
	10	Antibes	Port Vauban	43.585°N; 7.127°E	1-12/05/2015	Ulman et al. 2017
	11	Villefranche-sur-Mer	Port de Villefranche	43.698°N; 7.307°E	22-30/11/2016	Ulman et al. 2017
Italy	12	Alassio	Marina di Alassio	44.018°N; 8.192°E	27/05/2016	Unpublished
	13	Genoa	Lega Navale Italiana Genoa	44.400°N; 8.930°E	29/07/2013	Ferrario et al. 2017
	14	Santa Margherita Ligure	Marina di Santa Margherita Ligure	44.329°N; 9.213°E	06/07/2013	Ferrario et al. 2017
	15	La Spezia	Assonautica La Spezia	44.014°N; 9.827°E	11/06/2013	Ferrario et al. 2017
	16	Lerici	Porticciolo di Lerici	44.073°N; 9.908°E	4/07/2013	Ferrario et al. 2017
	17	Viareggio	Porto di Viareggio	43.863°N; 10.243°E	27/06/2013	Ferrario et al. 2017
	18	Piombino	Marina Terre Rosse	42.953°N; 10.545°E	26/07/2016	Unpublished
	19	Scarlino	Marina di Scarlino	42.885°N; 10.784°E	27/07/2016	Unpublished
	20	Punta Ala	Marina di Punta Ala	42.804°N; 10.732°E	27/07/2016	Unpublished
	21	Porto Torres	Marina Turritana	40.840°N; 8.402°E	11/06/2014	Ferrario et al. 2017
	22	Castelsardo	Porto di Castelsardo	40.912°N; 8.701°E	11/06/2014	Ferrario et al. 2017
	23	Porto Rotondo	Marina di Porto Rotondo	41.028°N; 9.545°E	9/06/2014	Ferrario et al. 2017
CENTRAL MEDITERRANEAN SEA						
Italy	24	Lido di Ostia	Porto Turistico di Roma	41.737°N; 12.250°E	12-19/07/2015	Ulman et al. 2017
	25	Ischia Island	Marina di Casamicciola; Marina di Sant'Angelo; Porto d'Ischia	40.748°N; 13.906°E 40.695°N; 13.893°E 40.743°N; 13.939°E	1-11/08/2015	Ulman et al. 2017
	26	Sorrento	Porto Turistico Marina Piccola	40.629°N; 14.375°E	22-29/07/2015	Ulman et al. 2017
	27	Palermo	Marina Villa Igiea	38.142°N; 13.370°E	26-29/07/2016	Ulman et al. 2017
	28	Palermo	Porto La Cala	38.120°N; 13.368°E	2-3/08/2016	Ulman et al. 2017
	29	Riposto	Porto dell'Etna	37.732°N; 15.208°E	17-28/09/2016	Ulman et al. 2017
	30	Siracusa	Porto Grande (Marina Yachting)	37.063°N; 15.284°E	15-16/08/2016	Ulman et al. 2017
	31	Marzamemi	Marina di Marzamemi	36.733°N; 15.119°E	08/10/2016	Ulman et al. 2017
	32	Marina di Ragusa	Porto Turistico Marina di Ragusa	36.781°N; 14.546°E	1-7/09/2016	Ulman et al. 2017
	33	Licata	Marina di Cala del Sole	37.097°N; 13.943°E	5-10/08/2016	Ulman et al. 2017
	34	Msida	Msida Yacht Marina	35.896°N; 14.493°E	1-8/07/2016	Ulman et al. 2017

Malta	35	Valletta	Grand Harbour Marina	35.890°N; 14.523°E	11-18/07/2016	Ulman et al. 2017
ADRIATIC SEA						
Italy	36	Chioggia	Porto Turistico San Felice	45.226°N; 12.294°E	10/07/2012	Unpublished
	37	Venice	Diporto Velico Veneziano	45.428°N; 12.365°E	11/07/2012	Unpublished
	38	Treporti	Marina Fiorita	45.471°N; 12.448°E	9/07/2012	Unpublished
EASTERN MEDITERRANEAN SEA						
Greece	39	Heraklion	Old Venetian Harbour	35.343°N; 25.136°E	1-15/11/2015	Ulman et al. 2017
	40	Agios Nikolaos	Agios Nikolaos Marina	35.187°N; 25.136°E	18-25/11/2015	Ulman et al. 2017
	41	Rhodes	Mandraki Port	36.449°N; 28.226°E	2-11/06/2016	Ulman et al. 2017
Turkey	42	Istanbul	Ataköy Marina	40.972°N; 28.875°E	20/08/2015	Unpublished
	43	Istanbul	Setur Kalamış Marina	40.976°N; 29.039°E	28/08/2015	Ulman et al. 2017
	44	Bodrum	Milta Bodrum Marina	37.034°N; 27.425°E	9-11/09/2015	Ulman et al. 2017
	45	Datça	Datça Marina	26.722°N; 27.689°E	10/10/2015; 13/05/2016	Ulman et al. 2017
	46	Marmaris	Setur Marmaris Netsel Marina	36.852°N; 28.276°E	14-18/09/2015	Ulman et al. 2017
	47	Fethiye	Eçe Marina	36.623°N; 29.101°E	19-24/09/2015	Ulman et al. 2017
	48	Finike	Setur Finike Marina	36.294°N; 30.149°E	18-27/05/2016	Ulman et al. 2017
Cyprus	49	Karpaz	Karpaz Gate Marina	35.558°N; 34.232°E	21-27/06/2016	Ulman et al. 2017
	50	Famagusta	Famagusta Port	35.123°N; 33.952°E	13-19/06/2016	Ulman et al. 2017

For the marinas sampled in the Ulman et al. (2017) study, a ‘modified’ rapid assessment (RAS) survey (Pedersen et al. 2003; Cohen et al. 2005; Ashton et al. 2006) was adopted, with an expanded collection time of approximately 8 hours per marina, or until it was considered no additional new species could be found. Marina samples were taken from the innermost, outermost and middle portions of the marina to ensure representative sampling. These samples were sorted on-site according to taxa, and then preserved in a 90% ethanol solution. The only exception to this preservation method being the ascidians, which were immediately preserved in seawater which was continually refreshed throughout the day to keep the sample alive, then when possible, placed in a freezer for 30 to 90 minutes with care taken not to freeze the sample; Next the sample was transferred to a 4% formalin/seawater solution for 48 hours for ‘fixing’ which is required for maintaining some rigidity to the structure of the specimens necessary for dissection, then preserved in a 90% ethanol solution. Photographs were also taken of much of the sampled biota *in situ* using either a SONY RXIII (with a Nauticam housing) or an Olympus TG-4. For both studies, samples were also collected from ladders, tires, ropes and buoys either by dislodging the samples using a paint scraper with a width of 6.35 cm or manually.

3.3.3 Identification

The collected preserved material were examined under a dissecting microscope, and, where needed, taxonomic slides were analyzed using an optical microscope; Additionally, the Tescan FESEM (Field Emission Scanning Electron Microscope) series Mira 3XMU for SEM pictures, with increasing magnification, at 6-19 mm working distance, using an accelerating voltage of 10 kV, with graphite metallization and detection by secondary electrons was used for the identification of select bryozoan species.

3.3.4 Statistical analyses

Total number of NIS (species richness) per marina, the dependent response variable

The total NIS per marina was calculated, after each species had their NIS status for the Mediterranean Sea verified (Clark & Johnston, 2009). The criteria used for evaluating a species is non-indigenous was taken from Chapman & Carlton (1991). This total value was used as the dependent variable which was tested against the abiotic factors explained below.

Abiotic factors

The factors included in this study were partially derived from previous studies on the topic conducted elsewhere which found the same factors to be significant (Clarke-Murray et al., 2014; Floerl & Inglis 2003; Foster et al., 2016; Peters et al., 2017), and partially derived from our own personal speculations or observations which were contrived during marina sampling and from interviews with local marina staff and boat owners. The abiotic factors tested here include environmental factors, marina specific factors, and proximity to major vectors and are highlighted in bold text below.

Environmental factors

Salinity was measured at each marina using a refractometer (Aqua fauna Model #8408). In brackish seas or along corridors connecting two water bodies, salinity is considered the most important factor for limiting the range or niche of species (Cognetti & Maltagliati 2000): in marinas that receive relevant freshwater inputs, settlement and reproduction of euryhaline species will be favoured (Floerl & Inglis 2003). **Proximity to**

freshwater source was codified as a binary variable (yes/no) and was deemed proximate if a source (river, spring or lagoon) was < 5 km from the marina and was able to affect the marina waters. We hypothesised that higher nutrient input due to riverine discharge may positively affect abundance and growth rates of fouling assemblages of select taxa, or alternatively, lower salinities would surpass the threshold tolerance of other taxa.

Temperature was measured using a thermometer for aquaria use, and the water was collected from 1 m depth to exclude the immediate warmer surface temperature layer. Temperature was found in other studies to be a good predictor of NIS richness, since this has a direct influence on reproductive success, i.e., most species have a minimum temperature requirement needed to trigger reproductive events (Brock-Morgan, 2010; Gallardo & Aldridge 2013); it is also the only factor here that accounts for the seasonality of different sampling dates.

General **primary productivity** was derived from a study that averaged primary productivity levels in g C m^{-3} from 2000-2009 using satellite data (Colella et al., 2016); Chlorophyll levels (a proxy of phytoplankton biomass) have been described as one of the most important variables for successful NIS establishment (Mattias Obst, pers. comm., Crete Alien Species Workshop, 2014).

The Köppen-Geiger climate classification (Peel et al., 2007) was used to test if similar climates had an influence on NIS richness; climate match is considered a potential factor as species have specific niche habitat requirements (Bax et al., 2003). The Mediterranean was split into ten **biogeographic sectors** following the subdivision proposed by Bianchi & Morri (2000); we added an additional 11th sector here to incorporate the Istanbul region (the Bosphorus Strait and Marmara Sea). The designations of these sectors account for the evolutionary histories of the areas combined with climatic variations.

Marina factors

A higher **number of berths** has been indicated elsewhere to correlate to higher NIS richness (Nall et al., 2015; Ros et al., 2013), as it can be a proxy for increased vessel traffic, and hence higher propagule pressure (Occhipinti-Ambrogi, 2007). **Total pier length** was measured in km and **marina area** was measured in km^2 (Google Earth Pro 2016) as NIS have repeatedly demonstrated better success in artificial habitats over natural ones (Airoldi et al., 2015; Glasby et al., 2007; Jimenez et al., 2017; Simkanin et al., 2017), thus additional habitat

could opportune more introduction events, and larger marina size has been shown to influence the establishment of NIS (Clark & Johnston, 2009; Clark & Johnston, 2005; Connell & Glasby, 1999); Peters et al., 2016). **Presence of floating pontoons** was codified as a binary variable (yes/no); the shallower portions of artificial substrates have shown to host higher NIS than their deeper counterparts (Dafforn et al., 2009), since they are separated from the seafloor, these habitats escape most predation (Bishop et al., 2015; Connell, 2001; Connell & Glasby, 1999; Simkanin et al., 2017). Finally, **marina opening length** was measured in km (Google Earth Pro, 2016); a partially-enclosed marina with a smaller opening length has shown to positively influence both the quantity and frequency of recruitment events due to the confinement of larval dispersal (Brock-Morgan, 2010; Floerl & Inglis, 2003; Foster et al., 2016).

Proximity to other major vectors

Proximity to aquaculture sites was codified as a binary variable (yes/no); an internet search was performed using the marina name, and also the names of the nearby towns, in combination with the terms “aquaculture”, “shellfish” or “fish farm” in the native language, and deemed proximate if < 5 km in distance from the marina. Some of these details were learned directly from field observations i.e., for Marina di Cala del Sole in Licata, Sicily (Italy), which was observed to have a small fish farm in the canal of the marina itself. Many fish farm localities stemmed from the Trujillo et al. (2012) report, and much of the Sicilian farms were learned from the Popescu (2010) source. Aquaculture facilities can facilitate both the availability and establishment of NIS by direct introduction for culturing, which can then develop self-sustaining populations nearby, or indirectly by hitchhiking on associated species on the cultured biota as epibionts (Naylor et al., 2001; Ruesink et al., 2005). In the EU, the introduction of NIS through aquaculture is controlled by Regulation 708/2007 and 1143/2014 which require specific permission to introduce a new species, but this does not protect from epibionts (European Commission, 2016).

Proximity to commercial harbours was codified as a binary variable (yes/no) and was deemed proximate if a harbour was < 5 km away. Ports are understood to be the main entry point for NIS (Gallardo & Aldridge, 2013; Minchin et al., 2006; Occhipinti-Ambrogi, 2007). This is because NIS can travel to ports via the major pathway being shipping and its two major vectors of transmission: in ballast water or as part of the biofouling (Seebens et al. 2016). Thus, harbours along with marinas have been advised as focal areas necessitating investigation for the early detection of NIS (Lehtiniemi et al., 2015).

Presence of a shipyard in the marina was codified as a binary variable (yes/no); boats generally get hauled-out of the water for annual maintenance which includes a professional high-pressure cleaning, and the application of new antifouling paint prior to the start of the tourist season. Recent laws enacted in most marinas now ensure that the biofouling waste removed from the hulls is disposed of separately (i.e., and not directly into the marina waters themselves), but we have personally observed that these regulations are not always respected. Although the establishment success of propagules released after cleaning operations has yet to be assessed (Verling et al., 2005), here we assume that in some cases, if disposal regulations are not respected, presence of a shipyard can increase propagule pressure and, ultimately, NIS richness in a marina.

Each marina's **distance to the Suez Canal** was calculated considering the shortest navigational route from the northern entrance of the canal in km. As the Suez Canal is an artificially created waterway, Indo-Pacific species traveling through here are considered NIS, either by natural establishment or through human-mediation. Over half of the multicellular NIS thriving in the Mediterranean more than likely has entered via this canal, which should be considered the major vector for marine introductions in the Mediterranean (Galil et al., 2015), hence we assume that those marinas in closer proximity to the Suez Canal may host more NIS.

3.3.5 Outline of statistical analyses applied to data

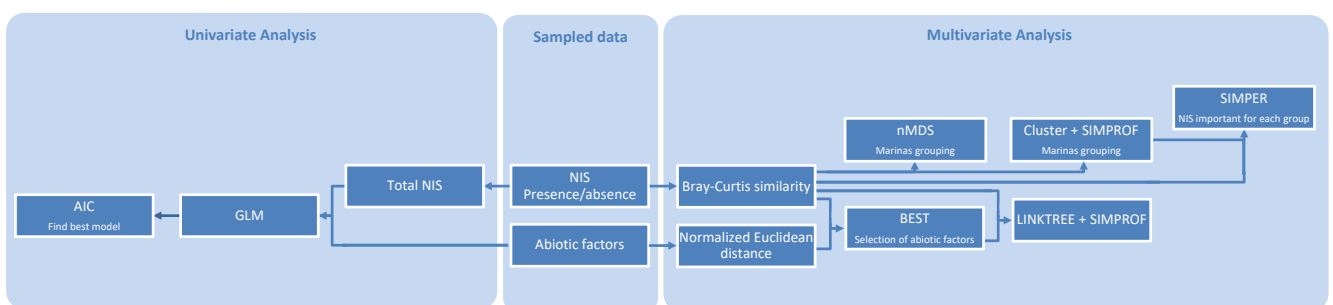


Figure 3.2 Flow chart of statistical analyses applied to both the univariate and multivariate analyses testing number of NIS against abiotic factors.

Univariate Analysis

The total NIS richness per marina, tested against the series of abiotic factors (Fig. 3.2), were used to test the null hypothesis that abiotic factors do not influence NIS richness in Mediterranean marinas. The nature of the relationships between total NIS richness per marina and the above mentioned abiotic factors considered (as explanatory variables) were visualized using scatterplots (for continuous variables) and boxplots (for categorical variables). Total NIS richness found in each marina was modelled as a function of the abiotic factors by means of generalized linear models (Fig. 3.2, GLMs) (McCullagh et al., 1983) in order to identify which of these influence an increase in NIS. GLMs are an extension of linear models allowing the incorporation of non-normal distributions of the response variable and transformations of the dependent variables to linearity (McCullagh & Nelder, 1983). Using the total number of NIS as a response variable, for this type of count data with non-negative values, a GLM with log link function and Poisson error distribution is recommended (McCullagh & Nelder, 1983). Data exploration was applied following Zuur et al. (2010). The presence of outliers were investigated using Cleveland dotplots, meanwhile collinearity was assessed using multi-panel scatterplots, Pearson correlation coefficients and variance inflation factors (VIF). Finally, the initial model applied contained all abiotic factors except for 'Total pier length' because this factor was found to be highly collinear with the 'Number of berths' factor. Therefore, the general model used was:

$$\begin{aligned}\log(\mu_i) &= \log(E(U_i)) \\ &= \beta_1 + \beta_2 \times \text{Salinity}_i + \beta_3 \times \text{Temperature}_i + \beta_4 \times \text{PrimaryProductivity}_i + \beta_5 \times \text{Area}_i + \beta_6 \times \text{NumberBerths}_i + \\ &\beta_7 \times \text{OpeningLength}_i + \beta_8 \times \text{DistanceSuezCanal}_i + \beta_9 \times \text{ProximityFreshwater}_i + \beta_{10} \times \text{PresenceShipyard}_i + \\ &\beta_{11} \times \text{VicinityAquaculture}_i + \beta_{12} \times \text{VicinityCommercialHarbours}_i + \beta_{13} \times \text{PresencePontoons}_i + \\ &\beta_{14} \times \text{BiogeographicSector}_i + \beta_{15} \times \text{ClimateType}_i\end{aligned}$$

Where μ_i is the expected number of NIS and βt is the parameter set relating the dependent variables to the response, using a log link function and a Poisson distribution for the response variable. Backward selection using the Akaike Information Criterion (AIC; Akaike, 1974) was used to find the optimal model. The AIC determines between adding or excluding each variable, creating a balance between the variability explained by each factor and the degrees of freedom introduced in the model (Akaike, 1974). Model validation was applied on the optimal model to verify the underlying assumptions (Zuur et al., 2013). Specifically, we plotted Pearson residuals versus fitted values, and also against each covariate in the model and those covariates not in the

model to investigate patterns. Additionally we assessed whether there was overdispersion (or underdispersion), and also used the Cook's distance value to check the presence of outliers in the model. All these analyses were conducted using R statistical computing software (R Core Team, 2016).

Multivariate Analysis

The multivariate structure of the entire assemblage of NIS found per marina and the series of abiotic factors were used (Fig. 3.2) to test the null hypothesis that abiotic factors do not influence similarities in NIS distribution patterns amongst Mediterranean marinas. Multivariate techniques suited for ecological data were used allowing for the investigation of each individual NIS' contribution to each marinas internal found assemblage, along with the total number of NIS (NIS richness). Thus, non-parametric approaches were selected by combining non-metric multidimensional scaling (nMDS) with hierarchical cluster (Clarke, 1993; Clarke & Warwick, 2001) to assess differences of the composition of the NIS assemblages within each marina.

Specifically, to incorporate the most influential abiotic factors in the multivariate structure of NIS, first the BEST, then LINKTREE routines were performed from the PRIMER v6 software (Clarke & Gorley 2006). A first assessment of the relationships between the multivariate structure of NIS and abiotic factors were provided by BEST, which were used to select the subset of abiotic factors that best correlated with the multivariate assemblage patterns of NIS. In order to carry out a stepwise search of each possible combination of abiotic factors, the BVSTEP procedure was run using Spearman's coefficient as a rank correlation method (Kendall, 1970). Subsequently, a global BEST match permutation test (using 999 permutations) was ran to test the degree of association between the multivariate structure of NIS and the subset of abiotic factors selected. The associated Pearson's correlation coefficient of pairs of abiotic factors was examined to identify strongly correlated factors, as only those with strong correlations would be included in the subsequent LINKTREE analysis. All subsets of variables strongly collinear (with values > 0.95 or < -0.95) were reduced to a single representative in the BEST run (Clarke & Warwick, 2001), thus removing one factor from the strongly collinear pair considered to have a lesser influence than the other. Next, these abiotic factors selected by BEST (i.e., which strongly correlated) were included as the independent (explanatory) variables in the subsequent LINKTREE procedure (De'ath, 2002). LINKTREE is a non-parametric multivariate form of classification and regression technique that works by constructing a hierarchical tree through successive dichotomies of sets of observations (marinas) using divisive clustering. Each division is characterized by the most influential variables, which can be a single or combination of variables (abiotic factors), and the procedure is repeated until all sites are sorted into groups sharing the same underlying factors and ranges of values that seem responsible for distinguishing each different internal NIS assemblage grouping. The

LINKTREE procedure is capable of distinguishing that an abiotic factor is important for the internal assemblage structuring of one group of samples, but not for another, even for groups with similar ranges of values.

For the overall multivariate testing technique, similarities between NIS assemblages were calculated using the Bray–Curtis similarity index, based on presence/absence data of NIS (Bray & Curtis 1957). Those abiotic factors with “Yes” or “No” categories (“Proximity to freshwater source”, “Presence of shipyard in the marina”, “Proximity to aquaculture facilities”, “Proximity to commercial harbours” and “Presence of floating pontoons”), were coded as ‘1’ when assigned to “Yes” and ‘0’ to when assigned to “No”. Accordingly, all qualitative abiotic factors with several categories (“Biogeographic sectors” and “Climate type”) were also coded as ‘1’ when assigned to marinas matching the corresponding category of the abiotic factor, and ‘0’ for those that did not. The abiotic factors similarity matrix used in the prior analysis was calculated between marinas using “Normalized Euclidean distance” (Clarke & Warwick, 2001).

The “similarity profile” permutation test (SIMPROF) (Clarke & Gorley 2006) tests for significant evidence of multivariate structure among samples, which have no pre-defined grouping, was combined with hierarchical cluster and LINKTREE to validate the identification and interpretation of clusters. The 5% significance level was conventionally applied, and 1000 permutations were run to calculate the mean similarity profile, with 999 permutations to generate the null distribution of the departure statistic, π . Subsequently, the contribution of each NIS to internal-group similarity was assessed for the groups identified by SIMPROF in the cluster using the SIMPER (SIMilarity PERcentages) procedure (Clarke & Warwick, 2001), identifying those NIS that are more important for each group of marinas.

3.4 Results

The full data-set containing the recorded NIS for each marina are presented as ‘Supplementary Data Table 3.1’. The abiotic factors measured or assessed for each marina are presented as ‘Supplementary Data Table 3.2’. The total number of NIS found in each marina are presented both by number (Table 3.2) and by taxa in Figure 3.3, which ranged from 2 in Villfranche-sur-Mer (France) and Alassio (Italy) to 27 NIS in Heraklion (Greece), followed by Palermo (Italy; 20 NIS), Famagusta and Karpaz Gate (Cyprus; 18 and 17 NIS, respectively), Port Camargue (France; 17 NIS), and Rhodes (Greece; 16 NIS). The SIMPER results showing similar taxa between marina groupings are shown in Appendix Table 3.3.

3.4.1 Qualitative analysis

Table 3.2 Number of NIS per marina, marina numbers from Table 2.1.

