INÊS MARIA FERREIRA DE MORAIS CERVEIRA

WEAKFISH *CYNOSCION REGALIS* (PISCES: SCIAENIDAE) (BLOCH & SCHNEIDER, 1801) ECOLOGY IN ITS NON-INDIGENOUS RANGE AND ITS POTENTIAL AS A NEW FISHING RESOURCE

UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologias
2018/2019

INÊS MARIA FERREIRA DE MORAIS CERVEIRA

WEAKFISH *CYNOSCION REGALIS* (PISCES: SCIAENIDAE) (BLOCH & SCHNEIDER, 1801) ECOLOGY IN ITS NON-INDIGENOUS RANGE AND ITS POTENTIAL AS A NEW FISHING RESOURCE

Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:
Professora Doutora Maria Alexandra Teodósio
Doutor Pedro Morais

UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologias
2018/2019

Declaração de autoria de trabalho

WEAKFISH *CYNOSCION REGALIS* (PISCES: SCIAENIDAE) (BLOCH & SCHNEIDER, 1801) ECOLOGY IN ITS NON-INDIGENOUS RANGE AND ITS POTENTIAL AS A NEW FISHING RESOURCE

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.

(Inês Maria Ferreira de Morais Cerveira)

Agradecimentos

À minha orientadora, Professora Doutora Alexandra Teodósio, pela orientação, pelo apoio e confiança que depositou em mim desde o início e ainda pelas oportunidades que me proporcionou.

Ao meu co-orientador, Doutor Pedro Morais, pelo seu aconselhamento, no trabalho e na vida, pela exigência e por nunca duvidar das minhas capacidades.

À equipa do laboratório ECOREACH do CCMR, em particular à Vânia Baptista, pela amizade, por todo o seu apoio em todas as fases deste trabalho e por me ter acolhido em sua casa como se fosse da família. À Joana Cruz pela ajuda e pelos conselhos, mas acima de tudo pelas horas de conversa e bom humor. Sem elas o trabalho teria sido mais difícil.

À Ester Dias do CIIMAR, pela sua orientação, paciência e compreensão que teve ao ajudar-me em todo o trabalho.

Ao Júlio André Cipriano da Setúbal Alive, pela sua amabilidade, por se ter disponibilizado a arranjar exemplares de corvinata-real e por nos ter ensinado a pescar no estuário do Sado.

Ao Chef Leonel Pereira do restaurante São Gabriel em Almancil, pela sua excentricidade, interesse e entusiasmo neste tema, que foram fundamentais para o desenvolvimento e divulgação deste trabalho.

Aos meus colegas de mestrado, em especial à Sara Pereira, pela sua amizade, companheirismo e pelos bons momentos experienciados.

À minha família, que me apoia e motiva sem questionar as minhas escolhas.

Ao Luis, pelo amor, por estar sempre comigo nos bons e maus momentos e sempre me incentivar a fazer o que mais gosto.

Resumo

As espécies invasoras podem interferir em processos ecológicos previamente estabelecidos num ecossistema, existindo assim uma preocupação acrescida com as espécies que possam prejudicar populações de espécies nativas com valor comercial. É o caso da corvinata-real *Cynoscion regalis* (Pisces: Sciaenidae), também conhecida por corvina americana ou rainha, uma espécie nativa da costa NE da América do Norte, que estabeleceu uma população invasora no estuário do Sado, tendo já sido registada em vários pontos da Península Ibérica. Os pescadores manifestaram a sua preocupação com a competição que possa existir entre a corvinata-real e os peixes nativos com valor comercial, contudo, não há evidências científicas que demonstrem que a corvinata-real está a competir com espécies nativas por alimento e espaço. Assim, o principal objetivo deste trabalho é fornecer as primeiras informações relativas à ecologia da população de corvinata-real no estuário do Sado, enquanto os seus objetivos específicos são: i) determinar a dieta e estratégia alimentar usada pela corvinata-real, através da análise de conteúdos estomacais, bem como de três espécies nativas com valor comercial – corvina-legítima *Argyrosomus regius*, robalo *Dicentrarchus labrax* e sargo *Diplodus sargus*; ii) inferir a sua posição trófica e a sobreposição de nicho trófico com estas três espécies nativas, através da análises de isótopos estáveis de carbono e azoto; iii) inferir o uso do habitat da corvinata-real através da análise da química de otólitos e averiguar a sobreposição de habitat com as três espécies nativas; iv) discutir os potenciais impactos desta invasão nos recursos pesqueiros do estuário do Sado, tendo em consideração a dieta e o habitat usado pela espécie ao longo do ciclo de vida; v) avaliar o potencial da corvinata-real como um novo recurso pesqueiro, através de inquéritos realizados a consumidores voluntários, e acções de sensibilização (parceria com chefs locais e publicação de dois comunicados de imprensa).

Os resultados dos conteúdos estomacais apontam que no estuário do Sado, a corvinata-real é predadora de crustáceos (53%) (principalmente mysidaceos, carídeos, isópodes e caranguejos), peixes (45%) (principalmente peixe-rei *Atherina* sp. e anchova *Engraulis encrasicolus* e cefalópodes (2%). Um caso de canibalismo foi verificado. Assim, esta espécie parece alimentar-se dos mesmos grupos de presas e em proporção semelhante ao que acontece na região nativa. A estratégia alimentar é generalista e a população aparenta ser composta por indivíduos especialistas, ou seja, indivíduos que têm preferência por diferentes presas, havendo assim uma baixa sobreposição de presas entre

indivíduos. Este foi certamente um fator importante que influenciou esta espécie a estabelecer-se nesta região.

Os resultados da análise de isótopos estáveis revelaram que a corvinata-real, a corvina-legítima e o robalo estão a alimentar-se no mesmo nível trófico, enquanto que o sargo está um nível trófico abaixo, uma indicação de que o sargo está a consumir presas diferentes ou em diferente proporção. Não houve uma sobreposição significativa de nicho isotópico, ou índice de Pianka (calculado com base no número e tipos de presas consumidas), entre a corvinata-real e as outras três espécies, rejeitando a hipótese de que a corvinata-real está a competir por alimento com estas espécies nativas. No entanto, a probabilidade de sobreposição de nicho foi maior com a corvina-legítima, indicando uma maior probabilidade de competição por alimento, possivelmente por serem espécies taxonomicamente próximas. A análise química dos otólitos revelou que identicamente à região nativa, a corvinata-real realiza um padrão anual de migrações, usando o estuário (até à zona de água doce) na primavera e no verão, e as áreas costeiras no inverno. Confirma-se, assim, a utilização simultânea do estuário pela corvinata-real, corvina-legitima, robalo e sargo, evidenciando a competição pelo uso do habitat.

Em relação aos potenciais impactos desta espécie noutros recursos pesqueiros, a corvinata-real poderá afectar a demografia das suas presas, algumas com valor comercial na região do estuário do Sado (ex: carapau *Trachurus trachurus*, anchova *Engraulis encrasicolus*, lula comum *Loligo vulgaris*, e choco comum *Sepia officinalis*. Poderá também competir com espécies nativas através da competição por alimento, uma vez que outras espécies também se alimentam das mesmas presas (ex: dourada *Sparus aurata*, sargo-do-senegal *Diplodus bellottii*, a anchova *Engraulis encrasicolus*, o carapau *Trahurus trachurus*, o choco comum *Sepia officinalis*, lula comum *Loligo vulgaris*, polvo comum *Octopus vulgaris*, golfinho-roaz *Tursiops truncatus*). Contudo é difícil responsabilizar a corvinata-real por algum impacto, uma vez que outros factores podem prejudicar espécies nativas, como a perda de habitat ou pesca excessiva. Há também a possibilidade de espécies nativas usarem a corvinata-real como alimento, uma questão que deverá ser abordada em estudos futuros com maiores amostragens de espécies nativas.

Os resultados dos inquéritos revelaram que esta espécie tem um grande potencial para ser bem aceite no mercado: grande parte dos consumidores demonstrou interesse em adquirir o peixe posteriormente; a maioria prefere peixe selvagem e por isso muitos optariam por adquirir a corvinata-real em vez de peixes nativos provenientes de aquacultura; reconhecem que a espécie está subvalorizada (5€ kg^{-1}) e estão dispostos a pagar em média mais 3€ kg^{-1} que o preço médio actual. Verificamos também que o principal motivo que levou os consumidores a preferirem outras espécies de peixes nativos (ex: corvina-legítima, dourada, sardinha) é a falta de informação sobre a espécie e o hábito de consumo. Assim, uma opção para contornar este conservacionismo dos consumidores será criar acções de divulgação que os informem sobre os benefícios de consumir uma espécie invasora, destacar a sua proveniência (mar), e promover o seu uso em pratos que melhorem o seu sabor e textura, nomeadamente pratos que envolvam o peixe partido, como caldeiradas ou massadas.

As acções de sensibilização criadas neste trabalho (parceria com chefs locais e publicação de comunicados de imprensa) demonstraram que existe interesse na corvinata-real e na temática das espécies invasoras, permitindo assim a promoção deste novo recurso-pesqueiro e a consciencialização dos portugueses sobre o aparecimento de espécies invasoras. Assim, a comercialização da corvinata-real tem potencial para ser optimizada e poderá vir a contribuir para a redução da população no estuário do Sado e minimizar os seus possíveis impactos. Contudo, é importante considerar os riscos e benefícios desta abordagem e incluir acções de monitorização e de divulgação que clarifiquem que os benefícios da sua comercialização não superam os benefícios do controlo da população a longo prazo.

Palavras-chave: invasões biológicas, sobreposição ecológica, isótopos estáveis, otólitos, Península Ibérica

Abstract

Weakfish *Cynoscion regalis,* a species native to the NE-coast of North America has established an invasive population in the Sado estuary, and since it is being captured in large numbers in Sado estuary and it is already being sold in fish markets, anglers expressed their concern with competition between weakfish and native prize fish; however, there are no scientific evidences showing that weakfish will outcompete native species for food and space. Therefore, we determined weakfish diet through stomach content analysis, and for three native prize fish: meagre, European bass, and white seabream. Results revealed that weakfish has a generalist feeding strategy and is preying on crustaceans (53%), fish (45%) and cephalopods (2%). We inferred trophic position through isotopic carbon and nitrogen content analysis in muscle and fin tissues, and weakfish, meagre and seabass are feeding at the same trophic level, and seabream a trophic level below. There was no significant isotopic niche overlap between weakfish and the three species, although it was higher for meagre. To infer competition for space, we determined weakfish habitat use through otolith chemistry analysis that revealed an annual pattern of migrations, using the estuary in spring/summer and coastal areas in winter, confirming the simultaneous use of the estuary among weakfish and the three natives.

To evaluate the potential of weakfish as a new fishing resource, we provided inquiries to consumers and created awareness events to engage the media and assess public interest on this matter. Inquiries revealed that consumers recognize weakfish potential, since they showed interest in acquiring the fish, would pay more than the current mean price, and would opt to buy weakfish instead of farmed native fish. The feedback from the awareness events was positive, indicating that invasive species subject attracts enough interest to promote weakfish successfully and increase society's awareness on invasive species.

Keywords: biological invasions, ecological overlap, stable isotopes, otoliths, Iberian Peninsula

Table of Contents

Index of Figures

Chapter I: Introduction

Chapter II: Insights into the ecology of weakfish *Cynoscion regalis* (Pisces: Sciaenidae) (Bloch & Schneider, 1801) in its non-native range

Chapter III: Weakfish as a new fishing resource: could weakfish be accepted by the Portuguese consumers?

Index of Tables

Chapter II: Insights into the ecology of weakfish *Cynoscion regalis* (Pisces: Sciaenidae) (Bloch & Schneider, 1801) in its non-native range

Chapter III: Weakfish as a new fishing resource: could weakfish be accepted by the Portuguese consumers?

List of Abbreviations

ASMAL – Algarve Mental Health Association

BPC – Between-phenotype component

eDNA – Environmental DNA

FO – Frequency of occurrence

IRMS – Isotope-ratio mass spectrometry

LA-ICPMS – Laser ablation inductively coupled plasma mass spectrometry

NW – Northeast

PSA – Prey specific abundance

SEA – Standard ellipse area

SEAc – Corrected standard Elipse Area

SIA – Stable Isotope Analysis

SIBER – Stable Isotope Bayesian Ellipses in R

UK – United Kingdom

US – United States

WPC – Within-phenotype component

Chapter I: Introduction

1. Biological invasions

1.1. Non-indigenous species and invasive species

Biological invasions and their impacts on the functioning of ecosystems has been a topic of profound debate and research over the last two decades (Sousa *et al.* 2011; Simberloff *et al.* 2013; Boltovskoy *et al.* 2018). The introduction of non-indigenous species may lead to the establishment of self-sustaining populations outside of their native range (Leppäkoski *et al.* 2002). However, non-indigenous species do not necessarily become invasive. A non-indigenous species is introduced through anthropogenic vectors (e.g., shipping, biofouling, canals, pet trade, aquaculture), which allows them to overcome biogeographical barriers that limit natural dispersal (Blackburn *et al.* 2014). After the introduction, individuals from a given species may perish or survive and establish a viable population. Intrinsic (e.g., phenotypic plasticity) and/or extrinsic factors (e.g., lack of natural enemies) may favour invasiveness or not, which may disrupt the established community dynamics, threatens biological diversity, and even economic loses (CBD 2017; Boltovskoy *et al.* 2018). There is no consensus about the definition of invasive species, since it is usually composed of subjective terms such as "negative impacts" or "threat to biodiversity" that may lead to different interpretations (Russell & Blackburn 2017), however, it is up to scientists to justify its application through scientific evidence (Colautti & MacIsaac 2004). It is relevant also to distinguish the invasion process from a natural range expansion which results from the natural movement of a species to surrounding areas of its distribution range when prevailing biotic and/or abiotic factors change (e.g., landscape fragmentation, global climate change, resource distribution, mating opportunities, predation risk, competition) (Gibbs *et al.* 2010).

