João Pedro da Silva Encarnação

The new aquatic fauna of southern Portugal and their potential as economic resources: A collaborative merge between science and society

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DECLARATION OF AUTHORSHIP

"The new aquatic fauna of southern Portugal and their potential as economic resources: A collaborative merge between science and society"

I declare that I am the author of this work, which is original and unpublished. The authors and works consulted are duly cited in the text and appear in the list of references.

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

João Encarnação

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ABSTRACT

Biological invasions are among the most challenging ecological and conservation riddles of our times, but still need to be faced with more pragmatism. Promote prevention, eradicate what can be eradicated, control and manage through time what is financially feasible, and innovate when it comes to deal with commercially valuable invasive species. This thesis aimed at merging traditional science with contributions from society, through citizen science, to track the spread, abundance, and putative impacts of non-indigenous marine species in south Portugal, but also evaluate the opportunities and constraints of integrating invasive species in the blue economy. During this four-year period, 8 non-indigenous species with invasive characteristics and 33 neonative species of subtropical origins were studied. Special attention was given to the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 because its invasiveness nature became evident during the development of this thesis. Thus, an in-depth study of its impacts on the food web was made, which disclosed their predatory behaviour upon fundamental prey of the Guadiana estuary food web, including native fish, bivalves, and other crustaceans. The citizen science campaign named New Marine Species of the Algarve (NEMA – Novas Espécies Marinhas do Algarve) has been a huge success and relied on the contributions of 260+ citizen scientists, 700+ validated records, belonging to 50+ marine species. Additionally, the commercial value of the Atlantic blue crab and weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) was evaluated in both sides of the Atlantic. A SWOT analysis evidenced that introducing widely distributed marine invasive species into the blue economy, if properly planned, can become an opportunity to support ecosystem and native resources management but also boost economic development in the less developed regions of Portugal, as are the areas surrounding the Guadiana River basin.

Keywords: biological invasions; neonative species; tropicalization; citizen science; blue economy

RESUMO

As invasões biológicas são um dos maiores desafios ecológicos e de conservação dos nossos tempos, mas ainda precisam de ser encaradas com mais pragmatismo. Promover a prevenção, erradicar o que pode ser erradicado, controlar e gerir ao longo do tempo o que é financeiramente viável e inovar quando se trata de lidar com espécies invasoras com valor comercial. Esta tese teve como objetivo fundir a ciência tradicional com contributos da sociedade, através da ciência cidadã, para monitorizar a dispersão, abundância e impactos putativos de espécies marinhas não-indígenas no sul de Portugal, mas também avaliar as oportunidades e constrangimentos da integração de espécies invasoras na economia azul. Durante este período de quatro anos, foram estudadas 8 espécies não-indígenas com características invasoras e 33 espécies neonativas de origem subtropical. Foi dada especial atenção ao caranguejo azul *Callinectes sapidus* Rathbun, 1896 pois o seu carácter invasor tornou-se evidente durante o desenvolvimento desta tese. Assim, foi efetuado um estudo aprofundado dos seus impactos na teia alimentar e revelado o seu comportamento predatório sobre presas fundamentais da teia alimentar do estuário do Guadiana, incluindo peixes nativos, bivalves e outros crustáceos. A campanha de ciência cidadã denominada Novas Espécies Marinhas do Algarve (NEMA) foi bastante bem-sucedida e contou com a contribuição de +260 cientistas cidadãos, +700 registos validados, pertencentes a +50 espécies marinhas. Adicionalmente, o valor comercial do caranguejo azul e da corvina americana *Cynoscion regalis* (Bloch & Schneider, 1801) foi avaliado nos dois lados do Atlântico. Uma análise SWOT evidenciou que a introdução na economia azul de espécies marinhas invasoras já amplamente distribuídas, se devidamente planeada, poderá ser uma oportunidade para uma melhor gestão dos ecossistemas e recursos nativos, mas também para impulsionar o desenvolvimento económico de regiões menos desenvolvidas de Portugal, como são as áreas circundantes da bacia do Guadiana.

Palavras-chave: invasões biológicas; espécies neonativas; tropicalização; ciência cidadã; economia azul

RESUMO ALARGADO

As invasões biológicas são um dos maiores desafios ecológicos e de conservação dos nossos tempos, mas ainda precisam de ser encaradas com mais pragmatismo. Promover mecanismos de prevenção e deteção precoce, erradicar o que pode ser erradicado, controlar e gerir ao longo do tempo o que é financeiramente viável e inovar no caso de espécies invasoras que podem proporcionar algum tipo de rendimento se forem integradas na economia. Esta tese teve como objetivo fundir a ciência tradicional com contributos da sociedade, através da ciência cidadã, de forma a monitorizar a disseminação, abundância e impactos putativos de espécies marinhas não-indígenas no sul de Portugal, mas também avaliar as oportunidades e constrangimentos da sua integração na economia azul. Durante este período de quatro anos, foram estudadas 8 espécies não-indígenas com características invasoras e 33 espécies neonativas de origem subtropical. Isto foi conseguido com campanhas de amostragem utilizando armadilhas bentónicas iscadas, redes de arrasto de fundo, censos visuais em mergulho, arrastos noturnos de plâncton, mas também com a implementação de uma campanha de ciência cidadã denominada Novas Espécies Marinhas do Algarve (NEMA).

As amostragens realizadas entre 2019 e 2022 resultaram na confirmação da presença de 7 espécies não-indígenas com características invasoras, como por exemplo, o caranguejo azul *Callinectes sapidus* Rathbun, 1896, o camarão *Palaemon macrodactylus* Rathbun, 1902, ou a medusa do mar Negro *Blackfordia virginica* Mayer, 1910, bem como os primeiros registos de mexilhão asiático *Arcuatula senhousia* (W. H. Benson, 1842) no sul de Portugal. Os censos visuais subaquáticos identificaram 13 espécies que podem ser classificadas como neonativas para Portugal continental, sendo um reflexo do processo de tropicalização das regiões temperadas. Algumas destas espécies foram já detetadas no Algarve, mas a maioria tinha somente sido reportada em literatura cinzenta. Registos de espécies como o rascasso da Madeira *Scorpaena maderensis* Valenciennes, 1833, o bodião *Thalassoma pavo* (Linnaeus, 1758), ou o verme-de-fogo *Hermodice carunculata* (Pallas, 1766), não tinham ainda sido

publicados em artigos científicos antes do decorrer desta tese de doutoramento. Os arrastos de plâncton evidenciaram também esta influência na costa Algarvia, nomeadamente com o caranguejo-ervilha africano *Afropinnotheres monodi* Manning, 1993 e o caranguejo-da-lama africano *Panopeus africanus* A. Milne-Edwards, 1867, que foram os dois taxa de Brachyura mais abundantes nos estuários do Arade e Guadiana nas amostragens de 2020.

Foi dada especial atenção ao caranguejo azul pois o seu carácter invasor tornou-se evidente durante o desenvolvimento desta tese. Com recurso a armadilhas bentónicas, foi possível desvendar, por exemplo, que a presença de juvenis aumenta durante os meses de inverno em especial no médio estuário do Guadiana, quando a salinidade é mais baixa devido ao maior caudal do rio, o que se explica pelas migrações para montante que realizam durante a fase juvenil. O movimento inverso para jusante, realizado por fêmeas aquando da desova, foi confirmado pelos registos de ciência cidadã NEMA. Foi também efetuado um estudo aprofundado sobre os impactos ecológicos do caranguejo azul, revelado o seu comportamento predatório sobre presas fundamentais da teia alimentar. Os dados de metagenómica mostraram que exploraram um conjunto diversificado de fontes alimentares, com destaque para peixes, moluscos e crustáceos, incluindo camarões, cracas e outras espécies de caranguejos. O seu caracter omnívoro foi evidente na dispersão das assinaturas isotópicas. Os dados de isótopos estáveis mostraram que peixes, camarões e bivalves foram as principais fontes de matéria orgânica que sustentam a biomassa do caranguejo azul no estuário do Guadiana. A sua posição trófica (4.3 ± 0.5) está entre as mais elevadas encontradas na zona não-nativa, o que pode ser explicado por uma alimentação baseada sobretudo em presas animais. A comparação das posições tróficas mostrou uma sobreposição considerável de nicho com o caranguejo verde europeu *Carcinus maenas* (Linnaeus, 1758) (30,13 ± 5,21%), o qual ainda assim apresentou uma amplitude de nicho potencialmente maior que o caranguejo azul.

A campanha de ciência cidadã NEMA foi excecionalmente bem-sucedida, apesar de ser uma ferramenta de baixo custo, que contou com a contribuição de +260 cientistas cidadãos, obteve +700 registos validados relativos a +50 espécies marinhas. Cerca de 40% dos registos foram relativos ao caranguejo azul, sobre o qual foi possível rastrear a sua dispersão ao longo da costa algarvia até Sagres e costa Oeste de Portugal. O NEMA contribuiu também para documentar o processo de tropicalização da biodiversidade marinha de Portugal Continental. O NEMA detetou 11 espécies neonativas cuja presença na região Algarvia, tanto quanto é do nosso conhecimento atual, ainda não tinha sido publicada em artigos científicos. Algumas espécies podem estar estabelecidas há alguns anos ou mesmo décadas, mas por outro lado,

alguns "recém-chegados", como o verme-de-fogo *Hermodice carunculata* ou o bodião *Thalassoma pavo*, aparentam estar a estabelecer-se.

O valor comercial do caranguejo azul e da corvina americana *Cynoscion regalis* (Bloch & Schneider, 1801) foi também avaliado nos dois lados do Atlântico. Uma análise SWOT evidenciou que a introdução na economia azul de espécies marinhas invasoras já amplamente distribuídas, se devidamente planeada, pode tornar-se numa oportunidade para apoiar a gestão dos ecossistemas e recursos nativos, mas também impulsionar o desenvolvimento económico de regiões menos desenvolvidas de Portugal, como são as áreas circundantes da bacia do Rio Guadiana. Uma gestão integrada destas, e outras, espécies invasoras através da sua apanha, poderá também contribuir para a Agenda 2030 das Nações Unidas para o Desenvolvimento Sustentável, nomeadamente para reduzir a pobreza, fome e melhorar a segurança alimentar (Objetivos 2 e 3), mas também promovendo a inovação no uso destas espécies invasoras, com uma utilização sustentável dos oceanos, mares e recursos marinhos nativos (Objetivos 9 e 14).

Durante o período desta tese de doutoramento, foram também feitos contributos importantes para grupos de peritos sobre invasoras, nomeadamente i) contribuição para os relatórios portugueses anuais do Grupo de Trabalho sobre Introdução e Transferência de Organismos Marinhos (WGITMO) do Conselho Internacional de Exploração do Mar (ICES), ii) parceiros de dados da Rede Europeia de Informação sobre Espécies Exóticas (EASIN) através do NEMA, iii) co-autor do plano de ação para o controlo do caranguejo azul, medusa do mar Negro, e corvina americana, em Portugal, no âmbito do projeto ALFCORAZUL (Fundo Ambiental).

LIST OF PAPERS

The following manuscripts were published, or are under preparation, as a result of this Ph.D. thesis.

Paper I (Chapter 2)	**Encarnação J**, Morais P, Baptista V, Cruz J, Teodósio MA (2019). New evidence of marine fauna tropicalization off the southwestern Iberian Peninsula (Southwest Europe). *Diversity* 11 (48). doi: 10.3390/d11040048.
Paper II (Chapter 2)	**Encarnação J**, Teodósio MA, Morais P (accepted). The arrival of a non-indigenous ecosystem engineer to a heavily invaded and flow-regulated estuary in Europe. *BioInvasions Records*.
Paper III (Chapter 3)	**Encarnação J**, Dias E, Engelen AH, Serrão E, Teodósio MA, Morais P (in preparation). Atlantic blue crab keeps native omnivory strategy on its path to invasion success, as disclosed by metagenomic and isotopic data.
Paper IV (Chapter 4)	**Encarnação J**, Teodósio MA, Morais P (2021). Citizen science and biological invasions: A review. *Frontiers in Environmental Science* 8:602980. doi: 10.3389/fenvs.2020.602980.
Paper V (Chapter 4)	**Encarnação J**, Baptista V, Teodósio MA, Morais P (2021). Low-cost citizen science effectively monitors the rapid expansion of a marine invasive species. *Frontiers in Environmental Science* 9:752705. doi: 10.3389/fenvs.2021.752705.
Paper VI (Chapter 4)	**Encarnação J**, Krug LA, Teodósio MA, Morais P (2022). Coastal countercurrents increase propagule pressure of an aquatic invasive species to an area where previous introductions failed. *Estuaries and Coasts* 45: 2504–2518. doi: 10.1007/s12237-022-01092-8.
Paper VII (Chapter 5)	**Encarnação J**, Teodósio MA, Morais P (in preparation). Marine invasive species in a blue economy: Opportunities and constraints in Portugal.

Related manuscripts published during the course of this Ph.D. thesis.

Paper VII	Vasconcelos P, Carvalho AN, Piló D, Pereira F, **Encarnação J**, Gaspar MB, Teodósio MA (2019). Recent and consecutive records of the Atlantic blue crab (*Callinectes sapidus* Rathbun, 1896): Rapid westward expansion and confirmed establishment along the southern coast of Portugal. *Thalassas: An International Journal of Marine Sciences* 35: 485-494. doi: 10.1007/s41208-019-00163-1.
Paper VIII	**Encarnação J**, Seyer T, Teodósio MA, Leitão F (2020). First record of the nudibranch *Tenellia adspersa* (Nordmann, 1845) in Portugal, associated with the invasive hydrozoan *Cordylophora caspia* (Pallas, 1771). *Diversity* 12 (214). doi: 10.3390/d12060214.
Paper IX	Baptista V, **Encarnação J**, Serrão EA, Wirtz P, Pestana LB, Faria S, Teodósio MA (2021). New records of fish species from the coast of Luanda, Angola. *Thalassas: An International Journal of Marine Sciences* 37: 803–811. doi: 10.1007/s41208-021-00297-1.
Paper X	Morais P, **Encarnação J**, Teodósio MA, Dias E (2021). Aliens from an underwater world. *Frontiers for Young Minds* 09:646539. doi: 10.3389/frym.2021.646539.
Paper XI	Chaouti A, Belattmania Z, Nadri A, Serrão EA, **Encarnação J**, Teodósio MA, Reani A, Sabour B (2022). The invasive Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its distributional range southward to Atlantic African shores: first records along the Atlantic coast of Morocco. *BioInvasions Records* 11(1): 227–237. doi: 10.3391/bir.2022.11.1.23.
Paper XII	González-Ortegón E, Berger S, **Encarnação J**, Chairi H, Morais P, Teodósio MA, Oliva-Paterna J, Schubart CD, Cuesta JA (2022). Free pass through the Pillars of Hercules? Genetic and historical insights into the recent expansion of the blue crab *Callinectes sapidus* west and east of the Strait of Gibraltar. *Frontiers in Marine Science* 9:918026. doi: 10.3389/fmars.2022.918026.
Paper XIII	Oficialdegui FJ, Zamora-Marín JM, Guareschi S, Anastácio PM, García-Murillo P, Ribeiro F, Miranda R, Cobo F, Gallardo B, García-Berthou E, Boix D, Arias A, Cuesta JA, Medina L, Almeida D, Banha F. Barca S, Biurrun I, Cabezas MP, Calero S, Campos JA, Capdevila-Argüelles L, Capinha C, Casals F, Clavero M, **Encarnação J**, Fernández-Delgado C, Franco J, Guillén A, Hermoso V, Machordom A, Martelo J, Mellado-Díaz A, Morcillo F, Oscoz J, Perdices A, Pou-Rovira Q, Rodríguez-Merino A, Ros M, Ruiz-Navarro A, Sánchez MI, Sánchez-Fernández D, Sánchez-González JR, Sánchez-Gullón E, Teodósio MA, Torralva M, Vieira-Lanero R, Oliva-Paterna FJ (2023). A horizon scan exercise for aquatic invasive alien species in Iberian inland waters. *Science of the Total Environment* 869: 161798. doi: 10.1016/j.scitotenv.2023.161798.

Paper XIV	Costa E, **Encarnação J**, Teodósio MA, Morais P (2023). Aquatic species shows asymmetric distribution range shifts in native and non-native areas. *Frontiers in Marine Science* 10:1158206. doi: 10.3389/fmars.2023.1158206.
Paper XV	Martins M, Abecasis D, Abrantes F, Aires T, Rautenbach S, Brahim K, Ebaye S, **Encarnação J**, Engelen AH, Gandega C, Magalhães V, Barusseau J, Freiwald A, Serrão E, de los Santos CB, Santos R (submitted). First assessment of sedimentary chronostratigraphy and eDNA analysis reveals potential blue carbon sources for the last centuries at Arguin island (Mauritania). *Biogeochemistry*.

TABLE OF CONTENTS

CHAPTER 1

General introduction

1.1 Non-indigenous species

The transport of species beyond their native area is happening for millennia since humans began travelling long distances (Hulme 2009), with iconic examples including the introduction of the crested porcupine *Hystrix cristata* Linnaeus 1758 by the Roman Empire into Italy (Trucchi and Sbordoni 2009). Globalization of trade may have started as early as the third millennium BCE with explorers connecting the Mediterranean Sea and Indian Ocean by the "silk roads" (Pollard 2014), but only boomed since the late fifteenth and early sixteenth centuries with the onset of intercontinental maritime trade and exchange of numerous commodities, including edible plants and domestic animals (Su 2013). During that time, maize, potato, and tomato were taken from South America to Europe, while chicken, pig, apple, coffee, and rice were transported to the Americas (Su 2013). The amount of non-indigenous species introduced both unintentionally and deliberately increased exponentially after the industrial revolution, particularly during the second half of the twentieth century (Hulme 2009; Keller et al. 2011; Seebens et al. 2017; Pyšek et al. 2020). Over the years, the lexicon used to refer to non-indigenous species has also increased, so numerous synonyms are found in the literature, including exotic, non-native, or alien species. A non-indigenous species is only classified as invasive, when it establishes itself successfully, spreads, the abundance increases, and causes ecological, economic, or public health impacts in the non-native range (IUCN 2000; Simberloff et al. 2005; Pyšek and Richardson 2010). A recently coined term – neonative species – intends to classify range-expanding species responding to human-induced environmental change, as a rise in sea temperatures, but such expansion cannot be a result of direct human intervention, as intentional or unintentional translocations (Essl et al. 2019).

The dispersion of non-indigenous species into new areas of the non-native range can be parsed out in seven pathways (Hulme et al. 2008):

i. Intentional release as a commodity;
ii. Intentional release as a biological control agent;
iii. Unintentional escape while intentionally introduced as a commodity;
iv. Unintentional introduction as a contaminant of a specific commodity;
v. Stowaway and unintentionally introduced through a transport vector;
vi. Unintentional introduction in a new area by dispersal along a human infrastructure corridor;
vii. Introduction in a new area by natural unaided dispersal.

In aquatic environments, non-indigenous species have been unintentionally introduced through several vectors:

i. Hitchhiking on other species. Floating seaweeds, as *Sargassum horneri* (Turner) C.Agardh, 1820 (Chromista, Fucales), may transport a variety of epibiotic eukaryotes to new regions (Kim et al. 2019);
ii. Biofouling human structures (ships or rafting). Hull-fouling species are a significant source of new introductions, mainly for aquatic species in the Arthropoda, Cnidaria, and Mollusca phyla (Godwin 2003; Drake and Lodge 2007). Rafting on plastic debris is also an emergent vector of trans-oceanic introductions (Carlton et al. 2017);
iii. Ballast water. Commercial ships regularly collect and release large amounts of seawater that contains a huge diversity of bacteria, phytoplankton, zooplankton, and other organisms in their larval phase, including vertebrates (McCarthy and Crowder 2000; Lockett and Gomon 2001; Choi et al. 2005; Sun et al. 2010);
iv. Aquatic corridors. Through maritime corridors, as the Suez Canal that connects the Red and Mediterranean seas (Katsanevakis et al. 2013; Havel et al. 2015); or river canals, as occurs with multiple river canals in central Europe (Leuven et al. 2009). The Suez Canal became a continuous source of Lessepsian fishes such as the bluespotted cornetfish *Fistularia commersonii* Rüppell, 1838 (Animalia, Syngnathiformes), the silver-cheeked toadfish *Lagocephalus sceleratus* (Gmelin, 1789) (Animalia, Tetraodontiformes), or the lionfish *Pterois miles* (Bennett, 1828) (Animalia, Perciformes) into the Mediterranean Sea (Katsanevakis et al. 2013; Kletou et al. 2016; D'Amen and Azzurro 2020).
v. Aquarium trade. Vertebrates, invertebrates, and algae are often released or transported alongside ornamental species and then released into the environment (Odom and Walters 2014; Patoka et al. 2020).

Intentional introductions of aquatic non-indigenous species have frequently been linked to fisheries, either recreational or commercial, or to aquaculture activities. For example, the Louisiana crayfish *Procambarus clarkii* (Girard, 1852) and the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) (Animalia, Decapoda) were initially introduced in Europe to restore freshwater fisheries following the collapse of native crayfish species (Savini et al. 2020). Freshwater fish, as the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Animalia, Salmoniformes), zander *Sander lucioperca* (Linnaeus, 1758) (Animalia, Perciformes), or

largemouth bass *Micropterus salmoides* (Lacepède, 1802) (Animalia, Perciformes) were initially stocked in freshwater systems for sport fishing, but also produced for stock enhancement or food production (Molony et al. 2003; Savini et al. 2020). In European coastal zones, the non-indigenous Pacific oyster *Magallana gigas* (Thunberg, 1793) (Animalia, Ostreida) and the Manila clam *Ruditapes philippinarum* (Adams & Reeve, 1850) (Animalia, Venerida) were intentionally introduced in marine aquacultures. Still, they also served as vectors of numerous unintentional introductions of other invertebrates and algae fouling their shells, on packaging material, or as their parasites (Humphreys et al. 2015; Lallias et al. 2015; Savini et al. 2020). Once introduced, either unintentionally or intentionally, several factors and traits dictate the success of a non-indigenous species in a new ecosystem.

The tolerance to a wide range of environmental variables dictates if introduced individuals survive the first days/weeks in the new environment (Keller et al. 2011). A second key-characteristic is a combination of evolutionary advantages related to reproduction – high fertility, early age at first maturity, and fast growth (Jeschke and Strayer 2006; Pyšek and Richardson 2010). The resistance of the receiving community towards non-indigenous species is vital in shaping community structure and succession, by which the priority effects hypothesis is intertwined with the abovementioned traits, since disturbed communities' succession greatly relies on which species is established first (Belyea and Lancaster 1999; Young 2001). Either during early life stages (seeds, larvae) or as adults, a high dispersal capability and mobility may allow a non-indigenous species to rapidly expand its distribution and colonize new ecosystems (Hastings et al. 2005; Ferreras and Galetto 2010; Keller et al. 2011). These species rarely encounter co-evolving predators, parasites, or pathogens in the non-native range (i.e., enemy release hypothesis), increasing the chances for establishment and growth (Keane and Crawley 2002; Colautti et al. 2004). Through time, native species may evolve to deal with non-indigenous species, learning how to prey on them or to incorporate them on their life cycle, in a co-adaptative evolution (DeRivera et al. 2005; Carrol 2007; Reichard et al., 2010). Establishment success is also dictated by propagule pressure, a widely recognizable factor that serves as an invasiveness predictor (Colautti et al. 2006; Simberloff 2009). Propagule pressure can be measured by the number of larvae, seeds, or individuals introduced over time, or number of introduction events (Colautti et al. 2006; Simberloff 2009). High propagule pressure enhances colonization success over time because it increases chances for population genetic variation and reduces bottleneck or founder effects, thus increasing the adaptability of the non-indigenous species to future environmental changes and facilitating the expansion to new

contiguous zones (Simberloff 2009). Nonetheless, it is critical to highlight that some successful invasive species across the world show minimal genetic diversity (e.g., Dlugosch and Parker 2008) being able to dodge the negative effects of founder effects (Simberloff 2009), not to mention clonal invasive species (e.g., Lui et al. 2005). Understanding why a non-indigenous species becomes a successful invader requires a deep knowledge on the functioning of the receiving ecosystem, but also the ecology of the introduced species, its interactions with native species, and which role it will have in the trophic web.

1.2 Impacts of invasive species

A thriving invasive species can be responsible for impacts across a wide range of areas, including ecological, economic, and public health (Ehrenfeld 2010; Pyšek and Richardson, 2010; Bédry et al. 2020; Pyšek et al. 2020; Fantle-Lepczyk et al. 2022). The most direct ecological impact translates into increasing predation by non-indigenous species, which have been responsible for significant declines in native species. The lionfish invasion of *Pterois volitans* (Linnaeus, 1758) (Animalia, Perciformes) and *Pterois miles* in the western Atlantic Ocean led to the decline of native fish (Green et al. 2012). The brown tree snake *Boiga irregularis* (Merrem, 1802) (Animalia, Squamata) in the island of Guam, western Pacific Ocean, caused a steep decline in over 10 native bird species (Savidge 1987). Competition for habitat and resources is a common mechanism by which many invasive terrestrial plants outcompete native species (Levine et al. 2003). Other ecological impacts may include bioturbation and physical alteration by ecosystem engineering species, such as earthworms, the Asian clam *Corbicula fluminea* (O. F. Müller, 1774) (Animalia, Venerida), or the crayfish *Procambarus clarkii* (Girard, 1852) (Animalia, Decapoda) (Addison 2009; Sousa et al. 2009; Souty-Grosset et al. 2016). The high density of non-indigenous species can also destabilize nutrient loading, namely through excessive excretion, as happened with the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Animalia, Cichliformes) in the Furnas reservoir in Brazil (Figueredo and Giani 2005).

Economic impacts are mainly related to invasive species that have a strong impact on native species of high commercial value, that disrupt the functioning of an entire ecosystem and impact ecosystem services, or cause damage to high-value infrastructure and/or require significant financial investment to eliminate them and restore the operation of such infrastructure (Connelly et al. 2007; Pejchar and Mooney 2009; Keller et al. 2011; Fantle-

Lepczyk et al. 2022). For example, the uncontrolled growth of the zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Animalia, Veneroida) has caused major economic damage. Zebra mussels can colonise and clog water pipes, including the cooling water intake line supply in nuclear and power plants, refineries, fish farms, water supply facilities, or ships, so cleaning and/or replacing this type of equipment is highly expensive (Connelly et al. 2007; Strayer 2009). In the United States, the costs related to invasive species increased from 2 billion US$ per year in 1960-1969, to 21 billion US$ per year in 2010-2020, totalling 1.22 trillion US$ between 1960 and 2020 (Fantle-Lepczyk et al. 2022). The sectors most affected were agriculture (> 500 billion US$), environmental (> 100 billion US$), forestry (> 40 billion US$), public and social welfare sectors (> 40 billion US$), and authorities-stakeholders (> 30 billion US$) (Fantle-Lepczyk et al. 2022). These impacts were mostly caused by mammals (> 230 billion US$) and plants (> 190 billion US$). In the European Union, the total economic impact of invasive species rises to at least 12.5 billion € per year according to documented costs (Kettunen et al. 2009). The invasive pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle 1970 (Animalia, Tylenchida) alone, can cause an estimated cost of 22 billion € in accumulated wood loss after 22 years in an unregulated spread scenario, and a reduction in social welfare of 218 million € by 2030 (Soliman et al. 2012).

The introduction of non-indigenous species may result in public health issues, either directly caused by the species, or by parasites and pathogens that are introduced alongside (Hulme 2014; Chinchio et al. 2020; Pyšek et al. 2020). Direct impacts usually occur with venomous stinging species, such as in the Mediterranean Sea with the lionfish *Pterois miles*, rabbitfishes *Siganus spp.* (Animalia, Acanthuriformes), the schyphomedusa *Rhopilema nomadica* Galil, Spanier & Ferguson, 1990 (Animalia, Rhizostomeae), or with poisonous species, such as the silver-cheeked toadfish *Lagocephalus sceleratus* (Bédry et al. 2020). Several introduced species are also vectors of parasites, such as fleas associated with infective carditis and bubonic plague, or mites that can transmit typhus (Hulme 2014). Several species of mosquitos of the genus *Aedes* (Meigen, 1818) (Animalia, Diptera), non-indigenous in Europe, can also be vector of diseases, already responsible for occurrences of Chikungunya fever in Italy in 2007, and dengue in Croatia in 2010 and in the Madeira Island (Portugal) in 2012 (Schaffner et al. 2013; Hulme 2014).

Nevertheless, many species that nowadays are perceived as native food commodities of Europe, as the olive tree *Olea europeaea* L. (Plantae, Lamiales) or carob tree *Ceratonia siliqua* L. (Plantae, Fabales), were introduced many centuries ago and naturalized over the years (Hall

2003; Mahdad and Gaouar 2023). Impacts of non-native species may also be perceived in opposite ways, as in the example of feral pigs across Hawaii, with hundreds of dollars of damage to crops and native forests in national parks, but they are also praised as a food source and recreational hunting for local communities (Pejchar and Mooney 2009). Such factors should be considered by managers in today's globalized world, especially when an invasive species may become a food source in areas of low income and food shortage.

1.3 Management of invasive species

The strategies to manage invasive species are intrinsically shaped by the environment where that species lives, so the approach is necessarily different for terrestrial areas, enclosed freshwater waterbodies, and open marine ecosystems (McCallum et al. 2004; Moorhouse and Macdonald 2015). The policy framework regarding the introduction and control of non-indigenous species at an international level is extensive, including instruments such as the Convention on Biological Diversity (CBD 2002), the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004), the European Marine Strategy Framework Directive (EC 2008), the creation of lists of concern for existing non-indigenous species (EU 2014) and prioritizing lists for potential new non-indigenous species (Tsiamis et al. 2020; Oficialdegui et al. 2023). Still, the implementation of such instruments and policies at a national level is not yet satisfactory, nor is it following the increasing number of new introductions (Keller et al. 2011; Pyšek et al. 2020). A growing consensus among the scientific community and policy makers, includes a more inclusive approach to both the prevention and the control of invasive species, making the common citizen a key-player in reducing the likelihood of new introductions and reducing the impacts of biological invasions, while directly working with scientific institutions (Courchamp et al. 2017; Johnson et al. 2020; Pyšek et al. 2020).

Containment, control, and remotion of invasive species in small freshwater waterbodies may be feasible and attain satisfactory results, as the examples of the zebra mussel *Dreissena polymorpha* in a small reservoir in south Portugal (Catita et al. 2020), or muskrats *Ondatra zibethicus* (Linnaeus, 1766) and coypus *Myocastor coypus* (Molina, 1782) (Animalia, Rodentia) in Britain (Gosling and Baker 1989). Still, when invasive species are already widespread across larger waterbodies, their control and even maintenance at low levels, are quite problematic and difficult to attain on the long-term, mainly due to unsustainable costs

(Pyšek et al. 2020; Simberloff 2021). Regarding marine environments, such efforts are substantially more complicated, namely due to inherent difficulty in managing a non-indigenous species in subtidal ecosystems, associated logistical problems, or even our ability to delimitate the invaded area and where to apply the management efforts (Simberloff 2021). In such scenarios, innovative mitigation measures must be studied and implemented to reduce impacts.

Invasive species with economic value should be used as a tool to increase awareness about biological invasions, while promoting its harvesting in areas where containment is no longer an option (Nuñez et al. 2012; Mancinelli et al. 2017; Ulman et al. 2022). Edible invasive species have a higher potential to be controlled with such approach and repurposed for human consumption. Nevertheless, such management approach must always be studied for each species, invasion timeline, and framed within the local socio-economic context (Nuñez et al. 2012; Pasko and Goldberg 2014). Biological invasions management should evaluate the cost-effectiveness of investments by comparing the expected long-term damage reductions with the costs of each intervention, be aware of counterproductive consequences of policies, which can induce unintended responses of stakeholders (e.g., triggering risk-increasing actions among economic sectors), plan spatial strategies for invasion control that can reduce long-term negative impacts and protect high-value economic resources or vast areas of ecological and economic value (Epanchin-Niell 2017).

1.4 Background and study area

The present Ph.D. thesis was developed in the Algarve region which forms the 140 km-long southern coast of Portugal (southwestern area of Europe). The existence of recreational and commercial harbours along this touristic coastal zone, as well as the proximity to major harbours in the Gulf of Cadiz, increases the arrival of novel non-indigenous marine species through ballast water or hull-fouling (Drake and Lodge 2004, 2007; Morais et al. 2017; González-Ortegón et al. 2020). Located in the Eastern Atlantic Ocean, the Algarve region borders three ecoregions – the Macaronesia archipelagos, the north-western African coast, and the Mediterranean Sea (Spalding et al. 2007) – which increments the chances for the establishment of neonative species due to changes in local climatic conditions and patterns.

Non-indigenous marine and estuarine species increased in the Algarve region since the beginning of the 21st century, with the subsequent increase of scientific reports in the late

2000's, either regarding non-indigenous species with invasive potential (e.g., Chícharo et al. 2009; Morais et al. 2009a) or neonative species (Abecasis et al. 2008). A second wave of reports seemed to have occurred after 2010, with non-indigenous species such as the seaweed *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, 1809 (Plantae, Bryopsidales), the hydrozoan *Cordylophora caspia* (Pallas, 1771) (Animalia, Anthoathecata), the weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) (Animalia, Perciformes), the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 (Animalia, Decapoda), and several sea squirts and barnacles being reported for both estuarine and coastal zones of the Algarve (Cunha et al. 2013; Ruiz 2015; Morais and Teodósio 2016; Seyer et al. 2017; Morais et al. 2019), along with neonative species as the brown mussel *Perna perna* (Linnaeus, 1758) (Animalia, Mytilida) or the Monrovia doctorfish *Acanthurus monroviae* Steindachner, 1876 (Animalia, Acanthuriformes) (Lourenço et al. 2012; Vasconcelos et al. 2018). Major contributions in this field have been done by our research group ECOREACH at the Centre of Marine Sciences of Algarve, namely in the Guadiana estuary (Eastern Algarve), which has become a case-study for biological invasions in the Iberian Peninsula, and one of the few case-studies in the world where the construction of a dam in the river mainstream is putatively linked with the onset of a series of estuarine biological invasions. The Alqueva dam started operating in February 2002, which became the biggest European dam and heavily reduced freshwater inflow to the estuary, resulting in changes in native species assemblages driven by a salinization of upper areas of the estuary (Chícharo et al. 2001, 2006; Morais 2008; Morais et al. 2009b; Encarnação et al. 2013). Such ecosystem changes seemed to have favoured the establishment of non-indigenous species, namely in estuarine areas where ecosystem balance has been more heavily disturbed by anthropogenic impacts and the existence of empty niches (Bunn and Arthington 2002; Havel et al. 2005; Paavola et al. 2005). This has been evident by the number of new non-indigenous species detected in the Guadiana estuary in recent years, many with invasive potential. The increase in such detections highlighted the need for more monitoring across the estuarine and coastal zones of Algarve, to which this doctoral thesis intends to contribute for. Additionally, it also intends to explore the potential of citizen science when studying marine non-indigenous species, following previous experiences of first records provided by fishers ahead of any detection by the scientific community (e.g., Morais and Teodósio 2016; Vasconcelos et al. 2018; Morais et al. 2019a).

1.5 Objectives of this thesis

This doctoral thesis is structured under four objectives:

i. Identify non-indigenous marine species in the Algarve region (south Portugal), and determine their distribution, abundance, and origin (Chapter 2 and 4);
ii. Evaluate the potential ecological impacts of the Atlantic blue crab in local food webs (Chapter 3);
iii. Implement a long-term citizen science campaign focused on monitoring non-indigenous marine fauna in Portugal, while informing the public on these species and potential public-health risks of some of them (Chapter 4);
iv. Evaluate if the commercial exploration of these potential new fishing resources is an effective solution to manage non-indigenous marine species with invasive potential in Portugal (Chapter 5).

1.6 References

Abecasis, D., Bentes, L., Ribeiro, J., Machado, D., Oliveira, F., Veiga, P., Gonçalves, J.M., and Erzini, K. (2008). First record of the Mediterranean parrotfish, *Sparisoma cretense* in Ria Formosa (south Portugal). *Marine Biodiversity Records* 1: e27. doi: 10.1017/S175526720600248X

Addison, J. (2009). Distribution and impacts of invasive earthworms in Canadian forest ecosystems. *Biological Invasions* 11: 59-79. doi: 10.1007/s10530-008-9320-4

Bédry, R., de Haro, L., Bentur, Y., Senechal, N., and Galil, B. (2021). Toxicological risks on the human health of populations living around the Mediterranean Sea linked to the invasion of non-indigenous marine species from the Red Sea: A review. *Toxicon* 191: 69-82. doi: 10.1016/j.toxicon.2020.12.012

Belyea L.R., and Lancaster, J. (1999). Assembly rules within a contingent ecology. *Oikos* 86(3): 402-416. doi: 10.2307/3546646

Bunn, S.E., and Arthington, A.H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* 30: 492-507. doi: 10.1007/s00267-002-2737-0

Carroll, S.P. (2007). Natives adapting to invasive species: ecology, genes, and the sustainability of conservation. *Ecological Research* 22(6): 892-901. doi: 10.1007/s11284-007-0352-5

Carlton, J.T., Chapman, J.W., Geller, J.B., Miller, J.A., Carlton, D.A., McCuller, M.I., Treneman, N.C, Steves, B.P., and Ruiz, G.M. (2017). Tsunami-driven rafting: Transoceanic species dispersal and implications for marine biogeography. *Science* 357(6358): 1402-1406. doi: 10.1126/science.aao1498

Catita, D., Gama, M., Azedo, R., Banha, F., Pinto, J., Ilhéu, A., and Anastácio, P.M. (2020). Detection and possible elimination of the first recorded population of the zebra mussel (*Dreissena polymorpha*) in Portugal from a reservoir. *Management of Biological Invasions* 11(3): 406-414. doi: 10.3391/mbi.2020.11.3.04

CBD - Convention on Biological Diversity (2002). Conference of the Parties (COP 6), decision VI/23, Netherlands.

Chícharo, M.A., Chícharo, L.M., Galvão, H., Barbosa, A., Marques, M.H., Andrade, J.P., Esteves, E., Miguel, C., and Gouveia, I. (2001). Status of the Guadiana estuary (South Portugal) during 1996-1998: An ecohydrological approach. *Aquatic Ecosystem Health & Management* 4(1): 73-89. doi: 10.1080/146349801753569298

Chícharo, M.A., Chícharo, L., and Morais, P. (2006). Inter-annual differences of ichthyofauna structure of the Guadiana estuary and adjacent coastal area (SE Portugal/SW Spain): Before and after Alqueva dam construction. *Estuarine, Coastal and Shelf Science* 70(1-2): 39-51. doi: 10.1016/j.ecss.2006.05.036

Chícharo, M.A., Leitão, T., Range, P., Gutierrez, C., Morales, J., Morais, P., and Chícharo, L. (2009). Alien species in the Guadiana Estuary (SE-Portugal/SW-Spain): *Blackfordia virginica* (Cnidaria, Hydrozoa) and *Palaemon macrodactylus* (Crustacea, Decapoda): Potential impacts and mitigation measures. *Aquatic Invasions* 4(3): 501-506. doi: 10.3391/ai.2009.4.3.11

Chinchio, E., Crotta, M., Romeo, C., Drewe, J.A., Guitian, J., and Ferrari, N. (2020). Invasive alien species and disease risk: An open challenge in public and animal health. *PLoS Pathogens* 16(10): e1008922. doi: 10.1371/journal.ppat.100892

Choi, K.-H., Kimmerer, W., Smith, G., Ruiz, G.M., and Lion, K. (2005). Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. *Journal of Plankton Research* 27(7): 707-714. doi: 10.1093/plankt/fbi044

Colautti, R.I., Ricciardi, A., Grigorovich, I.A., and MacIsaac, H.J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7(8): 721-733. doi: 10.1111/j.1461-0248.2004.00616.x

Colautti, R.I., Grigorovich, I.A., and MacIsaac, H.J. (2006). Propagule pressure: A null model for biological invasions. *Biological Invasions* 8: 1023-1037. doi: 10.1007/s10530-005-3735-y

Connelly, N.A., O'Neill, C.R., Knuth, B.A., and Brown, T.L. (2007). Economic impacts of zebra mussels on drinking water treatment and electric power generation facilities. *Environmental Management* 40(1): 105-112. doi: 10.1007/s00267-006-0296-5

Courchamp, F., Fournier, A., Bellard, C., Bertelsmeier, C., Bonnaud, E., Jeschke, J.M., and Russell, J.C. (2017). Invasion biology: Specific problems and possible solutions. *Trends in Ecology & Evolution* 32(1): 13-22. doi: 10.1016/j.tree.2016.11.001

Cunha, A.H., Paulo, D., Sousa, I., and Serrão, E. (2013). The rediscovery of *Caulerpa prolifera* in Ria Formosa, Portugal, 60 years after the previous record. *Cahiers de Biologie Marine* 54(3): 359-364.

D'Amen, M., and Azzurro, E. (2020). Lessepsian fish invasion in Mediterranean marine protected areas: A risk assessment under climate change scenarios. *ICES Journal of Marine Science* 77(1): 388-397. doi: 10.1093/icesjms/fsz207

DeRivera, C.E., Ruiz, G.M., Hines, A.H., and Jivoff, P. (2005). Biotic resistance to invasion: Native predator limits abundance and distribution of an introduced crab. *Ecology* 86(12): 3364-3376. doi: 10.1890/05-0479

Drake, J.M., and Lodge, D.M. (2004). Global hot spots of biological invasions: Evaluating options for ballast–water management. *Proceedings of the Royal Society of London. Series B* 271: 575-580. doi: 10.1098/rspb.2003.2629

Drake, J.M., and Lodge, D.M. (2007). Hull fouling is a risk factor for intercontinental species exchange in aquatic ecosystems. *Aquatic Invasions* 2(2): 121-131. doi: 10.3391/ai.2007.2.2.7

EC - European Commission (2008). Directive of the European Parliament and the Council establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Directive 2008/56/EC, OJ L 164, European Commission.

Ehrenfeld, J.G. (2010). Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics* 41: 59-80. doi: 10.1146/annurev-ecolsys-102209-144650

Encarnacão, J., Leitão, T., Morais, P., Piló, D., Range, P., Chícharo, L., and Chícharo, M.A. (2013). Effects of inter-annual freshwater inflow shifts on the community structure of

estuarine decapods. *Cahiers de Biologie Marine* 54(2): 181-189.

Epanchin-Niell, R.S. (2017). Economics of invasive species policy and management. *Biological Invasions* 19: 3333-3354. doi: 10.1007/s10530-017-1406-4

Essl, F., Dullinger, S., Genovesi, P., Hulme, P.E., Jeschke, J.M., Katsanevakis, S., Kühn, I., Lenzner, B., Pauchard, A., and Pyšek, P. (2019). A conceptual framework for range-expanding species that track human-induced environmental change. *BioScience* 69(11): 908-919. doi: 10.1093/biosci/biz101

EU - European Union (2014). Regulation No 1143/2014 of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species. Official Journal of the European Union, European Union. 35–55.

Fantle-Lepczyk, J.E., Haubrock, P.J., Kramer, A.M., Cuthbert, R.N., Turbelin, A.J., Crystal-Ornelas, R., Diagne, C., and Courchamp, F. (2022). Economic costs of biological invasions in the United States. *Science of the Total Environment* 806: 151318. doi: 10.1016/j.scitotenv.2021.151318

Ferreras, A.E., and Galetto, L. (2010). From seed production to seedling establishment: Important steps in an invasive process. *Acta Oecologica* 36(2): 211-218. doi: 10.1016/j.actao.2009.12.005

Figueredo, C.C., and Giani, A. (2005). Ecological interactions between Nile tilapia (*Oreochromis niloticus*, L.) and the phytoplanktonic community of the Furnas Reservoir (Brazil). *Freshwater Biology* 50(8): 1391-1403. doi: 10.1111/j.1365-2427.2005.01407.x

Godwin, L.S. (2003). Hull fouling of maritime vessels as a pathway for marine species invasions to the Hawaiian Islands. *Biofouling* 19(S1): 123-131. doi: 10.1080/0892701031000061750

González-Ortegón, E., Jenkins, S., Galil, B.S., Drake, P., and Cuesta, J.A. (2020). Accelerated invasion of decapod crustaceans in the southernmost point of the Atlantic coast of Europe: A non-natives' hot spot? *Biological Invasions* 22: 3487-3492. doi: 10.1007/s10530-020-02345-y

Gosling, L., and Baker, S. (1989). The eradication of muskrats and coypus from Britain. *Biological Journal of the Linnean Society* 38(1): 39-51. doi: 10.1111/j.1095-8312.1989.tb01561.x

Green, S.J., Akins, J.L., Maljković, A., and Côté, I.M. (2012). Invasive lionfish drive Atlantic coral reef fish declines. *PLoS ONE* 7(3): e32596. doi: 10.1371/journal.pone.0032596

Hall, M. (2003) Editorial: The native, naturalized and exotic – plants and animals in human history. *Landscape Research* 28(1): 5-9. doi: 10.1080/01426390306534

Hastings, A., Cuddington, K., Davies, K.F., Dugaw, C.J., Elmendorf, S., Freestone, A., Harrison, S., Holland, M., Lambrinos, J., and Malvadkar, U. (2005). The spatial spread of invasions: New developments in theory and evidence. *Ecology Letters* 8(1): 91-101. doi: 10.1111/j.1461-0248.2004.00687.x

Havel, J.E., Lee, C.E., and Zanden, M.J. (2005). Do reservoirs facilitate invasions into landscapes? *BioScience* 55(6): 518-525. doi: 10.1641/0006-3568(2005)055[0518:DRFIIL]2.0.CO;2

Havel, J.E., Kovalenko, K.E., Thomaz, S.M., Amalfitano, S., and Kats, L.B. (2015). Aquatic

invasive species: Challenges for the future. *Hydrobiologia* 750: 147-170. doi: 10.1007/s10750-014-2166-0

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., and Pergl, J. (2008). Grasping at the routes of biological invasions: A framework for integrating pathways into policy. *Journal of Applied Ecology* 45(2): 403-414. doi: 10.1111/j.1365-2664.2007.01442.x

Hulme, P.E. (2009). Trade, transport and trouble: Managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46(1): 10-18. doi: 10.1111/j.1365-2664.2008.01600.x

Hulme, P.E. (2014). Invasive species challenge the global response to emerging diseases. *Trends in Parasitology* 30(6): 267-270. doi: 10.1016/j.pt.2014.03.005

Humphreys, J., Harris, M.R., Herbert, R.J., Farrell, P., Jensen, A., and Cragg, S.M. (2015). Introduction, dispersal and naturalization of the Manila clam *Ruditapes philippinarum* in British estuaries, 1980–2010. *Journal of the Marine Biological Association of the United Kingdom* 95(6): 1163-1172. doi: 10.1017/S0025315415000132

IMO - International Maritime Organization (2004). International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM). International Maritime Organization, London.

IUCN - International Union for Conservation of Nature and Natural Resources (2000). IUCN guidelines for the prevention of biodiversity loss caused by alien invasive species. Species Survival Commission (SSC), Nairobi, Kenya. 21 pp.

Jeschke, J.M., and Strayer, D.L. (2006). Determinants of vertebrate invasion success in Europe and North America. *Global Change Biology* 12(9): 1608-1619. doi: 10.1111/j.1365-2486.2006.01213.x

Johnson, B.A., Mader, A.D., Dasgupta, R., and Kumar, P. (2020). Citizen science and invasive alien species: An analysis of citizen science initiatives using information and communications technology (ICT) to collect invasive alien species observations. *Global Ecology and Conservation* 21: e00812. doi: 10.1016/j.gecco.2019.e00812

Katsanevakis, S., Zenetos, A., Belchior, C., and Cardoso, A.C. (2013). Invading European Seas: Assessing pathways of introduction of marine aliens. *Ocean & Coastal Management* 76: 64-74. doi: 10.1016/j.ocecoaman.2013.02.024

Keane, R.M., and Crawley, M.J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17(4): 164-170. doi: 10.1016/S0169-5347(02)02499-0

Keller, R.P., Geist, J., Jeschke, J.M., and Kühn, I. (2011). Invasive species in Europe: Ecology, status, and policy. *Environmental Sciences Europe* 23(1): 1-17. doi: 10.1186/2190-4715-23-23

Kettunen, M., Genovesi, P., Gollasch, S., Pagad, S., Starfinger, U., ten Brink, P., and Shine, C. (2009). Technical support to EU strategy on invasive species (IAS) - Assessment of the impacts of IAS in Europe and the EU (final module report for the European Commission). Institute for European Environmental Policy (IEEP), Brussels, Belgium. 44 pp. + Annexes.

Kim, H.M., Jo, J., Park, C., Choi, B.-J., Lee, H.-G., and Kim, K.Y. (2019). Epibionts associated with floating *Sargassum horneri* in the Korea Strait. *Algae* 34(4): 303-313. doi: 10.4490/algae.2019.34.12.10

Kletou, D., Hall-Spencer, J.M., and Kleitou, P. (2016). A lionfish (*Pterois miles*) invasion has begun in the Mediterranean Sea. *Marine Biodiversity Records* 9(1): 1-7. doi: 10.1186/s41200-016-0065-y

Lallias, D., Boudry, P., Batista, F.M., Beaumont, A., King, J.W., Turner, J.R., and Lapègue, S. (2015). Invasion genetics of the Pacific oyster *Crassostrea gigas* in the British Isles inferred from microsatellite and mitochondrial markers. *Biological Invasions* 17: 2581-2595. doi: 10.1007/s10530-015-0896-1

Leuven, R.S., van der Velde, G., Baijens, I., Snijders, J., van der Zwart, C., Lenders, H.R., and bij de Vaate, A. (2009). The river Rhine: A global highway for dispersal of aquatic invasive species. *Biological Invasions* 11: 1989-2008. doi: 10.1007/s10530-009-9491-7

Levine, J.M., Vila, M., Antonio, C.M.D., Dukes, J.S., Grigulis, K., and Lavorel, S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1517): 775-781. doi: 10.1098/rspb.2003.2327

Lockett, M.M., and Gomon, M.F. (2001). Ship mediated fish invasions in Australia: Two new introductions and a consideration of two previous invasions. *Biological Invasions* 3: 187-192. doi: 10.1023/A:1014584201815

Lourenço, C.R., Nicastro, K.R., Serrão, E.A., and Zardi, G.I. (2012). First record of the brown mussel (*Perna perna*) from the European Atlantic coast. *Marine Biodiversity Records* 5: e39. doi: 10.1017/S1755267212000280

Lui, K., Thompson, F.L., and Eckert, C.G. (2005). Causes and consequences of extreme variation in reproductive strategy and vegetative growth among invasive populations of a clonal aquatic plant, *Butomus umbellatus* L. (Butomaceae). *Biological Invasions* 7: 427-444. doi: 10.1007/s10530-004-4063-3

Mahdad, Y.M., and Gaouar, S.B.S. (2023). Origin, distribution and domestication of the carob tree (*Ceratonia siliqua* L.). *Turkish Journal of Botany* 47(2): 89-96. doi: 10.55730/1300-008X.2748

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., and Ribeiro, F. (2017). On the Atlantic blue crab (*Callinectes sapidus* Rathbun 1896) in southern European coastal waters: Time to turn a threat into a resource? *Fisheries Research* 194: 1-8. doi: 10.1016/j.fishres.2017.05.002

McCallum, H.I., Kuris, A., Harvell, C.D., Lafferty, K.D., Smith, G.W., and Porter, J. (2004). Does terrestrial epidemiology apply to marine systems? *Trends in Ecology & Evolution* 19(11): 585-591. doi: 10.1016/j.tree.2004.08.009

McCarthy, H.P., and Crowder, L.B. (2000). An overlooked scale of global transport: Phytoplankton species richness in ships' ballast water. *Biological Invasions* 2(4): 321-322. doi: 10.1023/A:1011418432256

Molony, B.W., Lenanton, R., Jackson, G., and Norriss, J. (2003). Stock enhancement as a fisheries management tool. *Reviews in Fish Biology and Fisheries* 13: 409-432. doi: 10.1007/s11160-005-1886-7

Moorhouse, T.P., and Macdonald, D.W. (2015). Are invasives worse in freshwater than terrestrial ecosystems? *Wiley Interdisciplinary Reviews: Water* 2(1): 1-8. doi: 10.1002/wat2.1059

Morais, P. (2008). Review on the major ecosystem impacts caused by damming and watershed development in an Iberian basin (SW-Europe): Focus on the Guadiana estuary. *Annales de Limnologie-International Journal of Limnology* 44(2): 105-117. doi: 10.1051/limn:2008012

Morais, P., Teodósio, J., Reis, J., Chícharo, M.A., and Chícharo, L. (2009). The Asian clam *Corbicula fluminea* (Müller, 1774) in the Guadiana River Basin (southwestern Iberian Peninsula): Setting the record straight. *Aquatic Invasions* 4(4): 681-684. doi: 10.3391/ai.2009.4.4.14

Morais, P., Chícharo, M.A., and Chícharo, L. (2009b). Changes in a temperate estuary during the filling of the biggest European dam. *Science of the Total Environment* 407(7): 2245-2259. doi: 10.1016/j.scitotenv.2008.11.037

Morais, P., and Teodósio, M. (2016). The transatlantic introduction of weakfish *Cynoscion regalis* (Bloch & schneider, 1801)(sciaenidae, pisces) into Europe. *BioInvasions Records* 5(4): 259-265. doi: 10.3391/bir.2016.5.4.11

Morais, P., Cerveira, I., and Teodósio, M.A. (2017). An update on the invasion of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801)(Actinopterygii: Sciaenidae) into Europe. *Diversity* 9(4): 47. doi: 10.3390/d9040047

Morais, P., Gaspar, M., Garel, E., Baptista, V., Cruz, J., Cerveira, I., Leitao, F., and Teodosio, M. (2019). The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its non-native distribution into the Ria Formosa lagoon and the Guadiana estuary (SW-Iberian Peninsula, Europe). *BioInvasions Records* 8(1): 123-133. doi: 10.3391/bir.2019.8.1.14

Nuñez, M.A., Kuebbing, S., Dimarco, R.D., and Simberloff, D. (2012). Invasive species: To eat or not to eat, that is the question. *Conservation Letters* 5(5): 334-341. doi: 10.1111/j.1755-263X.2012.00250.x

Odom, R.L., and Walters, L.J. (2014). A safe alternative to invasive *Caulerpa taxifolia* (Chlorophtya)? Assessing aquarium-release invasion potential of aquarium strains of the macroalgal genus *Chaetomorpha* (Chlorophyta). *Biological Invasions* 16: 1589-1597. doi: 10.1007/s10530-013-0593-x

Oficialdegui, F.J., Zamora-Marín, J.M., Guareschi, S., Anastácio, P.M., García-Murillo, P., Ribeiro, F., Miranda, R., Cobo, F., Gallardo, B., García-Berthou, E., et al. (2023). A horizon scan exercise for aquatic invasive alien species in Iberian inland waters. *The Science of the Total Environment*: 161798. doi: 10.1016/j.scitotenv.2023.161798

Paavola, M., Olenin, S., and Leppäkoski, E. (2005). Are invasive species most successful in habitats of low native species richness across European brackish water seas? *Estuarine, Coastal and Shelf Science* 64(4): 738-750. doi: 10.1016/j.ecss.2005.03.021

Pasko, S., and Goldberg, J. (2014). Review of harvest incentives to control invasive species. *Management of Biological Invasions* 5(3): 263. doi: 10.3391/mbi.2014.5.3.10

Patoka, J., Prabowo, R.E., Petrtýl, M., Reynolds, J.D., Kuříková, P., Zámečníková-Wanma, B.P., and Kalous, L. (2020). Marine hitchhikers: A preliminary study on invertebrates unintentionally transported via the international pet trade. *NeoBiota* 61: 33-46. doi:

10.3897/neobiota.61.57682

Pejchar, L., and Mooney, H.A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution* 24(9): 497-504. doi: 10.1016/j.tree.2009.03.016

Pollard, E.A. (2014). "The Mediterranean and the Indian Ocean" in P. Horden and S. Kinoshita (eds) A Companion to Mediterranean History. John Wiley & Sons, New Jersey, U.S., 504 pp. doi: 10.1002/9781118519356.ch29

Pyšek, P., and Richardson, D.M. (2010). Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources* 35: 25-55. doi: 10.1146/annurev-environ-033009-095548

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W., Essl, F., Foxcroft, L.C., and Genovesi, P. (2020). Scientists' warning on invasive alien species. *Biological Reviews* 95(6): 1511-1534. doi: 10.1111/brv.12627

Reichard, M., Polačik, M., Tarkan, A.S., Spence, R., Gaygusuz, O., Ercan, E., Ondračková, M., and Smith, C. (2010). The bitter-ling–mussel coevolutionary relationship in areas of recent and ancient sympatry. *Evolution* 64: 3047–3056. doi: 10.1111/j.1558-5646.2010.01032.x

Ruiz, S.T. (2015). The status of the invasive sea squirts and barnacles found in the marinas and ports of Algarve, southern Portugal. Master thesis. Universidade do Algarve, Faro. 149 pp.

Savidge, J.A. (1987). Extinction of an island forest avifauna by an introduced snake. *Ecology* 68(3): 660-668. doi: 10.2307/1938471

Savini, D., Occhipinti–Ambrogi, A., Marchini, A., Tricarico, E., Gherardi, F., Olenin, S., and Gollasch, S. (2010). The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology* 26: 1-7. doi: 10.1111/j.1439-0426.2010.01503.x

Schaffner, F., Medlock, J., and Van Bortel (2013). Public health significance of invasive mosquitoes in Europe. *Clinical Microbiology and Infection* 19(8): 685-692. doi: 10.1111/1469-0691.12189

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., and Arianoutsou, M. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications* 8(1): 1-9. doi: 10.1038/ncomms14435

Seyer, T., Morais, P., Amorim, K., Leitão, F., Martins, F., and Teodósio, M.A. (2017). On the presence of the Ponto-Caspian hydrozoan *Cordylophora caspia* (Pallas, 1771) in an Iberian estuary: Highlights on the introduction vectors and invasion routes. *BioInvasions Records* 6(4): 331-337. doi: 10.3391/bir.2017.6.4.05

Simberloff, D., Parker, I.M., and Windle, P.N. (2005). Introduced species policy, management, and future research needs. *Frontiers in Ecology and the Environment* 3(1): 12-20. doi: 10.1890/1540-9295(2005)003[0012:ISPMAF]2.0.CO;2

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics* 40: 81-102. doi: 10.1146/annurev.ecolsys.110308.120304

Simberloff, D. (2021). Maintenance management and eradication of established aquatic

invaders. *Hydrobiologia* 848: 2399-2420. doi: 10.1007/s10750-020-04352-

Soliman, T., Mourits, M.C., Van Der Werf, W., Hengeveld, G.M., Robinet, C., and Lansink, A.G.O. (2012). Framework for modelling economic impacts of invasive species, applied to pine wood nematode in Europe. *PLoS ONE* 7(9): e45505. doi: 10.1371/journal.pone.0045505

Sousa, R., Gutiérrez, J.L., and Aldridge, D.C. (2009). Non-indigenous invasive bivalves as ecosystem engineers. *Biological Invasions* 11: 2367-2385. doi: 10.1007/s10530-009-9422-7

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., and Lourie, S.A. (2007). Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *BioScience* 57(7): 573-583. doi: 10.1641/B570707

Souty-Grosset, C., Anastacio, P.M., Aquiloni, L., Banha, F., Choquer, J., Chucholl, C., and Tricarico, E. (2016). The red swamp crayfish *Procambarus clarkii* in Europe: Impacts on aquatic ecosystems and human well-being. *Limnologica* 58: 78-93. doi: 10.1016/j.limno.2016.03.003

Strayer, D.L. (2009). Twenty years of zebra mussels: Lessons from the mollusk that made headlines. *Frontiers in Ecology and the Environment* 7(3): 135-141. doi: 10.1890/080020

Su, N.-Y. (2013). How to become a successful invader. *Florida Entomologist* 96(3): 765-769. doi: 10.1653/024.096.0309

Sun, B., Mouland, R., Way, C., and Rivkin, R.B. (2010). Redistribution of heterotrophic prokaryotes through ballast water: A case study from the west coast of Canada. *Aquatic Invasions* 5(1): 5-11. doi: 10.3391/ai.2010.5.1.2

Trucchi, E., and Sbordoni, V. (2009). Unveiling an ancient biological invasion: Molecular analysis of an old European alien, the crested porcupine (*Hystrix cristata*). *BMC Evolutionary Biology* 9: 1-10. doi: 10.1186/1471-2148-9-109

Tsiamis, K., Azzurro, E., Bariche, M., Çinar, M.E., Crocetta, F., De Clerck, O., Galil, B., Gómez, F., Hoffman, R., and Jensen, K.R. (2020). Prioritizing marine invasive alien species in the European Union through horizon scanning. *Aquatic Conservation: Marine and Freshwater Ecosystems* 30(4): 794-845. doi: 10.1002/aqc.3267

Ulman, A., Ali, F., Harris, H., Adel, M., Mabruk, S., Bariche, M., Candelmo, A., Chapman, J., Cicek, B., and Clements, K. (2022). Lessons from the western Atlantic lionfish invasion to inform management in the Mediterranean. *Frontiers in Marine Science* 9: 865162. doi: 10.3389/fmars.2022.865162

Vasconcelos, P., Carvalho, A.N., Moura, P., Ramos, J., and Gaspar, M.B. (2018). First record of *Acanthurus monroviae* (Osteichthyes: Perciformes: Acanthuridae) in southern Portugal, with notes on its recent distributional spread in the northeastern Atlantic and Mediterranean. *Marine Biodiversity* 48: 1673-1681. doi: 10.1007/s12526-017-0653-z

Young, T.P., Chase, J.M., and Huddleston, R.T. (2001). Community succession and assembly comparing, contrasting and combining paradigms in the context of ecological restoration. *Ecological Restoration* 19(1): 5-18. doi: 10.3368/er.19.1.5

CHAPTER 2

Assessment of non-indigenous marine species in south Portugal

Sections of the present chapter were published in the following peer-reviewed scientific articles under the objectives of this Ph.D. thesis:

- **Encarnação J**, Morais P, Baptista V, Cruz J, Teodósio MA (2019). New evidence of marine fauna tropicalization off the southwestern Iberian Peninsula (Southwest Europe). *Diversity* 11 (48). doi: 10.3390/d11040048.
- **Encarnação J**, Teodósio MA, Morais P (accepted). The arrival of a non-indigenous ecosystem engineer to a heavily invaded and flow-regulated estuary in Europe. *BioInvasions Records*.

2.1 Introduction

Biological invasions are a global ongoing process, and Portugal is no exception, with multiple examples across taxa and ecosystems – e.g., terrestrial plants, insects, or amphibians (Bella 2013; Duarte et al. 2020; Ginal et al. 2021), freshwater invertebrates and fish (Anastácio et al. 2019), and marine and estuarine fish, invertebrates, or algae (Chaínho et al. 2015). A successful non-indigenous species is generally characterized by high tolerance to a wide range of environmental variables, and by a combination of evolutionary advantages, as high fertility, early age of first maturity, and fast growth (Jeschke and Strayer 2006; Pyšek and Richardson 2010; Keller et al. 2011). Although widely used outside academia, the term 'invasive species', should only be applied to a non-indigenous species when it establishes itself successfully, spreads and increases in abundance, and causes ecological, economic, or public health impacts (IUCN 2000; Simberloff et al. 2005; Pyšek and Richardson 2010).

In the Algarve region (south Portugal), several marine non-indigenous species from different functional groups and inhabiting different ecosystems keep on being detected. Estuarine ecosystems, in particular the Guadiana estuary in eastern Algarve, seem to be a hotspot of non-indigenous species. The Guadiana estuary already features non-indigenous species from the pelagic compartment such as the planktonic species *Acartia tonsa* Dana, 1849 and *Blackfordia virginica* Mayer, 1910 (Chícharo et al. 2009; Cruz et al. 2017), while in the benthic compartment there are numerous infaunal and macrofaunal macroinvertebrates such as *Corbicula fluminea* (O. F. Müller, 1774), *Palaemon macrodactylus* Rathbun, 1902, *Synidotea laticauda* Benedict, 1897 (Chícharo et al. 2009; Morais et al. 2009; Nuño et al. 2018), biofouling species as *Cordylophora caspia* (Pallas, 1771) (Seyer et al. 2017), fish as *Fundulus heteroclitus* (Linnaeus, 1766) (Gonçalves et al. 2017), besides large nektonic and benthonic predators as the weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) and the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 (Morais and Teodósio 2016; Morais et al. 2019). These last two species seem to be expanding their distribution along coastal zones of Algarve (Morais et al. 2017; Vasconcelos et al. 2019). Additional non-indigenous species have been recorded along coastal and lagoonal systems of Algarve, as the algae *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, 1809, *Asparagopsis* spp., or *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim, 2009 (Cunha et al. 2013; Aires et al. 2016; Liulea 2021), the barnacle *Amphibalanus amphitrite* (Darwin, 1854) and the sea squirt *Styela plicata* (Lesueur, 1823) (Ruiz 2015), and bivalves as the Manila clam *Ruditapes philippinarum* (A. Adams &

Reeve, 1850) (Chiesa et al. 2017) or the Pacific oyster *Magallana gigas* (Thunberg, 1793) (Cardoso et al. 2013).

A second group of species include the so-called neonative species, which refers to range-expanding species responding to human-induced environmental change, when such range expansion is not a direct result of human intervention (Essl et al. 2019). In some temperate regions, the increase in seawater temperature is causing a poleward movement of species – a phenomenon known as the tropicalization of temperate regions (Bianchi and Morri 2003). Along the west and east coasts of Australia, dense kelp forests are disappearing, with records of 100 km range contractions on the west coast which are then followed by increases in the populations of subtropical and tropical herbivorous fish that suppress kelp recovery (Vergés et al. 2016; Wernberg et al. 2016; Zarco-Perello et al. 2017). Other important habitat-forming species, such as coral reefs, have been expanding their poleward distribution along the Japanese coast at a rate of up to 14 km $year^{-1}$ (Yamano et al. 2011), while tropical fish species are settling in these regions and withstanding the colder winter waters (Nakamura et al. 2013). In Portugal, reports of such species have also occurred both in the south coast – the Mediterranean parrotfish *Sparisoma cretense* (Linnaeus, 1758), the Monrovia doctorfish *Acanthurus monroviae* Steindachner, 1876, or the brown mussel *Perna perna* (Linnaeus, 1758) (Abecasis et al. 2008; Lourenço et al. 2012; Vasconcelos et al. 2018) – and the west coast (Horta e Costa and Gonçalves 2013; Horta e Costa et al. 2014).

Considering this increase of evidence in ongoing biodiversity changes, the objectives of this study consist in identifying non-indigenous marine fauna in southern Portugal, updating the knowledge on presence and distribution of both non-indigenous species and neonative (subtropical) species, relying on complementary sampling methods – benthic baited traps, bottom beam trawls, SCUBA diving visual census, and plankton night trawls.

2.2 Material and methods

2.2.1 Benthic baited traps

The benthic traps used were chosen and constructed considering one of the target study species – the non-indigenous Atlantic blue crab *Callinectes sapidus*. This type of baited fishing gear is used in south Portugal to catch the native green crab *Carcinus maenas* (Linnaeus, 1758), and in North America to catch the Atlantic blue crab (link). To assess the benthic communities of southern Portugal, and to collect Atlantic blue crabs for further analysis, this was the most

versatile fishing gear, as it can be operated by a single person, in small streams or across estuaries, from land, floating pontoons, or deployed from a boat (**Figure 2.1**). The traps had 50 cm in diameter and 30 cm height when opened, made of two steel circles, joined and closed at the bottom by 1 cm diameter fishing net, and baited with horse mackerel (**Figure 2.1A**). In addition, a 1 kg weight was attached to the bottom and a floater on the surface, depending on the sampling area, and current velocity (**Figure 2.1B**). The standard sampling procedure consisted of deploying the traps at least at 5 m from each other, and letting them sit at the bottom for 20 min. Then, the species were identified to the lowest taxonomic level possible and counted, before repeating the same procedure.