Locality	# NIS	Locality	# NIS
1. Alicante	10	26. Sorrento	8
2. Barcelona	11	27. Villa Igiea, Palermo	20
3. Cap d'Agde	8	28. La Cala, Palermo	16
4. La Grand-Motte	7	29. Riposto	13
5. Port Camargue	17	30. Siracusa	16
6. Saint Tropez	4	31. Marzamemi	11
7. Cogolin	6	32. Ragusa	14
8. Saint Maxime	3	33. Licata	11
9. Cannes	5	34. Msida	14
10. Antibes	5	35. Valletta	13
11. Villefranche	2	36. Chioggia	9
12. Alassio	2	37. Venezia	8
13. Genoa	5	38. Treporti	7
14. S. Margherita	7	39. Heraklion	27
15. La Spezia	7	40. Agios Nikolaos	12
16. Lerici	10	41. Rhodes	16
17. Viareggio	10	42. Ataköy, Istanbul	4
18. Piombino	3	43. Kalamış, Istanbul	4
19. Scarlino	7	44. Bodrum	12
20. Punta Ala	2	45. Datça	9
21. Porto Torres	10	46. Marmaris	6
22. Castelsardo	8	47. Fethiye	10
23. Porto Rotondo	3	48. Finike	14
24. Ostia, Rome	9	49. Karpaz	16
25. Ischia	5	50. Famagusta	17

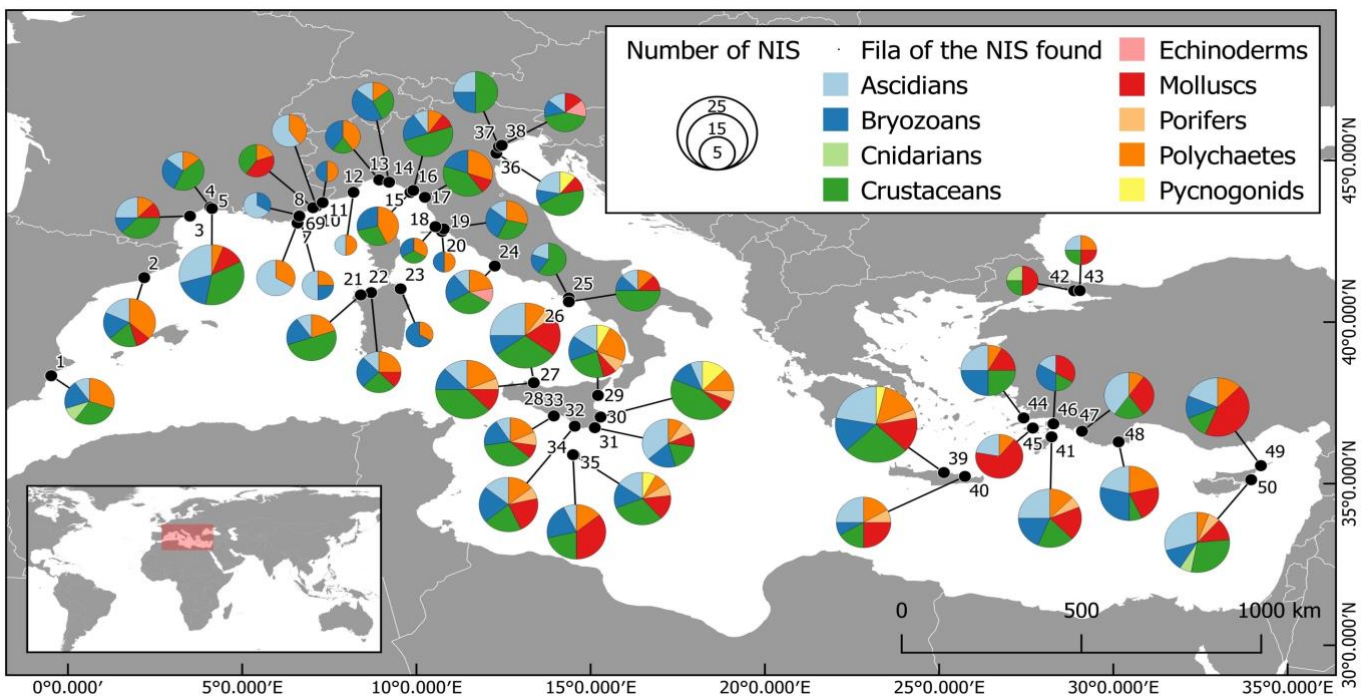


Figure 3.3 NIS records shown proportionately for each marina, by major taxon.

Generally, there was higher total NIS richness in the Eastern and Central Mediterranean than the Western Mediterranean. Overall, crustaceans, ascidians, bryozoans and polychaetes were the dominant taxa of NIS found in most marinas (Fig. 3.3). Additionally, there was a slightly higher number of NIS mollusc records in the Eastern Mediterranean, than other regions, aside from a couple marinas near aquaculture localities in France. Sponges were mainly restricted to the Central and Eastern Mediterranean.

Table 3.3 The most widespread NIS (Percentage of marinas found in).

Species	%	Species	%
<i>Styela plicata</i>	74	<i>Branchiomma bairdi</i>	30
<i>Hydroides elegans</i>	66	<i>Paraleucilla magna</i>	24
<i>Amathia verticillata</i>	62	<i>Asciella aspersa</i>	22
<i>Caprella scaura</i>	58	<i>Arcuatula senhousia</i>	22
<i>Celleporaria brunnea</i>	52	<i>Watersipora arcuata</i>	18
<i>Paranthura japonica</i>	52	<i>Ciona robusta</i>	16
<i>Brachidontes pharaonis</i>	34	<i>Tricellaria inopinata</i>	16
<i>Hydroides dirampha</i>	32	<i>Stenothoe georgiana</i>	16
<i>Mesanthura cf. romulea</i>	30	<i>Dendostrea cf. folium</i>	16
<i>Paracerceis sculpta</i>	30	<i>Magallana gigas</i>	16

The most widespread NIS found in this study (Table 3.3) were *Styela plicata* (Lesueur, 1823), *Hydroides elegans* (Haswell, 1883), *Amathia verticillata* (delle Chiaje, 1822), *Caprella scaura* Templeton, 1836, *Celleporaria brunnea* (Hincks, 1884), and *Paranthura japonica* Richardson, 1909.

3.4.2 Univariate analyses on total NIS richness in marinas

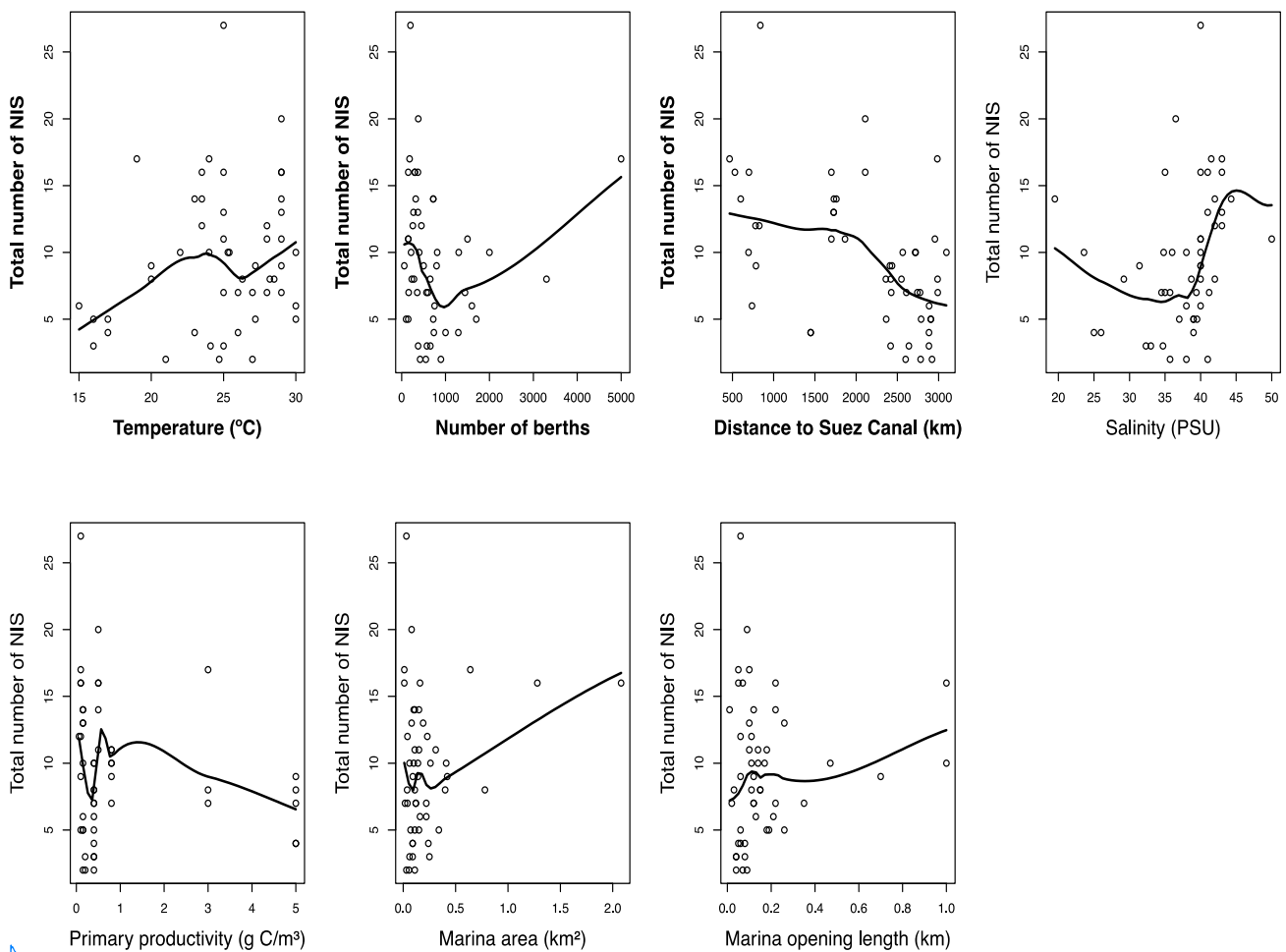


Figure 3.4 Scatter plots showing the relationship between the total NIS richness and each abiotic quantitative factor. To aid visual interpretation, a LOESS smoothing curve was added. Abiotic factors are ordered according to the strength of their relationship, with significant factors presented first in bold text.

From observing the relationships between the total NIS richness and the quantitative abiotic factors (Figure 3.4), the significant factors (shown in bold) included higher sea-surface water temperatures, which strongly influenced total NIS richness between 15-23°C and again from 26-30°C, although the pattern is non-linear, as it negatively influences total NIS richness between 24-26°C. The other significant factors were number of berths

(likely swayed by Port Camargue, and its 5000 berths, the largest marina in the Mediterranean), and proximity to the Suez Canal. Other factors showing no significant and weaker trends included salinities above 38 PSU, average primary productivity below 1 g C m⁻³, larger marina area and a larger marina opening length.

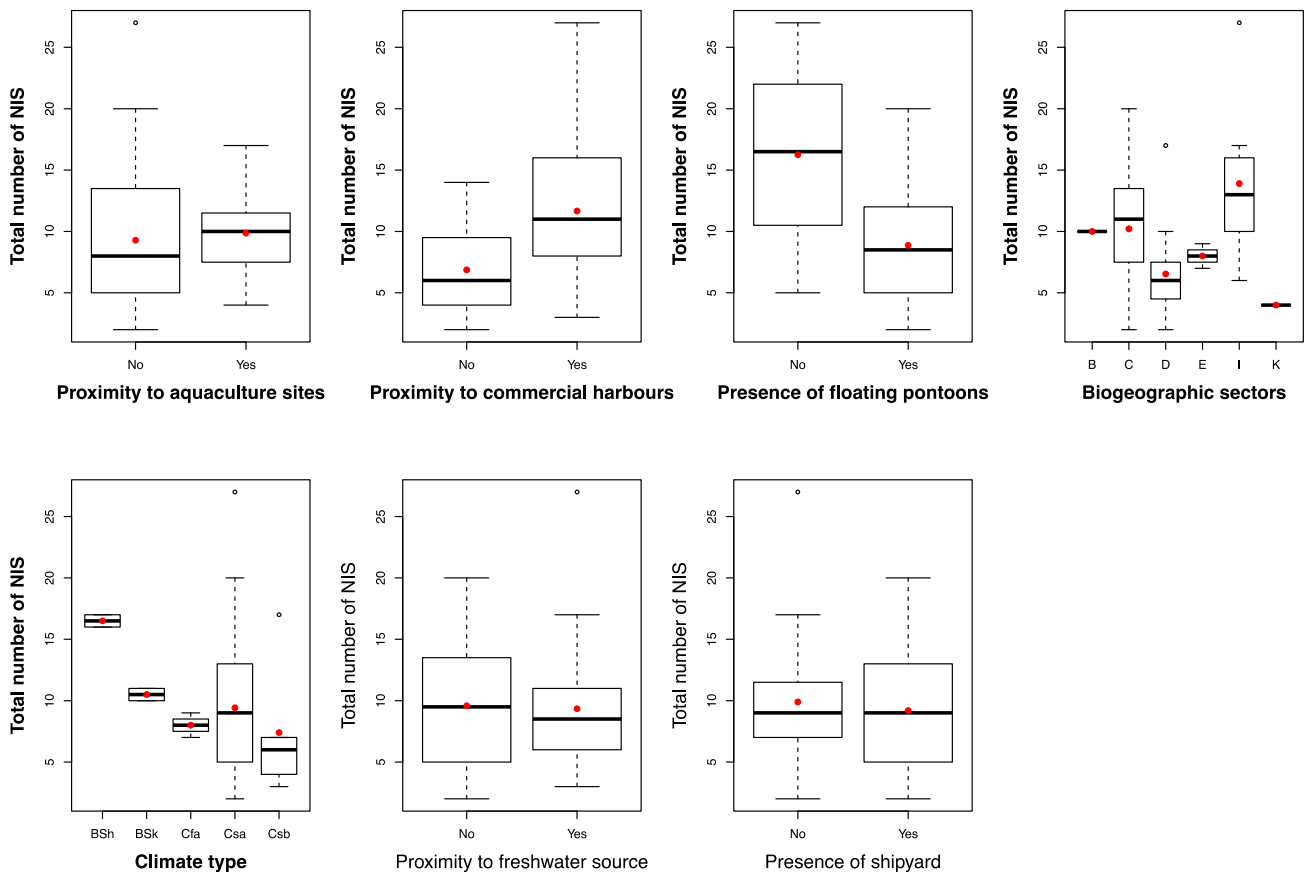


Figure 3.5 Boxplots representing the relationships between total NIS richness in marinas and each categorical qualitative abiotic factor. Red dots represent the mean, the black horizontal line in plots denotes the median of the data, and the black dots represent outliers.

From observing the trends between NIS richness and the categorical qualitative abiotic factors (Fig. 3.5), the most significant factors (shown in bold from Fig. 3.5) affecting higher total NIS richness in marinas were proximity to aquaculture sites, proximity to commercial harbours, absence of floating pontoons, pertaining to biogeographic sector I (representing the marinas in Turkey and Cyprus), and pertaining to climate type BSh (hot semi-arid climate for Cyprus); Whereas proximity to freshwater source and presence of shipyard sites showed no significant trend. Furthermore, pertaining to biogeographic sector K (Marmara Sea and the Bosphorus Strait pertaining to Istanbul), and to a lesser extent sectors D (southern France and western Italy) show a relationship towards lower NIS richness.

Regarding the GLM of the total NIS richness as a function of all the selected abiotic factors, the analyses of Pearson residuals confirms the goodness-of-fit of the models on the factors, so no additional transformation is necessary for their inclusion. The optimal model selected in the backward AIC procedure resulted in a model that considered the following eight abiotic factors as explanatory variables: temperature, number of berths, distance to Suez Canal, proximity to aquaculture sites, proximity to commercial harbours, presence of floating pontoons, biogeographic sectors and climate type. This model explains 72.1% of the variance observed for the total number of NIS. From analysing the coefficients of each term in the model (Table 3.4), total NIS richness seems to have an exponentially positive trend in relation to temperature and number of berths, and is also positively related with marinas in closer proximity to the Suez Canal. The marinas which are closer to commercial harbours have (on average) a higher number of NIS. Contrarily, if marinas have floating pontoons present or are further from aquaculture sites they have a lower number of NIS. Ordering the biogeographic sectors from high to low values in the total NIS richness resulted in Sectors I (the Ionian Sea and South Aegean), C (Balearic Sea to Tyrrhenian Sea), B (Algeria and Southern Spain), E (Northern Adriatic), D (Gulf of Lyon and Ligurian Sea) and K (Marmara Sea and Bosphorus Strait). Lastly, the number of NIS is higher in marinas with a climate type BSh (hot, semi-arid climate) and lower if they have BSk (arid, cold), or Cfa (temperate, without dry season).

Table 3.4 Coefficients from the Generalized Linear Model fitted to total number of NIS, using a log link function and a Poisson distribution. In categorical explanatory variables, estimates express the difference between each level of factors and the first level (which are considered in the intercept).

Coefficient	Estimate	Standard Error
Intercept*	303.155	0.82703
Temperature	0.0279	0.01788
Number of berths	0.00026	0.00007
Distance from the Suez Canal	-0.00093	0.00023
Proximity to aquaculture	-0.23349	0.14285
Proximity to commercial harbours	0.44002	0.11872
Presence of pontoons	-0.28360	0.17784
Biogeographic sector C	0.10509	0.43995
Biogeographic sector D	0.26092	0.48941
Biogeographic sector E	0.52156	0.57285
Biogeographic sector I	-0.81707	0.56081
Biogeographic sector K	-118.805	0.59735
Climate type BSk	112.741	0.45492
Climate type Csa	0.31884	0.21890
Climate type Csb	0.50441	0.32647

*Represents values regarding a marina with no vicinity to aquaculture sites and commercial harbours, and with no presence of pontoons, set in biogeographic sector B and climate type BSh.

3.4.3 Multivariate analyses based on NIS assemblage structure

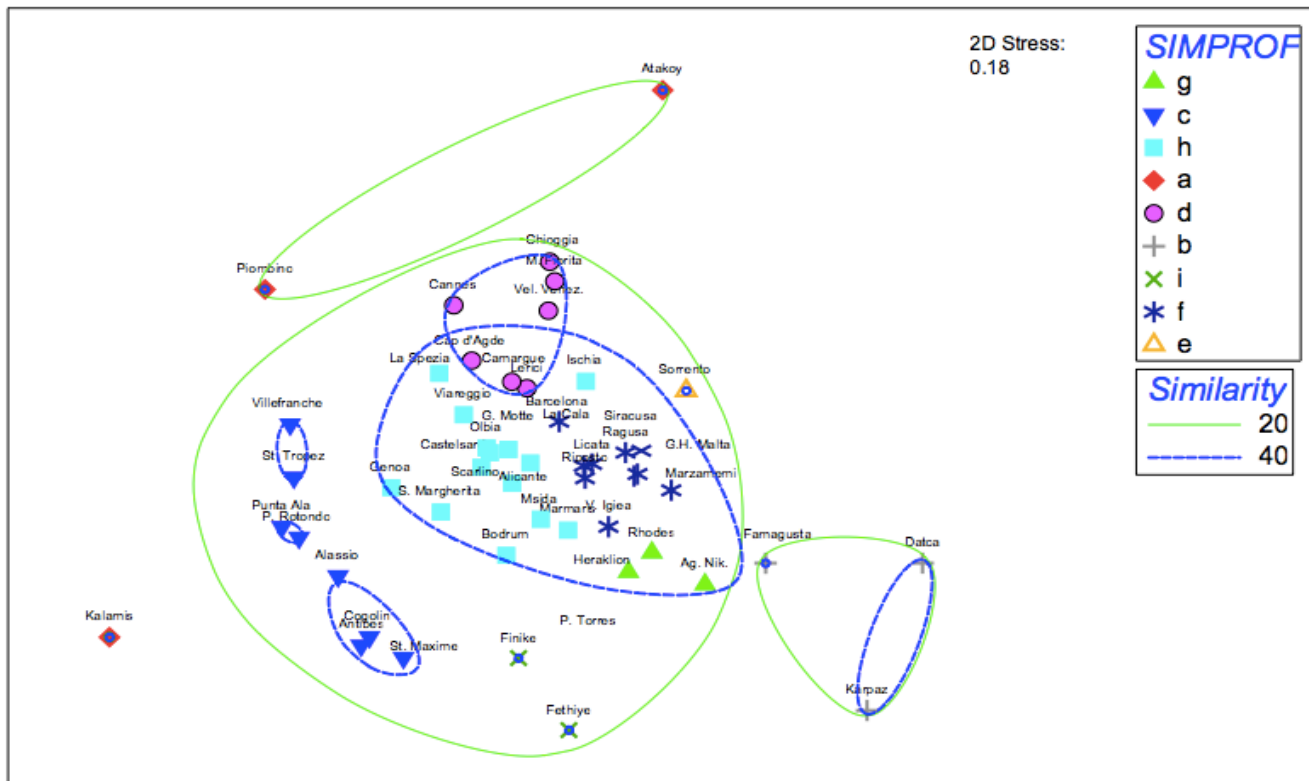


Figure 3.6 Two-dimensional nMDS plot of NIS similarities for marinas. SIMPROF test results were superimposed, identified with different symbols for the 9 groups (a-i) of marinas with significantly ($P < 0.05$) different NIS multivariate structure. Cluster results were also superimposed, groupings shown for similarity levels of 20% and 40%.

In Fig. 3.6, an unexpected combination of marinas sharing high similarities between their NIS assemblage compositions (over 50 %) and without significant differences between their NIS assemblage compositions (SIMPROF $P > 0.05$), despite considerable geographical distances between them; Group 'f' is composed of marinas from Sicily (Marina Villa Igiea, Siracusa, Marzamemi, Marina di Ragusa, Porto La Cala, Licata Cala del Sole, Riposto Porto dell'Etna), Spain (Port Vell, Barcelona) and Malta (Grand Harbour), with *S. plicata* and *A. verticillata* each contributing 11% to total similarities; *P. japonica* contributing 9%; *Branchiomma bairdi* (McIntosh, 1985) 8.7%; *C. scaura*, *Paracerceis sculpta* (Holmes, 1984), *Brachidontes pharaonis* (Fischer, 1870), *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, each contributing 8.5%, *C. brunnea* 6.5%, *H. elegans* 4.5%, *Hydroides dirampha* Mörch, 1863, 3.2% and *Microcosmus squamiger* Michaelsen, 1927, with 3.2%. Similarly, group 'g' is composed of the three Greek marinas (Agios Nikolaos, Heraklion and Rhodes Mandraki), which also showed high similarities (>50%) in their NIS communities without significant differences between them (SIMPROF $P > 0.05$), but in this case, Rhodes is quite distant geographically from the other two marinas in Crete (over 450 km), but less than 50 km from Marmaris in Turkey, which it showed to have no species in common with; for these Greek marinas, *S. plicata*, *Symplegma brakenhielmi* Michaelsen, 1904, *Celleporaria*

vermiformis (Waters, 1909), *B. pharaonis*, *P. magna*, *B. bairdi* and *H. elegans* each contributing 10% to similarities.

Alternatively, there are three groups of marinas that, while displaying lower NIS similarities (less than 35%) within each group, had significant different NIS assemblages from the other marinas (Appendix Table 3.4); These groups are 'a' (Kalamış, Ataköy and Piombino) with *Amphibalanus eburneus* (Gould, 1841), and *Ficopomatus enigmaticus* (Fauvel, 1923) each contributing 50%; 'b' (Datça and Karpaz Gate and Famagusta, Turkey and Cyprus, respectively) which had *B. pharaonis* contributing 20.3%, *Microcosmus exasperatus* Heller, 1878, *Cerithium scabridum* Philippi, 1848, *Dendostrea folium* Linnaeus, 1758, *Pinctada imbricata* (Gould, 1850) and *Septifer cumingii* Récluz, 1848, each contributing 7.5%, *Clavelina oblonga* Herdman, 1880 and *B. bairdi* 7.2% and *Herdmania momus* (Savigny, 1816), *Phallusia nigra* Savigny, 1816, *C. vermiformis* and *Ampithoe bizseli* Özeydinli & Coleman, 2012 each contributing 5.7% to species similarities; and 'i' (Fethiye Ece and Finike, both in Turkey) with *Diplosoma listerianum* (Milne Edwards, 1841), *S. plicata*, *S. brakenhielmi* and *H. dirampha* each contributing 25% to total similarities. The NIS assemblage found in Sorrento has a high similarity (40%) with other marinas, but was significantly different (SIMPROF $P < 0.05$) from the other marinas in group e.

The remaining groups identified by the SIMPROF test (*c*, *d*, *h*), shared high similarities in their NIS assemblages in their groupings (47-56%), and were significantly different from the other groups (ranging from 35-47%), and are composed of a mix of marinas from several countries (Appendix Table 3.4). The NIS assemblage composition of marinas from France and Italy are grouped in 'c' and 'd', meanwhile marinas in group 'h' span across the Mediterranean from Spain, France, Italy, Malta and Turkey. Group 'c' had NIS influences from *H. elegans* 57%, *S. plicata* 13.6%, *Ascidella aspersa* (Müller, 1776) 11.6%, and *C. brunnea* contributing 9.7%. Group 'd' has the following NIS contributing similarities: *C. scaura* and *P. japonica* (20.7%), *S. plicata* and *Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985 (13.5%), *Ianiropsis serricaudis* Gurjanova, 1936 (9.1%), *Magallana gigas* Thunberg, 1793 (6%), *H. elegans* (5.5%) and *Arcuatula senhousia* Benson, 1842 (5.3%). Group 'h' had *C. brunnea* contributing 19%, *A. verticillata* 15.9%, *H. elegans* 15.2%, *S. plicata* 13%, *C. scaura* 12.8%, *P. japonica* 8.9% and *H. dirampha* 6.5% to total similarities.