The invasion process is divided into four stages: transport, introduction, establishment, and spread. After introduction, the dynamics of a non-indigenous species is illustrated by five possible scenarios (Figure 1.1): A) a species is introduced but establishment fails; B) a species is introduced, establishes a population but remains non-invasive; C) a species becomes invasive after establishment; D) a species is introduced, the invasion process is triggered and fails due to intrinsic and/or external factors, however the species remains established; E) a species fails to establish even after becoming invasive at an initial stage. During the invasion process (C), a species tries to succeed in the new environment, and its abundance may remain low and unnoticed for an undetermined period of time – the lag phase (Mack *et al.* 2000; Crooks 2005), however certain conditions may trigger invasiveness and the population's abundance exponentially increases until reaching an equilibrium (Novak 2007).

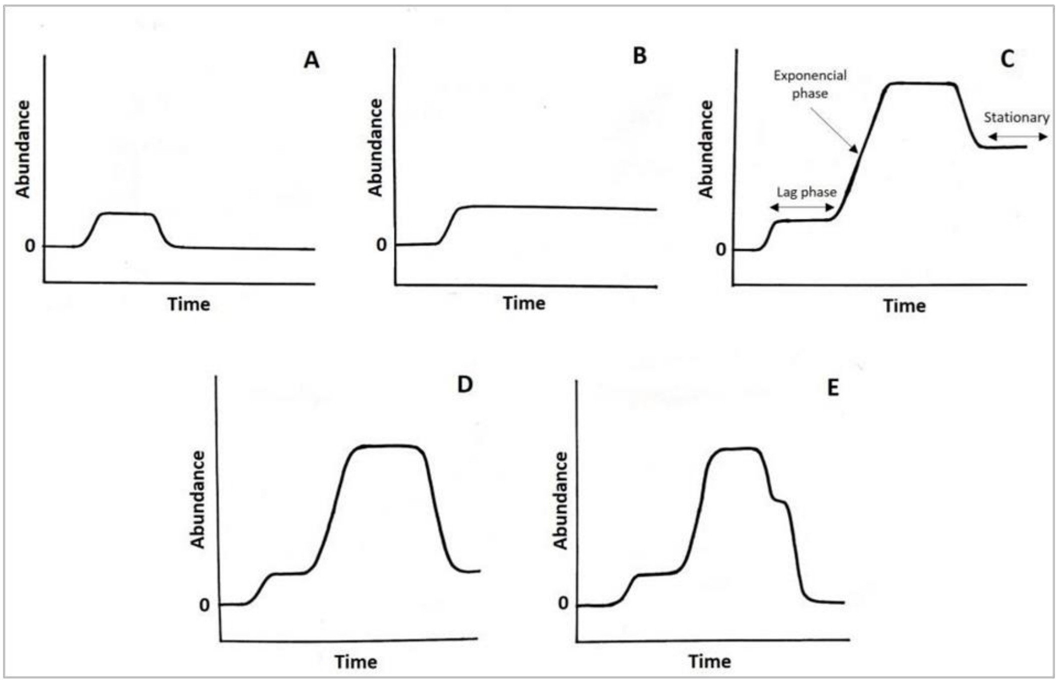


Figure 1.1. Five possible scenarios of non-indigenous species dynamics after introduction: A) establishment fails; B) established but species remains non-invasive; C) species becomes invasive after an exponential abundance increase, and then it reaches an equilibrium; D) invasion fails due to intrinsic and/or external factors, but species remains established; E) invasion and establishment fails after successful introduction.

1.2. Introduction vectors in aquatic systems

Biological invasions occur when organisms are transported and introduced into new regions through introduction vectors. These vectors may determine the distribution, frequency, and even the species that are introduced (Mack *et al.* 2000)). In aquatic ecosystems, the most common introduction vectors are shipping (Wonham *et al.* 2000; Keller *et al.* 2011; Bailey 2015), biofouling (Frey *et al.* 2014; Ruiz *et al.* 2015), canals (Carlton & Ruiz 2005), pet trade and public aquaria (Padilla & Williams 2004; Strecker *et al.* 2011), aquaculture (Welcomme 1991; Naylor *et al.* 2001), and intentional introductions (Bax *et al.* 2003).

Introduction through shipping is common for organisms transported in the hull of ships, in ballast water or dry ballast, cargo, decks, and anchors, usually carrying many specimens of several species to form a viable inoculum (Minchin & Gollasch 2002). Ballast water specifically can transport viruses, bacteria, plankton (including meroplankton), and sediment-associated species (Minchin & Gollasch 2002) because water-intake grids only prevent the entry of large biota (Wonham *et al.* 2000; Seebens *et al.* 2013). After the establishment of a population, natural dispersal may occur, as well as secondary introductions through intraregional ship transportation (Minchin & Gollasch 2002). Ballast water is considered the largest unintentional introduction vector of marine organisms, including some of the most problematic invasions, like the zebra mussel in North America or toxic dinoflagellates in Australia (Ruiz *et al.* 2015). The increased size of ships and their ballast water tanks, faster travels and frequent stops at different ports increase propagule pressure because organisms are less stressed, the diversity of species and number of individuals is greater as well as the potentially affected areas (Carlton 1996). So, defining areas where ballast water can be discharged safely and areas suitable for collecting water are among the many preventive measures that try to minimize this problem (IMO 2017). Biofouling introductions are usually associated with shipping and less commonly with rafting (e.g., tsunami wrecks from Japan reached the west coast of the US) which also facilitates transoceanic dispersal of a wide variety of sessile and mobile species (Carlton *et al.* 2017).

Figure 1.2. Ballast water discharge in the Sado estuary, Setúbal, Portugal.

Artificial canals that were created to shorten navigation routes are also a pathway for the introduction of non-indigenous species by connecting distinct biogeographical regions (e.g., Suez and Panama canals) (Azzurro *et al.* 2016) or facilitating the spread of non-indigenous species into other river basins, either naturally or not (Bij de Vaate *et al.* 2002).

Pet trade and public aquaria are also responsible for the introduction of fish, plants, and invertebrates (Cohen *et al.* 2007; Duggan 2010). They are easily accessible over the internet so transport and impacts can occur on a global scale and affect most aquatic ecosystems, except polar ecosystems. The release of pets in nature occurs because people consider it the least detrimental for the animal (Severinghaus & Chi 1999; Gertzen *et al.* 2008). Pet release occurs when people lack the interest in the animal, either because they became too aggressive, big, or fertile (Gertzen *et al.* 2008; Duggan 2010). Aquarium and ornamental trade usually choice healthy individuals that are more likely to withstand harvesting and transport harsh conditions, and yet survive and reproduce in aquariums at the final destination, being responsible for one-third of the worst aquatic invasions (Padilla & Williams 2004). The most efficient way to reduce aquarium trade introductions is prevention, which involves tracking every stage of pet trade, evaluate human behavior, species popularity (Gertzen *et al.* 2008), and create outreach strategies on environmental and legal consequences for those releasing pets in nature (Duggan 2010).

Intentional introductions may include species used in aquaculture, recreational angling (Savini *et al.* 2010), and biocontrol (Mack *et al.* 2000). Indeed, fish stocking for angling and aquaculture are the most significant pathways for non-indigenous fish introductions into European freshwaters during the 20th century (Welcomme 1991; Elvira & Almodóvar 2001). Species used in aquaculture are not necessarily deliberately introduced, but it usually occurs owing to fish escapees (Bartley 2011). Non-indigenous species are also used in biocontrol approaches, resulting in the attack and/or risk of extinction of non-targeted native species – sometimes even of species that should be preserved (Simberloff & Stiling 1996).

After introduction, a series of unintentional dispersal events might occur, either mediated by faunal transport – e.g., aquatic birds might transport cysts of non-indigenous species between different basins (Green & Fisher 2004); fish can disperse their parasites into other basins – e.g., the natural movements of eels in fresh, brackish, and coastal waters have accelerated dissemination and extended the range of the asian parasite *Anguillicoloides crassus* (Kuwahara, Niimi and Itagaki 1974) throughout Europe (Kirk

2003) – or by humans (e.g., biofouling of recreational boats, waders used by anglers or scientists) (Waterkeyn *et al.* 2010).

1.3. Invasiveness of aquatic species

Invasive species share traits and conditions that potentiate their invasiveness and that favor them in the prevailing abiotic and biotic characteristics of the non-indigenous ecosystem (Richardson & Pyšek 2006). To succeed, an invasive species must get through different stages: entering a means of transportation, survive the transport in conditions that allow it to successfully exit the transport vector, and then establish a population that may or may not become invasive.

Propagule pressure is a factor dictating establishment and eventually the invasiveness of a population. It is defined as the amount of nonindigenous individuals released in the new environment, whether in the adult or early-life stages (Johnston *et al.* 2009). There are two ways to achieve a high propagule pressure: a single introduction with a high abundance of individuals (propagule size) or successive introductions of fewer individuals (propagule number), and that could involve different temporal rates (Simberloff 2009). It represents the potential for introduction, so the higher the propagule pressure the higher the probability of introduction success and later on of invasion success (Johnston *et al.* 2009).

Some hypotheses emerged to explain what happens to a new species when it establishes into a new range. The Enemy-Release Hypothesis states that the release from natural enemies, as parasites or predators, could provide a competitive advantage to nonindigenous species, enhancing demographic expansion and invasiveness (Gendron *et al.* 2011). Parasites reduce host density and their body size, so leaving them behind is clearly beneficial (Torchin *et al.* 2001). Indeed, some non-indigenous populations are less parasitized than native populations (Torchin *et al.* 2003), likely owing to five main reasons: (1) most of the native parasitic fauna is left behind since only some non-indigenous hosts are being transported (Gendron *et al.* 2011); (2) non-indigenous species are usually introduced at an early-life stage, so chances of parasites co-introduction is reduced because parasites usually accumulate in larger and older organisms (Sasal *et al.* 1997); (3) non-indigenous species that survived until introduction are more likely to be less parasitized and/or are more resistant to diseases/parasites (Gendron *et al.* 2011); (4)

some parasites depend on intermediate hosts, but intermediate hosts are not necessarily introduced in tandem with the introduced species so the parasites will become extirpated (Torchin *et al.* 2003); (5) host-specific relation disables colonization by native parasites at the new location; however, this might be temporary since new host-parasite associations over time might occur (Krakau *et al.* 2006; Gendron *et al.* 2011).

Phenotypic plasticity also promotes invasiveness (Niu *et al.* 2012), by enabling a broad spectrum of behavioral, morphological, and physiological traits providing non-indigenous species with a competitive advantage in relation to native competitors and in a wider range of ecosystems (Knop & Reusser 2012; Weir & Salice 2012). Phenotypic plasticity enables non-indigenous species to maintain fitness in stressful conditions (robust species), to increase fitness in favorable conditions (opportunistic species), or both (robust and opportunistic species) (Richards *et al.* 2006). Some of the invasive species intrinsic traits are similar to traits of opportunistic species, which means that they can take the maximum advantage of the conditions provided to them. Some of these traits are linked with reproductive traits (e.g., number and size of eggs, parental care) (Bernardo 1996), higher growth rates (e.g., larger individuals are less susceptible to predation), higher number of generations (i.e., reaching a larger population faster than other native species), or dispersal capacity (e.g., swimming capacities, resistance to dissection), and even personality (Cote *et al.* 2010).

The receiving habitat also plays a key role in the invasion process. When a communities' equilibrium state is surpassed, either by press- or pulse-disturbances (Bengtsson 2002), a new equilibrium state has to be reached, enabling native opportunistic species or non-indigenous species to succeed within the community (*sensu* priority effect hypothesis) (Young *et al.* 2001). Also, while establishing in a new location, the lack of evolutionary history between the non-indigenous species and the native community may result in a lack of antipredator behavior from native species (Strauss *et al.* 2006; Sih *et al.* 2010). Some "naïve species" do not know how to deal with a new species and may even approach by curiosity which confers some advantage to the non-indigenous species (Zuberi *et al.* 2011).

1.4. Impacts of invasive species

Invasive aquatic species induce ecological (Mack *et al.* 2000; Strayer 2012; Gallardo *et al.* 2016) and evolutionary (Mooney & Cleland 2001; Grosholz 2002; Lee 2002) changes in recipient ecosystems. At a species-level, non-indigenous species can alter the demography of native species by competing for resources, by preying on them, or aggressiveness (Mooney & Cleland 2001; Crooks 2002b). At the community and ecosystem levels, non-indigenous species exert their impact through multiple mechanisms: (1) limiting resources (e.g., food, space, refuge habitats, mating partner) (Mack *et al.* 2000); (2) disrupting the established energy flow and food web dynamics (Crooks 2002b); (3) changing disturbance regimes (Bengtsson 2002); (4) altering the biotic and/or abiotic physical structure of the ecosystem (i.e., ecosystem engineers) (Crooks 2002a; Byers *et al.* 2012); (5) facilitate the introduction of other non-indigenous species and incrementing their impacts, a process known as invasional meltdown (Montgomery *et al.* 2011). However, each introduction is different and the impacts of non-indigenous species might not always be negative. In some cases, non-indigenous species may benefit native species by serving as food resource (Crooks 2002a, b), by establishing indirect or commensal relationships (Crooks 1998), and by serving as ecosystem engineers which may provide refuge habitats and modulate abiotic factors (Simberloff & Von Holle 1999; Crooks 2002a).

Impacts might also arise from hybridization between a non-indigenous species and a native species: (1) the creation of an invasive hybrid genotype leading to the formation of an hybrid more invasive than the introduced species (i.e, heterosis or hybrid vigor) can ultimately lead to the extirpation of the native parental species (Grosholz 2002); (2) the creation of sterile hybrids, that compete for resources with native species and might lead to a native population species to decline trough the waste of native gametes (Parker *et al.* 1999); (3) the loss of native genotypes trough introgressive hybridization, i.e., the stable integration of genetic material from one species into another through repeated backcrossing, until the "foreign" alleles become present throughout the recipient community (Baack & Rieseberg 2007; Schierenbeck 2011). At a longer time scale, repeated hybridization might homogenize biota across biogeographic realms and thus altering evolutionary pathways (Mack *et al.* 2000; Crooks 2002a).

Besides the ecological and evolutionary impacts on non-native species on ecosystems, they also induce economic impacts. In UK and Ireland combined, for example, the current estimate of annual cost reached 2.5 billion € (Kelly *et al.* 2013). Therefore, the detection and management of non-indigenous species is of paramount importance for the society.