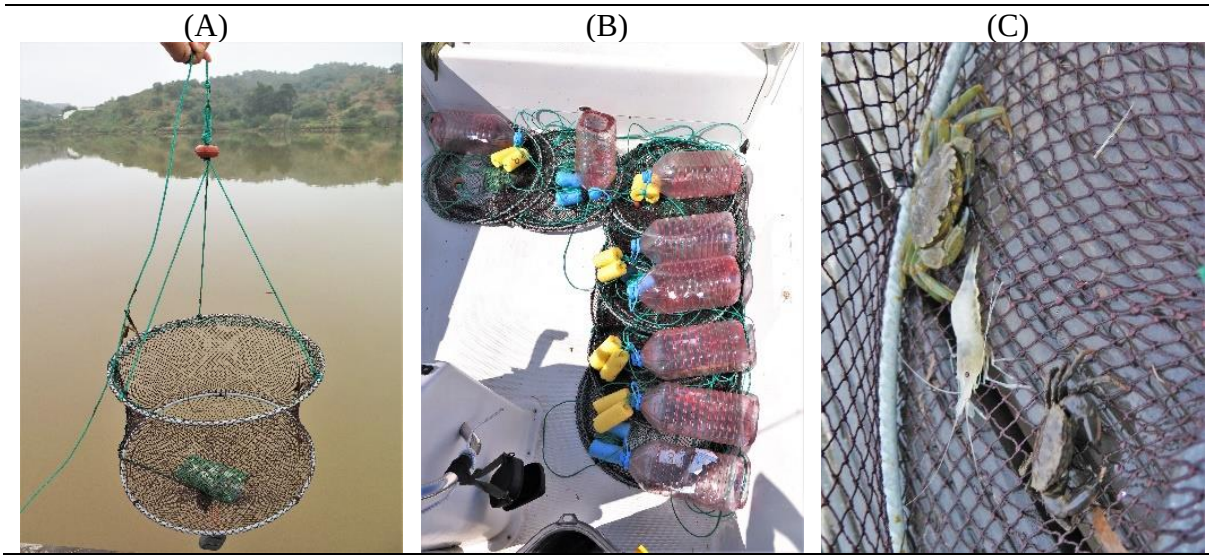


Figure 2.1 – (A) Example of a trap used in the Guadiana estuary from a floating pontoon; (B) set of eight traps while sampling in the Arade estuary from a boat; (C) native species in a trap in the Guadiana estuary. Photos by João Encarnação.

The sampling areas in the Algarve region (south Portugal) depended on access to the sampling area, available logistics, and were adapted along the experimental sampling period. These included the Arade estuary and the Alvor lagoon in western Algarve (**Figure 2.2A, B**), and the Guadiana estuary in eastern Algarve (**Figure 2.2A, C**). Experimental sampling took place in western Algarve between July and September 2020; in the Alvor lagoon sampling lasted for 5 days, traps were deployed 146 times (sum of the number of replicates), and they set in water for a total of 53 h (number of replicates deployed multiplied by the number of minutes in the water), while in the Arade estuary sampling lasted 7 days, traps were deployed 365 times, and stayed in water for a total of 121 h and 40 min. In the Guadiana estuary,

sampling also began in July 2020, but it extended until March 2022. In this eastern area of the Algarve, sampling elapsed for 29 days, traps were deployed 2839 times and stayed in water for 951 h and 40 min.

All organisms were identified to the lowest taxonomical level possible according to Whitehead et al. (1986), Haywood and Ryland (1990), Saldanha (1995), Ingle (1996), González-Ortegón and Cuesta (2006), and Gonçalves et al. (2013). Based on the number of individuals and the number of traps deployed, the number of individuals per trap, or per 100 traps, was calculated. Differences between factors were evaluated with two-way analysis of variance (ANOVA) and community structure was characterized with four ecological indexes: total number of species (S), total number of individuals (N), Margalef's species richness (d'), and Shannon's diversity index (H'). Statistical analyses were done in R, using the software R Studio version 2022.07.2+576 (RStudio Team, 2022), and in PRIMER 6 (Clarke and Gorley 2006).

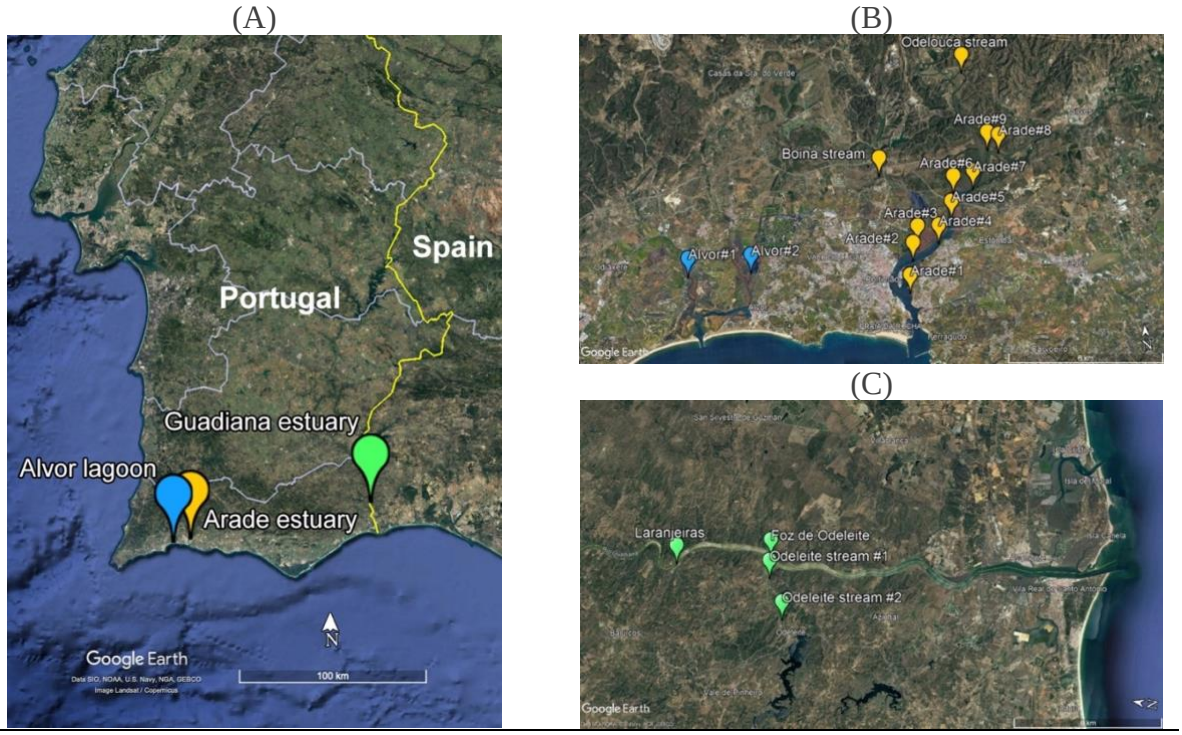


Figure 2.2 – (A) Location of the three areas where sampling was performed using benthic baited traps in Algarve (south Portugal); (B) location of sampling stations in the Alvor lagoon and Arade estuary in western Algarve and (C) in the Guadiana estuary in eastern Algarve.

2.2.2 Bottom beam trawls

Two sampling campaigns were performed in 2022, the first in April (spring-time) and the second in October (autumn-time), aimed at obtaining a detailed overview on the presence of non-indigenous species, but also the state of fishing resources in the Guadiana estuary (eastern Algarve). Sampling was made with a bottom beam trawl net, the net had conical-shape with a mouth opening of 4.2 m by 0.5 m and a length of 8 m. Mesh size was variable, decreasing between the mouth and the cod end of the net, from 20 mm to 10 mm respectively.

All trawls were performed during ebb tides, in the same direction as water flow. Sampling areas included the lower (14 trawls), middle (15 trawls), and higher (14 trawls) Guadiana estuary. The number and duration of trawls was variable, depending on the sampling location and abundance of organisms captured, but start and finish points were recorded using a Garmin GPS to calculate trawl distances. Trawls were performed in a boat equipped with an 80 hp engine, at a constant speed, between 2 and 5 knots (**Figure 2.3**). Trawl duration varied between 2 and 10 min and lasted on average 7.1 ± 1.8 min. Trawl distance varied between 106.1 m and 719.19 m, and was on average 481.9 ± 131.5 m, while the average sampled area per trawl was 2024.1 ± 552.5 m^2.

Figure 2.3 – Beam trawl being lowered into the Guadiana estuary.

All organisms were identified to the lowest taxonomical level possible according to Whitehead et al. (1986), Haywood and Ryland (1990), Saldanha (1995), Ingle (1996), González-Ortegón and Cuesta (2006), and Gonçalves et al. (2013). The density (density of individuals per m^2) was calculated based on the number of individuals and the trawl area. Differences between factors were evaluated with two-way analysis of variance (ANOVA) and community structure was characterized with four ecological indexes: total number of species (S), total number of individuals (N), Margalef's species richness (d'), and Shannon's diversity index (H'). Statistical analyses were done in R, using the software R Studio version 2022.07.2+576 (RStudio Team, 2022), and in PRIMER 6 (Clarke and Gorley 2006).

2.2.3 Underwater visual census

A series of dives were performed between July 2019 and May 2021 along the Algarve coast in south Portugal (**Figure 2.4**). These had the objective of assessing the presence or absence of invasive species, but also a broader group of species – the subtropical or neonative species. The sampling area was concentrated in the western Algarve, the most rocky area of the southern coast, while from Faro to the eastern Algarve, the coast is mainly a continuous sandy area. These campaigns included 30 SCUBA dives, and 7 snorkelling census, totalling 40 h and 12 min of diving, with an average dive time of 65.16 ± 25.59 min. The shallowest dive was in the Ria Formosa lagoon with a depth of 3 m, while the deepest dive was in the "Hermenegildo Capelo" wreck in Portimão with a maximum depth of 34 m.

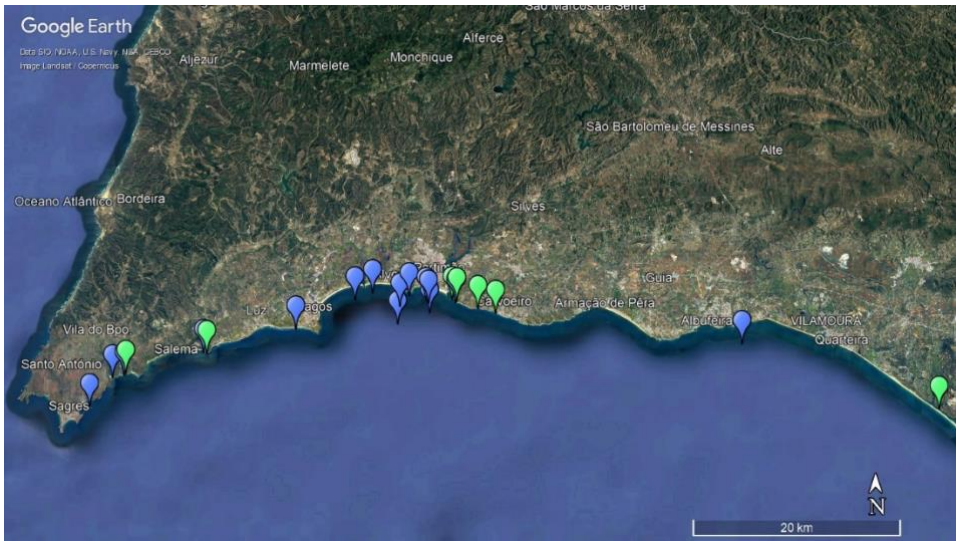


Figure 2.4 – Location of the SCUBA dives (blue icons) and snorkelling census (green icons) between July 2019 and May 2021, along the Algarve coast (south Portugal).

2.2.4 Plankton night trawls

The first objective of the plankton sampling was to assess the larvae dynamics of the non-indigenous Atlantic blue crab *Callinectes sapidus*, namely the re-ingress of the megalopa phase into estuarine areas of southern Portugal. In the native range, the whole reproductive cycle of the species is well described, but in the invaded range across Europe, the only study on this subject reports the finding of two megalopa in the Balearic Archipelago (Mediterranean Sea) in 2005 and 2011 (Png-Gonzalez et al. 2021).

The reproduction of the Atlantic blue crab initiates in oligohaline or mesohaline estuarine areas, where mating occurs, and females store the sperm for internal fertilization of eggs (Jivoff et al. 2007; Epifanio 2019). Following, the eggs brood into an external egg-mass and the females migrate to more saline areas, either lower estuarine or coastal zones, usually aided by selective vertical migrations during ebb tides, where the eggs hatch as planktonic larvae (Tankersley et al. 1998; Epifanio 2019). These seasonal migrations are due to physiological needs of the larvae, as the early life-stages need higher-salinity areas for a suitable development (Costlow and Bookhout 1959). The initial phases include seven zoeal stages, which are dispersed through the continental shelf by wind-driven surface currents during a 3-4-week period, until they metamorphose to megalopa, at which individuals settle on the bottom and then re-ingress into estuarine areas (Epifanio 2019). The Atlantic blue crab megalopa are strong swimmers, that undergo rhythmic vertical migrations to facilitate the transport into estuaries (Epifanio et al. 1984), aided by a capacity to detect chemical cues during settlement, which helps to avoid areas with higher abundance of predators (Welch et al. 1997; Diaz et al. 1999). The entrance in estuaries usually occur during flood tides when megalopa actively swim into the water column, and a peak in transport is usually registered when flood tides occur during the night (Olmi 1994; Forward et al. 2004).

Considering the species' reproductive cycle, the sampling design in south Portugal consisted of night-time 10-min surface tows, once every hour, during the 6 h period of a new moon flood tide. Sampling took place at the entrance of the two estuaries of southern Portugal – Arade (GPS: 37.1144 / -8.5242) and Guadiana (GPS: 37.1958 / -7.4133) – once per month in August, September, and October 2020. The tows were performed with a conical-shaped plankton net with an opening of 50 cm, 225 cm in length, a mesh of 500 µm, and equipped with a mechanical flowmeter (Hydro-Bios). Samples were immediately preserver in 4% formalin until further identification. In total, there were 36 samples, with a filtrated volume of

2429.0 m^3 (average of 134.9 ± 102.7 m^3) in the Arade estuary and 2004.6 m^3 (average of 111.4 ± 68.3 m^3) in the Guadiana estuary.

All Brachyura were identified to species level under a stereomicroscope (Leica MDG41), according to Ingle (1992), Paula (1996), Pessani et al. (1998), dos Santos and González-Gordillo (2004), González-Gordillo and Cuesta (2020). Based on the number of individuals and filtrated volumes, the density of individuals per m^3 was calculated. Differences between factors were evaluated with two-way analysis of variance (ANOVA) and community structure was characterized with four ecological indexes: total number of species (S), total number of individuals (N), Margalef's species richness (d'), and Shannon's diversity index (H'). Statistical analyses were done in R Studio version 2022.07.2+576 (RStudio Team, 2022), and in PRIMER 6 (Clarke and Gorley 2006).

2.3 Results

2.3.1 Benthic baited traps

During 2020 in the Alvor lagoon, benthic baited traps registered 5 species of fish, 4 species of gastropods, 3 species of crabs, and one taxon belonging to Paguroidea (**Figure 2.5**). During 2020 in the Arade estuary, there were 6 species of gastropods, 5 species of fish, 4 species of crabs, 2 species of shrimps, and one taxon each belonging to Paguroidea and Cephalopoda (**Figure 2.5**). In the Guadiana estuary, between 2020, 2021, and 2022, the number of species identified included 8 species of fish, 4 species of shrimps, 3 species of crabs, and one taxon each belonging to Bivalvia, Hydrozoa, and Reptilia (**Figure 2.5**).

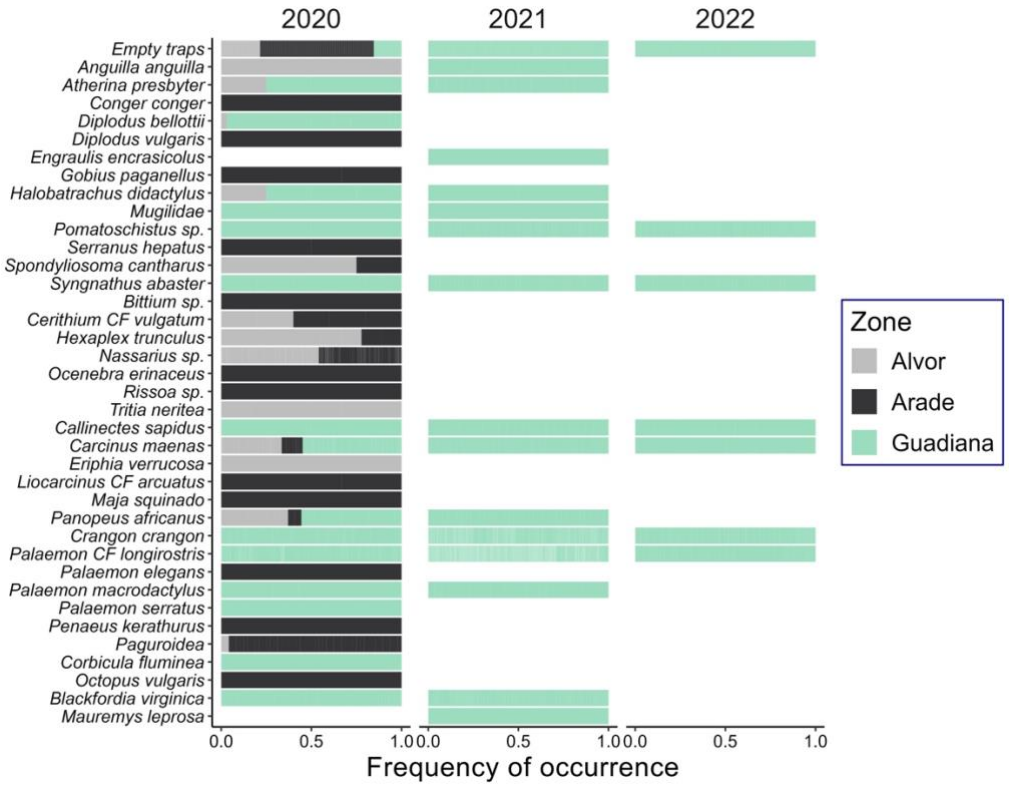


Figure 2.5 – List of species captured with benthic baited traps between 2020 and 2022, with their relative frequency of occurrence, in three sampling zones of Algarve, the Alvor Lagoon and the Arade and Guadiana estuaries.

In the Alvor lagoon (2020), the most abundant species were *Nassarius* sp. mud snails (350 ± 871 ind.100 traps^{-1}), followed by the European green crab *Carcinus maenas* and the African mud crab *Panopeus africanus* A. Milne-Edwards, 1867 (**Table 2.1**). In the Arade estuary (2020), the most common species were also the *Nassarius* sp. mud snails (119 ± 319 ind.100 traps^{-1}), followed by the Paguroidea hermit crabs and the European green crab *Carcinus maenas* (**Table 2.1**). In the Guadiana estuary, during the year 2020, the species with the higher average abundance was the shrimp *Palaemon longirostris* H. Milne Edwards, 1837 (523 ± 772 ind.100 traps^{-1}), followed by the Black Sea jellyfish *Blackfordia virginica* and the European green crab *Carcinus maenas* (**Table 2.1**). The shrimp *Palaemon longirostris* (331 ± 401 ind.100 traps^{-1}) was also the more abundant species during 2021 in the Guadiana estuary, followed by the brown shrimp *Crangon crangon* (Linnaeus, 1758) and the Black Sea jellyfish *Blackfordia virginica* (**Table 2.1**). In 2022, the most common species in the Guadiana estuary was the brown shrimp *Crangon crangon* (49 ± 116 ind.100 traps^{-1}), followed by the shrimp *Palaemon longirostris* and the goby *Pomatoschistus* sp. (**Table 2.1**). Empty traps were common in all zones, with 50.7% in Alvor (2020), 59.5% in Arade (2020), and 21.8% in the Guadiana in 2020, 14.9% in 2021, and 48.2% in 2022 (**Table 2.1**).

Table 2.1 – List of species identified in the Alvor lagoon, and Arade and Guadiana estuaries, during the sampling campaigns where benthic baited traps were used. Data is expressed as the percentage of empty trawls and percentage of individuals in each zone and year, and the average abundance (± SD) as the number of individuals per 100 traps.

	Alvor		**Arade**		**Guadiana**					
	2020		**2020**		**2020**		**2021**		**2022**	
	%	Avg	%	Avg	%	Avg	%	Avg	%	Avg
Empty traps	50.7	-	59.5	-	21.8	-	14.9	-	48.2	-
A. anguilla	0.2	1 ± 8	0	0	0	0	< 0.1	< 1	0	0
A. presbyter	0.2	1 ± 8	0	0	0.2	1 ± 14	0.2	1 ± 13	0	0
C. conger	0	0	0.2	< 1	0	0	0	0	0	0
D. bellottii	0.2	1 ± 8	0	0	1.9	13 ± 68	0	0	0	0
D. vulgaris	0	0	0.2	< 1	0	0	0	0	0	0
E. encrasicolus	0	0	0	0	0	0	< 0.1	< 1	0	0
G. paganellus	0	0	0.6	1 ± 9	0	0	0	0	0	0
H. didactylus	0.2	1 ± 8	0	0	0.2	1 ± 11	< 0.1	< 1	0	0
Mugilidae	0	0	0	0	0.1	< 1	< 0.1	< 1	0	0
Pomatoschistus sp.	0	0	0	0	0.1	1 ± 9	0.8	3 ± 20	21.6	24 ± 57
S. hepatus	0	0	0.4	1 ± 7	0	0	0	0	0	0
S. cantharus	0.5	2 ± 25	0.2	< 1	0	0	0	0	0	0
S. abaster	0	0	0	0	0.3	2 ± 14	0.5	2 ± 15	7.3	8 ± 28
Bittium sp.	0	0	0.2	< 1	0	0	0	0	0	0
C. CF vulgatum	0.3	1 ± 12	0.6	1 ± 9	0	0	0	0	0	0
H. trunculus	1.1	5 ± 21	0.4	1 ± 7	0	0	0	0	0	0
Nassarius sp.	83.1	350 ± 871	82.1	119 ± 319	0	0	0	0	0	0
O. erinaceus	0	0	0.2	< 1	0	0	0	0	0	0
Rissoa sp.	0	0	0.2	< 1	0	0	0	0	0	0
T. neritea	1.0	4 ± 23	0	0	0	0	0	0	0	0
*C. sapidus**	0	0	0	0	0.1	1 ± 9	0.3	1 ± 10	1.6	2 ± 15
C. maenas	11.2	30 ± 97	4.5	5 ± 39	6.8	32 ± 70	0.7	3 ± 21	1.1	1 ± 13
E. verrucosa	0.2	1 ± 8	0	0	0	0	0	0	0	0
L. CF *arcuatus*	0	0	0.6	1 ± 9	0	0	0	0	0	0
M. squinado	0	0	0.2	< 1	0	0	0	0	0	0
P. africanus	1.6	6 ± 27	0.4	1 ± 7	0.9	5 ± 24	0.4	2 ± 14	0	0
C. crangon	0	0	0	0	4.7	32 ± 117	13.0	54 ± 128	43.6	49 ± 116
P. longirostris	0	0	0	0	76.8	523 ± 772	80.3	331 ± 401	24.8	28 ± 110
P. elegans	0	0	0.2	< 1	0	0	0	0	0	0
*P. macrodactylus**	0	0	0	0	0.4	3 ± 19	0.3	1 ± 12	0	0
P. serratus	0	0	0	0	0.1	< 1	0	0	0	0
P. kerathurus	0	0	0.2	< 1	0	0	0	0	0	0
Paguroidea	0.3	1 ± 12	8.7	13 ± 45	0	0	0	0	0	0
*C. fluminea**	0	0	0	0	0.1	< 1	0	0	0	0
O. vulgaris	0	0	0.2	< 1	0	0	0	0	0	0
*B. virginica**	0	0	0	0	7.4	50 ± 219	3.2	13 ± 67	0	0
M. leprosa	0	0	0	0	0	0	< 0.1	< 1	0	0

* Non-indigenous species

During 2020, when the three sampling zones were sampled simultaneously, all the six ecological indices showed differences between zones, on average they were higher in the Guadiana estuary (**Table 2.2**). The greater differences ($p < 0.001$) were registered in the number

of species (S), number of individuals (N) and Shannon's diversity (H'), all increasing on average from Arade to Alvor to Guadiana, while Simpson's diversity (1-λ) increased on average from Alvor to Arade to Guadiana (**Table 2.2**). In the case of the Guadiana estuary, major differences were also registered for all ecological indices between years. From 2020 to 2022, there was a decreasing trend in S, N, and H', while species richness (d), Pielou's evenness (J'), and Simpson's diversity (1-λ) tended to increase, mainly in 2022 (**Table 2.2**).

Table 2.2 – Ecological indices (average ± SD) for the three sampling zones in 2020, 2021, and 2022, while using the benthic baited traps. One-way ANOVA results are presented for the year 2020, between the three sampling zones, and for the Guadiana estuary, between the three sampling years.

		S	**N**	**d**	**J'**	**H'**	**1-λ**
2020	Arade	0.49 ± 0.7	1.45 ± 3.3	0.24 ± 0.4	0.72 ± 0.2	0.04 ± 0.2	0.15 ± 0.3
	Alvor	0.64 ± 0.7	3.89 ± 7.9	0.23 ± 0.4	0.58 ± 0.3	0.05 ± 0.2	0.13 ± 0.3
	Guadiana	1.28 ± 1.0	6.87 ± 8.6	0.41 ± 0.4	0.76 ± 0.2	0.24 ± 0.3	0.29 ± 0.3
2021	Guadiana	1.30 ± 0.8	4.29 ± 4.7	0.44 ± 0.6	0.76 ± 0.2	0.21 ± 0.3	0.26 ± 0.3
2022	Guadiana	0.74 ± 0.7	1.24 ± 1.8	0.56 ± 0.6	0.91 ± 0.1	0.10 ± 0.2	0.39 ± 0.4
ANOVA *p* value	2020	< 0.001	< 0.001	0.0034	0.0127	< 0.001	< 0.001
	Guadiana	< 0.001	< 0.001	0.0574	< 0.001	< 0.001	< 0.001

The Guadiana estuary was the only sampling area where non-indigenous species were detected, namely the Atlantic blue crab *Callinectes sapidus*, the prawn *Palaemon macrodactylus*, the Black Sea jellyfish *Blackfordia virginica*, and the Asian clam *Corbicula fluminea* (**Figure 2.6**). Still, these were only 4.3% of the total number of specimens identified in the Guadiana estuary. Considering that 2847 traps (replicates) were deployed in the Guadiana estuary, the Atlantic blue crab was caught in 32 of them, representing a frequency of occurrence of 1.12% (34 individuals). The prawn *Palaemon macrodactylus* was identified in 29 traps (1.02%; 33 individuals), the Black Sea jellyfish *Blackfordia virginica* in 155 traps (5.44%; 403 individuals), and the Asian clam *Corbicula fluminea* in one trap (0.04%; 1 individual). These last two species, for its characteristics, one a planktonic jellyfish and the other an infauna bivalve, are considered accidental bycatch for the type of fishing gear being used. In the case of the Black Sea jellyfish, the results are still valid, but should be regarded as a vertical plankton tow and more carefully considered.

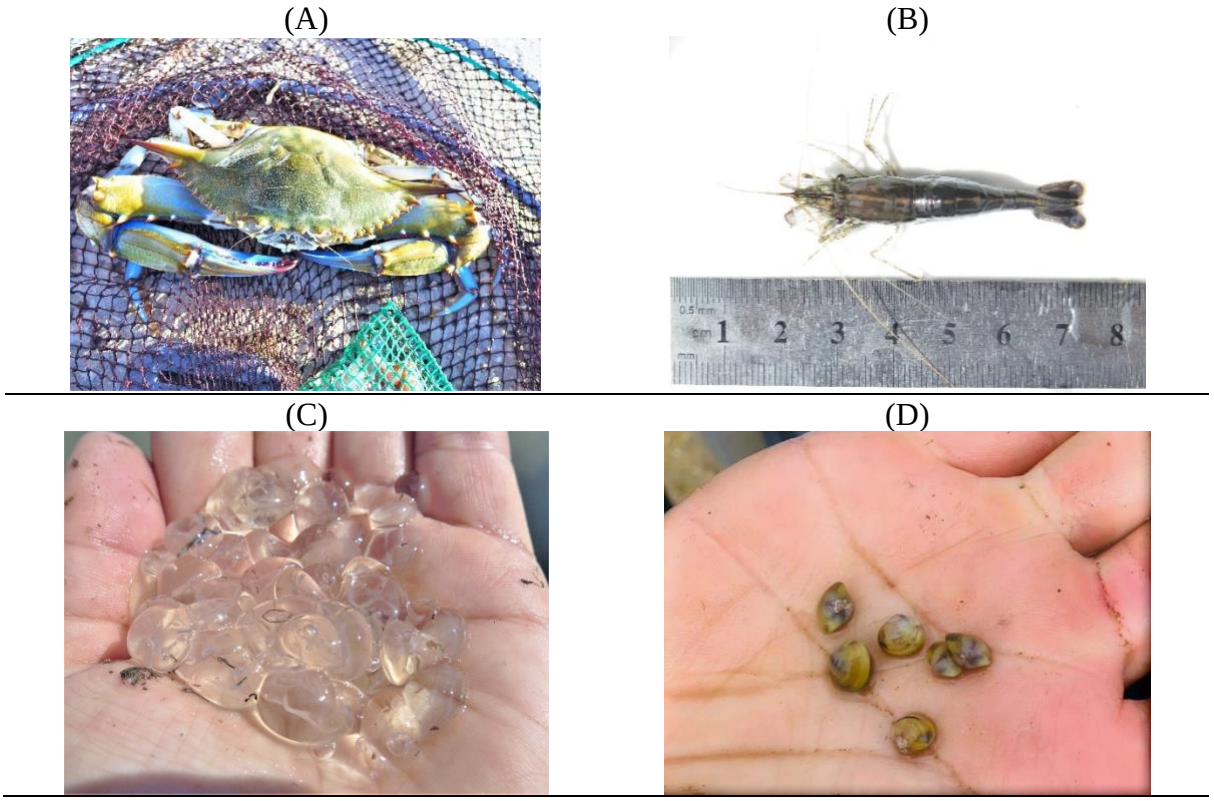


Figure 2.6 – The four non-indigenous species detected in the Guadiana estuary sampled with benthic baited traps: (A) the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896, (B) the prawn *Palaemon macrodactylus* Rathbun, 1902, (C) the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910, and (D) the Asian clam *Corbicula fluminea* (O. F. Müller, 1774).

Monthly changes in abundance were observed for the Atlantic blue crab (p= 0.013), prawn *Palaemon macrodactylus* (p< 0.001), and Black Sea jellyfish (p< 0.001). The Atlantic blue crab showed highest abundances in September 2020, April and May 2021, with a maximum abundance of 4.1 ± 22.6 ind.100 traps^{-1} in February 2022 (**Figure 2.7**). The prawn *Palaemon macrodactylus* was particularly abundant from September to November, with a maximum of 10.0 ± 35.4 ind.100 traps^{-1} in October 2020, while the Black Sea jellyfish was more abundant from August to October 2020, with a maximum of 93.9 ± 310.4 ind.100 traps^{-1} in August 2020 (**Figure 2.7**).

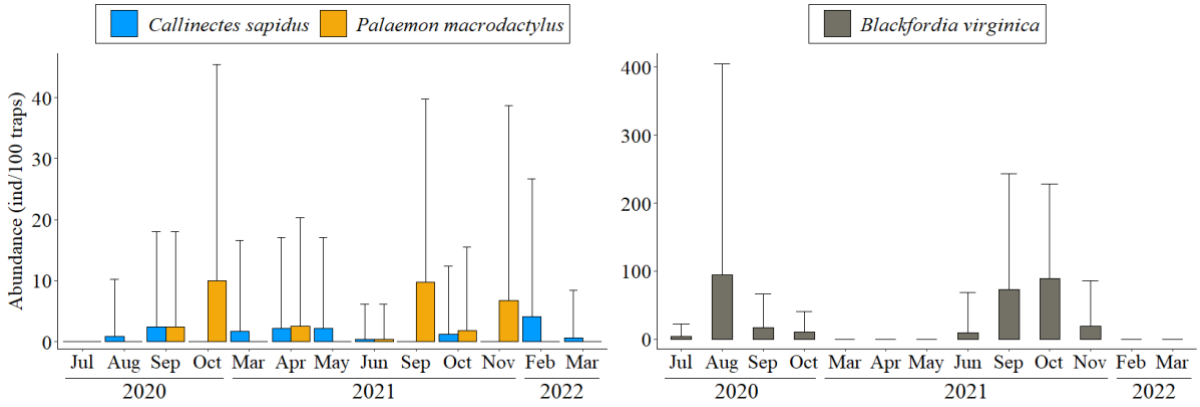


Figure 2.7 – Average abundance of non-indigenous species – the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896, the prawn *Palaemon macrodactylus* Rathbun, 1902, and the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910 – registered in the middle Guadiana estuary between 2020 and 2022 and sampled with benthic baited traps.

Atlantic blue crabs detected in the middle Guadiana estuary showed seasonal variations in length and weight between sex classes. Two-way ANOVA identified differences in both length and weight ($p \leq 0.01$), between seasons and also sexes. Seasonal differences in length ($p < 0.05$) were mainly registered between winter (105.0 ± 48.2 mm) and summer (177.0 ± 60.5 mm), and between winter and autumn (202.0 ± 10.6 mm). In the case of weight, these differences were even more accentuated ($p < 0.01$) between winter (99.5 ± 143.0 g) and summer (398.0 ± 227.0 g), and between winter and autumn (471.0 ± 49.5 g). Additional differences were identified between spring-autumn and spring-summer ($p < 0.05$).

On average, males were 24.6% larger and 69.6% heavier than females. During summer and early autumn (August, September, 2020; June, October, 2021), adult males comprised the majority of the catches (length 202.0 ± 18.9 mm; weight 485.5 ± 84.5 g), while during the other sampled months (February, March, April, May) size classes were smaller and females were more frequent – male length: 134.3 ± 54.6 mm; male weight: 189.5 ± 192.5 g; female length: 94.4 ± 26.1 mm; female weight: 51.0 ± 40.4 g (**Figure 2.8**).

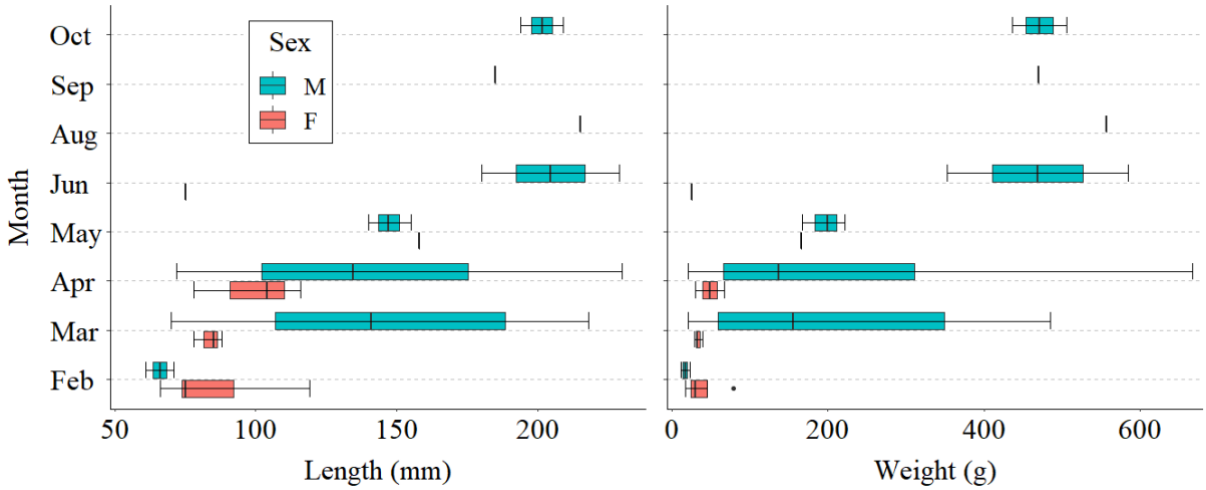


Figure 2.8 – Box-n-whisker plots of length and weight of male (*n*= 21) and female (*n*= 13) Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896, caught using benthic baited traps in the middle Guadiana estuary between 2020 and 2022. Lower and upper fences represent the 25th and 75th percentiles, and the median is in between. Whiskers represent 10th and 90th percentiles and dots the outliers.

2.3.2 Bottom beam trawls

During the spring sampling campaign of April 2022, bottom beam trawls led to the identification of 6 species of fish, 2 species of crabs, and 2 species of shrimps in the higher Guadiana estuary (Alcoutim) (**Figure 2.9, 2.10**); 6 species of fish, 2 species of shrimps, and 1 species of Hydrozoa in the middle estuary (Foz de Odeleite); and 10 species of fish, 2 species of crabs, 2 species of shrimps, and one species of Cephalopoda and Nudibranchia in the lower estuary (Vila Real de Santo António) (**Figure 2.10**).

Figure 2.9 – Catch of a bottom beam trawl performed in the higher Guadiana estuary (Alcoutim) in spring 2022.

The autumn sampling campaign in October 2022 identified 4 fish species, 2 shrimp species, and one species of Hydrozoa in the higher estuary; one species of shrimp, bivalve, and Hydrozoa in the middle estuary; and 2 species of fish, and one species of shrimp and Cephalopoda in the lower estuary (**Figure 2.10**).

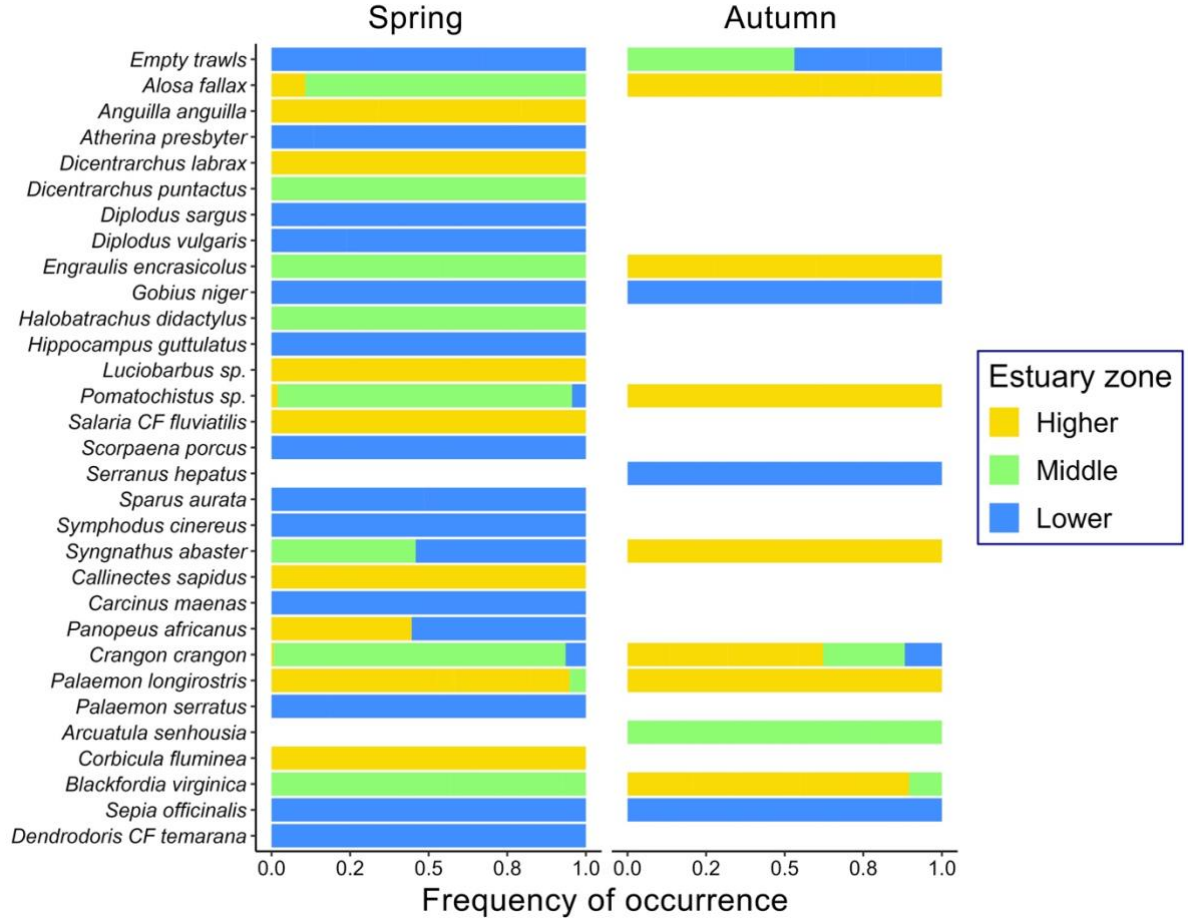


Figure 2.10 – List of species identified during the 2022 spring and autumn sampling campaigns with their relative frequency of occurrence between the three zones of the Guadiana estuary, while using the bottom beam trawl net.

In spring 2022,the shrimp *Palaemon longirostris* (5.60 ± 5.18 ind.100 m^{-2}) was the most abundant species, comprising 95.5% of individuals caught in the higher estuary, followed by the barbel *Luciobarbus* sp. (Almaça, 1967) (0.09 ± 0.11 ind.100 m^{-2}) and the European eel *Anguilla anguilla* (Linnaeus, 1758) (0.07 ± 0.10 ind.100 m^{-2}); while in the middle estuary the most abundant species were the brown shrimp *Crangon crangon* (1.55 ± 4.09 ind.100 m^{-2}), the goby *Pomatochistus* sp. (0.98 ± 2.53 ind.100 m^{-2}), and the shrimp *Palaemon longirostris* (0.27 ± 0.52 ind.100 m^{-2}); and in the lower estuary the most abundant species were the shrimp

Palaemon serratus (Pennant, 1777) (0.16 ± 0.33 ind.100 m^{-2}), the brown shrimp *Crangon crangon* (0.12 ± 0.29 ind.100 m^{-2}), and the white seabream *Diplodus sargus* (Linnaeus, 1758) (0.04 ± 0.11 ind.100 m^{-2}) (**Table 2.3**).

Table 2.3 – List of species identified during the 2022 spring and autumn sampling campaigns in the three zones of the Guadiana estuary, while using the bottom beam trawl net. Values are expressed as the percentage of empty trawls and percentage of individuals in each season and zone, and the average abundance (± SD) as the number of individuals per 100 m^2.

	Spring						**Autumn**					
	Higher		**Middle**		**Lower**		**Higher**		**Middle**		**Lower**	
	%	Avg	%	Avg	%	Avg	%	Avg	%	Avg	%	Avg
Empty trawls	0	-	0	-	42.9	-	0	-	28.6	-	57.1	-
A. fallax	0.1	< 0.1	1.5	< 0.1	0	0	0.4	< 0.1	0	0	0	0
A. anguilla	1.3	0.1 ± 0.1	0	0	0	0	0	0	0	0	0	0
A. presbyter	0	0	0	0	7.0	< 0.1	0	0	0	0	0	0
D. labrax	0.2	< 0.1	0	0	0	0	0	0	0	0	0	0
D. puntactus	0	0	0.2	< 0.1	0	0	0	0	0	0	0	0
D. sargus	0	0	0	0	8.1	< 0.1	0	0	0	0	0	0
D. vulgaris	0	0	0	0	4.0	< 0.1	0	0	0	0	0	0
E. encrasicolus	0	0	1.1	< 0.1	0	0	0.2	< 0.1	0	0	0	0
G. niger	0	0	0	0	3.0	< 0.1	0	0	0	0	74.0	0.1 ± 0.1
H. didactylus	0	0	0.6	< 0.1	0	0	0	0	0	0	0	0
H. guttulatus	0	0	0	0	1.0	< 0.1	0	0	0	0	0	0
Luciobarbus sp.	1.6	0.1 ± 0.1	0	0	0	0	0	0	0	0	0	0
Pomatochistus sp.	0.4	< 0.1	32.8	1.0 ± 2.5	10.1	< 0.1	0.2	< 0.1	0	0	0	0
S. CF *fluviatilis*	0.2	< 0.1	0	0	0	0	0	0	0	0	0	0
S. porcus	0	0	0	0	4.0	< 0.1	0	0	0	0	0	0
S. hepatus	0	0	0	0	0	0	0	0	0	0	9.7	< 0.1
S. aurata	0	0	0	0	2.0	< 0.1	0	0	0	0	0	0
S. cinereus	0	0	0	0	1.0	< 0.1	0	0	0	0	0	0
S. abaster	0	0	0.2	< 0.1	1.4	< 0.1	0.1	< 0.1	0	0	0	0
*C. sapidus**	0.1	< 0.1	0	0	0	0	0	0	0	0	0	0
C. maenas	0	0	0	0	1.0	< 0.1	0	0	0	0	0	0
P. africanus	0.1	< 0.1	0	0	2.0	< 0.1	0	0	0	0	0	0
C. crangon	0.2	< 0.1	51.9	1.6 ± 4.1	23.6	0.1 ± 0.3	0.5	< 0.1	1.7	< 0.1	9.4	< 0.1
P. longirostris	95.5	5.6 ± 5.2	9.0	0.3 ± 0.5	0	0	0.2	< 0.1	0	0	0	0
P. serratus	0	0	0	0	29.9	0.2 ± 0.3	0	0	0	0	0	0
*A. senhousia**	0	0	0	0	0	0	0	0	8.8	0.1 ± 0.2	0	0
*C. fluminea**	0.2	< 0.1	0	0	0	0	0	0	0	0	0	0
S. officinalis	0	0	0	0	1.0	< 0.1	0	0	0	0	6.9	< 0.1
*B. virginica**	0	0	2.8	0.1 ± 0.1	0	0	98.3	9.1 ± 5.6	89.5	1.1 ± 2.7	0	0
D. CF *temarana*	0	0	0	0	1.0	< 0.1	0	0	0	0	0	0

* Non-indigenous species

During the second sampling campaign in the autumn of 2022 on the higher estuary, the most abundant species, comprising 98.3% of individuals, was the Black Sea jellyfish *Blackfordia virginica* (9.09 ± 5.58 ind.100 m^{-2}), followed by the brown shrimp *Crangon crangon* (0.05 ± 0.05 ind.100 m^{-2}) and the twaite shad *Alosa fallax* (Lacepède, 1803) (0.03 ± 0.05 ind.100 m^{-2}); in the middle estuary the most abundant species, comprising 89.5% of individuals, was also the Black Sea jellyfish *Blackfordia virginica* (1.06 ± 2.67 ind.100 m^{-2}), followed by the Asian date mussel *Arcuatula senhousia* (W. H. Benson, 1842) (0.10 ± 0.19 ind.100 m^{-2}; **Figure 2.11**), and the brown shrimp *Crangon crangon* (0.02 ± 0.04 ind.100 m^{-2}); and in the lower estuary the most abundant species were black goby *Gobius niger* Lacepède, 1800 (0.07 ± 0.12 ind.100 m^{-2}), the brown comber *Serranus hepatus* (Linnaeus, 1758) (0.01 ± 0.03 ind.100 m^{-2}), and the brown shrimp *Crangon crangon* (0.01 ± 0.02 ind.100 m^{-2}) (**Table 2.3**). Morphometric details of *Arcuatula senhousia* individuals are presented in **Table 2.4**.

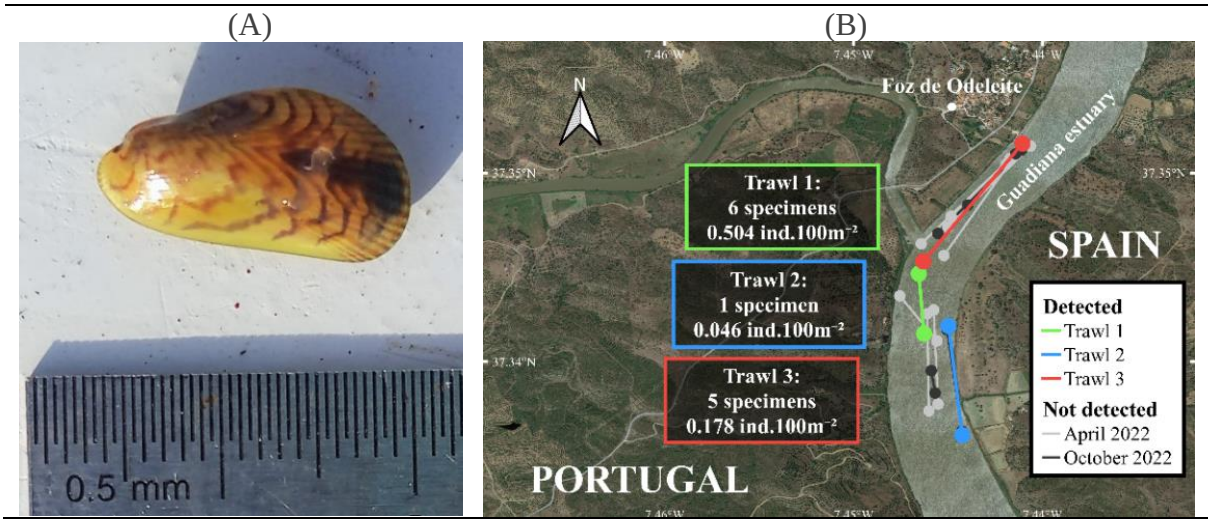


Figure 2.11 – (A) Photo of an Asian date mussel *Arcuatula senhousia* (W. H. Benson, 1842) specimen retrieved from the middle Guadiana estuary on October 7th, 2022. (B) Location of the bottom beam trawls performed in April and October 2022 in the middle Guadiana estuary. The number of specimens and abundance of the Asian date mussel is shown for the three trawls where they were collected in October 2022.

Table 2.4 – Morphometric details of the 12 Asian date mussels *Arcuatula senhousia* (W. H. Benson, 1842) specimens collected in the middle Guadiana estuary on October 7th, 2022.

Specimen	Tow number	Length (cm)	Width (cm)	Weight (g)	Notes
1	1	1.1	0.6	0.05*	* Broken shell
2	1	1.4	0.8	0.12*	* Broken shell
3	1	1.2	0.7	0.12	
4	1	0.7	0.5	0.02	
5	1	1.0	0.5	0.05	
6	1	1.5	0.8	0.20	
7	2	1.1	0.7	0.10	
8	3	1.6	0.9	0.30	
9	3	1.5	0.8	0.25	
10	3	1.4	0.8	0.21	
11	3	0.7	0.4	0.03	
12	3	1.4	0.8	0.18	

From the total number of bottom beam trawls performed, the number of empty trawls during the spring campaign was zero in the higher and middle estuary, but 42.9% in the lower estuary. During the autumn sampling campaign, the number of empty trawls was zero in the higher estuary, 28.6% in the middle estuary, and 57.1% in the lower estuary (**Table 2.3**).

Ecological indices displayed differences between seasons, but also between estuary zones (**Table 2.5**). The number of species (S) and Shannon's diversity (H'), both decreased on average between spring and autumn, while the number of individuals (N) decreased considerably from the higher to the lower estuary, and Pielou's evenness (J') showed a tendency to increase from the higher to the other two estuary zones (**Table 2.5**).

Table 2.5 – Ecological indices (average ± SD) from the 2022 spring and fall sampling campaigns in the three zones of the Guadiana estuary, while using the bottom beam trawl net. Nested ANOVA results are presented for differences between season, and between estuary zones within the two seasons.

		S	N	d	J'	H'	1-λ
Spring	Higher	3.14 ± 1.3	5.86 ± 5.4	3.61 ± 6.3	0.18 ± 0.1	0.22 ± 0.2	0.37 ± 0.7
	Middle	3.63 ± 2.2	2.99 ± 7.2	2.37 ± 5.9	0.60 ± 0.4	0.83 ± 0.6	0.44 ± 1.0
	Lower	3.00 ± 5.5	0.52 ± 1.2	1.72 ± 4.5	0.22 ± 0.4	0.45 ± 0.8	0.17 ± 0.5
Autumn	Higher	3.14 ± 1.7	9.25 ± 5.5	2.17 ± 2.9	0.15 ± 0.2	0.22 ± 0.3	0.21 ± 0.4
	Middle	1.00 ± 0.8	1.19 ± 2.7	0.07 ± 0.2	0.15 ± 0.3	0.11 ± 0.2	0.01 ± 0.0
	Lower	0.86 ± 1.1	0.10 ± 0.2	0	0.37 ± 0.5	0.25 ± 0.3	0
ANOVA *p* value	Seasons	0.051	0.778	0.147	0.186	0.033	0.105
	Season:Zones	0.473	0.002	0.498	< 0.001	0.173	0.206

2.3.3 Underwater visual census

A total of 103 species were registered during the underwater visual census between Vila do Bispo and the Ria Formosa lagoon in Faro (**Table 2.6**). Most were fish species, 67 of them were native and 13 considered neonative or with subtropical affinities.

Table 2.6 – Species detected and respective number of detections during the underwater visual census performed between July 2019 and May 2021 along the Algarve coast (south Portugal).

		West → East					
Origin	**Species**	**Vila do Bispo**	**Lagos**	**Lagoa**	**Portimão**	**Albufeira**	**Faro**
Native	*Alosa fallax*			1			
	Aphia minuta				1		
	Apogon imberbis		1				
	Argyrosomus regius				2		
	Atherina presbyter	3		6			1
	Balistes capriscus				4		
	Boops boops	3	1	1	14		
	Callionymus lyra	3		1	1		
	Centrolabrus exoletus	3	3	3	8	1	
	Chelidonichthys obscurus				1		
	Chelon labrosus	3		4		1	1
	Chromis chromis					1	
	Conger conger				8		
	Coris julis	7	3	6	19	1	
	Ctenolabrus rupestris	5	3	5	11	1	
	Dicentrarchus labrax	2		2	3		
	Dicentrarchus punctatus			1			
	Diplodus annularis		3		2	1	
	Diplodus cervinus	4	2	6	12		
	Diplodus puntazzo	3	1	6	14		
	Diplodus sargus	6	3	6	19	1	1
	Diplodus vulgaris	7	3	6	19	1	1
	Gobius bucchichi	2		4			
	Gobius cruentatus		3		9	1	
	Gobius gasteveni		1				1
	Gobius paganellus	5			1		
	Gobius xanthocephalus	1	3		6	1	
	Gobiusculus flavescens	4	2	1	1		
	Hippocampus hippocampus			1			
	Labrus bergylta	7	3	6	7	1	
	Labrus mixtus	1	2		2		
	Lepadogaster candolii		1				
	Microlipophrys canevae			1			
	Mullus surmuletus	4	1	2	5	1	
	Muraena helena		1		1		
	Oblada melanura	4		6	3		

Pagrus auriga		2		2		
Pagrus caeruleostictus		1				
Pagrus pagrus				1		
Parablennius gattorugine	4	2	2	7	1	
Parablennius incognitus	1			1		
Parablennius pilicornis	7	3	6	15	1	
Parablennius rouxi	2	2	1	5		
Parablennius sanguinolentus			1			
Phycis phycis				4		
Pomatomus saltatrix			1			
Pomatoschistus pictus	3		1			
Raja undulata				1		
Salaria pavo						1
Sarpa salpa	4		6			
Scomber colias	3	1	1	2		
Scophthalmus rhombus			1			
Scorpaena notata	1	2		15	1	
Scorpaena porcus	1	1	1	4		
Serranus cabrilla	5	3	1	14	1	
Serranus hepatus	2	1		3		
Sparus aurata				11		
Spondyliosoma cantharus		2	1	14	1	1
Symphodus bailloni	3	2	1	3	1	1
Symphodus melops	6		3	3	1	
Symphodus roissali			1			
Symphodus rostratus				1		
Symphodus sp.	1	1	6			
Trachinus draco			1	2		
Trachurus trachurus				4		
Tripterygion delaisi	5		6	3		
Trisopterus luscus		1	2	11		
Doriopsilla areolata	1					
Felimare cantabrica	1					
Felimare picta	1					
Felimare villafranca	2					
Flabellina affinis				1		
Luisella babai				1		
Paradoris indecora	1					
Maja squinado		1		1		
Necora puber	3	1	2	3		
Pagurus sp.						1
Palaemon serratus		1	1			
Palinurus elephas		1		1		
Scyllarus arctus	1					
Loligo vulgaris	2					
Octopus vulgaris	4		1		1	
Sepia officinalis	3		2	1	1	1
Cymodocea nodosa						1
Zostera noltii						1

Neonative	*Chromis chromis*	1	2		9		
	Dentex canariensis				1		
	Diplodus bellottii	1			11	1	1
	Labrus CF *merula*	1					
	Parapristipoma octolineatum		1				
	Plectorhinchus mediterraneus			1	1		
	Scorpaena maderensis		1		3		
	Serranus atricauda	3	2		5		
	Symphodus mediterraneus		2		5	1	
	Symphodus ocellatus					1	
	Symphodus rostratus				5		
	Thalassoma pavo			2	5	1	
	Umbrina canariensis				3		
Invasive	*Styela plicata*						1
	Amphibalanus amphitrite						1
	Callinectes sapidus			1			
	Branchiomma luctuosum						1
Cryptogenic	*Ecteinascidia turbinata*						1

Four invasive and one cryptogenic species were detected. A claw of the invasive Atlantic blue crab was found in the Pintadinho beach in Lagoa in February 2020. In the Ria Formosa lagoon in Faro, three other invasive species were recorded, namely the pleated sea squirt *Styela plicatai*, the striped barnacle *Amphibalanus amphitrite*, the fan worm *Branchiomma luctuosum* (Grube, 1870), and the cryptogenic mangrove tunicate *Ecteinascidia turbinata* Herdman, 1880 (**Figure 2.12**).

Figure 2.12 – Non-indigenous species detected in the Ria Formosa lagoon during the underwater visual census in July 2020. (A) The three invasive species recorded – the pleated sea squirt *Styela plicata* (Lesueur, 1823), striped barnacle *Amphibalanus amphitrite* (Darwin, 1854), and fan worm *Branchiomma luctuosum* (Grube, 1870); and (B) the cryptogenic mangrove tunicate *Ecteinascidia turbinata* Herdman, 1880.

2.3.4 Plankton night trawls

A total of 51 different brachyuran taxa were identified in the Arade estuary, and 49 taxa in the Guadiana estuary (**Figure 2.13**). In the Arade estuary, the three most abundant taxa were *Afropinnotheres monodi* Manning, 1993 (5.80 ± 13.22 ind.m^{-3}), *Panopeus africanus* (3.87 ± 14.35 ind.m^{-3}), and *Pilumnus hirtellus* (Linnaeus, 1761) (0.95 ± 1.64 ind.m^{-3}), while in the Guadiana estuary these were *Afropinnotheres monodi* (2.35 ± 5.53 ind.m^{-3}), *Panopeus africanus* (0.97 ± 1.92 ind.m^{-3}), and *Ethusa mascarone* (Herbst, 1785) (0.38 ± 1.03 ind.m^{-3}). Regarding the different larval stages in the three sampling months, zoeae I of *Panopeus africanus* (11.03 ± 24.00 ind.m^{-3}), zoeae II of *Afropinnotheres monodi* (1.60 ± 2.94 ind.m^{-3}), and zoeae I of *Pilumnus hirtellus* (0.22 ± 0.20 ind.m^{-3}), were the most abundant in the Arade estuary in August, September, and October 2020, respectively. In the Guadiana estuary, the most abundant were the zoeae I of *Panopeus africanus* (1.25 ± 1.61 ind.m^{-3} and 1.31 ± 2.23 ind.m^{-3}) in August and September, and the zoeae I of *Afropinnotheres monodi* (2.10 ± 4.94 ind.m^{-3}) in October 2020.

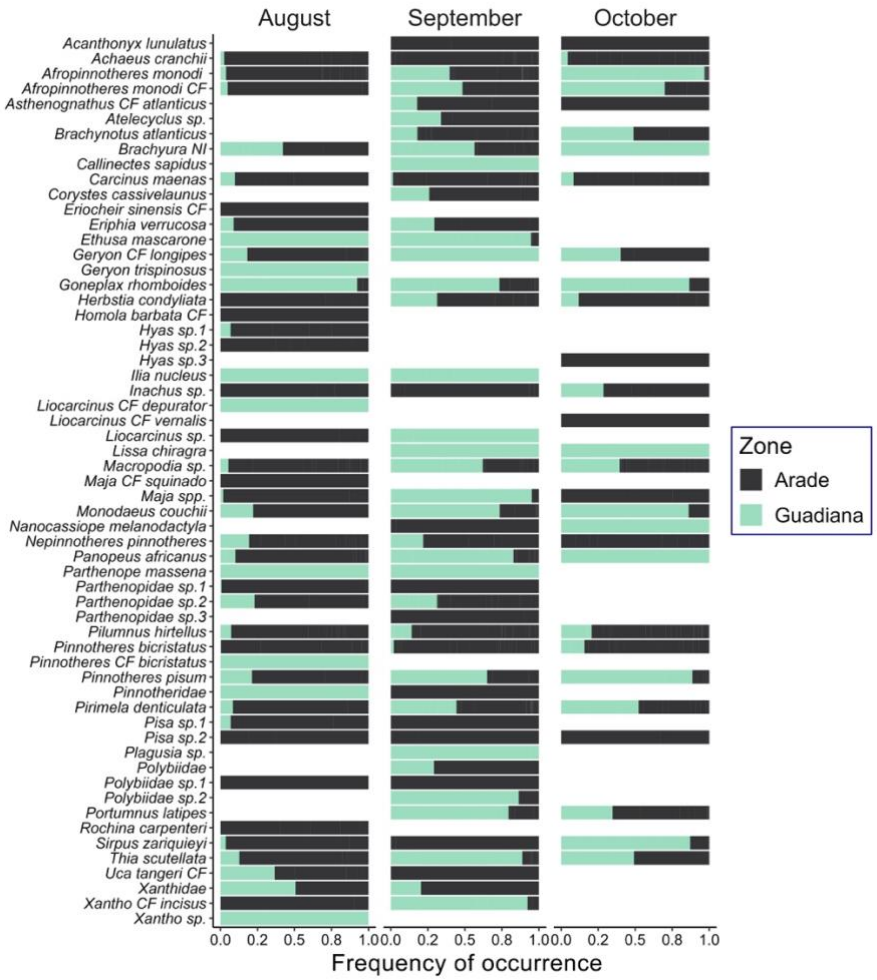


Figure 2.13 – List of brachyuran taxa identified during the 2020 plankton night trawls in the Arade and Guadiana estuaries, while using a 500 µm conical-shaped plankton net.

Ecological indices displayed differences between sampled estuaries, but also between the three sampling months of 2020 in the case of number of species (S) and Shannon's diversity (H'), and between sampling months in the case of Pielou's evenness (J') (**Table 2.7**). The number of species (S) decreased on average between the Arade and the Guadiana estuary, in the three sampling months. The month of September showed the higher average number of species, both in the Arade estuary (41.50 ± 11.98 ind.m^{-3}) and in the Guadiana estuary (29.33 ± 15.62 ind.m^{-3}). The Shannon's diversity index (H'), showed the most distinct differences, while the pattern was the same as for the number of species – values decreasing between the Arade and the Guadiana estuary, and higher values during the month of September (**Table 2.7**). An increase in the values of Pielou's evenness index (J') was registered form August, to September, to October, in both the Arade and Guadiana estuaries (**Table 2.7**).

Table 2.7 – Ecological indices (average ± SD) from the 2020 plankton night trawls in the Arade and Guadiana estuaries. Nested ANOVA results are presented for differences between locals, and between sampling months within the two locals.

		S	**N**	**d**	**J'**	**H'**	**1-λ**
Arade	August	27.00 ± 4.73	27.94 ± 50.05	16.41 ± 10.02	0.58 ± 0.09	1.91 ± 0.30	1.01 ± 0.35
	September	41.5 ± 11.98	9.69 ± 15.2	29.66 ± 12.76	0.78 ± 0.05	2.87 ± 0.14	1.24 ± 0.19
	October	23.17 ± 5.23	1.04 ± 0.38	43.94 ± 10.6	0.85 ± 0.06	2.65 ± 0.35	2.62 ± 0.39
Guadiana	August	15.83 ± 11.13	2.29 ± 1.98	20.36 ± 10.31	0.57 ± 0.16	1.46 ± 0.46	1.21 ± 0.50
	September	29.33 ± 15.62	9.20 ± 7.30	14.54 ± 5.23	0.72 ± 0.07	2.31 ± 0.71	0.95 ± 0.07
	October	12.00 ± 4.82	4.53 ± 9.26	137.23 ± 211.1	0.79 ± 0.11	1.90 ± 0.44	8.13 ± 11.34
ANOVA	Local	0.001	0.309	0.398	0.251	< 0.001	0.278
p value	Locals:Months	0.002	0.309	0.190	< 0.001	< 0.001	0.126

2.4 Discussion

2.4.1 Invasive species

The surveys performed between 2019 and 2022 culminated in the identification of 8 non-indigenous species with invasive characteristics: the Atlantic blue crab *Callinectes sapidus*, the prawn *Palaemon macrodactylus*, the Black Sea jellyfish *Blackfordia virginica*, the Asian clam *Corbicula fluminea*, the pleated sea squirt *Styela plicata*, the striped barnacle *Amphibalanus amphitrite*, the fan worm *Branchiomma luctuosum*, and the first records in southern Portugal of the Asian date mussel *Arcuatula senhousia*. Although the sampling effort was higher in the Guadiana estuary, the present results came to confirm the presence and establishment of species like the Atlantic blue crab and the prawn *Palaemon macrodactylus* in this estuary, since

previous studies relied solely on reports by local fishers in the first case (Morais et al. 2019), and in the second case the last reports of the species date back from 2009 (Encarnação et al. 2013).

The Atlantic blue crab was detected in the middle Guadiana estuary in August and September 2020, March-June and October 2021, and February and March 2022. In one beam trawl performed in Alcoutim (upper Guadiana estuary) one individual was caught in April 2022. During one underwater visual census, a claw of the species was also found in the coastal zone of Lagoa close to the entrance of the Arade estuary in February 2020. Although in low abundance, one stage V zoea of the Atlantic blue crab was detected in a plankton night trawl in the lower Guadiana estuary in September 2020. With the data collected using the benthic baited traps in the middle Guadiana estuary, it was possible to uncover several aspects related to seasonal variations in abundance, reproduction, and occurrence of the different size classes across the year. During the first months of the year, size classes were quite small, with the smallest individual weighting just 11.04 g and measuring 61 mm in carapace width. During February, March, and April samplings, from the total of 23 Atlantic blue crabs captured, 18 had less than 100 g, with an average of 40.21 ± 21.51 g and 87.50 ± 18.73 mm of carapace width. During the other months where Atlantic blue crabs were caught (May, June, August, September, October), the weight averaged 335.27 ± 187.89 g. The presence of small individuals in the middle Guadiana estuary during winter months, when salinity is the lowest due to higher river inflow, is in accordance with published data that describes upstream migrations of juvenile Atlantic blue crabs to lower salinity areas to avoid excessive predation during early-life phases (Posey et al. 2005; Epifanio 2019). Based on available growth rate data, these juveniles smaller than 90 mm should belong to the cohorts that hatched in early summer of previous the year, while smaller juveniles under 70 mm might have hatched in late summer (van Engel 1958; Ju et al. 2001). Estimations that Atlantic blue crabs attain sexual maturity between 82-89 mm in males, and 90-100 mm in females (Jivoff et al. 2007), indicate that these individuals reaching 90 mm width can be considered adults, and likely reaching sexual maturity. In the middle Guadiana estuary, 85% of total females were caught between February and April, averaging 88.64 ± 17.52 mm in carapace width likely because females only remain in these middle or upper areas of the estuary until they are sexually mature. After insemination, female Atlantic blue crabs tend to move downstream to more saline areas, including the coastal zone to spawn (Tankersley et al. 1998; Forward et al. 2003; Turner et al. 2003).