3.4.4 Analysis of abiotic factors

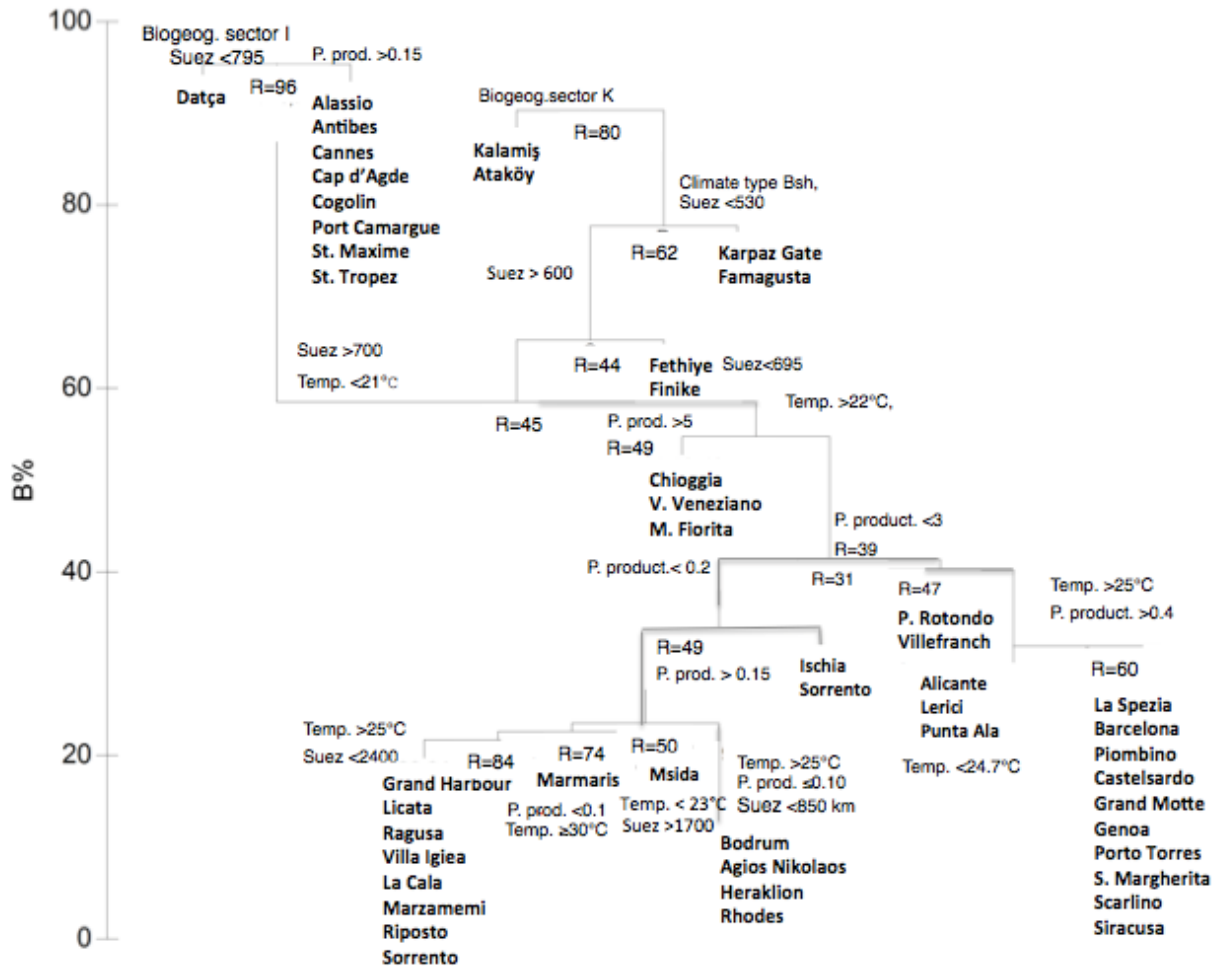


Figure 3.7 LINKTREE analysis results showing factors most responsible for separating marinas into groupings, according to their NIS assemblage compositions with the strongest differences between marina groups having a higher B% (absolute measure of group differences) from contributing factors (or pairs of factors if collinear). The plot displays only those divisions for which the SIMPROF test was significant ($p < 0.05$). For each split the ANOSIM test statistic (R: Rank similarity index) for each marina grouping is shown.

After each possible combination of abiotic factor pairs was tested, all were found to have low correlation (Pearson's correlation coefficient < 0.95) and therefore all were included in the successive BEST analysis to screen which combination(s) of factors better explain the multivariate patterns of NIS communities. The BEST results revealed that the following combinations of these six factors: 'Sea-surface temperature', 'Average primary productivity', 'pertain or not to Biogeographic region K', 'Proximity to commercial harbours', 'Proximity to Suez Canal', and 'pertain or not to Climate type BSh (hot semi-arid climate)' were the factors that positively correlated with the NIS assemblage structure ($Rho = 0.597$, $p < 0.001$), hence these factors were included for

testing in the subsequent LINKTREE analysis (Fig. 3.7). The factors not selected by the BEST screening and thus excluded from further analyses were the structural features of marinas (number of berths, total pier length, total area, presence of pontoons, presence of shipyard, marina opening distance, and exposure type), as well as proximity to freshwater source and to aquaculture site.

The results of the LINKTREE analysis (Fig. 3.7) show which of the above six main factors were responsible for grouping marinas based on their internal NIS assemblages (based on the Bray-Curtis similarity index). The groups are ordered by highest differences from the others groups at the top, and the results first grouped Datça, Turkey as the most dissimilar from the other marinas, for its biogeographic sector and proximity to the Suez Canal, along with its mean very low primary productivity; and with the same dissimilarity of 90%, all the French marinas along with Alassio, Italy (Italian Riviera adjacent to the French marinas) were grouped together according to their distance from the Suez, mean surface temperature $< 21^{\circ}\text{C}$ and primary productivity greater than 0.15 g C m^{-3} . The next grouping split the two marinas in Istanbul together (Kalamış and Ataköy by a dissimilarity value of 90%), as their NIS assemblages differed from the other groups due to their distinct biogeographic region, which distinctively differed from the other marinas in salinity and geography, as they are situated on the Bosphorus Strait. Next, the Famagusta and Karpaz Gate in Cyprus were grouped together with a 78% dissimilarity from the other groups, best explained by their proximity to the Suez Canal (465 and 530 km, respectively) and distinct biogeographic region being hot arid and dry. Next, Finike and Fethiye in Turkey were separated from the rest (with a dissimilarity of 65%) also due to their relatively short distance from the Suez Canal. The next group differed from the other groups by 55% which included the marinas from the Venice Lagoon in the Adriatic Sea with a much higher average primary production of over 5 g C m^{-3} . The next grouping had a dissimilarity of 41% and included Porto Rotondo and Villefranche-sur-Mer, with average primary productivity $< 3 \text{ g C m}^{-3}$. The subsequent grouping contained Ischia and Sorrento (which are in very close proximity) with a much lower primary productivity of $< 3 \text{ g C m}^{-3}$. Successively, there was an interesting assortment of marinas from the Western Mediterranean from Barcelona extending to the Central Mediterranean to Siracusa, Sicily with a dissimilarity of 28% due to slightly higher primary productivities of 0.4 g C m^{-3} . Lastly, a dissimilarity of 32% grouped the marinas of Sicily and Malta together due to water temperatures $> 24^{\circ}\text{C}$ and greater distances from the Suez Canal of $> 2400 \text{ km}$.

3.5 Discussion

We conducted a large-scale Mediterranean basin-wide study of 50 recreational marinas to test which abiotic factors influence the total number of NIS in marinas using a univariate analysis. Next, multivariate testing was performed to determine which factors influence similarities and differences between NIS patterns between marinas or groups of marinas. Subsequently, a hierarchical clustering was performed on those factors which best explain the groupings. A thorough analysis of the major causes underlying total NIS and their distributions need first be completed before effective management can be designed (Hopkins & Forrest 2008). These results certainly point to the magnitude of the biofouling vector in the spread of NIS in the Mediterranean, as many of these marinas are isolated habitats, yet many were shown here to be connected to other marinas via their fouling communities despite great distances, where boat travel is the only sensible explanation for the spreading of many species (although larval dispersal may also play a role for some taxa).

Contrary to the null hypothesis that there were no abiotic relationships contributing to higher NIS richness or similarities between NIS assemblages, several abiotic factors were shown to significantly affect both these analyses, so perhaps on a local scale, total NIS richness is influenced by certain factors which disappear when internal NIS assemblages are compared across the entire Mediterranean region. However, temperature and proximity to the Suez Canal were important factors for both tests. Average sea surface water temperature on sampling date is important for explaining which minimum temperatures must be reached before influencing higher total NIS, which is explained below. Proximity to the Suez Canal was prominent especially in the Eastern Mediterranean, for Erythraean species of Indo-Pacific origin, some of which then have spread westwards (Occhipinti-Ambrogi & Galil 2010; Tzomos et al. 2010). Many of these taxa are new to the region, especially those illustrated in the NIS compositions of the Cretan and Cypriot marinas; the Suez Canal vector of course is specific to the Mediterranean basin and urgently warrants some sort of specific targeted management (Galil et al., 2017), as its risk level for facilitating further invasions is assumed to be very high.

Our results indicate that at the Mediterranean scale, the underlying factors influencing higher NIS richness in marinas were due to water temperatures greater than 25°C, a higher number of berths (strongly influenced by Port Camargue), proximity to the Suez Canal, proximity to commercial harbours, absence of pontoons, climate type hot and dry (representing Cyprus) and biogeographic sector I (representing southern Turkey and Cyprus). Proximity to aquaculture sites was not found here correlate to be significant richness, even though a few NIS from the prominent aquaculture region in France (the Thau Lagoon surroundings) more than likely were arrived to nearby marinas of Port Camargue and Grand-Motte via this vector (i.e., *Aoroides longimerus* Ren & Zheng, 1996, *Balanus trigonus* Darwin, 1854, *C. brunnea*, *P. japonica*). However, proximity to commercial harbours did show a strong correlation, proving the importance of a major global vector for NIS transport

(shipping as a pathway and ballast water or biofouling as vectors to transport species). Many species were shown to have been transported via the biofouling vector having been found on boat-hulls, some examples of such include *A. verticillata*, *C. scaura*, *Dyspanopeus sayi* (Smith, 1869), *H. dirampha*, *H. elegans*, *P. magna* and *S. plicata* (see Ulman et al., 2017). For a locality to have high total numbers of NIS, it is thought to require many introduction events supplying new propagules. Unexpectedly, here, absence of floating pontoons correlated with higher total numbers of NIS, a counterintuitive result, also considering that most of our sampled marinas contained floating pontoons, in contrast with previous researches carried out in a non-Mediterranean context (Dafforn et al., 2009; Nall et al. 2015). We also found that a higher number of berths in marinas correlates with higher NIS richness; similarly to Nall et al. (2015) and Ros et al. (2013) studies, however, this factor is only going to be significant if the marina is popular among non-resident vessels, so that new propagules are brought in. A study carried out testing several of the same marina factors as this study (Foster et al., 2016) for NIS presences in UK marinas, found freshwater input, marina opening width and total seawall length to be significant factors; however, none of those factors were found significant here, or it may be that the role of climate and proximity to the Suez were overwhelming and masked these weaker factors. This indicates that different regions likely have different major contributing factors, thus management may need to be specially tailored for different subregions.

Factors found here to influence NIS assemblage similarities between marinas identified that environmental matching played the dominant role, specifically: temperature, primary productivity, biogeographic region and climate type, and additionally proximity to the Suez Canal also exerting a strong influence. From the nMDS plot, there is an unusual grouping of highly similar NIS assemblages found in marinas (40%) spanning from Spain to Sicily, which is explained in the LINKTREE by similar temperatures on sampling date above 25°C and similar primary productivities. We did expect to find a relationship between Saint-Tropez (France), Porto Rotondo (Italy), Palermo (Sicily) and Grand Harbour (Malta) in their NIS assemblages, since these are sister marinas sharing a popular sailing regatta each year (<http://tbsrace.com/>), but these results did not ensue, probably owing to the fact that racing vessels are kept exceptionally free of biofouling to enhance speed. We were also expecting to find high similarities in NIS assemblages between Finike (Turkey) and Agios Nikolaos (Crete, Greece) marinas as dozens of live -aboard vessels collectively relocated from Finike, Turkey to winter in Greece in late 2014 due to political instability in Turkey (personal communications with many boaters to the first author), but no relationship was found. However, some NIS present in Turkey, i.e. *C. brunnea* and *Paradella diana*e (Menzies, 1962), were found on boat-hulls which had just travelled to Greece, but were not yet found present in Turkey (Ulman et al., 2017), and perhaps needed more time to establish. A study from both US coasts by Lord et al. (2015) also found minimum temperature to be a key factor correlating to similar NIS assemblages between sites, with cargo shipping likely explaining regional distributions, and suggested

recreational boating and larval dispersal (not tested there) were the most likely factors shaping local distributions. Other marina factors (i.e., number of berths, marina size, presence of pontoons) were not shown here to influence similar NIS assemblages, contrarily to our results from the univariate analysis.

There were a few outlying marinas in relation to their distinctive NIS assemblages owing to a combination of select abiotic factors. The two Istanbul marinas are in a very dissimilar sector of the Mediterranean from the others, with much lower salinities of about 25 PPT. Ataköy, Istanbul had some well-known local NIS such as the sea snail *Rapana venosa* (Valenciennes, 1846); whereas Kalamış (Istanbul) and Marina Terre Rosse in Piombino, Tuscany, Italy (another marina outlier), were both dominated by the euryhaline serpulid *F. enigmaticus*, likely influenced by these lower salinity levels; *F. enigmaticus* is a well-known estuarine and transitional water ecosystem engineer in creating additional hard substrate, thus accelerating the success for other NIS and has been linked to triggering 'invasional meltdown' of local communities (Heiman & Micheli, 2010; Simberloff & Von Holle, 1999). Marina Terre Rosse is a unique marina located a little upstream from the sea inside a saltwater canal with limited water exchange, likely resulting in anoxic conditions. Other outliers include Sorrento (Italy), which was the only completely open marina, thus not confining larval settlement, and Finike with its salinity of 20 PSU, about half the Mediterranean average, due to a river positioned less than a kilometre from its entrance. Fethiye (Turkey) is another outlier as it has many sizeable fish farms in its bay, which may have directly contributed to its unique NIS assemblage by providing exceptionally high nutrient enrichment and/or by provisioning associated species. The marina in Rhodes (Greece) showed much affinity to the other Greek marinas despite being only 25 n.m. in distance from Netsel Marmaris Marina (Turkey), which it showed no affinity with, suggesting here that popular travel routes as opposed to distance likely influence similar communities, again pointing strength to the boating vector.

This study presents that 27 NIS coexist in one single marina, which is the highest number of macrozoobenthic NIS ever recorded in one artificial locality obtained from the Old Venetian Harbour, Crete, which is in rather close proximity to the Suez Canal comparatively to the other marinas (< 850 km) and is also located next to a major shipping port, both vectors shown here to influence higher total NIS richness. This Cretan marina and the other marinas with high NIS richness demonstrate that recreational marinas are certainly hot-spots for NIS in the Mediterranean, and along with both incoming and outgoing boating traffic, these marinas with high species richness can be considered important hubs for the transfer of NIS to other localities. Outside the Mediterranean, marinas with highest NIS richness were found in the NE United States with 18 species (Pederson et al. 2005), 18 from west Scotland (Nall et al., 2015), 16 from Madeira, Portugal (however, these were found cumulatively over a 6 year period; Canning-Clode et al., 2013); and 13 from England (Bishop et al., 2015; Foster et al., 2016), although these totals are not directly comparable, due to the variability in both sampling strategies and targeted taxa. Future sampling should consider standardizing either the targeted

species for pre-defined lists or ensure capabilities of local experts for unknown taxa so data can be comparable across borders.

Many of these NIS are widespread across the Mediterranean (Table 3.3), some of which have been known for decades, i.e., *A. verticillata* and *H. elegans*, and some of which have only recently appeared, i.e., *Watersipora arcuata* Banta, 1969 and *Stenothoe georgiana* Bynum & Fox, 1977 (Ferrario et al., 2015; Fernandez & Sanchez-Jerez, 2017). However, we stress that attention should also be paid to rare NIS only found here just in a few localities or less, such as the ascidians *Phallusia nigra* Savigny, 1816 and *Polyandrocarpa zorritensis* (Van Name, 1931); the molluscs *Chama asperella* Lamarck, 1819 and *Malleus regula* (Forsskål in Niebuhr, 1775); the sea spider *Achelia sawayai* Marcus, 1940, the isopod *Cymodoce* aff. *fuscina* Schotte & Kensley, 2005; the amphipod *Aoroides longimerus* and the crab *Charybdis (Gonioinfradens) paucidentatus* [A. Milne-Edwards, 1861] (Ulman et al. 2017).

Ongoing monitoring of Mediterranean marinas for both new NIS and their spreading would be relatively easy now that this initial baseline has been completed. While there are many NIS recorded in marinas, it is not yet understood how these hot-spots affect the natural biodiversity on a broader-scale (i.e., outside the marinas), as many of these species seem to be restricted to the artificial habitats of the marinas themselves due to both limited circulation and/or larval dispersal regimes, and due to surrounding habitats being unsuitable to most fouling species. However, some NIS have proven capable of colonizing numerous marinas across the Mediterranean Sea (up to 74% of marinas), even though many of the marinas have distinct underlying abiotic factors, which shows their potential for adapting to a wide-range of conditions, thus eventual establishment success to neighbouring natural habitats should not be ruled out. There is currently a huge gap in knowledge on biological traits (i.e., dispersal characteristics, space requirements, competition; Cardeccia et al., 2018) and biotic resistance (i.e., pathogens, parasites, competitors and native predators of NIS species) that has to be better understood before these factors can also be incorporated into bioinvasions modelling to make it more robust (Cardeccia et al., 2018; Lockwood et al., 2009; Simberloff & Von Holle, 1999). These biological interactions largely affect NIS population sizes and obviously ecosystem dynamics, but as this is a fairly new and emerging field of research, it requires targeted collaboration amongst scientists, which has already been initiated by some local initiatives, such as the LifeWatchGreece Research Infrastructure Project (see polytraits.lifewatchgreece.eu). It should also be considered that other abiotic factors not investigated here may also play a role in shaping NIS patterns and distributions in Mediterranean marinas; for example, we also hypothesise that pollution levels and dissolved oxygen would be interesting to test in subsequent studies, as high pollution levels cause a reduction in biodiversity, unless the species is adapted or tolerant to those conditions, as those sites favour opportunistic NIS (Bellou et al., 2016).

Now that the significance of Mediterranean marinas as hot-spots for NIS invasions has been evidenced, the biofouling vector necessitates management to help control the issue, as has already been initiated for aquaculture and ballast water, the other well-known vectors. The Mediterranean is indeed a hotspot both for recreational boaters and for NIS, and unique solutions are urgently needed to buffer from additional swarms of invaders. The next step for biofouling in the Mediterranean is to provide some effective pre-emptive regulations, as other countries have commenced (e.g., Australian Marine Conservation Society 2015; Ministry for Primary Industries 2017). As the Mediterranean is an enclosed sea, basin-wide management preventing entry of new invaders to the basin in theory should be relatively controllable, but would require imposed regulations on long-distance travelling boats, resulting from cooperation amongst the countries bordering the Strait of Gibraltar, the Suez Canal and the Bosphorus Strait; although such a collaboration is highly unlikely at present (Galil et al., 2015).

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Appendix Table 3.1 NIS records per Marina # 1-14 from Table 3.1, + for present, - for absent.

Marina #	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Asciidia														
<i>Asciidiella aspersa</i>	-	-	+	-	+	+	+	+	-	+	-	-	-	-
<i>Botrylloides violaceus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ciona robusta</i>	-	-	-	-	-	+	+	-	-	+	-	-	-	-
<i>Clavelina oblonga</i>	-	-	-	-	+	-	+	-	-	-	-	-	-	-
<i>Diplosoma listerianum</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Herdmania momus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microcosmus exasperatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microcosmus squamiger</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>Phallusia nigra</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Polyandrocarpa zorritensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Styela clava</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Styela plicata</i>	+	+	+	+	+	-	+	+	-	+	-	+	-	+
<i>Symplegma brakenhielmi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bryozoa														
<i>Amathia verticillata</i>	-	+	-	+	+	+	-	-	-	-	+	-	+	+
<i>Celloporaria brunnea</i>	+	-	-	+	+	-	-	+	-	-	-	-	+	+
<i>Celloporaria vermiformis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tricellaria inopinata</i>	-	-	+	-	+	-	-	-	-	-	-	-	-	-
<i>Parasmittina egyptiaca</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Watersipora arcuata</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+
<i>Hippopodina</i> sp. A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cnidaria														
<i>Cassiopea andromeda</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhopilema nomadica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oculina patagonica</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Crustacea														
<i>Ampithoe bizseli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amphibalanus eburneus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>Amphibalanus improvisus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aoroides longimerus</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Balanus trigonus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bemlos leptocheirus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Callinectes sapidus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Caprella scaura</i>	+	+	+	+	+	-	-	-	+	-	-	-	-	-
<i>Charybdis (Gonioinfradens)</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cymodoce</i> aff. <i>fuscina</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dyspanopeus sayi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ericthonius</i> cf. <i>pugnax</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Grandidierella japonica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ianiropsis serricaudis</i>	-	-	+	-	+	-	-	-	-	-	-	-	-	-
<i>Mesanthura</i> cf. <i>romulea</i>	+	-	-	-	-	-	-	-	-	-	-	-	+	+
<i>Paracerceis sculpta</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Paradella dianae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paranthura japonica</i>	+	+	+	+	+	-	-	-	+	-	-	-	-	-
<i>Portunus (Portunus) segnis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<i>Stenothoe georgiana</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Sphaeroma walkeri</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Echinoderma														
<i>Synaptula reciprocans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mollusca														
<i>Anadara transversa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Arcuatula senhousia</i>	-	+	-	-	+	-	-	-	+	-	-	-	-	-
<i>Brachidontes pharaonis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chama asperella</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chama pacifica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cerithium scabridum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Crassostrea angulata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dendostrea folium sensu lato</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Goniobranchus annulatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Magallana gigas</i>	-	-	+	-	+	-	-	-	+	-	-	-	-	-
<i>Malleus regula</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pinctada imbricata radiata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudochama cf. corbierei</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rapana venosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Saccostrea cf. cucullata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Saccostrea glomerata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Septifer cumingii</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Porifera														
<i>Paraleucilla magna</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polychaetea														
<i>Branchiomma bairdi</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ficopomatus enigmaticus</i>	+	-	-	-	-	-	+	-	-	-	-	-	-	-
<i>Hydroides brachyacantha sensu lato</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>Hydroides dirampha</i>	-	+	-	-	-	-	-	-	-	-	-	-	+	-
<i>Hydroides elegans</i>	+	+	+	+	+	+	+	-	+	+	+	+	+	+
<i>Hydroides heterocera</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudonereis anomala</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spirorbis marioni</i>	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>Spirobranchus tetraceros</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pycnogida														
<i>Ammothea hilgendorfi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Achelia sawayai sensu lato</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total NIS per marina	10	11	8	7	17	4	6	3	5	5	2	2	5	7

Appendix Table 3.1 (continued). NIS records per Marina # 15-28 from Table 3.1, + for present, - for absent.

Marina #	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Asciididae														
<i>Asciidiella aspersa</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>Botrylloides violaceus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ciona robusta</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	+
<i>Clavelina oblonga</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Diplosoma listerianum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Herdmania momus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microcosmus exasperatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microcosmus squamiger</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>Phallusia nigra</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Polyandrocarpa zorritensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Styela clava</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Styela plicata</i>	-	+	-	-	+	-	+	+	-	+	+	+	+	+
<i>Symplegma brakenhielmi</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Bryozoa														
<i>Amathia verticillata</i>	+	+	+	+	+	-	+	+	-	+	-	+	+	+
<i>Celleporaria brunnea</i>	-	-	+	-	+	+	+	+	+	+	+	-	+	+
<i>Celleporaria vermiformis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tricellaria inopinata</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>Parasmittina egyptiaca</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Watersipora arcuata</i>	-	-	-	-	-	-	-	-	+	-	-	-	-	-
<i>Hippopodina</i> sp. A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cnidaria														
<i>Cassiopea andromeda</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhopilema nomadica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oculina patagonica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crustacea														
<i>Ampithoe bizseli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amphibalanus eburneus</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Amphibalanus improvisus</i>	-	+	-	-	-	-	-	-	-	+	-	-	-	-
<i>Aoroides longimerus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Balanus trigonus</i>	-	-	-	-	-	-	-	-	-	-	-	+	+	+
<i>Bemlos leptocheirus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Callinectes sapidus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Caprella scaura</i>	+	+	+	-	+	-	+	-	-	+	+	+	+	+
<i>Charybdis (Gonioinfradens) paucidentatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cymodoce</i> aff. <i>fuscina</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dyspanopeus sayi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ericthonius pugnax</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Grandidierella japonica</i>	-	-	+	-	-	-	-	-	-	-	-	-	-	-
<i>Ianiropsis serricaudis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Mesanthura</i> cf. <i>romulea</i>	-	+	+	-	-	-	+	+	-	-	-	+	+	+
<i>Paracerceis sculpta</i>	-	-	-	-	-	-	+	-	-	-	+	-	+	+
<i>Paradella dianae</i>	-	-	-	-	-	-	+	-	-	-	-	-	+	-
<i>Paranthura japonica</i>	+	+	+	-	+	-	+	+	-	+	+	-	-	+
<i>Portunus (Portunus) segnis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<i>Stenothoe georgiana</i>	-	+	-	-	-	-	-	-	-	-	-	+	+	+
<i>Sphaeroma walkeri</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Echinoderma														
<i>Synaptula reciprocans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mollusca														
<i>Anadara transversa</i>	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>Arcuatula senhousia</i>	-	+	+	-	-	-	-	+	-	-	-	-	+	+
<i>Brachidontes pharaonis</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	+
<i>Chama asperella</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chama pacifica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cerithium scabridum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Crassostrea angulata</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>Dendostrea cf. folium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Goniobranchus annulatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Magallana gigas</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>Malleus regula</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pinctada imbricata radiata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudochama cf. corbierei</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rapana venosa</i>	-	-	-	-	-	-	-	-	-	-	-	+	-	-
<i>Saccostrea cf. culcullata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Saccostrea glomerata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Septifer cumingii</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Porifera														
<i>Paraleucilla magna</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Polychaetea														
<i>Branchiomma bairdi</i>	-	-	-	-	-	-	-	-	-	-	-	+	+	+
<i>Ficopomatus enigmaticus</i>	+	-	+	+	-	-	-	-	-	-	-	-	-	-
<i>Hydroides brachyacantha sensu lato</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Hydroides dirampha</i>	+	-	+	-	+	-	+	+	-	+	-	-	-	+
<i>Hydroides elegans</i>	+	+	+	-	+	+	+	+	+	+	-	-	+	+
<i>Hydroides heterocera</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudonereis anomala</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spirorbis marioni</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spirobranchus tetraceros sensu lato</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pycnogida														
<i>Ammothea hilgendorfi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Achelia sawayai sensu lato</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total NIS per marina	7	10	10	3	7	2	10	8	3	9	5	8	20	16

Appendix Table 3.1 (cont'd). NIS records for Marinas #29-40 from Table 3.1, + for present, - for absent.