1.5. Detection and Management of non-indigenous species

Management of invasive species requires organization and comprehension of the invasion process over time. Usually, by the time when the impacts of invasive species are noticed, the changes produced in the ecosystem are already irreversible and it substantially increases the management efforts and costs (Vilà *et al.* 2011). So, the optimal strategy to manage invasive species relies on prevention rather than on dealing with invasions' impacts (Figure 1.3). Management policies that are timely taken increase efficiency and save money, a process that can be divided in three main steps: (1) prevention; (2) early detection; (3) management (Figure. 1.3).

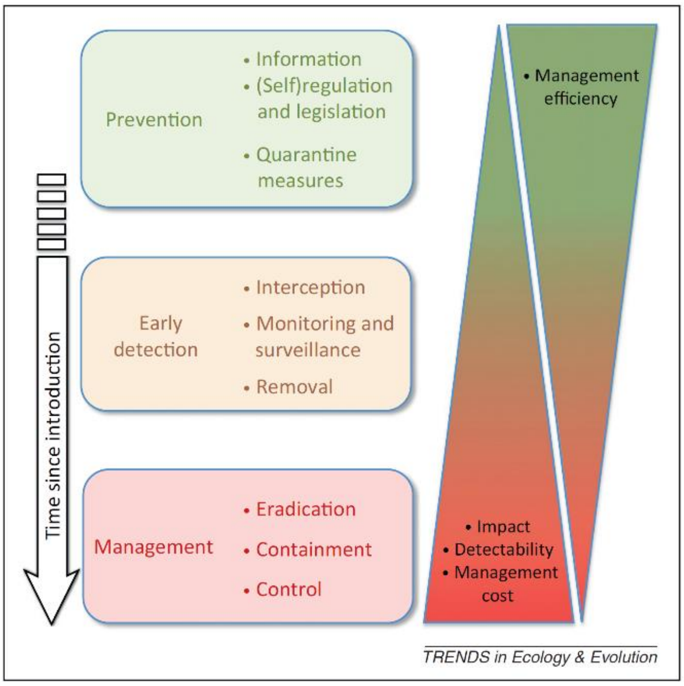


Figure 1.3. Schematic summary of the ideal management strategy of (potentially) invasive species over time. The more time elapses since lintroduction the higher the management costs, while management efficiency reduces management cost. Retrieved from (Simberloff *et al.* 2013).

Prevention is achieved by promoting management actions that aim at searching for species that are prone to cause significant economic impacts (Økland *et al.* 2011).

Such actions provide information related to risk prevention, which might involve informing the society on a potential threat, as it is the case of the "Protect Your Waters Program" (StopAquaticHitchhikers 2019). This program informs society on the risks posed by non-indigenous species and aims at preventing their introduction in and dispersal into the Great Lakes (North America). Prevention also concerns producing new policies, as the new restrictive ballast water policies (i.e., pathway constriction- ballast water treatment; interception- mid-ocean ballast water exchange) that aims at reducing propagule pressure (Simberloff *et al.* 2013).

Early-detection policies usually involve the detection of non-indigenous species at the site of entry (e.g., harbors), owing to active monitoring and surveillance on targeted (specific species control) or non-targeted species (multiple species) (Thomsen & Willerslev 2015). Non-targeted monitoring is becoming more efficient owing to environmental DNA (eDNA) analyses, which are obtained directly from an array of sample types (e.g., water, soil, sediment) that may contain DNA molecules from skin, mucous, sperm, secretions, eggs, faeces, urine, blood, and many others (Bohmann *et al.* 2014; Pedersen *et al.* 2015). The potential of this technique is enormous mainly due to their capability to screen several taxonomic groups simultaneously and because it even allows estimating species density (Thomsen & Willerslev 2015; Pawlowski *et al.* 2018). If early detected, species removal is ecologically less risky than later interventions, since interspecific relations within the invaded community were not established yet (Simberloff *et al.* 2013), and persistence can be monitored with this technique after the adoption of containment/eradication countermeasures (Pawlowski *et al.* 2018). Citizen science monitoring programs based on contributions of fishers, divers, and others could also be used along with traditional monitoring programs to help detect a species presence earlier – e.g., citizen observations documented lionfish 1–2 years earlier and more frequently than traditional reef fish monitoring programs (Scyphers *et al.* 2015).

When non-indigenous species are detected at the invasion stage, then the only procedure left is to manage the invasion mostly due to the lower probabilities of containing, controlling, and eradicating the invasion, all of which come at a greater financial cost (Figure 1.3). Eradication often impacts non-targeted native species (Caut *et al.* 2009); so, research must be conducted to avoid collateral impacts and minimize reinvasion (Simberloff *et al.* 2013). Upon species removal, active restoration is mandatory to re-establish native communities and avoid the risk of a community being dominated by opportunistic or non-indigenous species (Gaertner *et al.* 2012).

2. Objectives

Invasive species disrupt established ecological dynamics and special concern exist on those that may impair populations of native commercial species. This is the case of weakfish *Cynoscion regalis* (Pisces: Sciaenidae) (Bloch & Schneider, 1801) (Actinopterygii: Sciaenidae), a species native to the NW-Atlantic Ocean that established an invasive population in the Sado estuary (Portugal, Europe). Anglers are concerned with the competition between weakfish and prize fish (e.g., meagre, seabreams, seabass). However, there are no scientific evidence showing that weakfish will outcompete these native species for food and space. Nonetheless, weakfish has already been sold in some fish markets; so, it is possible that weakfish might represent a new fishing resource for local fishing communities, as it happens with other native species. Thus, the overall objective of this thesis is to provide the first comprehensive ecological dataset on an invasive weakfish population, while the specific objectives are to:

i) determine weakfish diet and trophic position through stomach content analysis;

ii) infer trophic position overlap with several prize fish – meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and white seabream *Diplodus sargus* (Linnaeus, 1758) – through carbon and nitrogen stable isotopes analyses;

iii) infer weakfish habitat use through the analysis of otolith chemistry, since this technique allows to retrospectively assign habitat along the entire life of each individual analysed;

iv) discuss potential impacts of weakfish invasion upon fishery resources from the Sado estuary, considering diet and habitat use;

v) evaluate the potential of weakfish as a new fishing resource through inquiries made to voluntary consumers.

Chapter II: Insights into the ecology of weakfish *Cynoscion regalis* (Pisces: Sciaenidae) (Bloch & Schneider, 1801) in its non-native range

1. Introduction

Invasive species may threaten recipient ecosystems and induce changes in native species abundance, ecological integrity, and ecosystem functioning (Vander Zanden *et al.* 1999). A species that successfully establish itself in a new ecosystem means that it has been able to integrate into the food web of its new habitat, and this often happens in the form of competition. In turn, through competition, the common preferred resources may become depleted (exploitative competition), or their access is blocked (interference competition), forcing the use of less preferred resources (Mooney & Cleland 2001; Amarasekare 2002). Competitive interactions are strongly influenced by the ecological strategies used by the novel species, such as feeding strategy. For example, a generalist invader will become more likely to adapt to a new environment since it relies on a wide variety of resources, minimizing direct competition (Sax & Brown 2000; Guzzo *et al.* 2013), while a novel specialist will compete directly with indigenous species for their preferred resources, possibly leading to natives exclusion (Juncos *et al.* 2015). Consequently, putative impacts (e.g., niche narrowing, changes in fitness, physiological condition, abundance of native competitors) (Curtis *et al.* 2017; Britton *et al.* 2018) are stronger when a native species resembles the invader, as functional similarity as they may use identical feeding strategies, prefer the same resources, and use the same spaces (Hardin 1960; Ricciardi & Atkinson 2004).

Weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) is native from the NE-coast of North America and has recently established invasive populations in the Iberian Peninsula (Morais & Teodósio 2016; Morais *et al.* 2017), and so far, there is no information on the ecology of the species in the new range. Weakfish was recorded for the first time in the Scheldt estuary (Belgium/The Netherlands) in 2009 (ANB 2011), however the species did not establish a population because temperature is often lower than its thermal limits (SeaTemperature 2018), since it ceases feeding at 7.9 ºC and dies at 3.3 ºC (Bigelow & Schroeder 1953). In the Iberian Peninsula, weakfish is present in the Gulf of Cádiz at least since 2011 (Bañón *et al.* 2017) and it has reached an invasive status in the Sado estuary at least since 2012 (MundoDaPesca 2014; Bañón *et al.* 2017). It was also captured in other locations along the Atlantic coast of Iberian Peninsula: Tagus and Mira estuary at least since 2013 or 2014, Praia da Vieira (Leiria) in 2015, Ría de Vigo in 2016, Ría del Barqueiro in 2016, Guadiana estuary and Olhos de Água in 2016, Ria Formosa in 2017 (Morais *et al.* 2017) (Figure 2.1). The existence of two viable populations in the Iberian Peninsula was hypothesized, one in the Sado estuary and the other in the Gulf of Cadiz (Morais *et al.* 2017). Both locations are near ports with intense transoceanic shipping traffic: the Sado estuary contains a harbour (Port of Setubal) and is located 60 km north of the 6th busiest transhipment port in Europe (Port of Sines) (APPA 2018), while Guadalquivir estuary is near a US military base, a shipyard, and a large port (Porto of Cádiz) in 30 km range, reinforcing the ballast water hypothesis as one of the possible introduction vectors for this species. Aquaculture escapees/release is another hypothesis for the introduction of weakfish in the Iberian Peninsula; however, there are no records of weakfish production on Portuguese and Spanish aquaculture facilities (Morais & Teodósio 2016). This last hypothesis can only be excluded after genetic analyses. In the meantime, two facts support the "ballast water hypothesis", (1) the short duration of transoceanic ship travel between the east coast of North America and the western Iberian Peninsula coast (nine days; SeaRates (2018)), and (2) the physiological plasticity of weakfish larvae and young-of-the-year, that may suggest that weakfish specimens circumvented ballast water exchange regulations (Able & Fahay 2010). Thus, if this hypothesis is correct, successful establishment of a weakfish population may have occurred through the rise of a viable population or through continuous and successful introductions via ballast water (Morais & Teodósio 2016). Although there are no published studies confirming the reproduction of this species in Iberian Peninsula, the size of the fish, abundance, and distribution seem to indicate it. Morais and Teodósio (2016) hypothesized three scenarios for pathways of weakfish introduction in Iberian Peninsula: (1) weakfish was introduced in three locations (Galicia, Sado estuary, and Gulf of Cádiz) followed by successful establishment, forming three localized populations; (2) weakfish was introduced in one of the extreme regions (Galicia or Gulf of Cadiz) followed by posterior dispersion; and (3) weakfish was introduced in a central area with posterior establishment and dispersal (Morais & Teodósio 2016). Since establishment of fish after introduction via ballast water is not the most common (Wonham *et al.* 2000; Padilla & Williams 2004) the first hypothesis (i.e., establishment in three sites) is very unlikely (Morais & Teodósio 2016). From the other two scenarios, the third was considered the most likely, since Sado estuary (the central area) is the site where anglers describe the species for the longest time, it has the highest number of non-indigenous marine species in Portugal (Chainho *et al.* 2015), the traffic of transoceanic ships is intense (Morais & Teodósio 2016; APSS 2018), and has the abiotic conditions to favour fish survival, growth (Lankford & Targett 1994; Able & Fahay 2010), establishment and subsequent transport and migration of larvae and adults to other regions (Morais & Teodósio 2016).

Figure 2.1. Locations where *Cynoscion regalis* (Bloch & Schneider, 1801) was reported in Europe: 1–Scheldt estuary, September 2009 (ANB 2011); 2—Gulf of Cádiz, 2011 (Bañón *et al.* 2017); 3—Sado estuary, in September 2014, although it was noticed in the area for "some" years before (MundoDaPesca 2014); 4—Tagus estuary, 2013 or 2014 (Abreu 2017); 5—Mira estuary, 2013 or 2014 (Abreu 2017); 6—Praia da Vieira, October 2015 (Gomes *et al.* 2017); 7—Ría de Vigo, June 2016 (Vigoe 2016); 8—Ría do Barqueiro, June 2016 (Vigoe 2016); 9—Guadiana estuary, June 2016 (Morais & Teodósio 2016); 10—Praia do Barranco das Belharucas, July 2016 (Morais & Teodósio 2016); 11—Ria Formosa (Jesus 2017). Map retrieved from Google Earth.

In the native area, weakfish adults can be found in shallow waters along open sandy shores and in larger bays and estuaries (FWC 2014). Weakfish is a multiple spawner and spawns in nearshore and estuarine areas after a spring-inshore migration, leaving again in autumn to overwintering grounds (Mercer 1989; Lowerre-Barbieri 1994). So, estuarine ecosystems are used as nursery areas where juveniles migrate from high to low salinity waters in the summer, and return to high salinities in the fall, leaving estuaries in December (Mercer 1989; Lowerre-Barbieri *et al.* 1996). Weakfish diet consists of a wide variety of prey, that can vary with its size and includes smaller fishes (e.g., anchovies, herring, jacks), crabs, amphipods, mysids, decapod shrimps, squids, shelled molluscs, and annelid worms (Bigelow & Schroeder 1953; Merriner 1975; Stickney *et al.* 1975).

In the Sado estuary, anglers expressed their concern about the large numbers of weakfish that were being captured and about the possibility of being harmful to native prize fish. However, there are no scientific evidence that weakfish is outcompeting with native species. Considering that this is a recent invasion and that known impacts tend to increase in time (Simberloff 2014), it becomes imperative to study this invasion and assess the need to take control measures. In the Sado estuary, meagre *Argyrosomus regius* (Asso, 1801) (the most abundant Sciaenidae in the Iberian Peninsula), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and white seabream *Diplodus sargus* (Linnaeus, 1758) are three native prize fish species commonly captured (Docapesca 2018). The ecological overlap between weakfish and these native species is probably high, owing to their feeding preferences (e.g., small fish, crabs, mollusks, annelids) and the timing when they use the estuary, either for reproduction, feeding or as nursery habitat (Table 1). Generally, these four species use estuaries during the warmer months, while meagre and weakfish may also compete for protection sites in holes and deep channels (Bigelow & Schroeder 1953; FAO 2018). Weakfish is smaller than meagre and seabass (Wilk 1978; Duncan *et al.* 2013; Froese & Pauly 2018), so weakfish larvae and juveniles may eventually serve as prey, however, their early maturation (Mercer 1989) might allow weakfish to reach a higher number of generations over time, counterbalancing the predatory effects exerted by the other species. Also, survival, growth, and fecundity of weakfish might be enhanced in the new range if their parasites were left behind (i.e., enemy-release hypothesis) (Torchin *et al.* 2001; Torchin *et al.* 2003; Colautti *et al.* 2004) and if native competitors show a naïve behavior towards them (Sih *et al.* 2010).