In October 2022, 12 specimens of the Asian date mussel *Arcuatula senhousia* were collected in the middle Guadiana estuary, close to the village of Foz de Odeleite, becoming the first records of this non-indigenous bivalve in southern Portugal. The Asian date mussel is an epifauna bivalve recorded in salinities between 5 and 18 (Yamamuro et al. 2010; Hosozawa et al. 2020), but able to colonize fully marine areas, as observed in the Solent (southern England) (Watson et al. 2021) and French localities along the Bay of Biscay (Massé et al. 2022). On the native range, the Asian date mussel may reach up to 2500 ind.m^{-2} (Morton 1974), but on invaded areas it has reached 10,000 ind.m^{-2} in Italy (Mistri et al. 2004), 16,000 ind.m^{-2} in New Zealand (Creese et al. 1997), and 190,000 ind.m^{-2} in Mission Bay (California, United States of America) which is among the highest ever recorded for marine bivalves (Crooks 1998). The detection of the Asian date mussel in only three trawls very close to each other, out of 43 trawls made along the estuary in 2022, shows that the species was recently introduced because it is not widespread, or its low density prevented its detection during previous sampling campaigns. In Portugal, the species was found in the Sado and Tejo estuaries in 2015 and 2018, respectively, both located in the western coast of Portugal (Cabral et al. 2020). In Spain, the presence of the Asian date mussel dates to 2006 in the Bidasoa estuary on the Atlantic border with France (Bachelet et al. 2009), and in the Mediterranean coast in 2014 in the Ebro delta (Soriano and Salgado 2014) and 2016 in a Barcelona's marina (Ulman et al. 2017). It is unlikely that the species has reached the Guadiana estuary by natural dispersion from any of these locations, so an additional introduction event must have occurred to the Guadiana estuary or Gulf of Cadiz. This small ecosystem engineer, that grows up to 28 mm in shell length, may form dense continuous mats of ovoid cocoons anchored on the sediment with a matrix of byssal threads (Morton 1974; Sousa et al. 2009). As an ecosystem engineer, the Asian date mussel also increases habitat complexity and, consequently, species richness and density, particularly of amphipods, gastropods, and polychaetes (Crooks 1998; Crooks and Khim 1999). The water filtration capacity of the species accelerates the transfer of suspended sediment and organic matter from the pelagic compartment into the benthic compartment through the production of faeces and pseudo-faeces. This process alters the sediment's nutrient balance and reduces the redox potential discontinuity layer impairing other taxa from living below the byssal mats (Morton 1974; Creese et al. 1997; Crooks 1998). The Asian date mussel may expand its distribution along the Guadiana estuary, both upstream and downstream of its current known location in the middle estuary. The impact of this new non-indigenous species upon other invasive bivalves, such as the Asian clam *Corbicula fluminea*, and native bivalve species in the Guadiana estuary will depend on its invasiveness. In the worst-case scenario, dense mats

of Asian date mussels could impair the survival of several notable native infaunal bivalves in the middle and lower estuary, such as *Scrobicularia plana* (da Costa, 1778), *Ruditapes decussatusi* (Linnaeus, 1758), and *Cerastoderma edule* (Linnaeus, 1758) (Conde et al. 2013).

The Guadiana estuary, and its brackish zone in particular, is a hotspot for non-indigenous species, where they occupy both the pelagic and benthic compartments while exploring different resources (Morais et al. 2009, 2017, 2019; Chícharo et al. 2009; Cruz et al. 2017; Seyer et al. 2017; Nuño et al. 2018). In the pelagic compartment we can find planktonic species (e.g., *Acartia tonsa*, *Blackfordia virginica*), while in the benthic compartment there are numerous infaunal and macrofaunal macroinvertebrates (*Palaemon macrodactylus*, *Synidotea laticauda*), including biofouling species (*Cordylophora caspia*), and bivalves (*Corbicula fluminea*, *Arcuatula senhousia*), besides large nektonic and benthonic predators (*Cynoscion regalis*, *Callinectes sapidus*). The constant low flow conditions set by the Alqueva dam since 2002, reshaped the ecosystem functioning of the Guadiana estuary, coinciding with the establishment of multiple non-indigenous species, and a more pronounced saline intrusion into upper zones of the estuary (Chícharo et al. 2006; Barbosa et al. 2010; Encarnação et al. 2013). The continuous appearance of new non-indigenous species, mainly in the middle Guadiana estuary, may be an indication of a continuous high propagule pressure to this region, but also a sign of ongoing ecological disturbances in the estuary and the availability of empty niches, facilitating the establishment of non-indigenous species. With a high concentration in one zone, facilitation and synergistic relationships between non-indigenous species may also start to develop, resulting in more accentuated impacts, a phenomenon called “invasion meltdown” (Simberloff and Von Holle 1999; Simberloff 2006; Havel et al. 2015). Such scenario needs further investigation in the Guadiana estuary but should not be overlooked.

2.4.2 Neonative species

The underwater visual census identified 13 species that can be classified as neonative for continental Portugal. These include *Chromis chromis* (Linnaeus, 1758), *Dentex canariensis* Steindachner, 1881, *Diplodus bellottii* (Steindachner, 1882), *Labrus* CF *merula* Linnaeus, 1758, *Parapristipoma octolineatum* (Valenciennes, 1833), *Plectorhinchus mediterraneus* (Guichenot, 1850), *Scorpaena maderensis* Valenciennes, 1833, *Serranus atricauda* Günther, 1874, *Symphodus mediterraneus* (Linnaeus, 1758), *Symphodus ocellatus* (Linnaeus, 1758), *Symphodus rostratus* (Bloch, 1791), *Thalassoma pavo* (Linnaeus, 1758), and *Umbrina*

canariensis Valenciennes, 1843. Some of these species may be present in south Portugal for many years, while others were only detected recently, and have been increasing in abundance and distribution. Such range-expansions may be related to the process of tropicalization of temperate regions (Bianchi and Morri 2003). Most of these neonative species had been reported in scientific publications and grey literature as being detected in south Portugal (Santos et al. 1996, 2002; Ribeiro et al. 2006; Monteiro et al. 2012), but the actual distribution and frequency of occurrence remains unknown.

Nevertheless, no records of the Madeira rockfish *Scorpaena maderensis* were found in the literature. The Madeira rockfish is a common benthic species in all the Macaronesian archipelagos and along the coasts of Morocco, Mauritania, and Senegal (Hureau and Litvinenko 1986). It is also present in the Mediterranean Sea, from Greek waters (Ahnelt 1983) to the central Mediterranean (La Mesa et al. 2005; Falzon 2011), and eastwards to the Balearic Islands (Cardona and Elices 2000). However, misidentifications might have hindered the report of this species earlier, as it occurs alongside with *Scorpaena porcus* Linnaeus, 1758 and *Scorpaena notata* Rafinesque, 1810, both in the Mediterranean Sea and off southern Portugal (La Mesa et al. 2005; Ribeiro et al. 2006). In Algarve, the four records, one in Lagos at 18 m deep, and three in Portimão between 17 and 29 m deep, along with three older personal observations in Portimão made between 2016 and 2019, confirms the presence of the Madeira rockfish in continental Portugal.

In the case of the ornate wrasse *Thalassoma pavo*, the only reference to its presence in continental Portugal was made in an internal report of a scientific survey made in the Sagres area in 2011 (Monteiro et al. 2012). The ornate wrasse is a charismatic and colourful species of the Macaronesian archipelagos, present throughout the west coast of Africa until Gabon, and across the Mediterranean Sea, where in the northern shores it is also becoming more common due to warming conditions (Guidetti et al. 2002). In this case, for its vibrant colours when compared to other fish species in continental Portugal, it's unlikely that the ornate wrasse has gone unnoticed or misidentified for a long period of time. In the present study, the species was detected three times in depths between 4 and 10 m in Lagoa and Albufeira, and five times in Portimão in sunken ships at depths between 26 and 29 m. The eight records made between 2019 and 2020, plus eight personal observations made between 2017 and 2019 in Portimão, its detection in 2011 in Sagres (Monteiro et al. 2012), and the lack of other records in the scientific literature suggest that the ornate wrasse arrived and established recently in the Algarve coast.

Along the southwestern and western coasts of the Iberian Peninsula, several algae species shifted northwards in the order of hundreds and thousands of kilometres (Lima et al. 2007; Nicastro et al. 2013). There are also examples of new species reported off southern Portugal, such as the brown mussel *Perna perna* (Linnaeus, 1758) (Lourenço et al. 2012), the parrotfish *Sparisoma cretense* (Linnaeus, 1758) (Abecasis et al. 2008), or the Monrovia doctorfish *Acanthurus monroviae* Steindachner, 1876 (Vasconcelos et al. 2018), which suggests that a wide array of species are shifting their northern distribution limit from northern Africa and the Macaronesia archipelagos to the Atlantic coasts of the Iberian Peninsula. In the current study, plankton trawls also evidenced such influence of neonative species in the Algarve coast, namely the African pea crab *Afropinnotheres monodi* and the African mud crab *Panopeus africas*, which were the two most abundant Brachyura taxa in the Arade and Guadiana estuaries in the 2020 zooplankton sampling campaigns. The African pea crab has started to be observed in the Gulf of Cádiz in 2003 and is now present in the Spanish coast between the Alboran Sea and Gulf of Cádiz, including in southern Portugal, and the Sado estuary in the west Portuguese coast (Subida et al. 2011; Pérez-Miguel et al. 2019a). This is an obligate symbiont of bivalves that inhabits the mantle cavity of mussels, clams, or cockles, making it a neonative species with invasive potential and hazardous effects in commercially important bivalve species under a range-expansion scenario (Drake et al. 2014; Pérez-Miguel et al. 2019b; Cuesta et al. 2020). The African mud crab *Panopeus africanus* is reported as a native species between the Mira estuary in the southwestern coast of Portugal, towards the Gulf of Cadiz, and along the Atlantic African coast until Angola (Rodríguez and Paula 1993). In 2013 the species has also been detected in Galicia, northwestern Spain, but likely due to a direct introduction from African shores hitchhiking ship hulls (Cuesta et al. 2016). Still, plankton studies in the southern coast of Portugal done between 1986 and 1994 (dos Santos 1999), and between 2006 and 2007 (Pochelon et al. 2017) never recorded the African mud crab, but in the Gulf of Cadiz the species was abundant in 1989-1992, both as adults and larvae (Rodríguez et al. 1997). The lack of more recent studies regarding larval abundances of Brachyura in the southern coast of Portugal hinders more accurate comparisons with the present results, but the fact that larvae of the African mud crab were among the most abundant crab species in the plankton tows show its high relevance within brachyuran diversity. Still, year-around sampling would be needed to confirm if this is a consistent pattern over-time, or if the sampling months of August, September, and October may correspond to the peak of the reproduction period of the species in Algarve, thus reflecting the high abundances found.

Overall, the location of the Algarve coast, in the south European Atlantic shelf ecoregion bordering three other ecoregions – Macaronesia archipelagos, the north-western African coast, and the Mediterranean Sea (Spalding et al. 2007) – increases the possibility for the appearance of species typically from other ecoregions. This further complicates the task of defining a clear line between which species should be considered "new" for a given location, or our ability to pinpoint the exact time when first specimens arrived. This could only be mitigated with more systematic, and recurrent large-scale surveys, but also by considering complementary sources of data on species distribution and presence, such as citizen science and local ecological knowledge (i.e., Azzurro et al. 2019; Giovos et al. 2019).

2.5 Conclusions

Surveys made in multiple aquatic ecosystems along the Algarve region identified eight non-indigenous species with invasive characteristics and confirmed the presence and establishment of the Atlantic blue crab *Callinectes sapidus* and the prawn *Palaemon macrodactylus* in the Guadiana estuary, a hotspot for non-indigenous species due to the salinization of the upper areas of the estuary. During this study, the first records in southern Portugal of the Asian date mussel *Arcuatula senhousia*, an ecosystem-engineer bivalve, were gathered in the middle Guadiana estuary. Underwater visual census and plankton trawls evidenced the on-going tropicalization of continental Portugal, which is particularly evident in the Algarve region. A total of 13 neonative species were identified, which for some of them, its presence in Algarve has never been reported in peer-reviewed scientific articles.

2.6 References

Abecasis, D., Bentes, L., Ribeiro, J., Machado, D., Oliveira, F., Veiga, P., Gonçalves, J.M., and Erzini, K. (2008). First record of the Mediterranean parrotfish, *Sparisoma cretense* in Ria Formosa (south Portugal). *Marine Biodiversity Records* 1: e27. doi: 10.1017/S175526720600248X

Ahnelt, H. (1983). Erstnachweis von *Scorpaena maderensis* Val., 1833 (Pisces, Scorpaenidae) für Kreta und Rhodos (Griechenland) mit Angaben zum bisher einzigen Fund dieser Art aus der Adria. *Annalen des Naturhistorischen Museums in Wien* 85B: 9-11.

Aires, T., Serrão, E.A., and Engelen, A.H. (2016). Host and environmental specificity in bacterial communities associated to two highly invasive marine species (genus *Asparagopsis*). *Frontiers in Microbiology* 7: 559. doi: 10.3389/fmicb.2016.00559

Anastácio, P.M., Ribeiro, F., Capinha, C., Banha, F., Gama, M., Filipe, A.F., Rebelo, R., and Sousa, R. (2019). Non-native freshwater fauna in Portugal: A review. *Science of the Total Environment* 650: 1923-1934. doi: 10.1016/j.scitotenv.2018.09.25

Azzurro, E., Bolognini, L., Dragičević, B., Drakulović, D., Dulčić, J., Fanelli, E., Grati, F., Kolitari, J., Lipej, L., and Magaletti, E. (2019). Detecting the occurrence of indigenous and non-indigenous megafauna through fishermen knowledge: A complementary tool to coastal and port surveys. *Marine Pollution Bulletin* 147: 229-236. doi: 10.1016/j.marpolbul.2018.01.016

Bachelet, G., Blanchet, H., Cottet, M., Dang, C., de Montaudouin, X., de Moura Queirós, A., Gouillieux, B., and Lavesque, N. (2009). A round-the-world tour almost completed: First records of the invasive mussel *Musculista senhousia* in the north-east Atlantic (southern Bay of Biscay). *Marine Biodiversity Records* 2: e119. doi: 10.1017/S1755267209001080

Barbosa, A.B., Domingues, R.B., and Galvão, H.M. (2010). Environmental forcing of phytoplankton in a Mediterranean Estuary (Guadiana Estuary, South-western Iberia): A decadal study of anthropogenic and climatic influences. *Estuaries and Coasts* 33: 324-341. doi: 10.1007/s12237-009-9200-x

Bella, S. (2013). New alien insect pests to Portugal on urban ornamental plants and additional data on recently introduced species. *Annales de la Société entomologique de France (N.S.): International Journal of Entomology* 49: 374-382. doi: 10.1080/00379271.2013.856210

Bianchi, C.N., and Morri, C. (2003). Global sea warming and "tropicalization" of the Mediterranean Sea: Biogeographic and ecological aspects. *Biogeographia–The Journal of Integrative Biogeography* 24(1). doi: 10.21426/B6110129

Cabral, S., Carvalho, F., Gaspar, M., Ramajal, J., Sá, E., Santos, C., Silva, G., Sousa, A., Costa, J.L., and Chainho, P. (2020). Non-indigenous species in soft-sediments: Are some estuaries more invaded than others? *Ecological Indicators* 110: 105640. doi: 10.1016/j.ecolind.2019.105640

Cardona, L., and Elices, M. (2000). Datos sobre la presencia en el litoral de Menorca (islas Baleares, Mediterráneo Occidental) de *Parablennius pilicornis* (Cuvier, 1829) y *Scorpaena maderensis* Valenciennes 1833. *Bolletí de la Societat d'Història Natural de les Balears* 43: 33–38.

Cardoso, J.F., Peralta, N.R., Machado, J.P., and van der Veer, H.W. (2013). Growth and

reproductive investment of introduced Pacific oysters *Crassostrea gigas* in southern European waters. *Estuarine, Coastal and Shelf Science* 118: 24-30. doi: 10.1016/j.ecss.2012.12.017

Chaínho, P., Fernandes, A., Amorim, A., Ávila, S.P., Canning-Clode, J., Castro, J.J., Costa, A.C., Costa, J.L., Cruz, T., and Gollasch, S. (2015). Non-indigenous species in Portuguese coastal areas, coastal lagoons, estuaries and islands. *Estuarine, Coastal and Shelf Science* 167: 199-211. doi: 10.1016/j.ecss.2015.06.019

Chícharo, M.A., Chícharo, L., and Morais, P. (2006). Inter-annual differences of ichthyofauna structure of the Guadiana estuary and adjacent coastal area (SE Portugal/SW Spain): Before and after Alqueva dam construction. *Estuarine, Coastal and Shelf Science* 70(1-2): 39-51. doi: 10.1016/j.ecss.2006.05.036

Chícharo, M.A., Leitão, T., Range, P., Gutierrez, C., Morales, J., Morais, P., and Chícharo, L. (2009). Alien species in the Guadiana Estuary (SE-Portugal/SW-Spain): *Blackfordia virginica* (Cnidaria, Hydrozoa) and *Palaemon macrodactylus* (Crustacea, Decapoda): Potential impacts and mitigation measures. *Aquatic Invasions* 4(3): 501-506. doi: 10.3391/ai.2009.4.3.11

Chiesa, S., Lucentini, L., Freitas, R., Marzano, F.N., Breda, S., Figueira, E., Caill-Milly, N., Herbert, R.J., Soares, A.M., and Argese, E. (2017). A history of invasion: COI phylogeny of Manila clam *Ruditapes philippinarum* in Europe. *Fisheries Research* 186: 25-35. doi: 10.1016/j.fishres.2016.07.024

Clarke, K.R., and Gorley, R.N. (2006). PRIMER v6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research). PRIMER-E, Plymouth.

Conde, A., Novais, J.M., and Domínguez, J. (2013). Characterization of an estuarine environment by means of an index based on intertidal macrofauna. *Marine Pollution Bulletin* 71(1-2): 129-138. doi: 10.1016/j.marpolbul.2013.03.024

Costlow Jr, J.D., and Bookhout, C. (1959). The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. *The Biological Bulletin* 116(3): 373-396. doi: 10.2307/1538947

Creese, R., Hooker, S., De Luca, S., and Wharton, Y. (1997). Ecology and environmental impact of *Musculista senhousia* (Moilusca: Bivalvia: Mytilidae) in Tamaki Estuary, Auckland, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 31(2): 225-236. doi: 10.1080/00288330.1997.9516760

Crooks, J.A. (1998). Habitat alteration and community-level effects of an exotic mussel, *Musculista senhousia*. *Marine Ecology Progress Series* 162: 137-152. doi: 10.3354/meps162137

Crooks, J.A., and Khim, H.S. (1999). Architectural vs. biological effects of a habitat-altering, exotic mussel, *Musculista senhousia*. *Journal of Experimental Marine Biology and Ecology* 240(1): 53-75. doi: 10.1016/S0022-0981(99)00041-6

Cruz, J., Teodósio, M.A., Ben-Hamadou, R., Chicharo, L., Garrido, S., Ré, P., and Santos, A.M.P. (2017). RNA:DNA ratios as a proxy of egg production rates of *Acartia*. *Estuarine, Coastal and Shelf Science* 187: 96-109. doi: 10.1016/j.ecss.2016.12.028

Cuesta, J.A., Almón, B., Pérez-Dieste, J., Trigo, J.E., and Bañón, R. (2016). Role of ships' hull fouling and tropicalization process on European carcinofauna: New records in Galician

waters (NW Spain). *Biological Invasions* 18: 619-630. doi: 10.1007/s10530-015-1034-9

Cuesta, J.A., Pérez-Miguel, M., González-Ortegón, E., Roque, D., and Drake, P. (2020). The prevalence of the pea crab *Afropinnotheres monodi* in mussels depending on the degree of habitat exposure: Implications for mussel culture. *Aquaculture* 520: 734772. doi: 10.1016/j.aquaculture.2019.734772

Cunha, A.H., Paulo, D., Sousa, I., and Serrão, E. (2013). The rediscovery of *Caulerpa prolifera* in Ria Formosa, Portugal, 60 years after the previous record. *Cahiers de Biologie Marine* 54(3): 359-364.

Diaz, H., Orihuela, B., Forward Jr, R.B., and Rittschof, D. (1999). Orientation of blue crab, *Callinectes sapidus* (Rathbun), megalopae: Responses to visual and chemical cues. *Journal of Experimental Marine Biology and Ecology* 233(1): 25-40. doi: 10.1016/S0022-0981(98)00121-X

dos Santos, A.M. (1999). Larvas de crustáceos decápodes ao largo da costa portuguesa. Ph.D. thesis. Universidade de Lisboa, Lisboa. 304 pp.

dos Santos, A.M., and González-Gordillo, J.I. (2004). Illustrated keys for the identification of the Pleocyemata (Crustacea: Decapoda) zoeal stages, from the coastal region of south-western Europe. *Journal of the Marine Biological Association of the United Kingdom* 84(1): 205-227. doi: 10.1017/S0025315404009075h

Drake, P., Marco-Herrero, E., Subida, M.D., Arias, A.M., and Cuesta, J.A. (2014). Host use pattern of the pea crab *Afropinnotheres monodi*: Potential effects on its reproductive success and geographical expansion. *Marine Ecology Progress Series* 498: 203-215. doi: 10.3354/meps10623

Duarte, L.N., Gomes, C.P., Marchante, H., and Marchante, E. (2020). Integrating knowledge of ecological succession into invasive alien plant management: A case study from Portugal. *Applied Vegetation Science* 23(3): 328-339. doi: 10.1111/avsc.12488

Encarnacão, J., Leitão, T., Morais, P., Piló, D., Range, P., Chícharo, L., and Chícharo, M.A. (2013). Effects of inter-annual freshwater inflow shifts on the community structure of estuarine decapods. *Cahiers de Biologie Marine* 54(2): 181-189.

Epifanio, C., Valenti, C., and Pembroke, A. (1984). Dispersal and recruitment of blue crab larvae in Delaware Bay, USA. *Estuarine, Coastal and Shelf Science* 18(1): 1-12. doi: 10.1016/0272-7714(84)90002-7

Epifanio, C.E. (2019). Early life history of the blue crab *Callinectes sapidus*: A review. *Journal of Shellfish Research* 38(1): 1-22. doi: 10.2983/035.038.0101

Essl, F., Dullinger, S., Genovesi, P., Hulme, P.E., Jeschke, J.M., Katsanevakis, S., Kühn, I., Lenzner, B., Pauchard, A., and Pyšek, P. (2019). A conceptual framework for range-expanding species that track human-induced environmental change. *BioScience* 69(11): 908-919. doi: 10.1093/biosci/biz101

Falzon, M.A. (2011). First records of *Scorpaena maderensis* (Pisces Scorpaeniformes Scorpaenidae) in inshore Maltese waters. *Naturalista Siciliano* 35: 419–423.

Forward Jr, R., Tankersley, R., and Pochelon, P. (2003). Circatidal activity rhythms in ovigerous blue crabs, *Callinectes sapidus*: Implications for ebb-tide transport during the spawning migration. *Marine Biology* 142: 67-76. doi: 10.1007/s00227-002-0923-5

Forward Jr, R., Cohen, J., Irvine, R., Lax, J., Mitchell, R., Schick, A., Smith, M., Thompson, J., and Venezia, J. (2004). Settlement of blue crab *Callinectes sapidus* megalopae in a North Carolina estuary. *Marine Ecology Progress Series* 269: 237-247. doi: 10.3354/meps269237

Horta e Costa, B., and Gonçalves, E.J. (2013). First occurrence of the Monrovia doctorfish *Acanthurus monroviae* (Perciformes: Acanthuridae) in European Atlantic waters. *Marine Biodiversity Records* 6: e20. doi: 10.1017/S1755267213000055

Horta e Costa, B., Assis, J., Franco, G., Erzini, K., Henriques, M., Gonçalves, E.J., and Caselle, J.E. (2014). Tropicalization of fish assemblages in temperate biogeographic transition zones. *Marine Ecology Progress Series* 504: 241-252. doi: 10.3354/meps10749

Keller, R.P., Geist, J., Jeschke, J.M., and Kühn, I. (2011). Invasive species in Europe: Ecology, status, and policy. *Environmental Sciences Europe* 23(1): 1-17. doi: 10.1186/2190-4715-23-23

Ginal, P., Moreira, F.D., Marques, R., Rebelo, R., and Rödder, D. (2021). Predicting terrestrial dispersal corridors of the invasive African clawed frog *Xenopus laevis* in Portugal. *NeoBiota* 64: 103-118. doi: 10.3897/neobiota.64.60004

Giovos, I., Kleitou, P., Poursanidis, D., Batjakas, I., Bernardi, G., Crocetta, F., Doumpas, N., Kalogirou, S., Kampouris, T.E., and Keramidas, I. (2019). Citizen-science for monitoring marine invasions and stimulating public engagement: A case project from the eastern Mediterranean. *Biological Invasions* 21: 3707-3721. doi: 10.1007/s10530-019-02083-w

Gonçalves, J.M.S., Veiga, P., Machado, D., Bentes, L., Monteiro, P., Afonso, C., Almeida, C., Oliveira, F., and Erzini, K. (2013). Biodiversidade do estuário do rio Arade: Peixes e invertebrados. Centre of Marine Sciences, Universidade do Algarve, Faro. 256 pp.

Gonçalves, R., Teodósio, M.A., Cruz, J., Ben-Hamadou, R., Correia, A.D., and Chícharo, L. (2017). Preliminary insight into winter native fish assemblages in Guadiana estuary salt marshes coping with environmental variability and non-indigenous fish introduction. *Fishes* 2(4): 19. doi: 10.3390/fishes2040019

González-Gordillo, J., and Cuesta, J. (2020). Pinnotheridae de Haan, 1833. *ICES Identification Leaflets for Plankton* No. 191. 17 pp. doi: 10.17895/ices.pub.5961

González-Ortegón, E., and Cuesta, J.A. (2006). An illustrated key to species of Palaemon and Palaemonetes (Crustacea: Decapoda: Caridea) from European waters, including the alien species *Palaemon macrodactylus*. *Journal of the Marine Biological Association of the United Kingdom* 86(1): 93-102. doi: 10.1017/S0025315406012896

Guidetti, P., Bianchi, C.N., La Mesa, G., Modena, M., Morri, C., Sara, G., and Vacchi, M. (2002). Abundance and size structure of *Thalassoma pavo* (Pisces: Labridae) in the western Mediterranean Sea: Variability at different spatial scales. *Journal of the Marine Biological Association of the United Kingdom* 82(3): 495-500. doi: 10.1017/S0025315402005775

Havel, J.E., Kovalenko, K.E., Thomaz, S.M., Amalfitano, S., and Kats, L.B. (2015). Aquatic invasive species: Challenges for the future. *Hydrobiologia* 750: 147-170. doi: 10.1007/s10750-014-2166-0

Haywood, P., and Ryland, J. (1990). The marine fauna of the British Isles and the north west

Europe. Vol 1. Introduction and protozoans to arthropods. Clarendon Press. Oxford, 671 pp.

Hosozawa, T., Kunii, H., Nakamura, M., Ojima, T., Sugiyama, Y., and Yamaguchi, K. (2020). Spatial, temporal and vertical variation of distribution and major habitats in Asian mussel (*Arcuatula senhousia*) in a brackish river along Sea of Japan. *Plankton and Benthos Research* 15(2): 121-131. doi: 10.3800/pbr.15.121

Hureau, J.C., and Litvinenko, N.I. (1986). "Scorpaenidae" in P.J.P. Whitehead, M.L. Bauchot, J.G. Hureau, J. Nelsen & E. Tortonese (eds) Fishes of the North-Eastern Atlantic and the Mediterranean, Paris: UNESCO, 1.211-211.229.

Ingle, R.W. (1996). "Shallow-water crabs." in R.S.K. Barnes & J.H. Crothers (eds) Synopses of the British Fauna (New Series). Nº 25 (2nd ed): Field Studies Council: Great Britain, 243 pp.

IUCN - International Union for Conservation of Nature and Natural Resources (2000). IUCN guidelines for the prevention of biodiversity loss caused by alien invasive species. Species Survival Commission (SSC), Nairobi, Kenya. 21 pp.

Jeschke, J.M., and Strayer, D.L. (2006). Determinants of vertebrate invasion success in Europe and North America. *Global Change Biology* 12(9): 1608-1619. doi: 10.1111/j.1365-2486.2006.01213.x

Jivoff, P.R., Hines, A.H., and Quackenbush, S. (2007). "Reproduction and embryonic development" in V.S. Kennedy & L.E. Cronin (eds) Blue crab: *Callinectes sapidus*, College Park, MD: Maryland Sea Grant, 255–298.

Ju, S.-J., Secor, D.H., and Harvey, H.R. (2001). Growth rate variability and lipofuscin accumulation rates in the blue crab *Callinectes sapidus*. *Marine Ecology Progress Series* 224: 197-205. doi: 10.3354/meps224197

La Mesa, M., La Mesa, G., and Micalizzi, M. (2005). Age and growth of madeira scorpionfish, *Scorpaena maderensis* Valenciennes, 1833, in the central Mediterranean. *Fisheries Research* 74(1-3): 265-272. doi: 10.1016/j.fishres.2005.01.018

Lima, F.P., Ribeiro, P.A., Queiroz, N., Hawkins, S.J., and Santos, A.M. (2007). Do distributional shifts of northern and southern species of algae match the warming pattern? *Global Change Biology* 13(12): 2592-2604. doi: 10.1111/j.1365-2486.2007.01451.x

Liulea, S. (2021). Invasion of the reds? Long-term shifts on intertidal seaweed at distribution limits. Master thesis. Universidade do Algarve, Faro. 40 pp

Lourenço, C.R., Nicastro, K.R., Serrão, E.A., and Zardi, G.I. (2012). First record of the brown mussel (*Perna perna*) from the European Atlantic coast. *Marine Biodiversity Records* 5: e39. doi: 10.1017/S1755267212000280

Massé, C., Jourde, J., Fichet, D., Sauriau, P.-G., Dartois, M., Ghillebaert, F., and Dancie, C. (2022). Northern range expansion of the Asian mussel *Arcuatula senhousia* (Benson, 1842) along the French Atlantic coasts. *BioInvasions Records* 11(3): 700–709. doi: 10.3391/bir.2022.11.3.12

Mistri, M., Rossi, R., and Fano, E. (2004). The spread of an alien bivalve (*Musculista senhousia*) in the Sacca di Goro Lagoon (Adriatic Sea, Italy). *Journal of Molluscan Studies* 70(3): 257-261. doi: 10.1093/mollus/70.3.257

Monteiro, P., Bentes, L., Sousa, I., Oliveira, F., Veiga, P., Rangel, M., Afonso, C., and Gonçalves, J.M.S. (2012). Biodiversidade marinha da costa sul de Sagres. Identificação e caracterização de biótopos. Relatório Interno Nº 2/2012 - MeshAtlantic. Universidade do Algarve, CCMAR, Faro. 48 pp.

Morais, P., Teodósio, J., Reis, J., Chícharo, M.A., and Chícharo, L. (2009). The Asian clam *Corbicula fluminea* (Müller, 1774) in the Guadiana River Basin (southwestern Iberian Peninsula): Setting the record straight. *Aquatic Invasions* 4(4): 681-684. doi: 10.3391/ai.2009.4.4.14

Morais, P., and Teodósio, M. (2016). The transatlantic introduction of weakfish *Cynoscion regalis* (Bloch & schneider, 1801)(sciaenidae, pisces) into Europe. *BioInvasions Records* 5(4): 259-265. doi: 10.3391/bir.2016.5.4.11

Morais, P., Cerveira, I., and Teodósio, M.A. (2017). An update on the invasion of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801)(Actinopterygii: Sciaenidae) into Europe. *Diversity* 9(4): 47. doi: 10.3390/d9040047

Morais, P., Gaspar, M., Garel, E., Baptista, V., Cruz, J., Cerveira, I., Leitao, F., and Teodosio, M. (2019). The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its non-native distribution into the Ria Formosa lagoon and the Guadiana estuary (SW-Iberian Peninsula, Europe). *BioInvasions Records* 8(1): 123-133. doi: 10.3391/bir.2019.8.1.14

Morton, B. (1974). Some aspects of the biology, population dynamics, and functional morphology of *Musculista senhausia* Benson (Bivalvia, Mytilidae). *Pacific Science* 28: 15 pp.

Nakamura, Y., Feary, D.A., Kanda, M., and Yamaoka, K. (2013). Tropical fishes dominate temperate reef fish communities within western Japan. *PLoS ONE* 8(12): e81107. doi: 10.1371/journal.pone.0081107

Nicastro, K.R., Zardi, G.I., Teixeira, S., Neiva, J., Serrão, E.A., and Pearson, G.A. (2013). Shift happens: Trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga *Fucus vesiculosus*. *BMC Biology* 11(6): 1-13. doi: 10.1186/1741-7007-11-6

Nuño, C., Peg, M., Mellado-Díaz, A., Sánchez-González, J.R., and Toro, M. (2018). First record of *Synidotea laticauda* Benedict, 1897 (Crustacea: Isopoda) in the Guadiana Estuary (SW Iberian Peninsula). *Limnetica* 37(2): 173-179. doi: 10.23818/limn.37.14

Olmi, E.J. (1994). Vertical migration of blue crab *Callinectes sapidus* megalopae: Implications for transport in estuaries. *Marine Ecology Progress Series* 113: 39.

Paula, J. (1996). A key and bibliography for the identification of zoeal stages of brachyuran crabs (Crustacea, Decapoda, Brachyura) from the Atlantic coast of Europe. *Journal of Plankton Research* 18(1): 17-27.

Pérez-Miguel, M., Drake, P., García Raso, J.E., Mamán Menéndez, L., Navas, J.I., and Cuesta, J.A. (2019a). European Pinnotheridae (Crustacea, Decapoda, Brachyura): Species, distribution, host use and DNA barcodes. *Marine Biodiversity* 49: 57-68. doi: 10.1007/s12526-017-0754-8

Pérez-Miguel, M., González-Ortegón, E., Drake, P., Navas, J.I., and Cuesta, J.A. (2019b). Temperature and salinity tolerance of the larval stages of the African pea crab *Afropinnotheres monodi* Manning, 1993: Implications for its dispersal along European

waters. *Aquatic Invasions* 14(2): 397-411. doi: 10.3391/ai.2019.14.2.15

Pessani, D., Burri, R., and Salton, L. (1998). A key for the identification of the known larval stages of the Mediterranean Brachyura. *Invertebrate Reproduction & Development* 33(2-3): 191-199. doi: 10.1080/07924259.1998.9652631

Png-Gonzalez, L., Papiol, V., Balbín, R., Cartes, J.E., and Carbonell, A. (2021). Larvae of the blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: Portunidae) in the Balearic Archipelago (NW Mediterranean Sea). *Marine Biodiversity Records* 14(1): 1-5. doi: 10.1186/s41200-021-00217-5

Pochelon, P.N., Pires, R.F., Dubert, J., Nolasco, R., Santos, A.M.P., Queiroga, H., and dos Santos, A. (2017). Decapod larvae distribution and species composition off the southern Portuguese coast. *Continental Shelf Research* 151: 53-61. doi: 10.1016/j.csr.2017.10.009

Posey, M.H., Alphin, T.D., Harwell, H., and Allen, B. (2005). Importance of low salinity areas for juvenile blue crabs, *Callinectes sapidus* Rathbun, in river-dominated estuaries of southeastern United States. *Journal of Experimental Marine Biology and Ecology* 319(1-2): 81-100. doi: 10.1016/j.jembe.2004.04.021

Ribeiro, J., Bentes, L., Coelho, R., Gonçalves, J.M., Lino, P.G., Monteiro, P., and Erzini, K. (2006). Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). *Estuarine, Coastal and Shelf Science* 67(3): 461-474. doi: 10.1016/j.ecss.2005.11.036

Rodríguez, A., and Paula, J. (1993). Larval and postlarval development of the mud crab *Panopeus africanus* A. Milne Edwards (Decapoda: Xanthidae) reared in the laboratory. *Journal of Crustacean Biology* 13(2): 296-308. doi: 10.1163/193724093X00084

Rodríguez, A., Drake, P., and Arias, A.M. (1997). Reproductive periods and larval abundance patterns of the crabs *Panopeus africanus* and *Uca tangeri* in a shallow inlet (SW Spain). *Marine Ecology Progress Series* 149: 133-142. doi: 10.3354/meps149133

RStudio Team (2022). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Ruiz, S.T. (2015). The status of the invasive sea squirts and barnacles found in the marinas and ports of Algarve, southern Portugal. Master thesis. Universidade do Algarve, Faro. 149 pp.

Saldanha, L. (1995). Fauna Submarina Atlântica. Publicações Europa-América, 364 pp.

Santos, M., Monteiro, C., and Lasserre, G. (1996). Faune ichtyologique comparée de deux récifs artificiels du littoral de la Ria Formosa (lagune Portugal): Résultats préliminaires. *Oceanologica Acta* 19(1): 89-97.

Santos, M., Gaspar, M., Vasconcelos, P., and Monteiro, C. (2002). Weight–length relationships for 50 selected fish species of the Algarve coast (southern Portugal). *Fisheries Research* 59(1-2): 289-295. doi: 10.1016/S0165-7836(01)00401-5

Seyer, T., Morais, P., Amorim, K., Leitão, F., Martins, F., and Teodósio, M.A. (2017). On the presence of the Ponto-Caspian hydrozoan *Cordylophora caspia* (Pallas, 1771) in an Iberian estuary: Highlights on the introduction vectors and invasion routes. *BioInvasions Records* 6(4): 331-337. doi: 10.3391/bir.2017.6.4.05

Simberloff, D., and Von Holle, B. (1999). Positive interactions of nonindigenous species: Invasional meltdown? *Biological Invasions* 1: 21-32. doi: 10.1023/A:1010086329619

Simberloff, D., Parker, I.M., and Windle, P.N. (2005). Introduced species policy, management, and future research needs. *Frontiers in Ecology and the Environment* 3(1): 12-20. doi: 10.1890/1540-9295(2005)003[0012:ISPMAF]2.0.CO;2

Simberloff, D. (2006). Invasional meltdown 6 years later: Important phenomenon, unfortunate metaphor, or both? *Ecology Letters* 9(8): 912-919. doi: 10.1111/j.1461-0248.2006.00939.x

Soriano, J.L., and Salgado, S.Q. (2014). Primeras citas de diversos moluscos marinos alóctonos en el Delta del Ebro (Cataluña, España). *Spira* 5: 149-151.

Sousa, R., Gutiérrez, J.L., and Aldridge, D.C. (2009). Non-indigenous invasive bivalves as ecosystem engineers. *Biological Invasions* 11: 2367-2385. doi: 10.1007/s10530-009-9422-7

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., and Lourie, S.A. (2007). Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *BioScience* 57(7): 573-583. doi: 10.1641/B570707

Subida, M.D., Arias, A.M., Drake, P., García-Raso, J.E., Rodríguez, A., and Cuesta, J.A. (2011). On the occurrence of *Afropinnotheres monodi* Manning, 1993 (Decapoda: Pinnotheridae) in European waters. *Journal of Crustacean Biology* 31(2): 367-369. doi: 10.1651/10-3381.1

Pyšek, P., and Richardson, D.M. (2010). Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources* 35: 25-55. doi: 10.1146/annurev-environ-033009-095548

Tankersley, R.A., Wieber, M.G., Sigala, M.A., and Kachurak, K.A. (1998). Migratory behavior of ovigerous blue crabs *Callinectes sapidus*: Evidence for selective tidal-stream transport. *The Biological Bulletin* 195(2): 168-173. doi: 10.2307/1542824

Turner, H.V., Wolcott, D.L., Wolcott, T.G., and Hines, A.H. (2003). Post-mating behavior, intramolt growth, and onset of migration to Chesapeake Bay spawning grounds by adult female blue crabs, *Callinectes sapidus* Rathbun. *Journal of Experimental Marine Biology and Ecology* 295(1): 107-130. doi: 10.1016/S0022-0981(03)00290-9

Ulman, A., Ferrario, J., Occhpinti-Ambrogi, A., Arvanitidis, C., Bandi, A., Bertolino, M., Bogi, C., Chatzigeorgiou, G., Çiçek, B.A., and Deidun, A. (2017). A massive update of non-indigenous species records in Mediterranean marinas. *PeerJ* 5: e3954. doi: 10.7717/peerj.3954

van Engel, W.A. (1958). The blue crab and its fishery in Chesapeake Bay. Part 1. Reproduction, early development, growth and migration. *Commercial Fisheries Review* 20(6): 6.

Vasconcelos, P., Carvalho, A.N., Moura, P., Ramos, J., and Gaspar, M.B. (2018). First record of *Acanthurus monroviae* (Osteichthyes: Perciformes: Acanthuridae) in southern Portugal, with notes on its recent distributional spread in the northeastern Atlantic and Mediterranean. *Marine Biodiversity* 48: 1673-1681. doi: 10.1007/s12526-017-0653-z

Vasconcelos, P., Carvalho, A.N., Piló, D., Pereira, F., Encarnação, J., Gaspar, M.B., and Teodósio, M.A. (2019). Recent and consecutive records of the Atlantic blue crab (*Callinectes sapidus* Rathbun, 1896): Rapid westward expansion and confirmed establishment along the Southern coast of Portugal. *Thalassas: An International Journal*

of Marine Sciences 35(2): 485-494. doi: 10.1007/s41208-019-00163-1

Vergés, A., Doropoulos, C., Malcolm, H.A., Skye, M., Garcia-Pizá, M., Marzinelli, E.M., Campbell, A.H., Ballesteros, E., Hoey, A.S., and Vila-Concejo, A. (2016). Long-term empirical evidence of ocean warming leading to tropicalization of fish communities, increased herbivory, and loss of kelp. *Proceedings of the National Academy of Sciences* 113(48): 13791-13796. doi: 10.1073/pnas.1610725113

Watson, G.J., Dyos, J., Barfield, P., Stebbing, P., and Dey, K.G. (2021). Evidence for self-sustaining populations of *Arcuatula senhousia* in the UK and a review of this species' potential impacts within Europe. *Scientific Reports* 11(1): 1-13. doi: 10.1038/s41598-021-86876-x

Welch, J.M., Rittschof, D., Bullock, T.M., and Forward Jr, R.B. (1997). Effects of chemical cues on settlement behavior of blue crab *Callinectes sapidus* postlarvae. *Marine Ecology Progress Series* 154: 143-153. doi: 10.3354/meps154143

Wernberg, T., Bennett, S., Babcock, R.C., De Bettignies, T., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C.J., and Hovey, R.K. (2016). Climate-driven regime shift of a temperate marine ecosystem. *Science* 353(6295): 169-172. doi: 10.1126/science.aad8745

Whitehead, P., Bauchot, M., Hureau, J., Nielsen, J., and Tortonese, E. (1986). Fishes of the North-Eastern and the Mediterranean. UNESCO, 1473 pp.

Yamamuro, M., Hiratsuka, J.i., and Ishitobi, Y. (2010). What prevents *Musculista senhousia* from constructing byssal thread mats in estuarine environments? A case study focusing on Lake Shinji and nearby estuarine waters. *Landscape and Ecological Engineering* 6: 23-28. doi: 10.1007/s11355-009-0074-7

Yamano, H., Sugihara, K., and Nomura, K. (2011). Rapid poleward range expansion of tropical reef corals in response to rising sea surface temperatures. *Geophysical Research Letters* 38(4). doi: 10.1029/2010GL046474

Zarco-Perello, S., Wernberg, T., Langlois, T.J., and Vanderklift, M.A. (2017). Tropicalization strengthens consumer pressure on habitat-forming seaweeds. *Scientific Reports* 7(1): 820. doi: 10.1038/s41598-017-00991-2

CHAPTER 3

Feeding habits of the invasive Atlantic blue crab

Sections of the present chapter are to be published in the following peer-reviewed scientific article under the objectives of this Ph.D. thesis:

- **Encarnação J**, Dias E, Engelen AH, Serrão E, Teodósio MA, Morais P (in preparation). Atlantic blue crab keeps native omnivory strategy on its path to invasion success, as disclosed by metagenomic and isotopic data.

3.1 Introduction

The feeding ecology of invasive species dictates not only which trophic levels are directly impacted but also provides a deeper understanding of the putative cascading effects across compartments of an ecosystem (Dias et al. 2014). Invasive species may disrupt food webs at different levels, including bioturbation and nutrient loading disruption (Figueredo and Giani 2005; Sousa et al. 2009), competition for habitat and resources, or predation (Savidge 1987; Levine et al. 2003; Green et al. 2012). Some species, such as the Asian clam *Corbicula fluminea* (O. F. Müller, 1774), may produce impacts at different levels, from alteration of habitat structure and competition for space, to impacts on organisms at the base of the food chain due to high filtration rates (Sousa et al. 2008; Dias et al. 2014). The Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853, for example, may physically impact the environment when damaging stream banks due to their burrowing activity, compete for habitat with native crab species, such as the European green crab *Carcinus maenas* (Linnaeus, 1758), or interfere with regular fishing activities upon their massive seasonal spawning migrations (Gilbey et al. 2008; Dittel and Epifanio 2009). Therefore, each species needs an assessment of their feeding habits in each of the areas where it was introduced, to infer potential ecological effects more accurately.

The invasive and fast spreading nature of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 in Mediterranean and eastern Atlantic ecosystems (Mancinelli et al. 2021; Clavero et al. 2022) is one of such cases that demands a detailed insight into their feeding ecology in more areas of its non-native range. In the native area, which extends from Nova Scotia in North America to Argentina in South America (Johnson 2015; Mancinelli et al. 2021), this species has significant ecological roles and commercial importance, supporting a large fishery industry across North America, with peak interest around Maryland and the Chesapeake Bay, with landings over 53,000 metric tons, worth more than 240 million US$ in 2021 (Millikin and Williams 1984; Guillory et al. 2001; NOAA 2023). The Atlantic blue crab also serves as a link between the benthic and pelagic compartments, particularly in estuarine food webs, by feeding on a variety of benthic prey, but also being preyed on by, for example, the striped bass *Morone saxatilis* (Walbaum, 1792) or redfish *Sciaenops ocellatus* (Linnaeus, 1766) (Laughlin 1982; Scharf et al. 2000; Overton et al. 2009). The Atlantic blue crab is an eurythermal and euryhaline species with high osmoregulatory capabilities (Tagatz 1968, 1971; Epifanio 2019), which, together with strong swimming capabilities, allows them to inhabit a wide variety of coastal and estuarine habitats, including areas of very low salinity (Orth and van Montfrans

1990; Williams et al. 1990; Posey et al. 2005; Scalici et al. 2022). These traits enable the species to explore multiple food sources throughout its different life stages and across ecosystems. As an omnivorous, detritivore, scavenger, and cannibalistic species, the Atlantic blue crab is an opportunistic predator with a high feeding adaptiveness (Laughlin 1982; Hines et al. 1990; Stoner and Buchanan 1990; Hsueh et al. 1992; Seitz et al. 2011). Animal prey may include fish, bivalves (oysters, clams, mussels), polychaetes, and other crustaceans (crabs, shrimps, amphipods) (Laughlin 1982; Stoner and Buchanan 1990; Fitz and Wiegert 1991; Hsueh et al. 1992). It may also consume vegetation, including mangrove plants, algae, or seagrass (Laughlin 1982; Ryer 1987; Stoner and Buchanan 1990; McClintock et al. 1991; Perkins-Visser et al. 1996; Reichmuth et al. 2009). The diet changes during ontogeny are quite significant. In Puerto Rico, while juveniles show a more diverse diet, including smaller prey such as amphipods, foraminifera, and polychaetes, larger crabs prefer fish, shrimp, molluscs, and crabs (Stoner and Buchanan 1990). In Florida, smaller crabs (< 31 mm) preferred detritus, plant material, and ostracods; the medium-sized crabs (31-60 mm) favoured fish, gastropods, xanthid crabs, and plant material; and larger crabs (> 60 mm) ate more bivalves than the previous groups along with fishes, xanthid crabs, and other blue crabs (Laughlin 1982). Diet is thought to also differ between sexes due to differences in claw morphology (Eggleston 1990) and because they often occupy different habitats within an ecosystem (Hines et al. 1987; Williams et al. 1990; Ramach et al. 2009). However, diet differences between sexes are evaluated seldomly, and feeding experiments have relied mostly on data from just one sex (e.g., Blundon and Kennedy 1982; Ebersole and Kennedy 1995; Mascaro et al. 2003; Prado et al. 2020).

In the invaded range, studies on the feeding habits of the Atlantic blue crab are limited to Mediterranean Sea populations. Off Spain, Italy, Croatia, and Turkey, the species occupies a relatively high trophic position despite its feeding plasticity and shows the same opportunistic and omnivorous behaviour as in the native range (Carrozzo et al. 2014; Mancinelli et al. 2016, 2017; Aslan and Polito 2021; Prado et al. 2022). Off Greece, the species also relies on a diverse diet (Kampouris et al. 2019), including commercially important bivalve species (*Cerastoderma glaucum* (Bruguière, 1789), *Chamelea gallina* (Linnaeus, 1758), *Modiolus barbatus* (Linnaeus, 1758)), crabs (*Carcinus aestuarii* Nardo, 1847), shrimps (*Penaeus* spp.), and fish (*Dicentrarchus labrax* (Linnaeus, 1758), *Solea solea* (Linnaeus, 1758), *Sparus aurata* Linnaeus, 1758). In the Ebro delta (Spain), it has been impacting local mussel (*Mytilus galloprovincialis* Lamarck, 1819) and oyster (*Magallana gigas* (Thunberg, 1793)) farms, as

well as native freshwater bivalves (e.g., Unionoida) or invasive *Corbicula* species (Ventura et al. 2018; Prado et al. 2020, 2022). In such studies, methods to infer on feeding habits have included visual observations in the field, laboratory feeding experiments, and stable isotope analysis.

Traditionally, studies about the diet of animals have relied on direct observations during feeding and analyses of gut content or faeces (e.g., Cherel et al. 2000; Balestrieri et al. 2011; Amundsen and Sánchez-Hernández 2019). However, these techniques are often unreliable, difficult, or impossible to use when dealing with small consumers and prey species living in aquatic ecosystems. The use of DNA sequencing to analyse the gut content of consumers has overcome part of such constraints, and shotgun metagenomics provides higher taxonomic resolution of prey from the gut content than other DNA sequencing techniques (Taberlet et al. 2012; Srivathsan et al. 2015; Paula et al. 2016). Metagenomic surveys have been revealing the feeding habits of organisms within multiple ecosystems, from marine fish like the yellowfin sea bream *Acanthopagrus latus* (Houttuyn, 1782) (Pan et al. 2021), to terrestrial mammals such as the endangered leopard cat *Prionailurus bengalensis* (Kerr 1792) (Woo et al. 2023), or the Raffles' banded langur *Presbytis femoralis* (Martin 1838) (Srivathsan et al. 2016). In terrestrial habitats, faecal samples are often used to study the diet of endangered species since it is a non-invasive technique (Srivathsan et al. 2015, 2016; Woo et al. 2023), while analysis of the stomach or intestine content is a more common approach for aquatic species (Pan et al. 2021). When sequencing a sample of the gut or stomach of the animal by shotgun DNA sequencing, it also allows analysing the bacterial microbiomes and parasite diversity (Paula et al. 2016).

The analyses of stable isotopes in the tissues of invasive species have allowed disclosing the sources of organic matter incorporated by individuals (Dias et al. 2014; Prado et al. 2022), therefore revealing a broader insight into the functioning of ecosystems than diet studies cannot provide. Carbon ($\delta^{13}C$: $^{13}C/^{12}C$) and nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) stable isotopes ratios are the most widely used to study energy flows in aquatic food webs (Hobson 1999; Fry 2002; Middelburg 2014; Dias et al. 2014, 2016; Prado et al. 2022). Isotopic signatures are a time-integrated reflection of the physical and metabolic processes occurring in natural ecosystems, enabling the study of energy transfer across the food web (Peterson and Fry 1987; Boecklen et al. 2011). Stable isotope ratios increase by about 0.4‰ $\delta^{13}C$ and 3.4‰ $\delta^{15}N$ per trophic level, a process called trophic fractionation that reflects the differences between consumers and their diets (Post 2002). For this reason, $\delta^{15}N$ is very useful in determining the trophic position of species across the food web and establishing relations between consumers and preys (Minagawa and Wada

1984; Post 2002). The isotopic composition is a measure of the assimilated diet, reflecting both long-term and short-term diets depending on the analysed tissue as well as turnover times (Peterson and Fry 1987; Dalerum and Angerbjörn 2005). Stable isotopes may also help distinguishing the origins of consumed organic matter, namely between ^{15}N-depleted terrestrial sources and ^{15}N-enriched aquatic sources (Peterson and Fry 1987; Cloern et al. 2002). The Δ^{13}C gradients may help distinguish between marine and freshwater food sources, as consumers in marine locations are on average more ^{13}C-enriched (Fry 2002; Dias et al. 2016).

The examples of biological invasions studies that relied on stable isotopes analysis, either in terrestrial ecosystems, freshwater watersheds, or marine environments is vast (e.g., Dias et al. 2014; McCue et al. 2020; Russel et al. 2020; Calizza et al. 2021). For example, the widespread invasive house mice *Mus musculus* Linnaeus 1758 appears to be exhausting its preferred preys in Antipodes Islands (New Zealand), which included native invertebrates, birds, and seabirds (Russel et al. 2020). In the Ebro Delta in Spain, the native glossy ibis *Plegadis falcinellus* (Linnaeus 1766) has incorporated the invasive apple snail *Pomacea maculata* Perry, 1810 in its diet in proportions between 26 and 40%, becoming a controlling agent against the apple snail (Bertolero and Navarro 2018). The use of ^{13}C and ^{15}N isotopes revealed the contributions of terrestrial-derived organic matter and phytoplankton in supporting the growth of the invasive Asian clam *Corbicula fluminea* along the salinity gradient of the Minho River in northwest Portugal (Dias et al. 2014). In Patagonia, it was possible to disclose that the invasive European green crab *Carcinus maenas* relied mostly on phytoplankton-derived sources from bivalve consumption which complemented nicely the information obtained from metabarcoding and stomach content analysis (Cordone et al. 2022).

The potential impacts of the Atlantic blue crab across Europe have only recently started to be assessed through stable isotope analysis. Assessments made in the Ionian Sea pointed to a trophic position higher than many native predator species, including the intertidal crab *Pachygrapsus marmoratus* (Fabricius, 1787), the warty crab *Eriphia verrucosa* (Forskål, 1775), or the gilthead sea bream *Sparus aurata* (Mancinelli et al. 2013; Carrozzo et al. 2014). In the Adriatic Sea, a study included the potential basal food sources (i.e., molluscs, crustaceans, fish) and found that the trophic position of the Atlantic blue crab was only surpassed by three fish and two gastropods which may suggest a high impact upon the food web, either through competition or predation (Mancinelli et al. 2016). In this area of the Mediterranean Sea, the invasive Atlantic blue crab has a high trophic flexibility, potentially adjusting its feeding habits according to spatial, temporal, and ontogenetic factors (Mancinelli

et al. 2017). In the Mediterranean coasts of Turkey, stable isotopic analysis showed seasonal niche overlap between the Atlantic blue crab and the intertidal crab *Pachygrapsus marmoratus* or the Baltic prawn *Palaemon adspersus* Rathke, 1836, while $\delta^{15}N$ indicated a trophic position consistently below the warty crab *Eriphia verrucosa* or the blotched picarel *Spicara maena* (Linnaeus, 1758) (Aslan and Polito 2021). The trophic position of the Atlantic blue crab was only below fish in the Ebro delta on the Mediterranean coast of Spain (Prado et al. 2022). Here, the sources of organic matter are quite diverse, including sediment organic matter, animal sources, and vegetation material contributing, on average, with 36%, 34%, and 24%, respectively, to the Atlantic blue crab biomass. These contributions change over time and habitats depending on prey availability; for example, at sites with a high abundance of bivalves, these may contribute up to 75% of their biomass (Prado et al. 2022).

Several hypotheses have been proposed to explain the invasiveness of a species, including better competitive advantages in feeding, reproduction, release from native-range enemies, or tolerance to a wider range of environmental variables (Levine et al. 2003; Colautti et al. 2004; Keller et al. 2011; Lenz et al. 2011). Here, we explore which food sources supported the exponential abundance increase of the Atlantic blue crab in the Guadiana estuary in less than four years since its first detection in 2017 (Morais et al. 2019; but also see results of **Chapters 2** and **4**). We hypothesized that the omnivorous and opportunistic behaviour of the Atlantic blue crab was the key to its invasive success in the Guadiana estuary, taking advantage of what is more readily available to support its biomass. Additionally, and due to the continuous appearance of new invasive species in the estuary, we hypothesized that such species could benefit the invasiveness of the Atlantic blue crab, for example the Black Sea jellyfish *Blackfordia virginica* Meyer, 1910 (Chícharo et al. 2009) or the Asian clam *Corbicula fluminea* (Morais et al. 2009a) through a process coined as "invasion meltdown" (Simberloff and Von Holle 1999; Simberloff 2006). Lastly, we hypothesize that the Atlantic blue crab has a competitive advantage towards native crabs – as already documented with a collapse of the European green crab *Carcinus maenas* in the Ebro Delta in eastern Spain (Clavero et al. 2022) – and their trophic niche size should be wider due to their omnivorous behaviour and overlap with those native species. We explored these hypotheses in the Guadiana estuary (Southwestern Iberian Peninsula, Europe), a flow-regulated estuary (Morais 2008) that went through marked changes in abiotic conditions (i.e., increased salinization of the estuary; Chícharo et al. 2006; Morais et al. 2009b, Barbosa et al. 2010; Encarnação et al. 2013) and biodiversity since the completion of the largest European water reservoir in February 2002,

with the establishment of several non-indigenous species mainly in the middle estuary (Morais 2008, Morais et al. 2009a, 2017, 2019; Chícharo et al. 2009; Cruz et al. 2017; Seyer et al. 2017; Nuño et al. 2018).

3.2 Material and Methods

3.2.1 Metagenomic analysis

Field sampling

Sampling took place in the middle Guadiana estuary, in the area of Foz de Odeleite in southeastern Portugal (southwestern Iberian Peninsula, Europe) in June 2021 and March 2022. The Atlantic blue crabs were collected with benthic baited traps (see **Section 2.2.1** for details) and additional specimens were obtained through local fishers operating in the same area. In both cases, specimens were quickly frozen at -20ºC after collection until sample extraction.

Sample preparation and analyses

Samples for metagenomic analysis were collected from the intestine content of Atlantic blue crabs, and DNA extraction was performed with the Quick-DNA^{TM} Miniprep Kit (D3024) from Zymo Research, following the manufacturer's protocol. After DNA extraction, samples were stored in DNA elution buffer and kept at -20 ºC until being shipped to BGI Genomics (link) for shotgun metagenomic analysis. From a total of 37 samples sent for shotgun analysis, quality control by BGI flagged most samples as "Level C", i.e., with low-grade quality for library construction and sequencing, and with an estimated success rate around 70.53% according to BGI. Twenty samples were analysed individually, i.e., one sample has DNA material from the intestine content of one individual, while 11 lower-concentration samples were pooled into three batches of samples. We set three pools of samples collected in 2021: Pool 1 (3 males), Pool 2 (4 males), Pool 3 (4 females). This has left us with 23 samples (20 individual samples, 3 pooled samples); however, 6 individual samples failed the library construction, so we present data from 17 samples (14 individual samples and 3 pooled samples).

Using the Geneious software, mate pair sequences were merged and megablast was used to annotate each sequence with standard settings returning the best single hit of each sequence. Then, the results of the annotation procedure were analysed, filtered, and hand-cured in four

steps. First, only hits of eukaryotic organisms were considered, and filtered by sequence length (≥ 150 bp) and percentage of identical basepairs (≥ 98%). Also, only results with more than one hit in more than one sample, or with three or more hits in the same sample were considered in the next steps. Second, a sequence of each taxon was manually checked in the Basic Local Alignment Search Tool (BLAST) of the US National Library of Medicine website (link). On each taxon, sequences were ordered by the higher percentage of identical sites and lower E-value (the number of hits one can expect to see by chance when searching the database). The top-ranked sequence was inserted in BLAST, or when several sequences presented the same percentage of identical sites and E-values, more than one sequence was tested whenever more doubts were found in the results. Third, in the BLAST tool, the options "Nucleotide collection (nr/nt)" and "Highly similar sequences (megablast)" were selected. Fourth, BLAST hits with 100% similarity were considered as priority, or alternatively either hits with more than 99% similarity or a closer percentage. The species with most hits was considered the best match. If in doubt, by checking the GBIF database, taxa occurring in the Iberian Peninsula were chosen over other taxa, and a classification by higher taxonomic level was considered, for example in cases of many hits in many species of the same genus or family. Changes in taxonomic classification can be consulted in **Table S1** of the Electronic Supplementary Material. From the 14 individual samples, one of them had a very small amount of hits and after the initial filtration and hand-curation, all the eukaryote taxa belonged to the host, so this sample was also discarded. The final hand-cured dataset was analysed to calculate the percentage of frequency of occurrence (FO) of each taxon across individual samples, excluding the pooled samples. The average was used to summarize data and standard deviation to represent data dispersion.

3.2.2 Stable isotope analysis

Field sampling

Sampling for stable isotope analysis also took place in the Foz de Odeleite area, in the middle Guadiana estuary, in 2021 and 2022. During both periods, sampling of potential sources of organic matter was performed one month before sampling of the Atlantic blue crabs to account for tissue turnover times (Llewellyn and La Peyre 2011; Mancinelli et al. 2013; Carrozzo et al. 2014). Potential sources of organic matter were collected at the end of April 2021 and February 2022, while the Atlantic blue crabs were sampled in June 2021 and March

2022. Atlantic blue crabs were collected with benthic baited traps (see **Section 2.2.1** for details) and additional specimens were bought from local fishers operating in the Foz de Odeleite area. In both cases, specimens were frozen at -20ºC after collection until muscle sample extraction.

A wide variety of putative sources of organic matter were sampled across the food web, which included particulate organic matter (POM, three replicates), sediment organic matter (SOM, three replicates), microphytobenthos (MPB, three replicates), and zooplankton. Potential macrofaunal prey were selected based on the feeding habits of the Atlantic blue crab across its native range (Laughlin 1982; Stoner and Buchanan 1990; Fitz and Wiegert 1991; Hsueh et al. 1992), which includes fish, bivalves, polychaetes, jellyfish, and other crustaceans (shrimps, isopods, amphipods, other crabs). Vegetation detritus and C3 and C4 plants were also collected (Laughlin 1982; Ryer 1987; Stoner and Buchanan 1990; McClintock et al. 1991; Perkins-Visser et al. 1996; Reichmuth et al. 2009). POM and zooplankton samples were retrieved at low and high tide to account for freshwater and marine influences in the estuary.

For POM determination, three 1.5 L replicates of sub-surface water were collected with a sterile container and kept refrigerated until further laboratory procedures. SOM samples were collected with a Petite Ponar hand dredge at a depth of 3 m, and each of the three replicates separated by 5 m distance. For MPB collection, three 50 cm PVC replicate tubes were suspended under a floating pontoon for two weeks to allow for colonization. Two weeks after deployment, MPB were scraped with a soft brush and passed to a sterile container with deionized water. Zooplankton was collected with a conical-shaped plankton net with a mouth opening of 40 cm, a mesh of 200 µm, and equipped with a mechanical flowmeter (Hydro-Bios), and samples were preserved in 70% ethanol upon collection. The number of tows and duration varied between sampling periods since the goal was to obtain enough material for analysis from a diverse group of taxa.

Potential prey was mainly collected with baited benthic traps (see **Section 2.2.1** for details) and then frozen at -20ºC until further analysis. A minimum of three specimens were collected for each species. Vegetation detritus brought by the river current and entangled in the traps were also collected for analysis, as well as riparian vegetation present along the margins. Most Atlantic blue crabs were captured by local fishers in the same area of the middle Guadiana estuary, along with specimens of the European eel (*Anguilla anguilla* (Linnaeus, 1758)) in 2022. In June 2021, together with the Atlantic blue crabs, 10 specimens of three native crab

species (*Afruca tangeri* (Eydoux, 1835), *Carcinus maenas*, and *Panopeus africanus* A. Milne-Edwards, 1867) were also collected to determine trophic positions and niche overlap.

Sample preparation and analysis

In the laboratory, POM water samples were sieved through a 200 μm sieve to remove debris and any zooplankton organisms. POM and MPB samples were then filtered through pre-combusted Whatman GF/F filters, and the filters dried in an oven (Binder WTB) at 60 ºC for 24 h and stored in sterile plastic bags inside a hermetic container with silica until further analysis. SOM samples were dried at 60 ºC for 24 h, placed in glass containers, and stored inside a hermetic container with silica until further analysis. Zooplankton taxa were sorted under a stereomicroscope (Leica MDG41), identified to the lowest taxonomic level possible, and dried at 60 ºC for 24 h. Zooplankton taxa used for isotopic analysis included copepods (*Acartia* sp., *Calanipeda aquaedulcis* Krichagin, 1873), mysis (*Mesopodopsis* CF *slabberi* (Van Beneden, 1861)), and amphipods (Melitidae). In the case of small sized species (e.g., copepods, amphipods, isopods), several specimens from the same species were pooled together in one sample to assure enough biomass for isotopic analysis.

Muscle samples were retrieved from fish (muscle without skin or bones), bivalves (adductor muscle or foot if the specimen was small), shrimps (tail muscle), and crabs (leg or claw muscle depending on the specimen size). In the case of polychaetes, jellyfish, isopods, and amphipods, the whole animal was used. These samples were dried at 60 ºC for 24 h and ground into a powder with a mortar and pestle. Plant material was cleaned with deionized water to remove debris and epiphytes, dried at 60 ºC for 24 h, and ground to a fine powder with a mixer mill for stable isotope analysis. After grounded to a fine powder, all samples were stored in glass containers, labelled, and stored inside a hermetic container with silica until isotopic analysis. Laboratory material was sterilized prior to sample collection and between specimens. The fish species collected as potential sources of organic matter were the European eel (*Anguilla anguilla*), sand smelt (*Atherina presbyter* Cuvier, 1829), gobies (*Pomatoschistus* sp.), and pipefish (*Syngnathus abaster* Risso, 1827). Bivalve species included the native clam *Scrobicularia plana* (da Costa, 1778) and oysters (*Magallana* sp.). Shrimps included the brown shrimp *Crangon crangon* (Linnaeus, 1758), the native *Palaemon longirostris* H. Milne Edwards, 1837, and the invasive *Palaemon macrodactylus* Rathbun, 1902. Crabs used as potential sources of organic matter included the fiddler crab *Afruca tangeri*, the European green

crab *Carcinus maenas*, and the mud crab *Panopeus africanus*. Other groups of animal taxa included polychaetes (Nereididae), jellyfish (the invasive *Blackfordia virginica*), four species of isopods (*Cyathura carinata* (Krøyer, 1847), *Lekanesphaera levii* (Argano & Ponticelli, 1981), *Saduriella losadai* Holthuis, 1964, *Synidotea laticauda* Benedict, 1897), and two species of amphipods (*Corophium multisetosum* Stock, 1952; *Gammarus* sp.). Plant material included *Ceratophyllum* CF *demersum* L., *Juncus* sp., *Potamogeton* sp., unidentified Hydrocharitaceae, and a mix of bottom vegetation detritus.

After the samples were processed at the Centre of Marine Sciences (CCMAR) in University of Algarve, they were shipped to MARINNOVA (University of Porto, Portugal) for isotopic analysis. Stable isotope ratios were measured using a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) via the Conflo IV Interface (MARINNOVA, University of Porto). Stable isotope ratios were reported in δ notation with Pee Dee Belemnite and air as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The analytical error, the average standard deviation of replicate reference material, was ± 0.1% for $\delta^{13}C$ and $\delta^{15}N$.

Data analyses

Corrections for the lipid content were applied to $\delta^{13}C$ values, as lipids are depleted in ^{13}C compared to protein and carbohydrates (DeNiro and Epstein 1978). Therefore, lipid content can bias bulk tissue $\delta^{13}C$ values and cause dietary information to be incorrectly interpreted (Logan et al. 2008). Zooplankton lipid content was corrected using the mass balance correction model proposed by Smyntek et al. (2007; Eq. 5). Zooplankton was also corrected for ethanol preservation (+0.4‰ $\delta^{13}C$, +0.6‰ $\delta^{15}N$) (Feuchtmayr and Grey 2003). The correction proposed by Bodin et al. (2007; Eq. 1) for the spider crab *Maja brachydactyla* Balss, 1922 was applied to the $\delta^{13}C$ values of all crab species. Lipid content in fish, bivalves, polychaetes, and other crustaceans, was corrected using the mass balance correction proposed by Hoffman and Sutton (2010; Eq. 6). This correction uses estimates of $C{:}N_{protein}$ and $\Delta\delta^{13}C_{lipid}$ that are similar to those from the muscle tissue found in fish (e.g. Sweeting et al. 2006) and other taxonomic groups as shrimps and zooplankton (Fry et al. 2003; Smyntek et al. 2007). No correction values were applied for POM, SOM, MPB, or plant material.