Marina #	29	30	31	32	33	34	35	36	37	38	39	40
Asciididae												
<i>Asciidella aspersa</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Botrylloides violaceus</i>	-	-	-	-	-	-	-	+	+	-	-	-
<i>Ciona robusta</i>	-	-	+	-	-	-	-	-	-	-	+	-
<i>Clavelina oblonga</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Diplosoma listerianum</i>	-	-	-	-	-	-	+	-	-	-	+	-
<i>Herdmania momus</i>	-	-	-	-	-	-	-	-	-	-	+	+
<i>Microcosmus exasperatus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microcosmus squamiger</i>	+	-	+	+	-	-	-	-	-	-	-	-
<i>Phallusia nigra</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Polyandrocarpa zorritensis</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Styela clava</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Styela plicata</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Symplegma brakenhielmi</i>	-	-	-	-	-	-	-	-	-	-	+	+
Bryozoa												
<i>Amathia verticillata</i>	+	+	+	+	+	+	+	-	+	-	+	-
<i>Celleporaria brunnea</i>	+	-	+	+	+	+	+	-	-	-	+	-
<i>Celleporaria vermiformis</i>	-	-	-	-	-	-	-	-	-	-	+	+
<i>Tricellaria inopinata</i>	-	-	-	-	-	-	-	+	+	+	+	-
<i>Parasmittina egyptiaca</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Watersipora arcuata</i>	-	+	-	+	-	+	-	-	-	-	-	-
<i>Hippopodina</i> sp. A	-	-	-	-	-	-	-	-	-	-	-	-
Cnidaria												
<i>Cassiopea andromeda</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhopilema nomadica</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oculina patagonica</i>	-	-	-	-	-	-	-	-	-	-	-	-
Crustacea												
<i>Ampithoe bizseli</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amphibalanus eburneus</i>	-	-	-	-	-	-	-	+	+	-	-	-
<i>Amphibalanus improvisus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aoroides longimerus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Balanus trigonus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bemlos leptocheirus</i>	-	-	-	-	-	-	-	-	-	-	+	+
<i>Callinectes sapidus</i>	-	+	-	-	-	-	-	-	-	-	-	-
<i>Caprella scaura</i>	+	+	-	+	+	+	+	+	+	+	+	+
<i>Charybdis (Gonioinfradens) paucidentatus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cymodoce aff. fuscina</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Dyspanopeus sayi</i>	-	+	-	-	-	-	-	-	-	-	+	-
<i>Ericthonius pugnax</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Grandidierella japonica</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ianiropsis serricaudis</i>	-	-	-	-	-	-	-	+	+	+	-	-
<i>Mesanthura cf. romulea</i>	-	+	-	-	-	+	-	-	-	-	-	-
<i>Paracerceis sculpta</i>	+	+	+	+	+	+	+	-	-	-	+	-
<i>Paradella diana</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paranthura japonica</i>	+	+	+	+	+	-	+	+	+	+	+	-
<i>Portunus (Portunus) segnis</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Stenothoe georgiana</i>	-	+	-	-	+	-	+	-	-	-	-	-

<i>Sphaeroma walkeri</i>	-	-	-	-	-	-	-	-	-	-	+	-
Echinoderma												
<i>Synaptula reciprocans</i>	-	-	-	-	-	-	-	-	-	-	-	-
Mollusca												
<i>Anadara transversa</i>	-	-	-	-	-	-	-	-	-	+	-	-
<i>Arcuatula senhousia</i>	-	-	-	+	-	-	-	+	-	-	-	-
<i>Brachidontes pharaonis</i>	+	+	+	+	+	-	+	-	-	-	+	+
<i>Chama asperella</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chama pacifica</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cerithium scabridum</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Crassostrea angulata</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dendostrea cf. folium</i>	-	-	-	-	-	+	+	-	-	-	+	-
<i>Goniobranchus annulatus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Magallana gigas</i>	-	-	-	-	-	+	-	-	-	+	-	-
<i>Malleus regula</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pinctada imbricata radiata</i>	-	-	-	-	-	+	-	-	-	-	+	+
<i>Pseudochama cf. corbierei</i>	-	-	-	+	-	-	-	-	-	-	-	-
<i>Rapana venosa</i>	-	-	-	-	-	+	-	-	-	-	-	-
<i>Saccostrea cf. culcullata</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Saccostrea glomerata</i>	-	-	-	-	-	+	-	-	-	-	-	-
<i>Septifer cumingii</i>	-	-	-	-	-	-	-	-	-	-	-	+
Porifera												
<i>Paraleucilla magna</i>	+	+	+	+	+	-	+	-	-	-	+	+
Polychaetea												
<i>Branchiomma bairdi</i>	+	+	+	+	+	-	-	-	-	-	+	+
<i>Ficopomatus enigmaticus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Hydroides brachyacantha sensu lato</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Hydroides dirampha</i>	+	-	-	+	-	+	+	-	-	-	+	-
<i>Hydroides elegans</i>	+	+	-	-	+	+	-	-	-	-	+	+
<i>Hydroides heterocera</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudonereis anomala</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spirorbis marioni</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spirobranchus tetraceros</i>	-	+	-	-	-	-	-	-	-	-	+	-
Pycnogida												
<i>Ammothea hilgendorfi</i>	-	-	-	-	-	-	-	+	-	-	-	-
<i>Achelia sawayai sensu lato</i>	+	+	-	-	-	-	+	-	-	-	-	-
Total NIS per marina	13	16	11	14	11	14	13	9	8	7	27	12

Appendix Table 3.1 (cont'd). NIS records per Marina #41-50, numbers from Table 3.1; + for present, - for absent.

Marina #	41	42	43	44	45	46	47	48	49	50
Asciididae										
<i>Asciidiella aspersa</i>	-	-	+	+	-	-	+	-	-	-
<i>Botrylloides violaceus</i>	-	-	-	-	-	-	-	-	-	-
<i>Ciona robusta</i>	+	-	-	-	-	-	-	-	-	-
<i>Clavelina oblonga</i>	-	-	-	-	+	-	-	-	-	+
<i>Diplosoma listerianum</i>	+	-	-	+	-	-	+	+	-	-
<i>Herdmania momus</i>	-	-	-	-	-	-	-	-	+	+
<i>Microcosmus exasperatus</i>	-	-	-	-	+	-	-	-	+	-
<i>Microcosmus squamiger</i>	-	-	-	-	-	-	-	-	-	-
<i>Phallusia nigra</i>	-	-	-	-	-	-	-	-	+	+
<i>Polyandrocarpa zorritensis</i>	-	-	-	-	-	-	-	-	-	-
<i>Styela clava</i>	-	-	-	-	-	-	-	-	-	-
<i>Styela plicata</i>	+	-	-	+	-	+	+	+	-	+
<i>Symplegma brakenhielmi</i>	+	-	-	-	-	-	+	+	-	+
Bryozoa										
<i>Amathia verticillata</i>	+	-	-	+	-	+	-	-	-	+
<i>Celleporaria brunnea</i>	-	-	-	+	-	+	-	+	-	-
<i>Celleporaria vermiformis</i>	+	-	-	-	-	-	-	-	+	+
<i>Tricellaria inopinata</i>	-	-	-	-	-	-	-	-	-	-
<i>Parasmittina egyptiaca</i>	-	-	-	-	-	-	-	+	+	-
<i>Watersipora arcuata</i>	-	-	-	+	-	-	-	+	-	-
<i>Hippopodina</i> sp. A	+	-	-	-	-	-	-	+	-	-
Cnidaria										
<i>Cassiopea andromeda</i>	-	-	-	-	-	-	-	-	-	+
<i>Rhopilema nomadica</i>	-	+	-	-	-	-	-	-	-	-
<i>Oculina patagonica</i>	-	-	-	-	-	-	-	-	-	-
Crustacea										
<i>Ampithoe bizseli</i>	-	-	-	-	-	-	-	-	+	+
<i>Amphibalanus eburneus</i>	-	+	-	-	-	-	-	+	-	-
<i>Amphibalanus improvisus</i>	-	-	+	-	-	-	-	-	-	-
<i>Aoroides longimerus</i>	-	-	-	-	-	-	-	-	-	-
<i>Balanus trigonus</i>	-	-	-	-	-	-	-	-	-	-
<i>Bemlos leptochairus</i>	-	-	-	-	-	-	-	-	-	-
<i>Callinectes sapidus</i>	-	-	-	+	-	-	-	-	-	-
<i>Caprella scaura</i>	-	-	-	+	-	+	-	-	-	-
<i>Charybdis (Gonioinfradens) paucidentatus</i>	-	-	-	-	-	-	-	-	-	+
<i>Cymodoce</i> aff. <i>fuscina</i>	-	-	-	-	-	-	-	-	-	-
<i>Dyspanopeus sayi</i>	-	-	-	-	-	-	-	-	-	-
<i>Ericthonius</i> cf. <i>pugnax</i>	-	-	-	-	-	-	-	-	-	-
<i>Grandidierella japonica</i>	-	-	-	-	-	-	-	-	-	-
<i>Ianiropsis serricaudis</i>	-	-	-	-	-	-	-	-	-	-
<i>Mesanthura</i> cf. <i>romulea</i>	+	-	-	-	-	-	-	-	+	+
<i>Paracerceis sculpta</i>	+	-	-	-	-	-	-	-	-	+
<i>Paradella diana</i>	-	-	-	-	-	-	+	-	-	-
<i>Paranthura japonica</i>	+	-	-	-	-	-	-	-	-	-
<i>Portunus (Portunus) segnis</i>	-	-	-	-	-	-	-	-	-	+

<i>Stenothoe georgiana</i>	-	-	-	-	-	-	-	-	-	-
<i>Sphaeroma walkeri</i>	-	-	-	-	-	-	+	-	-	-
Echinoderma										
<i>Synaptula reciprocans</i>	-	-	-	+	-	-	-	-	-	-
Mollusca										
<i>Anadara transversa</i>	-	-	-	-	-	-	-	-	-	-
<i>Arcuatula senhousia</i>	-	+	-	-	-	-	-	-	-	-
<i>Brachidontes pharaonis</i>	+	-	-	+	+	+	+	-	+	+
<i>Chama asperella</i>	-	-	-	-	-	-	-	-	-	+
<i>Chama pacifica</i>	-	-	-	-	-	-	-	-	+	-
<i>Cerithium scabridum</i>	-	-	-	-	+	-	-	+	+	-
<i>Crassostrea angulata</i>	-	-	-	-	-	-	-	-	-	-
<i>Dendostrea folium sensu lato</i>	+	-	-	-	+	+	-	+	+	-
<i>Goniobranchus annulatus</i>	+	-	-	-	-	-	-	+	-	-
<i>Magallana gigas</i>	-	-	-	+	-	-	+	-	-	-
<i>Malleus regula</i>	-	-	-	-	-	-	-	-	+	-
<i>Pinctada imbricata radiata</i>	-	-	-	-	+	-	-	-	+	-
<i>Pseudochama cf. corbierei</i>	-	-	-	-	-	-	-	-	-	-
<i>Rapana venosa</i>	-	+	-	-	+	-	-	-	-	-
<i>Saccostrea cf. culcullata</i>	-	-	+	-	-	-	+	-	-	-
<i>Saccostrea glomerata</i>	-	-	-	-	-	-	-	-	-	-
<i>Septifer cumingii</i>	-	-	-	-	+	-	-	-	+	-
Porifera										
<i>Paraleucilla magna</i>	+	-	-	-	-	-	-	-	-	+
Polychaetea										
<i>Branchiomma bairdi</i>	+	-	-	-	+	-	-	-	-	+
<i>Ficopomatus enigmaticus</i>	-	-	+	-	-	-	-	+	-	-
<i>Hydroides brachyacantha sensu lato</i>	-	-	-	-	-	-	-	-	-	-
<i>Hydroides dirampha</i>	-	-	-	-	-	-	+	+	-	-
<i>Hydroides elegans</i>	+	-	-	+	-	-	-	+	-	-
<i>Hydroides heterocera</i>	-	-	-	-	-	-	-	-	+	-
<i>Pseudonereis anomala</i>	-	-	-	-	-	-	-	-	+	-
<i>Spirorbis marioni</i>	-	-	-	-	-	-	-	-	-	-
<i>Spirobranchus tetraceros sensu lato</i>	-	-	-	-	-	-	-	-	-	-
Pycnogida										
<i>Ammothea hilgendorfi</i>	-	-	-	-	-	-	-	-	-	-
<i>Achelia sawayai sensu lato</i>	-	-	-	-	-	-	-	-	-	-
Total NIS per marina	16	4	4	12	9	6	10	14	16	17

Appendix Table 3.2 Total NIS and Abiotic factor results for Marina numbers 1-10.

Marina number from 3.1	1	2	3	4	5	6	7	8	9	10
Total # Alien species (NIS)	10	11	8	7	17	4	6	3	5	5
Salinity (ppt)	40	40	40	35	41.5	39	38	33	39	39
Water temp. (°C)	24	25	20	28	19	17	15	16	16	17
Avg. p. productivity (g C m ³)	0.4	0.8	3.0	3.0	3.0	0.4	0.4	0.4	0.15	0.15
Biogeographic sectors ¹	B	C	D	D	D	D	D	D	D	D
Marina area (km ²)	0.26	0.15	0.78	0.22	0.64	0.09	0.22	0.06	0.15	0.34
Total length piers (km)	5.5	2.92	16.34	5.83	18.56	2.57	5.5	2.05	4.56	7.4
Number of berths	810	151	3300	1443	5000	734	1600	375	720	0
Marina opening length (km)	0.14	0.18	0.11	0.12	0.1	0.06	0.13	0.08	0.26	0.18
Floating pontoons present	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Proximity to fw source	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Presence of shipyard	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Proximiy to aquaculture site	No	No	Yes	Yes	Yes	No	No	No	No	No
Proximity to commercial harbour	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	No
Distance to Suez Canal (km)	3095	2955	2988	2990	2985	4	2885	2883	2905	7
Climate type ²	BSk	BSk	Csa	Csb	Csb	Csb	Csb	Csb	Csa	Csa

¹Biogeographic sectors (Bianchi and Morri 2000): (B) Algeria and southern Spain; (C) Balearic Sea to Tyrrhenian Sea; and (D) Gulf of Lyon and Ligurian Sea.

²Climate type (Peel et al. 2007): Bsk- Arid, Steppe, Cold; Csa- temperate, dry, hot summer; and Csb-Temperate, dry, warm summer.

Appendix Table 3.2 (continued). Total NIS and Abiotic factor results for Marina numbers 11-20.

Marina number from 3.1	11	12	13	14	15	16	17	18	19	20
Total # NIS	2	2	5	7	7	10	10	3	5	2
Salinity	41	38	37	41.2	39.4	38	23.6	32.3	35.7	35.7
Water temp. (°C)	27	21	27.2	26	25	22	25.3	25	27	24.7
Avg. p. prod. (g C m ³)	0.2	0.15	0.4	0.4	0.8	0.8	0.8	0.4	0.4	0.4
Biogeographic sector ¹	D	D	D	D	D	D	D	C	C	C
Marina area (km ²)	0.03	0.055	0.07	0.12	0.04	0.14	0.41	0.25	0.12	0.11
Total pier length (km)	1.44	1.74	2.21	5.93	1.59	1.13	12.13	10.03	2.56	3.15
Number of berths	420	550	100	355	600	1300	2000	575	566	893
Marina opening (km)	0.04	0.07	0.19	0.35	0.22	0.47	0.17	0.04	0.12	0.09
Floating pontoons present	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Proximity to fw	No	No	No	No	No	No	Yes	Yes	Yes	No
Presence of shipyard	Yes	No	No	Yes	No	No	Yes	Yes	Yes	Yes
Proximiy to aquaculture site	No	No	No	No	Yes	Yes	No	No	No	No
Proximity to comm. harbour	No	No	Yes	No	Yes	Yes	No	Yes	No	No
Distance to Suez Canal	2920	2782	2788	2775	2743	2716	2722	2640	9	2600
Climate type ²	Csa	Csa	Csa	Csa	Csa	Csa	Csa	Csa	Csa	Csa

¹Biogeographic sectors (Bianchi and Morri 2000): (C) Balearic Sea to Tyrrhenian Sea; (D) Gulf of Lyon & Ligurian Sea.

²Climate type (Peel et al. 2007): Csa- temperate.

Appendix Table 3.2 (continued). Total NIS and Abiotic factor results for Marina numbers 21-30.

Marina # from Table 3.1	21	22	23	24	25	26	27	28	29	30
Total # NIS	10	8	3	9	5	8	20	16	13	16
Salinity (ppt)	34.8	38.7	34.7	40	39.5	42	36.5	40	43	35
Water temp. (°C)	25.4	26.3	24.1	29	30	28.5	29	29	29	29
Avg. primary prod. (g C m3)	0.4	0.4	0.2	0.8	0.1	0.4	0.5	0.5	0.15	0.5
Biogeographic sector ¹	C	C	C	C	C	C	C	C	C	C
Marina area (km ²)	0.06	0.11	0.09	0.14	0.11	0.4	0.08	0.16	0.08	0.01
Total pier length (km)	2.22	4.36	2.58	4.07	0.7	0.8	2.47	3.25	2.11	0.56
Number of berths	215	650	655	796	150	280	379	370	370	150
Marina opening (km)	0.11	0.15	0.04	0.06	0.06	0.15	0.09	0.22	0.1	1
Pontoons present	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Proximity to fw	Yes	No	No	Yes	No	No	No	No	No	No
Presence of shipyard	Yes	No	No	Yes	No	No	Yes	Yes	Yes	No
Proximiy to aquaculture site	No	No	No	No	No	No	No	No	No	No
Proximity to comm. harbour	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes
Distance to Suez Canal	2565	2550	2418	2435	2365	2360	2110	2110	1728	1700
Climate type ²	Csa	Csa	Csa	Csa	Csa	Csa	Csa	Csa	Csa	Csa

¹Biogeographic sectors (Bianchi and Morri 2000): (C) Balearic Sea to Tyrrhenian Sea.

²Climate type (Peel et al. 2007): Csa- temperate.

Appendix Table 3.2 (continued). Total NIS and Abiotic factor results for Marina numbers 31-40.

Marina # from Table 3.1	31	32	33	34	35	36	37	38	39	40
Total # NIS	11	14	11	14	13	9	8	7	27	12
Salinity (ppt)	50	44.3	40	42	41	31.4	29.2	3.	40	43
Water temp. (°C)	29	29	28	23	25	27.2	28.2	29	25	23.5
Avg. p. productivity (g C m3)	0.5	0.5	0.8	0.15	0.15	5.0	5.0	5.0	0.1	0.1
Biogeographic sector ¹	C	C	C	C	C	E	E	E	I	I
Marina area (km ²)	0.11	0.15	0.31	0.11	0.19	0.091	0.037	0.017	0.03	0.04
Total pier length (km)	2.35	3.38	5.19	3.22	3.61	3.06	1.49	0.89	1.27	1.41
Number of berths	150	720	1500	720	270	500	230	160	200	255
Marina opening (km)	0.1	0.12	0.14	0.22	0.26	0.7	0.03	0.02	0.06	0.06
Pontoons present	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Proximity to fw source	No	No	Yes	No	No	Yes	Yes	Yes	Yes	No
Presence of shipyard	No	Yes	Yes	No	Yes	No	No	No	No	Yes
Proximiy to aquaculture site	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Proximity to comm. harbour	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Distance to Suez Canal	1700	1760	1865	1730	1728	2409	2420	2426	838	780
Climate type ²	Csa	Csa	Csa	Csa	Csa	Cfa	Cfa	Cfa	Csa	Csa

¹Biogeographic sectors (Bianchi and Morri 2000): (C) Balearic Sea to Tyrrhenian Sea; (E) North Adriatic; (I) Ionian Sea & South Aegean.

²Climate type (Peel et al. 2007): Csa- temperate; Cfa-Temperate, without dry season, hot summer.

Appendix Table 3.2 (continued). Total NIS and Abiotic factor results for Marina numbers 41-50.

Marina # from Table 3.1	41	42	43	44	45	46	47	48	49	50
Total # NIS	16	4	4	12	9	6	10	14	16	17
Salinity (ppt)	41	26	25	42	40	40	36	19.5	43	43
Water temp. (°C)	23.5	23	26	28	20	30	30	23.5	25	24
Avg. p. productivity	0.1	5.0	5.0	0.06	0.1	0.15	0.15	0.15	0.1	0.1
Biogeographic sector ¹	I	K	K	I	I	I	I	I	I	I
Marina area (km ²)	1.28	0.09	0.24	0.23	0.42	0.16	0.1	0.1	2.08	0.01
Total pier length (km)	0.07	2.49	5.66	3.25	0.02	4.41	2.37	2.04	0.07	0.55
Number of berths	300	1000	1291	450	60	750	400	320	300	180
Marina opening (km)	0.05	0.08	0.05	0.11	0.12	0.21	1	0.01	0.07	0.05
Pontoons present	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Proximity to freshwater source	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Presence of shipyard	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	No
Proximiy to aquaculture	No	No	Yes	Yes	No	No	Yes	No	No	No
Proximity to harbour	Yes	No	No	No	No	No	No	No	Yes	Yes
Distance to Suez Canal (km)	700	1448	1450	825	785	737	695	600	530	465
Climate type ²	Csa	Csa	Csa	Csa	Csa	Csa	Csa	Csa	BSh	BSh

¹Biogeographic sectors (Bianchi and Morri 2000): (I) Ionian Sea & South Aegean; (K) Marmara Sea & Bosphorus Strait.

²Climate type (Peel et al. 2007): Csa- temperate; BSh- Arid, steppe, hot.