Table 2.1. Comparison between weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) and the different prize fish: meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and white seabream *Diplodus sargus* (Linnaeus, 1758), in respect to biology and habitat. 1- Bigelow and Schroeder (1953); 2- Froese and Pauly (2018); 3- FAO (2018); 4- Lowerre-Barbieri (1994); 5- Prista *et al.* (2009); 6- Shabana *et al.* (2012); 7- Lowerre-Barbieri *et al.* (1996); 8- Bigelow and Schroeder (1953); 9- Gil *et al.* (2013); 10- Mercer (1989); 11- Wassef and El Emary (1989); 12- Cabral and Costa (2001); 13- Abecasis *et al.* (2009).

Biology and habitat	**Weakfish**	**Meagre**	**European bass**	**White seabream**
Diet	Small fish (e.g., anchovies, herring or drums), crabs, amphipods, mysid and decapod shrimps, squids, shelled molluscs, and annelids. (1)	Fish, crabs, amphipods, mysid and decapod shrimps, shelled molluscs and annelids. (2)	Small fish, crabs, decapod shrimps, and cephalopods (e.g., cuttlefish). Feed on more fish as it gets older. (3)	Algae, crustaceans, molluscs, annelids, echinoderms and hydrozoans. Juveniles are omnivores and adults are carnivores. (12)
Maximum length	98 cm (2)	230 cm (2)	103 cm (2)	45 cm (2)
Maximum Weight	9 kg (2)	103 kg (2)	12 kg (2)	1.9 kg (2)
Maximum Age	9-12 years (4)	44 years (5)	30 years (2)	10 years (2)
First maturation	0-1 year-old Length at maturity varies within regions ~17.5 cm. (10)	~2 years old Males: 45 cm Females: 47 cm. (6)	Males: 4-7 years Females:5-8 years. (3)	2 years. (2)
Fecundity	Multiple spawner: undetermined fecundity. (7)	2.1–31.1 million eggs (1.0–1.7m females). (9)	Batch spawners: 230,000 – 809,000 eggs (33 cm fish). (11)	64,650 – 536,360 eggs. (2)
Spawning	Spring – Summer (8)	Spring – Summer (8)	Spring (3)	Winter (2)
Use of the estuary	Spring-late Summer/early Autumn. (8)	Spring-late Summer/early Autumn. (8)	Spring-late Autumn. (12)	Spring – Autumn. (13)

Therefore, we intend to test three hypotheses in this study: (i) weakfish feeding strategy is generalist which favors its establishment in the non-native region; (ii) weakfish is outcompeting the three native species mentioned above for food and space; and (iii) weakfish competition is higher with meagre, since they are taxonomically closer (both species are sciaenids fish) and likely occupy the same ecological niches. To test our hypotheses, we determined: (i) weakfish diet and feeding strategy through stomach content analysis, as well as for meagre, European bass, and white seabream; ii) trophic position through isotopic carbon and nitrogen content analysis in muscle and fin tissues for the four species to assess trophic overlap; (iii) infer weakfish habitat use through the analysis of otolith chemistry, to retrospectively assign habitat used along the entire life of each individual. In this way, we provide the first comprehensive dataset on the invasive weakfish population, that will allow us to perceive the level of ecological overlap between the invader and these prize native species, and discuss possible impacts on other fishery resources in Sado estuary.

2. Materials and Methods

2.1. Study area

The Sado estuary is located on the western coast of Portugal at 30 km south of Lisbon (37º25'-38º40'N, 7º40'- 8º5O'W) (Figure 2.2). It is about 20 km long and 4 km wide and the average depth is 8 m, while the maximum can reach more than 50 m (Martins *et al.* 2001). It has an area of ~180 km^2, of which one third is occupied by intertidal mudflats and salt marshes (Martins *et al.* 2001). Since only five tributaries provide freshwater to the estuary, salinity is usually high (above 29.5) during the whole year (Neves *et al.* 2008). The tidal amplitude is semi-diurnal, 4 m during spring tides and 1 m during neap tides (Rocha 1998). The estuary provides numerous habitats (e.g., seagrass meadows, mudflats, saltmarsh, dunes, ponds, puddles) and functions as a nursery area for numerous species (Harzen 1998). A deep-water port is located near the mouth of the estuary, the Port of Setubal, that comprises five terminals for public use and seven for private use, all intended for the carriage of liquid or solid bulk (APSS 2018).

Figure 2.2. Location of the Sado estuary on the western coast of Portugal. The open box comprises Port of Setúbal location. Maps retrieved from Google Earth.

2.2. Otolith chemistry

The otoliths were extracted via the branchial region with tweezers, cleaned with distilled water to remove adherent tissues, blotted in absorbent paper, and then let dry in an open plastic vial (Figure 2.3). A 500- μm transversal cross-section was cut near the otolith core to expose annual growth ring increments and perform chemistry analysis along a transect in the otolith. This transect will start in the core of the otolith and end in the posterior end of the otolith to include the entire life of the fish. A guideline was drawn over the inner face of the otolith to indicate the location of the nucleus in a transverse section of the sagitta (Figure 2.4). The inner side of the otolith is clearly distinguished by the sulcus acusticus, composed by the ostium (anterior) and cauda (posterior) (Figure 2.4). Right and left otoliths are distinguished by observing the inner side, with the ostium pointing to the left and cauda pointing to the right: the tip of the cauda will curl downwards in the right sagitta and upwards in the left sagitta. The line was drawn with a sharp pencil, under a stereo microscope, above the ending limit of the ostium (postostial lobe) and passing through the cauda in the dorsal-ventral plan (Figure 2.4).

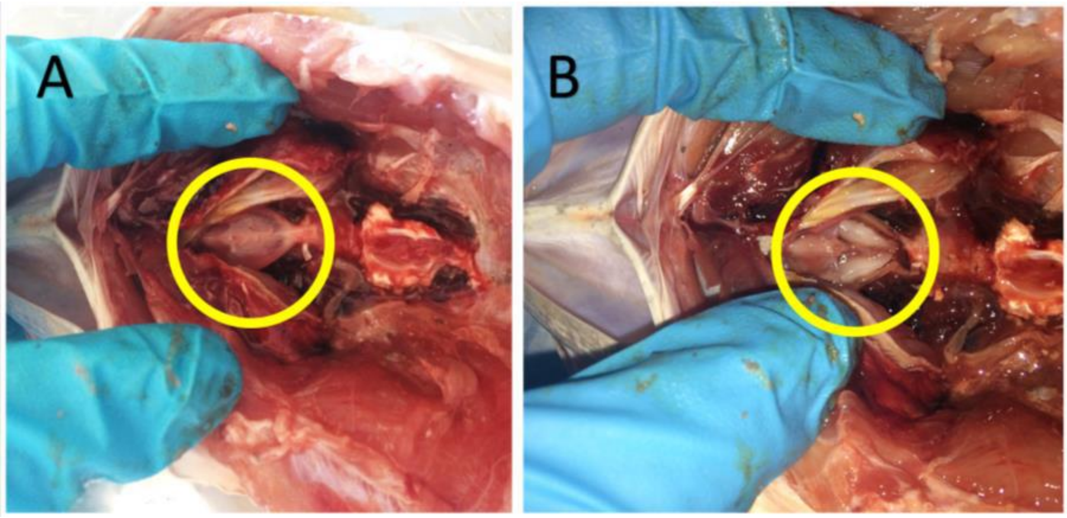


Figure 2.3. Ventral view of the optic capsules of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801). A: Optic capsule exposed. B: Otoliths exposed after removing part of the capsule and ready to be extracted.

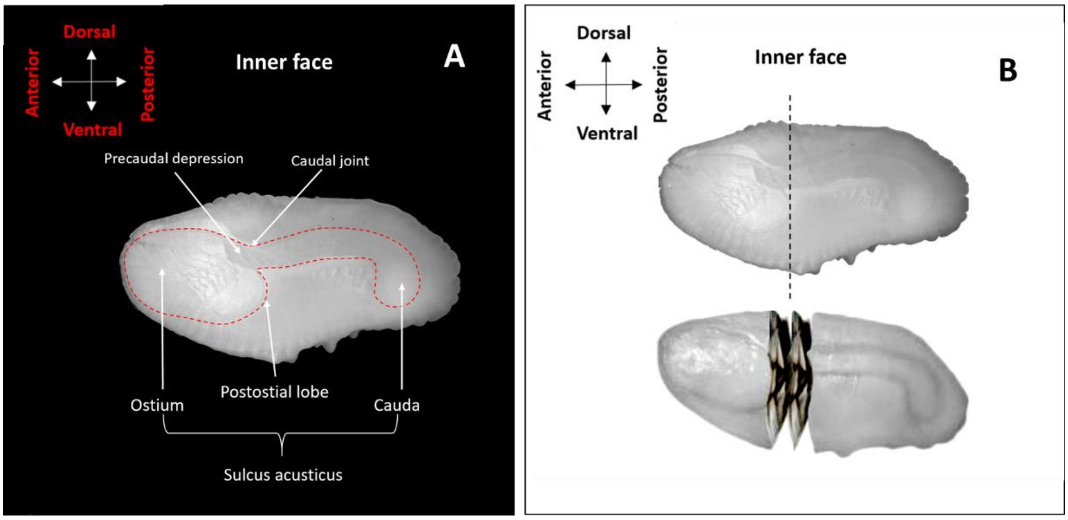


Figure 2.4. External morphology of the inner side of a right sagitta otolith of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801). On the left (A) the dashed line circumscribes the *sulcus acusticus* that comprises the ostium, the postostial lobe, precaudal depression, caudal joint and cauda. On the right (B), a schematic representation of the position of the section containing the nucleus. Arrows indicate otolith position in relation to the fish. Image adapted from (Béarez *et al.* 2016).

Each right otolith was placed in a disk-shaped mold (Ø 25mm), with the inner side facing down, and then epoxy resin (EpoThin resin and EpoThin hardener, Buehler) was poured into the mold, and let dry for at least 9 hours at room temperature. The right otoliths were sectioned transversely using a low-speed saw (Mecatome T180, Presi), equipped with a diamond cut-off disc (type LR Ø 100 mm with 0.5 mm thickness, Presi), and following the pencil guideline drawn on the otolith (Figure 2.5). The otolith sections were observed under a stereo microscope (Leica S8 APO) with dark-field polarization to check the distance between the nucleus and the otolith surface. The desired position is attained when the sulcal region displays a triangular shape pointing towards the core of the otolith (Figure 2.6). If the nucleus was not at the surface of the cut, the section was then sanded using a sequence of silicone carbide grinding papers, with decreasing degree of abrasiveness. Then, diamond suspension solutions of 9 µm, 3 µm, and 1 µm were used over polishing cloths to eliminate scratches from the otolith surface.

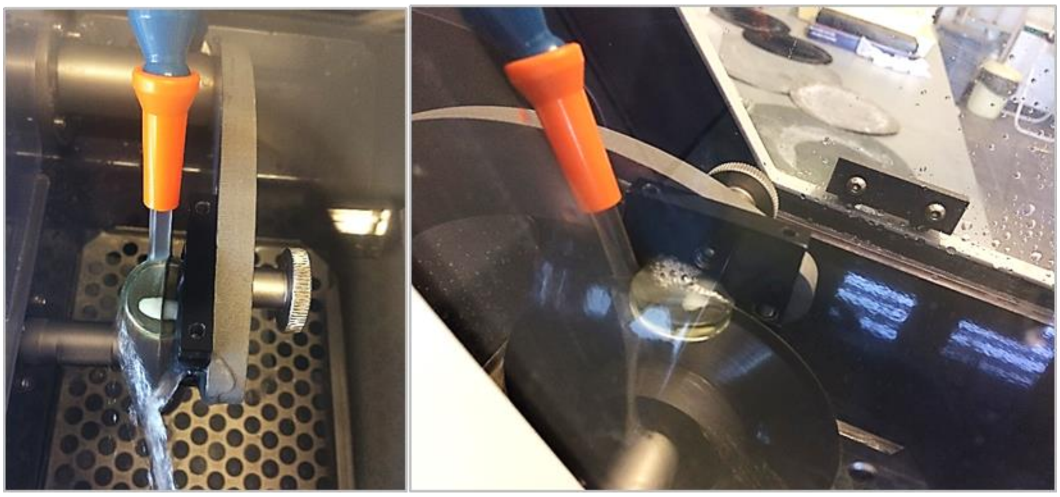


Figure 2.5. Sectioning a weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) otolith using a low-speed saw with a diamond-cut disc. Water was used to lubricate the saw and prevent overheating.

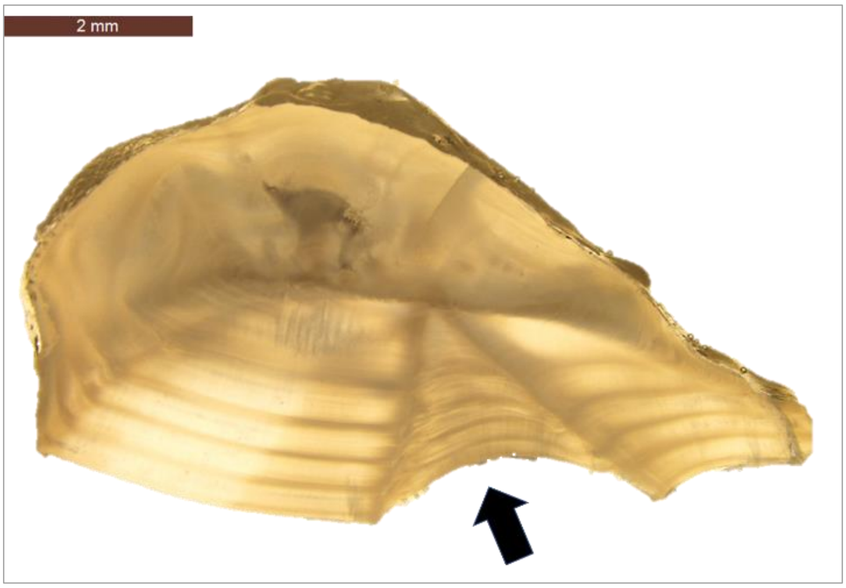


Figure 2.6. Section of a weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) sagitta otolith after being cut with a low speed saw. The sulcal region displays a triangular shape pointing towards the core of the otolith.