In the case of the consumer species, $\delta^{13}C$ and $\delta^{15}N$ values were corrected considering the trophic enrichment factors (TEF; or trophic fractionation). The values used in the present study

were calculated from the results of McCann and Jenson (2018) of muscle tissue of Atlantic blue crabs fed with clams and black sea bass (+0.56 ± 1.27‰ $\delta^{13}C$; +1.15 ± 1.05‰ $\delta^{15}N$).

Differences in values of $\delta^{13}C$ and $\delta^{15}N$ between the Atlantic blue crab and groups of sources of organic matter and species within those groups were tested with one-way analysis of variance (ANOVA) and post hoc Tukey HDS comparison tests. In order to assess which sources of organic matter were more likely to support the Atlantic blue crab in the Guadiana estuary, bi-plots using average $\delta^{13}C$ and $\delta^{15}N$ values of consumer and sources of organic matter were produced using average values at a species level, but also by larger taxonomic groups (e.g., fish, bivalves, crabs). These bi-plots, together with information on the feeding habits of the Atlantic blue crab (i.e., Laughlin 1982; Ryer 1987; Stoner and Buchanan 1990; Fitz and Wiegert 1991; McClintock et al. 1991; Hsueh et al. 1992; Perkins-Visser et al. 1996; Reichmuth et al. 2009), and results of metagenomic analysis (see **Section 3.2.1**) were used to select which potential sources of organic matter to include in Bayesian mixing models. Statistical analysis and plotting were performed in the RStudio platform (RStudio Team 2022).

The Bayesian MixSIAR model package (Stock and Semmens 2016) was used in the RStudio platform (RStudio Team 2022), to estimate the proportion of each potential source of organic matter for the Atlantic blue crab biomass. MixSIAR uses stable isotope signatures, including their standard error and tissue discrimination factors, from potential sources of organic matter as input variables to estimate the probability distributions of each organic matter source, allowing each source and TEF to be assigned with a normal distribution, producing a distribution of feasible solutions within 95% confidence intervals, while accounting for the uncertainty associated with multiple potential sources of organic matter (Parnell et al. 2010; Stock and Semmens 2016). The Markov Chain Monte Carlo methods (MCMC) in MixSIAR were set to run on 'very long' settings, i.e., chain length 1,000,000; burn-in 500,000; thin 500; and the number of chains set to 3. Values of TEF, calculated from the results of McCann and Jenson (2018), were included in the models in the discrimination files. In the 2021 model, the sex of Atlantic blue crabs was used as a factor.

The trophic position (TP) of the Atlantic blue crab and three other native brachyuran crabs was calculated following the equation proposed by Mancinelli et al. (2016):

$$TP_{\delta^{15}N} = \frac{(\delta^{15}N_{Consumer} - \delta^{15}N_{Baseline})}{D^{15}N + 1}$$

In this equation, $\delta^{15}N_{Consumer}$ is the nitrogen isotopic signature of the Atlantic blue crab, $\delta^{15}N_{Baseline}$ is the nitrogen isotopic signature of the baseline indicator, in this case bivalves (oysters) with an average value of 11.73‰. $\Delta^{15}N$ is the nitrogen trophic enrichment factor of 1.15‰, as previously calculated from the results of McCann and Jenson (2018). The λ is the trophic level of the baseline indicator, in this case $\lambda = 2$ for bivalves (oysters) as primary consumers.

The niche overlap between the four brachyuran crabs was evaluated using the package Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson et al. 2011). Ellipses were set to include 90% of the data (*p*.interval= 0.90). Estimation of overlap between ellipses based on their posterior distributions was done using the "bayesianOverlap" function, using 20 draws and a prediction interval of 90%. The area and percentage of overlap was then calculated for the cases where the ellipse of the Atlantic blue crab overlapped with the other crab species. Major niche width metrics were retrieved, namely total area of the convex hull encompassing the data points (TA), standard ellipse area (SEA), which contains proximately 40% of the data, thus representing the core isotopic niche area (Jackson et al. 2011), the corrected standard ellipse area (SEA_c), which is corrected for sample size and is considered more robust for smaller sample sizes (i.e., 10-30 observations) and/or when assumption of multivariate normality is not met (Jackson et al. 2011). The average will be used to summarize data and standard deviation to represent data dispersion.

3.3 Results

3.3.1 Metagenomic analysis

The outcome of analysed, filtered, and hand-cured metagenomic data was a total of 3238 hits, corresponding to 40 taxa, including bivalves, fish, other crabs, shrimps, barnacles, cnidarians, a gastropod, an ascidian, terrestrial plants, but also terrestrial mammals and pond turtles. The bivalve *Cerastoderma edule* (Linnaeus, 1758) was the species with the highest occurrence (938 hits), followed by the shrimp *Palaemon longirostris* (405 hits), the goby *Pomatoschistus* sp. (456 hits), and the oyster *Magallana gigas* (252 hits) (**Figure 3.1**).

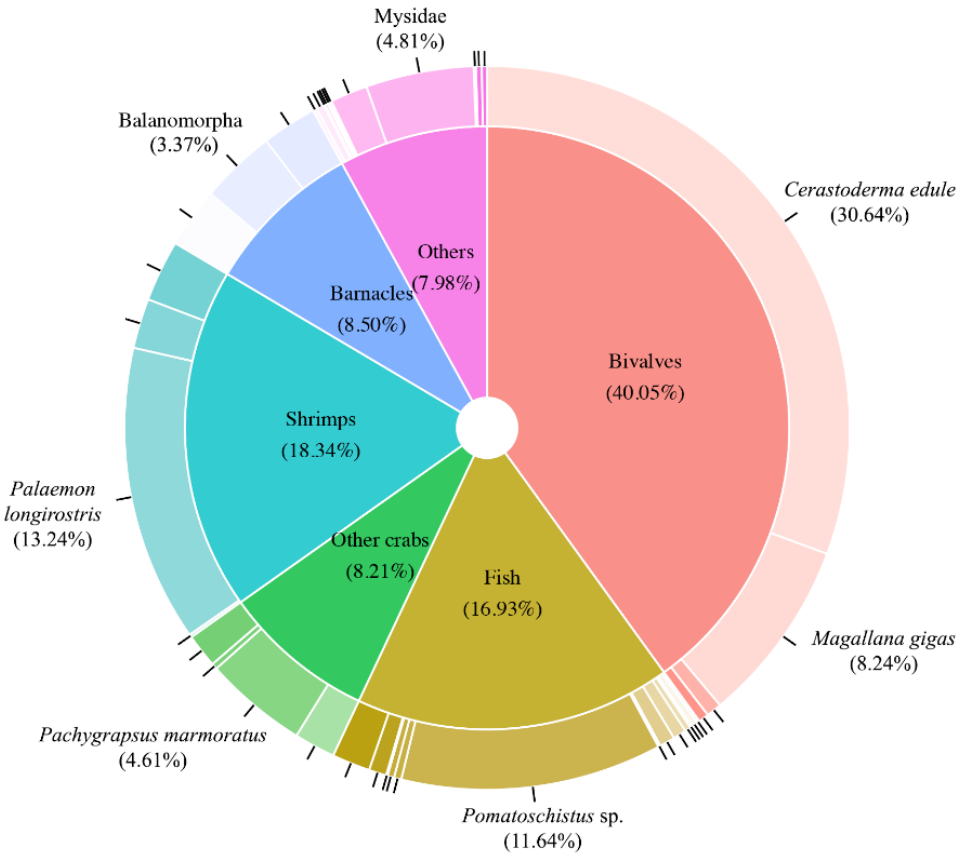


Figure 3.1 – Percentage of hits (3238 in total) from shotgun metagenomic analysis of the intestine content of Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896 collected in the Guadiana estuary in 2021 and 2022. The hits shown here are of the six larger groups and their respective taxa, after data was analysed, filtered, and hand-cured. Results include 13 individual samples and the three pooled samples.

Frequency of occurrence on the presence of prey species across individual samples showed that the crab *Pachygrapsus marmoratus* was the most frequent species in the Atlantic blue crab intestines, present in 12 out of 13 individual samples (FO= 92.3%). Following, there were five taxa with a frequency of occurrence of 84.6% (11 out of 13 samples) – Palaemonidae and Penaeus (shrimps), Grapsidae and *Panopeus* sp. (crabs), and Leptothecata or Ceriantharia (cnidaria). The next two taxa were the pipefish *Syngnathus* sp. (FO= 46.2%) and the oyster *Magallana gigas* (FO= 38.5%). Considering larger taxonomic groups, other crabs showed the highest frequency of occurrence (87.2 ± 4.4%), followed by shrimps (64.1 ± 35.5%), unidentified fish (38.5%), and oysters (26.9 ± 16.3%) (**Figure 3.2**).

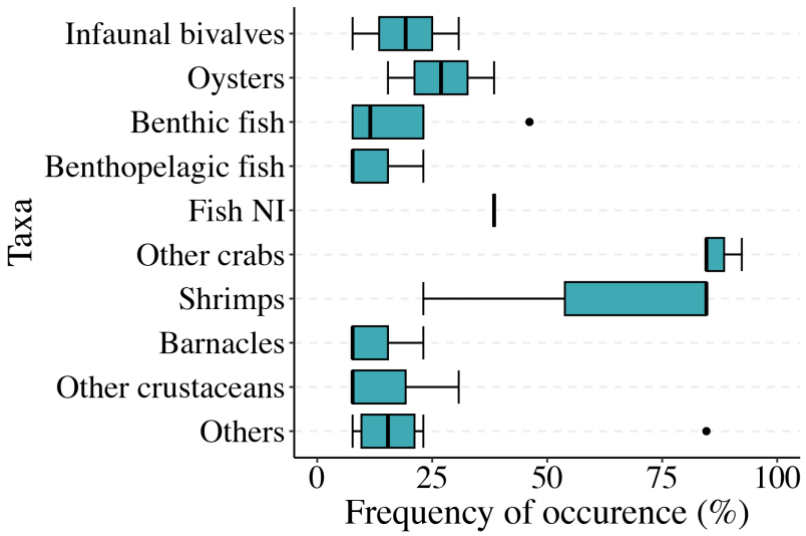


Figure 3.2 – Box-n-whisker plots of frequency of occurrence (%) of individual taxa grouped in larger taxonomic groups, obtained by shotgun metagenomic analysis of intestine content of Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896 collected in the Guadiana estuary in 2021 and 2022. Lower and upper fences represent the 25th and 75th percentiles, and the median is in between. Bars represent 10th and 90th percentiles and dots the outliers. Data shown was analysed, filtered, hand-cured, and does not include results from the three pooled samples.

3.3.2 Stable isotopes analysis

The carapace width of Atlantic blue crabs used for isotopic analysis was 155.5 ± 31.8mm (12 males: 163.0 ± 27.9mm; 10 females: 146.6 ± 35.3mm) in 2021, and 207.9 ± 16.7mm (20 males) in 2022. Isotopic values of the Atlantic blue crab were quite variable, particularly for carbon. Values of $\delta^{13}C$ ranged between -27.62 and -19.47‰, with an average (± SD) of -24.27 ± 1.62‰, while $\delta^{15}N$ ranged between 13.12 and 16.15‰, with an average (± SD) of 14.56 ± 0.70‰ (**Figure 3.2**). In 2021, when Atlantic blue crabs of both sexes were analysed, males were on average more ^{13}C-enriched than females (p= 0.0003), while no differences were detected for $\delta^{15}N$ (p= 0.6084).

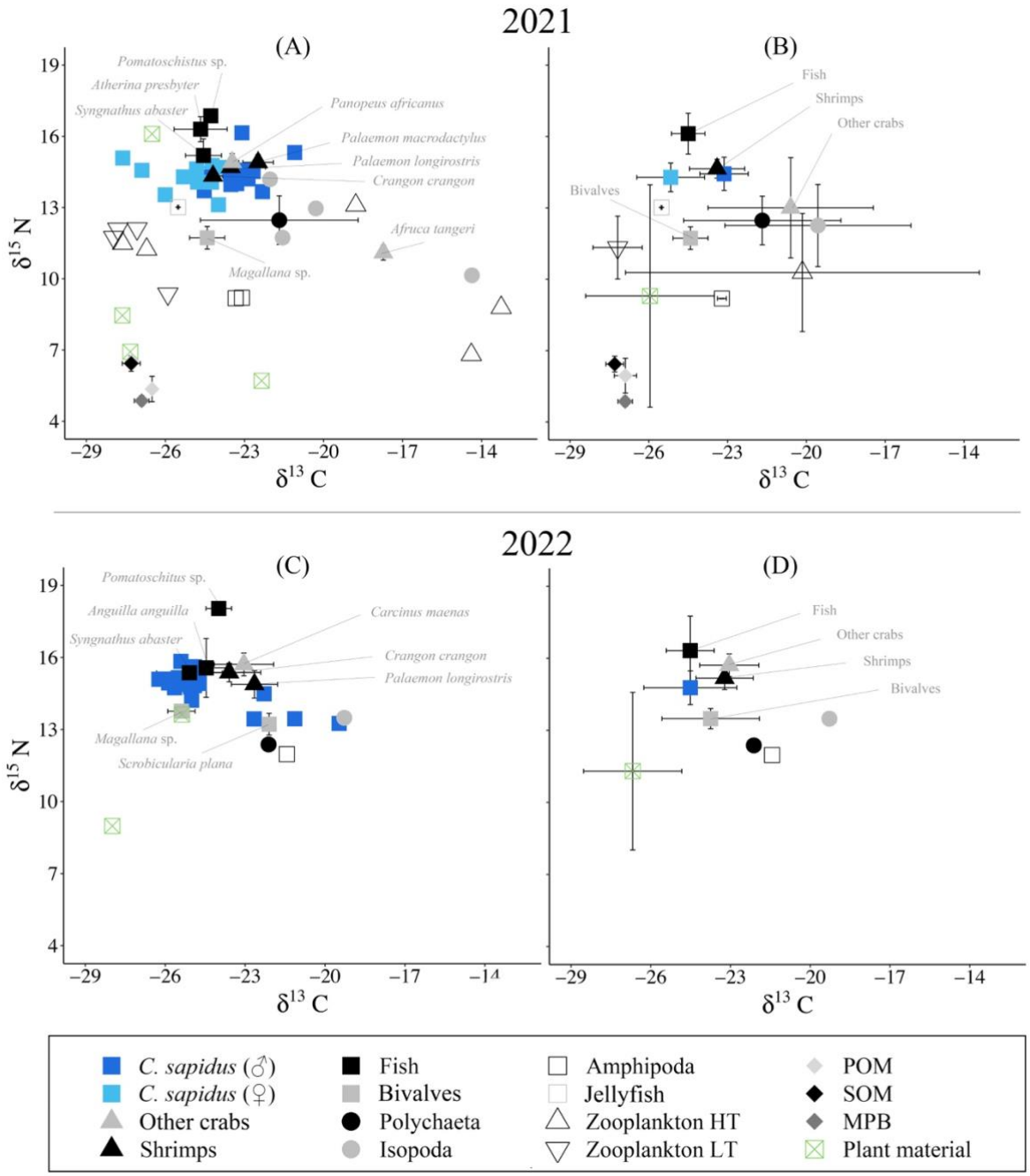


Figure 3.1 – Bi-plots of average (± SD) $\delta^{13}C$ and $\delta^{15}N$ values of the consumer species – the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 – and potential sources of organic matter as species and taxonomic groups for 2021 (A, B) and 2022 (C, D). Values of animal samples were corrected for lipid content, and ethanol preservation in the case of zooplankton. Values of the consumer species are corrected considering trophic enrichment factors (+0.56 ± 1.27‰ $\delta^{13}C$; +1.15 ± 1.05‰ $\delta^{15}N$), calculated from the results of McCann and Jenson (2018). Legend: HT– High Tide; LT– Low Tide; POM– Particulate Organic Matter; SOM– Sediment Organic Matter; MPB– Microphytobenthos.

Among potential food sources, fish showed the highest $\delta^{15}N$, 16.12 ± 0.86‰ in 2021 and 16.32 ± 1.44‰ in 2022, above most values of the Atlantic blue crab (overall value of 14.56 ± 0.70‰) (**Figure 3.1B, D**). The Atlantic blue crab $\delta^{15}N$ was higher ($p< 0.05$) than most taxonomic groups further lower on the trophic web, such as zooplankton (10.75 ± 2.01 ‰), bivalves (12.90 ± 0.97‰), polychaetes (12.44 ± 0.72‰), isopods (12.50 ± 1.59‰), and amphipods (10.12 ± 1.60‰). Most benthonic species, with the exception of *Afruca tangeri*, showed more intermediate $\delta^{15}N$ signatures that often overlapped with the Atlantic blue crab, as shrimps (14.84 ± 0.49‰), other crabs (*Carcinus maenas* 15.71 ± 0.47‰, *Panopeus africanus* 14.91 ± 0.36‰), and most fish species (*Syngnathus abaster* 15.28 ± 0.45‰, *Anguilla anguilla* 15.57 ± 1.22‰) (**Figure 3.2A**, **C**; **Table 3.1**). Differences in $\delta^{13}C$ between the Atlantic blue crab and potential food sources were not noticeable ($p> 0.05$), except Isopoda in 2022 ($p< 0.001$). Among species, differences in $\delta^{13}C$ were only detected between the Atlantic blue crab and *Afruca tangeri* in 2021 ($p< 0.001$), and *Carcinus maenas*, *Palaemon longirostris*, and *Scrobicularia plana* in 2022 ($p< 0.05$) (**Table 3.1**).

Table 3.1 – Average (± SD) $\delta^{13}C$ and $\delta^{15}N$ of the invasive consumer species (the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896), native crab species used in niche comparison collected in June 2021, and the potential sources of organic matter collected in late April 2021. In 2022, the consumer species was collected in March and its respective potential sources of organic matter collected in April. Values were corrected for lipid content, and ethanol preservation for zooplankton. Consumer species data are corrected considering trophic enrichment factors (+0.56 ± 1.27‰ $\delta^{13}C$; +1.15 ± 1.05‰ $\delta^{15}N$) according to McCann and Jenson (2018). Legend: HT– High Tide; LT– Low Tide; POM– Particulate Organic Matter; SOM– Sediment Organic Matter; MPB– Microphytobenthos.

Data	Category	Group	Species	$\delta^{13}C$	$\delta^{15}N$
2021	Invasive consumer	Atlantic blue crab	*Callinectes sapidus* (♂)	-23.1 ± 0.9	14.4 ± 0.7
			Callinectes sapidus (♀)	-25.2 ± 1.3	14.3 ± 0.6
	Niche comparison	Native crabs	*Afruca tangeri*	-17.8 ± 0.5	11.3 ± 0.4
			Carcinus maenas	-21.6 ± 1.6	14.5 ± 0.7
			Panopeus africanus	-23.1 ± 0.3	15.7 ± 0.2
	Animal sources of organic matter	Fish	*Atherina presbyter*	-24.7 ± 1.0	16.3 ± 0.5
			Pomatoschistus sp.	-24.3 ± 0.2	16.9 ± 0.1
			Syngnathus abaster	-24.6 ± 0.7	15.2 ± 0.7
		Bivalves	*Magallana* sp.	-24.4 ± 0.7	11.7 ± 0.5
		Polychaeta	*Nereididae* sp.	-21.7 ± 3.0	12.5 ± 1.0
		Crabs	*Afruca tangeri*	-17.7 ± 0.0	11.1 ± 0.3
			Panopeus africanus	-23.5 ± 0.2	14.9 ± 0.4
		Shrimps	*Crangon crangon*	-24.2 ± 0.4	14.3 ± 0.4
			Palaemon longirostris	-23.5 ± 1.3	14.7 ± 0.4
			Palaemon macrodactylus	-22.5 ± 0.6	14.9 ± 0.3

		Isopoda	*Cyathura carinata*	-22.0	14.2
			Lekanesphaera levii	-14.4	10.1
			Saduriella losadai	-20.3	13.0
			Synidotea laticauda	-21.6	11.7
		Amphipoda	*Corophium multisetosum*	-23.3	9.2
			Gammarus sp.	-23.1	9.2
		Jellyfish	*Blackfordia virginica*	-25.5 ± 0.0	13.0 ± 0.0
	POM	POM	POM HT	-26.5 ± 0.0	5.4 ± 0.5
			POM LT	-27.3 ± 0.1	6.5 ± 0.1
	SOM	SOM	SOM	-27.3 ± 0.3	6.4 ± 0.3
	MPB	MPB	MPB	-26.9 ± 0.3	4.9 ± 0.2
	Plant material	Plant material	*Juncus* sp.	-27.6	8.5
			Potamogeton sp.	-22.3	5.7
			Hydrocharitaceae	-26.5	16.1
			Vegetation detritus	-27.3	6.9
	Zooplankton	Zooplankton HT	*Acartia* sp.	-26.7	11.2
			Mesopodopsis CF *slabberi*	-27.6	11.5
			Gastropoda	-14.4	6.8
			Ostracoda	-13.3	8.8
			Paragnathia formica	-18.8	13.1
		Zooplankton LT	*Acartia* sp.	-27.8	12.1
			Calanipeda aquaedulcis	-27.1	12.1
			Melitidae	-25.9	9.4
			Mesopodopsis CF *slabberi*	-28.0	11.8
2022	Invasive consumer	Atlantic blue crab	*Callinectes sapidus* (♂)	-24.5 ± 1.7	14.8 ± 0.7
	Animal sources of organic matter	Fish	*Anguilla anguilla*	-24.5 ± 0.8	15.6 ± 1.2
			Pomatoschistus sp.	-24.0 ± 0.5	18.0 ± 0.3
			Syngnathus abaster	-25.1 ± 1.2	15.4 ± 0.1
		Bivalves	*Magallana* sp.	-25.4 ± 0.5	13.8 ± 0.1
			Scrobicularia plana	-22.1 ± 0.1	13.2 ± 0.5
		Polychaeta	*Nereididae* sp.	-22.1	12.4
		Other crabs	*Carcinus maenas*	-23.0 ± 1.1	15.7 ± 0.5
		Shrimps	*Crangon crangon*	-23.6 ± 1.2	15.4 ± 0.4
			Palaemon longirostris	-22.7 ± 0.9	14.9 ± 0.6
		Isopoda	*Saduriella losadai*	-19.3	13.5
		Amphipoda	*Gammarus* sp.	-21.4	12.0
	Plant material	Plant material	*Ceratophyllum* CF *demersum*	-25.4	13.6
			Vegetation detritus	-28.0	9.0

Bayesian MixSIAR models for 2021 and 2022 ran separately but using the same three groups of potential sources of organic matter: bivalves (oysters), fish, and shrimps. For bivalves collected in 2022, although there were two species that could be included in the models, only *Magallana* sp. was used because *Scrobicularia plana* was more ^{13}C-enriched ($p < 0.001$). In the case of other crab species, and although literature refers the consumption of other

crabs by the Atlantic blue crab (e.g., Laughlin 1982; Fitz and Wiegert 1991), this group was not used in the models. In the case of 2021, *Afruca tangeri* was isotopically different from *Panopeus africanus* ($p < 0.001$), being more ^{13}C-enriched, but also ^{15}N-deplected. When considering the use of only *Panopeus africanus* in 2021, or *Carcinus maenas* in 2022, in both cases the isotopic signatures of these crab species were very similar to shrimp species (**Figure 3.1**), and thus would only add uncertainty to the models.

In the case of the Atlantic blue crab, the high variability of isotopic signatures led to the exclusion from the models of two males and two females from the 2021 model, and two males from the 2022 model, using as threshold values of $\delta^{13}C$ and/or $\delta^{15}N$ outside 10% of the average of each year.

Bayesian MixSIAR model results from 2021 and 2022 showed similar, yet complementary results, with relevant contributions from all three potential food sources to the overall biomass of the Atlantic blue crab (**Figure 3.2**). While in 2021 shrimps composed a significant component of the Atlantic blue crab biomass, with a median proportion of 55.4% for males, in 2022 the value decreased to 28.1% (**Table 3.2**). Bivalves (oysters) were the second most important source of organic matter in both years, making up 45.4% of the sources of organic matter in 2022 (**Table 3.2**). In 2021, the main differences between sexes were the increase in the contribution of bivalves and fish to the biomass of female Atlantic blue crabs, and an increase in consumption of shrimps by males (**Table 3.2**). Fish were the prey that contributed the most to the biomass of female Atlantic blue crabs of 2021, with a higher proportion (35.2%) than males in either 2021 (18.9%) and 2022 (25.7%). Considering only the male specimens, a decrease between 2021 and 2022 was observed in the contribution of shrimps, in opposition to an increase of bivalves and fish (**Table 3.2**).

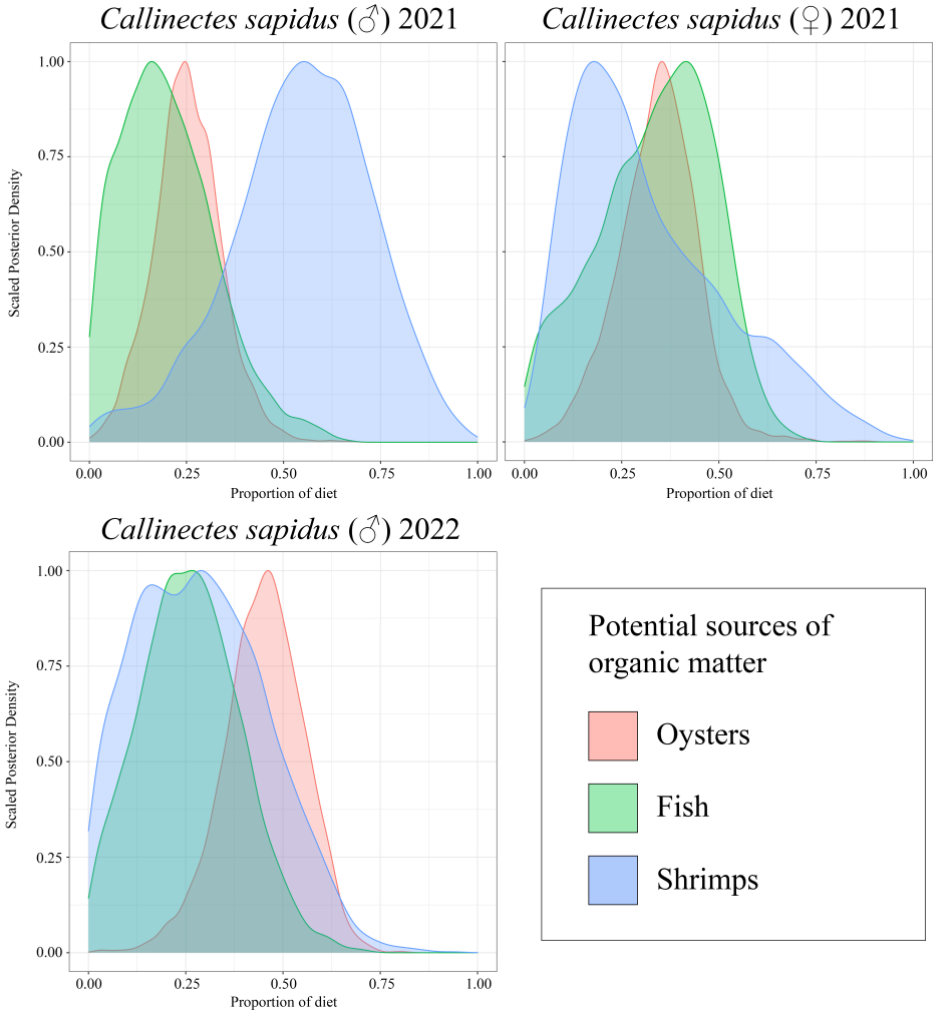


Figure 3.2 – Proportional contribution of each potential sources of organic matter to the Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896 collected in the middle Guadiana estuary (southeast Portugal) in 2021 and 2022, estimated from Bayesian MixSIAR models. Input values of $\delta^{13}C$ and $\delta^{15}N$ were corrected for lipid content. Trophic enrichment factors (+0.56 ± 1.27‰ $\delta^{13}C$; +1.15 ± 1.05‰ $\delta^{15}N$), calculated from the results of McCann and Jenson (2018), were considered in the discrimination factors.

Table 3.2 – Average (± SD) and median (50% quartile) proportion (%) of diet of the Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896 collected in the middle Guadiana estuary (southeast Portugal) in 2021 and 2022, estimated from Bayesian MixSIAR models.

	Callinectes sapidus (♂) 2021		*Callinectes sapidus* (♀) 2021		*Callinectes sapidus* (♂) 2022	
	Mean (± SD)	Median	Mean (± SD)	Median	Mean (± SD)	Median
Oysters	25.3 ± 8.7	25.0	34.2 ± 10.1	34.7	45.0 ± 10.3	45.4
Fish	20.2 ± 12.3	18.9	33.5 ± 14.8	35.2	26.2 ± 12.7	25.7
Shrimps	54.4 ± 18.1	55.4	32.3 ± 19.9	27.2	28.9 ± 16.6	28.1

Assessment of the trophic position of the four brachyuran crabs collected in the middle Guadiana estuary in June 2021 showed that three of them occupy a similarly high trophic position – *Panopeus africanus* (5.49 ± 0.20), *Carcinus maenas* (4.44 ± 0.64), *Callinectes sapidus* (4.32 ± 0.54) (**Table 3.3**; **Figure 3.3A**) – while *Afruca tangeri* had a significantly lower trophic position (1.61 ± 0.37; **Figure 3.3A**).

Niche overlap was observed between the Atlantic blue crab and two native brachyuran crabs (**Figure 3.3A**). The highest overlap was identified with *Carcinus maenas*, representing on average 30.13 ± 5.21% of the niche of both species. With *Panopeus africanus* the overlap was smaller, with an average of 5.62 ± 4.43%. The native *Carcinus maenas* was the crab species with the highest isotopic niche width (TA= 19.30; SEA_c= 4.19), followed by the non-indigenous *Callinectes sapidus* (TA= 14.12; SEA_c= 3.04), while *Panopeus africanus* and *Afruca tangeri* showed a very narrow niche width (SEA_c < 1) (**Figure 3.3B**).

Table 3.3 – Average (± SD) trophic position (TP) of the four species of brachyuran crabs collected in the middle Guadiana estuary in June 2021. Metrics to describe niche width include the total area of the convex hull (TA), the area of the standard ellipse (SEA), and the corrected standard ellipse area (SEA_c) as proposed by Jackson et al. 2011. Average (± SD) overlap area of ellipses and percentage of niche overlap between the Atlantic blue crab and the other three brachyuran crab species is presented.

Crab species	**Trophic position** (TP)	**TA**	**SEA** (‰^{2})	**SEA_c**	**Niche overlap** (‰^{2})	(%)
Callinectes sapidus	4.32 ± 0.54	14.12	2.91	3.04	NA	NA
Carcinus maenas	4.44 ± 0.64	19.30	3.73	4.19	7.57 ± 2.31	30.13 ± 5.21
Afruca tangeri	1.61 ± 0.37	2.55	0.49	0.55	0	0
Panopeus africanus	5.49 ± 0.20	1.27	0.24	0.28	0.73 ± 0.57	5.62 ± 4.43

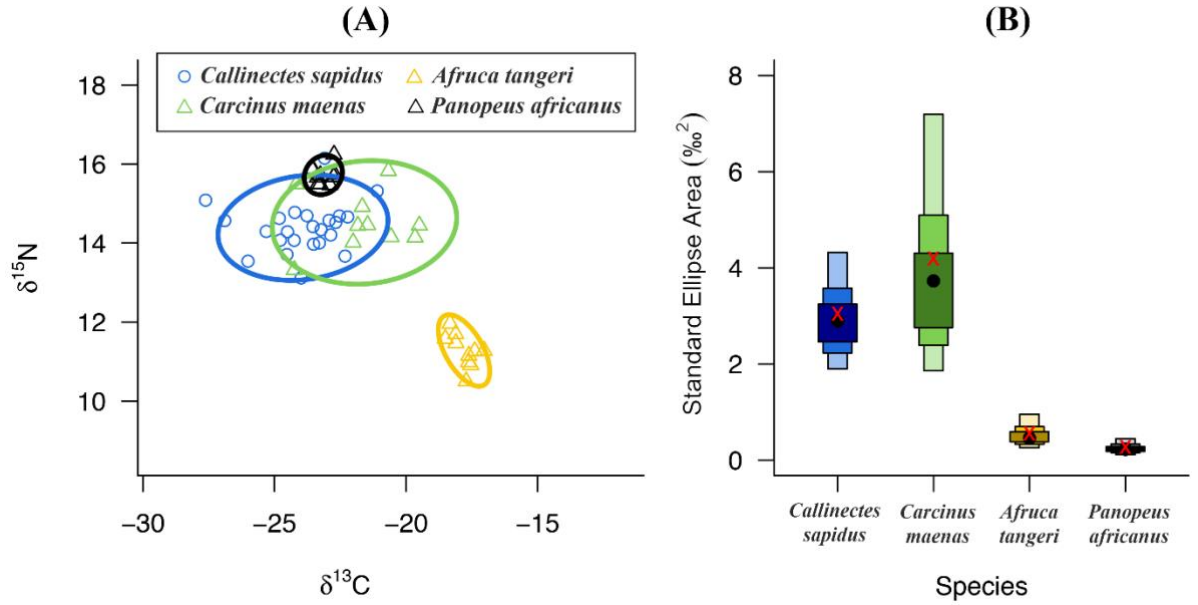


Figure 3.3 – (A) Stable Isotope Bayesian Ellipses in R (SIBER) results for the four brachyuran crab species collected in the middle Guadiana estuary in June 2021: *Callinectus sapidus* Rathbun, 1896, *Carcinus maenas* (Linnaeus, 1758), *Afruca tangeri* (Eydoux, 1835), and *Panopeus africanus* A. Milne-Edwards, 1867. Ellipses were set to include 90% of the data; (B) Standard Ellipses Areas (SEA) of the four brachyuran crab species. Black dots represent the mode, red crosses represent maximum likelihood estimated SEA_C, and the shaded boxes representing the 50%, 75% and 95% credible intervals from darker to lighter colours.

3.4 Discussion

The Atlantic blue crab *Callinectes sapidus* is a known omnivorous predator with an opportunistic feeding behaviour (Laughlin 1982; Hines et al. 1990; Stoner and Buchanan 1990; Hsueh et al. 1992; Seitz et al. 2011). This adaptiveness results in a capacity to explore the resources that are most available at any given time, which may change seasonally or across ontogeny (Laughlin 1982; Mancinelli et al. 2017; Prado et al. 2022). In our study, the metagenomic analysis confirmed the omnivorous nature of this species in the Guadiana estuary, while stable isotope analyses pinpointed the sources of organic matter they mostly rely on to support their biomass, namely bivalves, fish and other crustaceans, on similar proportions, with minor differences between males and females. The trophic position of the Atlantic blue crab (4.3 ± 0.5) was relatively high, while the comparison with native crab species showed a considerable niche overlap, particularly with the European green crab *Carcinus maenas*, which nevertheless presented a potentially larger niche width than the Atlantic blue crab. In the following sections, these aspects are discussed in more detail.

3.4.1 Disclosing the Atlantic blue crab diet through metagenomic analyses

DNA-sequencing based studies to infer on ecological interactions and feeding habits have become very popular, being used between terrestrial, freshwater, or marine ecosystems (Srivathsan et al. 2015; Paula et al. 2016; Pan et al. 2021; Dou et al. 2023). In the present study, results have shown a very diverse source of prey items for the Atlantic blue crabs of the Guadiana estuary, including bivalves, shrimps, benthic and benthopelagic fish. Still, a very laborious hand-curing process was needed to filter the initial results (446 365 hits) in order to reach a final dataset that could resemble the diversity of prey taxa available in the Guadiana estuary. Firstly, hits that did not belong to eukaryotic organisms were removed from the database (95.5%), which included bacteria and virus, as these were out of the scope of this study. In the resulting database, a large portion of hits were classified as belonging to the host (80.2%) – the Atlantic blue crab. From these only 32.9% were initially classified as *Callinectes sapidus*, and 3.1% as other *Callinectes* species. The other 44.1% were initially classified into 39 crustacean taxa, and after the hand-curing process, were reclassified as hits likely derived from host genetic material. The final database had 3238 hits (16.1% of the initial eukaryote database), which still included three crab taxa, namely Grapsidae (FO= 84.6%), *Panopeus* sp. (FO= 84.6%), and *Pachygrapsus marmoratus* (FO= 92.3%). The high frequency of occurrence of these three crab taxa, together with the large amount of other crab taxa that were discarded as host hits, shows that these results should be considered carefully, as the possibility that they are also due to host material should not be discarded.

Regarding frequencies of occurrence, following the previous three crab taxa, three other taxa showed values of 84.6% – Palaemonidae and Penaeus (shrimps), and a taxon that due to classification difficulties were left as either Leptothecata (Order) or Ceriantharia (Subclass) (both from the Phylum Cnidaria). Palaemonidae are represented by at least three species in the middle Guadiana estuary (*Palaemon longirostris*, *P. macrodactylus*, *P. serratus* (Pennant, 1777)), so they are likely to be an important prey for the Atlantic blue crab, namely *Palaemon longirostris* which is one of the most abundant shrimp species, along with the brown shrimp *Crangon crangon* (Encarnação et al. 2013). The later was also detected with four hits in the pooled samples. The species *Penaeus kerathurus* (Forskål, 1775) is also present in the Guadiana estuary, although its abundance seems to have declined considerably since the construction of the Alqueva dam in 2002 (Encarnação et al. 2013). In recent sampling campaigns (see **Sections 2.3.1** and **2.3.2**), the species has not been detected, so a frequency of occurrence of 84.6% seems a little high, and a misclassification with other shrimp species is a

possibility. Representatives of the Order Leptothecata (Phylum Cnidaria) in the Guadiana estuary include the invasive *Blackfordia virginica* (Chícharo et al. 2009), but other members of the Class Hydrozoa are also present, namely the invasive Ponto-Caspian hydrozoan *Cordylophora caspia* (Pallas, 1771) (Seyer et al. 2017). In the Class Scyphozoa, seasonal blooms of the native *Catostylus tagi* (Haeckel, 1869) also occur throughout the middle Guadiana estuary (Muha et al. 2017; Cruz et al. 2021). The Subclass Ceriantharia, commonly known as tube-dwelling anemones, are not well studied in the Guadiana estuary, but in the nearby coastal zone of Algarve, at least two species have been documented – *Cerianthus membranaceus* (Gmelin, 1791) and *Pachycerianthus solitarius* (Rapp, 1829) (BioDiversity4All 2023). Several species of the Subclass Hexacorallia also exist throughout the coastal zone of Algarve – *Anemonia viridis* (Forsskål, 1775), *Alicia mirabilis* Johnson, 1861, *Actinia* spp., *Aiptasia* spp., etc (BioDiversity4All 2023). From the twelve samples where these Cnidarians were detected, three samples were from June 2021 (21 hits) and nine from March 2022 (30 hits). March is not a time of the year when blooms of free living medusae are typically observed, as the invasive *Blackfordia virginica* or the native *Catostylus tagi*, which are rather more common in the months between June and October (J. Cruz, personal communication). Therefore, for the majority of these hits in the March 2022 samples, assuming the hits are indeed from Cnidarians, the most likely species that could have provided such results include anemones, or hydrozoans as the invasive *Cordylophora caspia* or other native species. Nevertheless, both *Blackfordia virginica* and *Catostylus tagi* have a benthic polyp stage (Wintzer et al. 2011; Gueroun et al. 2021), which despite their small size can still be consumed by benthic predators, as the Atlantic blue crab. In June 2021, medusae of the invasive *Blackfordia virginica* were already present in abundance in the Guadiana estuary (see **Section 2.3.1**), increasing the likelihood of becoming a prey for the Atlantic blue crab, as suggested in previous studies in the same study area by PCR methods (Cruz 2020). Between the Order Leptothecata and the Subclass Ceriantharia, the most likely representative in the middle Guadiana estuary is indeed the invasive *Blackfordia virginica*.

Fish presented an average frequency of occurrence of 16.7 ± 13.5%, with *Syngnathus* sp. showing the highest value (46.2%), followed by other non-identified fish (38.5%). In the middle Guadiana estuary, *Syngnathus abaster* is quite an abundant benthic fish (see **Sections 2.3.1** and **2.3.2**), so these results are in accordance with potential prey species for the Atlantic blue crab. Additionally, such predatory behaviour raises questions on potential impacts on other Syngnathids of the Algarve region, namely the endangered seahorses *Hippocampus* spp.,

more common in the nearby Ria Formosa lagoon, that have suffered a steep decline of populations in the last decades (Correia 2022), and where the Atlantic blue crab is also present since 2016 and increasing in abundance since then (Morais et al. 2019; Vasconcelos et al. 2019; but see **Chapter 4**). Regarding bivalves, these presented an average frequency of occurrence of 23.1 ± 14.0 %, with the oyster *Magallana gigas* having the highest frequency of occurrence among bivalves (38.5%), followed by the common cockle *Cerastoderma edule* (30.8%), while the clam *Scrobicularia plana* was present in 7.7% of samples. Oysters and the clam *Scrobicularia plana* are known species inhabiting the Guadiana estuary (Conde et al. 2013; López-Sanmartín et al. 2016). Still, the relatively high frequency of occurrence of *Cerastoderma edule* in Atlantic blue crabs caught in the middle Guadiana estuary is more prone to discussion. Typically, *Cerastoderma edule* is mostly found in more sandy areas of the lower estuary, while *Scrobicularia plana* is more abundant towards the middle estuary (Conde et al. 2013). There might be two explanations for the similar frequencies of occurrence of these two bivalve species as prey of the Atlantic blue crabs from the middle Guadiana estuary. An acoustic telemetry study done in the Guadiana estuary, simultaneously to this work, showed that the Atlantic blue crab can perform long and quick migrations along the estuary (Encarnação, unpublished data). Therefore, Atlantic blue crabs may move to lower sections of the estuary to feed on a food source that is more abundant and available there, as the bivalve *Cerastoderma edule* might be. On the other hand, several species have displayed a similar pattern in the Guadiana estuary in the last decades, namely an expansion towards upper areas of the estuary, due to continuous reduction of river flow and increasing salinization (Chícharo et al. 2006; Encarnação et al. 2013). If the middle Guadiana estuary becomes more saline, that may provide favourable environmental conditions for species most common of the river mouth to become a potential food source for Atlantic blue crabs inhabiting the middle estuary.

Overall, the present shotgun metagenomic results have shown a high diversity of potential prey from different taxonomic groups. Nevertheless, even after the whole process of filtering and hand-curation of results, many results of potential preys remain quite uncertain or dubious. The striking difference between the initial (eukaryotic) database, the many taxa initially identified that had no relation to the Portuguese marine biodiversity, not even the Atlantic Ocean, when compared to the final database, also adds uncertainty to the whole process of data processing and results reliability. One additional problem of this method is the possibility of hits resulting from secondary predation, i.e., positive identification of genetic material from species that were themselves prey items of the species the Atlantic blue crab

actively predated. Hypothetically, this could happen for example with the gobies *Pomatoschistus* sp., Pleuronectidae fish, or the brown shrimp *Crangon crangon*, that can prey on siphons of bivalves (Moreira et al. 1992; Mendonça et al. 2007), carrying the genetic material of bivalves such as *Scrobicularia plana* or *Cerastoderma edule* with them. Other fish, bivalves, or barnacle species can also be food sources for predatory fish such as the seabass *Dicentrarchus labrax* and many Sparidae, which may then be preyed upon by the Atlantic blue crab, either as juveniles or even adults.

Results such as wild boar *Sus scrofa* Linnaeus 1758 or pond turtles (Emydidae) among potential prey-items of the Atlantic blue crab, although unexpected, are also not impossible in the Guadiana estuary. Still, the present results only highlighted this potential prey in one individual sample (2 hits) and two pooled samples (5 hits) in the case of wild boar, and in one individual sample (1 hit) and another pooled sample (2 hits) in the case of Emydidae. The Atlantic blue crab is a known benthic scavenger (Holland et al. 1971; Laughlin 1982), so consumption of animal carcasses can occur, and wild boar are quite abundant along the Guadiana River basin. Representatives of Emydidae along the Guadiana River basin include the native Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812), detected on sampling campaigns in the Ribeira de Odeleite tributary (see **Section 2.3.1**), and although there is no strong evidence of predation by the Atlantic blue crab on pond turtles, such may happen with juvenile turtles (Davenport et al. 1992). Again, scavenging on carcasses is always a possibility.

Although very promising, shotgun-sequencing approaches should be carefully interpreted, while authors should resist to overinflate results (Alberdi et al. 2019). Issues with reference databases, degradation level of prey DNA, difficulties in separating consumer (host) and prey material at time of species classification, misclassification of closely related species, may lead to false-positives and pitfalls in interpretation of findings (Paula et al. 2016; Alberdi et al. 2019). The use of local custom-made library databases that could be cross-checked with biodiversity databases, along with a deep knowledge of the biodiversity of the study area, would increase reliability and replicability of results.

3.4.2 Sources of organic matter for the Atlantic blue crab

Variations in $\delta^{13}C$ found on Atlantic blue crabs of the Guadiana estuary, namely standard deviations of ± 0.9‰ in males and ± 1.6‰ in females during 2021 and ± 1.7‰ in males in

2022, also show the opportunistic nature of prey-selection and may reflect regular movements across the estuary to feed on different food sources. Atlantic blue crabs are excellent swimmers, even taking advantage of selective tidal-stream transport for longer migrations (Forward et al. 2003; Hench et al. 2004). As referred before, preliminary results of a telemetry study indicate quick migrations along the Guadiana estuary (Encarnação, unpublished data). Movements along the salinity gradient of the estuary may be reflected in a higher variation of $\delta^{13}C$, as food sources in more saline areas tend to be more ^{13}C-enriched due to the more enriched marine phytoplankton, which then is transmitted along the rest of the food web (Dehairs et al. 2000; Fry 2002; Dias et al. 2016; Prado et al. 2022). Individuals with isotopic signatures further away from the group average, namely the individuals excluded from the models, may have moved recently to the sampling area and were not in equilibrium with the local sources of organic matter.

The three groups of potential preys (bivalves, fish, shrimps) included in the MixSIAR models made up similar proportions to the overall diet, reflecting the omnivorous feeding of the Atlantic blue crab. While in 2021 males fed mainly on shrimps (> 50%), females preferred bivalves (oysters) and fish, while males collected in 2022 consumed mainly bivalves (oysters), which consisted of more than 45% of their diet. These three groups of potential prey were chosen due to the higher likelihood to integrate the diet of the Atlantic blue crab, either from general feeding habits of the consumer (Laughlin 1982; Hines et al. 1990; Stoner and Buchanan 1990; Hsueh et al. 1992; Seitz et al. 2011), and for representing some of the most common species found at the time in the study area (Chícharo et al. 2006; Encarnação et al. 2013; but also see results for Guadiana in **Chapter 2**). Input from metagenomic analysis (see **Section 3.3.1**) also confirmed the consumption of these three groups of potential prey, which was an important tool in the choice of these potential sources of organic matter as inputs of the models. The number of input sources was kept low (n= 3), so the models would run with the less amount of error and uncertainty, achieving a value of zero in the diagnostics of the models, namely the Gelman-Rubin and Geweke diagnostics.

Nevertheless, $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures and iso-plots produced show that other sources of organic matter may also be used by the Atlantic blue crab. Although other crab species were not included in the models, the similarity in isotopic signatures of *Panopeus africanus* and *Carcinus maenas* with shrimp species that were used in the models, indicate that they also can be a food source for the Atlantic blue crab. On the other hand, *Afruca tangeri* with its ^{13}C-enriched and ^{15}N-deplected signature, is not likely to be an important prey. In the

present results, plant material was not included in models as this group presented highly variable isotopic values, but some taxa showed values that could indicate it consumption by the Atlantic blue crab, namely Hydrocharitaceae and *Ceratophyllum* CF *demersum*. Both are submerged macrophytes commonly associated with freshwater ecosystems, which in the present case were collected in the middle Guadiana estuary, but as fragments carried by the tidal current. Vegetation is commonly reported as a food source for the Atlantic blue crab, but usually not the preferred one. Several authors suggest that on the lack of more nutritional animal food sources, the Atlantic blue crab readily feeds on detritus and vegetation material (Prado et al. 2022), or when unfitted for capturing live prey (Reichmuth et al. 2009). During the juvenile phase when Atlantic blue crabs are known to inhabit salt marshes and shallow-water macroalgal beds, such food items may constitute a significant proportion of diet, declining in contribution with growth and changes in habitat preferences and availability of more nutritional prey (Dittel et al. 2006).

Another taxon that needs further clarification on its potential role as a food source for the Atlantic blue crab in the Guadiana estuary are jellyfish. In the present study, this taxon was represented by the invasive *Blackfordia virginica*, that showed an isotopic signature slightly ^{15}N-depleted in comparison with the Atlantic blue crab. This invasive hydrozoan was firstly detected in the Guadiana estuary in 2008 and has been increasing in amount and intensity of blooms (Chícharo et al 2009; Muha et al. 2017; Encarnação personal observations; see results for Guadiana in **Chapter 2**). Consumption of jellyfish by the Atlantic blue crab has already been documented in North and South America (Farr 1978; Carman et al. 2017; Vitória et al. 2021), with preliminary results of genetic analysis positively identified *Blackfordia virginica* in the gut contents of crabs from the Guadiana estuary (Cruz 2020). So, this hypothesis should be further investigated in the Guadiana estuary since the seasonal availability of this resource can create the opportunity for the Atlantic blue crab to explore it. This was one of the non-indigenous species that could partially be supporting the invasiveness of the Atlantic blue crab, as initially hypothesised, but so far, its role in the diet of the Atlantic blue crab is not clear, or is only consumed occasionally, thus not supporting an invasion meltdown (Simberloff and Von Holle 1999; Simberloff 2006) at the moment. In the case of the non-native Asian clam *Corbicula fluminea*, we were not able to test its role in the food web, as no specimens were found in the middle Guadiana estuary during the present study. Although they were frequent in this area for some years, in what was apparently their lower distribution limit in the estuary (Morais et al. 2009a), the on-going salinization of this ecosystem (Chícharo et al. 2006;

Encarnação et al. 2013) may have shifted their distribution to upper areas of the Guadiana estuary, hindering its use in the present study as all samples for isotopic analysis should be collected in the same area, thus representing taxa living under the same environmental conditions.

3.4.3 Trophic position and niche overlap

In the Guadiana estuary, the trophic position estimate for the Atlantic blue crab (4.3 ± 0.5) is among the highest in the literature for the non-native area. Studies made along the Mediterranean Sea showed a wide array of trophic position values, varying between 2.98 ± 0.08 and 4.22 ± 0.19 in the Ionian Sea (Carrozzo et al. 2014), and averaging between 2.0 and 4.4 in the Aegean Sea (Aslan and Polito 2021), 3.73 ± 0.06 in the Adriatic Sea (Mancinelli et al. 2016), and 3.25 ± 0.17 between the Ionian and Adriatic Seas (Mancinelli et al. 2017).

The high proportion of shrimps and fish as primary sources of organic matter, as also disclosed by metagenomic data (frequencies of occurrence of 16.7 ± 13.5% for fish in general, 64.1 ± 35.5% for shrimps), may explain the high trophic position of the Atlantic blue crab in the Guadiana estuary. Such a high trophic position suggests a higher reliance on animal prey, rather than on plant material and detritus (German et al. 2013; Mancinelli et al. 2016, 2017, Prado et al. 2022). In the native range, an average value of 2.59 ± 0.38 was reported for an estuary in Texas, where no changes in trophic position were found between large and small Atlantic blue crabs (Hoeinghaus and Davis 2007). Such lower values come in concordance with previous studies, as these Atlantic blue crabs inhabiting salt marshes feed mainly on C_3 plants, benthic algae, and plant-derived detritus (Hoeinghaus and Davis 2007). In two New England tidal rivers, Atlantic blue crabs were found to be more generalist carnivores−omnivores, thus displaying a higher trophic level of 3.50 ± 0.35 (Taylor et al. 2022).

Differences in the trophic position of invasive Atlantic blue crab populations go beyond the inherent autoecological and synecological factors within each ecosystem. The trophic position of this species tends to increase in larger individuals – we only analysed adult individuals (155.5 ± 31.8 mm in 2021; 207.9 ± 16.7 mm in 2022) – because the consumption of molluscs and crustaceans, including other crabs, is higher than for younger life stages classes (Laughlin 1982). Data from the Ebro Delta (Spanish Mediterranean coast) shows how the

abundance fluctuations of primary food sources affected their trophic position. There, the trophic position was lower when they relied on bivalves as their primary source of organic matter (2.64 ± 0.20) and higher (3.11 ± 0.00) when they relied on additional animal sources (Prado et al. 2022). We opted to use a trophic enrichment factor explicitly calculated for the Atlantic blue crab, which is – so far – the only estimate available for this species (McCann and Jensen 2018). This trophic enrichment factor estimate was based on an experiment in which the Atlantic blue crab was fed with fish and clams to encompass a diverse diet. Aslan and Polito (2021) highlighted the potential effects of these methodological differences, namely on using benthic or pelagic baseline indicators, and different trophic enrichment factors, which across the Mediterranean Sea authors have used quite distinct values, ranging between 1.15 and 3.4 in the case of ^{15}N. Therefore, authors should make an effort to use standardized methods so to facilitate the comparison of results.

Competition for space and resources is one of the most cited impacts of invasive species (e.g., Crooks 2002; Grosholz 2002; Davis 2003; Britton et al. 2018). Comparisons of trophic positions showed a considerable niche overlap with the native European green crab *Carcinus maenas* (30.13 ± 5.21%). Nevertheless, the European green crab showed a potentially greater niche width (SEA_c= 4.19 ‰^{2}) than the Atlantic blue crab (SEA_c= 3.04 ‰^{2}) showing it may have some plasticity in the exploitation of available resources to cope with the competition with the Atlantic blue crab. Interestingly, the European green crab is an invasive species in the Atlantic blue crab native range. Across North America, the Atlantic blue crab showed to provide biotic resistance to the invasion of the European green crab, limiting its spread to areas where the native Atlantic blue crab is more abundant and where it readily preys upon the invader (DeRivera et al. 2005). Still, similar-sized crabs can be competitively similar, while larger Atlantic blue crabs take the advantage, especially at higher temperatures, while the European green crab may have the advantage at lower temperature zones (Rogers et al. 2018). The African mud crab *Panopeus africanus* and the west-African fiddler-crab *Afruca tangeri* have narrower niches than the Atlantic blue crab. Their isotopic signatures indicate a higher feeding specialization making them more susceptible to the putative impacts of the Atlantic blue crab. Although there is not much information on the ecology of the African mud crab specifically, small mud crab species are known to feed on a variety of benthic invertebrates, gastropods, barnacles, and bivalves (Ladwig 1999; Silliman et al. 2004; Premo et al. 2013). In the case of the mud crab *Panopeus herbstii* H. Milne Edwards, 1834, the species is able to compete alongside with the Atlantic blue crab to feed on oysters and mussels in North America

(Seed 1980; Bisker and Castagna 1987). In the Guadiana estuary, the African mud crab inhabits the same habitat and may exploit similar food sources as the Atlantic blue crab, thus partially sharing the same isotopic niche (5.62 ± 4.43%), becoming more prone to interspecific competition for food and habitat. The west-African fiddler crab is less susceptible to predation than the African mud crab because fiddler crabs occupy primarily intertidal areas and river margins and feed mostly on detritus (Wolfrath 1992; Ens et al. 1993; Moruf and Ojetayo 2017), which was evident in the differences between isotopic signatures. These two species of African crabs have been extending their distribution into European waters, together with the African pea crab *Afropinnotheres monodi* Manning, 1993, taking advantage of the numerous estuarine systems with muddy sediments and strong tidal amplitudes found along the Atlantic coasts of the Iberian Peninsula, which they do not find for example in most Mediterranean shores (Subida et al. 2011). To some extent, an increase in the abundance of the African mud crab, together with healthy populations of the European green crab, may increase the biotic resistance to the invasive Atlantic blue crab, at least during its juvenile phase.

3.5 Conclusions

The diet and sources of organic matter explored and incorporated into the biomass of the Atlantic blue crab in the Guadiana estuary helped explaining their invasiveness in this ecosystem. Metagenomic data showed that they exploited a diverse array of food sources – confirming their omnivorous and opportunistic trait – ranging between fish, molluscs, and crustaceans, including shrimps, barnacles, and potentially other crab species. Such omnivorous characteristic was evident in the dispersion of isotopic signatures. Stable isotope data confirmed that fish, shrimp, and bivalves were the major sources of organic matter supporting the biomass of the Atlantic blue crab. The present results were not enough to support the hypothesis of having previous invasive species facilitating the Atlantic blue crab invasion – i.e., an invasion meltdown. The Atlantic blue crab's trophic niche overlapped with two of three native crabs, as hypothesized, but did not have a wider trophic niche than the European green crab. Certainly, other factors intervene in the invasiveness of the Atlantic blue crab that should be explored in future studies, which will continue to provide critical feedback to environmental agencies in the Iberian Peninsula and along the non-native range.

3.6 References

Alberdi, A., Aizpurua, O., Bohmann, K., Gopalakrishnan, S., Lynggaard, C., Nielsen, M., and Gilbert, M.T.P. (2019). Promises and pitfalls of using high-throughput sequencing for diet analysis. *Molecular Ecology Resources* 19(2): 327-348. doi: 10.1111/1755-0998.12960

Amundsen, P.-A., and Sánchez-Hernández, J. (2019). Feeding studies take guts–critical review and recommendations of methods for stomach contents analysis in fish. *Journal of Fish Biology* 95(6): 1364-1373. doi: 10.1111/jfb.14151

Aslan, H., and Polito, M.J. (2021). Trophic ecology of the Atlantic blue crab *Callinectes sapidus* as an invasive non-native species in the Aegean Sea. *Biological Invasions* 23(7): 2289-2304. doi: 10.1007/s10530-021-02506-7

Balestrieri, A., Remonti, L., and Prigioni, C. (2011). Assessing carnivore diet by faecal samples and stomach contents: A case study with Alpine red foxes. *Open Life Sciences* 6(2): 283-292. doi: 10.2478/s11535-010-0106-1

Barbosa, A.B., Domingues, R.B., and Galvão, H.M. (2010). Environmental forcing of phytoplankton in a Mediterranean Estuary (Guadiana Estuary, South-western Iberia): A decadal study of anthropogenic and climatic influences. *Estuaries and Coasts* 33: 324-341. doi: 10.1007/s12237-009-9200-x

Bertolero, A., and Navarro, J. (2018). A native bird as a predator for the invasive apple snail, a novel rice field invader in Europe. *Aquatic Conservation: Marine and Freshwater Ecosystems* 28(5): 1099-1104. doi: 10.1002/aqc.2917

BioDiversity4All (2023). *Uma comunidade para naturalistas. Available at: www.biodiversity4all.org*. [Accessed March 20, 2023].

Bisker, R., and Castagna, M. (1987). Predation on single spat oysters *Crassostrea virginica* (Gmelin) by blue crabs *Callinectes sapidus* Rathbun and mud crabs *Panopeus herbstii* Milne-Edwards. *Journal of Shellfish Research* 6(1): 37.

Blundon, J.A., and Kennedy, V.S. (1982). Refuges for infaunal bivalves from blue crab, *Callinectes sapidus* (Rathbun), predation in Chesapeake Bay. *Journal of Experimental Marine Biology and Ecology* 65(1): 67-81. doi: 10.1016/0022-0981(82)90176-9

Bodin, N., Le Loc'h, F., and Hily, C. (2007). Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *Journal of Experimental Marine Biology and Ecology* 341(2): 168-175. doi: 10.1016/j.jembe.2006.09.008

Boecklen, W.J., Yarnes, C.T., Cook, B.A., and James, A.C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics* 42: 411-440. doi: 10.1146/annurev-ecolsys-102209-144726

Britton, J.R., Ruiz-Navarro, A., Verreycken, H., and Amat-Trigo, F. (2018). Trophic consequences of introduced species: Comparative impacts of increased interspecific versus intraspecific competitive interactions. *Functional Ecology* 32(2): 486-495. doi: 10.1111/1365-2435.12978

Calizza, E., Rossi, L., Careddu, G., Sporta Caputi, S., and Costantini, M.L. (2021). A novel approach to quantifying trophic interaction strengths and impact of invasive species in food webs. *Biological Invasions* 23: 2093-2107. doi: 10.1007/s10530-021-02490-y

Carman, M.R., Grunden, D.W., and Govindarajan, A.F. (2017). Species–specific crab predation on the hydrozoan clinging jellyfish *Gonionemus* sp.(Cnidaria, Hydrozoa), subsequent crab mortality, and possible ecological consequences. *PeerJ* 5: e3966. doi: 10.7717/peerj.3966

Carrozzo, L., Potenza, L., Carlino, P., Costantini, M.L., Rossi, L., and Mancinelli, G. (2014). Seasonal abundance and trophic position of the Atlantic blue crab *Callinectes sapidus* Rathbun 1896 in a Mediterranean coastal habitat. *Rendiconti Lincei* 25: 201-208. doi: 10.1007/s12210-014-0297-x

Cherel, Y., Weimerskirch, H., and Trouvé, C. (2000). Food and feeding ecology of the neritic-slope forager black-browed albatross and its relationships with commercial fisheries in Kerguelen waters. *Marine Ecology Progress Series* 207: 183-199. doi: 10.3354/meps207183

Chícharo, M.A., Chícharo, L., and Morais, P. (2006). Inter-annual differences of ichthyofauna structure of the Guadiana estuary and adjacent coastal area (SE Portugal/SW Spain): Before and after Alqueva dam construction. *Estuarine, Coastal and Shelf Science* 70(1-2): 39-51. doi: 10.1016/j.ecss.2006.05.036

Chícharo, M.A., Leitão, T., Range, P., Gutierrez, C., Morales, J., Morais, P., and Chícharo, L. (2009). Alien species in the Guadiana Estuary (SE-Portugal/SW-Spain): *Blackfordia virginica* (Cnidaria, Hydrozoa) and *Palaemon macrodactylus* (Crustacea, Decapoda): Potential impacts and mitigation measures. *Aquatic Invasions* 4(3): 501-506. doi: 10.3391/ai.2009.4.3.11

Cloern, J.E., Canuel, E.A., and Harris, D. (2002). Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography* 47(3): 713-729. doi: 10.4319/lo.2002.47.3.0713

Conde, A., Calvário, J., Sprung, M., Novais, J.M., and Domínguez, J. (2013). Converse effect of flooding on intertidal macrobenthic assemblages in the Guadiana estuary. *Journal of the Marine Biological Association of the United Kingdom* 93(6): 1431-1440. doi: 10.1017/S0025315413000453

Colautti, R.I., Ricciardi, A., Grigorovich, I.A., and MacIsaac, H.J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7(8): 721-733. doi: 10.1111/j.1461-0248.2004.00616.x

Cordone, G., Lozada, M., Vilacoba, E., Thalinger, B., Bigatti, G., Lijtmaer, D.A., Steinke, D., and Galván, D.E. (2022). Metabarcoding, direct stomach observation and stable isotope analysis reveal a highly diverse diet for the invasive green crab in Atlantic Patagonia. *Biological Invasions* 24: 505-526. doi: 10.1007/s10530-021-02659-5

Correia, M. (2022). Monitoring of seahorse populations, in the ria Formosa lagoon (Portugal), reveals steep fluctuations: Potential causes and future mitigations. *Proceedings of the Zoological Society* 75(2): 190-199. doi: 10.1007/s12595-021-00394-2

Crooks, J.A. (2002). Characterizing ecosystem-level consequences of biological invasions: The role of ecosystem engineers. *Oikos* 97(2): 153-166. doi: 10.1034/j.1600-0706.2002.970201.x

Cruz, J., Cerveira, I., Andrade, I., Baptista, V., and Teodósio, M.A. (2021). Prey selectivity and feeding rates of the scyphozoan *Catostylus tagi* (Haeckel, 1869). *Journal of Plankton Research* 43(6): 986-990. doi: 10.1093/plankt/fbab065

Cruz, J., Teodósio, M.A., Ben-Hamadou, R., Chicharo, L., Garrido, S., Ré, P., and Santos, A.M.P. (2017). RNA:DNA ratios as a proxy of egg production rates of *Acartia*. *Estuarine, Coastal and Shelf Science* 187: 96-109. doi: 10.1016/j.ecss.2016.12.028

Cruz, M. (2020). Jellyfish, a threat or an opportunity? The non-indigenous *Blackfordia virginica* as a potential food source for humans and aquatic organisms. Master thesis. Universidade do Algarve, Faro. 100 pp.

Dalerum, F., and Angerbjörn, A. (2005). Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144: 647-658. doi: 10.1007/s00442-005-0118-0

Davenport, J., Spikes, M., Thornton, S.M., and Kelly, B.O. (1992). Crab-eating in the diamondback terrapin *Malaclemys terrapin*: Dealing with dangerous prey. *Journal of the Marine Biological Association of the United Kingdom* 72(4): 835-848. doi: 10.1017/S0025315400060070

Davis, M.A. (2003). Biotic globalization: Does competition from introduced species threaten biodiversity? *BioScience* 53(5): 481-489. doi: 10.1641/0006-3568(2003)053[0481:BGDCFI]2.0.CO;2

Dehairs, F., Rao, R., Chandra Mohan, P., Raman, A., Marguillier, S., and Hellings, L. (2000). Tracing mangrove carbon in suspended matter and aquatic fauna of the Gautami–Godavari Delta, Bay of Bengal (India). *Hydrobiologia* 431: 225-241. doi: 10.1023/A:1004072310525

DeNiro, M.J., and Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42(5): 495-506. doi: 10.1016/0016-7037(78)90199-0

DeRivera, C.E., Ruiz, G.M., Hines, A.H., and Jivoff, P. (2005). Biotic resistance to invasion: Native predator limits abundance and distribution of an introduced crab. *Ecology* 86(12): 3364-3376. doi: 10.1890/05-0479

Dias, E., Morais, P., Antunes, C., and Hoffman, J.C. (2014). Linking terrestrial and benthic estuarine ecosystems: Organic matter sources supporting the high secondary production of a non-indigenous bivalve. *Biological Invasions* 16: 2163-2179. doi: 10.1007/s10530-014-0655-8

Dias, E., Morais, P., Cotter, A.M., Antunes, C., and Hoffman, J.C. (2016). Estuarine consumers utilize marine, estuarine and terrestrial organic matter and provide connectivity among these food webs. *Marine Ecology Progress Series* 554: 21-34. doi: 10.3354/meps11794

Dittel, A.I., Epifanio, C.E., and Fogel, M.L. (2006). Trophic relationships of juvenile blue crabs (*Callinectes sapidus*) in estuarine habitats. *Hydrobiologia* 568: 379-390. doi: 10.1007/s10750-006-0204-2

Dittel, A.I., and Epifanio, C.E. (2009). Invasion biology of the Chinese mitten crab *Eriochier sinensis*: A brief review. *Journal of Experimental Marine Biology and Ecology* 374(2): 79-92. doi: 10.1016/j.jembe.2009.04.012

Dou, H., Wang, M., Yin, X., Feng, L., and Yang, H. (2023). Can the Eurasian otter (*Lutra lutra*) be used as an effective sampler of fish diversity? Using molecular assessment of otter diet to survey fish communities. *Metabarcoding and Metagenomics* 7: 19-35. doi: 10.3897/mbmg.7.96733

Ebersole, E.L., and Kennedy, V.S. (1995). Prey preferences of blue crabs *Callinectes sapidus* feeding on three bivalve species. *Marine Ecology Progress Series* 118(1): 167-177. doi: 10.3354/meps118167

Eggleston, D.B. (1990). Functional responses of blue crabs *Callinectes sapidus* Rathbun feeding on juvenile oysters *Crassostrea virginica* (Gmelin): effects of predator sex and size, and prey size. *Journal of Experimental Marine Biology and Ecology* 143(1-2): 73-90. doi: 10.1016/0022-0981(90)90112-P

Encarnacão, J., Leitão, T., Morais, P., Piló, D., Range, P., Chícharo, L., and Chícharo, M.A. (2013). Effects of inter-annual freshwater inflow shifts on the community structure of estuarine decapods. *Cahiers de Biologie Marine* 54(2): 181-189.