Appendix Table 3.3 SIMPER Results for species similarities between marina groups.*Group g*

Average similarity: 55.63

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>S. plicata</i>	1.00	5.64	4.27	10.14	10.14
<i>S. brakenhelmi</i>	1.00	5.64	4.27	10.14	20.28
<i>C. vermiformis</i>	1.00	5.64	4.27	10.14	30.42
<i>B. pharaonis</i>	1.00	5.64	4.27	10.14	40.56
<i>P. magna</i>	1.00	5.64	4.27	10.14	50.70
<i>B. bairdi</i>	1.00	5.64	4.27	10.14	60.84
<i>H. elegans</i>	1.00	5.64	4.27	10.14	70.98
<i>H. momus</i>	0.67	1.71	0.58	3.07	74.06
<i>B. leptochirus</i>	0.67	1.71	0.58	3.07	77.13
<i>C. scaura</i>	0.67	1.71	0.58	3.07	80.20
<i>P. imbricata</i>	0.67	1.71	0.58	3.07	83.28
<i>C. intestinalis</i>	0.67	1.55	0.58	2.79	86.06
<i>D. listerianum</i>	0.67	1.55	0.58	2.79	88.85
<i>A. verticillata</i>	0.67	1.55	0.58	2.79	91.64

Group c

Average similarity: 41.76

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>H. elegans</i>	0.88	23.82	1.46	57.04	57.04
<i>S. plicata</i>	0.50	5.68	0.49	13.60	70.64
<i>A. aspersa</i>	0.50	4.86	0.51	11.65	82.29
<i>C. brunnea</i>	0.38	4.05	0.34	9.69	91.98

Group h

Average similarity: 55.80

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>C. brunnea</i>	0.93	10.58	2.12	18.97	18.97
<i>A. verticillata</i>	0.86	8.87	1.51	15.89	34.86
<i>H. elegans</i>	0.86	8.49	1.52	15.22	50.08
<i>S. plicata</i>	0.79	7.28	1.17	13.05	63.13
<i>C. scaura</i>	0.79	7.13	1.17	12.78	75.91
<i>P. japonica</i>	0.64	4.97	0.79	8.91	84.83
<i>H. dirampha</i>	0.57	3.65	0.65	6.53	91.36

Group a

Average similarity: 19.05

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>A. eburneus</i>	0.67	9.52	0.58	50.00	50.00
<i>F. enigmaticus</i>	0.67	9.52	0.58	50.00	100.00

Group d

Average similarity: 55.95

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>C. scaura</i>	1.00	11.59	4.24	20.71	20.71
<i>P. japonica</i>	1.00	11.59	4.24	20.71	41.41
<i>S. plicata</i>	0.86	7.58	1.45	13.54	54.95
<i>T. inopinata</i>	0.86	7.58	1.45	13.54	68.50

<i>I. serricaudis</i>	0.71	5.11	0.89	9.13	77.62
<i>M. gigas</i>	0.57	3.37	0.58	6.03	83.65
<i>H. elegans</i>	0.57	3.06	0.59	5.47	89.12
<i>A. senhousia</i>	0.57	2.97	0.59	5.31	94.43

Group b

Average similarity: 35.81

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>B. pharaonis</i>	1.00	7.25	6.96	20.25	20.25
<i>M. exasperatus</i>	0.67	2.67	0.58	7.45	27.69
<i>C. scabridum</i>	0.67	2.67	0.58	7.45	35.14
<i>D. folium</i>	0.67	2.67	0.58	7.45	42.58
<i>P. imbricata</i>	0.67	2.67	0.58	7.45	50.03
<i>S. cumingii</i>	0.67	2.67	0.58	7.45	57.48
<i>C. oblonga</i>	0.67	2.56	0.58	7.16	64.64
<i>B. bairdi</i>	0.67	2.56	0.58	7.16	71.80
<i>H. momus</i>	0.67	2.02	0.58	5.64	77.44
<i>P. nigra</i>	0.67	2.02	0.58	5.64	83.08
<i>C. vermiformis</i>	0.67	2.02	0.58	5.64	88.72
<i>A. bizseli</i>	0.67	2.02	0.58	5.64	94.36

Group i

Average similarity: 33.33

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>D. listerianum</i>	1.00	8.33	#####	25.00	25.00
<i>S. plicata</i>	1.00	8.33	#####	25.00	50.00
<i>S. brakenhielmi</i>	1.00	8.33	#####	25.00	75.00
<i>H. dirampha</i>	1.00	8.33	#####	25.00	100.00

Group f

Average similarity: 65.16

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>S. plicata</i>	1.00	7.33	7.48	11.25	11.25
<i>A. verticillata</i>	1.00	7.33	7.48	11.25	22.50
<i>P. japonica</i>	0.89	5.98	1.81	9.18	31.68
<i>B. bairdi</i>	0.89	5.66	1.77	8.69	40.37
<i>C. scaura</i>	0.89	5.55	1.78	8.51	48.88
<i>P. sculpta</i>	0.89	5.55	1.78	8.51	57.40
<i>B. pharaonis</i>	0.89	5.55	1.78	8.51	65.91
<i>P. magna</i>	0.89	5.55	1.78	8.51	74.43
<i>C. brunnea</i>	0.78	4.24	1.14	6.51	80.94
<i>H. elegans</i>	0.67	2.93	0.82	4.50	85.43
<i>H. dirampha</i>	0.56	2.08	0.61	3.20	88.63
<i>M. squamiger</i>	0.56	2.06	0.60	3.15	91.79

4 BOWED DOWN IN A SEA OF TROUBLES: THE ROLE OF RECREATIONAL BOATS IN THE SPREAD OF ALIEN SPECIES IN THE MEDITERRANEAN SEA

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4.1 Abstract

To determine the contribution of recreational boating to the spreading of non-indigenous species in the Mediterranean, a large-scale study on marinas and the boats in those marinas was undertaken. Approximately 600 boat owners were surveyed in total from 25 marinas across the Mediterranean on their boat specifics, antifouling practices and travel history in France, Italy, Malta, Greece, Turkey and Cyprus. Additionally, samples were taken from these same boat-hulls to first determine their NIS assemblages found in their biofouling, and then analyses were completed to determine which factors drive higher species richness on boat-hulls. Of all the sampled boats, 30% were visiting from other marinas averaging 67 travel days and visiting an average 7.5 other marinas, so spreading potential is high. About 4/5^{ths} of the vessels contained fouling, the remainder which were recently cleaned. Of all sampled vessels, 70% of vessels hosted NIS, the maximum carrying 11 NIS species. Higher species richness in marinas strongly correlated to higher NIS richness on their boats. Also, time since last hull-cleaning strongly influenced NIS richness on vessels, and the colonization of NIS has been shown to occur rapidly in the certain marinas. With in-water hull cleaning, the niche areas can go overlooked or even missed completely which often trigger more biofouling as antifouling coatings are usually not applied to these areas. Boat type, material, average cruising speed, and increased travel did not influence a higher incidence of NIS on boat-hulls. Additionally, all marinas in the Eastern Mediterranean region are of very high risk of spreading NIS as the boats sampled within them had between 70-85% dissimilar species with the marinas they were sampled in, showing the massive potential of new introductions to this region prompting urgent targeted management measures for at least routine monitoring and pontoon cleaning.

4.2 Introduction

The Mediterranean is a hotspot recreational boating traffic due to its favorable sunny climate which allows for year-round travel, its scenic and historical coastlines, its incredible diversity bordered by 22 countries, each with a different culture, and unlike other regions, boaters can reach new interesting destinations within a days travel. The Mediterranean also hosts the highest number of non-indigenous species (NIS) of any other sea on the planet (Galil 2009, Edelist et al. 2013), and these NIS can be spread to new localities as part of the biofouling community on boat-hulls.

Biofouling is defined by the International Maritime Organization as “the undesirable accumulation of microorganisms, plants, algae and animals on submerged structures, especially on ships’ hulls” (www.imo.org). It is becoming increasingly clear that the biofouling vector may be responsible for as many NIS introductions, if not more, than those caused by untreated ballast water (Hewitt et al., 2009; Sylvester et al., 2011). While international regulation on the treatment of ballast water entered force on September 8, 2017, management of the biofouling vector via the recreational sector is currently based only on voluntary guidelines directed at vessels under 24 m in length (IMO, 2012). Therefore the biofouling vector in the spread of NIS remains to be the largest unmanaged vector for NIS introductions (Clarke-Murray 2011, Zabin 2011), urgently requiring effective regulations based on sound science to inhibit further biological and economical harm.

Despite the Mediterranean being so popular for recreational boating, there is practically no data available on the habits or itineraries of this portion of the boating sector, except for a recent survey limited to Italian boaters (Ferrario et al. 2016). The only large-scale geographic databases on boater travel patterns relate to shipping for tracking all larger (commercial) vessels, and charge a hefty fee for usage, such as Marine Traffic (www.marinetraffic.com), whose data are used for identification, navigation and tracking purposes. However, data can only be generated for vessels mandated to have an ‘Automatic Identification System’ (AIS) tracking device equipped on the vessel, and satellites track these beacons. In 2000, the International Maritime Organization (IMO) put forth the Regulation 19 of Solas ChapterV (www.imo.org) requiring AIS to be fitted on all ships which are ≥ 300 gross tonnage (GT) which travel internationally, cargo ships ≥ 500 GT not engaging in international voyages, and all passenger ships, regardless of size. However, due to safety concerns, this data is not yet currently freely distributed. However, in an extremely creative novel attempt, such data has just been used to track illegal fisheries around the globe (Kroodsma et al., 2018), which will hopefully pave the way for other types of marine conservation measures. The majority of recreational boats fall under the minimum size requirements required to carry beacons, are thus their movements are not captured under the radar.

The monumental role of Mediterranean marinas as hotspots for NIS has recently been demonstrated (see Ulman et al. 2017). The role of recreational boats as the most probable means of transport for NIS was evidenced there with the recording of 20 individual cases of NIS found on boat-hulls which were not yet found in the same marinas and in most cases, even the country. The second output of this research study examined which abiotic factors influence higher species richness in marinas, and found proximity to other vectors (e.g., Suez Canal and commercial harbours) and temperature mainly to influence NIS success in individual marinas whereas environmental matching to better explain the larger-scale geographical patterns of NIS distribution (Ulman et al. In review).

This study, complements the previous outputs by examining various aspects of recreational boating behavior and their fouling in the Mediterranean further explore which aspects of boating influence higher NIS richness on vessels. First information on vessel cleaning and travel were obtained from surveys that were conducted in 25 marinas spanning from France to Cyprus. The data from the surveys were first coded and subsequently analyzed along with the variables relevant to NIS (e.g. total species number, estimated visible fouling percentage) found on the boat-hulls, in order to relate boating profiles to the fouling and NIS richness of their boats (Davidson et al. 2010, Clarke-Murray et al. 2011, Floerl et al. 2010). The lessons learned here can be used to support science-based decision-making for management of the biofouling vector via recreational boating.

4.3 Methods

Boaters were surveyed and their boats sampled from 25 marinas from April 2015 until November 2016 on a Mediterranean stretch spanning France to Cyprus. A period up to ten days were spent conducting the study at each marina. Information on marina names, geographic coordinates and sampling dates can be found in Ulman et al. (2017). In comparison to previously existing research on the recreational boating influence on the transport of NIS, this was one of the few studies (in addition to Mineur et al. 2008, Clarke-Murray 2011, Zabin et al. 2014) to survey the boaters (i.e., on their vessel characteristics, maintenance procedures, travel history and awareness) and directly sample the same boat-hulls for NIS. This strategy allows for the direct correlation between the results of the two and hence justly assess the potential strength of this vector. This type of approach is rarely attempted because it is extremely time-consuming to find boaters at leisure to converse with, who trust the motives of the study, and who also permit scraping of their boat-hulls (the latter which excludes all regatta racing boats). Particularly, this study focused on five aspects that we considered relevant

for assessing the strength of this vector: (i) vessel details, (ii) antifouling practices and cleaning frequency of hull, (iii) boater travel patterns, (iv) visual fouling percentage estimates, and (v) boat-hull sampling for NIS.

4.3.1 Boater Survey and Criteria

The recreational boater survey questionnaire used is available in Appendix Table 1. This questionnaire has built on that created by Clarke-Murray (2011) which focused on three major topics: boat specifics, antifouling practices and travel history. Additionally, questions were added pertaining to vessel horsepower (hp), average cruising speed, cost of antifouling applications, the sequence and time of visited marinas in the recent 12 months and their awareness of NIS. Question types were either multiple choice or open ended. Surveys were completed only after permission was granted from marina management to conduct our field research in the marina. Boaters were randomly targeted in the marinas when they seemed to be at ease (i.e., not while dining or tending to their vessel). An initial screening was first conducted with the boat owners/captains to ensure that their vessel had spent at least one night in another marina other than their home marina in the previous year before being selected for a survey and boat sampling, to ensure that their vessel posed a risk of spreading NIS.

Survey participation might have been biased towards boaters that travel more frequently, thus who were present at the marina during the study period. All interviews were conducted in person, and in either the native language of the boaters, or in English if they were fluent, and took approximately 20 minutes to complete. Surveys were available in the following languages: Spanish, French, Italian, Greek and Turkish.

Fishers, often present in marinas, were excluded from this study as they represent commercial activities, and hence are not here considered 'recreational' boaters. Additionally, we considered small fishing vessels to have a much lower chance of spreading NIS due to their travel patterns, which tend to go out to fish and then return back to their marina.

4.3.2 Boater travel patterns

Boaters were specifically asked to report in sequence the other marinas they had visited in the recent 12 months preceding the survey date, and the number of days moored in each locality. The area considered here as the “Western Mediterranean” extends from Gibraltar and Morocco in the west to the islands Corsica and Sardinia, Ligurian Sea, and Algeria in the east. The “Central Mediterranean” extends from the Tyrrhenian coast of Italy including Rome, Sicily, Malta and extending all the way around Italy to the Adriatic Sea including Slovenia, Croatia, and Montenegro, Tunisia and Libya. The “Eastern Mediterranean” extends from Greece as its western limitation, and includes Turkey, Cyprus, Syria, Palestine, Israel, Lebanon and Egypt. “Radar maps” are used to display the total number of surveyed boaters that travelled to either other sub-regions other than the subregion where the survey was conducted (the sampled region represented by the central point in the radar map, with the other visited subregions are represented by other axes) using the responses from the boater surveys.

4.3.3 Antifouling practices and cleaning frequency

There are two main methods of hull-cleaning. The first refers to a professional high-pressure cleaning when the boat is hauled out of the water at the dry dock, which is the most effective method at biofouling removal: the process is completed first by using paint scrapers if the fouling is heavy and then using a highly pressurized power washer effective at dislodging the remaining biota, and which always precedes the application of fresh antifouling coatings. The other method of cleaning occurs as the boat remains in the water, and is completed either by boat owner or professionals by scraping off the fouling biota using a scraper or sponge, contingent on the biota. The success of the in-water method in NIS removal is dependent on the effort exerted by the cleaner, which can be highly variable and often neglects hard to reach niche areas where NIS are present; this method of cleaning is usually performed in addition to professional cleaning as required by boat owners to help reduce fuel consumption brought on by additional drag of the fouling.

4.3.4 Visual fouling percentage inspection

Visual estimates were recorded for the percentage fouling of the entire boat-hull, and also for the percentage fouling of the ‘niche’ areas, which varied considerably from the overall hull-fouling. The niche areas include the

propeller, propeller shaft, keel, keel divisor, sea chests, ladders, and water jets, places which often go missed in-water cleanings, or tend to have accelerated fouling as antifouling coatings are seldom applied to them as the coating type has to be specialized (i.e., “*Prospeed*” is a brand-name of antifouling coating which can be applied to metal areas such as the propeller and engine shaft, but few boaters tend to use this). Motorboats have a much larger niche surface ratio than sailboats, so this additional niche fouling estimate was only recorded for sailboats if their small niche (propellers, keel etc.) areas were visibly fouled, the most common area for them being the divisor in the rudder found on some sailboat models which had a tendency to trap species between the two panels, and which is impossible to properly clean in-water. This estimate accounted for the biofouling from macroinvertebrates, and not for the presence of biofilm, the first stage in the succession process (Hilary et al. 2009). Photographs of the boat-hulls were taken using the Olympus TG-4 to validate visible fouling estimates.

4.3.5 Boat-hull sampling

Fouling samples were only collected from boats which already had a survey completed with the owner/crew to identify exactly which NIS they were carriers of. Samples were collected from boat-hulls either when the boat was being serviced at the dry-dock for its cleaning and antifouling applications, or in water via snorkeling, or by scuba diving. Samples were dislodged from the hull using a paint scraper (6.35 cm width), and directly collected into a small net made for aquaria use (6.35 cm width by 7.35 length with a mesh size of 0.5 cm) wherever biofouling was found. All aspects of the hull were inspected from the waterline to the keel (where applicable), including the propeller, propeller shaft, water jets, rudder, ladder and sea chests, however, the grate was never removed. If the vessel contained a high degree of biofouling, samples were taken from many different areas with no limit in sample size with the intent on collecting all available taxa. Samples from each vessel were immediately preserved in a 90% ethanol solution.

4.3.6 Taxonomic identification

Both sessile and mobile macroinvertebrates were collected and identified in this study. Unicellular organisms, plants and macroalgae, were not collected nor examined. All boat-hull samples were identified at the Ecology Laboratory at the University of Pavia with the exception of a few samples/taxa which were either sent or brought to specialized experts for taxonomic verification. The preserved specimens were observed under a dissecting microscope and, where needed, taxonomic slides were prepared and analyzed under an optical

microscope. Photographs of magnified specimens or morphological parts were taken directly from the microscopes using the Olympus TG-4 camera (i.e., for serpulids and crustaceans).

4.3.7 NIS verification

The definition of NIS used here is (EEA 2012): “An organism introduced outside its natural past or present distribution range by human agency, either directly or indirectly”. Thus, these new arrivals must be assisted by anthropogenically-enabled facilitation. There is a lot of uncertainty plaguing invasion science, namely in assigning the “NIS” status correctly (Marchini et al., 2015b) and the transfer vector for NIS to a new region, as this is usually done by best reasoning from expert judgement (Katsanevakis and Moustekas 2018), and can be attributed to a number of vectors common to the area near first records. The statuses of the NIS found from this study are discussed in detail in Ulman et al. (2017).

4.3.8 Statistical analyses

Univariate analysis

The total NIS richness per boat, tested against the series of boat factors, were used to test the null hypothesis that boat type, travel and cleaning are not correlated to NIS richness on boats. There were ten boat factors tested here which included some of the results about the surveyed boats derived from the survey results (i.e., boat specifics such as boat type[sail or motor], boat length in m, hull construction [fiberglass, wood or other], and average cruising speed); antifouling and cleaning frequency in months since last application; travel (in number of days spent outside home marina in the last year and number of marinas visited); and from the sampling results the visible hull and niche fouling estimates (as %) were used. All factors were first tested for collinearity, of which there were none, therefore all ten factors were run in the GLM. The nature of the relationships between total NIS richness found on the boat-hulls (the response variable) and boat factors (as explanatory variables) were visualized using scatterplots (for continuous variables) by means of generalized linear models (Fig. 2, GLMs) (McCullagh & Nelder, 1983) in order to identify which of these influence the increment of NIS. GLMs are an extension of linear models allowing the incorporation of non-normal distributions of the response variable and transformations of the dependent variables to linearity (McCullagh & Nelder, 1983). For this type of count data with non-negative values, a GLM with log link function and Poisson

error distribution is recommended (McCullagh & Nelder, 1983). Data exploration was applied following Zuur, Ieno & Elphick (2010). The presence of outliers were investigated using Cleveland dotplots, meanwhile collinearity was assessed using multi-panel scatterplots, Pearson correlation coefficients and variance inflation factors (VIF). Finally, the initial model applied contained all boat factors. Therefore, the general model used was:

$$\begin{aligned} \log(\mu_i) &= \log(E(U_i)) \\ &= \beta_1 + \beta_2 \times \text{BoatType}_i + \beta_3 \times \text{BoatLength}_i + \beta_4 \times \text{Avg.CruisingCspeed}_i + \beta_5 \times \text{HullType}_i + \beta_6 \times \text{TimeSinceLastPaint}_i + \\ &\beta_7 \times \text{TimeSinceLastClean}_i + \beta_8 \times \text{DaysSpentTraveling}_i + \beta_9 \times \text{MarinasVisited}_i + \beta_{10} \times \text{VisibleFoulingHullEstimate}_i + \\ &\beta_{11} \times \text{VisibleFoulingNicheEstimate}_i \end{aligned}$$

Where μ_i is the expected number of NIS and βt is the parameter set relating the dependent variable to the response, using a log link function and a Poisson distribution for the response variable. Backward selection using the Akaike Information Criterion (AIC; Akaike, 1974) was used to find the optimal model. The AIC determines between adding or excluding each variable, creating a balance between the variability explained by each factor and the degrees of freedom introduced in the model (Akaike, 1974). Model validation was applied on the optimal model to verify the underlying assumptions (Zuur, Hilbe & Ieno, 2013). Specifically, we plotted Pearson residuals versus fitted values, and also against each covariate in the model and those covariates not in the model to investigate patterns. Additionally, we assessed whether there was overdispersion (or underdispersion), and also used the Cook's distance value to check the presence of outliers in the model. All this analyses were conducted by R statistical computing software (R Core Team, 2016).

Correlation between marinas and boat NIS

To determine if there was a relationship between total NIS found in the marina, the maximum NIS found on boats from the same marinas and the percentage of boats that were hosting at least 1 NIS, Spearman's correlation was used.

Multivariate analyses

SIMPER (SIMilarity PERcentages) and nMDS (non-metric multidimensional scaling plot) analyses were carried out, through the software PRIMER version 6.1.13 (Clarke 1993; Clarke and Gorley 2006), to compare NIS similarities between all sampled boats and their relative sampled marina. For the marina and boat NIS data, presence/absence values were used. The distances among centroids of each marina and boats within each marina were plotted on a non-metric multidimensional scaling graph (nMDS), based on the Bray–Curtis resemblance matrix. Next a SIMPER (SIMilarity PERcentages) analysis was performed to check the dissimilarity percentages between the same marinas and boats data, to determine the marinas captured in this study where new NIS introductions are of higher probability.

4.4 Results

Just over 600 boaters completed the survey from 25 Mediterranean marinas, with approximately 25 completed per marina. Not all captains/crew that completed surveys could have their boats sampled since some left rather quickly before the vessel sampling could be organized. Two marinas were deemed too unhealthy to snorkel in, one attributed to boaters illegally dumped their untreated waste into the marina on the sampling date and the other due to the town wastewater being disposed of in the marina itself. In total, 585 vessels were sampled (Table 4.1). Even those that were recently cleaned were inspected to determine any rapid growth or missed areas, however, 105 vessels or 18% of those sampled were completely free of fouling.

Table 4.1 Sampling overall results: number and percentages of boats sampled and their statuses.		
Sampling outcome	Total	%
# vessels surveyed	601	-
# vessels sampled	585	97%
# vessels clean hull = no fouling	105	18%
# fouled vessels	480	82%
# sampled vessels hosting at least 1 NIS	413	70%

Of all sampled vessels, 70% were hosting at least 1 NIS, 12% contained fouling but did not host any NIS and 18% were free of fouling. The number of NIS per vessel averaged 2.1 NIS per vessel. One boat in Licata, Sicily, hosted the maximum of 11 NIS species.

4.4.1 Vessel characteristics

For vessel type, there was a slightly higher proportion of motorboats to sailboats in the ‘Western’ region (53%), less than half motorboats to sailboats in the ‘Central’ (45%), while the ‘Eastern’ subregion had ¼ motorboats and ¾ sailboats. Most mega-yachts tended to stay in the very affluent and expensive marinas located in the west (such as Saint Tropez, Antibes, Monaco etc.), although owners and crew stated that they occasionally travel also to the Central and Eastern Mediterranean, as demanded by charter customers.

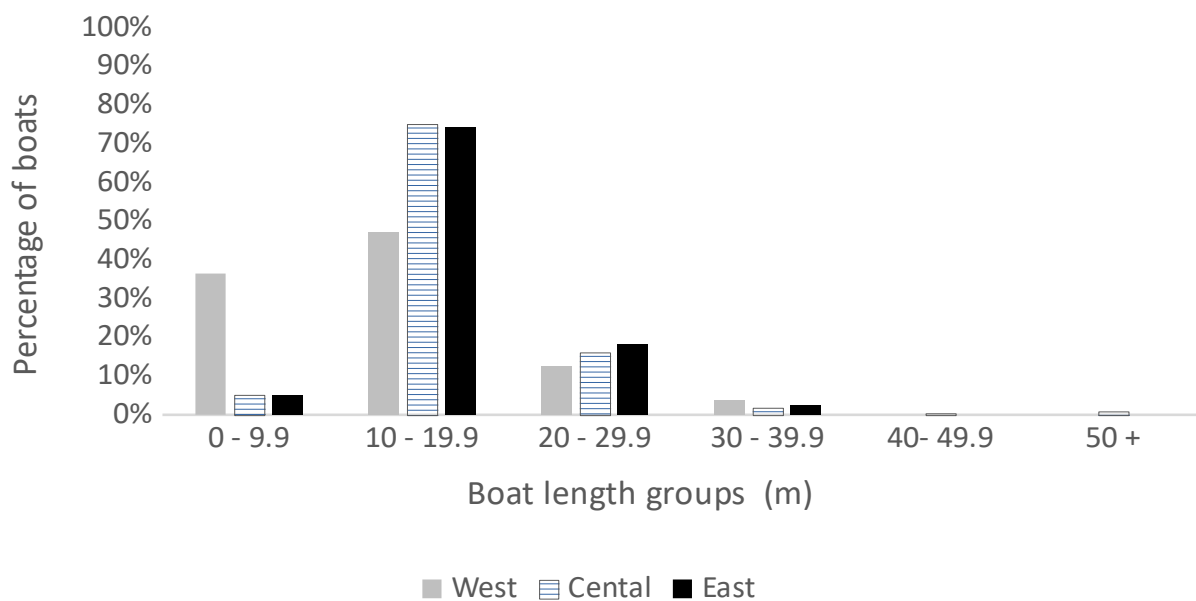


Figure 4.1 Percentages of lengths of sampled boats (m) represented for each subregion (Western n=166); Central (n=210); and Eastern (n=205).

The sampled sailboats ranged from 7 m to 55 m in length, the largest of which was a luxury sailboat which was sampled in Ischia Harbour, its home marina being in Palma, Mallorca. The motorboats ranged from 6 m to 32 m in length. In the Eastern, Central and Mediterranean, sampled vessels had mean lengths of 13.5 m, 16.3 m and 15.8 m, respectively. Most vessels had their hull structures coated in fibreglass, whereas the remainder were constructed of wood, which increased in prominence in the Eastern Mediterranean with 18% of vessels, 10% in the Central Mediterranean and only 4% in the Western Mediterranean. The higher incidence of wooden vessels in the Eastern Mediterranean is owed to the Turkish traditional wooden “*Gulet*” sailboat which typically has two or three masts and are normally constructed in either Bodrum or Marmaris, Turkey. Many of these wooden vessels also have a layer of fibreglass coating applied to them to prolong protection. There were also a few vessels constructed from aluminium, steel and even one from concrete. Horsepower (hp) ranged from 5 to 5,500 hp and average cruising speed ranged from 5 to 32 knots per hour (1 mile= 0.87 knots).

4.4.2 Travel duration and patterns

The Western Mediterranean had the highest proportion (34%, n=160) of non-resident vessels surveyed in its marinas, followed by 27% in the Central Mediterranean, and 23% in the Eastern Mediterranean (n=173).