After exposing the otoliths' core, we analysed the concentration of Sr isotopes ratios ($^{87}Sr/^{86}Sr$) to distinguish marine and freshwater signals from the Sr voltage output given in the analysis (Phillis *et al.* 2018). Strontium isotope ratios and the intensity of the Sr ion beam (Sr V, used as a proxy for Sr concentration) were done at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry with a multiple collection laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Strontium voltage at the end of the life of the fish will give us an indication of the habitat conditions at time of death (marine, freshwater, brackish) and serve as a reference to interpret the life history of weakfish. The concentration of Sr in the water is generally higher in sea than in freshwater, and since Sr is mostly incorporated into the otolith as function of its concentration in the water, then it is possible to assign the habitats used by a fish along their entire life. Higher Sr voltages in the otolith also correspond to higher salinity environments. Incorporation of Sr in the otolith matrix is possible because Sr has a similar ionic radius to Calcium (Ca), a major constituent of the otolith matrix which is made of calcium carbonate ($CaCO_3$) and otolin.

2.3. Diet

The guts of thirty-three weakfish, five meagres, five seabasses, and five white seabreams were individually frozen in small vials after their dissection while still fresh. The guts of each fish was thawed at room temperature and opened with scissors to reveal their content. Only stomach contents were included in the analysis, except for *Diplodus sargus* that does not show morphological or chemical differentiation of the stomach (Quignard 1966). Preys were identified to the lowest taxonomic level possible using a stereo microscope (Leica S8 APO) according to the following identification manuals: fish – "Fishes of North-Eastern Atlantic and the Mediterranean" by Whitehead *et al*. (1985; 1986a; 1986b), crustaceans – "Crustáceos Decápodes Ibéricos" by Alvarez (1968), molluscs – "Conchas Marinhas de Portugal" by Macedo *et al.* (1999). In cases were preys could not be identified to the species level, they were assumed as a non-identified species, so that all preys could be plotted at the same taxonomic level. For example, if four fish in an advanced digestion state are found in the stomachs of several predators, and the species could not be identified, they will be considered as four individuals of the same species.

Then, the prey-specific abundance (Pi) and the frequency of occurrence (Fi) were calculated using the new Costello graphical approach (Amundsen *et al.* 1996) (Figure 2.7). Prey-specific abundance (equation 1) is defined as the percentage a prey taxon comprises of all prey items, in only those predators in which the actual prey occurs (Amundsen *et al.* 1996),

$$Pi = (\sum Si / \sum Sti) \times 100 \text{ (equation 1)}$$

where, Pi is the prey-specific abundance of prey i, Si is the stomach content (number) comprised of prey i, and *Sti* the total stomach content in only those predators with prey *i* in their stomach. The frequency of occurrence (Fi) (equation 2) of a given prey type is defined as the number of stomachs in which that prey occurs, expressed as a frequency of the total number of stomachs in which prey are present (Amundsen *et al.* 1996).

$$F_i = N_i / N \text{ (equation 2)}$$

where *Ni* is the number of predators with prey *i* in their stomach, and *N* is the total number of predators with stomach contents.

The original Costello method (Costello 1990) assesses the prey importance for a certain predator population – if it occurs rarely of dominantly - and its feeding strategy, i.e., if the population is generalist and has a wide niche breadth or if it is a specialist and its niche is narrow. Since we are dealing with more than one individual predator, the new Costello approach by Amundsen *et al.* (1996) came to distinguish a niche from a single individual from a niche of a whole population. This means that a population with a narrow niche must necessarily be composed by individuals with small and specialized niches, while a population with a broad niche might include individuals with narrow, wide or a combination of both niche widths. Therefore, the new approach relies on two components for the niche width: the high "between-phenotype component" (BPC) that consists of specialized individuals with little or no overlap in resource use (differences between individuals), and the high "within-phenotype component" (WPC) composed of generalists, each exploiting a wide range of overlapping resources (behavioral or physiological flexibility) (Amundsen *et al.* 1996; Giller 2012).

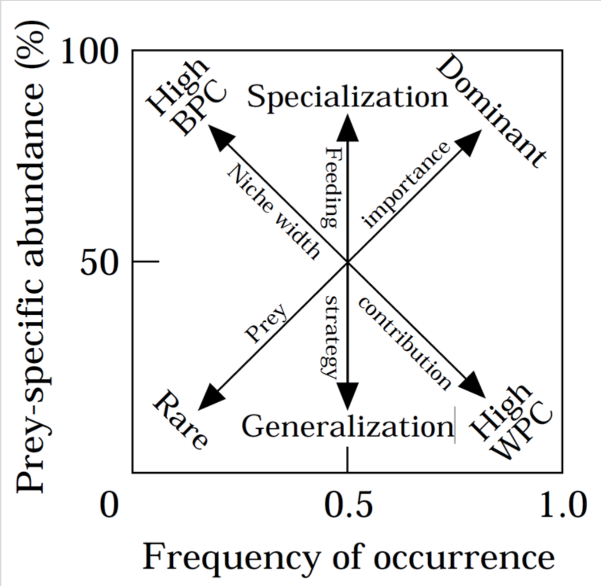


Figure 2.7. The New-Costello diagram where it is possible to infer a species feeding strategy, niche width contribution (BPC - between phenotype component, WPC - high within phenotype component) and prey importance. Diagram retrieved from Amundsen *et al.* (1996).

To complement this information, the trophic niche breadth of each predator species (equation 3) was evaluated using the normalized Shannon's index (see Shannon and Weaver (1963) for the original definition, and Colwell and Futuyma (1971) for its use as a niche-breath index). The niche breadth of predator species *i* was obtained based on the relative abundance of prey:

$$h_i = -(lnN)^{-1} \sum_{k=1}^{N} p_{i,k}\, lnp_{i,k} \quad \text{(equation 3)}$$

where:

h_i = Shannon-Weaver measure of niche breadth

$p_{i,k}$ = proportion of individuals from predator species *i* consuming prey taxon *k*

N = total number of prey taxon groups

The Shannon-Weaver index range from 0 to ∞, so the normalized index provides values from 0 to 1, where 0 indicates that a predator is a specialist and preys upon one prey type, and 1 when the predator is a generalist and preys on all the available preys.

A niche overlap index was also used to measure the feeding overlap between weakfish and the other three predator species in Sado estuary, i.e., the intensity of exploitative competition (indirect competition by using the same resources). So, we used the Pianka Index (Pianka (1973) (equation 4), a similar and symmetrical measure of one proposed by MacArthur and Levins (1967), and obtained by:

$$\hat{O}_{ij} = \frac{\sum_{i}^{n} \hat{p}_{ij}\hat{p}_{ik}}{\sqrt{\sum_{i}^{n} \hat{p}_{ij}^{2} \sum_{i}^{n} \hat{p}_{ij}^{2}}} \text{ (equation 4)}$$

where:

O_{jk} = Pianka's measure of niche overlap between species *j* and species *k*

p_{ij} = Proportion prey *i* is of the total preys consumed by species *j*

p_{ik} = Proportion prey *i* is of the total preys consumed by species *k*

n = Total number of prey taxon groups

This index also ranges from 0 to 1, where 0 indicates no resource use in common by both predator species and 1 indicates complete overlap (Krebs 1998). We considered a significant overlap to be > 0.6, the same used by Schoener (1968).

2.4. Carbon and nitrogen stable isotopes

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes emerged as useful tools to disclose the structure and dynamics of estuarine food webs because they provide time-integrated information about the trophic relationships and energy flow through food webs. The $\delta^{15}N$ of an organism is typically enriched by 3-4‰ relative to its diet and is usually used to determine the trophic position of an organism (Minagawa & Wada 1984). The $\delta^{13}C$ changes little as carbon moves through the food web (1-2‰), being used to evaluate the sources of energy used by an organism (Peterson & Fry 1987).

For stable isotope analysis, we used fin and muscle tissues due to their differences in isotopic incorporation rates (turnover rates). They will incorporate consumer diet at different time scales, so the analysis of two tissues might provide us information about the temporal dynamics of resource use (Vander Zanden *et al.* 2015). Fin has a fasters turnover rate than muscle, so it should reflect putative short-term changes in the consumers' diet (Phillips & Eldridge 2006; Cano-Rocabayera *et al.* 2015).

Muscle (dorsal region) and fin tissue samples were extracted from thirty-three weakfish, five meagers, five seabasses, and five white seabreams. Tissues were placed in a drying oven at 60 °C until dry and then ground to a fine powder, with a mortar and pestle for posterior stable isotopes analyses. Since fin tissue samples were ground including the fin rays, samples had to be acidified to reduce variability caused by the presence of inorganic carbonate. Fish scales are typically enriched in ^{13}C by 1–2‰ relative to that in the muscle (Cano-Rocabayera *et al.* 2015) so a similar relationship might exist between fin and muscle (Hayden *et al.* 2017). Therefore, acidification took place before stable isotope analysis (SIA) by carefully applying one drop-by-drop of HCl (1 M) upon the sample, until no further CO^2 development was visible (Jacob *et al.* 2005). Then, samples were dried again at 60°C without rinsing to minimize loss of dissolved organic matter and ground again to facilitate the transfer of samples into tin capsules for weighting.

Figure 2.8. Extraction of muscle from the dorsal region of weakfish *Cynosicion regalis* (Bloch & Schneider, 1801) for carbon and nitrogen stable isotope analysis.

Stable isotope ratios were measured using a Thermo Scientific Delta V Advantage IRMS via a Conflo IV interface (Marinnova, University of Porto). Stable isotope ratios are reported in δ notation (δX): $\delta X = (Rsample/Rstandard - 1) \times 10^3$, where X is the C or N stable isotope, R is the ratio of heavy:light stable isotopes (Fry 2006). Pee Dee Belemnite and air are standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The analytical error, the mean standard deviation of replicate reference material is usually ±0.1‰ for $\delta^{13}C$ and $\delta^{15}N$. Those samples with an SD between replicates (i.e., the two sub-samples of the same sample) >0.3 ‰ $\delta^{13}C$ or $\delta^{15}N$ were repeated for both stable isotopes, to control for sample quality processing.

We used the T-test after assessing the test's assumptions – i.e., normal distribution and heteroscedasticity – to check for significant differences in the contents of carbon and nitrogen isotopes between tissues (fin and muscle). When the assumptions were not met, we used the analogous non-parametric test, the Kruskal-Wallis. All statistical analyses were done using R (version 3.4.2). Statistical significance was set at 0.05.

The $\delta^{13}C$ and $\delta^{15}N$ data was used to calculate the trophic niche size of each fish species, since these isotopes are influenced by factors that include growth rate and metabolism (Jackson *et al.* 2011). The analysis was performed using the R package 'Stable Isotope Bayesian Ellipses in R' (SIBER). This model describes a population's isotopic niche by plotting and measuring the standard ellipse area (SEA) of isotope bi-plots, a bivariate measure of the distribution of individuals in isotopic space, revealing the population's typical resource use. The ellipse areas (‰^{2}) were calculated with probabilistic methods by testing 10,000 probability interactions and were corrected (SEAc) for low sample sizes (<30) (Jackson *et al.* 2012). Then the overlap between two ellipses is calculated using a single point value (SEA_C metric) of each ellipse (Jackson *et al.* 2011). The values are then used to calculate the degree of isotopic niche overlap, representing a quantitative measure of dietary similarity between populations. We considered a significant overlap to be >0.6 (Schoener 1968).

3. Results

Weakfish samples were mainly composed of females (61%), and length varied between 27.2 cm and 60.0 cm. Most individuals belonged to the [30-40[cm size class (60%) (Figure 2.9). All five meagres sample were males and four of them (80%) belonged to the 40 cm length class. Four of the five seabasses were females and three (60%) were in the 30 cm class, while all seabreams fitted in the 20 cm size class of which four were females.

3.1. Weakfish - otolith chemistry

The oldest weakfish individual was five years-old, being also the largest and heaviest (TL= 54.8 cm; TW= 2.160 kg). Strontium voltage changed along the otolith from 1.3 to 4.7 V, from values ranging coastal habitat use (>3.5 V) and estuarine/freshwater habitat use (<3.5 V). and multiple annual Sr voltage peaks were registered (Figure 2.10).

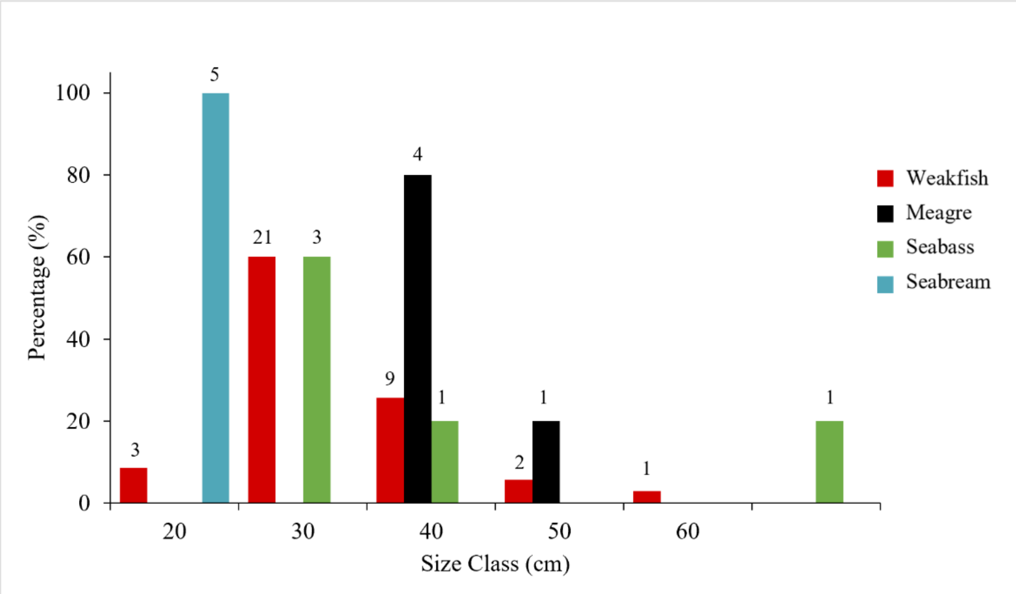


Figure 2.9. Size class distribution (%) of weakfish *Cynosicion regalis* (Bloch & Schneider, 1801), meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and seabream *Diplodus sargus* (Linnaeus, 1758) samples. The numbers on top of each bar represent the number of individuals (n).