Ens, B., Klaassen, M., and Zwarts, L. (1993). Flocking and feeding in the fiddler crab *(Uca tangeri*): Prey availability as risk-taking behaviour. *Netherlands Journal of Sea Research* 31(4): 477-494.

Epifanio, C.E. (2019). Early life history of the blue crab *Callinectes sapidus*: A review. *Journal of Shellfish Research* 38(1): 1-22. doi: 10.2983/035.038.0101

Farr, J.A. (1978). Blue crab predation on jellyfish. *Florida Scientist* 41(4): 217-219.

Feuchtmayr, H., and Grey, J. (2003). Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in Mass Spectrometry* 17(23): 2605-2610. doi: 10.1002/rcm.1227

Figueredo, C.C., and Giani, A. (2005). Ecological interactions between Nile tilapia (*Oreochromis niloticus*, L.) and the phytoplanktonic community of the Furnas Reservoir (Brazil). *Freshwater Biology* 50(8): 1391-1403. doi: 10.1111/j.1365-2427.2005.01407.x

Fitz, H.C., and Wiegert, R.G. (1991). Utilization of the intertidal zone of a salt marsh by the blue crab *Callinectes sapidus*: density, return frequency, and feeding habits. *Marine Ecology Progress Series* 76: 249-260.

Forward Jr, R., Tankersley, R., and Pochelon, P. (2003). Circatidal activity rhythms in ovigerous blue crabs, *Callinectes sapidus*: Implications for ebb-tide transport during the spawning migration. *Marine Biology* 142: 67-76. doi: 10.1007/s00227-002-0923-5

Fry, B. (2002). Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. *Estuaries* 25(2): 264-271. doi: 10.1007/BF02691313

Fry, B., Baltz, D.M., Benfield, M.C., Fleeger, J.W., Gace, A., Haas, H.L., and Quiñones-Rivera, Z.J. (2003). Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. *Estuaries* 26(1): 82-97. doi: 10.1007/BF02691696

Germain, L.R., Koch, P.L., Harvey, J., and McCarthy, M.D. (2013). Nitrogen isotope fractionation in amino acids from harbor seals: Implications for compound-specific trophic position calculations. *Marine Ecology Progress Series* 482: 265-277. doi: 10.3354/meps10257

Gilbey, V., Attrill, M.J., and Coleman, R.A. (2008). Juvenile Chinese mitten crabs (*Eriocheir sinensis*) in the Thames estuary: distribution, movement and possible interactions with the native crab *Carcinus maenas*. *Biological Invasions* 10: 67-77. doi: 10.1007/s10530-

007-9110-4

Green, S.J., Akins, J.L., Maljković, A., and Côté, I.M. (2012). Invasive lionfish drive Atlantic coral reef fish declines. *PLoS ONE* 7(3): e32596. doi: 10.1371/journal.pone.0032596

Grosholz, E. (2002). Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology & Evolution* 17(1): 22-27. doi: 10.1016/S0169-5347(01)02358-8

Gueroun, S.K., Torres, T.M., dos Santos, A., Vasco-Rodrigues, N., Canning-Clode, J., and Andrade, C. (2021). *Catostylus tagi* (Class: Scyphozoa, Order: Discomedusae, Suborder: Rhizostomida, Family: Catostylidae) life cycle and first insight into its ecology. *PeerJ* 9: e12056. doi: 10.7717/peerj.12056

Guillory, V., Perry, H.M., Steel, P., Wagner, T., Keithly, W., Pellegrin, B., Petterson, J., Floyd, T., Buckson, B., Hartman, L., et al. (2001). The blue crab fishery of the Gulf of Mexico, United States: A regional management plan. Gulf States Marine Fisheries Commission, Mississippi. 301 pp

Hench, J.L., Forward Jr, R.B., Carr, S.D., Rittschof, D., and Luettich Jr, R.A. (2004). Testing a selective tidal-stream transport model: observations of female blue crab (*Callinectes sapidus*) vertical migration during the spawning season. *Limnology and Oceanography* 49(5): 1857-1870. doi: 10.4319/lo.2004.49.5.1857

Hines, A.H., Lipcius, R.N., and Haddon, A.M. (1987). Population dynamics and habitat partitioning by size, sex, and molt stage of blue crabs *Callinectes sapidus* in a subestuary of central Chesapeake Bay. *Marine Ecology Progress Series* 36(1): 55-64.

Hines, A.H., Haddon, A.M., and Wiechert, L.A. (1990). Guild structure and foraging impact of blue crabs and epibenthic fish in a subestuary of Chesapeake Bay. *Marine Ecology Progress Series*.

Hobson, K.A. (1999). Tracing origins and migration of wildlife using stable isotopes: A review. *Oecologia* 120: 314-326. doi: 10.1007/s004420050865

Hoeinghaus, D.J., and Davis III, S.E. (2007). Size-based trophic shifts of saltmarsh dwelling blue crabs elucidated by dual stable C and N isotope analyses. *Marine Ecology Progress Series* 334: 199-204. doi: 10.3354/meps334199

Hoffman, J.C., and Sutton, T.T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep Sea Research I* 57(8): 956-964. doi: 10.1016/j.dsr.2010.05.003

Holland, J., Aldridge, D.C., and Kirk, S. (1971). Effects of temperature and salinity on growth, food conversion, survival and temperature resistance of juvenile blue crabs, *Callinectes sapidus* Rathbun. Texas A&M University, Texas. 190 pp

Hsueh, P.-W., McClintock, J.B., and Hopkins, T.S. (1992). Comparative study of the diets of the blue crabs *Callinectes similis* and *C. sapidus* from a mud-bottom habitat in Mobile Bay, Alabama. *Journal of Crustacean Biology* 12(4): 615-619. doi: 10.1163/193724092X00094

Jackson, A.L., Inger, R., Parnell, A.C., and Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80(3): 595-602. doi: 10.1111/j.1365-2656.2011.01806.x

Johnson, D.S. (2015). The savory swimmer swims north: A northern range extension of the blue crab *Callinectes sapidus*? *Journal of Crustacean Biology* 35(1): 105-110. doi:

10.1163/1937240X-00002293

Kampouris, T.E., Porter, J.S., and Sanderson, W.G. (2019). *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae): An assessment on its diet and foraging behaviour, Thermaikos Gulf, NW Aegean Sea, Greece: Evidence for ecological and economic impacts. *Crustacean Research* 48: 23-37. doi: 10.18353/crustacea.48.0_23

Keller, R.P., Geist, J., Jeschke, J.M., and Kühn, I. (2011). Invasive species in Europe: Ecology, status, and policy. *Environmental Sciences Europe* 23(1): 1-17. doi: 10.1186/2190-4715-23-23

Ladwig, C.A. (1999). Food choices of mud crabs (Family Xanthidae) in Chesapeake Bay. Ph.D. thesis. The George Washington University, Washington, DC. 103+ pp

Laughlin, R.A. (1982). Feeding habits of the blue crab, *Callinectes sapidus* Rathbun, in the Apalachicola estuary, Florida. *Bulletin of Marine Science* 32(4): 807-822.

Lenz, M., da Gama, B.A.P., Gerner, N.V., Gobin, J., Gröner, F., Harry, A., Jenkins, S.R., Kraufvelin, P., Mummelthei, C., and Sareyka, J. (2011). Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: Results from a globally replicated study. *Environmental Research* 111: 943–952. doi: 10.1016/j.envres.2011.05.001

Levine, J.M., Vila, M., Antonio, C.M.D., Dukes, J.S., Grigulis, K., and Lavorel, S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1517): 775-781. doi: 10.1098/rspb.2003.2327

Llewellyn, C., and La Peyre, M. (2011). Evaluating ecological equivalence of created marshes: Comparing structural indicators with stable isotope indicators of blue crab trophic support. *Estuaries and Coasts* 34: 172-184. doi: 10.1016/j.ecss.2013.06.008

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., and Lutcavage, M.E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: Comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*: 838-846. doi: 10.1111/j.1365-2656.2008.01394.x

López-Sanmartín, M., López-Fernández, J., Cunha, M., De la Herrán, R., and Navas, J. (2016). Ostreid herpesvirus in wild oysters from the Huelva coast (SW Spain). *Diseases of Aquatic Organisms* 120(3): 231-240. doi: 10.3354/dao03031

Mancinelli, G., Carrozzo, L., Costantini, M.L., Rossi, L., Marini, G., and Pinna, M. (2013). Occurrence of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 in two Mediterranean coastal habitats: Temporary visitor or permanent resident? *Estuarine, Coastal and Shelf Science* 135: 46-56. doi: 10.1016/j.ecss.2013.06.008

Mancinelli, G., Glamuzina, B., Petrić, M., Carrozzo, L., Glamuzina, L., Zotti, M., Raho, D., and Vizzini, S. (2016). The trophic position of the Atlantic blue crab *Callinectes sapidus* Rathbun 1896 in the food web of Parila Lagoon (South Eastern Adriatic, Croatia): A first assessment using stable isotopes. *Mediterranean Marine Science* 17(3): 634-643. doi: 10.12681/mms.1724

Mancinelli, G., Guerra, M.T., Alujević, K., Raho, D., Zotti, M., and Vizzini, S. (2017). Trophic flexibility of the Atlantic blue crab *Callinectes sapidus* in invaded coastal systems of the Apulia region (SE Italy): A stable isotope analysis. *Estuarine, Coastal and Shelf Science*

198: 421-431. doi: 10.1016/j.ecss.2017.03.013

Mancinelli, G., Bardelli, R., and Zenetos, A. (2021). A global occurrence database of the Atlantic blue crab *Callinectes sapidus*. *Scientific Data* 8(1): 111. doi: 10.1038/s41597-021-00888-w

Mascaro, M., Hidalgo, L.E., Chiappa-Carrara, X., and Simoes, N. (2003). Size-selective foraging behaviour of blue crabs, *Callinectes sapidus* (Rathbun), when feeding on mobile prey: active and passive components of predation. *Marine and Freshwater Behaviour and Physiology* 36(3): 143-159. doi: 10.1080/10236240310001603224

McCann, M., and Jensen, O. (2018). Laboratory experiments to determine trophic enrichment factors of stables isotope and fatty acid biomarkers in the blue crab, *Callinectes sapidus*. Distributed by: Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC), Harte Research Institute. *Texas A&M University–Corpus Christi*. doi: 10.7266/N76971K2

McClintock, J.B., Klinger, T.S., Marion, K., and Hsueh, P. (1991). Digestive carbohydrases of the blue crab *Callinectes sapidus* (Rathbun): Implications in utilization of plant-derived detritus as a trophic resource. *Journal of Experimental Marine Biology and Ecology* 148(2): 233-239. doi: 10.1016/0022-0981(91)90084-A

McCue, M.D., Javal, M., Clusella-Trullas, S., Le Roux, J.J., Jackson, M.C., Ellis, A.G., Richardson, D.M., Valentine, A.J., and Terblanche, J.S. (2020). Using stable isotope analysis to answer fundamental questions in invasion ecology: Progress and prospects. *Methods in Ecology and Evolution* 11(2): 196-214. doi: 10.1111/2041-210X.13327

Mendonça, V., Raffaelli, D., Boyle, P., and Emes, C. (2007). The ecological role of overwintering fish in the food web of the Culbin Sands lagoon ecosystem, NE Scotland: Identifying major trophic links and testing effects of the fish *Pomatoschistus microps* (Pallas) on benthic invertebrates. *Scientia Marina* 71(4): 649-660. doi: 10.3989/scimar.2007.71n4649

Middelburg, J. (2014). Stable isotopes dissect aquatic food webs from the top to the bottom. *Biogeosciences* 11(8): 2357-2371. doi: 10.5194/bg-11-2357-2014

Millikin, M.R. (1984). Synopsis of biological data on the blue crab, *Callinectes sapidus* Rathbun. NOAA Technical Report NMFS 1. US Department of Commerce, Washington. 47 pp

Minagawa, M., and Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta* 48(5): 1135-1140. doi: 10.1016/0016-7037(84)90204-7

Morais, P. (2008). Review on the major ecosystem impacts caused by damming and watershed development in an Iberian basin (SW-Europe): Focus on the Guadiana estuary. *Annales de Limnologie-International Journal of Limnology* 44(2): 105-117. doi: 10.1051/limn:2008012

Morais, P., Teodósio, J., Reis, J., Chícharo, M.A., and Chícharo, L. (2009a). The Asian clam *Corbicula fluminea* (Müller, 1774) in the Guadiana River Basin (southwestern Iberian Peninsula): Setting the record straight. *Aquatic Invasions* 4(4): 681-684. doi: 10.3391/ai.2009.4.4.14

Morais, P., Chícharo, M.A., and Chícharo, L. (2009b). Changes in a temperate estuary during

the filling of the biggest European dam. *Science of the Total Environment* 407(7): 2245-2259. doi: 10.1016/j.scitotenv.2008.11.037

Morais, P., Cerveira, I., and Teodósio, M.A. (2017). An update on the invasion of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801)(Actinopterygii: Sciaenidae) into Europe. *Diversity* 9(4): 47. doi: 10.3390/d9040047

Morais, P., Gaspar, M., Garel, E., Baptista, V., Cruz, J., Cerveira, I., Leitao, F., and Teodosio, M. (2019). The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its non-native distribution into the Ria Formosa lagoon and the Guadiana estuary (SW-Iberian Peninsula, Europe). *BioInvasions Records* 8(1): 123-133. doi: 10.3391/bir.2019.8.1.14

Moreira, F., Assis, C., Almeida, P., Costa, J., and Costa, M. (1992). Trophic relationships in the community of the upper Tagus estuary (Portugal): A preliminary approach. *Estuarine, Coastal and Shelf Science* 34(6): 617-623. doi: 10.1016/S0272-7714(05)80066-6

Moruf, R.O., and Ojetayo, T.A. (2017). Biology of the West African fiddler crab, *Uca tangeri* (Eydoux, 1835)(Decapoda: Ocypodidae) from a mangrove wetland in Lagos, Nigeria. *International Journal of Aquatic Biology* 5(4): 263-267. doi: 10.22034/ijab.v5i4.328

Muha, T.P., Teodósio, M.A., and Ben-Hamadou, R. (2017). Impact assessment of non-indigenous jellyfish species on the estuarine community dynamic: A model of medusa phase. *Estuarine, Coastal and Shelf Science* 187: 249-259. doi: 10.1016/j.ecss.2016.10.040

NOAA - National Oceanic and Atmospheric Administration (2023). *Fisheries One Stop Shop (FOSS). Available at: https://www.fisheries.noaa.gov/foss/f?p=215:200:16121283799345:Mail:::::*. [Accessed April 27, 2023].

Nuño, C., Peg, M., Mellado-Díaz, A., Sánchez-González, J.R., and Toro, M. (2018). First record of *Synidotea laticauda* Benedict, 1897 (Crustacea: Isopoda) in the Guadiana Estuary (SW Iberian Peninsula). *Limnetica* 37(2): 173-179. doi: 10.23818/limn.37.14

Orth, R.J., and van Montfrans, J. (1990). Utilization of marsh and seagrass habitats by early stages of *Callinectes sapidus*: A latitudinal perspective. *Bulletin of Marine Science* 46(1): 126-144.

Overton, A.S., Margraf, F.J., and May, E.B. (2009). Spatial and temporal patterns in the diet of striped bass in Chesapeake Bay. *Transactions of the American Fisheries Society* 138(4): 915-926. doi: 10.1577/T07-261.1

Pan, W., Qin, C., Zuo, T., Yu, G., Zhu, W., Ma, H., and Xi, S. (2021). Is metagenomic analysis an effective way to analyze fish feeding habits? A case of the yellowfin sea bream *Acanthopagrus latus* (Houttuyn) in Daya Bay. *Frontiers in Marine Science* 8: 634651. doi: 10.3389/fmars.2021.634651

Parnell, A.C., Inger, R., Bearhop, S., and Jackson, A.L. (2010). Source partitioning using stable isotopes: Coping with too much variation. *PLoS ONE* 5(3): e9672. doi: 10.1371/journal.pone.0009672

Paula, D.P., Linard, B., Crampton-Platt, A., Srivathsan, A., Timmermans, M.J., Sujii, E.R., Pires, C.S., Souza, L.M., Andow, D.A., and Vogler, A.P. (2016). Uncovering trophic interactions in arthropod predators through DNA shotgun-sequencing of gut contents.

PLoS ONE 11(9): e0161841. doi: 10.1371/journal.pone.0161841

Perkins-Visser, E., Wolcott, T.G., and Wolcott, D.L. (1996). Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathbun). *Journal of Experimental Marine Biology and Ecology* 198(2): 155-173. doi: 10.1016/0022-0981(96)00014-7

Peterson, B.J., and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18(1): 293-320. doi: 10.1146/annurev.es.18.110187.001453

Posey, M.H., Alphin, T.D., Harwell, H., and Allen, B. (2005). Importance of low salinity areas for juvenile blue crabs, *Callinectes sapidus* Rathbun, in river-dominated estuaries of southeastern United States. *Journal of Experimental Marine Biology and Ecology* 319(1-2): 81-100. doi: 10.1016/j.jembe.2004.04.021

Post, D.M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* 83(3): 703-718. doi: 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Prado, P., Peñas, A., Ibáñez, C., Cabanes, P., Jornet, L., Álvarez, N., and Caiola, N. (2020). Prey size and species preferences in the invasive blue crab, *Callinectes sapidus*: Potential effects in marine and freshwater ecosystems. *Estuarine, Coastal and Shelf Science* 245: 106997. doi: 10.1016/j.ecss.2020.106997

Prado, P., Ibáñez, C., Chen, L., and Caiola, N. (2022). Feeding habits and short-term mobility patterns of blue crab, *Callinectes sapidus*, across invaded habitats of the Ebro Delta subjected to contrasting salinity. *Estuaries and Coasts* 45(3): 839-855. doi: 10.1007/s12237-021-01004-2

Premo, K.M., and Tyler, A.C. (2013). Threat of predation alters the ability of benthic invertebrates to modify sediment biogeochemistry and benthic microalgal abundance. *Marine Ecology Progress Series* 494: 29-39. doi: 10.3354/meps10561

Ramach, S., Darnell, M.Z., Avissar, N., and Rittschof, D. (2009). Habitat use and population dynamics of blue crabs, *Callinectes sapidus*, in a high-salinity embayment. *Journal of Shellfish Research* 28(3): 635-640. doi: 10.2983/035.028.0328

Reichmuth, J.M., Roudez, R., Glover, T., and Weis, J.S. (2009). Differences in prey capture behavior in populations of blue crab (*Callinectes sapidus* Rathbun) from contaminated and clean estuaries in New Jersey. *Estuaries and Coasts* 32: 298-308. doi: 10.1007/s12237-008-9130-z

Rogers, T.L., Gouhier, T.C., and Kimbro, D.L. (2018). Temperature dependency of intraguild predation between native and invasive crabs. *Ecology* 99(4): 885-895. doi: 10.1002/ecy.2157

RStudio Team (2022). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Russell, J.C., Peace, J.E., Houghton, M.J., Bury, S.J., and Bodey, T.W. (2020). Systematic prey preference by introduced mice exhausts the ecosystem on Antipodes Island. *Biological Invasions* 22: 1265-1278. doi: 10.1007/s10530-019-02194-4

Ryer, C.H. (1987). Temporal patterns of feeding by blue crabs (*Callinectes sapidus*) in a tidal-marsh creek and adjacent seagrass meadow in the lower Chesapeake Bay. *Estuaries* 10: 136-140. doi: 10.2307/1352178

Savidge, J.A. (1987). Extinction of an island forest avifauna by an introduced snake. *Ecology* 68(3): 660-668. doi: 10.2307/1938471

Scalici, M., Chiesa, S., Mancinelli, G., Rontani, P.M., Voccia, A., and Nonnis Marzano, F. (2022). Euryhaline aliens invading Italian inland waters: The case of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896. *Applied Sciences* 12(9): 4666. doi: 10.3390/app12094666

Scharf, F.S., and Schlicht, K.K. (2000). Feeding habits of red drum (Sciaenops ocellatus) in Galveston Bay, Texas: Seasonal diet variation and predator-prey size relationships. *Estuaries* 23(1): 128-139. doi: 10.2307/1353230

Seed, R. (1980). Predator-prey relationships between the mud crab *Panopeus herbstii*, the blue crab, *Callinectes sapidus* and the Atlantic ribbed mussel *Geukensia* (= *Modiolus*) *demissa*. *Estuarine and Coastal Marine Science* 11(4): 445-458. doi: 10.1016/S0302-3524(80)80067-3

Seitz, R.D., Knick, K.E., and Westphal, M. (2011). Diet selectivity of juvenile blue crabs (*Callinectes sapidus*) in Chesapeake Bay. *Integrative and Comparative Biology* 51(4): 598-607. doi: 10.1093/icb/icr098

Seyer, T., Morais, P., Amorim, K., Leitão, F., Martins, F., and Teodósio, M.A. (2017). On the presence of the Ponto-Caspian hydrozoan *Cordylophora caspia* (Pallas, 1771) in an Iberian estuary: Highlights on the introduction vectors and invasion routes. *BioInvasions Records* 6(4): 331-337. doi: 10.3391/bir.2017.6.4.05

Silliman, B.R., Layman, C.A., Geyer, K., and Zieman, J.C. (2004). Predation by the black-clawed mud crab, *Panopeus herbstii*, in mid-Atlantic salt marshes: Further evidence for top-down control of marsh grass production. *Estuaries* 27: 188-196. doi: 10.1007/BF02803375

Simberloff, D., and Von Holle, B. (1999). Positive interactions of nonindigenous species: Invasional meltdown? *Biological Invasions* 1: 21-32. doi: 10.1023/A:1010086329619

Simberloff, D. (2006). Invasional meltdown 6 years later: Important phenomenon, unfortunate metaphor, or both? *Ecology Letters* 9(8): 912-919. doi: 10.1111/j.1461-0248.2006.00939.x

Smyntek, P.M., Teece, M.A., Schulz, K.L., and Thackeray, S.J. (2007). A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. *Limnology and Oceanography* 52(5): 2135-2146. doi: 10.4319/lo.2007.52.5.2135

Sousa, R., Gutiérrez, J.L., and Aldridge, D.C. (2009). Non-indigenous invasive bivalves as ecosystem engineers. *Biological Invasions* 11: 2367-2385. doi: 10.1007/s10530-009-9422-7

Srivathsan, A., Sha, J.C., Vogler, A.P., and Meier, R. (2015). Comparing the effectiveness of metagenomics and metabarcoding for diet analysis of a leaf-feeding monkey (*Pygathrix nemaeus*). *Molecular Ecology Resources* 15(2): 250-261. doi: 10.1111/1755-0998.12302

Srivathsan, A., Ang, A., Vogler, A.P., and Meier, R. (2016). Fecal metagenomics for the simultaneous assessment of diet, parasites, and population genetics of an understudied primate. *Frontiers in Zoology* 13: 1-13. doi: 10.1186/s12983-016-0150-4

Stock, B., and Semmens, B. (2016). MixSIAR GUI User Manual. Version 3.1. https://github.com/brianstock/MixSIAR/. doi: 10.5281/zenodo.47719

Stoner, A.W., and Buchanan, B.A. (1990). Ontogeny and overlap in the diets of four tropical *Callinectes species*. *Bulletin of Marine Science* 46(1): 3-12.

Subida, M.D., Arias, A.M., Drake, P., García-Raso, J.E., Rodríguez, A., and Cuesta, J.A. (2011). On the occurrence of *Afropinnotheres monodi* Manning, 1993 (Decapoda: Pinnotheridae) in European waters. *Journal of Crustacean Biology* 31(2): 367-369. doi: 10.1651/10-3381.1

Sweeting, C., Polunin, N., and Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry* 20(4): 595-601. doi: 10.1002/rcm.2347

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., and Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* 21(8): 2045-2050. doi: 10.1111/j.1365-294X.2012.05470.x

Tagatz, M.E. (1968). Growth of juvenile blue crabs, *Callinectes sapidus* Rathbun, in the St. Johns River, Florida. *Fishery Bulletin* 67(2): 281-288.

Tagatz, M.E. (1971). Osmoregulatory ability of blue crabs in different temperature-salinity combinations. *Chesapeake Science* 12(1): 14-17. doi: 10.2307/1350497

Taylor, D.L., Fehon, M.M., Cribari, K.J., and Scro, A.K. (2022). Blue crab *Callinectes sapidus* dietary habits and predation on juvenile winter flounder *Pseudopleuronectes americanus* in southern New England tidal rivers. *Marine Ecology Progress Series* 681: 145-167. doi: 10.3354/meps13909

Vasconcelos, P., Carvalho, A.N., Piló, D., Pereira, F., Encarnação, J., Gaspar, M.B., and Teodósio, M.A. (2019). Recent and consecutive records of the Atlantic blue crab (*Callinectes sapidus* Rathbun, 1896): Rapid westward expansion and confirmed establishment along the Southern Coast of Portugal. *Thalassas: An International Journal of Marine Sciences* 35(2): 485-494. doi: 10.1007/s41208-019-00163-1

Ventura, M.P., Salgado, S.Q., De Arenas, J.H.N., Cano, J.V., Mata, P.R., and Soriano, J.L. (2018). Predation of the blue crab *Callinectes sapidus* Rathbun, 1896 on freshwater bivalves (Unionidae & Corbiculidae) in eastern Iberian Peninsula. *Folia Conchyliologica* 47.

Vitória, T.L., Silveira, S., and Nagata, R.M. (2021). Predation of portunid crabs *Callinectes sapidus* and *Callinectes ornatus* (Crustacea, Decapoda) on the medusa *Lychnorhiza lucerna* (Cnidaria, Scyphozoa). *Ocean and Coastal Research* 69: e21002. doi: 10.1590/2675-2824069.20-323tlv

Williams, A.H., Coen, L.D., and Stoelting, M.S. (1990). Seasonal abundance, distribution, and habitat selection of juvenile *Callinectes sapidus* (Rathbun) in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* 137(3): 165-183. doi: 10.1016/0022-0981(90)90183-D

Wintzer, A.P., Meek, M.H., Moyle, P.B., and May, B. (2011). Ecological insights into the polyp stage of non-native hydrozoans in the San Francisco Estuary. *Aquatic Ecology* 45: 151-161. doi: 10.1007/s10452-010-9343-7

Wolfrath, B. (1992). Burrowing of the fiddler crab *Uca tangeri* in the Ria Formosa in Portugal and its influence on sediment structure. *Marine Ecology Progress Series* 85: 237-243.

Woo, C., Kumari, P., Eo, K.Y., Lee, W.-S., Kimura, J., and Yamamoto, N. (2023). Combining vertebrate mitochondrial 12S rRNA gene sequencing and shotgun metagenomic sequencing to investigate the diet of the leopard cat (*Prionailurus bengalensis*) in Korea. *PLoS ONE* 18(1): e0281245. doi: 10.1371/journal.pone.0281245

CHAPTER 4

Citizen science and biological invasions

Sections of the present chapter were published in the following peer-reviewed scientific articles under the objectives of this Ph.D. thesis:

- **Encarnação J**, Teodósio MA, Morais P (2021). Citizen science and biological invasions: A review. *Frontiers in Environmental Science* 8:602980. Doi: 10.3389/fenvs.2020.602980.
- **Encarnação J**, Baptista V, Teodósio MA, Morais P (2021). Low-cost citizen science effectively monitors the rapid expansion of a marine invasive species. *Frontiers in Environmental Science* 9:752705. Doi: 10.3389/fenvs.2021.752705.
- **Encarnação J**, Krug LA, Teodósio MA, Morais P (2022). Coastal countercurrents increase propagule pressure of an aquatic invasive species to an area where previous introductions failed. *Estuaries and Coasts*. Doi: 10.1007/s12237-022-01092-8.

4.1 Introduction

Biological invasions usually go unnoticed by the scientific community during the initial period of low abundance (Simberloff and Rejmánek 2011). A similar process is observed with their impacts, which are only acknowledged by the scientific community and public when impacts are already significant (Pyšek and Richardson 2010; Simberloff 2011). Recognizably, prevention, early detection, and localized containment are the most effective measures to minimize the impact of non-indigenous species, including invasive species (Pyšek and Richardson 2010). Given the widespread nature of non-indigenous species, collaborative approaches should be considered towards reducing the time of first detection.

Given the pervasive nature of biological invasions, citizen science emerged as an additional tool for earlier detection and management of biological invasions. Citizen scientists – as individuals or communities – collect and analyse data, helping to conduct research, generate new hypothesis, or solving unanswered questions (Eitzel et al., 2017). The Oxford English Dictionary defines citizen science as "scientific work undertaken by members of the general public, often in collaboration with or under the direction of scientists and scientific institutions" (Simpson and Weiner 2014). One of the main advantages of citizen science is the ability to cover larger geographical areas, at a significantly lower cost when compared to traditional scientific surveys (Carr 2004; Crall et al., 2010; Tulloch et al., 2013; Pocock et al., 2017; Simoniello et al., 2019). Citizen science can significantly reduce the time until the first detection of a non-indigenous species and track its dispersion with a wide network of citizen scientists. Therefore, eradication, containment, and mitigation measures may occur earlier and eventually be more effective (Gallo and Waitt, 2011; Pocock et al., 2017; Eritja et al., 2019).

A more traditional approach to citizen science projects consists of recruiting citizen scientists, provide them informative materials, develop training sessions, and then finishing with the participatory activities in the field, supervised, or in collaboration with scientists. One of such examples consisted of a group of 12 citizens that received a 15-min training session before the one-day field sampling campaign with scientists to document the spread of the hemlock woolly adelgid *Adelges tsugae* Annand, 1924 (Animalia, Hemiptera) in a forest in Massachusetts (United States) (Fitzpatrick et al., 2009). This type of approach, named bioblitz, is increasingly popular. These are defined as participatory actions that quickly and intensively survey a given area to provide a biodiversity snapshot (Robinson et al., 2013). During the "Marine Invasive Species Bioblitz" in Sitka (Alaska, United States), participants received training on the identification of several target non-indigenous species which resulted in the

detection of a 1000-km northward expansion of the invasive tunicate *Didemnum vexillum* Kott, 2002 (Animalia, Aplousobranchia) during the 2-days sampling (Cohen et al., 2011). The development of user-friendly tools and metrics enables the participation of a wider range of people. For example, the Metric of Aquatic Invertebrates for Volunteers (MAIV) enabled elementary and middle school students from Southern Portugal to evaluate the ecological status of streams (Pinto et al., 2020). In terrestrial ecosystems, the "Invaders of Texas" project in the United States aims at monitoring invasive plants across the state. Every year, hundreds of citizens receive training through frequent workshops and online training programs to identify several new locations where the giant reed *Arundo donax* L. (Plantae, Poales) is present in Texas. In many locations, the new discoveries were done without support from scientific literature or species lists. Then data were submitted to the "Invaders of Texas" database and later validated by professional scientists through photographic evidence (Gallo and Waitt, 2011).

There are numerous examples of first detections in aquatic ecosystems – e.g., two gelatinous non-indigenous species in the Western Mediterranean Sea (Boero et al., 2009) or freshwater crayfish across Greece and Italy (Faraone et al., 2017; Perdikaris et al., 2017). One of the more successful projects aimed at the early detection and monitoring of the invasive European green crab *Carcinus maenas* (Linnaeus, 1758) (Animalia, Decapoda) along the northern Pacific coast of the United States. Here, trained citizen scientists deployed baited traps and carry visual surveys along 3,000 km of shoreline. Citizen scientists documented the expansion of the European green crab across the state of Washington, which was later confirmed by scientists with rapid assessment surveys (Grason et al., 2018). Such kind of data collection over broad geographical areas may also help predict range expansions through species distribution modelling – e.g., two non-indigenous species present of insects in Sweden (Widenfalk et al., 2014) and several invasive plant species in the United States (Crall et al., 2015) and Portugal (César de Sá et al., 2019). Such citizen science data can also complement other databases and help to predict range expansions due to climate change at a global level, as with the case of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 (Animalia, Decapoda) (Costa et al. 2023).

Extremely relevant information may also arise from sporadic reports made by informed citizens, not necessarily associated with formal scientific projects. Such reports may contribute to specific citizen science projects, as the single record reports of two non-indigenous fish species present in the Mediterranean Sea – the sergeant major *Abudefduf saxatilis* (Linnaeus,

1758) (Animalia, Perciformes) record submitted to the project "Seawatchers" (Azzurro et al., 2013), while the white-spotted puffer *Arothron hispidus* (Linnaeus, 1758) (Animalia, Tetraodontiformes) record was uploaded to the Facebook group "Mediterranean Marine Life" (Bariche et al., 2018). Sporadic reports may also trigger the onset of a citizen science project. The first record of Asian bush mosquito *Aedes japonicus* (Theobald, 1901) (Animalia, Diptera) in Spain was submitted to the project "Mosquito Alert", triggering scientific surveys that confirmed the presence of the species and suggesting that the establishment had occurred a long time ago (Eritja et al., 2019).

Similarly, the first records of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) (Animalia, Perciformes) and the Atlantic blue crab (Animalia, Decapoda) in southern Portugal were reported by fishermen to scientists (Morais and Teodósio, 2016; Morais et al. 2019), revealing the necessity of more communication between scientists and local citizens. Therefore, the objectives of this study are to implement a citizen science monitoring campaign to anticipate the detection of new marine and estuarine faunal species in the Algarve, while increasing the public's awareness to non-indigenous and neonative species, particularly in the cases they pose health risks, and being an auxiliary tool to assess if some of these new species can become new fishing resources.

4.2 Material and methods

4.2.1 Creating the NEMA campaign

Following past experiences in collaborations with local citizens, which resulted in noteworthy detections of new marine invasive species in south Portugal, such as the weakfish (Morais and Teodósio 2016; Morais et al. 2017) or the Atlantic blue crab (Morais et al. 2019), the establishment of a citizen science campaign was a necessity. This would foster the collaboration between civil society and the scientific community, increasing the exchange of knowledge between the two.

The initial approach was to create an online campaign that relied mostly on eye-catching outreach materials across social media channels, covering the topics of biological invasions with special emphasis on non-indigenous species present in south Portugal. This had the goal of drawing attention of the public on these topics, especially citizens with most contact with the ocean, either professionally or recreationally, and therefore recruiting potential participants that could provide records of non-indigenous species present in south Portugal. The campaign

was meant to be kept as simple as possible, i.e., the participatory activities should not overload participants with too many tasks or demand a long-term commitment, allowing citizens with different experiences, motivations, and expectations to custom-tailor their participation and commitment with the campaign. For example, citizen scientists are generally uncomfortable in making advanced scientific decisions, regardless of their motivations and engagement, so, such kind of requests may limit the number of submitted records (Gallo and Waitt 2011).

The first step was the creation of the brand and identity of the campaign. The name and logo can define the success and reach of a campaign, being especially important in the modern-day era of social media networks (Icha and Agwu 2015; Lee 2019). The chosen name was "Novas Espécies Marinhas do Algarve" (NEMA or NEMAlgarve; New Marine Species of Algarve), reflecting the scope of the campaign, while not restricting to any specific group of non-indigenous species. Secondly, the image of the NEMA campaign would need to incorporate at least one charismatic non-indigenous species present of the Algarve. The logo was kindly designed in collaboration with Dr. Sarita Camacho, as part of her graduation internship in Communication and Design at the University of Algarve. The taxa chosen for the logo include emblematic species in the Algarve while encompassing different taxonomic groups, as the invasive Atlantic blue crab, the native bloom-forming jellyfish *Catostylus tagi* (Haeckel, 1869), or subtropical species like the ornate wrasse *Thalassoma pavo* (Linnaeus, 1758) (**Figure 4.1**). The design matches with the institutional image of the research centre (CCMAR – Centre of Marine Sciences; www.ccmar.ualg.pt), namely colour and font type, to increase credibility, facilitate outreach, and ultimately the number of submitted records.

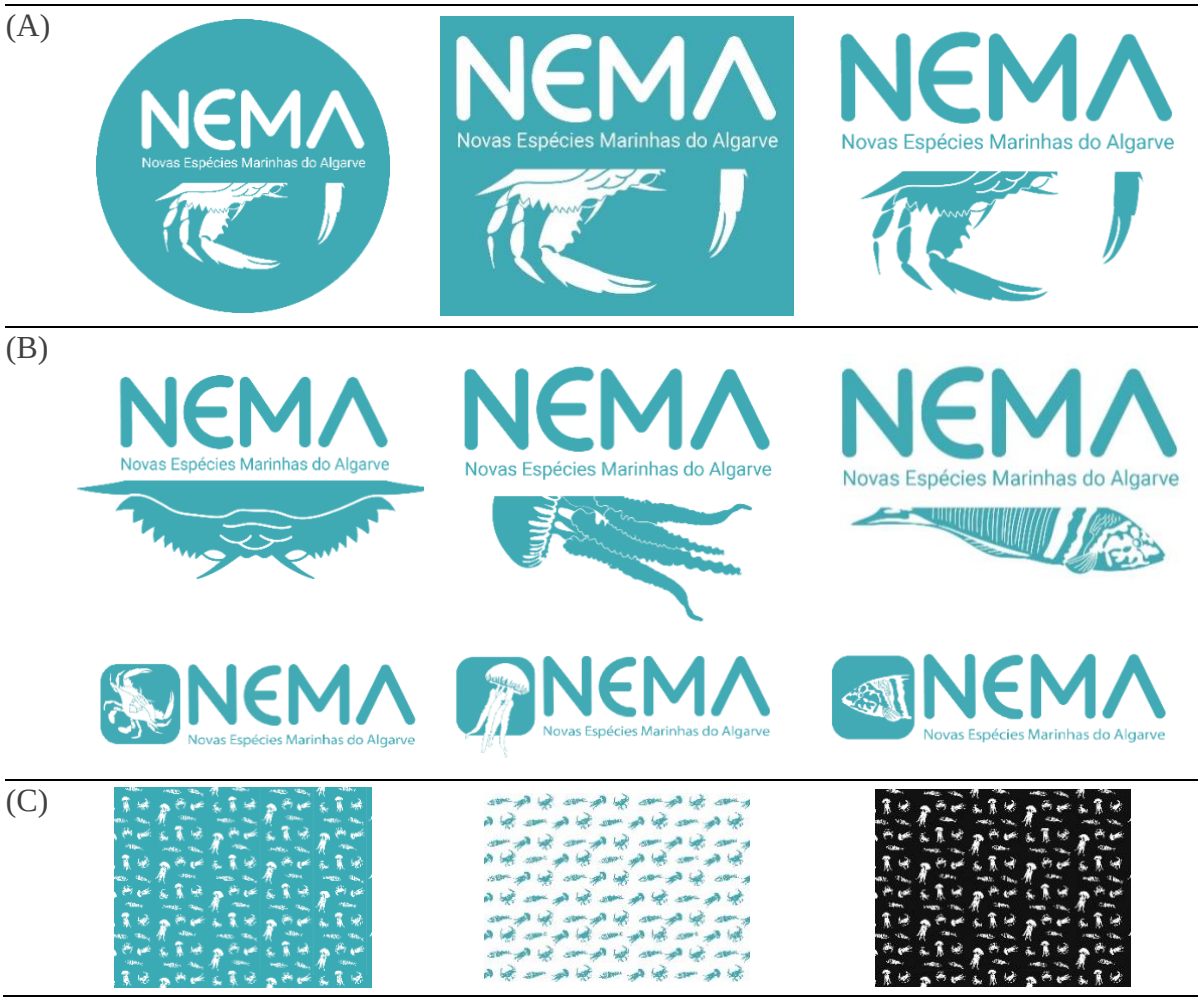


Figure 4.1 – Brand image of the "Novas Espécies Marinhas do Algarve" (NEMA) citizen science campaign. (A) Main logo's incorporating the invasive Atlantic blue crab *Callinectes sapidus* Rathbun, 1896. (B) Complementary logo's incorporating the Atlantic blue crab *Callinectes sapidus*, the jellyfish *Catostylus tagi* (Haeckel, 1869), and the ornate wrasse *Thalassoma pavo* (Linnaeus, 1758). (C) Background patterns including the three species.

4.2.2 Communication, promotion, and data collection

In April 2019, with the brand image defined, NEMA was officially launched. Accounts in the three main social media networks were created – Facebook (www.facebook.com/NEMAlgarve), Instagram (www.instagram.com/NEMAlgarve), and Twitter (www.twitter.com/NEMAlgarve) – to promote NEMA and reach a high number of citizens in the shortest period possible. Additional communication channels were created, as a dedicated email account (NEMAlgarve@gmail.com), a YouTube channel (www.youtube.com/@NEMAlgarve), and a project page on BioDiversity4All (www.biodiversity4all.org/projects/nemalgarve). The BioDiversity4All citizen science platform, the Portuguese version of iNaturalist, intended to provide a user-friendly platform where citizens could independently upload their non-indigenous species presence records.

Also, it was chosen to gather all the validated records received across all the other communication channels, so they would be publicly available for consultation. This was achieved by creating a NEMA account on BioDiversity4All and uploading such records and acknowledging the citizen that sent each record. As an intended low-cost campaign, during the first year of NEMA, only the above free web tools were used. Nevertheless, there was a necessity to centralize and gather all the information in one place, so a website was also developed and launched in May 2020: www.NEMAlgarve.com.

To stimulate the participation of citizens and showcase potential species of interest for the campaign, a selection of underwater photographs was made from personal archives, complemented by additional photographs from other sources when needed. These were used to complement social media and BioDiversity4All pages (**Figure 4.2**), but also used as posters in call-for-action activities and online publications.

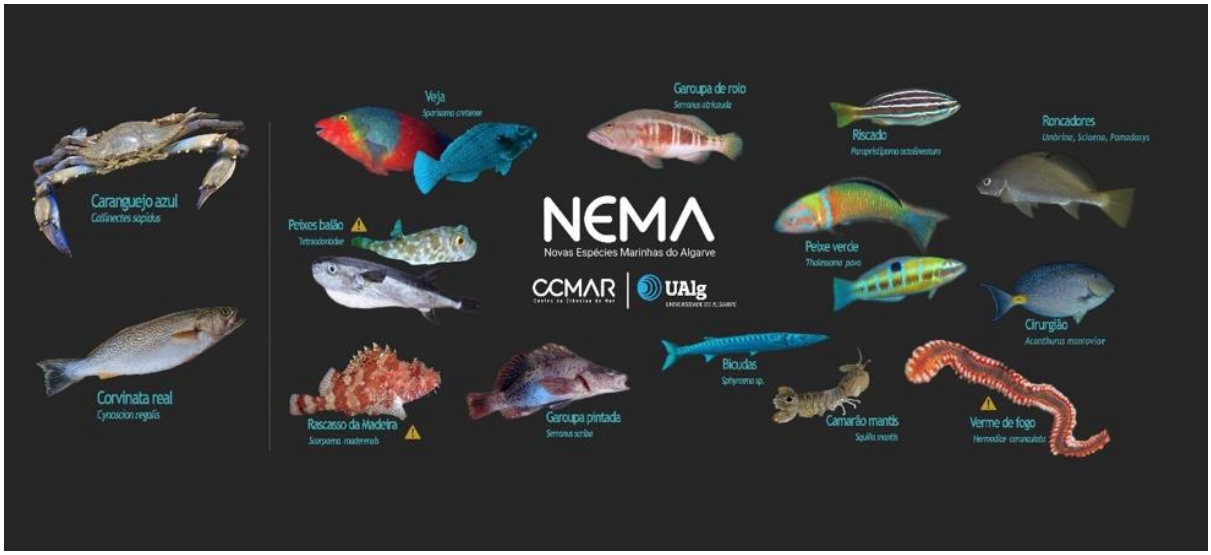


Figure 4.2 – Composition of images of species of interest in the "Novas Espécies Marinhas do Algarve" (NEMA) citizen science campaign used to complement social media and BioDiversity4All pages. All species photographs by João Encarnação, except for *Cynoscion regalis* (Bloch & Schneider, 1801) by Pedro Morais and *Squilla mantis* (Linnaeus, 1758) by João Carlos Oliveira.

A primary outreach approach in social media was the use of posters with species of interest (**Figure 4.3**), while explaining the objectives of NEMA, how citizens could participate, details needed to validate a record, and contact information. A Portuguese version of this poster was used in most instances. Species carrying poison or some sort of toxicity, as pufferfishes (Tetraodontidae), the bearded fireworm (*Hermodice carunculata* (Pallas, 1766)), or the

Madeira rockfish (*Scorpaena maderensis* Valenciennes, 1833), were flagged with a danger sign to let the public know that there could be some danger if those species were eaten or badly handled (Haddad Jr and Barreiros 2007; Guardone et al. 2018; Verdes et al. 2018).

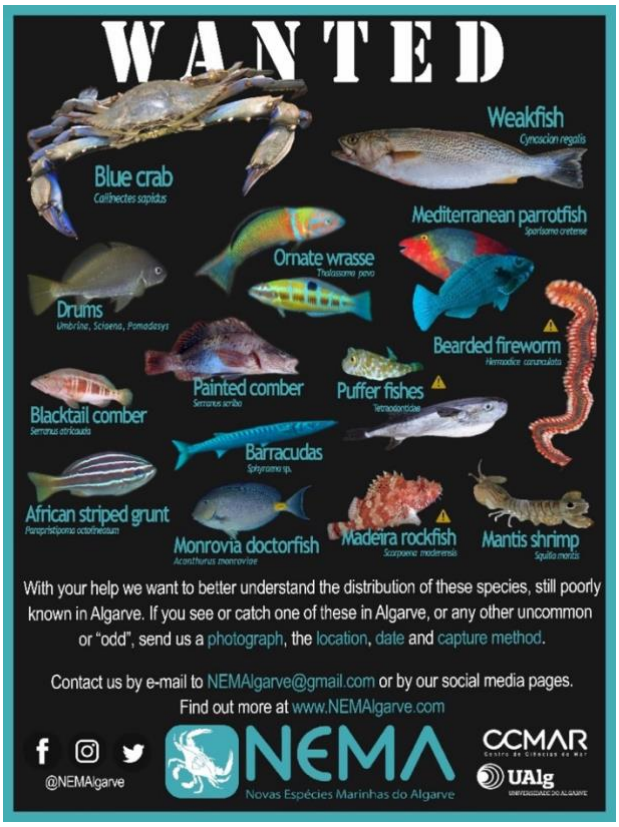


Figure 4.3 – Poster used in call-for-action activities and online publications, showcasing potential species of interest for the "Novas Espécies Marinhas do Algarve" (NEMA) citizen science campaign. All species photographs by João Encarnação, except for *Cynoscion regalis* (Bloch & Schneider, 1801) by Pedro Morais and *Squilla mantis* (Linnaeus, 1758) by João Carlos Oliveira. A Portuguese version of this poster was used in most instances.

Social media has proven to be very effective in reaching potential volunteers to participate in the NEMA campaign. Facebook offered the most useful tools to reach a higher number of citizens, namely the numerous groups related to fishing and general ocean activities, where the NEMA campaign could be actively disseminated. Once a month, from June to October 2019 and sporadically afterwards until August 2021, the "WANTED" poster (**Figure 4.3**) was used as an outreach tool to engage with the public on several Facebook groups. On average, we reached out to 26.9 ± 7.7 Facebook groups *per* month in this period.

When contacted about a potential record, citizens scientists were asked to provide information about the species of interest and for five details about their observation: 1) a

photograph of the specimen(s); 2) date of observation; 3) location; 4) method of capture or observation, and, whenever possible, 5) the inclusion of an object to serve as a scale in the photograph. Only records that included, at least, one photograph to allow the species identification, date of observation and a detailed location were considered valid and included in NEMA's database. The direct communication channels provided the opportunity to obtain all the details to validate records and permission to add it to the database.

Regular publications were made with the records submitted by citizen scientists to acknowledge their contribution, but also to further promote the participation of other citizens, while showcasing the details needed to validate a record (**Figure 4.4**). To increase outreach, publications were often shared by the research centre (CCMAR) on their social media accounts.

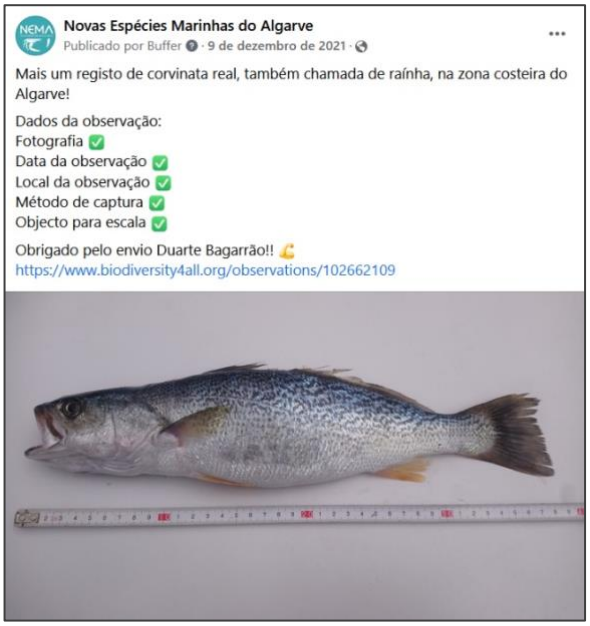


Figure 4.4 – Example of publication on NEMA's Facebook page (also replicated in Instagram and Twitter) using a record of a weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) submitted by D. Bagarrão.

After nearly four years (April 2019-December 2022), NEMA's database had 619 validated submissions, from 2808 specimens belonging to 41 taxa, gathered by 241 citizen scientists (**Table S2**, Electronic Supplementary Material). In social media, NEMA reached more than 5600 followers by the end of December 2022 (Facebook: 5023; Instagram: 575; Twitter: 71).

Facebook was the social media platform most used by citizen scientists to contact NEMA – 38.8% of the total of 534 validated records received until December 2022 (**Figure 4.5**).

NEMA's Facebook account received 29.4% of these records (*n*= 157), either through private message or as comments in the page's publications, and the other 9.4% (*n*= 50) were gathered in Facebook groups, or as a direct response to our explanatory publications in these groups. Only one record was reported through Instagram, while NEMA's Twitter account received no submissions. Records uploaded on BioDiversity4All accounted for 21.2% of the records (*n*= 113), despite that most first contacts were also made through social media. NEMA's email account received 4.9% of the records (*n*= 26) and the website 1.9% (*n*= 10). The remaining records (33.1%, *n*= 177) were personal communications sent by private message or personal communications (**Figure 4.5**). These mostly included records sent by Mr. Ricardo Gonçalves (20.9%, *n*= 101), a professional fisherman from the Guadiana estuary. After the records of weakfish and Atlantic blue crab published in Morais and Teodósio (2016) and Morais et al. (2019), the NEMA database incorporated all these records of non-indigenous species from fishers already in collaboration with the research group before the launch of NEMA. These were the so-called informed citizens, who were already interested in the topic of non-indigenous species and informed on the importance of reporting such information. Additionally, sporadic personal observations (*n*= 85) gathered by the author outside sampling campaigns, were also included in this database, identified as observer ECOREACH (**Table S2**, Electronic Supplementary Material).

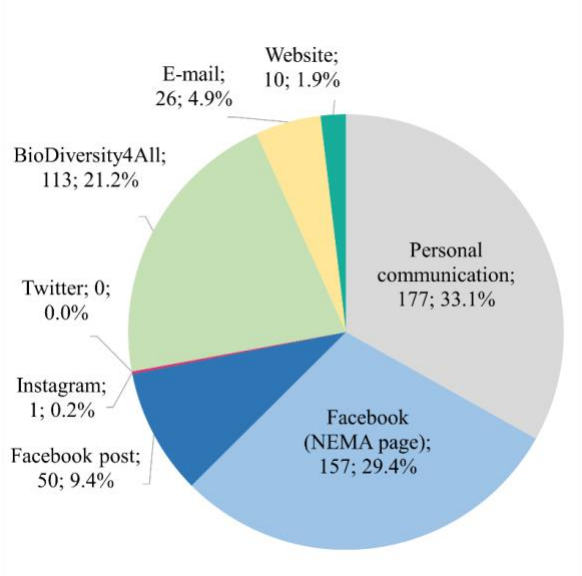


Figure 4.5 – Contribution of each communication platform used by citizens to submit records to the NEMA citizen science campaign until December 2022.

Facebook is the most popular social media account of NEMA, so several metrics were retrieved to assess the impact of social media outreach on the submission of records. Until early 2020, Facebook made available several metrics, such as daily followers, daily total impressions, daily total consumers, or page reach, among many others. Unfortunately,

Facebook changed how pages work and are displayed during 2020, and drastically reduced the number available metrics. For a reason beyond our understanding, there was also a gap in data between April and mid-August 2020 (**Figure 4.6**). For these reasons, this assessment only includes the number of followers and the page reach, i.e., number of people that saw any content of the page or about the page, which was the metric with more data thorough the study period. By December 2022, NEMA's Facebook page had close to 5023 followers, registering three significant maximums of page reach – 54631 people on August 04th, 2021; 127155 people on May 16th, 2022; and 108198 people on May 17th, 2022 (**Figure 4.6**). Some level of correspondence between the page reach, Facebook group publications, and media articles or interviews, could be identified for several times, namely during the first year of NEMA with the monthly publications in Facebook groups, or in early August 2021 with the several media interactions (**Figure 4.6**).

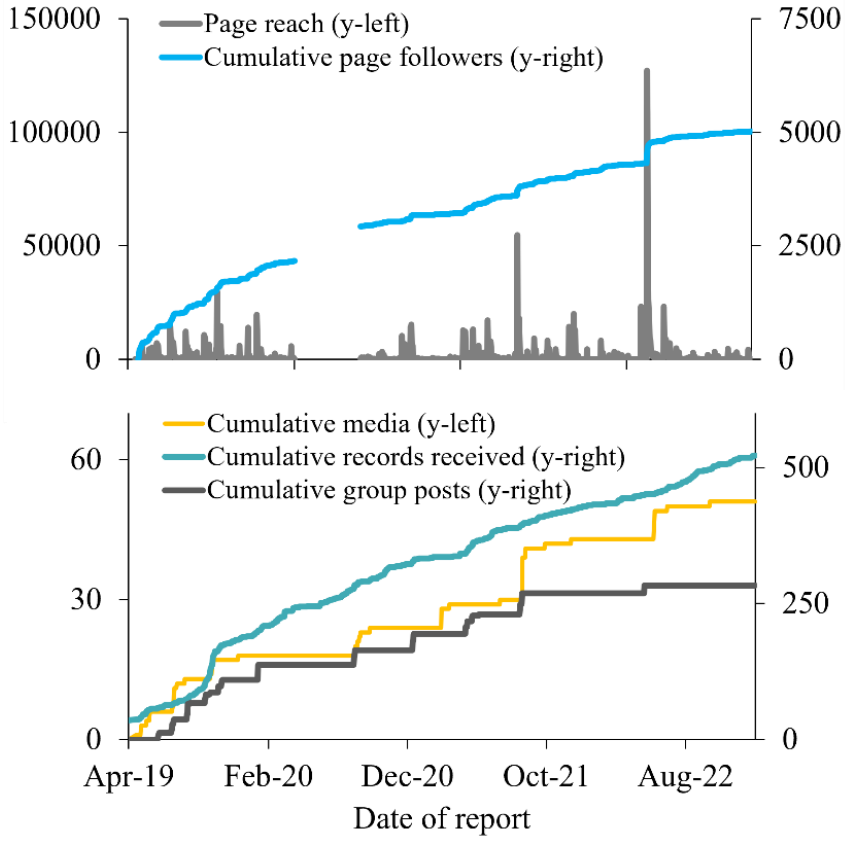


Figure 4.6 – Reach of NEMA's Facebook page (number of people that saw any content of the page or about the page) and the cumulative number of followers of NEMA's Facebook page (top figure); Cumulative number of media interactions regarding the NEMA campaign and invasive species (online and newspaper articles, radio, and television interviews), cumulative number of valid records received, and cumulative Facebook group monthly posts (bottom figure). Data between April 2019 and December 2022.

The success and visibility of the campaign on social media, led to a growing interest from traditional media on NEMA and citizen science, invasive and neonative species (e.g., Atlantic blue crab, bearded fireworm), research in the Algarve, etc. (**Figure 4.7**).

Figure 4.7 – Word cloud based on keywords used by traditional media in titles of news and published articles.

Until December 2022, this translated into five interviews for national television (**Figure 4.8**), three live interviews for national radio stations, and 46 online news articles, of which three were also published in printed version (**Table S3**, Electronic Supplementary Material).

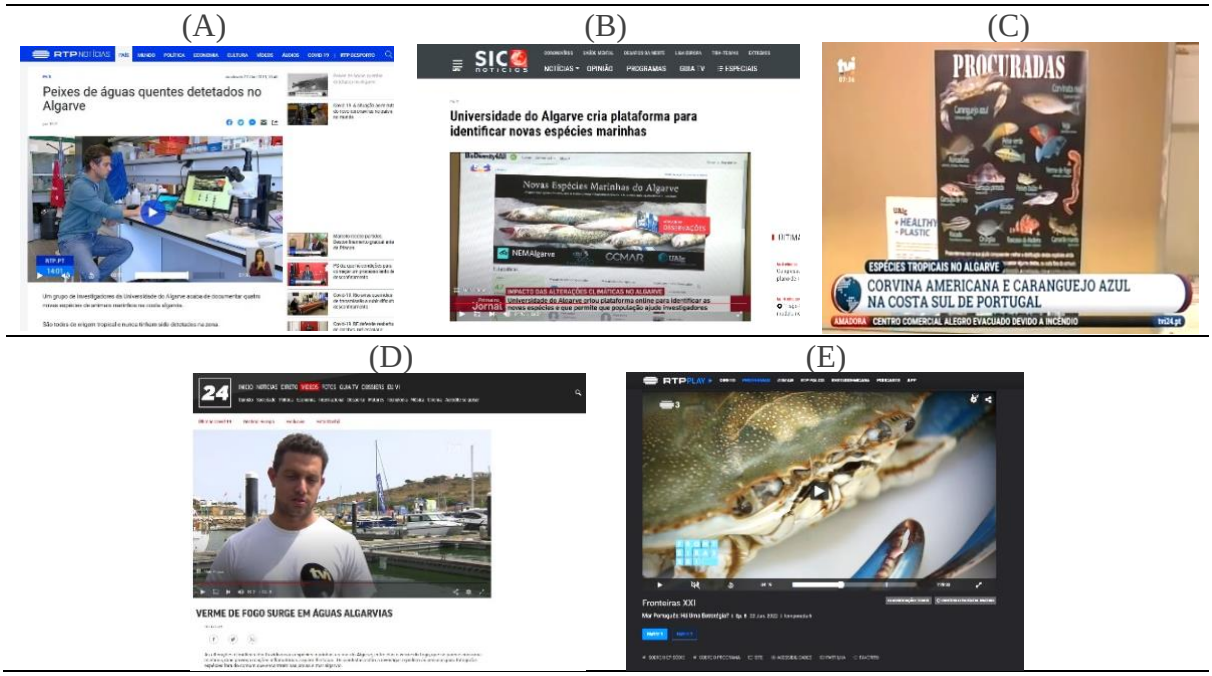


Figure 4.8 – Interviews given for national television channels about the NEMA campaign and invasive or subtropical species in south Portugal: (A) RTP1 on April 27th, 2019; (B) SIC on May 16th, 2019; (C) TVI on August 17th, 2020; (D) TVI on August 16th, 2021; I RTP3 on June 22nd, 2022. For a full list of interactions with media channels, see Table S3 of the Electronic Supplementary Material.

4.2.3 Cost-effectiveness of NEMA

A cost-benefit analysis of the NEMA campaign was performed, based on the costs of producing and running all the outreach platforms, and on retrieving the same records submitted by citizen scientists and informed citizens. Based on their background and the amount of data provided, the citizens recruited during the NEMA campaign will be referred to as "citizen scientists", while citizens already in collaboration with the research group before the launch of NEMA will be referred to as "informed citizens", and results will subsequently be presented in separate when adequate. For this cost-benefit exercise, data from the first year of NEMA (April 2019 – March 2020) on the Atlantic blue crab was used, but also the records provided by the informed citizens since the last records published in Morais et al. (2019), hereafter referred to as "pre-NEMA". To estimate the hypothetical costs, we would have in running NEMA, the amount of time invested in each task was indexed to the 2020 daily stipend of a Ph.D. fellowship financed by the Foundation for Science and Technology (FCT, Portugal) – i.e., 51.20 € per workday, and compared it with service quotes from three companies. For this cost-benefit analysis we only considered statistics from our outreach actions in NEMA's Facebook account. Therefore, the following costs of NEMA are an estimation of the hypothetical costs calculated upon the time invested in each task, based only on the above daily stipend of a Ph.D. fellowship.

This analysis was based on three components. The first component consisted in giving a cost to creating NEMA's communication channels, i.e., the campaign's accounts on social media (Facebook, Instagram, and Twitter), BioDiversity4All, and email. The website of NEMA (www.NEMAlgarve.com) was launched in May 2020, so it did not influence the outreach and outcomes of the first year of NEMA and was not considered in this cost-benefit analysis. We spent seven workdays to create the initial platforms and their content, and then compared its cost, indexed to the Ph.D. fellowship, with the cost of outsourcing the production of a website, mobile app, and create the social media accounts to obtain the same information provided by citizen scientists and informed citizens.

The second component consisted in giving a cost to the time spent in handling NEMA's social media accounts, i.e., to create, publish, and follow-up each publication. We spent, on average, 1.5 h with each publication: 30 min for designing the publication, 20 min for publishing it, and 40 min for following up the publication, retrieving relevant information, or communicating with people that actively engaged with it. Then, we compared the cost of the

total number of publications, indexed to the Ph.D. fellowship, with the cost of hiring a professional social media manager.

The third component consisted in calculating the expenses we would have in going to the field and collect the same information provided by citizen scientists or informed citizens. In a real situation, we would have needed to go to the field multiple times to increase the chances of finding an Atlantic blue crab, but due to the unforeseen nature of fieldwork, we can only calculate the minimum cost to retrieve the same record (one specimen or group of specimens) as the one made by a citizen. The cost was calculated as the money spent by one scientist to travel to the same location from the university campus, considering a car that spends 6 € 100 km^{-1} of gas (considering a diesel car with an average consumption of 4 l 100 km^{-1} and a diesel price of 1.5 € l^{-1}), toll costs for a class 1 car (ViaLivre 2021), plus the daily stipend of the Ph.D. fellowship. Distances were estimated with Google Maps, between the university campus in Faro (37.0428, -7.9735) and the closest road to the record site (GPS positions available in **Table S2**, Electronic Supplementary Material). For records in the vicinities of Faro (between Albufeira and Tavira), no toll costs were included (n= 22). No costs related to boat renting and fuel, nor equipment depreciation were included in this analysis. The cost per trip was then divided by the number of specimens in each record to obtain the cost per individual. Data is described by its range (minimum-maximum), the mean, and standard deviation was used as a measure of data dispersion. Lastly, this value was compared with the cost associated to the time spent in retrieving the total number of records through NEMA's communication channels, indexed to the Ph.D. fellowship. We invested 10 min per observation, on average, to retrieve all the necessary parameters.

4.2.4 Tropicalization of the Iberian Peninsula

In order to assess the usefulness of the records of neonative species gathered by NEMA, under a climate change and tropicalization scenario, a comparison with data from the GBIF (2023) database until December 2022 was performed. Records of the 31 neonative species recorded by NEMA were retrieved from GBIF with the following criteria: had the date of record, comprised until December 2022; had GPS coordinates; and were located inside a polygon that comprised the African coast since Gabon, including the Macaronesian archipelagos and the Mediterranean Sea, until the German shores in the North Sea, formed by the coordinates 45.84934/-2.8644, 45.84934/60.33601, -33.14784/60.33601, -33.14784/-

2.8644. Although some species may have a distribution outside this area, this criterion intended to focus the assessment on the north-east Atlantic Ocean, including continental Portugal, the Macaronesian archipelagos, the Mediterranean Sea, and the western African coast. Although more than one species of *Kyphosus* and *Sphyraena* were recorded by NEMA, data retrieved from GBIF considered the genera, therefore all species from these two genus withing the above criteria. Following, this data was checked for repeated records and doubtful records, and if needed those were removed.

To assess how scientific institutions can keep track of these neonative species in the Algarve region (south Portugal) in the last decades, a search for records published in scientific literature was carried out for each of the 27 neonative species identified by NEMA. In comparison with the total number of taxa that comprised the species list that NEMA identified (*n*= 31), for this assessment, *Kyphosus* was only considered at genus level, and only *Sphyraena* that were undoubtedly identified to species level were considered (two species), while *Centrostephanus longispinus* was not reported for the Algarve region.

4.3 Results

4.3.1 Invasive species records

Until December 2022, the NEMA campaign gathered records of three invasive species – the Atlantic blue crab *Callinectes sapidus*, the weakfish *Cynoscion regalis*, and the macroalga *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, 1809 (**Figure 4.9**; **Table S2**, Electronic Supplementary Material). The Atlantic blue crab was by far the most reported species in the entire campaign with 249 records (40.2%), on a total of 1986 individuals, submitted by 118 citizen scientists and informed citizens. Most records of this species were on the Algarve region in the south of Portugal (97.2%), but NEMA also received three records of one individual each in the vicinities of the Mértola town (Guadiana upper estuary, Alentejo region), three other records of one individual each the Mira estuary (south-western Portugal), and one record of one individual from the Sesimbra peninsula (western Portugal) (**Figure 4.9**).

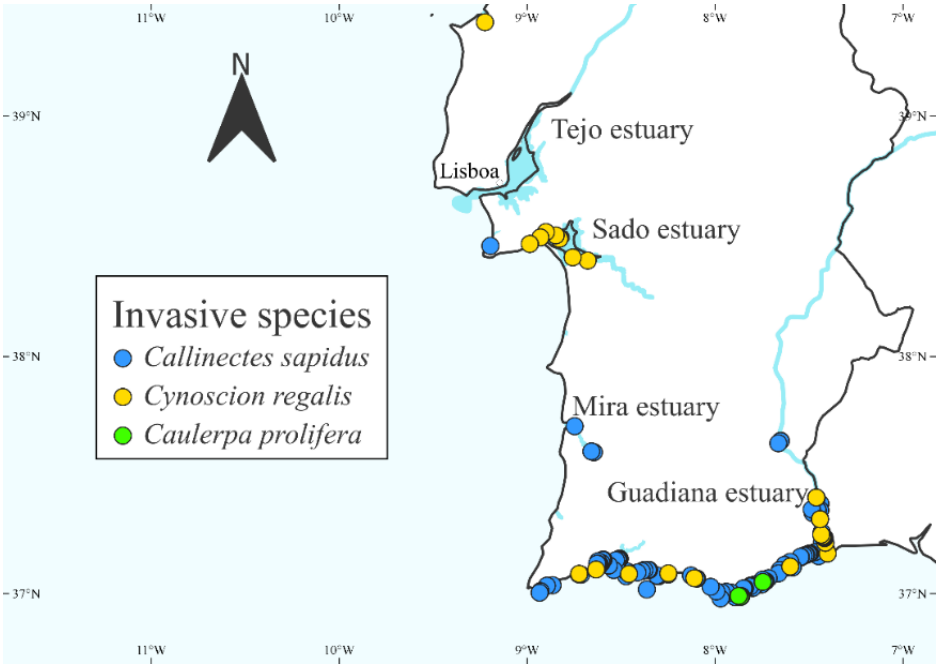


Figure 4.9 – Distribution of the three invasive species records submitted to the NEMA citizen science campaign until December 2022. For a detailed list, see Table S2 of the Electronic Supplementary Material.

Most Atlantic blue crab records were collected with a fishing gear (56.4%; 141 records; 1850 individuals), mostly with fishing nets (34.8%; 87 records; 1648 individuals), but also by hand (16.8%, 42 records; 48 individuals) or found dead (20.8%, 52 records; 55 individuals or remains) (**Figure 4.10**). The Guadiana estuary and the contributions made by Mr. Ricardo Gonçalves represent a particular sub-set of records. This informed citizen alone reported 1769 Atlantic blue crab specimens, all captured along a 12 km stretch of the middle Guadiana estuary, close to the village of Foz de Odeleite. These represent 37.3% of the Atlantic blue crab records in the NEMA database. Most of these specimens were males (61.2%, n= 1083) and females only accounted for 6.4% (n= 114), while the remaining specimens were not sexed (32.3%, n= 572). Two months stood out – September 2019 (125 males, 6 females, 508 unsexed specimens) and March 2020 (456 males, 0 females). In 2019, the maximum daily catch was 105 specimens (September 17), and it reached 110 and 130 specimens in 2020 during two consecutive days, March 4, and March 5, respectively. No similar amount of daily catches were ever reported anywhere in Portugal. On the total records from the Guadiana estuary, most were done in the middle and lower sections of the estuary, but three reports in Mértola, at 70 km from the river mouth in the Alentejo region, were also submitted to NEMA (**Figure 4.9**).