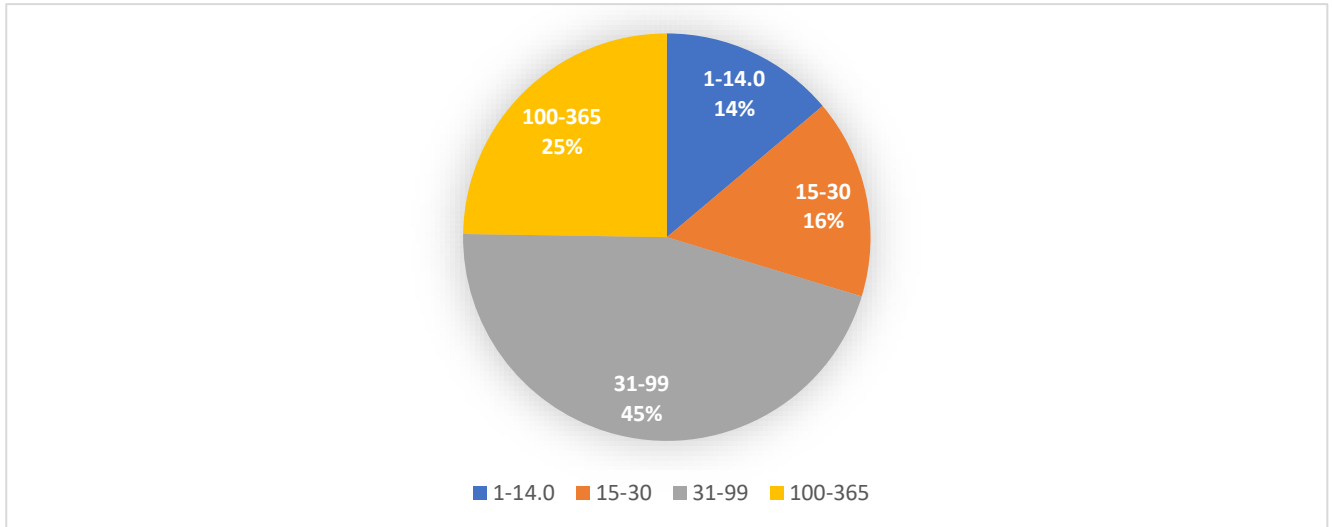


Figure 4.2 The number of days boaters spent traveling in the most recent year from survey data, also expressed as percentages of respondents below days (n=474).

In the most recent year of travel for the boaters, nearly half the respondents (46%) travelled at least 30 days in the previous year, 25% travelled between 100-365 days, while less than 15% used their boat only two weeks or less per year (Fig. 4.2). The boaters traveled 67.5 days on average in the last year, and visited an average of 7.5 marinas each, or over 4,200 marinas cumulatively in their last year of travel. The main season for recreational boating extends from May to September, and if people have less vacation time, they generally use their boats for a period sometime between July and August.

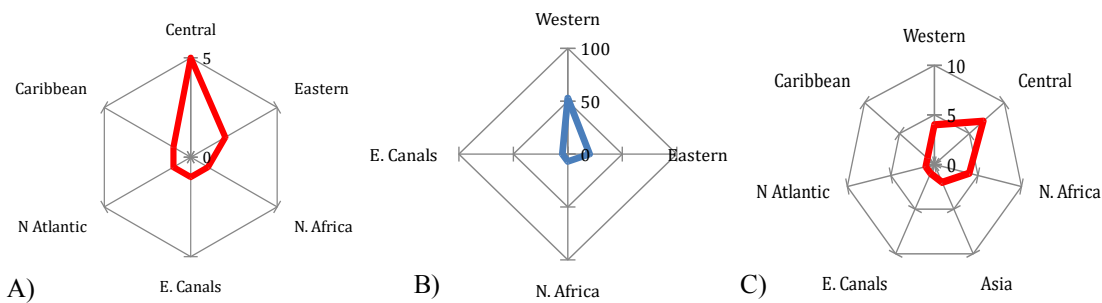


Figure 4.3 A-C. Radar maps showing recent traveled subregions based only on those who had left the subregion where they survey took place, based on # of boaters from each subregion: A) Western Mediterranean; B) Central Mediterranean and C) Eastern Mediterranean. E. Canals stands for European navigable rivers and channels (e.g., the Rhone, the Rhine, the Seine the Sonne and the Danube rivers).

The percentage of boaters that travelled externally outside of the subregion where the surveys took place in the recent year was 8% for boaters sampled in the Western (Figure 4.3A), 42% for the Central (Figure 4.3B) and 10% in the Eastern Mediterranean (Figure 4.3C). The much higher percentage from the 'Central Mediterranean' is attributable to the 'Western Mediterranean' region being in very close proximity to it.

The boats sampled in the Eastern Mediterranean showed examples of recreational boats arriving to the Mediterranean from each and every possible entrance, with boats from the Caribbean, the North Atlantic using the Strait of Gibraltar; from South Africa by ship, boats from Asia entering through the Red Sea and then up through Suez Canal; and boats arriving from the North Sea and Northern Europe entering either along the Eastern Atlantic and through the Gibraltar Strait, through the western or eastern European canals and rivers, the latter which goes through the Black Sea and then through the Turkish Straits (Figure 4.4). There was one case of boat owner from Dubai coming to the Greek Islands who had to transport their vessel via land across Saudi Arabia as their boating insurance company did not cover the high level of pirating which is prevalent around north-east Africa.

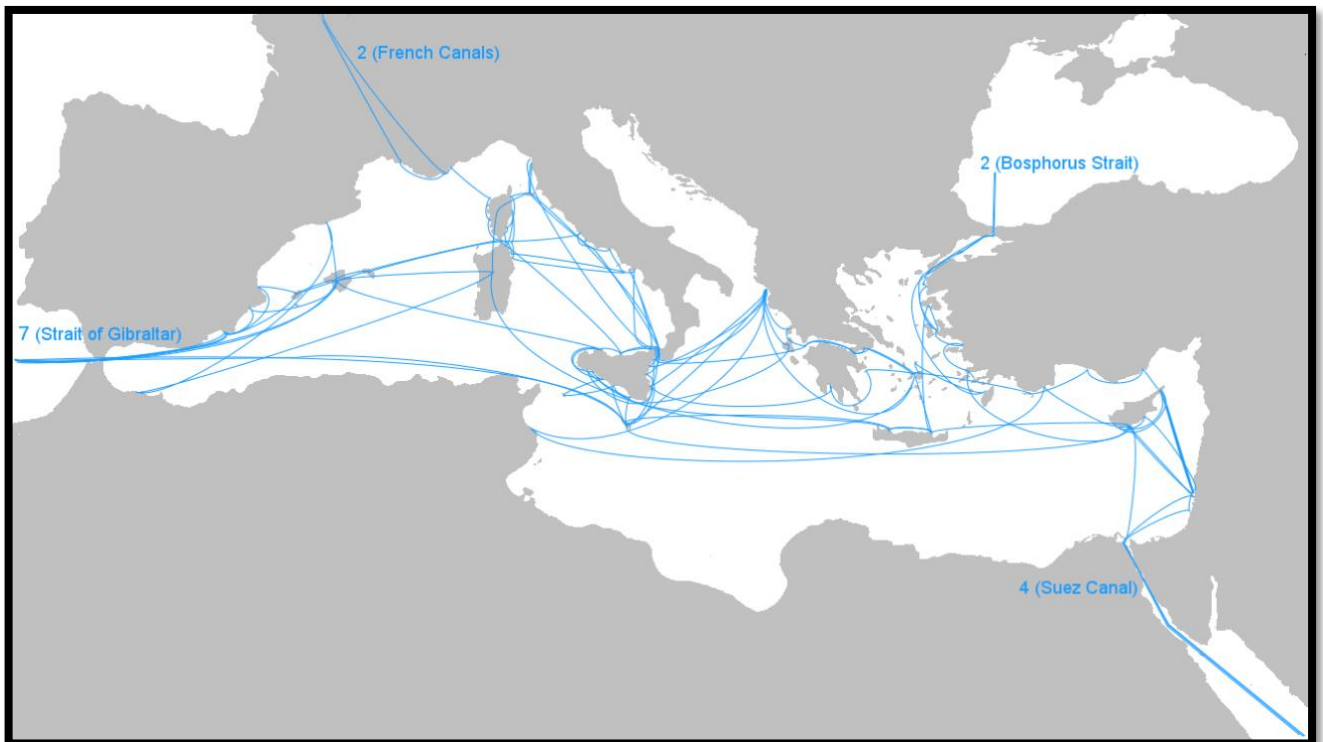


Figure 4.4 Map showing the vessels that entered the Mediterranean Sea and the routes that they took in their recent year of travel until they were surveyed for this study (n=15). Additionally, the four possible gateways to the Mediterranean Sea are shown and numbered from captured travel respondents.

The most popular localities for boating are mainly represented by various Mediterranean islands, e.g., Corsica, the Balearic Islands (the most popular being the four largest: Mallorca, Menorca, Ibiza and Formentera), Porquerolles in the Western Mediterranean; the Aeolian Islands, Sardinia, Malta and Gozo in the Central Mediterranean; as well as the Greek Dodecanese, including especially Rhodes and Kos in the Eastern Mediterranean (Table 4.2). Marmaris and Finike (both Turkey) are the most popular non-island localities captured from these results.

Table 4.2 Most popular localities visited by boaters in each region from boater surveys, from summing the total days each vessel spent visiting each locality during their recent 12 months from the survey date.

Western Mediterranean		Central Mediterranean		Eastern Mediterranean	
Locality	# days	Locality	# days	Locality	# days
1. Corsica	2141	1. Aeolian Islands	1942	1. Marmaris	2202
2. Balearic Islands	2093	2. Malta	1116	2. Dodecanese	1999
3. Porquerolles	588	3. Sardinia	981	3. Finike	1489
4. Monaco	471	4. Gozo	875	4. Rhodes	1357
5. Saint Tropez	454	5. Egadi Islands	734	5. Gokova	995
6. Camargue	440	6. Amalfi coast	489	6. Bodrum	937
7. Barcelona	387	7. Palermo	421	7. Kekova	906
8. Antibes	350	8. Salerno	396	8. Fethiye	889
9. Cap d'Agde	300	9. Siracusa	372	9. Kos	613
10. Valencia	293	10. Sciacca	244	10. Datça	553

4.4.3 Antifouling and cleaning

Nearly ¾ of boaters (72%) apply antifouling to their boat-hulls once per year, which was similar across all subregions, followed by 17% who apply antifouling every second year, 8% every three years, and 3% who only apply antifouling in intervals longer than 3 years.

Table 4.3 Hull-cleaning frequency from boater survey results.

# Cleanings per year	Western (n=147)	Central (n=181)	Eastern (n=184)	Average
0	12%	10%	16%	13%
1	74%	67%	55%	65%
2	7%	17%	18%	14%
3	5%	4%	7%	5%
4	0%	0%	1%	0%
5+	1%	2%	3%	2%

The majority (2/3) of vessels are cleaned once per year, while an additional 20% are clean more frequently, from two times per year or up to five times per year if the fouling was excessive (Table 4.3). Before a vessel can apply a new coat of antifouling paint, it must be cleaned professionally at the dry dock, but additional cleanings are made in water to reduce drag for the purpose of saving on fuel consumption.

The cost of applying an antifouling coating ranges considerably dependent on the boat size and if applied personally or professionally. For example, the paint for a small boat ≤ 10 m in length can cost as little as 80 €, but still requires an additional cost for the boat to be hauled out and back into the water. Generally, when boats are professionally cleaned in the marina at the dry-dock, they are invoiced a total price which includes the haul-out, the cleaning (which always must precede new antifouling applications), the antifouling application itself, any other special paints for the propeller etc., and then the return hoist back into the water. The priciest of these total professional cleaning package was for the previously mentioned luxury 55 m sailboat, with a total cost of 45,000 €; Due to this extremely high cost, this particular boat owner chose to only undergo this procedure once per two years rather than the recommended once per year.

While many boat-hulls appeared to have little or no visible fouling on them, a considerable amount of boat-hulls were very highly visibly fouled. For example, 15% of boats in the “Western Mediterranean” had over 50% of their hulls visibly fouled, and 12% in both the Central and Eastern Mediterranean. Over 1/3 (37%) of vessels in the “Western Mediterranean” had over 25% of their boat-hull visible fouled, 16% in the “Central Mediterranean” and 21% in the “Eastern”, averaging $\frac{1}{4}$ of sampled boats across the Mediterranean (Figure 4.5).

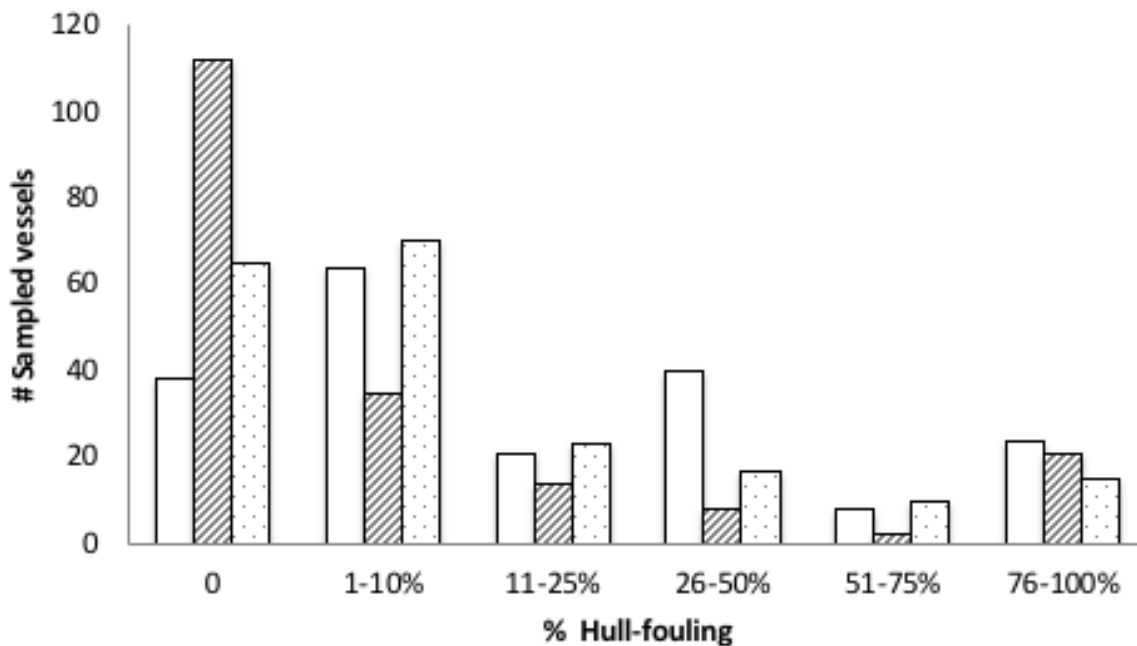


Figure 4.5 Visual estimate of percentage of biofouling from sampled boat-hulls, white bars represent “Western Mediterranean”, diagonal lines represent “Central Mediterranean” and polka-dots represent “Eastern Mediterranean”.

Many boats had only a tiny fraction of visible fouling in their niche areas, in many cases not even warranting a 1% estimate (say a small clump on a large boat) yet were still found to host many NIS, even up to 8 NIS. Many vessels having 10% or less visible fouling in their niche areas hosted as many NIS as vessels containing very high fouling, demonstrating that small clumps of fouling can contain many associated NIS within it. Most sailboats also had visible fouling only in niche areas, especially in the crack found in the middle of the rudder on some models.

4.4.4 NIS levels on boats

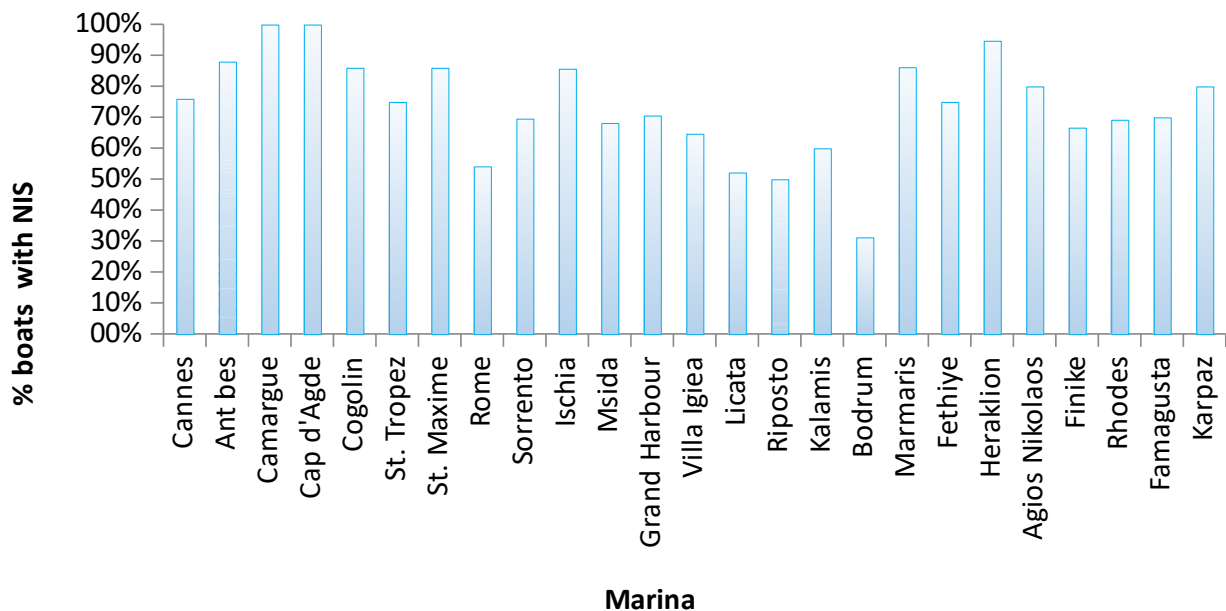


Figure 4.6 Percentage of sampled boats from each marina hosting at least 1 NIS on their boat-hulls (n=516). This analysis excluded clean boat-hulls.

In both Port Camargue and Cap d'Agde, 100% of the fouled boats were hosting at least 1 NIS. Additionally, 95% of fouled boats in Heraklion contained at least 1 NIS (Figure 4.6), 88% in Antibes, 86% in Marines de Cogolin, Saint Maxime, Ischia and Marmaris. The lowest percentage of boats hosting NIS was in Bodrum, with 31% of boats hosting at least 1 NIS, and Riposto with 50%. Over 2/3 of all sampled boats containing some fouling hosted at least 1 NIS. The maximum NIS was a boat from Licata, Sicily, host 11. A much higher proportion of boats sampled in the Western Mediterranean hosted NIS (88%), compared to 74% in the Eastern Mediterranean and 63% in the Central Mediterranean.

Table 4.4 Most abundant NIS found on sampled vessels from this study (n= 413, vessels containing NIS).

<i>Amathia verticillata</i>	47.7%
<i>Hydroides elegans</i>	41.2%
<i>Caprella scaura</i>	36.6%
<i>Celleporaria brunnea</i>	26.9%
<i>Branchiomma bairdi</i>	18.2%
<i>Paranthura japonica</i>	16.5%
<i>Styela plicata</i> and <i>Hydroides dirampha</i>	16.2%
<i>Paracerceis sculpta</i>	12.4%
<i>Amphibalanus eburneus</i> and <i>Celleporaria vermiformis</i>	4.1%

The most abundant non-indigenous taxa found on the surveyed boats (Table 4.4) included four crustaceans (*Caprella scaura* Templeton, 1836; *Paranthura japonica* Richardson, 1909; *Paracerceis sculpta* [Holmes, 1904]; and the barnacle *Amphibalanus eburneus* [Gould, 1841]), three bryozoans (*Amathia verticillata* [delle Chiaje, 1822]; *Celleporaria brunnea* [Hincks, 1884]; and *Celleporaria vermiformis* [Waters, 1909]); three polychaetes (*Hydroides elegans* [Haswell, 1803]; *Hydroides dirampha* Mörch, 1863, and *Branchiomma bairdi* [Mcintosh, 1885]); and one ascidian (*Styela plicata* [Leseur, 1823]).

Univariate analyses

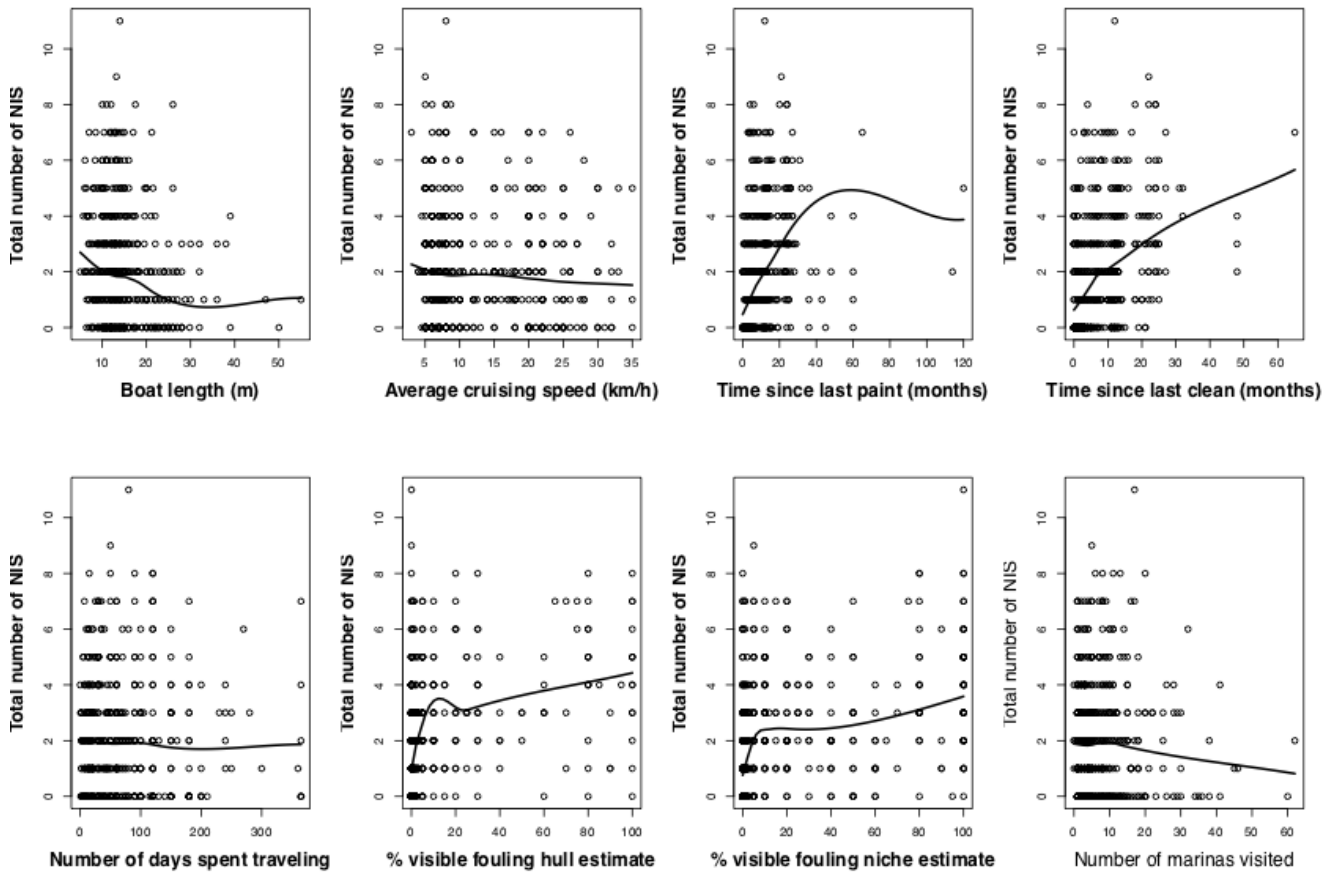


Figure 4.7 GLM results for boat factors influencing total number of NIS on boats. Significant factors (the first seven are in bold print).

Of the ten factors tested to determine which influence total species richness on boat-hulls, three factors were insignificant (boat type, hull construction type and number of marinas visited), while the other factors (in bold print in Fig. 4.7, Table 4.5) together explain 19.2% of the observed variance.

Table 4.5 Coefficients from a generalized linear model fitted to total number of NIS, using a log link function and a Poisson distribution.