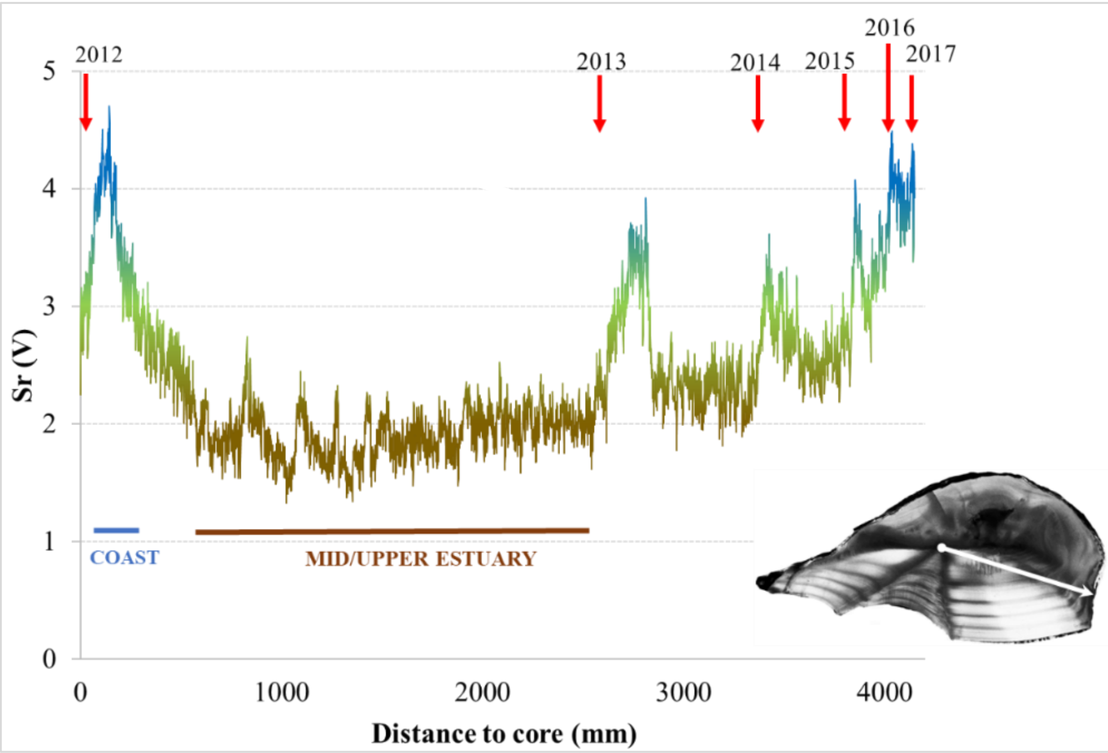


Figure 2.10. Otolith strontium isotopes ($^{87}Sr/^{86}Sr$) transect of a five-year-old weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) using LA-ICPMS. The laser transect starts at the otolith core and extends to the posterior end of the otolith.

3.2. Diet

Diet composition

Weakfish *Cynoscion regalis*

Seventeen prey taxonomic groups were identified in the stomachs of weakfish, and 24% of the stomachs (n= 8) were empty (Table 2.3). Crustaceans represent 53% of the diet of this species (n= 33), composed by mysids (38%), decapods (10%) that include carideans, and brachyurans, and isopods (6%) (Table 2.2). Fish also represent a large part of this species diet (45%), where sand smelt *Atherina* sp. was the most consumed (31%), followed by European anchovy *Engraulis encrasicolus* (9%), seabreams *Diplodus* sp., *Gobius niger*, horse mackerel *Trachurus trachurus* and even weakfish *Cynoscion regalis* (each species representing 1%). The remaining fish species were not identified due to their advanced state of digestion (3%). Lastly, cephalopods (2%), one cuttlefish *Sepia* sp. and one squid from Loliginidae family were also part of the weakfish diet.

Meagre *Argyrosomus regius*

Seven prey types were identified in the stomachs of meagre, and 20% of the stomachs (n= 1) were empty. Meagre preyed mainly on crustaceans (62%): mysids, decapods that included carideans and brachyurans (Table 2.2). One anchovy was also eaten (3%). Ten unidentified structures, similar to egg masses and corresponding to 34% of the total items found, were present in the stomachs of two meagres (five in each one).

European bass *Dicentrarchus labrax*

Five prey types were identified in the stomachs of European bass, and none of the stomachs were empty. This species preyed only on crustaceans, that included brachyura decapods, mainly Portunidae (65%) and *Upogebia* c.f. *tipica* (30%) (Table 2.2).

White seabream *Diplodus sargus*

Six prey types were identified in the stomachs of white seabream and none of the stomachs were empty. This species preyed mainly on bivalves (95%) and crustaceans (5%), that included a Portunidae crab and a Balanidae (Table 2.2).

Table 2.2. Number of preys found in the stomachs of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801), meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and white seabream *Diplodus sargus* (Linnaeus, 1758).

			Weakfish		**Meagre**		**European bass**		**White seabream**	
Taxonomic Group		**Prey species**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Osteichthyes	Atherinidae	*Atherina* sp.	48	31	0	0	0	0	0	0
	Sparidae	*Diplodus* sp.	1	1	0	0	0	0	0	0
	Carangidae	*Trachurus trachurus*	1	1	0	0	0	0	0	0
	Engraulidae	*Engraulis encrasicolus*	14	9	1	3	0	0	0	0
	Sciaenidae	*Cynoscion regalis*	1	1	0	0	0	0	0	0
	Gobiidae	*Gobius niger*	1	1	0	0	0	0	0	0
	Unidentified	Unidentified fish	4	3	0	0	0	0	0	0
Cruastacea	Cirolanidae	*Eurydice pulchra*	7	5	0	0	0	0	0	0
	Sphaeromatidae	Unidentified	2	1	0	0	0	0	1	0
	Crangonidae	*Crangon crangon*	2	1	2	7	0	0	0	0
	Palaemonidae	*Palaemon serratus*	1	1	5	17	0	0	0	0
	Mysida	Unidentified	58	38	8	28	0	0	0	0
	Caridea	Unidentified	10	6	1	3	0	0	0	0
	Upogebiidae	*Upogebia* c.f. *tipica*	0	0	0	0	6	30	0	0
	Portunidae	*Liocarcinus navigator*	0	0	2	7	0	0	0	0
	Pilumnidae	*Pillumnus hirtellus*	0	0	0	0	2	10	11	3
	Carcinidae	*Portumnus latipes*	0	0	0	0	1	5	0	0
	Portunidae	Unidentified	1	1	0	0	10	50	0	0
	Brachyura	Unidentified	1	1	0	0	1	5	0	0
	Balanidae	*Balanus* sp.	0	0	0	0	0	0	6	2
Mollusca	Semelidae	*Ervilia castanea*	0	0	0	0	0	0	333	93
	Veneridae	*Ruditapes decussatus*	0	0	0	0	0	0	1	0
	Mytilidae	*Mitylus edulis*	0	0	0	0	0	0	5	1
Cephalopoda	Sepiidae	*Sepia* sp.	1	1	0	0	0	0	0	0
	Loliginidae	Unidentified	1	1	0	0	0	0	0	0
Unidentified	Unidentified	Unidentified	0	0	10	34	0	0	0	0
Total			**154**	**100**	**29**	**100**	**20**	**100**	**357**	**100**

Table 2.3. Comparison of mean prey taxa per species (weakfish *Cynoscion regalis* (Bloch & Schneider, 1801), meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and white seabream *Diplodus sargus* (Linnaeus, 1758), mean abundance of prey per species, and Shannon-Weaver index based on relative abundance of taxonomic prey groups.

Species	Vacuity rate (%)	Prey taxonomic groups (N)	Prey taxa per fish (mean ± SD)	Prey abundance per fish (mean ± SD)	Shannon-Weaver Index	Pianka measure of overlap with weakfish
Weakfish	24	17	1.4 ± 1.0	4.7 ± 9.9	0.6	-
Meagre	20	7	1.8 ± 1.5	5.8 ± 5.7	0.8	0.45
European bass	0	5	1.2 ± 0.4	4.0 ± 2.6	0.8	0.01
White seabream	0	6	1.8 ± 1.2	71.4 ± 55.8	0.2	0.00

Feeding Strategy and niche breadth

Weakfish seems to have a mixed feeding strategy, with different degrees of specialization and generalization of varying prey types (Figure 2.11). Prey points located on the upper part of the Costello diagram are indicative of specialization in those preys (*C. regalis*, *G. niger*, mysids, *E. encrasicolus*, *Atherina* sp.), while the points located on the upper left (low FO and high PSA) indicate a specialization of individual predators from this population. *Atherina* sp. was the most frequent prey (consumed in greater numbers by a larger part of the predators) indicating greater importance (dominant prey importance) and contribution of this prey to weakfish diet. Preys points positioned in the lower part of the diagram indicate generalization in those preys (consumed in lower abundances), while the points on the lower left (low PSA and low FO) indicate that low proportions of most preys were included in the diet of these individuals – i.e., rare prey importance. Therefore, the concentration of prey points along the y-axis is indicative of prey items that were present in the stomachs of individual weakfish but that were rarely seen in the stomachs of more than a single animal.

Weakfish has a broad niche width since the prey points are located along or below the axis from the upper left to the lower right corner. This is confirmed by the Shannon-Weaver index (0.62) that indicates a more generalist feeding behavior, although it is not a full generalist. Weakfish is the species with most prey taxonomic groups consumed (17 taxa), most likely because weakfish is the sample with most of individuals (n= 33) in comparison with the other species (n= 5). However, the average number of taxonomic groups per predator is low and similar among all (Table 2.4), indicating a clear difference in the type of preys consumed between weakfish predators. So, in addition to the broad trophic niche, this population comprises individuals with narrow niche widths (specialized individuals) so with a low overlap among them and a high between phenotype component.

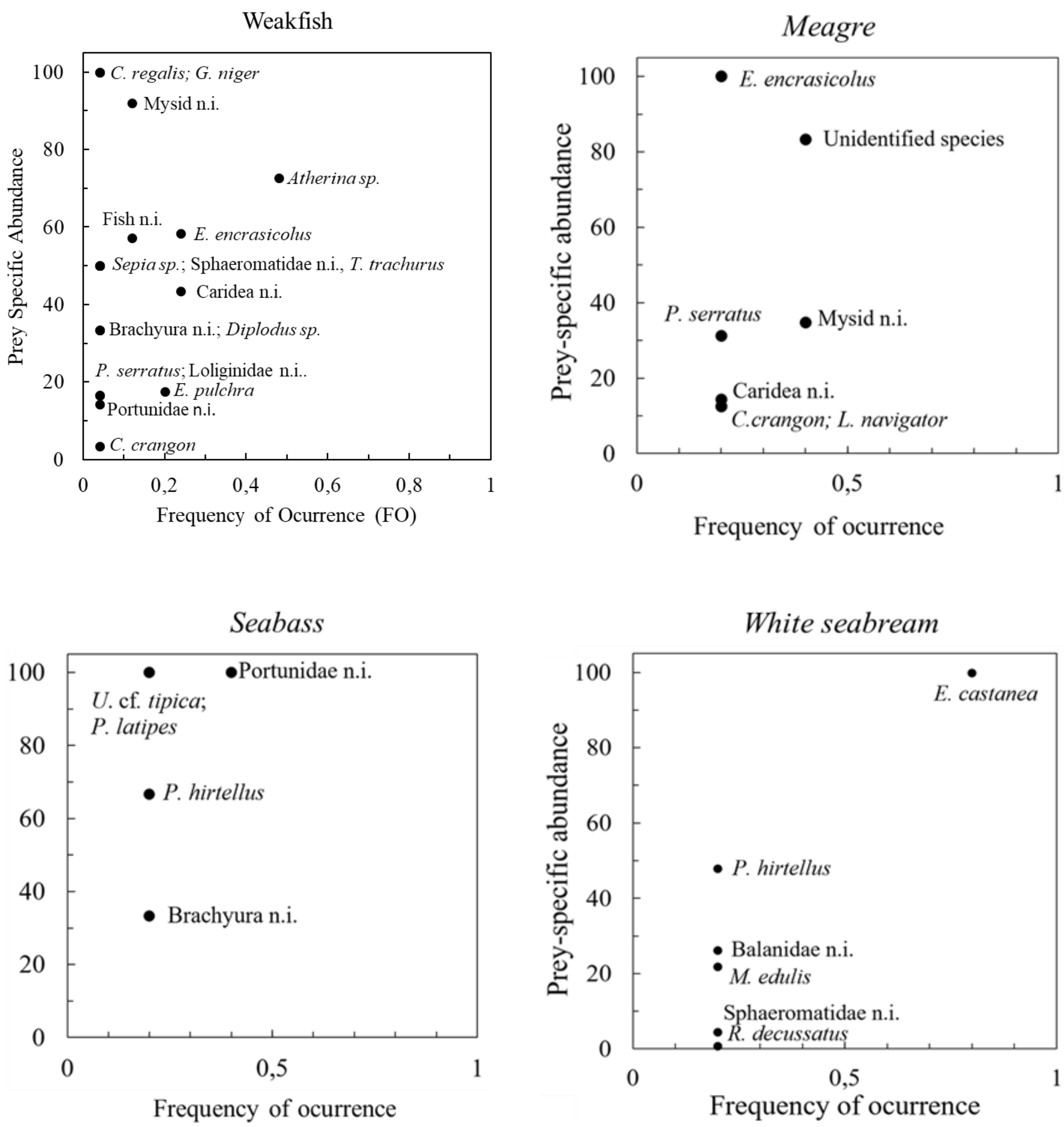


Figure 2.11. The feeding strategy diagram of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801), meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and white seabream *Diplodus sargus* (Linnaeus, 1758) according to the new diagram approach of the Costello method by Amundsen *et al.* (1996). Prey-specific abundance was plotted against the frequency of

occurrence of prey in the diet of the predator. The preys whose species could not be identified were considered as one single species (e.g., “mysid n.i.” is a single species belonging to the Mysida order).