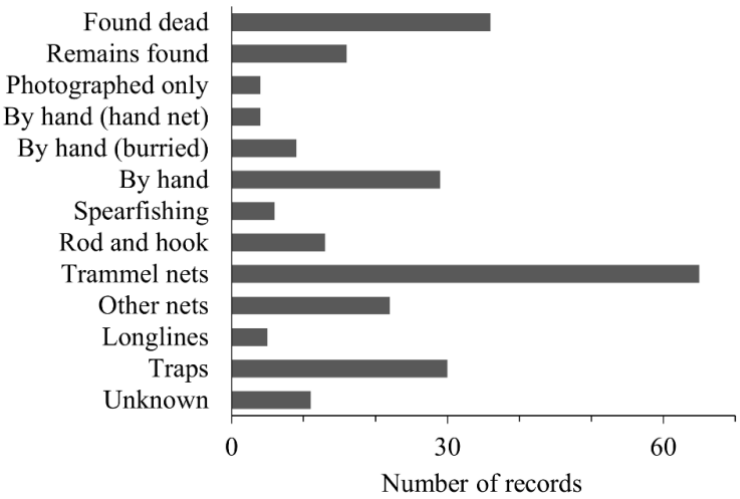


Figure 4.10 – Observation method for reports of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896, submitted to the NEMA citizen science campaign until December 2022.

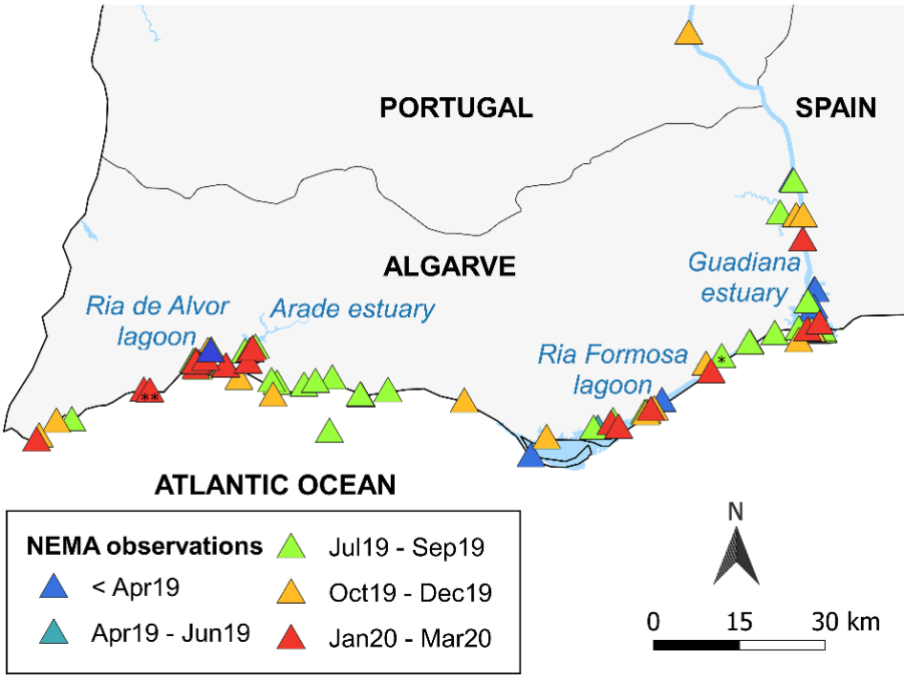
The first year of the NEMA campaign – April 2019 to March 2020 – was particularly fruitful in records of the Atlantic blue crab along southern Portugal. Citizen scientists alone contributed with 77 valid observations about 109 Atlantic blue crabs – 44.0% males (n= 48), 43.1% females (n= 47), 12.8% unsexed (n= 14). Observations made in estuarine ecosystems (Ria de Alvor, Arade estuary, Ria Formosa, and Guadiana estuary) accounted for 50.6% (n= 39) of the observations – 61.7% males (n= 29), 34.0% females (n= 16), 4.3% unsexed (n= 2). Observations made in coastal areas represented 49.4% (n= 38) of all records – 30.6% males (n= 19), 50.0% females (n= 31), 19.4% unsexed (n= 12). There were differences in the proportion of sexes between coastal and estuarine areas during the non-reproductive (p= 0.044) and reproductive periods (p= 0.065) (**Table 4.1**). In both cases, females were more frequent in coastal areas (31 specimens) than in estuarine ecosystems (16 specimens).

Table 4.1 – Chi-square test results, applied to 2×2 contingency tables, to assess differences in proportions of sexes between reproductive periods and ecosystems where Atlantic blue crabs *Callinectes sapidus* Rathbun, 1896 were observed by citizen scientists during the first year of NEMA.

	Value	**df**	***p* value**
Coastal ecosystems (Non-reproductive vs. Reproductive)	1.247	1	0.264
Estuarine ecosystems (Non-reproductive vs. Reproductive)	0.916	1	0.339
Non-reproductive period (Coastal vs. Estuarine)	4.071	1	0.044
Reproductive period (Coastal vs. Estuarine)	3.399	1	0.065

In the first 3 months of NEMA (April–June 2019), only one Atlantic blue crab specimen was reported. Nonetheless, five specimens captured during July 2019 extended the known distribution westwards by over 50 km, from Faro to the Arade estuary in Portimão (**Figure 4.11**). Two specimens captured in Ria de Alvor (one male, one female) further extended the distribution westwards by 8 km in August 2019. On September 19, 2019, one male specimen captured near the beach of Zavial further extended the western distribution limit by 23 km (**Figure 4.11A**). Between August and November 2019, 16 observations (20 specimens: 7 males, 7 females, and 6 unsexed) confirmed the establishment of the Atlantic blue crab in the area between Albufeira and Alvor (green and orange symbols in **Figure 4.11A**). In the same period, between Faro and Vila Real de Santo António, 19 observations were made (21 specimens: 3 males, 13 females 4 unsexed) of which nine females have washed ashore in the beaches close to the mouth of the Guadiana estuary (**Figure 4.11A**). In August and September 2019, ovigerous females were reported (**Figure 4.11B**), one found dead in a beach close to the mouth of the Guadiana estuary (observation #34), two inside the Ria Formosa lagoon (observations #26 and #41), one in the Arade estuary (observation #17), and another one in the coastal zone of Portimão (observation #31). It is worth mentioning that two non-ovigerous females were captured at night while swimming at the surface on August 27, 2019, and October 2, 2019 (observations #22 and #42). In December 2019, a single observation (observation #66) reported one male and six females in the lower Guadiana estuary near Vila Real de Santo António, and one additional female was captured in the harbour of Sagres (observation #63). This last record extended the western distribution limit by another 4.5 km (**Figure 4.11A**). In January 2020, one fisherman made three observations on subtidal areas off Alvor and Lagos and mentioned that the Atlantic blue crab was a "frequent" bycatch. Two of these observations narrowed the gap of records made between Alvor and Sagres (**Figure 4.11A**). One of such observations reported 8 males and 3 females, all captured at night with a fishing net set near the Porto de Mós beach (Lagos, January 17, 2020) (**Figure 4.11C**). During the first 3 months of 2020, 10 observations confirmed the presence of the species in vicinities of Ria de Alvor and the Arade estuary (red symbols in **Figure 4.11A**). The entire south coast of the Algarve was formally colonized by the Atlantic blue crab when a female specimen was recorded in the Mareta beach (Sagres) on March 3, 2020 (observation #86, westernmost red symbol in **Figure 4.11A**).

(A)



(B)*

(C)**

Figure 4.11 – (A) Observations documenting the expansion of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 from East to Western Algarve until the end of March 2020. Information submitted by informed citizens and citizen scientists to the NEMA citizen science campaign. Each icon represents an observation that may include more than one specimen. (B) An ovigerous Atlantic blue crab specimen collected in the Ria Formosa lagoon on September 20, 2019 (observation #41 submitted by D. Barragão). (C) Atlantic blue crabs collected off Lagos on January 17, 2019 (observation #70 submitted by V. Gomes). For a detailed list, see Table S2, Electronic Supplementary Material.

Between May 2020 and December 2022, an average of 3.3 ± 2.7 records per month, and a total of 232 Atlantic blue crabs were submitted to NEMA. Some months stood out regarding the number of individuals registered – September 2020 (5 records; 52 individuals); March 2021 (7 records; 69 individuals); June 2021 (4 records; 24 individuals); July 2022 (10 records; 21 individuals); August 2022 (11 records; 12 individuals).

Regarding the weakfish, the NEMA campaign gathered 34 records (59 individuals) of this invasive fish species (**Figure 4.9**), 24 of them in the Algarve region (39 individuals), 9 in the Sado estuary and nearby zones (19 individuals), and 1 individual in the Óbitos lagoon, north of Lisbon (**Table S2**, Electronic Supplementary Material). Although most records were in the Guadiana estuary (18 records; 33 individuals), captures were also reported in the coastal zones of Tavira, Loulé, Albufeira, Lagoa, Portimão, and Lagos. Several records of male and female weakfish with developed gonads were reported in the Guadiana estuary during spring and early summer months (April, May, June 2021; July 2022), showing that the species may be using this estuarine system to spawn (**Figure 4.12**).

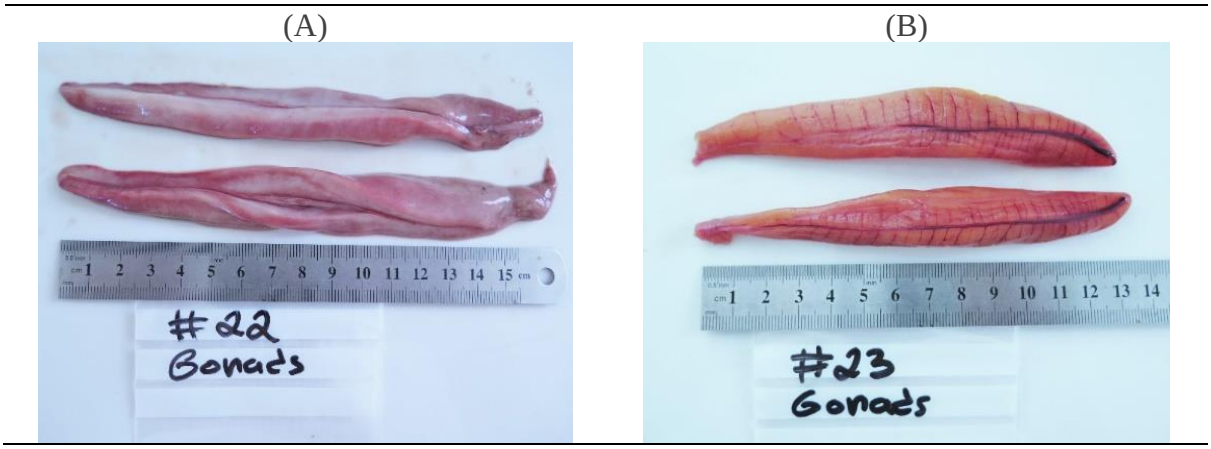


Figure 4.12 – (A) Male and (B) female gonads of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) caught in the Guadiana estuary in May 2021, reported by fisher R. Gonçalves to the NEMA campaign.

In central Portugal, besides the validated records collected by NEMA in the Sado estuary, additional information in social media also showed the state of this invasion. Numerous reports of the weakfish as being a very frequent catch, both in the Sado estuary, but also in the Tejo estuary, were witnessed in fishing groups across Facebook. The species was commonly labelled as a very aggressive predator, a "pest", and even with reports of it preying upon whole specimens of the native seabass *Dicentrarchus labrax* (Linnaeus, 1758) (**Figure 4.13**).

(A) (B)

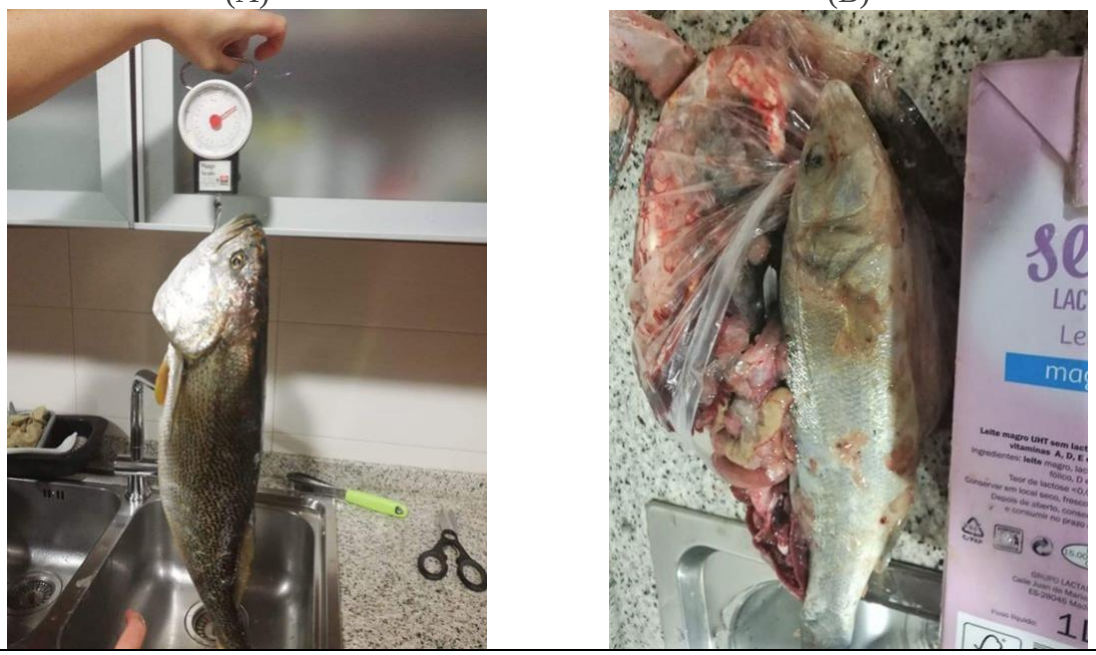


Figure 4.13 – (A) A weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) with a weight of 3 kg caught in the Sado estuary in 2019, and (B) its respective gut content, including an adult seabass *Dicentrarchus labrax* (Linnaeus, 1758), reported by angler N. Moreira to the NEMA campaign.

4.3.2 Neonative species records

A total of 31 taxa that could be classified as neonative, or with affinities to more subtropical zones, were reported to the NEMA campaign until December 2022 (**Table 4.2**). With 55 reports, the blacktail comber *Serranus atricauda* Günther, 1874 was the most reported species, followed by the damselfish *Chromis chromis* (Linnaeus, 1758) (28 reports), the African striped grunt *Parapristipoma octolineatum* (Valenciennes, 1833) (23 reports), the Canary drum *Umbrina canariensis* Valenciennes, 1843 (22 reports), the painted comber *Serranus scriba* (Linnaeus, 1758) (21 reports), and the bearded fireworm *Hermodice carunculata* (19 reports) (**Table S2**, Electronic Supplementary Material). Regarding the bearded fireworm, one additional personal observation was made in September 2018 at 16m depth in the Portimão area which, at the start of this thesis, was the first record in continental Portugal. Later on, a record submitted in 2020 by Tiago Gomes to the NEMA campaign, came to show its presence in Albufeira already in 2015.

Table 4.2 – List of neonative species reported to the NEMA campaign until December 2022 for the Algarve region (south Portugal) and west coast of Portugal. Results are shown as the number of records submitted, and the number of specimens between brackets. For a detailed list, see Table S2 of the Electronic Supplementary Material.

Zone	Species	2005-2016	2017	2018	2019	2020	2021	2022	Total
Algarve region (south Portugal)	*Chromis chromis*	10(22)	2(46)	3(66)	2(69)	6(20)	1(6)		24(229)
	Dentex canariensis	5(5)							5(5)
	Fistularia petimba					1(1)			1(1)
	Hermodice carunculata	1(1)		1(1)	3(3)	3(22)	8(8)	3(3)	19(38)
	Kyphosus CF *bigibbus*						1(1)		1(1)
	Kyphosus CF *vaigiensis*						1(1)		1(1)
	Kyphosus sp.						1(1)	2(2)	3(3)
	Parapristipoma octolineatum	6(7)	6(73)		2(2)	4(7)	1(1)	3(3)	22(93)
	Plectorhinchus mediterraneus	4(4)	3(31)		2(3)	3(3)	2(2)		14(43)
	Pomadasys incisus	1(1)			2(3)	8(12)	2(2)	2(2)	15(20)
	Pseudocaranx dentex			1(1)					1(1)
	Sciaena umbra					1(1)	1(1)	1(1)	3(3)
	Scorpaena maderensis			2(2)	2(2)	1(1)	2(2)		7(7)
	Scyllarides latus						1(1)		1(1)
	Seriola rivoliana	3(7)	1(1)						4(8)
	Serranus atricauda	15(16)	10(11)	4(4)	2(2)	2(2)	4(4)	2(2)	39(41)
	Serranus scriba				2(2)	7(8)	5(5)	7(7)	21(22)
	Sparisoma cretense	1(2)	1(2)				1(2)	1(1)	4(7)
	Sphoeroides marmoratus	3(3)						2(2)	5(5)
	Sphoeroides pachygaster					3(3)		1(1)	4(4)
	Sphyraena sphyraena					1(1)			1(1)
	Sphyraena viridensis	1(1)					1(4)	1(1)	3(6)
	Sphyraena sp.					1(12)			1(12)
	Squilla mantis		2(2)		4(5)		2(2)		8(9)
	Stromateus fiatola				1(1)				1(1)
	Symphodus mediterraneus	1(1)				2(2)	2(3)		5(6)
	Symphodus rostratus					3(3)	1(1)		4(4)
	Thalassoma pavo	1(1)			4(6)	2(2)	6(7)		13(16)
	Trachinotus ovatus	3(7)	1(1)				1(1)		5(9)
	Umbrina canariensis	5(12)	1(1)	1(1)	4(7)	8(37)	3(12)		22(70)
West coast of Portugal	*Centrostephanus longispinus*						2(2)	1(1)	3(3)
	Chromis chromis				1(1)	1(2)		2(2)	4(5)
	Kyphosus CF *bigibbus*						1(1)		1(1)
	Kyphosus sp.						4(4)	2(3)	6(7)
	Parapristipoma octolineatum							1(1)	1(1)
	Scorpaena maderensis	2(2)				1(1)		2(2)	5(5)
	Serranus atricauda					1(1)	5(5)	10(10)	16(16)
	Sphoeroides marmoratus							1(1)	1(1)
	Sphoeroides pachygaster							1(1)	1(1)
	Sphyraena viridensis							1(1)	1(1)
	Squilla mantis					2(2)	1(1)		3(3)
	Symphodus mediterraneus	1(1)					1(1)	2(2)	4(4)

Some of these species may pose public health risks, due to venomous, poisonous, or toxic characteristics, namely *Hermodice carunculata* (**Figure 4.14**), *Scorpaena maderensis*, *Sphoeroides marmoratus* (Lowe, 1838), *Sphoeroides pachygaster* (Müller & Troschel, 1848). In these cases, outreach campaigns are of upmost importance, while citizen science also contributes to increase awareness.

Figure 4.14 – A specimen of the bearded fireworm *Hermodice carunculata* (Pallas, 1766) in the Ria Formosa lagoon in July 2019, reported by J. Augusto to the NEMA campaign.

4.3.3 A low-cost citizen science campaign

Based on the number of hours we spent in launching and handling NEMA, the corresponding cost during its first year would have summed up to 3,751.47 € (**Figure 4.15**). Hiring the services of professionals to develop and handle all the digital platforms plus gathering the same number of records of the Atlantic blue crab in the field during this one-year period, would have cost between 29,815.58 € (lowest quotations) and 153,485.58 € (highest quotations) (**Figure 4.15**).

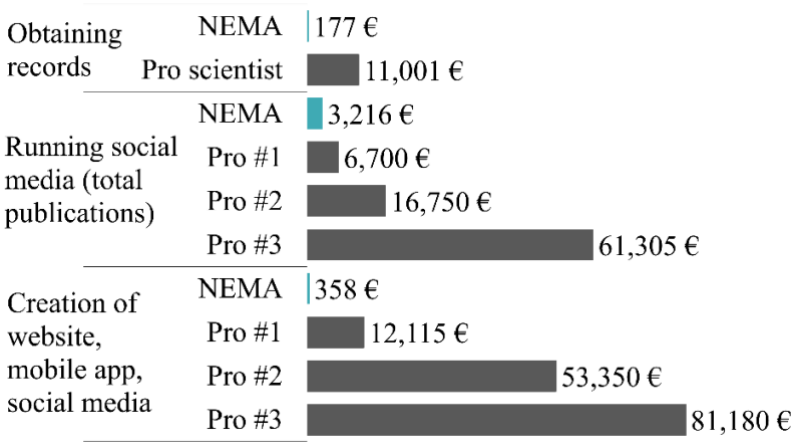


Figure 4.15 – Cost comparison on the first year of activity of the NEMA citizen science campaign, comparing the cost of obtaining the records through the campaign, versus a professional scientist going to the field to retrieve the same 166 records; the corresponding cost to the time spent running NEMA's social media ourselves, versus the three quotations provided for a professional social media manager to handle the same 335 publications on NEMA's Facebook account during its first year; and the costs for creation of the various communications channels used by NEMA, versus three quotations from professionals.

The service quotes from three software developers to build a website and a smartphone app with basic features (i.e., submission of a photograph, location, date, and contact of the citizen scientist) were quite distinct – 12,115 €; 55,350 €; and 81,180 €. Although they are free to create and use, the cost associated with the time spent in creating NEMA's social media accounts, e-mail, and project page on Biodiversity4All would be only 358.40 € (**Figure 4.15**).

Between April 2019 and the end of March 2020, we made a total of 335 publications on Facebook – 198 publications on NEMA's Facebook account and 137 explanatory publications on Facebook groups. Considering a value of 9.60 € per publication (hourly stipend times 1.5 h per publication), the cost of media handling associated with these publications would correspond to a total of 3,216 € for this first year of NEMA (**Figure 4.15**). For the same 355 publications, service quotes provided by professional social media managers were at 20 €, 50 €, and 183 € per publication, which would result in a total of 6,700 €, 16,750 €, and 61,305 € respectively (**Figure 4.15**).

The total cost for a NEMA scientist to go to the field and make the same 166 records (1747 Atlantic blue crabs) would have reached 11,000.58 €. The records made before NEMA would sum up to a minimum of 2,015.61 €, while during the first year of NEMA, the total minimum cost would have been sum 8,984.96 €, namely 4,965.06 € for records made by citizen scientists and 4,019.90 € for records made by informed citizens. This represents an average

minimum savings of 748.75 ± 505.77 € month^{-1} during NEMA's first year. The maximum cost per individual was 75.73 € for the westernmost record (record #86, Mareta beach, Sagres) and averaged 36.59 ± 28.05 € individual^{-1} (**Figure 4.16**). The cost per individual was on average higher for observations provided by citizen scientists (6.59–75.73 € individual^{-1}, 58.99 ± 16.20 € individual^{-1}) than informed citizens (0.52–68.06 € individual^{-1}, 13.64 ± 16.73 € individual^{-1}) because records made by informed citizens were mostly made in the Guadiana estuary and many individuals were reported in most communications (**Figure 4.16**). The minimum average cost per trip for a NEMA scientist to obtain the same record (one individual or several) as those made by citizen scientists was 64.35 ± 6.42 € trip^{-1}. This value was similar to the cost to obtain the same record as of informed citizens (68.23 ± 0.56 € trip^{-1}) since all these observations were done in the middle and lower Guadiana estuary (**Figure 4.16**). By investing our time in handling the digital communication channels to retrieve the 166 observations submitted by citizens scientists and informed citizens, we saved 177.07 € (**Figure 4.15**).

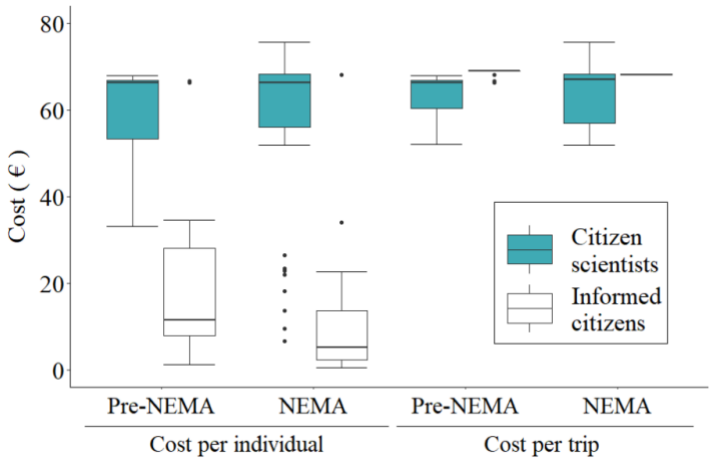


Figure 4.16 – Minimum cost for a scientist to obtain the same 166 observations (1747 Atlantic blue crabs) submitted by informed citizens and citizen scientists before (April 2018–March 2019) and during the NEMA campaign (April 2019–March 2020). These are minimum costs, as they only include transportation costs and the daily value of a Ph.D. fellowship of one scientist.

4.3.4 Outreach actions

The NEMA campaign participated in several seminars and workshops, national and international conferences (**Table 4.3**), to increase scientific literacy while disclosing its scientific results. Increasing engagement with citizen scientists was also fostered with underwater photoblitzs and awareness campaigns in southern Portugal.

Table 4.3 – Presentations given in seminars and workshops, national and international conferences regarding the implementation of the NEMA campaign and its scientific results.

Date	Title of presentation	Location	Organizing entity
07/10/2019	"A low-cost citizen science campaign increased the knowledge on two invasive marine species in south Portugal"	Cascais, Portugal	42nd Mediterranean Science Commission (CIESM) Congress
05/11/2019	"Disclosing new evidence of marine fauna tropicalization with the help of citizen science"	Mobile, USA	25th Coastal and Estuarine Research Federation (CERF) Conference
12/12/2019	"As novas espécies marinhas do Algarve"	Faro, Portugal	Conselho de Região Hidrográfica do Algarve, APA
10/10/2020	"Novas Espécies Marinhas do Algarve - O caso do projecto NEMA"	Faro, Portugal	Centro de Ciência Viva do Algarve, SNEI 2020
09/07/2021	"Projeto NEMA – Novas ocorrências nas costas algarvias e utilização da ciência-cidadã"	Online, Portugal	LIFE-INVASAQUA Project, Centro de Ciências do Mar (CCMAR)
04/10/2021	"Ciência cidadã e tropicalização da fauna marinha em Portugal"	Online, Portugal	Liga para a Proteção da Natureza (LPN)
04/11/2021	"Low-cost citizen science effectively monitors the rapid expansion of a marine invasive species"	Online, USA	26th Coastal and Estuarine Research Federation (CERF) Conference
21/03/2022	"Espécies invasoras no Algarve e o contributo da ciência cidadã"	Online, Portugal	Escola Profissional de Ciências Geográficas, Lisboa
27/03/2022	"O caranguejo azul e plataformas de ciência cidadã"	Castro Marim, Portugal	Instituto da Conservação da Natureza e das Florestas (ICNF), Universidade do Algarve
03/06/2022	"Ciência cidadã em meio marinho e a campanha NEMA no Algarve"	Loulé, Portugal	LIFE-INVASAQUA Project, Centro de Ciências do Mar (CCMAR)
15/10/2022	"Inovações no uso de espécies invasoras algarvias no estuário do Guadiana"	Castro Marim, Portugal	ATLAZUL Project, Universidade do Algarve
19/10/2022	"Espécies invasoras marinhas como novo recurso pesqueiro em Portugal"	Isla Cristina, Spain	ATLAZUL Project, Junta de Andalucía
01/06/2023	"Ciência cidadã em meio marinho e a campanha NEMA no Algarve"	Faro, Portugal	Centro de Ciências do Mar (CCMAR), LIFE-INVASAQUA Project
06/06/2023	"Ciência cidadã em meio marinho e a campanha NEMA no Algarve"	Faro, Portugal	Sustainable Horizons Project, Universidade do Algarve
26/06/2023	"Low-cost citizen science effectively monitors the rapid expansion of a marine invasive species"	Faro, Portugal	UP BLUE BRITE project, FLAD and Universidade do Algarve

In October 2020, two underwater photoblitz were organized in Algarve, the first in collaboration with the SubNauta dive centre in Portimão, and the second with the EasyDivers dive centre in Albufeira (**Figure 4.17**). These events were also part of the first national week on invasive species, an awareness week in Portugal with hundreds of activities regarding all sorts of invasive species (link). A photoblitz is not a photography competition, its ultimate goal is to document the biodiversity of a given location through photography in a short period of time. In 2020, these photoblitz had a general objective of documenting any non-indigenous marine species in Algarve, gathering over 20 participants. In total, 6 neonative species were documented (*Chromis chromis, Thalassoma pavo, Symphodus mediterraneus* (Linnaeus, 1758), *Symphodus ocellatus* (Linnaeus, 1758), *Symphodus rostratus* (Bloch, 1791), *Serranus atricauda*), without any record of invasive species.

(A) (B)

(C) (D)

Figure 4.17 – Posters of the 2020 NEMA underwater photoblitz in (A) Portimão and (B) Albufeira, and (C, D) respective participants.

In 2021, during the second national and first Iberian week on invasive species (link), NEMA organized an event to showcase the gastronomic potential of the invasive weakfish (**Figure 4.18A**). This was achieved in collaboration with Chef Leonel Pereira's CHECKin restaurant in Faro (www.checkinfaro.pt), where the Chef prepared a dish entitled "Roasted weakfish filet, with vegetable schmear and sea plankton" **(Figure 4.18B**). The dish was very well accepted by customers, making it into the permanent menu of the restaurant.

(A) (B)

Figure 4.18 – (A) Poster of the 2021 NEMA event, organised in collaboration with Chef Leonel Pereira's CHECKin restaurant in Faro, under the scope of the second national, and first Iberian, week on invasive species, and (B) the dish "Roasted weakfish filet, with vegetable schmear and sea plankton" produced by Chef Leonel Pereira.

During the summer of 2021, NEMA organized the second edition of its underwater photoblitzs. This time, with five associated diving centres across the Algarve: EasyDivers in Albufeira, DiveSpot in Armação de Pêra, PortiSub and SubNauta in Portimão, and WeDive in Lagos (**Figure 4.19**). In this edition, the focus-species was the bearded fireworm *Hermodice carunculata*, gathering over 30 participants. No specimens of the target species were detected in these photoblitz, but 6 other neonative species (*Chromis chromis*, *Thalassoma pavo*, *Symphodus mediterraneus*, *Symphodus ocellatus*, *Symphodus rostratus*, *Serranus atricauda*) were documented by the participants.

Figure 4.19 – (A) Poster of the 2021 NEMA underwater photoblitz, and participants of events organized during the summer of 2021 in collaboration with the dive centres Portisub, EasyDivers, SubNauta, WeDive, and Divespot, respectively (B–F).

4.3.5 Tropicalization of the Iberian Peninsula

The NEMA (*n*= 314; 31 species) and GBIF (*n*= 29251; 27 taxa) records are presented in **Figure 4.20**. The pattern of species distribution retrieved from GBIF showed one clear pattern – most records were located southern of mainland Portugal and in the Mediterranean Sea. In mainland Portugal, while the NEMA campaign received 314 records from 31 neonative species in just four years, the GBIF database had only 13 of these species (298 records): *Centrostephanus longispinus* (Philippi, 1845), *Chromis chromis*, *Fistularia petimba* Lacepède, 1803, *Plectorhinchus mediterraneus* (Guichenot, 1850), *Scorpaena maderensis*, *Scyllarides latus* (Latreille, 1803), *Seriola rivoliana* Valenciennes, 1833, *Serranus atricauda*, *Sphoeroides marmoratus*, *Sphoeroides pachygaster*, *Symphodus mediterraneus*, *Symphodus rostratus*, and *Trachinotus ovatus* (Linnaeus, 1758). In this data subset of GBIF, 90% of the records (*n*= 269) were from the Arrábida area (central Portugal), where the Professor Luiz Saldanha Marine Park is located. Additionally, 82% of GBIF records (*n*= 243) in this Arrábida area belonged to four species – *Chromis chromis*, *Serranus atricauda*, *Symphodus mediterraneus*, *and Symphodus rostratus*. The number of GBIF records in the northern Iberian Peninsula and further north in the Atlantic Ocean were greatly reduced, with only 45 records (**Figure 4.20**), belonging to 11 species: *Kyphosus* spp., *Parapristipoma octolineatum*, *Sciaena umbra* Linnaeus, 1758,

Serranus scriba, *Sphoeroides pachygaster*, *Sphyraena* spp., *Squilla mantis* (Linnaeus, 1758), *Thalassoma pavo*, *Trachinotus ovatus*, *Umbrina canariensis*.

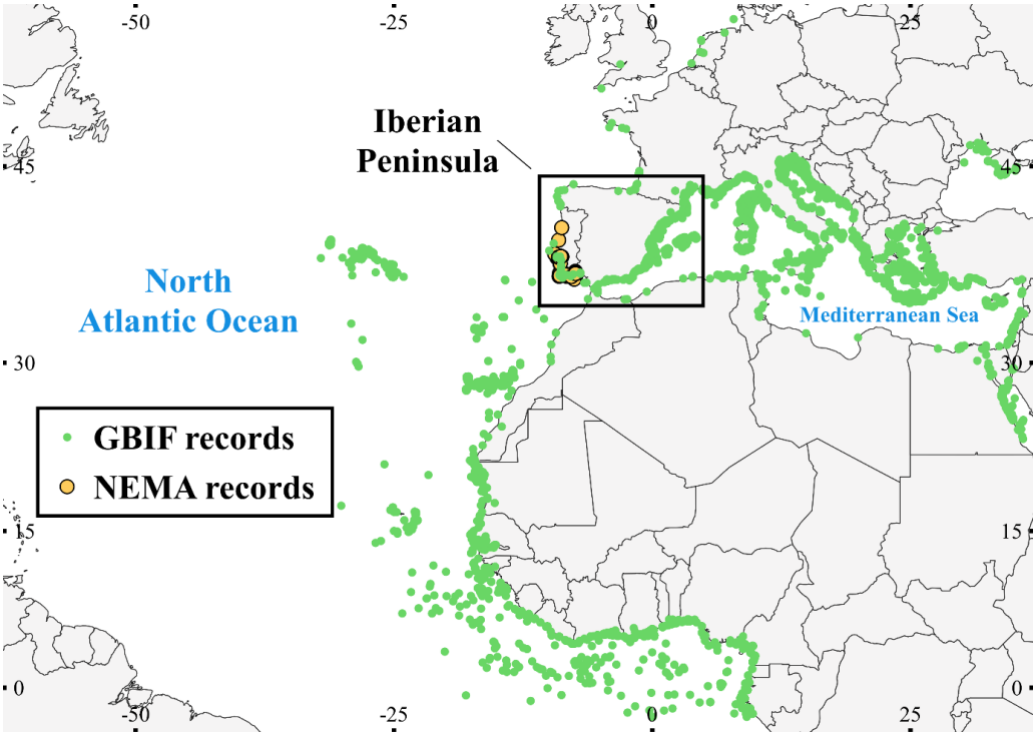


Figure 4.20 – Distribution of the 31 neonative species records received by the NEMA citizen science campaign, and the records retrieved from the GBIF database.

Considering these 27 neonative species, 13 (48.1%) were previously reported in scientific articles, 9 (33.3%) were reported in grey literature (master thesis, scientific reports) of which only *Squilla mantis*, *Symphodus mediterraneus*, and *Symphodus rostratus* were subsequently published in scientific articles (**Table 4.4**). Still, no published records were found for five (18.5%) neonative species – *Fistularia petimba*, *Hermodice carunculata*, *Sciaena umbra*, *Seriola rivoliana*, and *Sphyraena viridensis* Cuvier, 1829. To these five species, six other can be added which were only found in grey literature – *Pseudocaranx dentex* (Bloch & Schneider, 1801), *Scorpaena maderensis*, *Scyllarides latus*, *Serranus atricauda*, *Sphoeroides pachygaster*, and *Thalassoma pavo*.

Table 4.4 – Neonative species identified by the NEMA citizen science campaign in south Portugal until December 2022 (total number of records, date and location of first detection), and the respective records found in scientific literature, including grey literature.

Species	NEMA			Scientific literature				
	n	Date	Location	Date	Location	Author	Notes	Subsequent records
Chromis chromis	24	2008	Faro	1990-1994?	Faro-Olhão?	Santos et al. 1996		Santos and Monteiro 1998; Santos et al. 2005; Gonçalves et al. 2004, 2007, 2008, 2010; Erzini et al. 2008; Monteiro et al. 2012; Capaz 2013; Běláčková 2019; Macé 2022
Dentex canariensis	5	2014	Portimão	1990-1994?	Faro-Olhão?	Santos et al. 1996		Santos and Monteiro 1998; Santos et al. 2002a; Gonçalves et al. 2007, 2008; Macé 2022
Fistularia petimba	1	2020	Armação de Pêra	X	X	X		X
Hermodice carunculata	19	2015	Albufeira	X	X	X		X
Kyphosus spp.	5	2021	Vila do Bispo; Faro; VRSA	2004	Faro	Canas et al. 2005	One *K. sectator*	X
Parapristipoma octolineatum	22	2005	VRSA	?	Portimão-Faro?	Erzini et al. 1998		Santos et al. 2002a, 2002b; Gonçalves et al. 2007, 2008, 2010
Plectorhinchus mediterraneus	14	2010	VRSA	1990-1994?	Faro-Olhão?	Santos et al. 1996		Santos and Monteiro 1998; Santos et al. 2002b, 2005; Gonçalves et al. 2004, 2007, 2008, 2010; Monteiro et al. 2012
Pomadasys incisus	15	2006	VRSA	1990-1994?	Faro-Olhão?	Santos et al. 1996		Santos et al. 2005; Capaz 2013
Pseudocaranx dentex	1	2018	Albufeira	2018	Sagres?	Běláčková 2019	Master thesis	X
Sciaena umbra	3	2020	Tavira	X	X	X		X
Scorpaena maderensis	7	2018	Vila do Bispo	2015-2016?	Lagos, Tavira	Costa 2016	Master thesis	X
Scyllarides latus	1	2021	Lagos	2009-2010?	Portimão-Lagos?	Gonçalves et al. 2010	Scientific report	X
Seriola rivoliana	4	2014	Portimão	X	X	X		X
Serranus atricauda	39	2014	Portimão	2003-2004?	Albufeira	Gonçalves et al. 2004	Scientific report	Gonçalves et al. 2007, 2010; Monteiro et al. 2012; Běláčková 2019; Macé 2022
Serranus scriba	21	2019	VRSA	1990-1994?	Faro-Olhão?	Santos et al. 1996		Erzini et al. 1996; Santos et al. 2005
Sparisoma cretense	4	2013	Vila do Bispo	2005?	Faro	Abecasis et al. 2008		X
Sphoeroides marmoratus	5	2012	Lagos	1989?	Faro?	Monteiro 1989 in Santos et al. 1996	As *S. spengleri*	Gonçalves et al. 2004 (as *S. spengleri*); Gonçalves et al. 2007 (as *S. marmoratus* and *S. spengleri*)
Sphoeroides pachygaster	4	2020	Vila do Bispo	2009	Portimão	Carrasco 2009	Master thesis	Silva and Borges 2014 (as *S. cutaneus*)
Sphyraena sphyraena	1	2020	Loulé	1990-1994?	Olhão	Santos et al. 1996		X

Sphyraena viridensis	3	2014	Albufeira	X	X	X		X
Squilla mantis	8	2017	Armação de Pêra	2013	Sagres	Chaves and Silva 2013	Scientific report	Silva and Borges 2014; Vasconcelos et al. 2017
Stromateus fiatola	1	2019	Castro Marim	2000-2001?	Castro Marim	Veiga et al. 2006		Santos et al. 2002a
Symphodus mediterraneus	5	2012	Portimão	2003-2004?	Albufeira	Gonçalves et al. 2004	Scientific report	Gonçalves et al. 2007, 2008, 2010; Erzini et al. 2008; Monteiro et al. 2012
Symphodus rostratus	4	2020	Portimão	2003-2004?	Albufeira	Gonçalves et al. 2004	Scientific report	Ribeiro et al. 2006; Gonçalves et al. 2004, 2007, 2008, 2010; Monteiro et al. 2012
Thalassoma pavo	13	2011	Armação de Pêra	2009-2010?	Portimão-Lagos?	Gonçalves et al. 2010	Scientific report	Monteiro et al. 2012
Trachinotus ovatus	5	2005	Portimão	1990-1994?	Olhão	Santos et al. 1996		Erzini et al. 1996, 1998; Santos et al. 2002b
Umbrina canariensis	22	2006	VRSA	1990-1994?	Faro-Olhão?	Santos et al. 1996		Santos and Monteiro 1997, 1998

4.4 Discussion

4.4.1 Running the NEMA campaign

Detecting the presence of aquatic non-indigenous species after their introduction is extremely challenging and, in most cases, they only become noticed when an invasive status is reached (Mehta et al., 2007; Pyšek and Richardson, 2010). This has been tackled across the globe with rapid assessment surveys in artificial structures (Collin et al., 2015) or systematic surveys with fishing gears (Yamada et al., 2015; Poirier et al., 2017), but also using new technologies (e.g., eDNA analyses) that enhance the success of detecting non-indigenous species present with low abundances (Rees et al., 2014). However, implementing eDNA monitoring programs is unfeasible in most regions due to the financial costs associated with such technologies. In some cases, citizen sciences campaigns may mitigate the lack of intensive monitoring programs. For example, several successful citizen science campaigns have focused on crustaceans (e.g., Asian shore crab *Hemigrapsus sanguineus* (De Haan, 1835) and the European green crab *Carcinus maenas* (Delaney et al., 2008; Grason et al., 2018)), algae (e.g., *Caulerpa taxifolia* (M.Vahl) C.Agardh, 1817 (Ellul et al., 2019)), or fish (e.g., lionfish *Pterois miles* (Bennett, 1828) (Azzurro et al., 2017; Giovos et al., 2018)).

The running costs of citizen science campaigns are usually unavailable, making it impossible to analyse and validate the cost-benefit of such approaches. During NEMA's first year, the campaign relied mostly on social media to communicate with potential citizen scientists. Social media provides a dual-communication channel with citizen scientists, i.e.,

allows promoting the project while providing updates on recent discoveries, increasing scientific literacy, and interact directly with citizen scientists. Direct communication with citizen scientists on social media, mainly on Facebook, provided valuable records of several invasive and neonative species in the Algarve, in particular the Atlantic blue crab. Relying on free digital platforms (social media, e-mail, and citizen science platforms) meant running NEMA with no associated costs during its first year and save over 11,000 € for the total of 166 Atlantic blue crab observations received (8,900 € for observations made only in the first year period). This is the minimum amount of money that we would need to obtain the exact same information on the field and with just one scientist. Assuming that every field trip would result in one record.

Other successful citizen science projects relied on dedicated websites and/or smartphone apps (Gallo and Waitt, 2011; Azzurro et al., 2013, 2019; Zenetos et al., 2013; Marchante et al., 2017; Eritja et al., 2019). Such technologies are extremely costly to produce and maintain. Additionally, NEMA is being implemented as a long-term detection campaign, and such web platforms also require recurring annual fees. NEMA's approach to engage with citizen scientists mostly through online outreach is still a time-consuming methodology, that requires constant communication with participants and all the tasks associated with social media handling. If the time invested would result in a direct cost to create all the platforms and handling the social media pages ourselves, the correspondent cost during this first year of NEMA would have summed up to 3,574 €, which is still much lower than hiring professionals (18,815 €–142,485 €).

Outreach is a present-day necessity of most scientific projects, in order to increase scientific literacy and communication between academia and society. As a citizen science campaign, NEMA has these objectives as top priority, alongside with increasing the scientific knowledge on non-indigenous marine species in Portugal. Social media showed to be a valuable ally in reaching a higher audience, with over 5600 followers in four years. Participatory actions, as the underwater photoblitzs organized by NEMA in the summer of 2020 and 2021, resulted in a higher awareness about biological invasion, not only to the participants, but also the general society due to parallel media coverage of such events. Specific awareness actions directed at non-indigenous species with some sort of associated danger, as the underwater photoblitzs of 2021 about the bearded fireworm, are of the upmost importance to local populations dealing with new species about whose they did not had any previous

knowledge. Outreach and awareness were amplified by the several news pieces and articles in traditional media, along with the presentations given in scientific events and others.

The NEMA citizen science campaign has demonstrated the value of citizen science in tracking biological invasions, while also showing the value of a set of low-cost tools that can be used to replicate this approach in other regions of the world. The high engagement of citizen scientists allowed to monitor the expansion of invasive species, along with range-expanding neonative species, while providing relevant clues for future research hypotheses.

4.4.2 Invasive species

Species records gathered by NEMA greatly increased the scientific knowledge on the presence and distribution of non-indigenous marine species in Portugal, particularly in the Algarve region. Regarding the invasive Atlantic blue crab *Callinectes sapidus*, in about one year (2019–2020) the spread of this species across the Algarve was monitored and its known distribution was extended by over 140 km until Sagres in western Algarve, since previous published studies (Morais et al., 2019; Vasconcelos et al., 2019). One advantage of citizen science and modern-day technologies is the possibility to recover records older than the launch-date of the citizen science campaign itself, as happened with NEMA. Such an example was one record of an Atlantic blue crab in Alvor in 2018, showing the presence of the species in western Algarve by that time and about 65 km towards west since the last published records, at that time, in Ria Formosa in 2019 (Vasconcelos et al., 2019). Furthermore, the records in the western coast of Portugal in 2019 and 2020, along with two possible new records in Ria de Aveiro (north of Lisbon) from 2018 and 2019 (Izquierdo-Gómez 2022), could indicate a higher propagule pressure from the southern Algarve coast and therefore a possible new wave of colonization by the Atlantic blue crab into this western coast of Portugal, since previous introductions seemed to fail until recently (Gaudêncio and Guerra 1979; Ribeiro and Veríssimo 2014).

Data from the Atlantic blue crab also showed that citizen scientists have different engagement levels, yet equally valuable to monitor biological invasions. Without a wide network of citizen scientists, tracking the westward expansion of the Atlantic blue crab would not have been possible. Without an informed citizen from the Guadiana estuary (fisher Mr. Gonçalves), we could not obtain precious fine-scale information about the presence of the species in this estuary for an extended period. Therefore, all connections should be nourished.

NEMA also obtained interesting details about the ecology of the Atlantic blue crab. Two females were reported to be swimming at the surface during the night close to the coast, which is a typical behaviour of ovigerous females that perform vertical migrations at night during the spawning periods (Tankersley et al., 1998; Aguilar et al., 2005; Forward et al., 2005). NEMA's data also showed that female Atlantic blue crabs were more common in coastal areas throughout the year, and not only during the supposed reproductive period (August–October). Ovigerous females in coastal areas were only recorded once off Portimão, but the other three ovigerous individuals were found in the lower Arade estuary and Ria Formosa. The high mobility of Atlantic blue crabs and its fast adaptation to environmental conditions, namely salinity, are key factors for the selection of spawning areas (Forward et al., 2003; Aguilar et al., 2005). These preliminary results show that spawning areas in Algarve seem to include both the lower section of estuaries and coastal areas.

After the sporadic records of the weakfish *Cynoscion regalis* in the Algarve region gathered between 2016 and 2017 (Morais and Teodósio 2016; Morais et al. 2017), the NEMA records came to confirm the presence of the species in the region, with 24 records (39 individuals) between July 2019 and December 2022. Taking into consideration the records of the weakfish gathered by NEMA in western Portugal (10 records; 20 individuals), along with many other online reports, the species is unequivocally established in central Portugal, namely in the Sado and Tejo estuaries, where recreational fishers already label it as a 'pest'. In the south of Portugal, the invasion scenario seems different, with fewer and more sporadic reports, although the Guadiana estuary seems to have an ecologically important role for the species as a possible spawning area during Spring. The species seems to follow a preference for estuarine ecosystems in Portugal, or at least a seasonal estuarine residence, as documented in its native range (Lankford and Targett 1994; Thorrold et al. 1998; Litvin et al. 2014; Turnure et al. 2015). Of the 24 records received by NEMA in the Algarve region, only 6 were in coastal areas, but three of them seemed to be small individuals (records #5, #6, and #36, see **Table S2** of the Electronic Supplementary Material) captured in the area between Lagoa and Lagos. These observations of smaller individuals in coastal areas may show the dispersal and emigration of younger weakfish to the so-called offshore wintering areas, as described in the East coast of North America in areas such as the Delaware Bay and other estuarine areas (Thorrold et al. 2001; Litvin et al. 2014; Turnure et al. 2015). Later on, as adults, they return to estuarine systems to spawn, even displaying a high spawn site fidelity (Thorrold et al. 2001; Krause et al. 2020). In this subject, the Guadiana estuary may be becoming a spawning ground with

higher importance. Although the abundance of the species in the northern estuaries of Sado and Tejo seems to be much higher, such detailed information of population characteristics is still completely unknown and highly needed in the near future to understand the reasons behind the differences in abundance between these two areas of the Portuguese coast.

4.4.3 Neonative species and tropicalization

The NEMA campaign was able to detect 11 neonative species whose presence in the Algarve region, as far as our best present knowledge and until the beginning of this Ph.D., had not yet been published in a peer-reviewed scientific paper – *Fistularia petimba*, *Hermodice carunculata*, *Pseudocaranx dentex*, *Sciaena umbra*, *Scorpaena maderensis*, *Scyllarides latus*, *Seriola rivoliana*, *Serranus atricauda*, *Sphoeroides pachygaster*, *Sphyraena viridensis*, and *Thalassoma pavo*. The Algarve's location at the intersection of three ecoregions (Spalding et al. 2007) not only increases the chances of new neonative species appearance under a climate change scenario, but also complicates the task of defining which species should be considered "new", or when exactly a certain species started to appear in Algarve. The amount of neonative species records that NEMA gathered in such a short period of time, by far surpasses our own capacity to detect such species by traditional scientific campaigns, as employed during this study (see **Chapter 2**), and in some cases what is being published in scientific literature and public databases. Citizen science has then the ability to overcome part of such constraints with insufficient funding and logistics of scientific institutions, resulting in important advances in knowledge across extended geographical areas at considerably lower costs (Carr 2004; Crall et al. 2010; Tulloch et al. 2013; Pocock et al. 2017; Simoniello et al. 2019).

The tropicalization of temperate regions (Bianchi and Morri 2003) is particularly well documented in the Mediterranean Sea since the early 1990s, where several southern warmer-water fish species have been recorded in northern coasts (Bianchi and Morri 1994, 2003; Bianchi et al. 2018; Azzurro et al. 2019). Some of the neonative species being recorded in southern Portugal by the NEMA campaign, are also showing such range expansions in the Mediterranean Sea. For example, the ornate wrasse *Thalassoma pavo* (Bianchi and Morri 1994; Francour et al. 1994; Vacchi et al. 2001; Bianchi et al. 2018), the parrotfish *Sparisoma cretense* (Linnaeus, 1758) (Bianchi and Morri 1994; Dulčić and Pallaoro 2001; Azzurro et al. 2011; Bianchi et al. 2018), or the bearded fireworm *Hermodice carunculata* (Righi et al. 2020; Krželj et al. 2020; Simonini et al. 2021), are becoming more frequent in the northern shores of the

Mediterranean Sea. Here, citizen science has also been a fundamental tool in keeping track of such changes (e.g., Azzurro et al. 2011, 2019; Giovos et al. 2019; Krželj et al. 2020).

In continental Portugal, scientific publications on the presence and distribution of the 31 neonative taxa identified by NEMA are still very insufficient. From the information gathered in this study, published records are either very patchy and associated with areas of the country where scientific campaigns take place more often, or dispersed in grey literature. One such example in the dataset retrieved from GBIF, with a concentration of records in the Arrábida area, where the Professor Luiz Saldanha Marine Park is located, and where a high sampling effort takes place by scientific institutions (e.g., Henriques et al. 2007; Batista et al. 2011; Cunha et al. 2014; Horta e Costa 2014). Similarly in southern Portugal, a high number of records published in scientific papers are derived from studies done in the vicinities of Faro, where universities and research institutes are located (e.g., Santos et al. 1996, 2005; Erzini et al. 1996; Santos and Monteiro 1998; Ribeiro et al. 2006), while towards western Algarve records were mostly found in grey literature (e.g., Gonçalves et al. 2008, 2010; Carrasco 2009; Monteiro et al. 2012; Costa 2016; Běláčková 2019). Furthermore, most publications present their results as tables of species lists, providing very little detail on their records, missing on fundamental information about methodologies as date of each sampling campaign, or presenting the results only by very large areas, resulting in difficulties in gathering accurate information of these neonative records (see **Table 4.4**).

Climate change and sea warming may lead to several local extinctions, particularly in areas closer to the poles and the tropics, while temperate regions will witness constant changes in species composition and recurrent turnover of communities (Cheung et al. 2009; Molinos et al. 2015). In temperate regions such as Portugal, changes in fauna distribution may be characterized by year-to-decade oscillations, which increases the difficulty in clearly detecting long-term changes caused by climate change in coastal zones (Henriques et al. 2007; Horta e Costa et al. 2014). While some temperate species may become less frequent, the arrival of subtropical species in a sea warming scenario, will also provide new opportunities for fisheries in exploring such species with commercial value (Vinagre et al. 2011).

Several evidence point to an ongoing tropicalization of continental Portugal's marine biodiversity, potentially exacerbated by more common and long-lasting marine heatwaves (Fragkopoulou et al. 2023). Some species may be more frequent and established for several years or even decades, shown by the number of published records in southern Portugal and

number of NEMA records, namely *Chromis chromis*, *Parapristipoma octolineatum*, *Plectorhinchus mediterraneus*, *Serranus atricauda*, *Symphodus mediterraneus*, or *Symphodus rostratus*. On the other side, "newcomers" such as *Hermodice carunculata* or *Thalassoma pavo* may become established, while colder winter temperatures may dictate the long-term success in colonizing northern areas of the Portuguese coast. In particular, warmer winters may be of particular importance for these species to persist and gradually expand their distribution (Horta e Costa et al. 2014). Still, this process may have been overlooked for some time by the scientific community in Portugal, as sporadic records of subtropical species (e.g., Abecasis et al. 2008; Vasconcelos et al. 2018) may not be core evidence of significant changes when seen individually, but when the pattern is observed across a broader group of species – such as the ones here under analysis – the tropicalization phenomenon becomes evident. The contribution of NEMA and citizen science shows the need for more frequent and large-scale scientific surveys, to allow a more comprehensive study of these increasingly rapid changes.

4.5 Conclusions

The NEMA citizen science campaign was the first of its kind in Portugal, exclusively dedicated to marine non-indigenous species. The campaign was very successful and relied on the contributions of 260+ citizen scientists, 700+ validated records, belonging to 50+ marine species. From these, about 40% were records of the invasive Atlantic blue crab *Callinectes sapidus*, which enabled us to track its expansion along the Algarve coast, but also gather records in the west coast of Portugal, while providing relevant clues about ecological aspects for future research hypotheses. An important contribution from NEMA also included records of 11 neonative species whose presence in the Algarve region, as far as our best present knowledge and until the beginning of this Ph.D., had not yet been published in a peer-reviewed scientific paper. The tropicalization process may have been overlooked for some time by the scientific community in Portugal, as sporadic records of subtropical species may not be core evidence of significant changes when seen individually, but when the pattern is observed across a broader group of species the tropicalization phenomenon becomes evident. The present results, both regarding the Atlantic blue crab and the neonative species, also supported and complemented those obtained in Chapter 2.

4.6 References

Abecasis, D., Bentes, L., Ribeiro, J., Machado, D., Oliveira, F., Veiga, P., Gonçalves, J.M., and Erzini, K. (2008). First record of the Mediterranean parrotfish, *Sparisoma cretense* in Ria Formosa (south Portugal). *Marine Biodiversity Records* 1: e27. doi: 10.1017/S175526720600248X

Aguilar, R., Hines, A.H., Wolcott, T., Wolcott, D., Kramer, M., and Lipcius, R. (2005). The timing and route of movement and migration of post-copulatory female blue crabs, *Callinectes sapidus* Rathbun, from the upper Chesapeake Bay. *Journal of Experimental Marine Biology and Ecology* 319(1-2): 117-128. doi: 10.1016/j.jembe.2004.08.030

Azzurro, E., Moschella, P., and Maynou, F. (2011). Tracking signals of change in Mediterranean fish diversity based on local ecological knowledge. *PloS one* 6(9): e24885. doi: 10.1371/journal.pone.0024885

Azzurro, E., Broglio, E., Maynou, F., and Bariche, M. (2013). Citizen science detects the undetected: The case of *Abudefduf saxatilis* from the Mediterranean Sea. *Management of Biological Invasions* 4: 167-170. doi: 10.3391/mbi.2013.4.2.10

Azzurro, E., Stancanelli, B., Di Martino, V., and Bariche, M. (2017). Range expansion of the common lionfish *Pterois miles* (Bennett, 1828) in the Mediterranean Sea: An unwanted new guest for Italian waters. *BioInvasions Records* 6(2): 95-98. doi: 10.3391/bir.2017.6.2.01

Azzurro, E., Bolognini, L., Dragičević, B., Drakulović, D., Dulčić, J., Fanelli, E., Grati, F., Kolitari, J., Lipej, L., and Magaletti, E. (2019). Detecting the occurrence of indigenous and non-indigenous megafauna through fishermen knowledge: A complementary tool to coastal and port surveys. *Marine Pollution Bulletin* 147: 229-236. doi: 10.1016/j.marpolbul.2018.01.016

Bariche, M., Constantinou, C., and Sayar, N. (2018). First confirmed record of the white-spotted puffer *Arothron hispidus* (Linnaeus, 1758) in the Mediterranean Sea. *BioInvasions Records* 7(4): 433-436. doi: 10.3391/bir.2018.7.4.13

Batista, M.I., Baeta, F., Costa, M.J., and Cabral, H.N. (2011). MPA as management tools for small-scale fisheries: The case study of Arrábida Marine Protected Area (Portugal). *Ocean & Coastal Management* 54(2): 137-147. doi: 10.1016/j.ocecoaman.2010.10.032

Běláčková, A. (2019). Evaluation of the reserve effect in a Marine Protected Area in Sagres (PNSACV). Master thesis. Universidade do Algarve, Faro. 72 pp

Bianchi, C., and Morri, C. (1994). Southern species in the Ligurian Sea (northern Mediterranean): New records and a review. *Bollettino dei Musei e degli Istituti Biologici dell'Università di Genova* 58(59): 181-197.

Bianchi, C.N., and Morri, C. (2003). Global sea warming and "tropicalization" of the Mediterranean Sea: Biogeographic and ecological aspects. *Biogeographia–The Journal of Integrative Biogeography* 24(1). doi: 10.21426/B6110129

Bianchi, C.N., Caroli, F., Guidetti, P., and Morri, C. (2018). Seawater warming at the northern reach for southern species: Gulf of Genoa, NW Mediterranean. *Journal of the Marine Biological Association of the United Kingdom* 98(1): 1-12. doi: 10.1017/S0025315417000819

Boero, F., Putti, M., Trainito, E., Prontera, E., Piraino, S., and Shiganova, T.A. (2009). First records of *Mnemiopsis leidyi* (Ctenophora) from the Ligurian, Thyrrhenian and Ionian Seas (Western Mediterranean) and first record of *Phyllorhiza punctata* (Cnidaria) from the Western Mediterranean. *Aquatic Invasions* 4(4): 675-680. doi: 10.3391/ai.2009.4.4.13

Canas, A., Vasconcelos, P., Lino, P.G., and Santos, M.N. (2005). Northernmost record of *Kyphosus sectator* (Osteichthyes: Perciformes: Kyphosidae) in the north-eastern Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 85(6): 1535-1537. doi: 10.1017/S0025315405012750

Capaz, J.C. (2013). Estrutura de comunidades de peixes, amostradas por SVSI - Sistema de vídeo subaquático iscado, na Costa Sul de Portugal. Master thesis. Universidade do Algarve, Faro. 154 pp

Carr, A.J. (2004). Why do we all need community science? *Society & Natural Resources* 17: 841–849. doi: 10.1080/08941920490493846

Carrasco, N.C. (2009). Quantificação das capturas acessórias e rejeições de arrasto de crustáceos na costa do Algarve. Master thesis. Universidade do Algarve, Faro. 51 pp

César de Sá, N., Marchante, H., Marchante, E., Cabral, J.A., Honrado, J.P., and Vicente, J.R. (2019). Can citizen science data guide the surveillance of invasive plants? A model-based test with Acacia trees in Portugal. *Biological Invasions* 21(6): 2127-2141. doi: 10.1007/s10530-019-01962-6

Chaves, C., and Silva, C. (2013). Crustáceos-2013, Jun/Julho. Relatórios de Campanha. IPMA, Lisboa. 24 pp

Cheung, W.W., Lam, V.W., Sarmiento, J.L., Kearney, K., Watson, R., and Pauly, D. (2009). Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries* 10(3): 235-251. doi: 10.1111/j.1467-2979.2008.00315.x

Cohen, C.S., McCann, L., Davis, T., Shaw, L., and Ruiz, G.M. (2011). Discovery and significance of the colonial tunicate *Didemnum vexillum* in Alaska. *Aquatic Invasions* 6: 263–271. doi: 10.3391/ai.2011.6.3.03

Collin, S., Tweddle, J., and Shucksmith, R. (2015). Rapid assessment of marine non-native species in the Shetland Islands, Scotland. *BioInvasions Records* 4(3): 147-155. doi: 10.3391/bir.2015.4.3.01

Costa, E.F., Encarnação, J., Teodósio, M.A., and Morais, P. (2023). Aquatic species shows asymmetric distribution range shifts in native and non-native areas. *Frontiers in Marine Science* 10: 1158206. doi: 10.3389/fmars.2023.1158206

Costa, M. (2016). Benthic communities in shipwrecks along the Portuguese continental coast. Master thesis. Universidade do Algarve, Faro. 100 pp

Crall, A.W., Newman, G.J., Jarnevich, C.S., Stohlgren, T.J., Waller, D.M., and Graham, J. (2010). Improving and integrating data on invasive species collected by citizen scientists. *Biological Invasions* 12: 3419-3428. doi: 10.1007/s10530-015-0885-4

Crall, A.W., Jarnevich, C.S., Young, N.E., Panke, B.J., Renz, M., and Stohlgren, T.J. (2015). Citizen science contributes to our knowledge of invasive plant species distributions. *Biological Invasions* 17(8): 2415-2427. doi: 10.1007/s10530-015-0885-4

Cunha, A.H., Erzini, K., Serrão, E.A., Gonçalves, E., Borges, R., Henriques, M., Henriques, V., Guerra, M., Duarte, C., and Marbá, N. (2014). Biomares, a LIFE project to restore and manage the biodiversity of Prof. Luiz Saldanha Marine Park. *Journal of Coastal Conservation* 18: 643-655. doi: 10.1007/s11852-014-0336-x

Delaney, D.G., Sperling, C.D., Adams, C.S., and Leung, B. (2008). Marine invasive species: Validation of citizen science and implications for national monitoring networks. *Biological Invasions* 10: 117-128. doi: 10.1007/s10530-007-9114-0

Dulčić, J., and Pallaoro, A. (2001). Some new data on *Xyrichthys novacula* (Linnaeus, 1758) and *Sparisoma* (*Euscarus*) *cretense* (Linnaeus, 1758) from the eastern Adriatic. *Annales, Series Historia Naturalis* 11.

Eitzel, M., Cappadonna, J., Santos-Lang, C., Duerr, R., West, S.E., Virapongse, A., Kyba, C., Bowser, A., Cooper, C., and Sforzi, A. (2017). Citizen science terminology matters: Exploring key terms. *Citizen Science: Theory and Practice* 2: 1-20. doi: 10.5334/cstp.96

Ellul, T., Evans, J., and Schembri, P.J. (2019). Invasion alert: rapid range expansion of *Caulerpa taxifolia* var. *distichophylla* in Maltese waters (central Mediterranean). *BioInvasions Record* 8(2): 208–217. doi: 10.3391/bir.2019.8.2.02

Eritja, R., Ruiz-Arrondo, I., Delacour-Estrella, S., Schaffner, F., Álvarez-Chachero, J., Bengoa, M., Puig, M.-Á., Melero-Alcíbar, R., Oltra, A., and Bartumeus, F. (2019). First detection of *Aedes japonicus* in Spain: An unexpected finding triggered by citizen science. *Parasites & Vectors* 12(1): 1-9. doi: 10.1186/s13071-019-3317-y

Erzini, K., Gonçalves, J.M., Bentes, L., Lino, P.G., and Cruz, J. (1996). Species and size selectivity in a Portuguese multispecies artisanal long-line fishery. *ICES Journal of Marine Science* 53(5): 811-819. doi: 10.1006/jmsc.1996.0102

Erzini, K., Gonçalves, J.M., Bentes, L., Lino, P.G., and Ribeiro, J. (1998). Species and size selectivity in a 'red' sea bream longline 'métier' in the Algarve (southern Portugal). *Aquatic Living Resources* 11(1): 1-11. doi: 10.1016/S0990-7440(99)80025-4

Erzini, K., Bentes, L., Coelho, R., Lino, P.G., Monteiro, P., Ribeiro, J., and Gonçalves, J. (2008). Catches in ghost-fishing octopus and fish traps in the northeastern Atlantic Ocean (Algarve, Portugal). *Fishery Bulletin* 106: 321–327.

Faraone, F.P., Giacalone, G., Canale, D.E., D'Angelo, S., Favaccio, G., Garozzo, V., Giancontieri, G.L., Isgrò, C., Melfi, R., and Morello, B. (2017). Tracking the invasion of the red swamp crayfish *Procambarus clarkii* (Girard, 1852)(Decapoda Cambaridae) in Sicily: A "citizen science" approach. *Biogeographia–The Journal of Integrative Biogeography* 32(1). doi: 10.21426/B632135512

Fitzpatrick, M.C., Preisser, E.L., Ellison, A.M., and Elkinton, J.S. (2009). Observer bias and the detection of low-density populations. *Ecological Applications* 19(7): 1673-1679. doi: 10.1890/09-0265.1

Forward Jr, R., Tankersley, R., and Pochelon, P. (2003). Circatidal activity rhythms in ovigerous blue crabs, *Callinectes sapidus*: Implications for ebb-tide transport during the spawning migration. *Marine Biology* 142: 67-76. doi: 10.1007/s00227-002-0923-5

Forward Jr, R.B., Cohen, J.H., Darnell, M.Z., and Saal, A. (2005). The circatidal rhythm in vertical swimming of female blue crabs, *Callinectes sapidus*, during their spawning migration: A reconsideration. *Journal of Shellfish Research* 24(2): 587-590. doi:

10.2983/0730-8000(2005)24[587:TCRIVS]2.0.CO;2

Fragkopoulou, E., Sen Gupta, A., Costello, M.J., Wernberg, T., Araújo, M.B., Serrão, E., De Clerck, O., Assis, J. (2023). Marine biodiversity exposed to prolonged and intense subsurface heatwaves. *Nature Climate Change*. doi: 10.1038/s41558-023-01790-6

Francour, P., Boudouresque, C.-F., Harmelin, J., Harmelin-Vivien, M., and Quignard, J. (1994). Are the Mediterranean waters becoming warmer? Information from biological indicators. *Marine Pollution Bulletin* 28(9): 523-526. doi: 10.1016/0025-326X(94)90071-X

Gallo, T., and Waitt, D. (2011). Creating a successful citizen science model to detect and report invasive species. *BioScience* 61(6): 459-465. doi: 10.1525/bio.2011.61.6.8

Gaudêncio, M., and Guerra, T. (1979). Note sur la presence de *Callinectes sapidus* Rathbun 1896 (Crustacea Decapoda Brachyura) dans l'estuaire du Taje. *Boletim do Instituto Nacional de Investigação das Pescas* 2: 67-73.

GBIF - The Global Biodiversity Information Facility (2023). *GBIF Home Page. Available from: https://www.gbif.org*. [Accessed February 14, 2023].