Coefficient	Estimate	Standard Error
Intercept*	0.56972	0.10707
Boat length (m)	-0.01614	0.00577
Average cruising speed (km/hr)	-0.00952	0.00451
Time since last paint (months)	0.00610	0.00259
Time since last clean (months)	0.01536	0.00392
Number of days spent traveling	0.00100	0.00047
% visible fouling hull estimate	0.00290	0.00126
% visible fouling other	0.00622	0.00099

Correlation analyses

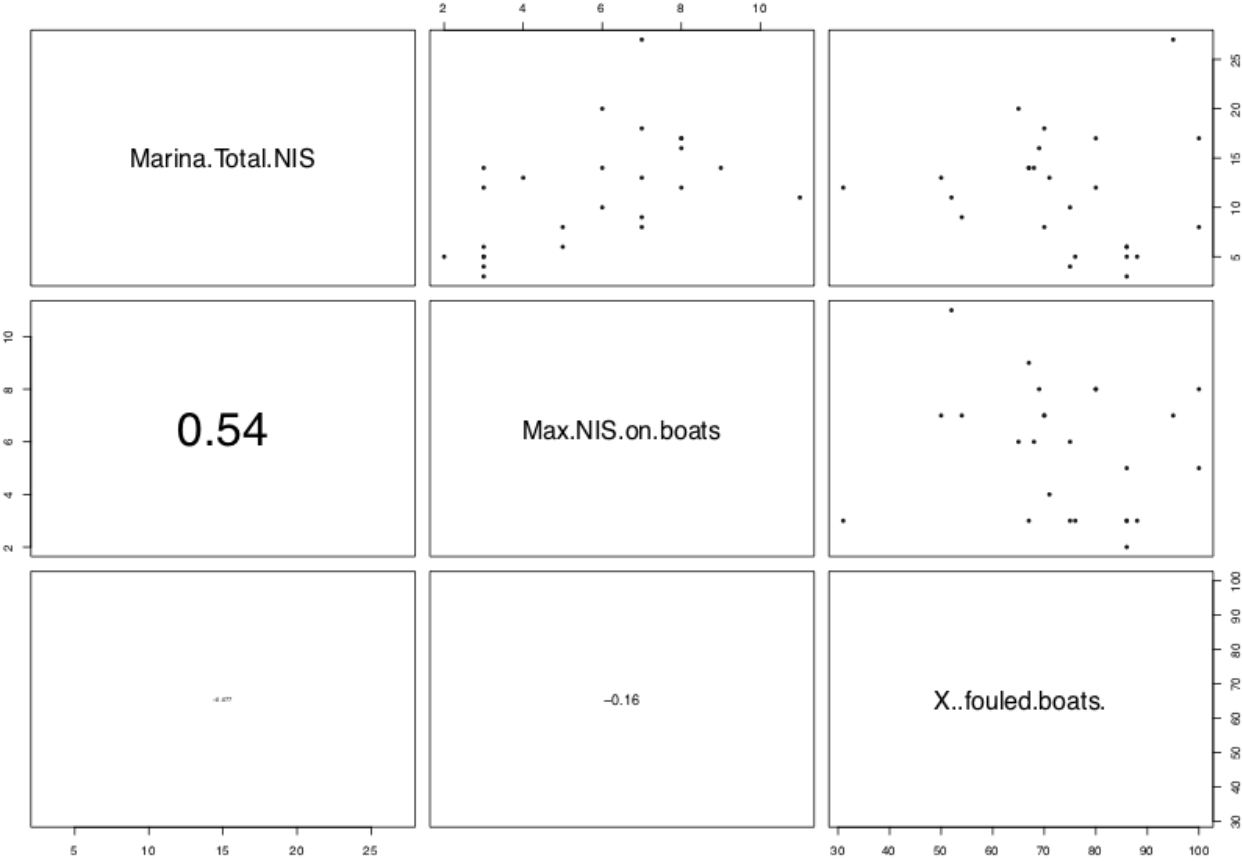


Figure 4.8 Correlation test between total number of NIS found in marina, maximum # of NIS found on the boats from the same marinas and percentage of boats hosting at least 1 NIS.

The correlation model found a significant relationship (Fig.4.8, n=25, p <0.01) between total NIS richness found in marinas and the total NIS richness found on boats in the same marinas, but did not find a relationship between the factors mentioned above and the percentage of fouled boats hosting at least 1 NIS.

Table 4.7 Interesting cases of boats which were recently painted and thus professionally cleaned when dry, (D) or cleaned in water (IW), yet still hosting NIS, % fouling taken by visual estimation while sampling.

Marina locality	Last clean in weeks	Cleaning type: Dry or In Water	% fouling hull	% fouling niche	# NIS	NIS
Cannes	1	IW	0	50	3	<i>C. robusta</i> , <i>P. japonica</i> and <i>H. elegans</i>
Camargue	4	D	100	100	4	<i>S. plicata</i> , <i>H. elegans</i> , <i>C. scaura</i> and <i>P. japonica</i>
Cap d'Agde	4	D	0	30	3	<i>C. scaura</i> , <i>H. elegans</i> , <i>P. japonica</i>
Sorrento	8	D	0	25	3	<i>C. scaura</i> , <i>A. verticillata</i> , <i>H. elegans</i>
Sorrento	3	D	2	2	3	<i>C. scaura</i> , <i>A. verticillata</i> , <i>H. elegans</i>
Sorrento	10	D	20	20	7	<i>C. scaura</i> , <i>S. georgiana</i> , <i>S. plicata</i>
Sorrento	2	D	0	1	2	<i>A. verticillata</i> , <i>T. inopinata</i>
Ischia	8	D	0	5	2	<i>A. verticillata</i> , <i>H. elegans</i>
Ischia	4	D	0	1	2	<i>C. scaura</i>
Rome	2	IW	0	10	5	<i>A. verticillata</i> , <i>H. dirampha</i> , <i>H. elegans</i> , <i>C. brunnea</i> , <i>C. scaura</i>
Rome	.5	IW	0	10	1	<i>P. japonica</i>
V.I., Palermo	1	IW	0	10	4	<i>B. bairdi</i> , <i>H. elegans</i> , <i>C. brunnea</i> , <i>A. verticillata</i>
Licata	1	D	2	NA	3	<i>H. elegans</i> , <i>A. verticillata</i> , <i>C. brunnea</i>
Famagusta	1.3	IW	10	30	5	<i>P. nigra</i> , <i>P. magna</i> , <i>H. elegans</i> , <i>C. lepadiformis</i> , <i>A. verticillata</i>
Famagusta	1	IW	0	2	2	<i>P. magna</i> , <i>C. vermiformis</i>
Karpaz	1	IW	0	2	3	<i>B. bairdi</i> , <i>D. folium</i> and <i>M. regula</i>
Marmaris	4	IW	40	50	3	<i>C. scaura</i> , <i>H. elegans</i> , <i>A. verticillata</i>
Fethiye	.5	IW	0	1	3	<i>S. walkeri</i> , <i>S. plicata</i> , <i>B. pharaonis</i>
Fethiye	1	IW	30	NA	5	<i>S. walkeri</i> , <i>H. dirampha</i> , <i>H. elegans</i> , <i>B. pharaonis</i> , <i>D. folium</i>
Fethiye	1	IW	2	NA	1	<i>B. pharaonis</i>
Heraklion	1	IW	40	NA	3	<i>C. scaura</i> , <i>P. sculpta</i> , <i>H. elegans</i>
Heraklion	8	IW	2	NA	6	<i>S. walkeri</i> , <i>P. sculpta</i> , <i>H. elegans</i> , <i>H. dirampha</i> , <i>H. brachyacantha</i> , <i>C. brunnea</i>
Heraklion	2	IW	5	NA	7	<i>H. elegans</i> , <i>C. scaura</i> , <i>S. walkeri</i> , <i>P. diana</i> , <i>P. sculpta</i> , <i>C. aff. fuscina</i> , <i>A. verticillata</i>
Ag. Nikolaos	6	IW	2	40	2	<i>H. elegans</i> , <i>H. dirampha</i>

Table 4.7 points to interesting cases of either recently painted (and hence professionally cleaned) or recently cleaned in water boats, still hosting NIS. In most of these cases where the boats were cleaned in water, the niche areas were generally missed, as these can be hard to reach, and thus hard to clean. As for the boats which were recently cleaned at the dry dock, this means the cleaning would have been 100% effective as this always precedes the application of new antifouling coatings, therefore the species which were found on those vessels must have rapid growth and surprisingly contained an assortment of taxa groups, not limited to highly mobile taxa (such as crustaceans), but also contained bryozoans, serpulids and ascidians. After just one week

of being professionally cleaned at the dry dock, the encrusting bryozoan *Celleporaria brunnea*, *Hydroides elegans* and *Amathia verticillata* were found on a vessel in Licata, Sicily; we hypothesize that in this circumstance, the vessel must not have been thoroughly cleaned here as it is very unlikely for larvae to settle and grow into recognizable specimens or colonies so rapidly. Also very interestingly, *Styela plicata* and *Hydroides elegans* were found on vessels which had just been professionally cleaned at the dry dock after just a four week duration in Port Camargue and Cap d'Agde, France. *Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985 and *Amathia verticillata* were also found to 'supposedly' have very quick growth on a boat from Sorrento which had just been professionally dry cleaned two weeks prior to sampling. Boaters in Rome reported that in summer *A. verticillata* can grow large colonies in a few weeks' time; that NIS was actually rampant there during our study.

4.4.5 Sonic boom antifouling application

There were three vessels that had applied sonic boom in addition to their antifouling paint. This method sends ultra-sonic acoustic waves through the hull in regular intervals in order to deter the attachment of fouling biota. The first of these surveyed boats applied it two years prior for a cost of US\$ 1600 and had a high level of fouling when sampled (70% hull-fouling, 80% in niche areas), mostly by serpulids, bryozoans and barnacles and was hosting 3 NIS. The second boat with sonic boom had it installed two months prior and was fouled 40% with green algae and 2% with serpulids and barnacles and was hosting 1 NIS. However, the owners were told this method was effective in repelling algae for 2 years and 1 year for barnacles/serpulids. The third boat with this device had applied it three years prior and had only 1% of fouling on their hull consisting mainly of serpulids and was hosting 2 NIS. The sonic boom antifouling system can only be applied to vessels with steel hulls.

4.4.6 Boat NIS vs. Marina NIS

In this study, 75 NIS were identified in marinas and 48 NIS were identified on boat hulls. The highest NIS richness in a marina was Heraklion with 27 NIS and from boat-hulls from Licata, Sicily with 11 NIS on a single boat hull.

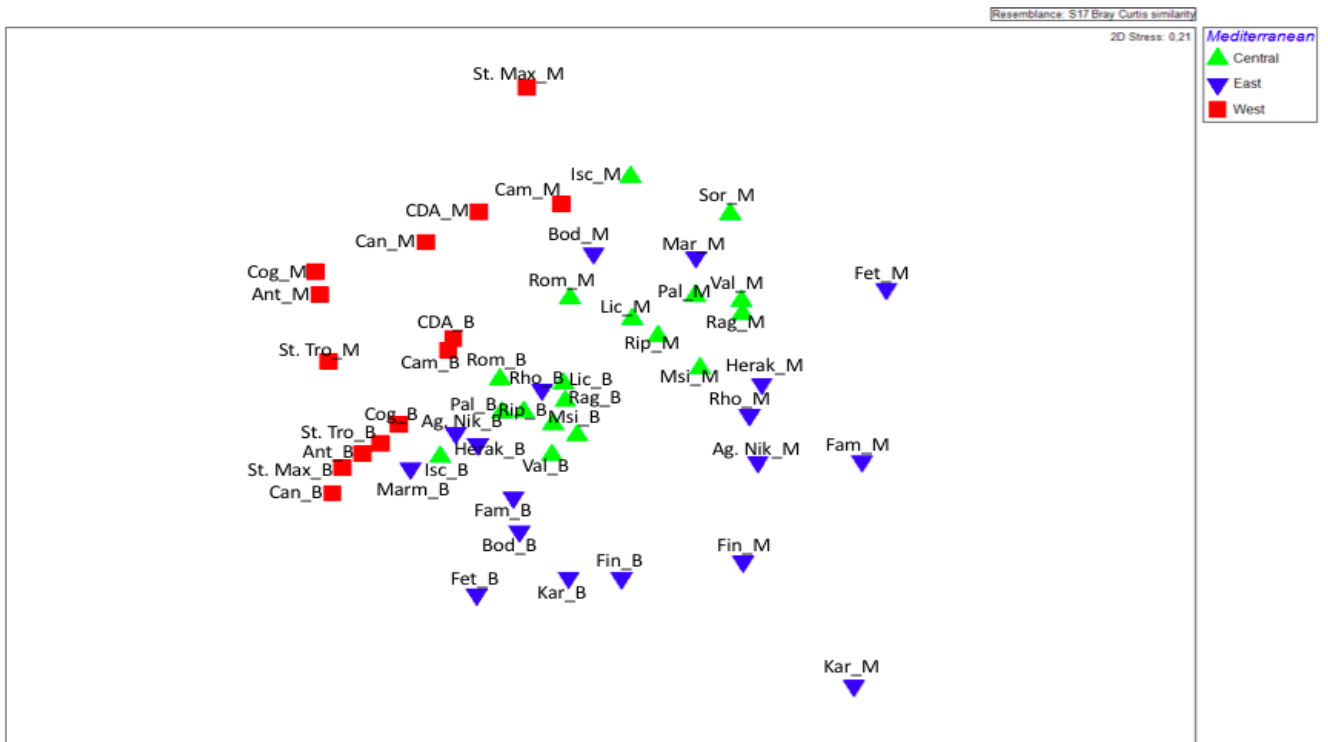


Figure 4.9 nMDS plot of similarities between NIS composition in Mediterranean marinas (M) compared to boat-hulls (B) sampled from those same marinas. For clarity, boat data in each marina are represented by their centroid.

From the nMDS plot (Figure 4.9), it is clear that in every instance, the boats in the marinas have different NIS compositions as what is in the marinas themselves. The marinas and boats with the most dissimilar NIS compositions (ranging from 70-83%) between marinas and boats included all marinas in the Eastern Mediterranean subregion (i.e., Marmaris, Finike, Karpaz, Famagusta, Agios Nikolaos, Heraklion and Fethiye, Marmaris, Heraklion, Rhodes). In the Central Mediterranean, the Maltese marinas were also quite high (over 70%), along with Sorrento and Ragusa. In the Western Mediterranean, St. Maxime was the highest at 82%, followed by Cogolin, both located in the Gulf of St. Tropez. Spearman's correlation coefficient comparing the dissimilarities between NIS richness from pairs of marinas and from pairs of boat-hulls in marinas ($n=300$) was $r_s = 0.41$, showing NIS on boats and in the same marinas to be only weakly-to-moderately correlated.

4.4.7 Awareness of NIS

From the survey results, boaters were least aware of NIS in the Western Mediterranean region with only 1/3 of respondents having heard of the issue. Awareness increased to half the boaters (50%) in the Central Mediterranean, and increased even greater to 63% in the Eastern Mediterranean region. It was not specified here that the NIS had to be of a marine nature.

Of those respondents that claimed they were aware of the issue, they were additionally asked what species (if any) they knew of. The main NIS identified for the Western Mediterranean region was *Caulerpa taxifolia* (M.Vahl) C.Agardh, 1817, which made national headlines in mainstream media in France as it was considered to be released directly into the sea from the Oceanographic Museum of Monaco in 1984, and within just one decade was reported to occur in about 30 km², displacing native species (Meinesz et al. 1993); jellyfish were also mentioned to have recently become a nuisance in the region, and one boater named the mauve stinger *Pelagia noctiluca* (Forsskål, 1775) species. For fouling NIS, surprisingly only two boaters named serpulids (calcareous tubeworms) being an issue, despite most boats being fouled with serpulids. One respondent complained about the “new long algae”, in fact were referring to the spaghetti bryozoan *Amathia verticillata*.

The main NIS identified by the boaters from the Central Mediterranean region (Italy and Malta) were both *Caulerpa taxifolia* and barracuda, referring to a new species of barracuda which has been newly described as *Sphyræna intermedia* Pastore, 2009, not present before in the area and differing morphologically from the two naturally occurring species of barracuda in the Mediterranean (*Sphyræna sphyræna* and *Sphyræna viridensis*). Jellyfish were the third most cited NIS by the Central Mediterranean respondents. For fouling species, four respondents mentioned some combination of barnacles, algae and mussels. A couple people mentioned the spaghetti bryozoan '*Amathia verticillata*' from Ostia Marina in Rome (where it was exceptionally abundant at the time of the survey).

The Eastern Mediterranean had the highest awareness of NIS, most being aware of fish species. The silver-cheeked toadfish (*Lagocephalus sceleratus* [Gmelin, 1789]) was the most cited known NIS in the area; this species is a highly poisonous species of pufferfish that has received much media attention mostly to warn locals about the toxicity of this species as consuming this species has led to several deaths in the region (Bentur et al. 2008). *Lagocephalus sceleratus* exploded in abundance in the mid-to-late 2000s, can weigh over

7 kg and is known to decimate local cephalopod populations as juveniles and fish populations as adults (Ulman et al. 2015). The second most mentioned NIS in the 'Eastern' region is the newly invasive Indo-Pacific devil firefish (locally known as lionfish, *Pterois miles* [Bennett,1828]) which has been rapidly increasing its range in the area since 2012; also a poisonous species, however, once its spines are removed, it is safe to eat and may be a substitute to offset the declining fisheries in the region (Alford & Wood 2017). Due to its extremely high fecundity, spawning every four days (Kletou et al. 2016), it is feared that this lionfish species will come to dominate the entire basin, just like they have recently done in the Caribbean. A couple respondents were aware of a new moray eel to the area, and also invasive rabbitfish (*Siganus* spp.), the blue-spotted cornetfish (*Fistularia commersonii* Rupell, 1838) and squirrelfish (locally referred to as 'German' fish due to its red stripes). A few respondents were aware of particular freshwater or terrestrial NIS particular to their home countries, particularly Japanese knotweed, the grey squirrel and crayfish in England. One mistakenly identified the Mediterranean monk seal as a NIS, although this is rather an endangered species.

4.5 Discussion

This study reveals the capacity of the recreational boating sector in facilitating the spread of NIS around the Mediterranean by comparing the boaters habits to their hulls. This basin is already by far, the most NIS rich region on the planet, and also has excessive movement, hosting the second highest concentration of global recreational boating traffic (Cappato 2011). The boaters surveyed here travel considerably, averaging visiting 7.5 other marinas aside from their home marina each year with the maximum visiting 60 marinas in the past year, and travel frequently, spending an average over 67 days vessel⁻¹ year⁻¹ traveling, which, mostly occurs in summer, when environmental cues trigger spawning and establishment events, increasing opportunities for successful introductions. The majority of boat owners (72%) do apply new antifouling coatings annually, and many also perform subsequent in water cleanings as demanded, yet, over 2/3 (70%) of the sampled boats here containing biofouling are carriers of NIS, which is an extremely important finding, starkly contrasting a large-scale study from Western Canada and another from California both showing only 25% of their sampled vessels to host NIS (Clarke-Murray et al. 2011; Zabin et al. 2014). Additionally, boats are shown here to enter the Mediterranean basin via each possible entrance.

To better understand the factors influencing higher NIS richness on boat-hulls, boat data, antifouling and cleaning regimes were tested along with visible fouling estimates and travel frequency. Of these, boat length, average cruising speed, time since last professional cleaning, time since last in-water cleaning, visible fouling estimates of both hull and niche areas and number of days spent traveling contributed to higher species

richness on boats; whereas boat type, hull material and number of marinas visited were not shown here to have an influence. From the correlation analysis, higher species richness in marinas was found correlate with higher species richness on boats but not affecting the percentage of fouled boats in the marina.

Another key finding from this study is that even though some boats had zero visible hull-fouling when sampled, their niche areas (such as the propeller, propeller shaft, water vents, ladder etc.) were often densely fouled and there was a very strong correlation with the amount of estimated biofouling found in the niche areas of the hulls and a higher NIS richness on the same boats. Thus, inspection of the niche areas was found here a better predictor for finding NIS on boats than just inspecting the hull. There were even several cases when these niche areas contained only 1% fouling and were still found hosting up to 8 NIS. Hence, we suggest here, that dockside 'Level of Fouling' assessments which have been proposed (Floerl 2002), which have inspectors conduct a mini-inspection of the boat-hulls level of fouling using a pole-camera to assess the risk level of boats carrying NIS when a boat arrives to a new region, is thus not a good predictor for screening risk profiles in the Mediterranean context as many NIS can and do hide in the niche areas which may go missed by such assessments. The many boats which were found to contain NIS shortly after having an in-water cleaning could be resultant from lazy, poor or inefficient cleaners, hard to reach or completely missed niche areas. For example, many boaters clean the waterline themselves regularly but usually ignore the niche areas, as their primary concern is to reduce drag for fuel conservation, thus, in-water cleaning is much less effective than professional cleaning when the boat is dry at removing NIS. The sonic boom method is supposed to deter biofouling for at least two years but was shown here to be ineffectual.

The introduction of a new species to the Mediterranean needs just one vessel, while its first establishment is then dependent on similar environmental conditions between the previous and the new locality (Ulman et al., in review). It is these rare and new invaders and their potential spreading that is of extreme importance to track. Findings from this study show that NIS communities found on boats and in the same marinas are only moderately correlated. There is a high number of uncommon species, especially in the Eastern Mediterranean on boats, which have not yet spread to the marina. For example, the marina in the Eastern Mediterranean with the highest NIS dissimilarity percentage between the marina and the boats therein has the largest potential for NIS transfers; Karpaz Gate at the northern tip of Cyprus is a brand new marina which only opened in 2013 and already hosts 17 NIS, many species not recorded yet in any other marina studied here such as: *Pinctada radiata* (Leach, 1814), *Malleus regula* (Forsskål in Niebuhr, 1775), *Hydroides heterocera* (Grube, 1868) and *Pseudonereis anomala* Gravier, 1900. On boats sampled within this marina, the following species were not yet found in the marina warranting not only routine monitoring but also a targeted management plan: *Ampithoe*

bizseli Özeydinli & Coleman, 2012, *Amphibalanus eburneus* (Gould, 1841), *Balanus trigonus* Darwin, 1854, *Styela canopus* (Savigny, 1816) and *Hydroides homoceros* (Pixell, 1913). The Eastern Mediterranean is the subregion of greatest concern for the spread of new NIS, due to ever-increasing introductions stemming from the Suez Canal (another major vector of spread) and climate change making the Mediterranean more akin to the environment of the Red Sea over time. Although our sample size for boaters traveling to/from the Red Sea was small (n=4), Israel and Cyprus were their first visited countries which may be a helpful observation to aid develop future management measures.

The most abundant NIS found on boat-hulls, namely the spaghetti bryozoan *Amathia verticillata*, is an old invader in the Mediterranean, being first recorded in Naples in the early 19th century (Dell Chiaje, 1822). However, it appears to recently have spread considerably, and has been associated with many crustaceans that attach to it which may use it as a niche habitat, thus it is likely enabling the spread of such as several other abundantly found species here to other boats and marinas including *Caprella scaura*, *Pacacerceis sculpta* and *Paranthura japonica* (Marchini et al. 2015), all recent Mediterranean invaders. *P. sculpta* was first recorded in Tunisia in 1978 (Rezig, 1978), *P. japonica* was first reported in the Lagoon of Venice in 1983 (Cesari & Pellizzato 1985) and *Caprella scaura* was also first recorded also in the Lagoon of Venice in 1994 (Mizzan 1999). Fouling serpulids like *Hydroides elegans* and *Hydroides dirampha* are very common in artificial substrates and are more difficult to remove due to their calcareous encasing. The bryozoan *Celleporaria brunnea* is a new invader, only being first recorded in the basin in 2004 from Turkey (Kocak 2007), but now reported from all corners, especially from artificial habitats such as marinas and boat-hulls (see Ulman et al. 2017 and references therein).

Another issue to note is that overall awareness of NIS on boats and in marinas is almost non-existent despite boaters spending sometimes exorbitant amounts of money routinely on new antifouling coatings. The overall tendency for boaters in the Eastern Mediterranean to be slightly more aware of NIS than other subregions is understandable as this subregion recently has been heavily impacted by a couple extremely poisonous NIS within the last decade which have attracted much interest from local media such as the silver-cheeked toadfish (Kletou et al. 2016), and the new lionfish invader. Education to boaters about their role and involvement in facilitating the spread of NIS urgently needs to be initiated and/or improved upon (Marchini et al. 2017).

As the European Union is soon to propose regulations to control the spread of NIS via the biofouling vector, we strongly suggest that all 23 countries bordering the basin be mandated to help control the spread of additional

NIS. The development of a basin-wide strategy involving routine sampling in NIS hotspots (namely marinas, shipping ports and aquaculture localities) and additionally increased biofouling removal from pontoons from those marinas with high NIS richness, and mandated dry dock cleaning for vessels traveling from marinas rich in NIS would help control additional spreading within the basin. As prevention is deemed the best method for inhibiting new introductions, it is the entrances located at the Strait of Gibraltar and the Suez Canal that are of greatest concern for future management of the issue, as NIS on boats entering through European Canals and the Turkish Straits would have a much lower chance of species survival as they travel through freshwater and/or low salinity environments. Such salinity extremes may be the best currently available measures to eliminate biota from entering from the Suez Canal and Gibraltar Strait. Thus, to deter new migrants from entering the basin, an effective screening technique and applicable quarantine measures (e.g., either via power washing or freshwater immersions) for incoming vessels would need to be initiated in both the Gibraltar Strait and Suez Canal entrances.