Similarly, meagre also seems to have a mixed feeding strategy, with specialization of individual predators on the European anchovy and an unidentified species; and the inclusion of the other prey types consumed by few individuals in lower abundance, indicating their rare prey contribution (Figure 2.11). The high Shannon-Weaver index (0.84) reveals a broad niche width, but the low number of prey taxa per fish also suggests that this population is composed of individuals with narrow niche widths (specialized individuals) or a combination of specialist and generalist individuals, with low overlap among them (high BPC).

Seabass Shannon-Weaver index was high (0.77) which reveals its broad niche width, with individual specialization in a Portunidae crab species, *Portumnus latipes* and *Upogebia* cf. *tipica* (Figure 2.11). Like weakfish and meagre, mostly BPC seems to contribute to the niche width (low prey taxa per fish) (Table 2.3). In turn, the white seabream presents a small niche breadth index (0.19) meaning that this predator population consumes just a few prey species, namely the clam *Ervilia castanea*. The other preys were consumed less frequently (Figure 2.11).

In relation to the most consumed items in number (Table 2.2), weakfish preyed mainly on mysids, sand smelt *Atherina* sp., European anchovy, eucarideans, and isopods, while meagre seems to prefer mysids, carideans (*Palaemon serratus*, *Crangon crangon*) and portunids (*Liocarcinus navigator*), while seabream is mainly eating bivalves (*Ervilia castanea*). So, and based only in stomach contents, meagre seems to be the most likely species to overlap with weakfish.

Niche Overlap

The highest overlap measure with weakfish belongs to meagre (Pianka index= 0.45) because most of the prey consumed by meagre were also eaten by weakfish (*Engraulis encrasicolus*, mysida sp., *Crangon crangon*, *Palaemon serratus*, Caridea). The overlap between weakfish and European bass was very low (Pianka index=0,01), the common preys were unidentified brachyurids and portunids, while there was no overlap between weakfish and white seabream (Pianka index= 0).

3.3. Carbon and Nitrogen stable isotopes ratios and derived metrics

There were no significant differences between the $\delta^{13}C$ and $\delta^{15}N$ of muscle and fin tissues for any species, except for the European bass that had significant differences in $\delta^{13}C$ content between tissues ($t(8)= 2.48$, $p= 0.05$). Weakfish, meagre, and European bass isotopic niches indicate that they are feeding at the same high trophic level, and that white seabream is at least a trophic level below of weakfish (~3.2‰) (Figure 2.12). European bass had the highest $\delta^{15}N$ standard deviation for both tissues (Table 2.4), indicating high intraspecific variability in $\delta^{15}N$ (Figure 2.12).

The SEA of weakfish was smaller than meagre (probability of SEA meagre < SEA weakfish= 0.4, p= 0.95) and European bass (probability of SEA European bass < SEA weakfish= 0.0, p= 0.95) and larger than seabream (probability of SEA white seabream < SEA weakfish= 80.6, p= 0.95)(Table 2.5). The species with the highest probability of SEA overlap with weakfish was meagre (fin 22.3%, muscle 20.5%), followed by European bass and white seabream (Table 2.6).

Table 2.4. $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of fin and muscle tissues (mean ± SD) of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) (red), meagre *Argyrosomus regius* (Asso, 1801) (black), European bass *Dicentrarchus labrax* (Linnaeus, 1758) (green), and white seabream *Diplodus sargus* (Linnaeus, 1758) (blue). Diff. is the mean difference between tissues (‰).

	$\delta^{13}C$ (‰)		
	Fin	**Muscle**	**Diff.**
Weakfish	-15.7 ± 1.3	-17.0 ± 0.9	1.3
Meagre	-15.8 ± 1.4	-18.1 ± 1.5	2.3
European bass	-16.3 ± 1-0	-18.0 ± 1.2	1.7
White seabream	-15.3 ± 1.0	-17.6 ± 0.2	2.3
	$\delta^{15}N$ (‰)		
Weakfish	14.5 ± 0.7	14.6 ± 0.7	0.1
Meagre	15.5 ± 1.4	16.3 ± 1.3	0.8
European bass	16.9 ± 3.3	16.7 ± 3.5	0.2
White seabream	11.3 ± 0.4	11.5 ± 0.6	0.2

Table 2.5. Ellipse metrics (p= 0.95) statistics (TA- total area, SEA- Standard Elipse Area, SEA_c- corrected Standard Elipse Area) calculated based on the $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values of fin and muscle tissues of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) (red), meagre *Argyrosomus regius* (Asso, 1801) (black), European bass *Dicentrarchus labrax* (Linnaeus, 1758) (green), and white seabream *Diplodus sargus* (Linnaeus, 1758) (blue).

	Fin			**Muscle**		
	TA	**SEA**	**SEA_c**	**TA**	**SEA**	**SEAc**
Meagre	3.3	3.3	4.4	4.0	3.6	4.8
Weakfish	5.4	1.5	1.5	4.5	1.4	1.5
European bass	10.6	11.0	14.7	10.1	11.4	15.2
White seabream	0.8	0.9	1.2	0.5	0.5	0.6

Table 2.6. Probability of overlap (%) of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) with meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758) and white seabream *Diplodus sargus* (Linnaeus, 1758), of fin and muscle samples (p= 0.95).

	Fin	**Muscle**
Weakfish vs. Meagre	22.3	20.5
Weakfish vs. European bass	10.3	9
Weakfish vs. White seabream	0.0	0.2

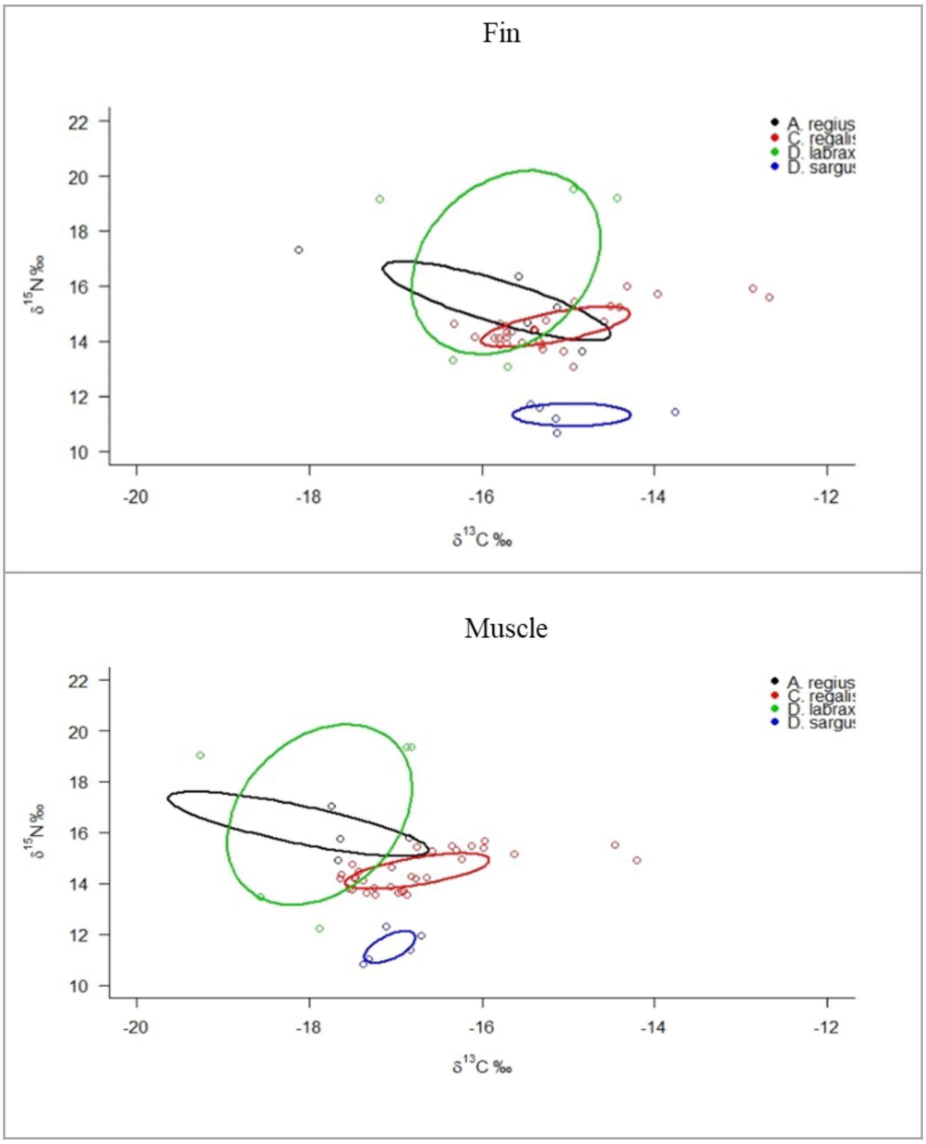


Figure 2.12. δ13C (‰) and δ15N (‰) stable isotope ratios bi-plot with overlaid standard ellipses, created by SIBER analysis, for weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) (red), meagre *Argyrosomus regius* (Asso, 1801) (black), European bass *Dicentrarchus labrax* (Linnaeus, 1758) (green), and white seabream *Diplodus sargus* (Linnaeus, 1758) (blue). Top: fin samples. Down: muscle samples.

4. Discussion

The oldest weakfish individual was five years old, which means that this species is present in the Iberian Peninsula at least since 2012, which confirms anglers sightings (MundoDaPesca 2014; Bañón *et al.* 2017). The growth pattern of the otoliths depends on abiotic and biotic factors, and it is common a higher growth rate in the summer and a slower growth rate in the winter (Morales-Nin 1992; Panfili *et al.* 2002). The peaks in the concentration of Sr match the zones of slower growth, indicating that weakfish uses coastal areas during winter, shifting to estuarine waters in spring and summer, signalized by the lowest values of Sr voltage. So, this fish likely hatched during late summer or fall, since it happened right before the first Sr peak correspondent to the winter season, in the low estuary or under the influence of the estuary in nearshore areas. It used coastal waters in the winter and returned inshore in spring to the brackish/freshwater part of the estuary to spend the summer. This pattern was repeated every year, indicating a consistent seasonal migration pattern between inshore and offshore waters. This pattern is similar to what happens in the native range, where spawning occurs between March and October, depending on the region (influenced by temperature and photoperiod) and the size of the fish (Wilk 1978; Shepherd & Brunswick 1984; Lowerre-Barbieri 1994). It occurs in nearshore and estuarine areas after a spring inshore migration (Bigelow & Schroeder 1953; Mercer 1989; Lowerre-Barbieri *et al.* 1996). In the Chesapeake Bay and Delaware Bay, juvenile weakfish move between high and low salinity areas throughout the summer and return to high salinity waters in fall, to leave the estuaries in December to overwintering grounds (Mercer 1989). This implies the simultaneous use of the Sado estuary by meagre (FAO 2018), European bass (Cabral & Costa 2001) and white seabream (Abecasis *et al.* 2009), and competition for space during this period.

Stomach content analysis provides useful taxonomic and quantitative information about prey items intake at a given moment, giving an important insight into the trophic interactions occurring in the estuarine environment (Pasquaud *et al.* 2008). Studies in the native range describe weakfish diet as mainly composed by crustaceans like penaeid and mysid shrimps, and fish like anchovies and clupeids, especially herrings (Merriner 1975; Stickney *et al.* 1975). However, its diet varies regionally according with what is most readily available, so crabs, amphipods, decapod shrimps, squids, shelled mollusks, and annelid worms are also part of the diet of this species in the native region (Merriner 1975; Stickney *et al.* 1975; Bowman *et al.* 2000; Nemerson & Able 2004; Willis *et al.* 2015). In the Sado estuary, weakfish preyed on crustaceans (53%) (mainly mysids but also carideans, isopods, and crabs), fish (45%) (mainly sand smelt *Atherina* sp. and European anchovy *Engraulis encrasicolus*), and cephalopods (2%). Therefore, weakfish seems to feed on the same prey groups, but consuming the species present in the invaded range. Mysid shrimps were consumed in similar proportions to the native area (Merriner 1975; Stickney *et al.* 1975). Clupeids were not identified despite being the most abundant family in the Sado estuary (França *et al.* 2011). In turn, the Atherinidae and Engraulidae family are more abundant in this region (Neves *et al.* 2008; França *et al.* 2011) and might be substituting clupeid species. Cannibalism was observed in one individual confirming what it also happens in the native range, which might affect the abundance of weakfish juveniles (Thomas 1971; Merriner 1975; Lowerre-Barbieri 1994). Studies describing ontogenic changes in the diet of weakfish mention that younger individuals prey mostly on crustaceans while older individuals prey on fish of several size ranges (Merriner 1975; Chao 1977; Hartman & Brandt 1995); however it is impossible to make such evaluation in this study due to the low number of individuals analyzed (n= 33) and poor homogeneity of length classes.

Our study points to a mixed feeding strategy of weakfish, with different individuals preying on different items (specialists), which agrees with a study performed in the native range (survey from North Carolina to Florida) where weakfish showed a mixed feeding strategy with occasional opportunistic feeding behavior (Willis *et al.* (2015). Other studies suggest that weakfish is flexible in prey selection across different geographic areas (Perlmutter *et al.* 1956; Merriner 1975) and that the abundance of a preferred food item is not limiting their survival and growth (Merriner 1975). Therefore, the first hypothesis tested in this work is supported, and this strategy was certainly an important factor influencing the success in the establishment of weakfish in the new range, as it is common for invasive aquatic species (Sax & Brown 2000; Guzzo *et al.* 2013).