Giovos, I., Kleitou, P., Paravas, V., Marmara, D., Romanidis-Kyriakidis, G., and Poursanidis, D. (2018). Citizen scientists monitoring the establishment and expansion of *Pterois miles* (Bennett, 1828) in the Aegean Sea, Greece. *Cahiers de Biologie Marine* 59: 359-365. doi: 10.21411/CBM.A.8DFA67CE

Giovos, I., Kleitou, P., Poursanidis, D., Batjakas, I., Bernardi, G., Crocetta, F., Doumpas, N., Kalogirou, S., Kampouris, T.E., and Keramidas, I. (2019). Citizen-science for monitoring marine invasions and stimulating public engagement: A case project from the eastern Mediterranean. *Biological invasions* 21: 3707-3721. doi: 10.1007/s10530-019-02083-w

Gonçalves, J., Monteiro, P., Coelho, R., Afonso, C., Ribeiro, J., Almeida, C., Veiga, P., Machado, D., Berecibar, E., Oliveira, F., et al. (2004). Mapeamento de biocenoses marinhas da reserva ecológica nacional submarina entre Albufeira e Vale do Lobo - Relatório Final. CCDR Algarve. Centro de Ciências do Mar, Universidade do Algarve, Faro. 217 pp

Gonçalves, J., Monteiro, P., Coelho, R., Afonso, C., Almeida, C., Veiga, P., Machado, M., Machado, D., Oliveira, F., Ribeiro, J., et al. (2007). Cartografia e caracterização das biocenoses marinhas da Reserva Ecológica Nacional Submarina entre a Galé e a barra Nova do Ancão - Relatório Final. CCDR Algarve. Centro de Ciências do Mar, Universidade do Algarve, Faro. 360 pp

Gonçalves, J., Monteiro, P., Afonso, C., Almeida, C., Oliveira, F., Rangel, M., Ribeiro, J., Machado, M., Veiga, P., Abecasis, D., et al. (2008). Cartografia e caracterização das biocenoses marinhas da Reserva Ecológica Nacional Submarina entre a Galé e a foz do rio Arade - Relatório Final. CCDR Algarve. Centro de Ciências do Mar, Universidade do Algarve, Faro. 179 pp

Gonçalves, J., Monteiro, P., Afonso, C., Oliveira, F., Rangel, M., Machado, M., Veiga, P., Leite, L., Sousa, I., Bentes, L., et al. (2010). Cartografia e caracterização das biocenoses marinhas da Reserva Ecológica Nacional Submarina entre a foz do Rio Arade e a Ponta da Piedade - Relatório Final. ARH Algarve. Centro de Ciências do Mar, Universidade do Algarve, Faro. 170 pp

Grason, E.W., McDonald, P.S., Adams, J., Litle, K., Apple, J.K., and Pleus, A. (2018). Citizen science program detects range expansion of the globally invasive European green crab in Washington State. *Management of Biological Invasions* 9(1): 39-47 doi: 10.3391/mbi.2018.9.1.04

Guardone, L., Gasperetti, L., Maneschi, A., Ricci, E., Susini, F., Guidi, A., and Armani, A. (2018). Toxic invasive pufferfish (Tetraodontidae family) along Italian coasts: Assessment of an emerging public health risk. *Food Control* 91: 330-338. doi: 10.1016/j.foodcont.2018.04.005

Haddad Jr, V., and Barreiros, J.P. (2007). Animais Marinhos dos Açores: Perigosos e Venenosos. Blu Edições, Ilha Terceira, Portugal. 58 pp

Henriques, M., Gonçalves, E.J., and Almada, V.C. (2007). Rapid shifts in a marine fish assemblage follow fluctuations in winter sea conditions. *Marine Ecology Progress Series* 340: 259-270. doi: 10.3354/meps340259

Horta e Costa, B., Assis, J., Franco, G., Erzini, K., Henriques, M., Gonçalves, E.J., and Caselle, J.E. (2014). Tropicalization of fish assemblages in temperate biogeographic transition zones. *Marine Ecology Progress Series* 504: 241-252. doi: 10.3354/meps10749

Icha, O., and Agwu, E. (2015). Effectiveness of social media networks as a strategic tool for organizational marketing management. *Journal of Internet Banking and Commerce* S2(006). doi: 10.4172/1204-5357.S2-006

Izquierdo-Gómez, D. (2022). Synergistic use of facebook, online questionnaires and local ecological knowledge to detect and reconstruct the bioinvasion of the Iberian Peninsula by *Callinectes sapidus* Rathbun, 1896. *Biological Invasions* 24(4): 1059-1082. doi: 10.1007/s10530-021-02696-0

Krause, J.R., Hightower, J.E., Buckel, J.A., Turnure, J.T., Grothues, T.M., Manderson, J.P., Rosendale, J.E., and Pessutti, J.P. (2020). Using acoustic telemetry to estimate weakfish survival rates along the US East Coast. *Marine and Coastal Fisheries* 12(5): 241-257. doi: 10.1002/mcf2.10095

Krželj, M., Cerrano, C., and Di Camillo, C.G. (2020). Enhancing diversity knowledge through Marine citizen science and social platforms: The case of *Hermodice carunculata* (Annelida, Polychaeta). *Diversity* 12(8): 311. doi: 10.3390/d12080311

Lankford, T.E., and Targett, T.E. (1994). Suitability of estuarine nursery zones for juvenile weakfish (*Cynoscion regalis*): Effects of temperature and salinity on feeding, growth and survival. *Marine Biology* 119: 611-620. doi: 10.1007/BF00354325

Lee, S. (2019). Does brand logo matter in social media marketing? The moderating role of brand logo and brand equity in brand evaluation. *Journal of Digital & Social Media Marketing* 6(4): 371-383.

Litvin, S.Y., Weinstein, M.P., and Guida, V.G. (2014). Habitat utilization patterns determine the physiological condition of *Cynoscion regalis* during estuarine residency. *Marine Ecology Progress Series* 510: 87-99. doi: 10.3354/meps10913

Macé, J. (2022). Assessment of the fish communities of the largest coastal reef of the Algarve (Portugal) to contribute to a future Marine Protected Area. Master thesis. Universidade do Algarve, Faro. 54 pp

Marchante, H., Morais, M.C., Gamela, A., and Marchante, E. (2017). Using a WebMapping platform to engage volunteers to collect data on invasive plants distribution. *Transactions in GIS* 21(2): 238-252. doi: 10.1111/tgis.12198

Mehta, S.V., Haight, R.G., Homans, F.R., Polasky, S., and Venette, R.C. (2007). Optimal detection and control strategies for invasive species management. *Ecological Economics* 61(2-3): 237-245. doi: 10.1016/j.ecolecon.2006.10.024

Molinos, J.G., Halpern, B.S., Schoeman, D.S., Brown, C.J., Kiessling, W., Moore, P.J., Pandolfi, J.M., Poloczanska, E.S., Richardson, A.J., and Burrows, M.T. (2016). Climate velocity and the future global redistribution of marine biodiversity. *Nature Climate Change* 6(1): 83-88. doi: 10.1038/NCLIMATE2769

Monteiro, P., Bentes, L., Sousa, I., Oliveira, F., Veiga, P., Rangel, M., Afonso, C., and Gonçalves, J. (2012). Biodiversidade marinha da costa sul de Sagres. Identificação e caracterização de biótopos. Relatório Interno Nº 2/2012 - MeshAtlantic. Centro de Ciências do Mar, Universidade do Algarve, Faro. 48 pp

Morais, P., and Teodósio, M. (2016). The transatlantic introduction of weakfish *Cynoscion regalis* (Bloch & schneider, 1801)(sciaenidae, pisces) into Europe. *BioInvasions Records* 5(4): 259-265. doi: 10.3391/bir.2016.5.4.11

Morais, P., Cerveira, I., and Teodósio, M.A. (2017). An update on the invasion of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801)(Actinopterygii: Sciaenidae) into Europe. *Diversity* 9(4): 47. doi: 10.3390/d9040047

Morais, P., Gaspar, M., Garel, E., Baptista, V., Cruz, J., Cerveira, I., Leitao, F., and Teodosio, M. (2019). The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its non-native distribution into the Ria Formosa lagoon and the Guadiana estuary (SW-Iberian Peninsula, Europe). *BioInvasions Records* 8(1): 123-133. doi: 10.3391/bir.2019.8.1.14

Perdikaris, C., Konstantinidis, E., Georgiadis, C., and Kouba, A. (2017). Freshwater crayfish distribution update and maps for Greece: Combining literature and citizen-science data. *Knowledge & Management of Aquatic Ecosystems* 418: 12. doi: 10.1051/kmae/2017042

Pinto, P., Oliveira-Junior, J.M.B., Leitão, F., Morais, M.M., Chícharo, L., Vaz, P., Delgado, S.M., Voreadou, C., Morales, E.A., and Teodósio, M.A. (2020). Development of a metric of aquatic invertebrates for volunteers (MAIV): A simple and friendly biotic metric to assess ecological quality of streams. *Water* 12(3): 654. doi: 10.3390/w12030654

Pocock, M.J., Roy, H.E., Fox, R., Ellis, W.N., and Botham, M. (2017). Citizen science and invasive alien species: Predicting the detection of the oak processionary moth *Thaumetopoea processionea* by moth recorders. *Biological Conservation* 208: 146-154. doi: 10.1016/j.biocon.2016.04.010

Poirier, L.A., Ramsay, A.P., St-Hilaire, S., and Quijón, P.A. (2017). Revisiting recent history: Records of occurrence and expansion of the European green crab across Prince Edward Island, Atlantic Canada. *Marine Biodiversity Records* 10: 1-11. doi: 10.1186/s41200-017-0132-z

Pyšek, P., and Richardson, D.M. (2010). Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources* 35: 25-55. doi: 10.1146/annurev-environ-033009-095548

Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R., and Gough, K.C. (2014). The

detection of aquatic animal species using environmental DNA–a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology* 51(5): 1450-1459. doi: 10.1111/1365-2664.12306

Ribeiro, F., and Veríssimo, A. (2014). A new record of *Callinectes sapidus* in a western European estuary (Portuguese coast). *Marine Biodiversity Records* 7(E36). doi: 10.1017/S1755267214000384

Ribeiro, J., Bentes, L., Coelho, R., Gonçalves, J.M., Lino, P.G., Monteiro, P., and Erzini, K. (2006). Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). *Estuarine, Coastal and Shelf Science* 67(3): 461-474. doi: 10.1016/j.ecss.2005.11.036

Righi, S., Prevedelli, D., and Simonini, R. (2020). Ecology, distribution and expansion of a Mediterranean native invader, the fireworm *Hermodice carunculata* (Annelida). *Mediterranean Marine Science* 21(3): 558-574. doi: 10.12681/mms.23117

Robinson, L.D., Tweddle, J.C., Postles, M.C., West, S.E., and Sewell, J. (2013). *Guide to running a BioBlitz 2.0. Available at: https://www.bnhc.org.uk/communicate/guide-to-running-a-bioblitz-2-0/.* [Accessed August 23, 2022].

Santos, M.N., Monteiro, C., and Lasserre, G. (1996). Faune ichtyologique comparée de deux récifs artificiels du littoral de la Ria Formosa (lagune Portugal): résultats préliminaires. *Oceanologica Acta* 19(1): 89-97.

Santos, M.N., and Monteiro, C.C. (1997). The Olhao artificial reef system (south Portugal): Fish assemblages and fishing yield. *Fisheries Research* 30(1-2): 33-41. doi: 10.1016/S0165-7836(96)00567-X

Santos, M.N., and Monteiro, C.C. (1998). Comparison of the catch and fishing yield from an artificial reef system and neighbouring areas off Faro (Algarve, south Portugal). *Fisheries Research* 39(1): 55-65. doi: 10.1016/S0165-7836(98)00169-6

Santos, M.N., Saldanha, H.J., and Garcia, A. (2002a). Observations on by-catch from a tuna trap fishery off the Algarve (Southern Portugal). *Collective Volume of Scientific Papers - ICCAT* 54(5): 1726-1732.

Santos, M.N, Gaspar, M.B., Vasconcelos, P., and Monteiro, C.C. (2002b). Weight–length relationships for 50 selected fish species of the Algarve coast (southern Portugal). *Fisheries Research* 59(1-2): 289-295. doi: 10.1016/S0165-7836(01)00401-5

Santos, M.N., Monteiro, C.C., and Lasserre, G. (2005). Observations and trends on the intra-annual variation of the fish assemblages on two artificial reefs in Algarve coastal waters (southern Portugal). *Scientia Marina* 69(3): 415-426. doi: 10.3989/scimar.2005.69n3415

Silva, C., and Borges, M.F. (2014). Crustáceos-2014, Julho. Relatórios de Campanha. IPMA, Lisboa. 24 pp

Simberloff, D. (2011). How common are invasion-induced ecosystem impacts? *Biological Invasions* 13(5): 1255-1268. doi: 10.1007/s10530-011-9956-3

Simberloff, D., and Rejmánek, M. (2011). Encyclopedia of biological invasions. University of California Press, Berkeley, CA. 792 pp.

Simoniello, C., Jencks, J., Lauro, F.M., Loftis, J.D., Weslawski, J.M., Deja, K., Forrest, D.R., Gossett, S., Jeffries, T.C., and Jensen, R.M. (2019). Citizen-science for the future:

Advisory case studies from around the globe. *Frontiers in Marine Science* 6: 225. doi: 10.3389/fmars.2019.00225

Simonini, R., Righi, S., Zanetti, F., Fai, S., and Prevedelli, D. (2021). Development and catch efficiency of an attracting device to collect and monitor the invasive fireworm *Hermodice carunculata* in the Mediterranean Sea. *Mediterranean Marine Science* 22(3): 706-714. doi: 10.12681/mms.26916

Simpson, J.A., and Weiner, E.S.C. (2014). "Citizen science" in The Oxford English dictionary. Clarendon Press, Oxford, UK.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., and Lourie, S.A. (2007). Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *BioScience* 57(7): 573-583. doi: 10.1641/B570707

Tankersley, R.A., Wieber, M.G., Sigala, M.A., and Kachurak, K.A. (1998). Migratory behavior of ovigerous blue crabs *Callinectes sapidus*: Evidence for selective tidal-stream transport. *The Biological Bulletin* 195(2): 168-173. doi: 10.2307/1542824

Thorrold, S.R., Jones, C.M., Swart, P.K., and Targett, T.E. (1998). Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. *Marine Ecology Progress Series* 173: 253-265. doi: 10.3354/meps173253

Thorrold, S.R., Latkoczy, C., Swart, P.K., and Jones, C.M. (2001). Natal homing in a marine fish metapopulation. *Science* 291(5502): 297-299. doi: 10.1126/science.291.5502.297

Tulloch, A.I., Possingham, H.P., Joseph, L.N., Szabo, J., and Martin, T.G. (2013). Realising the full potential of citizen science monitoring programs. *Biological Conservation* 165: 128-138. doi: 10.1016/j.biocon.2013.05.025

Turnure, J.T., Grothues, T.M., and Able, K.W. (2015). Seasonal residency of adult weakfish (*Cynoscion regalis*) in a small temperate estuary based on acoustic telemetry: A local perspective of a coast wide phenomenon. *Environmental Biology of Fishes* 98: 1207-1221. doi: 10.1007/s10641-014-0353-5

Vacchi, M., Morri, C., Modena, M., La Mesa, G., and Bianchi, C.N. (2001). Temperature changes and warm-water species in the Ligurian Sea: The case of the ornate wrasse *Thalassoma pavo* (Linnaeus, 1758). *Archo Oceanography Limnology* 22: 149-154.

Vasconcelos, P., Carvalho, A.N., Pilo, D., Gaspar, M.B., and Cristo, M. (2017). First record of the spottail mantis shrimp, *Squilla mantis* (Stomatopoda, Squillidae), in the Ria Formosa lagoon (Algarve coast, southern Portugal). *Crustaceana* 90(13): 1665-1671. doi: 10.1163/15685403-00003717

Vasconcelos, P., Carvalho, A.N., Moura, P., Ramos, J., and Gaspar, M.B. (2018). First record of *Acanthurus monroviae* (Osteichthyes: Perciformes: Acanthuridae) in southern Portugal, with notes on its recent distributional spread in the northeastern Atlantic and Mediterranean. *Marine Biodiversity* 48: 1673-1681. doi: 10.1007/s12526-017-0653-z

Vasconcelos, P., Carvalho, A.N., Piló, D., Pereira, F., Encarnação, J., Gaspar, M.B., and Teodósio, M.A. (2019). Recent and consecutive records of the Atlantic blue crab (*Callinectes sapidus* Rathbun, 1896): Rapid westward expansion and confirmed establishment along the Southern Coast of Portugal. *Thalassas: An International Journal*

of Marine Sciences 35(2): 485-494. doi: 10.1007/s41208-019-00163-1

Veiga, P., Vieira, L., Bexiga, C., Sá, R., and Erzini, K. (2006). Structure and temporal variations of fish assemblages of the Castro Marim salt marsh, southern Portugal. *Estuarine, Coastal and Shelf Science* 70(1-2): 27-38. doi: 10.1016/j.ecss.2006.05.037

Verdes, A., Simpson, D., and Holford, M. (2018). Are fireworms venomous? Evidence for the convergent evolution of toxin homologs in three species of fireworms (Annelida, Amphinomidae). *Genome Biology and Evolution* 10(1): 249-268. doi: 10.1093/gbe/evx279

ViaLivre (2021). *ViaLivre. Available at: https://www.vialivre.pt/pt-pt/portagens/portagens-a22/.* [Accessed September 01, 2021].

Vinagre, C., Santos, F.D., Cabral, H., and Costa, M.J. (2011). Impact of climate warming upon the fish assemblages of the Portuguese coast under different scenarios. *Regional Environmental Change* 11: 779-789. doi: 10.1007/s10113-011-0215-z

Widenfalk, L., Ahrné, K., and Berggren, A. (2014). Using citizen-reported data to predict distributions of two non-native insect species in Sweden. *Ecosphere* 5(12): 1-16. doi: 10.1890/ES14-00212.1

Yamada, S.B., Peterson, W.T., and Kosro, P.M. (2015). Biological and physical ocean indicators predict the success of an invasive crab, *Carcinus maenas*, in the northern California Current. *Marine Ecology Progress Series* 537: 175-189. doi: 10.3354/meps11431

Zenetos, A., Koutsogiannopoulos, D., Ovalis, P., and Poursanidis, D. (2013). The role played by citizen scientists in monitoring marine alien species in Greece. *Cahiers de Biologie Marine* 54: 419-426.

CHAPTER 5

Invasive species in a blue economy: Opportunities and constraints

Sections of the present chapter are to be published in the following peer-reviewed scientific articles under the objectives of this Ph.D. thesis:

- **Encarnação J**, Teodósio MA, Morais P (in preparation). Marine invasive species in a blue economy: Opportunities and constraints in Portugal.

5.1 Introduction

In a globalized world, biological invasions are common and must be faced with pragmatism. Therefore, the encouragement of prevention and implementation of early-detection mechanisms are being complemented with a more pragmatic perspective by eradicating what can be eradicated, control and manage through time what is financially feasible, and innovate when it comes to deal with invasive species that may provide ecological or economic benefits. Still, multiple caveats may arise from harvesting and commercializing invasive species, mainly because the creation of a market may encourage people to maintain such species or even recreate such market in previously uninvaded ecosystems (Nuñez et al. 2012). To reduce these possibilities, such management approaches must also be supported by strong scientific monitoring and outreach campaigns to ensure the best practices among stakeholders (Nuñez et al. 2012; Pasko and Goldberg 2014; Carballo-Cárdenas and Tobi 2016; Cerveira et al. 2022).

Edible marine invasive species have the potential to be more easily integrated in local economies. In the Caribbean Sea, the invasive lionfish *Pterois miles* (Bennett, 1828) is probably one of the most emblematic examples. The growing populations of this invasive species have been monitored with citizen science initiatives, controlled locally through fishing and spearfishing, and the fish sold in markets and restaurants (Barbour et al. 2011; Carballo-Cardenas et al. 2016; Clements et al. 2021). Still, these efforts need a long-term commitment to maintain local populations under control (Barbour et al. 2011). The buzz around lionfish has been such that it has become the highlight dish of many local restaurants and even deserving the publication of a cookbook dedicated to this species (Fendt 2014; Ferguson and Akins 2016; Smith et al. 2023). Across the Eastern Mediterranean Sea, a similar invasion process is underway, while researchers and managers are urged to take measures, many learned from the Western Atlantic experience, including routine removals by spearfishing, encouraging the development of recreational and commercial lionfish fisheries, and engage local communities and resource users in achieving multiple objectives, as promoting lionfish removals, market-development, research, and public education (Ulman et al. 2022). The European native shore crabs *Carcinus* spp. have become global invaders, with established populations in the Pacific and Atlantic coasts of North America, Australia, South Africa, and Japan (Ens et al. 2022). Across North America, the European green crab *Carcinus maenas* (Linnaeus, 1758) has rapidly became a source of concern due to their rapid expansion (Grason et al. 2018; Ens et al. 2022). Nevertheless, potential uses for this invader have been suggested and tested – production of

soft-shell green crab, a potentially valuable product, comparable to the traditional North American Atlantic blue crab soft-shell (St-Hilaire 2016); using the species to produce fermented condiments, similar to other commercial fish sauce products (Greiner et al. 2021); by extracting bio-products as chitin and protein isolate (Khiari et al. 2020); or even to flavour a whiskey produced in New Hampshire (Osborne 2022).

The negative economic impacts of invasive species are frequently highlighted, and for obvious reasons; for example, the economic impact of invasive species surpassed 21 billion US$ annually between 2010 and 2020 in North America (Fantle-Lepczyk et al. 2022). Still, an assessment of all possible trade-offs should be undertaken to evaluate the economic losses and putative benefits in the form of ecosystem services. For example, in South Africa, invasive plants like wattles (*Acacia* spp.), eucalypts (*Eucalyptus* sp.), or pine (*Pinus* sp.) can negatively impact ecosystem services because of the excess use of water in comparison to native species, because they set the conditions for severe wildfires which result in higher rates of soil erosion, or by invading beach areas and altering areas that are sought-after for ecotourism (Pejchar and Mooney 2009). Concurrently, these species provide multiple goods like firewood and charcoal (valued at up to 143 million US$), building materials, and paper products, while sequestering more carbon than native plants (a service valued in 24 million US$ in potentially tradable stored carbon) (Pejchar and Mooney 2009). In Hawaii, feral pigs can damage crops by eating roots and trampling the soil, resulting in increased soil erosion and risk of more severe floods, while having an important cultural heritage due to spiritual and religious values for local culture, providing subsistence food, and creased recreation and tourism hunting (Pejchar and Mooney 2009). In the southern Iberian Peninsula, including the Mediterranean coasts of Spain until southern Portugal mainland and islands in the Atlantic Ocean, the invasive algae *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim, 2009, has become a severe issue for the environment, fisheries, and tourism (García-Gómez et al. 2020, 2021; Faria et al. 2022; Liulea 2021; Liulea et al. 2023). However, this may be used in biogas production, composting, production of bioplastic materials, and pharmaceutical formulations with anti-inflammatory, antimicrobial, and antidiabetic properties (Barcellos et al. 2023). So, the commercial valorisation of this algae can drive private investors to contribute for the removal of the biomass from public beaches, decreasing the cost to local authorities that nowadays have to collect and discard such biomass from touristic beaches.

In 2016, fishers from southern Portugal found two species native from the west Atlantic – the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896, and the weakfish *Cynoscion regalis*

(Bloch & Schneider, 1801) – which soon became new fishing resources (Morais and Teodósio 2016; Morais et al. 2017, 2019; Vasconcelos et al. 2019; Cerveira et al. 2021). Across their native range, these two species support valuable fisheries, despite the recent low historical values in landings, and are deeply embedded in the recreational and commercial fishing culture (Wilk 1979; Guillory et al. 2001; Paolisso 2007; ASMFC 2016, 2019).

The Atlantic blue crab supports large commercial and recreational fisheries across North America, from the Northwest Atlantic shores (e.g., Chesapeake Bay, Maryland; South Carolina) towards the Gulf of Mexico (e.g., coasts of Florida; Alabama; Louisiana), being harvested both as hard- and soft-shell crabs (Millikin and Williams 1984; Orth and van Montfrans 1990; Guillory et al. 2001). In 2021 alone, landings have summed over 53,000 metric tons, worth more than 240 million US$ (NOAA 2023). The Chesapeake Bay area likely hosts the largest Atlantic blue crab fisheries in North America and, as in other native regions, it's overexploited with over 60% of the fishable population harvested between 1998 and 2002 (Miller et al., 2005). The Chesapeake Bay's landings accounted for over 75% of the United States landings until 1950, but it has decreased to less than 50% in the late 1990s (Stagg and Whilden 1997; Guillory et al. 2001). In this region alone, in the early 1990s the Atlantic blue crab fishery was a 52,000 metric tons industry, worth 72 million US$, but declined to 28,000 metric tons and 61 million US$ fishery by 2004 (Zohar et al. 2008). Across the non-native range, several fisheries have emerged, namely in the Mediterranean Sea. For example, in Turkey during the yearly 2000s about 200 metric tons were sold annually (Nehring 2011), with further reports of 77 metric tons in 2009 (Ayas and Ozogul 2011). The average selling price in Turkish markets in 2020 ranged from 0.34 to 2.24 € kg^{-1}, and up to 9 € kg^{-1} for exported Atlantic blue crabs (Öndes and Gökçe 2021). In the northern Aegean Sea, annual landings reached 84 metric tons in the Thermaikos Gulf in 2010, and 34 metric tons in the Vistonikos Gulf in 2015 (Kevrekidis and Antoniadou 2018). In the Ebro delta in the Spanish Mediterranean coast, landings reached over 35 metric tons by the end of 2019 (Clavero et al. 2022). In southern Portugal, although the Atlantic blue crab was first recorded in 2016 (Morais et al. 2019), by the summer of 2018 the species had already reached marked values of up to 14 € kg^{-1} on a wholesale market in Vila Real the Santo António by the mouth of the Guadiana River, or 15 € kg^{-1} on Olhão's public market in 2019 (Vasconcelos et al. 2019). In the native range, at Daytona Beach, Florida, selling prices for hard-shell crabs may range between 25–35 US$ (22.5–31.5 €) for a dozen of medium size crabs, to 50–75 US$ (45–67.5 €) for large size crabs, while a dozen of soft-shell crabs may cost up to 80 US$ (72 €) (Stoney Farms 2023).

Weakfish is another species widely recognisable in North America, both among commercial recreational fishers (Wilk 1979; ASMFC 2016, 2019). Commercial landings show a maximum over 8,000 metric tons in 1982, and a minimum of 48 metric tons in 2018 (ASMFC 2019; NOAA 2023). Among recreational landings, values peaked in 1987 with over 9,000 metric tons, and by 2011 decreased to a minimum of 46 metric tons (ASMFC 2019). In 2021, annual landings in the United States summed 88 metric tons worth over 380,000 US$ in commercial fisheries, while recreational landings reached 243 metric tons (NOAA 2023). In the native range, weakfish is overfished, and the stock's declining trend has led the IUCN to classify the species as 'Endangered' since 2019 (Barbieri and Barbieri 2020). In the Eastern Atlantic, across the south and western Iberian Peninsula, the non-indigenous weakfish established several populations along the Gulf of Cadiz, southern Portugal, and the Sado and Tejo estuaries in western Portugal (Morais and Teodósio 2016; Bañón et al. 2017; Morais et al. 2017; Cerveira et al. 2021; González et al. 2021; but also see **Chapter 4**). In western Portugal, the weakfish was being sold from 3 to 10 € kg^{-1} in the Setúbal fish market, while consumers reported that they would be willing to pay 8.3 ± 6.2 € kg^{-1} (Cerveira et al. 2022). In 2019 across the native range, the average retail price was 3.95 US$ kg^{-1} (~ 3.5 € kg^{-1}), and according to the Fulton Fish Market, fresh weakfish were sold for 26.76 US$ kg^{-1} (~ 24 € kg^{-1}) (Cerveira et al. 2022). These species have then an economic value, both in native and non-native ranges, contributing for the blue economy around marine fishery resources.

Blue economy has been defined as the sustainable use of ocean's living- and non-living resources for economic growth, improved livelihoods and jobs, and ocean ecosystem health is a societal challenge driven by science, education, and entrepreneurship (Smith-Godfrey 2016; Lee et al. 2020). The blue economy concept overarches numerous activity clusters, such as fisheries, aquaculture, biotechnology, transport and its infrastructure, renewable energy, tourism, and coastal protection – and just to cite a few. Coastal marine fisheries have been a core staple of the southern Portugal economy for centuries, namely the Algarve region. Without surprise, the Algarve's seafood has become a hallmark of the tourism industry since its inception in 1960s. Yet, the overexploitation of important fisheries during the second half of the 20th century (e.g., sardines, tuna) is a legacy that still poses challenges to current generations, while also coping with the impacts of climate change (Leitão 2015; Leitão et al. 2018; Baptista et al. 2018). Introducing invasive species in the blue economy, if properly planned, is an opportunity to support ecosystem and natural resource management while simultaneously boosting economic development and environmental awareness (Pasko and

Goldberg 2014). Therefore, this study has three objectives: 1) evaluate the potential economic impact of two non-indigenous marine species in southern Portugal, the Atlantic blue crab and weakfish, by comparing national wholesale landings of commercially valuable native species and these two non-indigenous species; 2) assess their economic value in the native and non-native ranges, and potential impacts in the non-native range; 3) evaluate if the integration of these new fishery resources into the blue economy of the non-native range has the potential to support the management of non-indigenous marine species in Portugal.

5.2 Material and methods

5.2.1 Effects of marine invasive species in native species stocks

Using Portuguese wholesale market statistics between 2011 and 2022 (supplied by DocaPesca), an assessment was carried out on the potential effects of the introduction of the Atlantic blue crab and weakfish in mainland Portugal. This study was divided into two regions where the two non-indigenous species are established and most abundant – the Atlantic blue crab is more abundant in southern Portugal, while weakfish in more abundant off the mid-western Portuguese coast, namely in the Tejo and Sado estuaries. The landings statistics of native species, with economic value in Portugal, and that could be more affected by each non-indigenous species, were analysed in each study zone.

In the case of the Atlantic blue crab, data from wholesale fishing markets of the Algarve region (n= 4) were analysed because the species is abundant and spread along the entire region (Morais et al. 2019; Vasconcelos et al. 2019; but see **Chapters 2** and **4**). This data was compared with landings of the native European green crab *Carcinus maenas* sold in Algarve's wholesale markets (n= 7). In 2017 and 2018, the Atlantic blue crab began being caught in the Guadiana estuary, but it was sold as European green crab in wholesale markets (R. Gonçalves, personal communication), until 2019 when the species was officially included in the list of wholesale market species. The landings of this native crab were compared with the non-indigenous Atlantic blue to assess competition and predation effect, since the European green crab is one of the most abundant and widely distributed estuarine crabs in Portugal (Sprung 2001; Monteiro et al. 2022; Portela et al. 2023). Also, data from the Ebro Delta in Spain showed the collapse of native crab populations after the establishment of the invasive Atlantic blue crab (Clavero et al. 2022).

Regarding weakfish, data from wholesale fishing markets surrounding the Tejo and Sado estuaries were analysed (*n*= 6), where the species is very abundant throughout the year (Morais and Teodósio 2016; Morais et al. 2017; Cerveira et al. 2021; but also see **Chapter 4**), and compared with landings of three native species – cuttlefish *Sepia officinalis* Linnaeus, 1758, meagre *Argyrosomus regius* (Asso, 1801), and seabass *Dicentrarchus labrax* (Linnaeus, 1758). Data from these three species were obtained from wholesale markets located in the same region (*n*= 13). The two native fish, meagre and seabream, may compete for resources – food and space – as already reported for the Sado estuary (Cerveira et al. 2021). Cuttlefish is a potential weakfish prey and a gastronomic hallmark of the region, particularly in the Sado estuary. As a control dataset, landings of the four native species were analysed at a national level for the remaining wholesale markets (*n*= 38), i.e., excluding the markets analysed in the two regions of interest, the Algarve and the Tejo-Sado.

The temporal daily landings trend for the five native species were assessed with the Pearson correlation test and respective significance value (*p*-value) for the time-series available in each case and by study region – green crab, meagre, seabass, and cuttlefish, between 2011-2022; Atlantic blue crab between 2019-2022; weakfish between 2016-2022. Correlation plots of landings between each invasive species and native species for the two study regions were produced for the periods since each invasive species was detected – the Atlantic blue crab since 2016 in the Algarve region (Morais et al. 2019), and the weakfish since 2014 in the region of the Tejo and Sado estuaries (Morais et al. 2017). For each pair of comparisons, the Pearson correlation coefficient and significance value (*p*-value) of the test were calculated. Statistical analyses and plots were produced in R, using the software R Studio version 2022.07.2+576 (RStudio Team, 2022).

5.2.2 Market value of the Atlantic blue crab and the weakfish in native and non-native areas

The wholesale market prices and landings of the Atlantic blue crab and weakfish in Portugal and the United States, were compared to assess differences in their market value. Portuguese wholesale market statistics were supplied by DocaPesca. Wholesale landings and value for the Atlantic blue crab in the Algarve comprise data from all wholesale markets in the Algarve region (*n*= 4) due to the species conspicuous distribution in the region (Morais et al. 2019; Vasconcelos et al. 2019; but see **Chapters 2** and **4**). Weakfish wholesale market prices

from wholesale fishing markets surrounding the Tejo and Sado estuaries were analysed considered (n= 6) to encompass the areas where the species is currently very abundant throughout the year (Morais and Teodósio 2016; Morais et al. 2017; Cerveira et al. 2021; but also see **Chapter 4**). Fishery landings statistics from the United States were retrieved from the National Oceanic and Atmospheric Administration website (NOAA 2023), for the period 2011-2021 (data from 2022 is not available yet). The temporal changes in market value and landings were assessed with the Pearson correlation test and respective significance value (p-value) for the time-series available in each variable. Additional information on sale prices to the public in the United States was also gathered from the internet, and in Portugal from reports made to the NEMA citizen science campaign (see **Chapter 4**), and from personal communications and observations.

5.2.3 SWOT analysis

The strengths, weaknesses, opportunities, and threats (SWOT) analysis is a strategic planning tool widely used in business planning (Benzaghta et al. 2021), but also applied to environmental sciences (Paliwal 2006; Sayyed et al. 2013), including participatory fisheries management (Viegas et al. 2014; Murillas-Maza et al. 2021) and native and non-indigenous species management (García-Arberas et al. 2009; de Leaniz et al. 2010; Mancinelli et al. 2017; Benyahkoub et al. 2021).

This SWOT analysis was developed on the specific topic of the commercial valorisation of marine invasive species in Portugal, and their potential integration into the blue economy as a management tool. Information was gathered from the literature on native and invaded zones, conversations with experts (scientists, managers) from both the native and invaded range, and personal interactions with recreational and professional fishers (local ecological knowledge) from the invaded range and gathered through the NEMA citizen science campaign (see **Chapter 4)**.

5.3 Results

5.3.1 Effects of invasive species in native species stocks

Wholesale market records of the Atlantic blue crab began in April 2019 in the Algarve (5 markets), and in June 2016 for weakfish in wholesale fishing markets surrounding the Tejo

and Sado estuaries (6 markets). In the Algarve, both green and Atlantic blue crabs showed no significant trends in daily landings (**Figure 5.1A**). Between 2019-2022, a total of 2786.5 kg of Atlantic blue crabs were sold, with a monthly average of 58.1 ± 88.6 kg. Daily maximums reached 86.0 kg (March 03, 2020), 103.4 kg (March 09, 2020) and or 87.9 kg (February 14, 2022) (**Figure 5.1A**). The correlation between landings of the two specie did not show a clear influence of one species upon the other (**Figure 5.2A**).

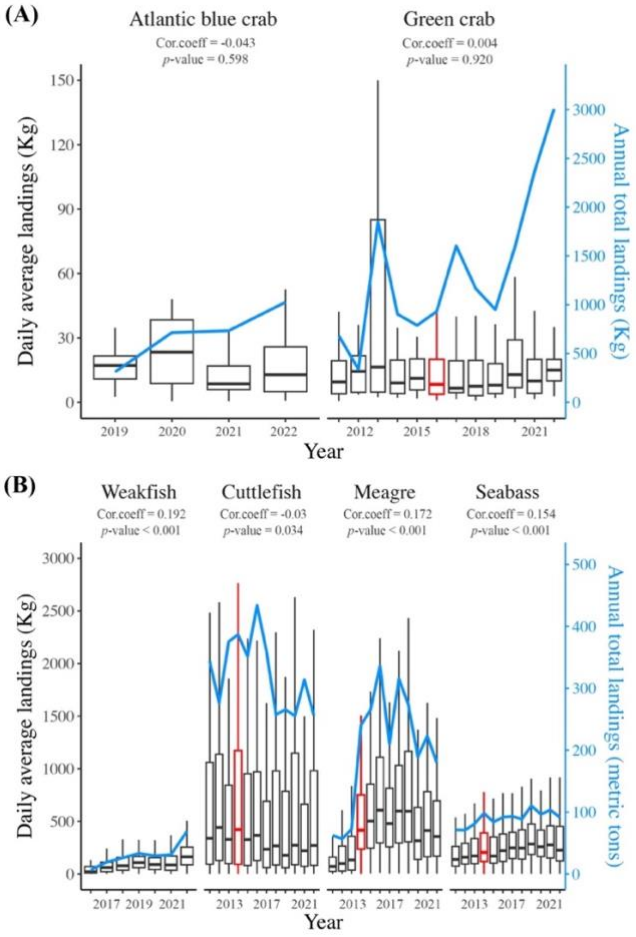


Figure 5.1 – Daily average of wholesale market landings (black boxplots) and annual total landings (blue line) for (A) the invasive Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 and native European green crab *Carcinus maenas* (Linnaeus, 1758) in five markets in the Algarve, and for (B) weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) and three native species – cuttlefish *Sepia officinalis* Linnaeus, 1758, meagre *Argyrosomus regius* (Asso, 1801), and seabass *Dicentrarchus labrax* (Linnaeus, 1758), from six markets surrounding the Tejo and Sado estuaries. In the boxplots, lower and upper fences represent the 25th and 75th percentiles, and the median is in between. Bars represent 10th and 90th percentiles, and outliers are not disclosed for improved viewing. Red boxplots indicate the year of first detection of each invasive species – 2016 for the Atlantic blue crab in the Algarve (Morais et al. 2019), and 2014 for weakfish in the Sado estuary (Morais and Teodósio 2016). Wholesale market statistics kindly supplied by DocaPesca. The Pearson's correlation coefficient and respective p-value are provided for each species.

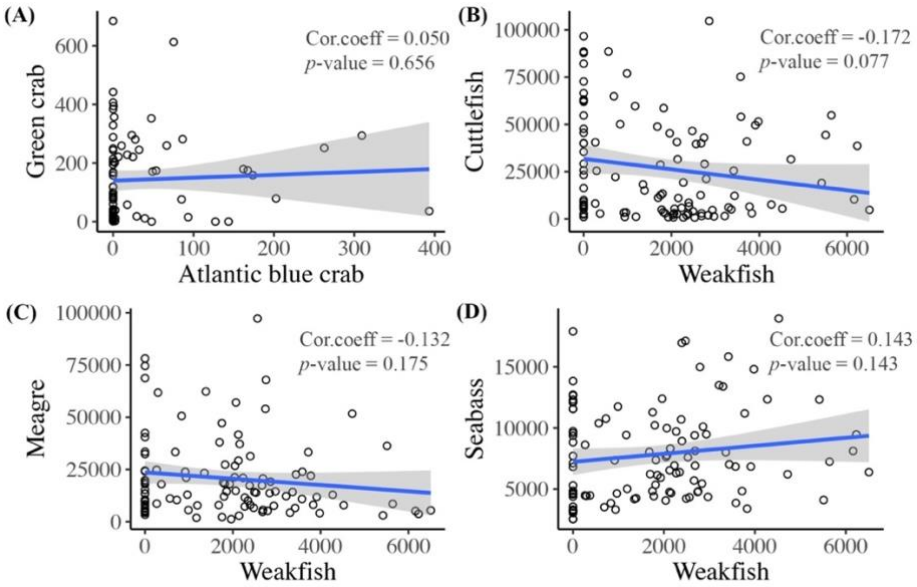


Figure 5.2 – Correlation plots of wholesale market landings between (A) the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896, and the native European green crab *Carcinus maenas* (Linnaeus, 1758) in five markets in the Algarve; and between weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) and the native species (B) cuttlefish *Sepia officinalis* Linnaeus, 1758, (C) meagre *Argyrosomus regius* (Asso, 1801), and (D) seabass *Dicentrarchus labrax* (Linnaeus, 1758) in six markets surrounding the Tejo and Sado estuaries. In each study case, data comprises the period after the invasive species were detected in each region – 2016 for the Atlantic blue crab in the Algarve (Morais et al. 2019), and 2014 for weakfish in the Sado estuary (Morais and Teodósio 2016) – although market sales only began later. Wholesale market statistics kindly supplied by DocaPesca. The Pearson's correlation coefficient and respective p-value are provided for each comparison.

Weakfish landings in the Sado and Tejo estuaries increased significantly in the period 2016-2022, surpassing a total of 214 metric tons, with a monthly average of 2554.9 ± 2108.4 kg (**Figure 5.1B**). In 2022, daily landings were particularly high – 7019 kg (January 11), 3756 kg (January 20), 2827 kg (February 11), 1915 kg (April 06). January 2022 alone accounted for 7.5 % of the total weakfish landings – over 16 metric tons – since the species began being sold in that region. Regarding the three native species in the Sado and Tejo estuaries, only cuttlefish showed a noticeable decrease in landings since 2011 (p= 0.033), while meagre and seabass showed an overall increase (**Figure 5.1B**). The cuttlefish decrease showed a moderate correlation (coefficient= -0.172; p= 0.077) with the increase of the invasive weakfish (**Figure 5.2B**), while meagre and seabass showed no statistically significant correlations (**Figure 5.2C** and **D**).

National landings (control dataset) of the four native species showed an increasing trend in the case of the green crab and the seabass ($p\leq$ 0.05), a small decrease in landings of the meagre, and no temporal changes in the case of the cuttlefish (**Figure 5.3**).

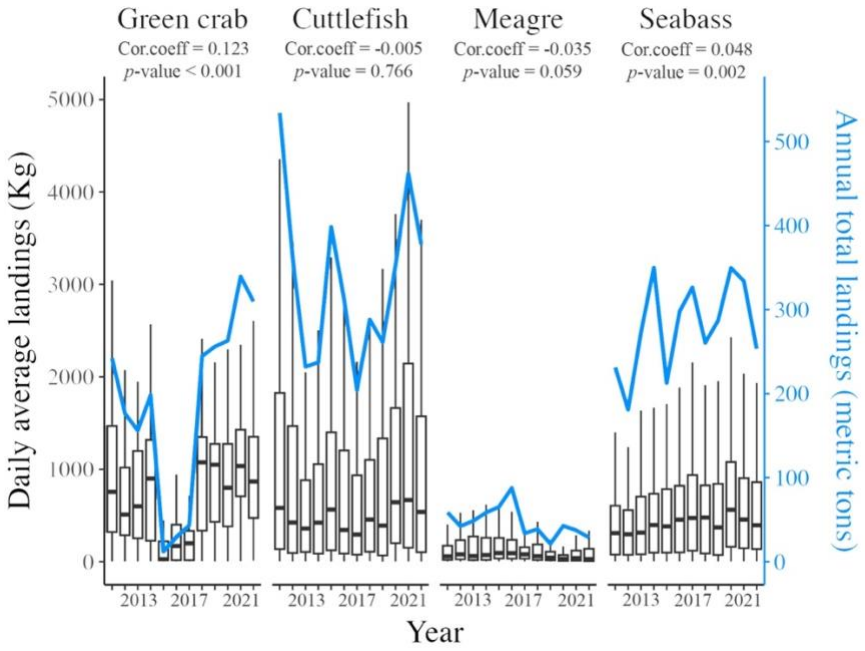


Figure 5.3 – Control dataset of national daily wholesale market landings (black boxplots) and annual total landings (blue line) for four native species, European green crab *Carcinus maenas* (Linnaeus, 1758), cuttlefish *Sepia officinalis* Linnaeus, 1758, meagre *Argyrosomus regius* (Asso, 1801), and seabass *Dicentrarchus labrax* (Linnaeus, 1758). Dataset includes national landings for all wholesale markets excluding the markets in the two regions under analysis for the invasive-native species dataset (*n*= 38). In the boxplots, lower and upper fences represent the 25th and 75th percentiles, and the median is in between. Bars represent 10th and 90th percentiles, and outliers are not disclosed for improved viewing. Wholesale market statistics kindly supplied by DocaPesca. The Pearson's correlation coefficient and respective *p*-value are provided for each species.

5.3.2 Market value of the Atlantic blue crab and the weakfish

The Atlantic blue crab started being sold in Algarve's wholesale markets in 2019 and prices have steadily increased since then, with a maximum of 8125 € of gross sales in 2022 for a total 1026 kg (**Figure 5.3**). The average selling price has also increased from 7.9 ± 1.2 € kg^{-1} in 2019 to 9.5 ± 3.2 € kg^{-1} in 2022. In four years, the total landings were 2786.5 kg corresponding to a total value of over 22k € at wholesale markets. In the United States, landings have been decreasing during the analysed period, from 91,906 metric tons in 2011 to 53,526 metric tons in 2021 (**Figure 5.3**). Inversely, gross sale values have been increasing, from over 184 million US$ in 2011 to over 240 million US$ in 2021.

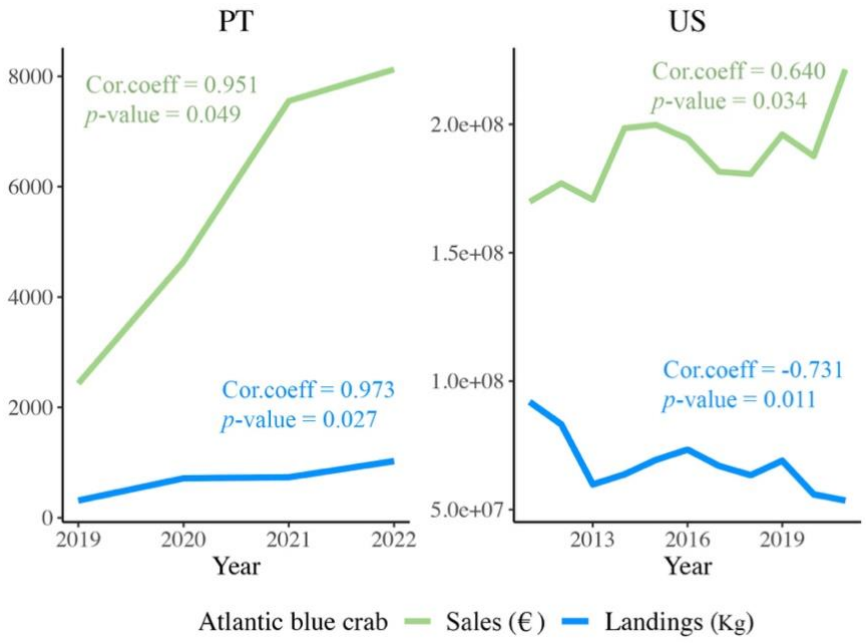


Figure 5.3 – Yearly gross sales and landings of the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 in wholesale markets in Algarve (southern Portugal, PT) between 2019-2022, and the United States (US) between 2011-2021. Portuguese landings statistics were supplied by DocaPesca, and data from the United States was retrieved from NOAA (2023). The Pearson's correlation coefficient and respective *p*-value are provided for each variable.

Weakfish landings increased 10.7 times in the Sado and Tejo estuaries from 6440 kg in 2016 to 68,983 kg in 2022 (**Figure 5.4**), corresponding to gross sales of 4946 € in 2016 and 151,789 € in 2022, the highest revenue on record. The average selling price has increased from 1.1 ± 0.8 € kg^{-1} in 2016 to 2.7 ± 1.1 € kg^{-1} in 2022. In six years, 214 metric tons of weakfish were landed and sold for a total of over 458k € at wholesale markets. In the United States, commercial landings maxed in 2013 with 161 metric tons, and it has been decreasing since then, with a minimum of 48 metric tons in 2018, and 88 metric tons in 2021 (**Figure 5.4**). Gross sales of commercial landings followed a similar pattern, with a maximum of 576,728 US$ in 2013 and a minimum of 201,415 US$ in 2018. Interestingly, data on recreational landings in the native range is also available. These were always higher than commercial landings, with a maximum of 337 metric tons in 2012 and a minimum of 243 metric tons in 2021 (**Figure 5.4**).

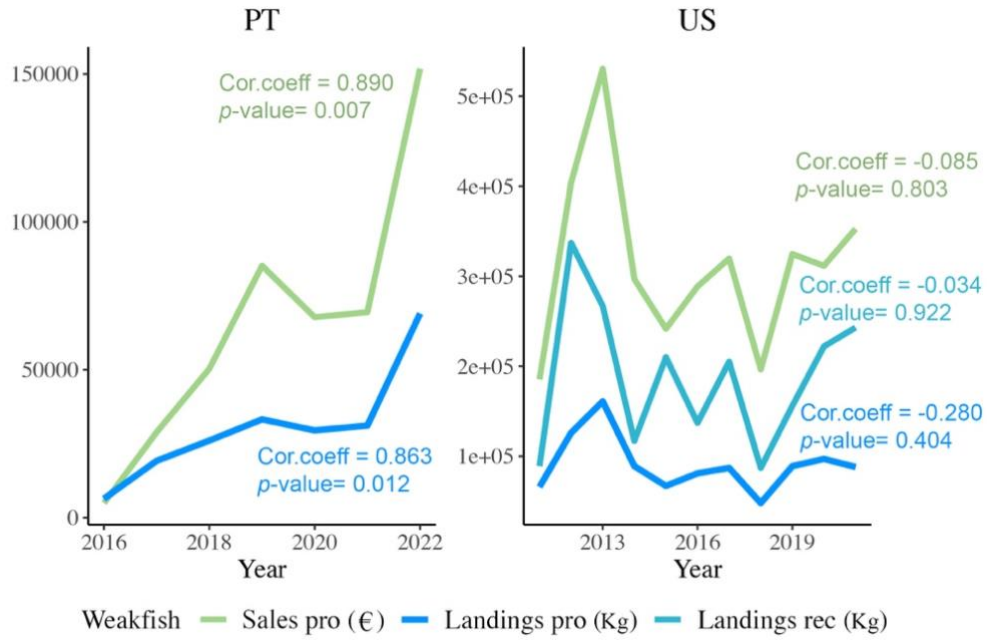


Figure 5.4 – Yearly gross sales and landings of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) in wholesale markets (pro) in the Sado and Tejo estuaries in Portugal (PT) between 2016-2022, and the United States (US) between 2011-2021, including recreational fishing landings (rec). Portuguese landings statistics from DocaPesca, and United States data retrieved from NOAA (2023). The Pearson's correlation coefficient and respective p-value are provided for each variable.

The outreach efforts of the NEMA citizen science campaign (see **Chapter 4**) gathered relevant information on the commercialization of the Atlantic blue crab to complement information from public markets across the Algarve. The first record sent by citizen scientists was in September 2018 about Atlantic blue crabs being sold in Vila Real de Santo António's market (at the mouth of the Guadiana River, Eastern Algarve) for 22 € kg^{-1} (A. Paco, personal communication; **Figure 5.5A**). In August 2019, we received a record mentioning that Atlantic blue crabs were being sold in a restaurant in the town of Odeleite, close to the middle Guadiana estuary (J. Machado, personal communication). By January 2020, a market at Albufeira (central Algarve) was selling Atlantic blue crabs (P. Norberto, personal communication). In multiple occasions, between February and April 2020, a large market in Olhão was selling Atlantic blue crabs for 8 to 11 € kg^{-1} (Intermarché Olhão, personal communication). By March 2022, a shellfish restaurant in Portimão (Western Algarve) had live Atlantic blue crabs to be selected by customers and prepared in the restaurant (J. Encarnação, personal observation). Regarding weakfish, selling prices ranged between 8 and 12 € kg^{-1} in public markets located between Olhão and Portimão (**Figure 5.5B**), although most fish were caught in the Sado

estuary and transported to southern Portugal's fish markets (J. Encarnação, personal observations).

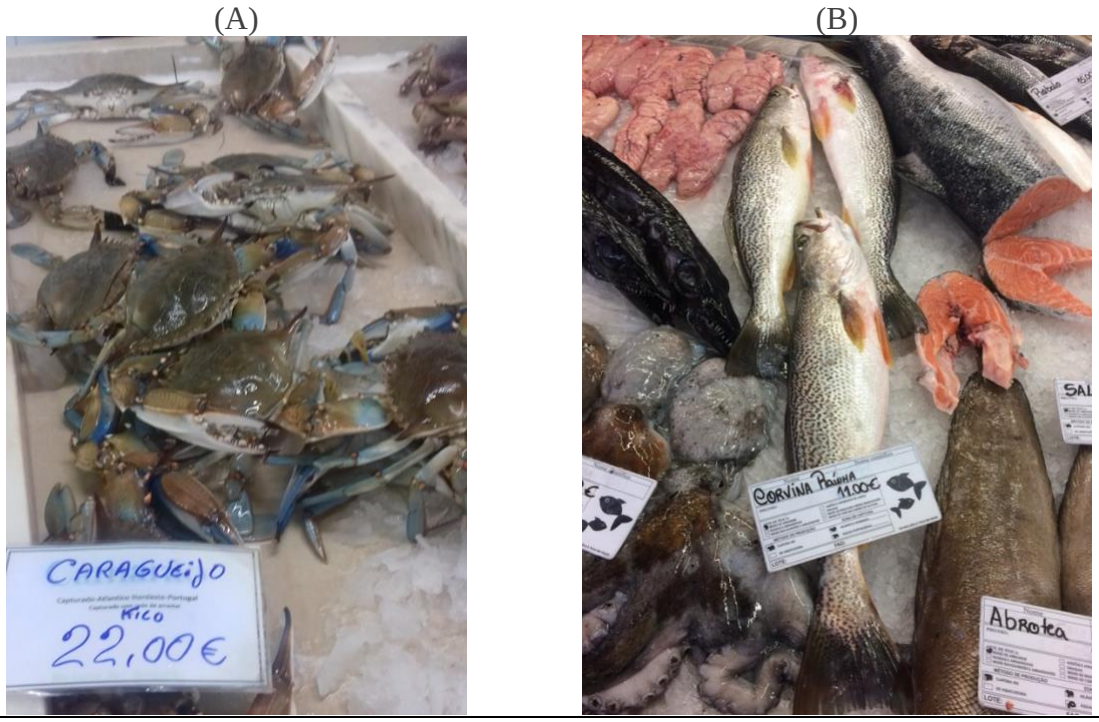


Figure 5.5 – (A) Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 at the Vila Real de Santo António market being sold for 22 € kg^{-1} in September 2018. (B) Weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) at a Portimão market being sold for 11 € kg^{-1} in March 2019. Photos by A. Paco and J. Encarnação, respectively.

5.3.3 SWOT analysis

Strengths

The increasing number of non-indigenous species and expansion of established invasive species pose serious challenges for conservation, particularly in marine and estuarine ecosystems where control and mitigation are more difficult. In such ecosystems where containment is virtually impossible, the commercial harvesting of invasive species may benefit conservation actions by becoming a target fishery, thus helping to at least sustain a lower standing stock. Additionally, locally caught seafood, for its quality and freshness, will always be preferred over other sources of mass-produced products, particularly in cultures where fresh seafood is the primary choice, like Portugal (Cerveira et al., 2021).

The introduction of edible invasive species into the blue economy is easier than for other species, like the macroalga *Rugulopteryx okamurae* in southern Iberian Peninsula which needs an elaborate production chain to be in place, which is not the case of edible marine invasive species. Unsurprisingly, the Atlantic blue crab and weakfish quickly found their way into the blue economy in Portugal without the help of environmental stakeholders, and despite their efforts (Cerveira et al. 2021). Environmental stakeholders should therefore pursue reaching out to economic stakeholders and highlight the importance of controlling invasive species and how important they can be to mitigate an environmental problem.

Various marine invasive species commercially exploited in Portugal have high market values in their native and invaded areas. This is the case of the Atlantic blue crab and weakfish, but also the Manila clam *Ruditapes philippinarum* (A. Adams & Reeve, 1850) in Portugal, which attain high prices for the final consumer 15-23 € kg^{-1}, 3-11 € kg^{-1}, 8-12 € kg^{-1}, respectively (Ramajal et al. 2016; Vasconcelos et al. 2019; Cerveira et al. 2021; Encarnação, personal observations). This helps explaining why edible invasive species can quickly weave into the local economy.

Invasive species are often highly fertile and have a fast growth which may sustain a stable and productive economic activity all-year around. Traditional harvesting equipment might need adaptations, but local fishers can quickly adapt to these new fisheries due to the similarity of fishing techniques used on similar native species combined with extensive information from the native area. A Guadiana fisherman proactively studies how US fishers capture the Atlantic blue crab, by analysing online resources about their fishing gears and fishing periods.

The extensive knowledge available in the scientific literature from the native and non-native areas, combined with years of fishing experience and processing techniques, add an increasing interest to new edible invasive species for commercial harvesting. This is particularly true in southern Europe, including in Portugal where seafood is consumed in high quantities, so the novelty of a new product in the market – available fresh and caught locally – may excite consumers. Awareness campaigns involving media partners and citizen science campaigns with the public have shown its importance to increase environmental literacy, communication of best practices when dealing with invasive species, and why consuming them may be important for local ecosystems (Carballo-Cárdenas and Tobi 2016; Giovos et al. 2019; Cerveira et al. 2021).

Weaknesses

The commercial harvesting of invasive species may be prohibited or highly conditioned depending on national or international laws and regulations about the classification of invasive species. Such legal framework can reduce commercial harvesting or profitability due to limited licensing, therefore delaying the launch of putative new economic activities.

The Atlantic blue crab and the weakfish are still understudied in Portugal, despite its recent introduction owing to lack of funding. Information about the full extent of the species distribution, temporal use of estuarine ecosystems, as well as response to climatic variability and extreme weather events is scarce or non-existent. This can undermine the willingness to invest in their commercial exploitation due to overall uncertainty of business projections.

Other edible invasive species that are not traditionally used in western cultures, as the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910, need a high investment in marketing, commercial harvesting techniques, processing, and development of supply chains. For such species their integration into the blue economy as a new food resource is certainly more complicated and probably would only fill a niche of the market (i.e., gourmet cuisine).

The established fishing industry in Portugal still resists to innovation, is limited by outdated maritime support structures, makes poor use of existing distribution channels, and investments on marketing are shy; all these hinders even the implementation of new but basic business models because only few investors surpass the more traditional business model of harvesting for human consumption.

Opportunities

Harvesting invasive species while prioritizing the conservation of native species through targeted fishing practices is an opportunity to contribute to the United Nations 2030 Agenda for Sustainable Development goals, mainly goal 1 (“End poverty in all its forms everywhere”) and goal 2 (“End hunger, achieve food security and improved nutrition and promote sustainable agriculture”), goal 9 (“Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation”), and goal 14 (“Conserve and sustainably use the oceans, seas and marine resources for sustainable development”). Therefore, the appearance of a new fishery, even if an invasive species, may represent an opportunity to alleviate the fishing

pressure upon native species and diversify the income sources of local fishers and other sectors of the economy linked with fisheries (e.g., fishing supplies, restaurants, gastronomic tourism).

The Guadiana estuary showcases two examples. There, and for now, there is only the possibility to incorporate two of the numerous non-indigenous species into the blue economy, the Atlantic blue crab and weakfish (Morais and Teodósio 2016; Morais et al. 2017, 2019). These two species may support a small-scale and regulated local artisanal fishery, uplifting the economy of one of the poorest regions in the European Union, while eventually trying to control these non-indigenous populations. New business opportunities, with new products and uses, can arise from exploiting some of these invasive species. In North America, soft-shell Atlantic blue crabs are greatly appreciated, however this type of product is still unknown in Portugal, thus it may represent an opportunity to add value to this fishery and associated economic activities (e.g., gastronomic tourism with a new gastronomic delicacy). Other edible species in the Guadiana estuary, as the Black Sea jellyfish and Asian clam *Corbicula fluminea* (O. F. Müller, 1774) have been tested in a restaurant in the Algarve, but had low acceptance by consumers, contrary to what is observed in far-east countries. Also, fishing-related tourism activities could be explored, either by recreational fishing, or activities in collaboration with commercial fishing where tourists participate in the fishing activity.

Non-edible invasive species, as the invasive macroalga *Rugulopteryx okamurae*, are becoming a serious problem in south Portugal because it covers underwater reefs and when ashore they sprawl through touristic beaches and must be removed (Liulea 2021; Liulea et al. 2023; Encarnação, personal observations). Harvesting and valorisation of subproducts minimizes some of the problems while bringing revenue to a very specialized industry (Barcellos et al. 2023) without the inherent constraints brought by the intent to perpetuate the fisheries of edible invasive species and intentional introductions (see section below). In cases of large accumulations of biomass, bringing the interest of private investors will also reduce the burden to the public sector that on many occasions is forced to invest in cleaning of touristic zones to avoid catastrophic economic loses.

Access to public or European funding to launch a new business or request an economic feasibility assessment certainly benefits from this business–ecological framework, where an impactful invasive species is being harvested with private investment, while being beneficial to the ecosystem. European states are being urged to fight invasive species impacts, so

combining economic growth and science is an opportunity that deserves being explored more efficiently.

Threats

Promoting and creating a market demand for an edible invasive species must be carefully planned and assessed. The prospect of economic profits pressure managers to perpetuate such fishery combined with intentional introductions into new areas (Nuñez et al. 2012; Pasko and Goldberg 2014). Such approach should only be considered if an invasive species is already widespread across a region or country, accompanied by well-structured awareness campaigns, and monitoring by local authorities (Nuñez et al. 2012; Pasko and Goldberg 2014; Cerveira et al. 2021).

Some invasive species experience large fluctuations in abundance over time, and some populations may collapse dramatically in a short period and without an obvious reason (Simberloff and Gibbons 2004). Such unforeseen scenarios may threaten any commercial activities that became overdependent on invasive species. Uncertainties in the legal framework regarding the commercialization of invasive species can discourage potential investors, or promote the illegal, unreported, and unregulated harvesting of such species when market value is already high. The reduced amount of transboundary cooperation is also a threat on scaling up solutions and may undermine regional progresses in managing invasive species.

SWOT matrix

The information gathered previously is now compiled in the following SWOT matrix on the topic of commercial valorisation of marine invasive species, under a framework of control and mitigation of impacts in southern Portugal.

Strengths	**Weaknesses**
▪ Many invasive species have a high market value in the native and non-native areas. ▪ The economic valorisation of invasive species will promote its harvesting, as a higher value fresh and locally caught seafood. ▪ The Atlantic blue crab and weakfish can be harvested all-year around and require no or minimal gear adaptations. ▪ There is extensive literature knowledge from the native areas about the species and their fisheries. ▪ Biological invasions gather extensive attention by the media and curiosity from the public, thus facilitating scientific literacy programs and the implementation of management best practices.	▪ The legal framework may reduce scalability of the commercial harvesting approach. ▪ The scientific knowledge of most invasive species in Portugal is reduced which undermines private investment. ▪ Non-edible invasive species for the western culture, like jellyfish and freshwater clams, need a high investment not only in transport and processing but especially in marketing the product. ▪ Resistance to innovation and surpass the business model based on harvesting only for direct human consumption.
Opportunities	**Threats**
▪ Transition to new fisheries (invasive species) may ease the harvesting pressure of native species. ▪ Fisheries of invasive species are seen as an economic opportunity in low-income areas. ▪ Recreational fishing and gastronomic tourism are innovative strategies to add value to invasive species. ▪ Access to scientific and investment funding may benefit from this business–ecological framework. ▪ Harvesting invasive species with the best fishing practices can contribute to the United Nations 2030 Agenda for Sustainable Development goals: Goal 1 (End poverty in all its forms everywhere), Goal 2 (End hunger, achieve food security and improved nutrition and promote sustainable agriculture), Goal 9 (Build resilient infrastructure, promote sustainable industrialization, and foster innovation), and Goal 14 (Conserve and sustainably use the oceans, seas and marine resources).	▪ Highly profitable invasive species fisheries add pressure on managers to perpetuate the fishery instead of reducing the population's standing stock. ▪ Increase of intentional introductions into new areas to take advantage of a profitable new resource. ▪ Populations of invasive species can collapse suddenly and naturally, which threatens businesses targeting only one species. ▪ Uncertainties in the legal framework may discourage investors, or promote the illegal, unreported, and unregulated harvesting. ▪ Regional progress is undermined in areas where resources are shared by multiple countries since transboundary cooperation is slow, minimal, or inexistent.

5.4 Discussion

The Atlantic blue crab and weakfish are valuable fisheries across their native range, deeply embedded in the recreational and commercial fishing culture of North America (Wilk 1979; Guillory et al. 2001; Paolisso 2007; ASMFC 2016, 2019). While in their native range these species are overfished, they have established booming populations in Portugal (Morais and Teodósio 2016; Morais et al. 2017, 2019; Vasconcelos et al. 2019; Cerveira et al. 2021; but also see **Chapters 2** and **4**) and across the Gulf of Cadiz (Bañón et al. 2017; González-Ortegón et al. 2022). Their widespread occurrence demands an active commitment to tackle these two invasions, which already made their way into the blue economy quite rapidly.

Marine invasions are nearly impossible to control and to limit their spread, meaning that they should be managed more pragmatically by scientists and environmental agencies, with the support of economic stakeholders (Pyšek and Richardson 2010). Marine invasive species that are widely distributed and established should be allowed by law to be introduced into the blue economy, and not merely by force of circumstances which in certain situations may lead to serious humanitarian and public health problems – e.g., illegal collection of *Ruditapes phillipinarum* in the Tagus estuary by undocumented immigrants, often under the mercy of illegal immigration networks, that distribute this shellfish without adequate sanitary control in Portugal and abroad. This is not the case of the Atlantic blue crab or the weakfish so far, as there is not yet any legislation or legal recommendations on harvesting and commercialization of these two non-indigenous species. We advocate that the legislation of certain countries – including Portugal – should be adapted to incorporate this holistic approach instead of prohibiting the commercialization of invasive species owing solely to its invasive status (Kleitou et al. 2021a). When properly planned, harvesting invasive species can become an opportunity to mitigate ecosystem impacts, alleviate the pressure upon native fisheries, and boost economic development – most importantly in economic depressed regions (Pasko and Goldberg 2014). The integration of a non-indigenous species into the blue economy should be assessed on a case-by-case approach by considering the species invasiveness potential, the invaded ecosystem (e.g., environmental quality), the likelihood for deliberate introductions, the cost of adapting harvest methods to explore a new resource, and the well-being of local population and livelihoods, social status, expectations towards resources management (Howard 2019; Shackleton et al. 2019), and the threat of the activity to be controlled by criminal networks.

The Portuguese wholesale market landings showed that these two invasive species are being caught by professional fishers and sold across wholesale markets since 2019 in the case of the Atlantic blue crab, and at least since 2016 in the case of weakfish. The comparison between the commercialization of these species in the native and non-native ranges highlighted that in Portugal they are still an emerging fishery, although summing over 22k € for the Atlantic blue crab in four years, and almost half a million euros during a seven year period for weakfish. In the following sections, these two aspects are analysed in detail.

5.4.1 Changes in landings and market value between native and non-native ranges

The landings of the Atlantic blue crab in the Algarve have been steadily increasing since 2019, with a total 2786.5 kg in just four years. There was no evident decline in the landings of the native European green crab in the Algarve since the highest value on record for the analysed period was obtained in 2022 (three metric tons). In the Ebro Delta, in the Spanish Mediterranean coast, where the Atlantic blue crab is established for a longer period of time – at least since 2012 – it was observed a dramatic decline of the European green crab, from a sales' maximum above 750 kg in 2016 to close to zero since 2018 (Clavero et al. 2022). Although the European green crab has a low to no commercial value in Portugal (Portela et al. 2023), this species is widely used in the Algarve in recreational and professional fisheries, despite some prohibitions on their use as bait (Leitão et al. 2021) and the annual landings here demonstrate that the species maintains a high commercial demand. Therefore, monitoring the landings of the European green crab should continue to be done in the following years, as more Atlantic blue crab landing data is reported and particularly if the increasing trend continues.