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5 GENERAL DISCUSSION

5.1 Summary & Synthesis

The Mediterranean is historically known for being “the cradle of civilization”, has recently become a magnet for new marine bioinvasions. Although marine NIS have been studied by select experts in many countries, marinas as hot-spots for NIS have largely been overlooked. However, interest has slowly been improving within the last decade. Also, the contribution of recreational boating as a major vector of spread of NIS in the Mediterranean Sea has also gone overlooked prior to this study. This deficiency is addressed here, by sampling marinas for their fouling communities from both marinas as well as active recreational boat-hulls (when permissions allowed for) across the entire Northern Mediterranean, in order to assess subregional differences. All present macroinvertebrates taxa were also identified (with the help of many experts). The results revealed numerous new NIS records for the Mediterranean basin, sub-regions and countries, a fact which clearly demonstrates marinas as one of the most important hot-spots both primary introductions and as hubs for secondary transfers.

The findings of so many new records for NIS in marinas certainly suggests recreational boats/biofouling as the main vector of introduction, but other vectors may also contribute NIS to marinas, especially if they are in close proximity, such as major harbours and aquaculture sites. To address this knowledge gap, this study which also sampled boat-hulls for NIS provides additional proof for the strength of the recreational boating vector due to many cases of NIS found on visiting vessels, not yet present in those marinas or in the countries. Additionally, through also surveying the boaters on their recent travel history, it was learned most likely where these vessels picked up these NIS for transport, adding fresh insight on this topic (Ulman et al. 2017).

This study also provides much-needed knowledge in the Mediterranean context on which abiotic factors influence NIS richness in marinas; and while a few of these factors have previously been tested elsewhere, they have never been tested with such a large sample size of marinas and with this many NIS (74 NIS in total were tested), thus making the multivariate analyses performed to be robust. Additionally, many of the factors tested here were novel to bioinvasion research (i.e., climate type, presence of shipyard in marina, proximity to freshwater source), and also unique to the Mediterranean basin (i.e., proximity to the Suez Canal).

The chapter on boating revealed very interesting information. The majority of boats do apply antifouling once per year; also the majority of boaters can be considered very frequent travelers, and most of their boats host NIS species. Also there was a strong correlation between marinas and the boats within those marinas to both have high NIS richness, therefore these marinas can be considered having a high risk for future spreading NIS and are in need of frequent monitoring. It was also deduced that the biofouling process can occur very rapidly, especially from the marinas having higher NIS richness. Niche areas are often missed during in water cleanings, and visual inspections of overall fouling percentages are not a suitable predictor for the risk level boats pose as many NIS are found hiding in the niche areas. A better predictor is to determine if boats have spent sufficient time in highly fouled marinas, and this work provides a good assessment of which marinas have higher fouling and also reveals why they are better hosts for NIS.

5.2 Management Implications

Before an effective management strategy for combatting NIS can be designed, a thorough understanding of NIS settlement success, distribution, vectors of spread need be understood (Bax et al. 2001). This work provides a first assessment of non-indigenous fouling invertebrates in 50 Mediterranean marinas which reveals that the magnitude of the phenomenon can no longer be ignored and urgently calls for mandatory action. Also, understanding which factors affect distribution is fundamental for the both averting and managing the potential impacts of NIS (Simkanin et al. 2017).

The current global leaders in applying some form of management to the recreational boating vector are from Australia; due to its isolation, NIS are considered there a massive threat to biosecurity (Bulleri & Airoldi 2005; Bulleri & Chapman 2004; Ferrario et al. 2017), and Canada (which has suffered losses in Great Lakes mainly), which comparatively has very few marine NIS as compared to the Mediterranean (Simard et al. 2017) and an extremely generous budget dedicated to the issue (Brett van Poorten, pers. comm. 2017).

The European Guidelines suggest as part of their 'voluntary measures' that recreational vessels apply a new coating of antifouling paint at least once a year, which most vessels undertake anyways to avoid drag and decrease fuel consumption (IMO 2012). The results of this study clearly show that it can take as little as 2-3 months before NIS can establish on a newly painted vessel so this recommendation is insufficient. Therefore, it is highly suggested that vessels should have a dry professional cleaning before they visit a new country. There

are currently four methods for in water hull-cleaning (Floerl et al. 2010): brush systems, underwater jet, heat treatment and encapsulation, the latter two which are currently under development, but thus far, none are able to remove all of the biofouling in the niche areas.

Therefore, until the technology is improved upon, in water cleaning is not recommended alone to deter biofouling, as many boaters do this in marinas where marina personnel turns a blind eye, releasing many NIS propagules into the confined artificial habitat. As the Mediterranean Sea has 23 countries inside or surrounding it, management of this vector will require active participation and dialogue amongst countries in monitoring for new NIS. Based on the results of this research, it is highly recommended that at the very least, as a brave first step in tackling this issue would be that incoming vessels passing through both the Strait of Gibraltar and through the Suez Canal are visually inspected and hauled-out for a professional cleaning as necessary to deter further introductions.

5.3 Future Research Directions

As the influx of NIS continues to increase, so then should the resources contributing to the scientific discipline addressing this issue. However, In Europe, there is a major funding shortfall for marine bioinvasion research, despite it being one of the major stressors affecting local marine biodiversity and the relevant economy.

Routine monitoring does not have to be costly as some may imagine. In fact, this study was done on an extremely modest budget, where most of the attributed costs came from lodging. If sampling is completed by local scientists, then costs could be negligent. We denote the success of this project in finding appropriate experts help to identify all taxa groups, and encourage others to do the same.

Marine bioinvasions are poorly treated in the media, and when they are, they mainly deal with a few species which directly impact human health (poisonous or venomous fish and jellyfish), but environmental impacts of marine NIS are rarely reported in the news (Campbell et al. 2017; Marchini et al. 2017). As mentioned in Chapter 4, awareness of NIS for both citizens and boaters is almost non-existent except for the silver-cheeked toadfish in the Eastern Mediterranean and the new lionfish, due to its peculiarity which the media finds of interest. A study from Tasmania found that despite a general tendency for boaters to report a higher level of

awareness of non-indigenous species, most poorly understood the issue along with the threats they bring (Campbell et al. 2017).

The lessons learned here can potentially be used to help marina design and ecological engineering in the future to reduce the likelihood of NIS establishment (Carlton, Ruiz 2005). For example, since it was found that NIS richness in marinas was mainly dependent on the following combination of factors (proximity to Suez Canal, proximity to shipping ports, minimum temperatures, etc.), it is recommended that at the very least, the marinas at the north end of the Suez, and its most popular hubs are routinely monitored by trained staff to be able to promptly advise interested parties on newcoming NIS, especially those thought to be invasive.

5.3.1 Future outputs from this research

An additional aim from this study is to create a 'field identification guide for NIS in the Mediterranean' using photos of Mediterranean NIS collected from this research to assist others on completing similar and comparable assessments in the Mediterranean.

Some additional research directions which would be beneficial for a better understanding of this vector and/or its management include (but are not limited to) the following:

5.3.2 Taxonomy and identification

- Clarify native ranges for the many cryptogenic species currently in the Mediterranean, many of which are likely non-indigenous; and
- Build online citizen science apps or websites with experienced researchers helping to catalogue biofouling species.

5.3.3 Marina as hot spots for spreading

- Frequent assessments of marinas with high NIS richness to better understand the sequence and spreading of NIS;
- Marinas in countries in closer proximity to the Suez Canal (i.e., Lebanon, Syria, Palestine, Tunisia and Egypt) should be properly investigated for NIS;
- Using the abiotic factors found here to influence NIS distributions, build a model which estimate NIS richness in southern Mediterranean marinas; and
- Install several long-withstanding underwater video cameras and/or settlement plates at various places in marinas at the north end of the Suez Canal.

5.3.4 Boating as a vector for spread

- A study that tests the sonic boom method of antifouling and its effects on particular types of NIS settlement is of great interest;
- Attachment and drag of the worrisome known local 'invasive' NIS in the Mediterranean should be better studied;
- Build a model of 'spatial connectivity' using boaters popular travel routes along with NIS found in those marinas;
- Build a future scenario model of likely NIS distributions for 5 and 10 years into the future;
- Monitor incoming vessels to the Mediterranean at the tip of the Suez Canal and Strait of Gibraltar; and
- Another possibility for future monitoring would be to use environmental DNA (e-DNA) combined to metabarcoding techniques to determine which species are present in either the biofouling of vessels, or in marinas. At present, this can only test a small set of species, which need to already have their genomes sequenced.

5.4.1 Scientific significance

Chapter 2 demonstrates the importance of marinas at hot spots for NIS, with 27 as the most numerous NIS ever recorded in one artificially created locality. The chapter also reports over 50 new country records for NIS, along with taxonomic characters used to identify each species and photographs to help other scientists correctly identify these species. All these records are to be deposited in the WORMS database to improve on the known distributions of each species and make this data available to the scientific community. Additionally, the chapter demonstrates part of the bioinvasion process showing dozens of examples of recreational boats carrying new NIS taxa, with the chance of seeding these marina under optimal environmental conditions.

Chapter 3 exemplifies the strong importance of the Suez Canal in influencing both NIS success in individual marinas and affecting similarities between marinas. While due to its anthropogenic creation, the Suez Canal is a vector of transfer itself, but it also can expedite increased introductions through increased shipping traffic via ballast water and biofouling. Environmental matching especially due to sampled water temperature and average primary productivity were found to affect community similarities between marinas whereas proximity to other vectors and water temperature were found to influence individual success. These results can be applied to help direct future management since it shows that marinas near other vectors present a higher risk of having more NIS, and that vessels coming from regions with similar environmental conditions likely have a higher chance of establishing in the new marina, thus showing how to screen vessels on entrance to a new marina or region and direct them to be professionally cleaned as necessary.

Chapter 4 reveals a great deal of indispensable information about the recreational boating sector and its role and/or risk level in the spread of NIS in the Mediterranean, which was much needed as no data has previously been collected here on this sector. Most alarmingly, over 2/3 of sampled boats were hosting NIS, however, boaters are generally unaware of the issue and that they are contributing to the issue. Moreover, all sampled boats in marinas closest to major aquaculture regions and most vessels next to commercial shipping ports were highly infected with NIS. This shows that major vectors in close proximity to each other exacerbate the issue by increasing the available pool of NIS; whereas aquaculture sites and commercial shipping ports are known introduction sources, both commercial shipping ports and recreational marinas provide the means to transfer the NIS wherever they may go. Thus, marinas in close proximity to other major vectors, and the boats therein should be considered of higher-risk and have tougher screening applied to them when vessels are traveling from these sites. There is a strong relationship between marinas with high NIS richness and boats

within those marinas having higher NIS richness, with frequency of travel also showing an influence showing that time spent in festered areas increase the chance and hence risk level or spreading NIS. Time since last professional and in-water cleaning were also strong influences for higher NIS richness on boats, and also the visual fouling estimates proved to be good predictors of higher NIS richness, but care must be taken to inspect niche areas as well, as even a little clump can host a community. Also, quite a few interesting cases are presented where NIS colonize a boat either shortly after a professional cleaning signifying rapid NIS growth or very shortly after an in-water cleaning showing that the latter is not effective at getting rid of NIS.

5.4.2 Study answers

Here, the main research questions presented in Chapter 1 are answered here from an analysis of the study results.

1. Are Mediterranean marinas hotspots for marine bioinvasions?

Mediterranean marinas are certainly hotspots for marina bioinvasions. Evidence of this is provided by Port Heraklion in Crete hosting a total of 27 NIS. From a review of published research, this marina appears to be the most NIS ever recorded in one artificial locality. Additionally, NIS were recorded in all sampled marinas with a higher incidence in the eastern and central portions of the marina, and declining in the western region.

2. Which NIS are present on boat-hulls, and do these differ from the NIS found in the same marinas?

While the most abundantly found NIS were common to both marinas and boats, there are many examples of NIS on boats which were not yet present either in the marina or even yet in the country (See the 19 species this applies to in Table 2.3) providing ample evidence for the introduction of new NIS via recreational boats. These special cases should be used for subsequent monitoring determine if these NIS fouling species are successful in colonizing the marina substrate in the future, and can be included in future baseline assessments.

3. Do recreational boats in the Mediterranean carry a substantial amount of NIS?

It was very surprising to find that 75% of all sampled vessels were found to host NIS species. The highest amount of NIS found on one boat-hull was 8, which was recorded from several boats from both the Eastern and Western Mediterranean.

4. Which abiotic factors (or combinations thereof) contribute to total NIS richness in individual marinas?

The following factors were significant in shaping NIS richness in marinas: sea surface temperature, number of berths, proximity to Suez Canal, proximity to aquaculture sites, proximity to commercial harbours, absence of pontoons, biogeographic sector and climate type.

5. Which underlying factors shape similar NIS distribution patterns between marinas across the Mediterranean?

Here, environmental matching played the dominant role (i.e., mainly water temperature, biogeographic region, primary productivity) along with proximity to the Suez Canal.

6. Which marinas or subregions present the greatest risk for the additional spreading of alien species to new localities?

The factors found to shape similarities of NIS assemblages across marinas contrasted from the previous results, owing almost entirely to environmental factors rather than proximity to known vectors of introduction; here a combination of temperature, primary productivity, biogeographic region, climate type and additionally proximity to the Suez Canal were found to be significant influences.

7. Which factors influence boats to have higher species richness in their biofouling composition?

The factors found to influence some boats to host higher NIS richness's than others pertained to if they were found in marinas also containing a high number of NIS, and also frequency of travel, alluding to increased biofouling on boat-hulls being influenced by time spent in such marinas

tainted with many NIS, which gives rise to more opportunities for settlement and spreading to occur. Boat length, time since last antifouling application and last in-water cleaning, along with average cruising speed, and visible fouling estimates were all found to be contributing factors towards higher NIS on boats, which can be topics used to screen incoming vessels.

8. Are boaters cleaning and painting their boats often enough to prevent the growth of biofouling?

According to the current guidelines to prevent the spread of biofouling organisms, the major recommendation is that boats apply new antifouling coatings to their boat-hulls once per year. The vast majority of surveyed boaters (72%) are indeed already doing this as it is the norm to apply a new coating at the commencement of each boating season. However, this study has shown that many boats can become infested with NIS in as little as six weeks, so this current recommendation is certainly not adequate in deterring the spread of NIS in the Mediterranean from recreational boats.

9. Does increased boat travel relate to higher NIS richness on boats?

While increased boat travel in terms of number of days spent away from home marina was found to correlate to higher NIS richness, the number of visited marinas did not. This shows that time is important as it provides a higher likelihood of a vessel becoming colonized with new NIS, but visiting more marinas does not have the same likelihood as the season has to be favourable for reproductive events and the marina has to have different NIS than the vessel.

10. What are boaters awareness levels of non-indigenous species?

Despite boaters paying sometimes exorbitant amounts of money to rid their boat hulls of biofouling growth preceding new antifouling applications nearly each year, most boaters are generally unaware that the biota they host usually contain NIS and that they are contributing to the risk of spreading NIS. Boaters are more aware of a few certain cases that have been popularized in the media of their countries, such as *Caulerpa taxifolia* in France and *Lagocephalus sceleratus* in the Eastern Mediterranean. Education of NIS transport and its associated environmental, economic, and health

risks urgently needs to be initiated in recreational marinas, which can start with simple catchy awareness posters to engage the average boater.

11. What recommendations can this research give for future management of this vector?

As prevention is considered the best key for non-indigenous species since they are nearly impossible to eradicate once they have established in the marine realm, this response is divided into two parts. The first is a response in how to prevent additional spreading within the Mediterranean once a species is already present, and the other refers to how to prevent or reduce new introductions into the Mediterranean basin.

To reduce the level of further spreading within the basin, here I recommend that marinas especially in the eastern portion of the basin, along with marinas in close proximity to other major vectors such as aquaculture sites and major shipping ports undergo routine monitoring to detect new introductions. This monitoring should be completed at least once annually and preferably in peak summer months, when fouling populations are most successful and abundant. A concise Mediterranean key identification book needs to be first made available to help educate non specialists to undertake this work, although several taxa will always require verification from experts due to their size and/or confusion with other species, which applies especially to crustaceans, ascidians, bryozoans and molluscs. Perhaps a few key experts should routinely be used to send samples to avoid misidentification. Additionally, incoming boats to new marinas should be screened to determine firstly (a) if they had visited any marinas considered of high-risk (situated in close proximity to other vectors or already with many known NIS), and secondly (b) if they have any fouling with great care to also inspect the niche areas, and if there is either a positive response to (a) or to (b), the boats should be professionally cleaned either out of the water or in water using an extremely high-pressured in-water cleaning technique using a special quarantined area which would reduce the costs associated with the boat haul-out and haul back into the water. This extra cost would not be appreciated the boaters, and would likely have to be imposed as an mandatory regulation and perhaps subsidized as a new form of environmental tax.

To prevent new introductions from entering the Mediterranean basin, this solution at a first glance seems much more controllable as the basin is arguably a totally enclosed system with the exception of a couple extremely narrow entrances. However, political collaboration between a few countries would be necessary which may necessitate formal regulations imposed by a global organization such as the International Maritime Organization or the United Nations. As per the

entrance routes, the European Canals are not considered a threat for marine bioinvasions as they are comprised of freshwater rivers, dams and locks which all act as quarantines inhibiting the survival of marine NIS during the passage. The only other entrances from the North are through the Turkish Straits connecting the Black Sea to the Mediterranean Sea, which include the Bosphorus Strait and the Dardanelles; here the Black Sea and the Bosphorus Strait have an average salinity nearly half that of the Mediterranean so only select species with wider salinity niche tolerance ranges have the possibility of survival (i.e., barnacles), but boats coming from this route should be carefully inspected for new NIS. Next there is the anthropogenically created Suez Canal which is an extremely dangerous vector on its own, as it bridges the Mediterranean to the Red Sea and its seemingly endless supply of biota of Indo-Pacific origins, which is the area hosting the most marine biodiversity on the planet. Due to its recent expansion, the volatility of this vector and its history of colonizing the Eastern Mediterranean, the greatest care should be placed here where all incoming vessels to the Mediterranean need be screened for biofouling and if any is detected, either professionally cleaned placed or placed in a freshwater quarantine, not yet an option but an ingenious idea. If the Egyptian government was to place a freshwater lock at the northern end of the Suez, this would greatly reduce the chances of transferring non-native biota from the Red Sea to the Mediterranean. Lastly, there is the Strait of Gibraltar, also very narrow, however shared by Morocco and Gibraltar. Boat (hulls_ entering through this route should be visually inspected either underwater or by pole-cam (camera attached to a pole to submerge underwater) and mandatorily cleaned if any fouling is detected.

5.4.2 Conclusions

Non-indigenous species are considered a huge threat to the marine realm which can negatively affect native biodiversity, ecosystems and even human health. As globalization continues to intensify (Bax et al. 2003), climate change continues to make the Mediterranean and Red Sea more alike, a pattern to be aggravated by the most recent 2015 expansion of the Suez Canal (Meyerson & Mooney 2007), resulting in likely a future homogenization of the basin. Before NIS can be properly managed, the scale of marine invasions must first be understood, which this research has substantially contributed to. The number of NIS detections in the Mediterranean continues to increase each year, and will continue to do so, especially after this research exposed some concentrated localities and an extensive amount of new records found both in marinas on recreational vessels. This research signifies the importance of marinas as hotspots in need of sequential monitoring to be able to continue to map the spread of many worrisome species so that any taxa that are deemed 'invasive' (i.e., having negative effects on human health or the economy) can be frequently monitored.

This research demonstrates that Mediterranean marinas host many more NIS than previously imagined, and that recreational boating is the most likely culprit for transporting many of these NIS to new marinas, especially as approximately 2/3 of the NIS identified in marinas were also found in the biofouling assemblages on the boat-hulls. Interestingly, approximately half of the total NIS species identified here lack larval stages, which displays the strength of the biofouling vector via recreational boating. Thus, in the Mediterranean context, recreational boating should now be considered a major vector for the spread of alien species, in need of appropriate effective management measures. This work also demonstrates the pivotal role that recreational boats play in supplying new propagules to marinas they are visiting, especially in the 20 cases of boats documented here hosting NIS not yet found in the corresponding marina and in many cases not even the country. Nearly ¾ of all boats inspected here were found to host NIS species, with even a higher proportion in the Western Mediterranean, which is an astounding result. Additionally, the majority of these Mediterranean-faring vessels are quite active both in the extent of their travel routes and in their duration of stays, exposing their risk level to be high in the possibility of facilitating subsequent spreading. It is now also understood that in the Mediterranean, proximity to other major vectors (the Suez Canal, commercial ports and aquaculture sites) in combination with sea surface temperature are the most influential factors affecting NIS richness in marinas, whereas community similarities between marinas were more influenced by environmental matching in addition to the Suez Canal vector. As each subregion in the Mediterranean is unique, management initiatives will have to be carefully sculpted here to inhibit further spreading and to deter the onslaught of further invasions, especially due to the Mediterranean basins nearly totally enclosed structure, the sheer volume of vessel traffic and the additional stressors being brought on by global change making the Mediterranean and the Red Sea more akin.

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RESUME EN FRANCAIS

Les écosystèmes marins sont bouleversés par de nombreux phénomènes tels que la surpêche, la pollution, le changement climatique et les espèces invasives, dont les impacts conjugués affectent négativement leurs structures et fonctionnements. Il est urgent d'évaluer le rôle de la navigation de plaisance comme facilitateur des invasions biologiques, et particulièrement pour la mer Méditerranée qui accueille deux tiers du trafic mondial de bateaux affrétés, et constitue le point chaud de la problématique des espèces non indigènes. Cette thèse se propose de combler ce besoin en accomplissant la première étude du rôle de la navigation de plaisance dans la propagation des ENI, par le bio-encrassement des marinas et coques de bateaux, à l'échelle du bassin méditerranéen. Une recherche minutieuse d'ENI a été conduite dans 34 marinas à travers la Méditerranée (s'étalant de l'Espagne à la Turquie), en ciblant les macro-invertébrés pour déterminer si les marinas constituaient bien des points chauds en ENI. Puis, des entretiens ont été conduits avec des propriétaires et/ou capitaines sur les caractéristiques de leurs bateaux, dont les opérations de nettoyage de la coque, les peintures, et leurs historiques de trajets récents. Des échantillons biologiques de bio-encrassement ont ensuite été collectés sur environ 600 bateaux, pour lesquels le capitaine/propriétaire avait été interrogé, afin de corrélérer les deux sources d'information. Les résultats de cette évaluation des marinas à l'échelle de la Méditerranée ont ensuite été combinés avec des données existantes sur les ENI présents dans les marinas italiennes, portant le nombre de marinas échantillonnées à 50. L'ensemble de ces données a été utilisé dans des analyses statistiques multivariées afin d'identifier les principaux facteurs abiotiques contribuant à la richesse en ENI et les similarités entre les différentes marinas.

Mots clés : [espèces envahissantes: espèces non indigènes (ENI): navigation de plaisance: marina: Méditerranée: vecteur]

ENGLISH ABSTRACT

Recreational boating as a major vector of spread of alien species around the Mediterranean

Many stressors, such as climate change, overfishing, pollution and biological invasions, are currently devastating the marine domain. The role of recreational boating in facilitating marine bioinvasions urgently necessitated a proper evaluation, especially in the Mediterranean Sea which hosts 2/3 of global charter boat traffic and is also *the* global hotspot for alien species. This study addresses this shortfall by completing the first-ever Mediterranean basin-wide study investigating the influence of recreational boats in the transfer of NIS from biofouling both in marinas and from boat-hulls. First, a thorough investigation of NIS was conducted in 34 marinas across the Mediterranean (spanning from Spain to Turkey), targeting benthic macroinvertebrates. All marinas were found to host NIS, ranging from 2 to 27 per marina. This first output of this research provides a massive update of new NIS records and updated species distributions for the Mediterranean, and presents three new species in the Mediterranean basin, 51 new NIS country records and 20 new subregional records, which can now be fed into models and databases to gain a better comprehension of the composition and scale of NIS colonizing marina habitats. It was realized that almost 80% of sampled fouled vessels were found to host at least 1 NIS, while 11 was the maximum NIS found on one boat-hull. It was also found that recreational vessels visiting new marinas sometimes carry NIS not yet present neither in that marina nor in the country in which they are visiting, thus providing ample evidence of recreational boating supplying new NIS to marinas. The results of this large-scale Mediterranean marina assessment were combined with other existing data on NIS in Italian marinas for a total sample size of 50 marinas, which were then used to feed both univariate and multivariate statistical tests aimed at identifying which abiotic factors mainly contribute to total species richness of NIS in marinas and also which factors contribute to similar NIS assemblages between marinas. The results revealed that a higher species richness of NIS in Mediterranean marinas was influenced by the following

factors: water temperatures above 25°C, a higher number of berths, absence of floating pontoons, proximity to the Suez Canal and proximity to commercial harbours. Whereas the similarities between NIS assemblages amongst marinas were more influenced by environmental factors such as temperature, biogeographical region, climate type, primary productivity and again proximity to the Suez Canal. The significance of the Suez Canal as a prominent factor in both analyses coincides with the general trend of higher total NIS found in the Eastern Mediterranean strongly influencing NIS distributions. The results presented within this thesis, adding to those marinas surveyed from around the world, form a robust case that recreational boating provides an extremely important pathway in facilitating primary NIS introduction events and their associated secondary spread to other coastal areas as 'stepping stone' habitats.

Keywords : [invasive species, non-indigenous species NIS, recreational boating, marina, Mediterranean, vector]