In Portugal, the wholesale market value of the Atlantic blue crab surpassed 8k € in 2022 and summed over 22k € between 2019-2022. Such values are incomparable to the million-dollar industry that the species supports in North America (Steele and Bert 1998; Perry et al. 2022). Despite the stocks being overfished in the native range and under restricted management plans (Eggleston et al. 2004; Miller et al. 2005; ASMFC 2016, 2019; Barbieri and Barbieri 2020), the commercial landings surpassed 240 million US$ in 2021. There, landings data from 2011 till 2021 show that commercialization has followed the typical market demand dynamics, with prices rising as the amount of product keeps decreasing. In Portugal, selling prices keep rising as landings also increase, with an average price of 9.5 ± 3.2 € kg^{-1} in 2022, with a maximum of 20.5 € kg^{-1} registered in April 2022, at wholesale markets. In public markets, Atlantic blue crabs have already been sold for over 20 € kg^{-1} already in 2018. In other non-native areas, prices are lower and range between 0.34 and 2.24 € kg^{-1} in Turkey (Öndes and Gökçe 2021), ~1.5 € kg^{-1} in Tunisia (Baggi 2022), 2 € kg^{-1} in France (Minguez 2021), 7–9 € kg^{-1} in Spain (Escudero 2021), or up to 15 € kg^{-1} in Italy (Foraggi 2023). Overall, prices are higher in Europe, and they seem to increase from Eastern Mediterranean countries towards the Gulf of Cadiz. The higher prices observed in Portugal are probably linked to the "novelty factor" and because Portugal is the country with the third highest seafood consumption *per* capita in the world (Almeida et al. 2015), therefore being prone to accepting this new seafood product.

Weakfish has a considerable fishery established in the western Portuguese coast, namely in the Tagus and Sado estuaries. In 2016-2022, landings surpassed 214 metric tons, with a monthly average of 2.5 ± 2.1 metric tons. The yearly landings of three native species analysed to investigate putative impacts cause by weakfish (cuttlefish, meagre, seabass) fluctuated between 56 and 434 metric tons, but only cuttlefish landings decreased noticeably with the increase of weakfish landings (coefficient= -0.172; p= 0.077). In the Sado estuary, cuttlefish and other cephalopods were present in the gut content of weakfish (Cerveira et al. 2021), and cephalopods in general are regular prey items of other *Cynoscion* species (Lucena et al. 2000; Raymundo-Huizar et al. 2005; Talavera 2020). Cuttlefish landings have decreased in this area, particularly since 2016, while landings increased in the national control dataset since 2017, reinforcing the putative impact of weakfish in the food web of the Tagus and Sado estuaries. The cuttlefish has a commercial and cultural importance that is deeply embedded in the populations surrounding the Sado estuary, supporting a year-around mostly artisanal fishery both inside the estuary and adjacent coastal zone (Serrano 1992; Neves et al. 2009). Cuttlefish has also an ecological importance, being one of the most important prey for the bottlenose dolphin *Tursiops truncatus* (Montagu, 1821) population of the Sado estuary, which are the only resident dolphin population in continental Portugal, and one of the smallest known populations (dos Santos et al. 2007). Although meagre and seabass landings are not correlated with weakfish landings for the analysed period, meagre's landings are decreasing since 2016, at least four years after the introduction of weakfish (Morais and Teodósio 2016; Morais et al. 2017). In 2016, weakfish annual landings surpassed 6 metric tons, confirming that the species was already well established, and landings have been increasing since then, with an annual maximum of 68 metric tons in 2022. In the Sado estuary, weakfish display a high level of niche overlap with meagre (54.7%) and seabass (71.8%), which in case of food limitation will result in competition between weakfish and these two native species (Cerveira et al. 2021). From the present landings data, this might be happening since 2016 in the case of the meagre, due to potential competition for food and habitat, but so far not noticeable with the seabass.

Weakfish sales on wholesale markets surrounding the Tagus and Sado estuaries reached a maximum annual value of 150k € in 2022, with a total of ~68 metric tons of landed fish. In the analysed dataset (2011-2021), commercial landings in the United States were lower than this value for two years. In 2018, only 48 metric tons of weakfish were landed and sold for ~213k US$ (~192k €). There, recreational landings are more relevant, with 243 metric tons captured in 2021, and an annual average of 188 ± 79 metric tons. In Portugal, recreational

fishing statistics do not exist, but North America's data clearly highlight the potential of weakfish for recreation activities and tourism in Portugal. In Spain, the only reported artisanal fisheries are located near the Guadalquivir estuary in the Gulf of Cadiz (Bañón et al. 2017), but extensive fisheries statistics or its worth are not widely available. Still, at wholesale markets in Sanlúcar de Barrameda, a total of ~10 metric tons were sold at an average price of 0.87 € kg^{-1} in May 2023 (ATLAZUL 2023). This average price is lower than in western Portugal, where average price at wholesale markets increased from 1.1 ± 0.8 € kg^{-1} in 2016, to 2.7 ± 1.1 € kg^{-1} in 2022 (this study), with prices to the public ranging between 3–10 € kg^{-1} in Setúbal's fish market (Cerveira et al. 2022), or 8–12 € kg^{-1} in southern Portugal (this study).

5.4.2. Wholesale markets mislabelling issues

Commercial fishing statistics are a valuable source of data for fisheries analysis for a country and along a species' distribution range for stock assessment and fishery management (Kleisner et al. 2013; Pauly and Zeller 2016). Still, the misidentification and mislabelling of species results in false landings estimates, making fisheries management inaccurate and challenging to interpret (Garcia-Vazquez et al. 2009; Gordoa et al. 2017; Giovos et al. 2020; Blanco-Fernandez et al. 2022). Mislabelling may be deliberately done to obscure illegal fishing of protected species, such as tuna or elasmobranchs (Gordoa et al. 2017; Giovos et al. 2020). In other cases, mislabelling is often involuntary when species are morphologically similar and overlap the same fishing grounds – e.g., *Merluccius albidus* (Mitchill, 1818) and *Merluccius bilinearis* (Mitchill, 1814) in the Northwest Atlantic (Garcia-Vazquez et al. 2009); *Merluccius senegalensis* Cadenat, 1950 and *Merluccius polli* Cadenat, 1950 in the Eastern Atlantic (Blanco-Fernandez et al. 2022).

Regarding the Portuguese wholesale landings data, several uncertainties became evident when analysing the Atlantic blue crab and weakfish data, which then raised questions on the rest of the dataset about native species. When a new non-indigenous species enters the supply chain, it is understandable that mislabelling may occur due to delays in creating and implementing information about the new species. In southern Portugal, the Atlantic blue crab was first detected in 2016 in the Ria Formosa lagoon and in 2017 in the Guadiana estuary (Morais et al. 2019), being sold in wholesale markets as European green crab during 2018 (R. Gonçalves, personal communication), until it was officially included in the species list of wholesale markets and correctly labelled since 2019. In the Algarve, the maximum of the

European green crab landings occurred in 2022 and coincided with an increase of landings in the national control dataset, so mislabelling of the Atlantic blue crab is likely to have stopped in 2019.

The Portuguese weakfish dataset raised two main concerns, which led this study to focus exclusively on the Tagus and Sado estuaries. First, the weakfish dataset started with many landings in 2011 – which continued until 2022 – in northwestern Portugal, in wholesale markets as Póvoa do Varzim or Vila do Conde, which are about 70 km from the northern Portuguese boarder with Spain. Such landings make little sense because the main fishing areas are located around the Tagus and Sado estuaries (Morais et al. 2017; Cerveira et al. 2021, 2022), and Gulf of Cadiz (Bañón et al. 2017; ATLAZUL 2023), with no reports of other relevant weakfish fisheries in the Iberian Peninsula apart from sporadic records (Gomes et al. 2017; Morais et al. 2017; Bañón et al. 2018). Second, the Portuguese wholesale landings has no records of the species being sold in the wholesale market of Vila Real de Santo António, which is the primary selling market for fishers from the Guadiana estuary in southeastern Portugal. Here, weakfish is sold regularly as meagre (R. Gonçalves, personal communication), which is something that has also been reported in wholesale markets near the Guadalquivir estuary (Bañón et al. 2017). Despite some morphological similarities between weakfish and meagre, they are quite distinguishable. The weakfish mislabelling does not result in a higher selling price as if it was the native meagre (Bañón et al. 2017; R. Gonçalves, personal communication), so the reason for this continuous mislabelling is intriguing. These observations raise the question about the sudden increase in landings of the native meagre in the Tagus and Sado estuaries after 2014 since it may account for mislabelled weakfish. This potential mislabelling may then have become less frequent, thus raising the question if there was an actual decrease in landings of the native meagre after 2016 potentially driven by the weakfish population boom, or a sign of correct labelling of weakfish over time. Still, the national control dataset also indicates a decrease in meagre landings since 2016.

The analysed data provides critical information about the increase of the Atlantic blue crab and weakfish landings in Portugal and their great potential in the blue economy, but they are still emerging fisheries in comparison to the quantity and value they attain in their native range. The putative negative impacts seemed more evident upon cuttlefish in the Tagus and Sado area, but it should be considered with caution. Future *in-loco* assessments at wholesale markets, particularly in the case of the weakfish in southern and northern Portugal, could help unravelling the causes of mislabelling in landings, while other factors can be responsible for

the fluctuations in native species landings, besides the introduction of the non-indigenous species. In the case of the Atlantic blue crab, more data are needed to better assess temporal trends in landings.

5.4.3 Integration of marine invasive species into the blue economy to promote their management

The concept of blue economy, also referred as oceans economy, was introduced in the United Nations Conference on Sustainable Development held in Rio de Janeiro in 2012. The concept included the improvement of human wellbeing and social equity, while significantly reducing environmental risks and ecological scarcities, optimization of the use of natural marine resources within ecological limits, sourcing and usage of local raw materials, promoting economic and trade activities that consider the conservation and sustainable use and management of marine biodiversity, while also supporting sustainable livelihoods and food security of local populations (UNCTAD 2014). Invasive species, due to their potential negative ecological and economic impacts (Early et al. 2016; Pyšek et al. 2020; Cuthbert et al. 2021), urgently need practical and pragmatic management strategies. Aquatic invasive species that are already widely distributed and established, should be allowed to integrate the blue economy, and if properly planned, their harvesting can become an opportunity to support ecosystem and native resources management, but also boost economic development, particularly in less developed regions (Pasko and Goldberg 2014).

In the case of Portugal, the SWOT analysis showed that the inclusion of marine invasive species into the blue economy has several strengths and opportunities. Many invasive species – as the Atlantic blue crab and the weakfish – have a high market value in their native ranges and a long history of harvesting, transformation, and available literature and expertise on best practices, since harvesting towards innovative uses, promotion, and commercialization. In the non-native range, this is an opportunity to promote a transition in fisheries, particularly in highly-invaded and low-income areas, which can reduce fishing pressure on native species, while helping to control non-indigenous species. In Portugal, several native estuarine and marine fisheries are extirpated – e.g., European sea sturgeon *Acipenser sturio* Linnaues, 1758 in the Guadiana estuary (Almaça and Elvira 2000; Castanheira 2021) – or overexploited – e.g., allis shad *Alosa alosa* (Linnaues, 1758) (Mota et al. 2016); European pilchard *Sardina pilchardus* (Walbaum 1792) (Almeida et al. 2014; Monteiro 2017) – a situation similar to many

other areas around the globe (Kleisner et al. 2013; Link and Watson 2019). The Guadiana estuary in southeastern Portugal, is a case-study of such reality, where a decline in native species is observed alongside the arrival of an increasing number of non-indigenous species (Chícharo et al. 2006, 2009; Morais et al. 2009, 2017, 2019). Environmental literacy and citizen science campaigns as NEMA (see **Chapter 4**) will also contribute to showcase opportunities that some non-indigenous species may bring to the local economy, while promoting participation of the public and disseminating best practices. Across the Mediterranean and Caribbean seas, citizen science projects are also merging scientific research with invasive species outreach activities to tackle the expansion and impacts of marine invasive species such as lionfish *Pterois* spp., the Atlantic blue crab, or *Lagocephalus* pufferfishes (Carballo-Cárdenas and Tobi 2016; Giovos et al. 2019; Kleitou et al. 2021b). The economy of the Algarve region has a strong connection to the so-called 'beach and sun' tourism, but with many emerging activities carried out in the coastal zone or associated with marine environment. This willingness to be innovative has already resulted in collaborations between the University of Algarve and local restaurants with well-known chefs showcasing the culinary uses of non-indigenous species present in the Guadiana estuary, namely the Atlantic blue crab, weakfish, and jellyfish species. These are also opportunities for outreach actions, as happened in 2021, during the second national and first Iberian week about invasive species, where the NEMA citizen science campaign organized an event to showcase the gastronomic potential of the invasive weakfish (link). This was achieved in collaboration with Chef Leonel Pereira's CHECKin restaurant in Faro, where a dish entitled "Roasted weakfish filet, with vegetable schmear and sea plankton" was prepared and available for clients during that week. The dish was very well accepted, making it into the permanent menu of the restaurant. In October 2022, during the blue innovation festival of the Guadiana estuary organized by the University of Algarve under the scope of the ATLAZUL project (link), the Vila Real de Santo António tourism school prepared the lunch for the 50+ participants of this scientific and outreach event using the Atlantic blue crab and weakfish as main ingredients.

Expert knowledge transfer is one strength of dealing with non-indigenous species, where the collaboration between scientists across the native and non-native range of the species will benefit control and management of species that became invasive but will also potentiate innovative ways of economic development in the non-native range. This is the case of the collaboration between the University of Algarve (Portugal) and the University of Maryland Baltimore County (United States), which under the scope of the UP BLUE BRITE project,

funded by the Luso-American Development Foundation (FLAD), aims at launching the basis for a future production of soft-shell Atlantic blue crab in the Algarve, by linking academia, the aquaculture industry, and entrepreneurs from the native and non-native ranges (link).

The SWOT analysis also highlighted several weaknesses and threats of such approach. The present-day weaknesses are mainly related to uncertainties in the legal framework related to non-indigenous and invasive species commercialization, lack of in-depth knowledge on distribution and biological aspects in the non-native range, which can create uncertainty in private investors and business projections, and a low emphasis in more innovative products, besides the harvesting for human consumption or the most traditional seafoods. Most of these weaknesses can be tackled with more investment in science, and more active collaboration with international partners, both in academia and economic stakeholders. In the Algarve region, and particularly with the case of the Atlantic blue crab, this is being implemented for example with the UP BLUE BRITE project, bringing together experts from the native range – scientists and aquaculture industry – and experts with multiple expertise from the species' non-native range, namely from Spain, Croatia, Turkey, Tunisia or Egypt. Uncertainties in the legal framework may be both a present-day weakness, and also represent a threat in the future if unforeseen changes are implemented without consultation of the stakeholders that may have made investments in businesses that somehow rely on non-indigenous species. In the European Union in general, invasive species are already subject to an extensive regulatory framework, between guidelines, international laws, and instruments, such as the ones included in the Convention on Biological Diversity (CBD 2002), the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004), the European Marine Strategy Framework Directive (EC 2008), lists of concern for existing non-indigenous species (EU 2014), and prioritizing lists for potential new non-indigenous species (Tsiamis et al. 2020; Oficialdegui et al. 2023). As referred, each invasive species, or functional group of invasive species, should be evaluated carefully and separately to avoid "one-size-fits-all" approaches. In the case of Portugal, the national framework is mainly regulated by the National List of Invasive Species, where the included species become subject to strict rules, such as, prohibition on introduction into the wild or restocking, prohibition on keeping, giving away, buying, selling, offering for sale, transporting, cultivating, breeding, or using as an ornamental plant or pet animal, prohibition on returning specimens to the wild that have been caught, etc. (Decree-Law n.º 92/2019, link). Still, some species have become subject to an exception regime, mainly due to their national economic importance, as for example the Pacific oyster *Magallana gigas*

(Thunberg, 1793), or the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). In other cases, alongside its introduction in the National List of Invasive Species, national management plans are being developed and implemented while considering the harvesting and commercialization of such invasive species, as the Louisiana crawfish *Procambarus clarkii* (Girard, 1852), the Atlantic blue crab, weakfish, and Black Sea jellyfish (Resolution of the Council of Ministers n.º 133/2021; Morais et al. 2022). Still, such regulatory processes are lengthy, and usually result in overcomplicated administrative procedures to legalize the harvesting and transport of invasive species, include new fees for permits and licences, and superfluous routine paperwork – as the example of the Louisiana crawfish with the Resolution of the Council of Ministers n.º 133/2021 (link). Such approach will likely discourage commercial and recreational fishers, investors, and even promote the illegal, unreported, and unregulated harvesting of such species, when the outcome should be to encourage everyone to harvest as many invasive species from the wild as possible. Across North America, several programs regularly promote the harvesting of invasive species, including recreational and commercial fishing tournaments, for example with Asian carp species, lionfish, or pythons (Chapman et al. 2016; Hoag 2021; Malpica-Cruz et al. 2016). Here, invasive species have also been subject to bounty programs to quickly engage the public in harvesting actions, as with the northern pikeminnow *Ptychocheilus oregonensis* (Richardson, 1836), the black carp *Mylopharyngodon piceus* (Richardson, 1846), suckermouth armoured catfishes *Pterygoplichthys*, or nutria *Myocastor coypus* (Molina 1782) (Dedah et al. 2010; Pasko and Goldberg 2014; Kroboth et al. 2019; Hay et al. 2022). So, harvesting of widely distributed invasive species should be encouraged by decision makers, to increase participation from a broader spectrum of the society. This will also result in less public money being spent by states and local authorities in the control and mitigation of ecological and economic impacts.

In many cases, a more achievable reality passes by building ecosystems capable of delivering key ecosystem services, rather than battling to restore ecosystems free of invasive species (Pyšek and Richardson 2010). Adaptation to changing ecosystems is needed, and in many cases non-indigenous species will continue to be part of such reality, so scientists and managers should seek a middle-ground in the native/non-indigenous species debate and management, while seeking to maintain ecosystem services in the best condition possible, while considering all socio-ecological trade-offs (Pejchar and Mooney 2009; Shackelford et al. 2013; Kleitou et al. 2021a).

5.5 Conclusions

The Atlantic blue crab *Callinectes sapidus* and weakfish *Cynoscion regalis* are valuable fisheries across their native range, deeply embedded in the recreational and commercial fishing culture, although being overfished. This study showed that weakfish may have negative impacts in the fisheries of valuable national resources where it is most established as an invasive species, particularly upon cuttlefish in the Sado estuary area. In the case of the Atlantic blue crab, more robust datasets are needed in the upcoming years to better assess their potential impacts on other fishing resources. Comparisons with landings from the native range of Atlantic blue crab and weakfish, showed that in Portugal they are still an emerging fishery, but also highlighted their potential to be integrated in the blue economy. The SWOT analysis evidenced that the introduction into the blue economy of widely distributed invasive species, if properly planned, can become an opportunity to support ecosystem and native resources management, but also boost economic development in the less developed regions of Portugal, as are the areas surrounding the Guadiana River basin.

5.6 References

Almaça, C., and Elvira, B. (2000). Past and present distribution of *Acipenser sturio* L., 1758 on the Iberian Peninsula. *Boletín-Instituto Español de Oceanografía* 16(1/4): 11-16.

Almeida, C., Vaz, S., Cabral, H., and Ziegler, F. (2014). Environmental assessment of sardine (*Sardina pilchardus*) purse seine fishery in Portugal with LCA methodology including biological impact categories. *The International Journal of Life Cycle Assessment* 19: 297-306. doi: 10.1007/s11367-013-0646-5

Almeida, C., Karadzic, V., and Vaz, S. (2015). The seafood market in Portugal: Driving forces and consequences. *Marine Policy* 61: 87-94. doi: 10.1016/j.marpol.2015.07.012

ASMFC - Atlantic States Marine Fisheries Commission (2016). Weakfish benchmark stock assessment and peer review report. Atlantic States Marine Fisheries Commission, Virginia. 270 pp

ASMFC - Atlantic States Marine Fisheries Commission (2019). Weakfish stock assessment update report. Atlantic States Marine Fisheries Commission, Arlington, VA. 93 pp

ATLAZUL (2023). *Entregable 1.2.4 - Potencial de los productos del mar en el sector gastronómico - IFAPA. Available at: https://atlazul.eu/resultados/*. [Accessed July 04, 2023].

Ayas, D., and Ozogul, Y. (2011). The effects of sex and seasonality on the metal levels of different muscle tissues of mature Atlantic blue crabs (*Callinectes sapidus*) in Mersin Bay, north-eastern Mediterranean. *International Journal of Food Science & Technology* 46(10): 2030-2034. doi: 10.1111/j.1365-2621.2011.02713.x

Baggi, G. (2022). *Love 'em and loathe 'em: Mediterranean grapples with tasty, voracious, invasive crabs. Available at: https://pulitzercenter.org/stories/love-em-and-loathe-em-mediterranean-grapples-tasty-voracious-invasive-crabs*. [Accessed July 04, 2023].

Bañón, R., Arias, A., Arana, D., and Cuesta, J.A. (2017). Identification of a non-native *Cynoscion* species (Perciformes: Sciaenidae) from the Gulf of Cádiz (southwestern Spain) and data on its current status. *Scientia Marina* 81(1): 19-26. doi: 10.3989/scimar.04494.21A

Bañón, R., Barros-García, D., Gómez, D., Berta-Ríos, M., and De Carlos, A. (2018). Evidence of a rapid colonization of the Atlantic European waters by the non-native weakfish *Cynoscion regalis* (Perciformes: Sciaenidae). *Marine Biodiversity* 48(4): 2237-2242. doi: 10.1007/s12526-017-0738-8

Baptista, V., Silva, P.L., Relvas, P., Teodósio, M.A., and Leitão, F. (2018). Sea surface temperature variability along the Portuguese coast since 1950. *International Journal of Climatology* 38(3): 1145-1160. doi: 10.1002/joc.5231

Barbieri, S., and Barbieri, L. (2020). *Cynoscion regalis. The IUCN Red List of Threatened Species 2020. Available at: https://www.iucnredlist.org/species/46104933/49226925*. [Accessed July 04, 2023].

Barbour, A.B., Allen, M.S., Frazer, T.K., and Sherman, K.D. (2011). Evaluating the potential efficacy of invasive lionfish (*Pterois volitans*) removals. *PloS ONE* 6(5): e19666. doi: 10.1371/journal.pone.0019666

Barcellos, L., Pham, C.K., Menezes, G., Bettencourt, R., Rocha, N., Carvalho, M., and

Felgueiras, H.P. (2023). A concise review on the potential applications of *Rugulopteryx okamurae* macroalgae. *Marine Drugs* 21(1): 40. doi: 10.3390/md21010040

Benyahkoub, Y., Wariaghli, F., Fekhaoui, M., and Yahyaoui, A. (2021). SWOT analysis of Louisiana crayfish *Procambarus clarkii* (Girard, 1852) valorization in Morocco. *Aquaculture, Aquarium, Conservation & Legislation* 14(2): 874-883.

Benzaghta, M.A., Elwalda, A., Mousa, M.M., Erkan, I., and Rahman, M. (2021). SWOT analysis applications: An integrative literature review. *Journal of Global Business Insights* 6(1): 55-73. doi: 10.5038/2640-6489.6.1.1148

Blanco-Fernandez, C., Erzini, K., Rodriguez-Diego, S., Alba-Gonzalez, P., Thiam, N., Sow, F.N., Diallo, M., Viðarsson, J.R., Fernandez-Vidal, D., and Goncalves, J. (2022). Two fish in a pod. Mislabelling on board threatens sustainability in mixed fisheries. *Frontiers in Marine Science* 9: 841667. doi: 10.3389/fmars.2022.841667

Carballo-Cárdenas, E.C., and Tobi, H. (2016). Citizen science regarding invasive lionfish in Dutch Caribbean MPAs: Drivers and barriers to participation. *Ocean & Coastal Management* 133: 114-127. doi: 10.1016/j.ocecoaman.2016.09.014

Castanheira, J.M.C. (2021). A pesca no Algarve medieval. Master thesis. Universidade do Algarve, Faro. 193 pp

CBD - Convention on Biological Diversity (2002). Conference of the Parties (COP 6), decision VI/23, Netherlands.

Cerveira, I., Dias, E., Baptista, V., Teodósio, M.A., and Morais, P. (2021). Invasive fish keeps native feeding strategy despite high niche overlap with a congener species. *Regional Studies in Marine Science* 47: 101969. doi: 10.1016/j.rsma.2021.101969

Cerveira, I., Baptista, V., Teodósio, M.A., and Morais, P. (2022). What's for dinner? Assessing the value of an edible invasive species and outreach actions to promote its consumption. *Biological Invasions*: 1-15. doi: 10.1007/s10530-021-02685-3

Chapman, D.C., Chen, D., Hoover, J.J., Du, H., Phelps, Q.E., Shen, L., Wang, C., Wei, Q., and Zhang, H. (2016). Bigheaded carps of the Yangtze and Mississippi rivers: Biology, status, and management. *American Fisheries Society Symposium* 84: 113-126.

Chícharo, M.A., Chícharo, L., and Morais, P. (2006). Inter-annual differences of ichthyofauna structure of the Guadiana estuary and adjacent coastal area (SE Portugal/SW Spain): Before and after Alqueva dam construction. *Estuarine, Coastal and Shelf Science* 70(1-2): 39-51. doi: 10.1016/j.ecss.2006.05.036

Chícharo, M.A., Leitão, T., Range, P., Gutierrez, C., Morales, J., Morais, P., and Chícharo, L. (2009). Alien species in the Guadiana Estuary (SE-Portugal/SW-Spain): *Blackfordia virginica* (Cnidaria, Hydrozoa) and *Palaemon macrodactylus* (Crustacea, Decapoda): Potential impacts and mitigation measures. *Aquatic Invasions* 4(3): 501-506. doi: 10.3391/ai.2009.4.3.11

Clavero, M., Franch, N., Bernardo-Madrid, R., López, V., Abelló, P., Queral, J.M., and Mancinelli, G. (2022). Severe, rapid and widespread impacts of an Atlantic blue crab invasion. *Marine Pollution Bulletin* 176: 113479. doi: 10.1016/j.marpolbul.2022.113479

Clements, K.R., Karp, P., Harris, H.E., Ali, F., Candelmo, A., Rodríguez, S.J., Balcázar-Escalera, C., Fogg, A.Q., Green, S.J., and Solomon, J.N. (2021). The role of citizen

science in the research and management of invasive lionfish across the Western Atlantic. *Diversity* 13(12): 673. doi: 10.3390/d13120673

Cuthbert, R.N., Pattison, Z., Taylor, N.G., Verbrugge, L., Diagne, C., Ahmed, D.A., Leroy, B., Angulo, E., Briski, E., and Capinha, C. (2021). Global economic costs of aquatic invasive alien species. *Science of the Total Environment* 775: 145238. doi: 10.1016/j.scitotenv.2021.145238

De Leaniz, C.G., Gajardo, G., and Consuegra, S. (2010). From best to pest: Changing perspectives on the impact of exotic salmonids in the southern hemisphere. *Systematics and Biodiversity* 8(4): 447-459. doi: 10.1080/14772000.2010.537706

Dedah, C., Kazmierczak, R.F., and Keithly, W.R. (2010). The role of bounties and human behavior on Louisiana nutria harvests. *Journal of Agricultural and Applied Economics* 42(1): 133-142. doi: 10.1017/S1074070800003345

dos Santos, M.E., Coniglione, C., and Louro, S. (2007). Feeding behaviour of the bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) in the Sado estuary, Portugal, and a review of its prey species. *Revista Brasileira de Zoociências* 9(1): 31-39.

Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., Gonzalez, P., Grosholz, E.D., Ibañez, I., and Miller, L.P. (2016). Global threats from invasive alien species in the twenty-first century and national response capacities. *Nature Communications* 7(1): 12485. doi: 10.1038/ncomms12485

EC - European Commission (2008). Directive of the European Parliament and the Council establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Directive 2008/56/EC, OJ L 164, European Commission.

Eggleston, D.B., Johnson, E.G., and Hightower, J.E. (2004). Population dynamics and stock assessment of the blue crab in North Carolina. North Carolina Fishery Resource Grant Program, Raleigh, NC. 252 pp

Ens, N.J., Harvey, B., Davies, M.M., Thomson, H.M., Meyers, K.J., Yakimishyn, J., Lee, L.C., McCord, M.E., and Gerwing, T.G. (2022). The Green Wave: reviewing the environmental impacts of the invasive European green crab (Carcinus maenas) and potential management approaches. *Environmental Reviews* 30(2): 306-322.

Escudero, M. (2021). *Cangrejo azul: La especie invasora que se convirtió en manjar*. *Available at: https://elpais.com/gastronomia/el-comidista/2021/02/11/articulo/1613065996_811867.html*. [Accessed July 04, 2023].

EU - European Union (2014). Regulation No 1143/2014 of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species. Official Journal of the European Union, European Union. 35–55

Fantle-Lepczyk, J.E., Haubrock, P.J., Kramer, A.M., Cuthbert, R.N., Turbelin, A.J., Crystal-Ornelas, R., Diagne, C., and Courchamp, F. (2022). Economic costs of biological invasions in the United States. *Science of the Total Environment* 806: 151318. doi: 10.1016/j.scitotenv.2021.151318

Faria, J., Prestes, A.C., Moreu, I., Cacabelos, E., and Martins, G.M. (2022). Dramatic changes in the structure of shallow-water marine benthic communities following the invasion by *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in Azores (NE Atlantic). *Marine*

Pollution Bulletin 175: 113358. doi: 10.1016/j.marpolbul.2022.113358

Fendt, L. (2014). *The lionfish hunters. Availble at: https://ticotimes.net/2014/10/09/the-lionfish-hunters*. [Accessed July 04, 2023].

Ferguson, T., and Akins, L. (2016). *Lionfish Cookbook 2nd Edition. Available at: https://www.reef.org/products/lionfish-cookbook-2nd-edition*. [Accessed July 04, 2023].

Foraggi, O. (2023). *Granchio blu "infesta" la acque e finisce sulle nostre tavole: Quanto costa e cosa sta succedendo. Available at: https://www.investireoggi.it/news/granchio-blu-infesta-acque-finisce-sulle-tavole-quanto-costa/*. [Accessed July 04, 2023].

García-Arberas, L., Rallo, A., and Antón, A. (2009). The future of the indigenous freshwater crayfish *Austropotamobius italicus* in Basque Country streams: Is it possible to survive being an inconvenient species? *Knowledge and Management of Aquatic Ecosystems* 394-395(19): 11 pp. doi: 10.1051/kmae/2010015

García-Gómez, J.C., Sempere-Valverde, J., González, A.R., Martínez-Chacón, M., Olaya-Ponzone, L., Sánchez-Moyano, E., Ostalé-Valriberas, E., and Megina, C. (2020). From exotic to invasive in record time: The extreme impact of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in the strait of Gibraltar. *Science of the Total Environment* 704: 135408. doi: 10.1016/j.scitotenv.2019.135408

García-Gómez, J.C., Florido, M., Olaya-Ponzone, L., Rey Díaz de Rada, J., Donázar-Aramendía, I., Chacón, M., Quintero, J.J., Magariño, S., and Megina, C. (2021). Monitoring extreme impacts of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in El Estrecho Natural Park (Biosphere Reserve). Showing radical changes in the underwater seascape. *Frontiers in Ecology and Evolution* 9: 639161. doi: 10.3389/fevo.2021.639161

Garcia-Vazquez, E., Horreo, J.L., Campo, D., Machado-Schiaffino, G., Bista, I., Triantafyllidis, A., and Juanes, F. (2009). Mislabeling of two commercial North American hake species suggests underreported exploitation of offshore hake. *Transactions of the American Fisheries Society* 138(4): 790-796. doi: 10.1577/T08-169.1

Giovos, I., Kleitou, P., Poursanidis, D., Batjakas, I., Bernardi, G., Crocetta, F., Doumpas, N., Kalogirou, S., Kampouris, T.E., and Keramidas, I. (2019). Citizen-science for monitoring marine invasions and stimulating public engagement: A case project from the eastern Mediterranean. *Biological invasions* 21: 3707-3721. doi: 10.1007/s10530-019-02083-w

Giovos, I., Arculeo, M., Doumpas, N., Katsada, D., Maximiadi, M., Mitsou, E., Paravas, V., Aga-Spyridopoulou, R.N., Stoilas, V.-O., Tiralongo, F., et al. (2020). Assessing multiple sources of data to detect illegal fishing, trade and mislabelling of elasmobranchs in Greek markets. *Marine Policy* 112: 103730. doi: 10.1016/j.marpol.2019.103730

Gomes, P., Vieira, A.R., Oliveira, R., Silva, H., Martins, R., and Carneiro, M. (2017). First record of *Cynoscion regalis* (Pisces, Sciaenidae) in Portuguese continental waters. *Journal of Fish Biology* 90(6): 2470-2474. doi: 10.1111/jfb.13318

González, G., Vilas, C., Baldó, F., Laiz, I., Fernández-Delgado, C., Fowler, A., Jenkins, S., Acosta-Morillas, V., Cuesta, J.A., and González-Ortegón, E. (2021). Recruitment of the exotic weakfish at its southernmost limit in Europe: A preliminary assessment. *29th Biennal CERF (conference paper)*.

González-Ortegón, E., Berger, S., Encarnação, J., Chairi, H., Morais, P., Teodósio, M.A., Oliva-Paterna, F.J., Schubart, C.D., and Cuesta, J.A. (2022). Free pass through the pillars of Hercules? Genetic and historical insights into the recent expansion of the Atlantic blue crab *Callinectes sapidus* to the West and the East of the Strait of Gibraltar. *Frontiers in Marine Science* 9: 918026. doi: 10.3389/fmars.2022.918026

Gordoa, A., Carreras, G., Sanz, N., and Viñas, J. (2017). Tuna species substitution in the Spanish commercial chain: A knock-on effect. *PloS ONE* 12(1): e0170809. doi: 10.1371/journal.pone.0170809

Grason, E.W., McDonald, P.S., Adams, J., Litle, K., Apple, J.K., and Pleus, A. (2018). Citizen science program detects range expansion of the globally invasive European green crab in Washington State. *Management of Biological Invasions* 9(1): 39-47 doi: 10.3391/mbi.2018.9.1.04

Greiner, D.M., Skonberg, D.I., Perkins, L.B., and Perry, J.J. (2021). Use of invasive green crab *Carcinus maenas* for production of a fermented condiment. *Foods* 10(4): 659. doi: 10.3390/foods10040659

Guillory, V., Perry, H.M., Steel, P., Wagner, T., Keithly, W., Pellegrin, B., Petterson, J., Floyd, T., Buckson, B., Hartman, L., et al. (2001). The blue crab fishery of the Gulf of Mexico, United States: A regional management plan. Gulf States Marine Fisheries Commission, Mississippi. 301 pp

Hay, A., Riggins, C.L., Heard, T., Garoutte, C., Rodriguez, Y., Fillipone, F., Smith, K.K., Menchaca, N., Williamson, J., and Perkin, J.S. (2022). Movement and mortality of invasive suckermouth armored catfish during a spearfishing control experiment. *Biological Invasions* 24(10): 3119-3131. doi: 10.1007/s10530-022-02834-2

Hoag, H. (2014). Invasive-species control: Bounty hunters. *Nature* 513(7518): 294. doi: 10.1038/513294a

Howard, P.L. (2019). Human adaptation to invasive species: A conceptual framework based on a case study metasynthesis. *Ambio* 48(12): 1401-1430. doi: 10.1007/s13280-019-01297-5

IMO - International Maritime Organization (2004). International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM). International Maritime Organization, London.

Kevrekidis, K., and Antoniadou, C. (2018). Abundance and population structure of the blue crab *Callinectes sapidus* (Decapoda, Portunidae) in Thermaikos Gulf (Methoni Bay), northern Aegean Sea. *Crustaceana* 91(6): 641-657. doi: 10.1163/15685403-00003795

Khiari, Z., Kelloway, S., and Mason, B. (2020). Turning invasive green crab (*Carcinus maenas*) into opportunity: recovery of chitin and protein isolate through isoelectric solubilization/precipitation. *Waste and Biomass Valorization* 11: 133-142. doi: 10.1007/s12649-018-0398-3

Kleisner, K., Zeller, D., Froese, R., and Pauly, D. (2013). Using global catch data for inferences on the world's marine fisheries. *Fish and Fisheries* 14(3): 293-311. doi: 10.1111/j.1467-2979.2012.00469.x

Kleitou, P., Crocetta, F., Giakoumi, S., Giovos, I., Hall-Spencer, J.M., Kalogirou, S., Kletou, D., Moutopoulos, D.K., and Rees, S. (2021a). Fishery reforms for the management of

non-indigenous species. *Journal of Environmental Management* 280: 111690. doi: 10.1016/j.jenvman.2020.111690

Kleitou, P., Rees, S., Cecconi, F., Kletou, D., Savva, I., Cai, L.L., and Hall-Spencer, J.M. (2021b). Regular monitoring and targeted removals can control lionfish in Mediterranean Marine Protected Areas. *Aquatic Conservation: Marine and Freshwater Ecosystems* 31(10): 2870-2882. doi: 10.1002/aqc.3669

Kroboth, P., Cox, C., Chapman, D.C., and Whitledge, G.W. (2019). Black carp in North America: A description of range, habitats, time of year, and methods of reported captures. *North American Journal of Fisheries Management* 39(5): 1046-1055. doi: 10.1002/nafm.10340

Lee, K.-H., Noh, J., and Khim, J.S. (2020). The Blue Economy and the United Nations' sustainable development goals: Challenges and opportunities. *Environment International* 137: 105528. doi: 10.1016/j.envint.2020.105528

Leitão, F. (2015). Landing profiles of Portuguese fisheries: Assessing the state of stocks. *Fisheries Management and Ecology* 22(2): 152-163. doi: 10.1111/fme.12112

Leitão, F., Maharaj, R.R., Vieira, V.M., Teodósio, A., and Cheung, W.W. (2018). The effect of regional sea surface temperature rise on fisheries along the Portuguese Iberian Atlantic coast. *Aquatic Conservation: Marine and Freshwater Ecosystems* 28(6): 1351-1359. doi: 10.1002/aqc.2947

Leitão, F., Bueno-Pardo, J., Ovelheiro, A., Monteiro, J.N., Nobre, D., and Teodósio, A.M. (2021). Effect of bait type on the octopus fishery in Algarve, Southern Portugal. *Ocean & Coastal Management* 207: 105587. doi: 10.1016/j.ocecoaman.2021.105587

Link, J.S., and Watson, R.A. (2019). Global ecosystem overfishing: Clear delineation within real limits to production. *Science Advances* 5(6): eaav0474. doi: 10.1126/sciadv.aav0474

Liulea, S. (2021). Invasion of the reds? Long-term shifts on intertidal seaweed at distribuition limits. Master thesis. Universidade do Algarve, Faro. 40 pp

Liulea, S., Serrão, E.A., and Santos, R. (2023). Spread and impact of the invasive brown algae *Rugulopteryx okamurae* on the Algarve coast, Southern Portugal (NE Atlantic). *Preprint at SSRN*. doi: 10.2139/ssrn.4446622

Lucena, F.M., Vaske, T., Ellis, J.R., and O'brien, C.M. (2000). Seasonal variation in the diets of bluefish, *Pomatomus saltatrix* (Pomatomidae) and striped weakfish, *Cynoscion guatucupa* (Sciaenidae) in southern Brazil: Implications of food partitioning. *Environmental Biology of Fishes* 57: 423-434. doi: 10.1023/A:1007604424423

Malpica-Cruz, L., Chaves, L.C., and Côté, I.M. (2016). Managing marine invasive species through public participation: Lionfish derbies as a case study. *Marine Policy* 74: 158-164. doi: 10.1016/j.marpol.2016.09.027

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., and Ribeiro, F. (2017). On the Atlantic blue crab (*Callinectes sapidus* Rathbun 1896) in southern European coastal waters: Time to turn a threat into a resource? *Fisheries Research* 194: 1-8. doi: 10.1016/j.fishres.2017.05.002

Miller, T.J., Martell, S.J., Bunnell, D.B., David, G., Fegley, L., Sharov, A., Bonzek, C.F., Hewitt, D., Hoenig, J., and Lipcius, R. (2005). Stock assessment of the blue crab in

Chesapeake Bay 2005. University of Maryland Center for Environmental Science, Annapolis, MD. 177 pp

Millikin, M.R. (1984). Synopsis of biological data on the blue crab, *Callinectes sapidus* Rathbun. NOAA Technical Report NMFS 1. US Department of Commerce, Washington. 47 pp

Minguez, A. (2021). *On French Mediterranean, invasive blue crab wreaks havoc on local molluscs. Available at: https://www.reuters.com/world/europe/french-mediterranean-invasive-blue-crab-wreaks-havoc-local-molluscs-2021-08-31/.* [Accessed July 04, 2023].

Monteiro, J.N., Ovelheiro, A., Ventaneira, A.M., Vieira, V., Teodósio, M.A., and Leitão, F. (2022). Variability in *Carcinus maenas* fecundity along lagoons and estuaries of the Portuguese coast. *Estuaries and Coasts* 45: 1716–1727. doi: 10.1007/s12237-021-01035-9

Monteiro, P.V. (2017). The purse seine fishing of sardine in Portuguese waters: A difficult compromise between fish stock sustainability and fishing effort. *Reviews in Fisheries Science & Aquaculture* 25(3): 218-229. doi: 10.1080/23308249.2016.1269720

Morais, P., Teodósio, J., Reis, J., Chícharo, M.A., and Chícharo, L. (2009). The Asian clam *Corbicula fluminea* (Müller, 1774) in the Guadiana River Basin (southwestern Iberian Peninsula): Setting the record straight. *Aquatic Invasions* 4(4): 681-684. doi: 10.3391/ai.2009.4.4.14

Morais, P., and Teodósio, M. (2016). The transatlantic introduction of weakfish *Cynoscion regalis* (Bloch & schneider, 1801)(sciaenidae, pisces) into Europe. *BioInvasions Records* 5(4): 259-265. doi: 10.3391/bir.2016.5.4.11

Morais, P., Cerveira, I., and Teodósio, M.A. (2017). An update on the invasion of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801)(Actinopterygii: Sciaenidae) into Europe. *Diversity* 9(4): 47. doi: 10.3390/d9040047

Morais, P., Gaspar, M., Garel, E., Baptista, V., Cruz, J., Cerveira, I., Leitao, F., and Teodosio, M. (2019). The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its non-native distribution into the Ria Formosa lagoon and the Guadiana estuary (SW-Iberian Peninsula, Europe). *BioInvasions Records* 8(1): 123-133. doi: 10.3391/bir.2019.8.1.14

Morais, P., Encarnação, J., and Teodósio, M.A. (2022). Plano de ação para o controlo em Portugal continental de espécies invasoras aquáticas, aforreca negra, corvina americana e caranguejo azul (ALFCORAZUL). Relatório elaborado no âmbito do programa Ambiental e Ação Climática - Fundo Ambiental. *Diário da República* Aviso n.º 9678/2021: 52 pp.

Mota, M., Rochard, E., and Antunes, C. (2016). Status of the diadromous fish of the Iberian Peninsula: Past, present and trends. *Limnetica* 35(1): 1-18. doi: 10.23818/limn.35.01

Murillas-Maza, A., García-Allut, A., Aranda, M., Pazos, C., Iriondo, A., and Montero, M. (2021). Enhancing the participation of small-scale fishing sector in decision-making: Good practices from Atlantic in EU. *Marine Policy* 129: 104533. doi: 10.1016/j.marpol.2021.104533

Nehring, S. (2011). "Invasion history and success of the American blue crab Callinectes sapidus in European and adjacent waters" in B. Galil, P. Clark & J. Carlton (eds) In the

wrong place - Alien marine crustaceans: Distribution, biology and impacts, 6. Dordrecht: Springer, 607-624. doi: 10.1007/978-94-007-0591-3_21

Neves, A., Cabral, H., Sequeira, V., Figueiredo, I., Moura, T., and Gordo, L. (2009). Distribution patterns and reproduction of the cuttlefish, *Sepia officinalis* in the Sado estuary (Portugal). *Journal of the Marine Biological Association of the United Kingdom* 89(3): 579-584. doi: 10.1017/S0025315409002677

NOAA - National Oceanic and Atmospheric Administration (2023). *Fisheries One Stop Shop (FOSS). Available at: www.fisheries.noaa.gov/foss/f?p=215:200:16121283799345:Mail::::*. [Accessed April 27, 2023].

Nuñez, M.A., Kuebbing, S., Dimarco, R.D., and Simberloff, D. (2012). Invasive species: To eat or not to eat, that is the question. *Conservation Letters* 5(5): 334-341. doi: 10.1111/j.1755-263X.2012.00250.x

Oficialdegui, F.J., Zamora-Marín, J.M., Guareschi, S., Anastácio, P.M., García-Murillo, P., Ribeiro, F., Miranda, R., Cobo, F., Gallardo, B., García-Berthou, E., et al. (2023). A horizon scan exercise for aquatic invasive alien species in Iberian inland waters. *The Science of the Total Environment*: 161798. doi: 10.1016/j.scitotenv.2023.161798

Öndes, F., and Gökçe, G. (2021). Distribution and fishery of the invasive blue crab (*Callinectes sapidus*) in Turkey based on local ecological knowledge of fishers. *Journal of Anatolian Environmental and Animal Sciences* 6(3): 325-332. doi: 10.35229/jaes.891379

Orth, R.J., and van Montfrans, J. (1990). Utilization of marsh and seagrass habitats by early stages of *Callinectes sapidus*: A latitudinal perspective. *Bulletin of Marine Science* 46(1): 126-144.

Osborne, M. (2022). *New Hampshire distillery makes whiskey out of invasive crabs. Available at: https://www.smithsonianmag.com/smart-news/new-hampshire-distillery-makes-whiskey-out-of-invasive-crabs-180980414/*. [Accessed July 04, 2023].

Paliwal, R. (2006). EIA practice in India and its evaluation using SWOT analysis. *Environmental Impact Assessment Review* 26(5): 492-510. doi: 10.1016/j.eiar.2006.01.004

Paolisso, M. (2007). Taste the traditions: Crabs, crab cakes, and the Chesapeake Bay blue crab fishery. *American Anthropologist* 109(4): 654-665. doi: 10.1525/aa.2007.109.4.654

Pasko, S., and Goldberg, J. (2014). Review of harvest incentives to control invasive species. *Management of Biological Invasions* 5(3): 263. doi: 10.3391/mbi.2014.5.3.10

Pauly, D., and Zeller, D. (2016). Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. *Nature Communications* 7(1): 10244. doi: 10.1038/ncomms10244

Pejchar, L., and Mooney, H.A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution* 24(9): 497-504. doi: 10.1016/j.tree.2009.03.016

Perry, H.M., Anderson, J., and Collins, L. (2022). Status and management of the blue crab fishery in the Gulf of Mexico. *North American Journal of Fisheries Management* 42(1): 164-179. doi: 10.1002/nafm.10727

Portela, T., Cruz, D., Monteiro, R., Correia, M.J., Costa, J.L., and Domingos, I. (2023). The population dynamics of *Carcinus maenas* (Crustacea: Portunoidea) in a coastal lagoon

(Portugal, SW). *Scientia Marina* 87(2): e068-e068. doi: 10.3989/scimar.05353.068

Pyšek, P., and Richardson, D.M. (2010). Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources* 35: 25-55. doi: 10.1146/annurev-environ-033009-095548

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W., Essl, F., Foxcroft, L.C., and Genovesi, P. (2020). Scientists' warning on invasive alien species. *Biological Reviews* 95(6): 1511-1534. doi: 10.1111/brv.12627

Ramajal, J., Picard, D., Costa, J., Carvalho, F., Gaspar, M., and Chainho, P. (2016). "Amêijoa-japonesa, uma nova realidade no estuário do Rio Tejo: Pesca e pressão social e impacto socio-económico" in L.C. da Fonseca, A.C. Garcia, S.D. Pereira & M.A.C. Rodrigues (eds) Entre Rios e Mares: um Património de Ambientes, Histórias e Saberes, Rio de Janeiro: UERJ, 17-30.

Raymundo-Huizar, A.R., Pérez-España, H., Mascaró, M., and Chiappa-Carrara, X. (2005). Feeding habits of the dwarf weakfish (*Cynoscion nannus*) off the coasts of Jalisco and Colima, Mexico. *Fishery Bulletin* 103(2): 453-460.

RStudio Team (2022). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Sayyed, M. (2013). SWOT analysis of Tandooreh National Park (NE Iran) for sustainable ecotourism. *Proceedings of the International Academy of Ecology and Environmental Sciences* 3(4): 296.

Serrano, M.D. (1992). Contribuição para o conhecimento da biologia e da pescaria do choco, *Sepia officinalis* (Linnaeus, 1758) no estuário do Sado e zona costeira adjacente. Relatório Técnico Científico, INIP, Lisboa. 26 pp

Shackelford, N., Hobbs, R.J., Heller, N.E., Hallett, L.M., and Seastedt, T.R. (2013). Finding a middle-ground: The native/non-native debate. *Biological Conservation* 158: 55-62. doi: 10.1016/j.biocon.2012.08.020

Shackleton, R.T., Shackleton, C.M., and Kull, C.A. (2019). The role of invasive alien species in shaping local livelihoods and human well-being: A review. *Journal of Environmental Management* 229: 145-157. doi: 10.1016/j.jenvman.2018.05.007

Simberloff, D., and Gibbons, L. (2004). Now you see them, now you don't! – Population crashes of established introduced species. *Biological Invasions* 6: 161-172. doi: 10.1023/B:BINV.0000022133.49752.46

Smith, N., Burgess, K., Clements, K.R., Burgess, J.C., Lavoie, A., and Solomon, J.N. (2023). Serving conservation from reef to plate: Barriers and opportunities for invasive lionfish consumption in restaurants. *Aquatic Conservation: Marine and Freshwater Ecosystems*. doi: 10.1002/aqc.3933

Smith-Godfrey, S. (2016). Defining the blue economy. *Maritime affairs: Journal of the national maritime foundation of India* 12(1): 58-64. doi: 10.1080/09733159.2016.1175131

Sprung, M. (2001). "Larval abundance and recruitment of *Carcinus maenas* L. close to its southern geographic limit: A case of match and mismatch" in J.P.M. Paula, A.A.V. Flores & C.H.J.M. Fransen (eds) Advances in Decapod Crustacean Research. Developments in Hydrobiology, 154. Dordrecht: Springer, 153-158. doi: 10.1007/978-

94-017-0645-2_16

Stagg, C., and Whilden, M. (1997). The history of Chesapeake Bay's blue crab (*Callinectes sapidus*): Fisheries and management. *Investigaciones Marinas* 25(1): 93-104.

Steele, P., and Bert, T.M. (1998). The Florida blue crab fishery: History, status, and management. *Journal of Shellfish Research* 17: 441-450.

St-Hilaire, S. (2016). Assessing the potential for a soft-shell green crab industry in PEI. Master thesis. University of Prince Edward Island, Charlottetown. 41 pp

Stoney Farms (2023). *Daytona's #1 stop for seafood. Availabe at: https://www.stoneyfarmscrabshop.com/menu/*. [Accessed July 04, 2023].

Talavera, D.A. (2020). Ecología trófica de la cachema *Cynoscion analis* (Jenyns, 1842) procedente de la pesca artesalan en las zonas de Santa Rosa, Chimbote y Callao. Bachelon thesis. Universidad Científica del Sur, Lima. 57 pp

Tsiamis, K., Azzurro, E., Bariche, M., Çinar, M.E., Crocetta, F., De Clerck, O., Galil, B., Gómez, F., Hoffman, R., and Jensen, K.R. (2020). Prioritizing marine invasive alien species in the European Union through horizon scanning. *Aquatic Conservation: Marine and Freshwater Ecosystems* 30(4): 794-845. doi: 10.1002/aqc.3267

Ulman, A., Ali, F., Harris, H., Adel, M., Mabruk, S., Bariche, M., Candelmo, A., Chapman, J., Cicek, B., and Clements, K. (2022). Lessons from the western Atlantic lionfish invasion to inform management in the Mediterranean. *Frontiers in Marine Science* 9: 865162. doi: 10.3389/fmars.2022.865162

UNCTAD - United Nations Conference on Trade and Development (2014). The Oceans Economy: Opportunities and challenges for small island developing states. United Nations, New York. 40 pp

Vasconcelos, P., Carvalho, A.N., Piló, D., Pereira, F., Encarnação, J., Gaspar, M.B., and Teodósio, M.A. (2019). Recent and consecutive records of the Atlantic blue crab (*Callinectes sapidus* Rathbun, 1896): Rapid westward expansion and confirmed establishment along the Southern Coast of Portugal. *Thalassas: An International Journal of Marine Sciences* 35(2): 485-494. doi: 10.1007/s41208-019-00163-1

Viegas, M.D.C., Moniz, A.B., and Santos, P.T. (2014). Artisanal fishermen contribution for the integrated and sustainable coastal management – application of strategic SWOT analysis. *Procedia - Social and Behavioral Sciences* 120: 257-267. doi: 10.1016/j.sbspro.2014.02.103

Wilk, S.J. (1979). Biological and fisheries data on weakfish, *Cynoscion regalis* (Blochand Schneider). NOAA, US Department of Commerce, Highlands, NJ. 62 pp

Zohar, Y., Hines, A.H., Zmora, O., Johnson, E.G., Lipcius, R.N., Seitz, R.D., Eggleston, D.B., Place, A.R., Schott, E.J., and Stubblefield, J.D. (2008). The Chesapeake Bay blue crab (*Callinectes sapidus*): A multidisciplinary approach to responsible stock replenishment. *Reviews in Fisheries Science* 16(1-3): 24-34. doi: 10.1080/10641260701681623

CHAPTER 6

General conclusions and future perspectives

This Ph.D. thesis incremented the scientific knowledge on non-indigenous marine species, spearheaded the use of citizen science in the research of non-indigenous marine species in Portugal, and evaluated how marine invasive species can be managed if integrated into the blue economy. Numerous parts of this thesis have already been published in international peer-reviewed articles and presented in international conferences and national outreach events as oral communications and posters. In this last chapter, the general conclusions drawn from this thesis are presented and framed with a perspective focused on future endeavours in the form of recommendations to a wide audience, from resource managers to the general public.

6.1 General conclusions

Biological invasions are a global problem, and scientists still struggle to monitor and keep-up with the rapid changes in the distribution of known invasive species, while facing continuous added challenges with constant new introductions, their range expansion, and their inherent ecological and economic impacts, which are now often exacerbated by climate change. Most non-indigenous marine species detected during the sampling campaigns undertaken during this Ph.D. had already been reported for some years in southern Portugal (**Chapter 2**). Still, the first specimens of the Asian date mussel *Arcuatula senhousia* (W. H. Benson, 1842) in southern Portugal were sampled in the middle Guadiana estuary in 2022. The establishment of other invasive species in this same area was also confirmed during this study, namely the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 or the prawn *Palaemon*

macrodactylus Rathbun, 1902. The numerous non-indigenous species present in the middle Guadiana estuary may be a consequence of facilitation and synergistic relationships between them, resulting in a greater abundance and diversity of non-indigenous species, along more accentuated impacts in an estuary already under several anthropogenic stressors. Such scenario of an "invasion meltdown" (sensu Simberloff and Von Holle 1999) needs further investigation in the Guadiana estuary and should not be overlooked. A total of 13 neonative species were also identified, and for some of them their presence in Algarve has never been accounted for in peer-reviewed scientific articles until this thesis. The ongoing tropicalization of the southern Portuguese coast was evident, independently of the sampling methodologies or life stages of the neonative species found in the Algarve.

Due to the invasiveness nature of the Atlantic blue crab, this species deserved a special attention during the development of this thesis, with an in-depth study of its impacts upon the Guadiana estuary food web (**Chapter 3**). Shotgun metagenomic analyses delivered an overview on main groups of prey species, which encompassed fish, molluscs, and crustaceans, including shrimps, barnacles, and other crab species, confirming the omnivorous and opportunistic traits of the Atlantic blue crab. The species with more hits were the bivalve *Cerastoderma edule* (Linnaeus, 1758), followed by the shrimp *Palaemon longirostris* H. Milne Edwards, 1837, the fish *Pomatoschistus* sp., and the oyster *Magallana gigas* (Thunberg, 1793). Several challenges arose while processing the data obtained with this methodology, namely difficulties in separating consumer (host) and prey outputs or finding the most correct species-match in reference databases. So, results were interpreted in a conservative manner because false-positives can lead to pitfalls in the interpretation of data (Alberdi et al. 2019). Nevertheless, metagenomics guided the selection of the most likely groups of preys to be used in the stable isotopes mixing models. These models showed that fish, shrimps, and bivalves were the three groups of prey functioning as the major sources of organic matter responsible for supporting the biomass of the Atlantic blue crab in the Guadiana estuary. The choice for using three groups of prey in the mixing models was due to the dispersion or overlap of isotopic signatures of other potential sources of organic matter, so that including these other sources in models would increase uncertainty and error. Other putative sources of organic matter could include other crab species which presented identical isotopic signatures as shrimps, submerged macrophytes plants, and jellyfish (invasive *Blackfordia virginica* Mayer, 1910). The Atlantic blue crab's trophic position (4.3 ± 0.5) was among the highest found in the non-native range likely because their diet was mainly based on animal prey. The comparison of trophic positions

showed a considerable niche overlap with two of the three native crabs, which in the case of the European green crab *Carcinus maenas* (Linnaeus, 1758) was on average 30.13 ± 5.21%.

Biological invasions usually go unnoticed by the scientific community during the introduction and establishment period (Simberloff and Rejmánek 2011), so innovative approaches are needed to tackle such widespread problem. Citizen science has gained popularity as an additional tool to anticipate first detections, monitor the spread of non-indigenous species, and increase the engagement with the general population in tandem with environmental literacy (Delaney et al. 2008; Crall et al. 2010; Azzurro et al. 2013). The launch of the NEMA citizen science campaign in 2019, at the onset of this thesis, proved to be very successful, being the first of its kind in Portugal exclusively dedicated to marine non-indigenous species (**Chapter 4**). Since then, the campaign has relied on the contributions from 260+ citizen scientists, 700+ validated records, with reports of three invasive species (Atlantic blue crab, weakfish *Cynoscion regalis* (Bloch & Schneider, 1801), macroalga *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, 1809), and 31 neonative species. The Atlantic blue crab was by far the most reported species in the entire campaign until 2022, with 249 records (40.2%) regarding 1986 individuals, submitted by 118 citizen scientists and informed citizens. During the first year of NEMA alone, this campaign tracked the expansion of the Atlantic blue crab along the 140 kms of the Algarve coast and towards the west coast of Portugal. The NEMA campaign detected numerous neonative marine species, reinforcing the findings of Chapter 2. This participative approach identified 11 neonative species whose presence in the Algarve region has, to the best of our knowledge, not yet been published in peer-reviewed scientific articles until the onset of this thesis – *Fistularia petimba* Lacepède, 1803, *Hermodice carunculata* (Pallas, 1766), *Pseudocaranx dentex* (Bloch & Schneider, 1801), *Sciaena umbra* Linnaeus, 1758, *Scorpaena maderensis* (Valenciennes, 1833), *Scyllarides latus* (Latreille, 1803), *Seriola rivoliana* Valenciennes, 1833, *Serranus atricauda* Günther, 1874, *Sphoeroides pachygaster* (Müller & Troschel, 1848), *Sphyraena viridensis* Cuvier, 1829, and *Thalassoma pavo* (Linnaeus, 1758). Multidimensional evidence has proven the ongoing tropicalization of continental Portugal's marine biodiversity, and while some species may have been established for some years or even decades, some "newcomers", such as the fireworm *Hermodice carunculata* or the ornate wrasse *Thalassoma pavo*, appear to be establishing themselves along the south coast of Portugal. The tropicalization of temperate regions is well documented in the Mediterranean Sea since the early 1990s (Bianchi and Morri 2003), but the location of the Algarve at the intersection of three ecoregions in the Atlantic Ocean (Spalding et al. 2007)

makes it a prime location to study the effects of climate change and the tropicalization phenomenon. This process may have been overlooked for some time by the scientific community in Portugal, as sporadic records of subtropical species may not be core evidence of significant changes when seen individually, but when the pattern is observed across a broader group of species, as the records gathered during this thesis, the tropicalization phenomenon becomes clear. While some temperate species may become less frequent, the arrival of subtropical species in a sea warming scenario, will also provide new opportunities to explore new fisheries with commercial value (Vinagre et al. 2011).

Marine invasions are nearly impossible to control and limit their spread, meaning that they should be managed more pragmatically by scientists, environmental agencies, and economic stakeholders (Pyšek and Richardson 2010). Edible marine invasive species that are established and widely distributed should be considered a priority when considering the integration of invasive species into the blue economy, as part of a well-though management strategy. This topic was the focus of **Chapter 5**, where the landings and commercial value of two invasive species in Portugal – the Atlantic blue crab and weakfish – was compared between their native and non-native ranges to assess their potential for the blue economy, while discussing the opportunities and constraints for the economic exploitation of marine invasive species. In the Algarve region, the wholesale market landings of the Atlantic blue crab have been steadily increasing since 2019, with a total 2786.5 kg (over 22k € in value) in just four years, and an average selling price at wholesale markets of 9.5 ± 3.2 € kg^{-1} in 2022. The weakfish is mostly fished in the Tejo and Sado estuaries in western Portugal and in six years the total landings in this area alone surpassed 214 metric tons (over 458k € in value), at an average selling price at wholesale markets of 2.7 ± 1.1 € kg^{-1} in 2022. Weakfish may have negative impacts in the fisheries of valuable local resources, namely upon cuttlefish in the Sado estuary since a negative correlation in landings was detected between these two species. Data from landings in the native range showed that these two non-indigenous species are still an emerging fishery in Portugal in comparison to the quantity and value they attain in their native range. Interestingly, this fact also highlights their potential to be integrated into the Portuguese blue economy. The SWOT analysis showed that the introduction into the blue economy of widely distributed invasive species, if properly planned, can become an opportunity to support the management of ecosystems and native resources, while boosting the economic development of the less development regions of Portugal, as those along the Guadiana River basin. In southern Portugal, integrating invasive species in the blue economy to promote their

management has several strengths, including the high market value they already attain which will promote its harvesting, while the extensive literature and knowledge from native areas will benefit their management and fisheries. Opportunities include the possibility to partially replace overfished native resources by the new fisheries provided via such invasive species, provide new economic opportunities in low-income areas, while innovation may arise in areas such as recreational fishing and gastronomic tourism. Non-edible invasive species of high concern, like the algae *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim, 2009, can also become a new resource of subproducts, thus helping on the removal of excess biomass. Potential weaknesses and threats include uncertainties in the legal framework about invasive species harvesting and commercialisation, limited knowledge on the distribution and seasonal abundances of invasive species in Portugal, and that invasive species populations can collapse suddenly and naturally, which threatens businesses overdependent on one species. A highly profitable invasive species fisheries can also add pressure on managers to perpetuate the fishery instead of reducing their abundance, and the risk of new intentional introductions into new areas to recreate a profitable new resource.

6.2 Future perspectives and recommendations

Allowing an invasive species to be integrated in a local economy is subject to extended debate among the scientific community (Pejchar and Mooney 2009; Nuñez et al. 2012; Pasko and Goldberg 2014; Mancinelli et al. 2017; Meadows and Sims 2023). The strategy should try to eradicate what can be eradicated, control and manage through time what is financially feasible, but also evaluate the possibility of commercial valorisation as a managing tool when it comes to deal with commercially valuable invasive species. Early detection and eradication should be the priority in areas and ecosystems where this is physically and economically attainable (Pyšek and Richardson 2010). But when an invasive species is already widely established and distributed on an ecosystem where such measures are impossible to implement, then scientists and environmental agencies should promote pragmatic management strategies. Mitigation measures should include incentives to promote multispecies fisheries targeting invasive species, which can help compensating putative losses caused by such invasive species through their commercial exploitation, while reducing the pressure upon overexploited native species. This approach needs to be studied on a case-by-case situation, considering not only the ecological characteristics of the non-indigenous species and invasion timeline, but also the surrounding socio-economic scenario. Innovative products derived from invasive species are

also critical, either from non-edible species as the algae *Rugulopteryx okamurae* or by differentiated products with an added-value as soft-shell Atlantic blue crabs, or introducing non-traditional species into European markets, such as the Black Sea jellyfish *Blackfordia virginica*, in partnership with renowned chefs.

Traditional harvesting and exploitation of marine products will be the quickest way to incorporate edible marine invasive species into the blue economy, while trying to mitigate impacts and control the spread of invasive species. The integration of invasive species into the blue economy also contributes for the diversification of food supplies and food security, contribution for several goals of the United Nations' 2030 Agenda for Sustainable Development (e.g., Goals 1, 2, 9, and 14). Promoting best practices and increasing scientific literacy related to biological invasions is of the utmost importance and should always be implemented by governmental entities in collaboration with academia. This thesis has undoubtedly proved the relevance of citizen science in the context of marine invasive species and the critical role it plays in engaging a wider audience in the management of biological invasions and increasing ocean literacy.

6.3 References

Alberdi, A., Aizpurua, O., Bohmann, K., Gopalakrishnan, S., Lynggaard, C., Nielsen, M., and Gilbert, M.T.P. (2019). Promises and pitfalls of using high-throughput sequencing for diet analysis. *Molecular Ecology Resources* 19(2): 327-348. doi: 10.1111/1755-0998.12960

Azzurro, E., Broglio, E., Maynou, F., and Bariche, M. (2013). Citizen science detects the undetected: The case of *Abudefduf saxatilis* from the Mediterranean Sea. *Management of Biological Invasions* 4: 167-170. doi: 10.3391/mbi.2013.4.2.10

Bianchi, C.N., and Morri, C. (2003). Global sea warming and "tropicalization" of the Mediterranean Sea: Biogeographic and ecological aspects. *Biogeographia–The Journal of Integrative Biogeography* 24(1). doi: 10.21426/B6110129

Crall, A.W., Newman, G.J., Jarnevich, C.S., Stohlgren, T.J., Waller, D.M., and Graham, J. (2010). Improving and integrating data on invasive species collected by citizen scientists. *Biological Invasions* 12: 3419-3428. doi: 10.1007/s10530-015-0885-4

Delaney, D.G., Sperling, C.D., Adams, C.S., and Leung, B. (2008). Marine invasive species: Validation of citizen science and implications for national monitoring networks. *Biological Invasions* 10: 117-128. doi: 10.1007/s10530-007-9114-0

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., and Ribeiro, F. (2017). On the Atlantic blue crab (*Callinectes sapidus* Rathbun 1896) in southern European coastal waters: Time to turn a threat into a resource? *Fisheries Research* 194: 1-8. doi: 10.1016/j.fishres.2017.05.002

Meadows, B., and Sims, C. (2023). Can we love invasive species to death? Creating efficient markets for invasive species harvests. *Environmental and Resource Economics* 85(2): 443-477. doi: 10.1007/s10640-023-00772-8

Nuñez, M.A., Kuebbing, S., Dimarco, R.D., and Simberloff, D. (2012). Invasive species: To eat or not to eat, that is the question. *Conservation Letters* 5(5): 334-341. doi: 10.1111/j.1755-263X.2012.00250.x

Pasko, S., and Goldberg, J. (2014). Review of harvest incentives to control invasive species. *Management of Biological Invasions* 5(3): 263. doi: 10.3391/mbi.2014.5.3.10

Pejchar, L., and Mooney, H.A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution* 24(9): 497-504. doi: 10.1016/j.tree.2009.03.016

Pyšek, P., and Richardson, D.M. (2010). Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources* 35: 25-55. doi: 10.1146/annurev-environ-033009-095548

Simberloff, D., and Von Holle, B. (1999). Positive interactions of nonindigenous species: Invasional meltdown? *Biological Invasions* 1: 21-32. doi: 10.1023/A:1010086329619

Simberloff, D., and Rejmánek, M. (2011). Encyclopedia of biological invasions. University of California Press, Berkeley, CA. 792 pp.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., and Lourie, S.A. (2007). Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *BioScience* 57(7): 573-583. doi: 10.1641/B570707

Vinagre, C., Santos, F.D., Cabral, H., and Costa, M.J. (2011). Impact of climate warming upon the fish assemblages of the Portuguese coast under different scenarios. *Regional Environmental Change* 11: 779-789. doi: 10.1007/s10113-011-0215-z