Stable isotope analysis shows that weakfish, meagre, and seabass are feeding at the same trophic level, while seabream is a trophic level below, indicating the consumption of distinct food preys or different proportions of the same prey (Bearhop *et al.* 2004). There were no significant differences in $\delta^{15}N$ between the two tissues in all species, indicating that there were no changes in trophic level in the time preceding sampling (Hayden *et al.* 2017). However, European bass had the highest $\delta^{15}N$ standard deviation for both tissues, indicating high intraspecific variability in $\delta^{15}N$, that may lead to variable positioning across different trophic levels depending on the individual or variation in trophic position of prey organisms (Vander Zanden *et al.* 1997). This difference might be explained by the size range of seabass (42,5-70 cm) or by its dietary diversity (Bearhop *et al.* 2004). These two components tend to be related since larger fish can eat larger preys. This relationship between size and $\delta^{15}N$ was already demonstrated for *Dicentrarchus labrax* (Pasquaud *et al.* 2008). No significant differences in $\delta^{13}C$ between tissues of weakfish, meagre, and white seabream indicate that there were no changes in resource use in the last 2 to 4 months (Vander Zanden *et al.* 2015). Differences in $\delta^{13}C$ in seabass might be explained either by a difference in tissue turnover rates after a change in diet, or an inherent difference between tissues due to their structure (Hayden *et al.* 2017), either in lipid concentration (Andvik *et al.* 2010) or carbon content (Cano-Rocabayera *et al.* 2015). However, this last hypothesis is less probable since lipid correction and acidification were conducted before the analysis.

There was no significant isotopic niche overlap or a measure of overlap (Pianka index) between weakfish and the other three species, which does not support our hypothesis that weakfish is outcompeting the other three native species for food and space. However, both these measures were higher between weakfish and meagre, an indication that competition for food is higher with meagre (Guzzo *et al.* 2013; Britton *et al.* 2018) than with European bass and white seabream, which supports our third hypothesis. This is most probably related to the taxonomic proximity between the two Sciaenidae species, that lead them to seek the same type of food, to have similar feeding strategies, and to look for the same spaces. An interesting fact is that no parasites were observed in the guts of weakfish, while nematodes parasites were observed in the small samples of meagre and European bass, leading us to suggest that the incidence of endoparasites on weakfish is likely very reduced. This fact reinforces the enemy release hypothesis as a possible advantage for the establishment of weakfish (Torchin *et al.* 2003).

As widely recognized, invasive species can induce ecological and evolutionary consequences in the recipient communities and ecosystem (Lee 2002; Simberloff 2014; Gallardo *et al.* 2016). At the species level, weakfish may affect the demography of native species through competition for resources, aggressiveness, or predation (Mooney & Cleland 2001; Crooks 2002a). We already confirmed that these effects may affect meagre: the overlapping diet, the search for the same refuges, and even interfering in the reproduction since these species are known for competing through sounds during spawning (Bigelow & Schroeder 1953; Lagardère & Mariani 2006). Concerning predation, no meagres were found among the weakfish preys; however, this hypothesis cannot be ruled out. One *Diplodus* sp. specimen was found in the stomach of one weakfish, suggesting that *Diplodus sargus* and *Diplodus bellottii*, another common species commercialized in the region of Sado estuary, can be preyed by weakfish.

Weakfish may have direct impacts on the demography of its preys, such as the sand smelt, horse mackerel, European anchovy, black goby, mysid shrimps, carideans, brachyurans, isopods, and even cephalopods (e.g., squid, cuttlefish). Of these prey, horse mackerel, squid, and cuttlefish are the ones with greater economic importance in the Sado estuary (Docapesca 2018). Weakfish may also have an indirect impact on other native fishery resources by competing for food, possibly creating a cascading effect. Many of the weakfish preys are also preyed by many other species that use the estuary during the same period (spring, summer, early autumn), such as guilthead seabream *Sparus aurata* (Linnaeus, 1758), the Senegal seabream *Diplodus belloti* (Steindachner, 1882), the anchovy *Engraulis encrasicolus* (Linnaeus, 1758), horse mackerel *Trahurus trachurus* (Linnaeus, 1758), blackspot seabream *Pagellus bogaraveo* (Brünnich, 1768) and axillary seabream *Pagellus acarne* (Risso, 1827), common cuttlefish *Sepia officinalis* (Linnaeus, 1758), common squid *Loligo vulgaris* Lamarck, 1798, common octopus *Octopus vulgaris* Cuvier, 1797, and even the common bottlenose dolphin *Tursiops truncates* (Montagu, 1821). These are just a few examples of native species that may be affected by weakfish invasion since the impact upon other species is difficult to diagnose because other concurrent deleterious factors might act simultaneously. It is also possible that native species could be taking advantage of this invasion and use weakfish as a food source. One of the most recurring concerns among fishers about weakfish is the overlap between their prey with those of the common bottlenose dolphin. However, it could be possible that dolphins are consuming the new Sciaenidae, which is one of the preys types of this dolphin species (Santos *et al.* 2007; Froese & Pauly 2018).

Our study presents one limitation, namely the low sample size of analyzed native fish. However, other studies on the diet of these native species are available, an increase in sample size would allow us to see if meagre or seabass could be preying on weakfish larvae or juveniles since both species are piscivorous (Pasquaud *et al.* 2008; FAO 2018; Froese & Pauly 2018). Additionally, only one sample was performed (in June 2017), which may bias the type of prey found in the stomach contents and feeding strategy due to different abundances of prey at different times of the year in the estuary. However, the main objective of this work was accomplished, which was to provide the first ecological assessment about weakfish in the invaded range, and provide a solid base for future studies.

5. Conclusion

In the Sado estuary, weakfish showed a generalist feeding strategy since it preys on multiple prey types (broad niche width), but this population appears to be composed by specialists – i.e., different individuals preying on different prey items and with little prey overlap among them. This was certainly an important factor influencing weakfish to establish and persist in this region. Concerning competition for food, we found that the weakfish, meagre, and European bass are feeding on the same trophic high level, whereas the white seabream is one trophic level lower confirming that it feeds on different preys than the others. We found no significant isotopic niche overlap, or Piaka index, between the weakfish and the other three species, rejecting the initial hypothesis that weakfish would be competing for food with these three species. Yet, the probability of niche overlap was higher between weakfish and meagre, validating the hypothesis that competition for food is higher with meagre, quite possibly because they are taxonomically close species. Similarly to what happens in the native region, weakfish performs annual migrations, using the brackish/reshwater part of the estuary in spring and summer and coastal areas in winter. Thus, weakfish uses the Sado estuary simultaneously with meagre, European bass and white seabream, evidencing some degree of competition for habitat use. The information provided in this work is serving as a basis for other ongoing studies investigating possible competition between weakfish and other native species. It might also be used to support management policies about fisheries and control of this invasive species, a subject that will be approached in the next chapter.

Chapter III: Weakfish as a new fishing resource: could weakfish be accepted by the Portuguese consumers?

1. Introduction

Non-indigenous species benefit from a wide array of competitive advantages compared to native species, including the absence of predators and naïve preys (Colautti *et al.* 2004). So, the consumption of invasive species by humans, the top predator on Earth, emerged as an approach to control and reduce edible non-indigenous populations (Roman 2006; Lai 2015; Orth & Schmitt 2018). Although challenging, humans are driving species to extinction or populations to extirpation or collapse owing to their persistent and voracious appetite (Nuñez *et al.* 2012). The consumption of invasive species has become popular in the US as a means to control the invasive lionfish *Pterois volitans* (Linnaeus, 1758) and *Pterois miles* (Benett, 1828) through a campaign whose slogan was "Eat the lionfish" (NOAA 2011), and through the publication of the cookbook *The Lionfish Cookbook: The Caribbean's New Delicacy* (Ferguson & Akins 2010).

Several chefs all over the world intend to promote a similar approach with other invasive species. Chef Bun Lai from Miya's Sushi restaurant (Connecticut, USA) wants to convince the world that invasive species can be delicious, like the Asian sea squirt *Styela clava* Herdman, 1881, the European green crab *Carcinus maenas* (Linnaeus, 1758), and earthworms, among many others (Lai 2015). In the UK, celebrity Chef Gordon Ramsey featured the Chinese mitten crab *Eriocheir sinensis* H. Milne-Edwards, 1853, captured in the Thames river in one of his TV shows (Ramsey 2009), while the Eastern grey squirrel *Sciurus carolinensis* Gmelin, 1788 was included in the seasonal menu of "The Jugged Hare" restaurant in London to promote its consumption while advocating for the conservation of the European red squirrel (Hyslop 2015). In the US, the invasive Asian carp – a collective designation for the Bighead Carp *Hypophthalmichthys nobilis* (Richardson, 1845), Black Carp *Mylopharyngodon piceus* (Richardson, 1846), Grass Carp *Ctenopharyngodon Idella* (Valenciennes, 1844), and Silver Carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) – were introduced in the Mississippi River Basin and fished to feed disadvantaged people through the campaign "Target Hunger Now!" (McCloud 2011). However, there are still just a few examples reflecting the potential of fishing and eating aquatic invasive species to control their populations.

Fishing invasive species as a means to minimize putative ecological impacts might be more effective in countries with higher fish consumption rates, like Portugal, which rank third in the list of world fish consumers (EUMOFA 2016). On average, Portuguese eat more than 55 kg of fish *per capita* in one year, twice the European Union's average (EUMOFA 2016). Most seafood consumed in Portugal is sold fresh, without being processed or preserved (Almeida *et al.* 2015). Fresh seafood requires constant supply and various other means to guarantee the fish quality, which would be unfeasible to sustain without consumers being willing to pay a higher price. Also, 63% of the consumed fish in Portugal is imported, 35% is fished locally, and only 2% is produced in national aquaculture facilities (WWF-Mediterranean 2017). Portuguese consumers prefer wild species to farmed species (Cardoso *et al.* 2013; Fernandes 2017), likely due to consumers' skepticism about aquaculture, which often relies on preconceived ideas transmitted between consumers that farmed fish is of lower quality (Ramalho & Teresa Dinis 2011). All these aspects could play in favor when introducing a new wild fish species into the Portuguese food market. But, what if this new species is an invasive species?

Weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) is one of the most recently introduced fish in Europe, and specifically in the Iberian Peninsula (Morais & Teodósio 2016; Morais *et al.* 2017). This species is native from the Northwest Atlantic where it has supported local fisheries at least since the late 1800s (ASMFC 2016). Currently, the stock is considered depleted since 2003 owing to overfishing and increased natural mortality observed since the mid-1990s (ASMFC 2017), being under an Interstate Fishery Management Plan developed by the Atlantic States Marine Fisheries Commission (ASMFC 2016). In 2015, the last year for which weakfish price data is available, the average price per pound was $1.79 (Fissues 2019). However, a 3-pound (1.36 kg) fresh weakfish is sold online for 36.40$ (26.76$ kg^{-1}) (Fultonfishmarket.com 2019).

In the Iberian Peninsula, and particularly in Portugal, weakfish have been sold in at least three fish markets. In Setúbal (30 km south of Lisbon), the species is captured in Sado estuary and sold for 3 to 10 € kg^{-1} depending if they are smaller or bigger specimens, respectively, and for 5 € kg^{-1}in Tavira and Olhão – some of the fish sold in these fish markets might come from the Sado estuary. In our opinion, weakfish is underappreciated, largely because this species is unknown to Portuguese consumers. So, could weakfish be accepted as a new fishing resource by Portuguese consumers?

Given the general preference of Portuguese for fresh seafood we hypothesized that Portuguese fish consumers would give a good evaluation to weakfish in terms of its appearance, flavor, and texture, and would prefer the invasive weakfish over native or imported species produced in aquaculture (e.g., gilthead seabream, salmon). We also hypothesized that the current average selling price (5 € kg^{-1}) could be under-priced, since similar wild native species, like meagre, seabass, and gilthead seabream are sold in Portugal for no less than 12 € kg^{-1}, 25 € kg^{-1}, and 25 € kg^{-1}, respectively. Likely lack of information about the species may explain the lower prices. Increasing weakfish's selling price would encourage fishers and fish vendors to capture and promote the species so that a fair price tag could reflect weakfish's quality.

When introducing a new species into an established culinary culture, as the Portuguese, customers must be curious to taste something new while thinking that the investment in such product is worthy. We hypothesized that for being a wild fish and that can easily be sold fresh, there is a good chance of acceptance of this new species by Portuguese consumers. To test this hypothesis, we provided some weakfish specimens to Portuguese consumers and conducted an inquiry to evaluate their opinion on the fish. If opinions are positive, the development of a commercial harvest plan to control weakfish population density should be considered.

The resilience that always exists to try new food products might be broken by promoting its positive attributes (e.g., health benefits, environmental protection, origin) (Sanjuán-López *et al.* 2011; Nuñez *et al.* 2012), and by providing information that familiarizes the customer with the product. Thus, outreach actions can be created to promote the new fish species and increase consumers' awareness which will hopefully lead consumers to be willing to buy this new product for a fair price (Varble & Secchi 2013). We hypothesized that the creation of awareness events to inform the public about invasive species (e.g., the benefits of consuming invasive species to the environment and to the local economy, the negative impacts exerted by aquatic non-indigenous species on ecosystems) and raise awareness about the benefits of eating weakfish to ultimately encourage consumers to buy weakfish if they see them on the market. Therefore, the main objectives of this work were to (1) evaluate the potential of weakfish as a new fishing resource by perceiving consumers receptivity to a new fish species, and (2) promote weakfish consumption through a series of activities, as food tasting sessions, social media outreach, and press releases.

2. Materials and methods

2.1. Questionnaire survey

A total of twenty-four specimens of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) were bought at Setúbal fish market on June 2017. These fish were given to a panel of 30 consumers who prepared and ate the fish in their homes to later evaluate their opinion about weakfish and its potential as a fishing resource. Consumers were chosen randomly considering their gender and age group (under 25, 26-50, 51-65, above 65). The fish were provided fresh and without gut contents. Each consumer replied to a survey containing eleven questions (Figure 3.1).

The Chi-square test was used on discrete data to test the null hypothesis (Table 3.1) after assessing the test's assumptions – i.e., normal distribution and heteroskedasticity. When the assumptions were not met, we used the analogous non-parametric test, the Wilcoxon Signed Rank test. All statistical analyses were done using R (version 3.4.2). Statistical significance was set at 0.05.

Survey to consumers

Name: ____

Age: __________ Contact: ______________________________

1) Appearance: Bad ☐ Indifferent ☐ Good ☐
Flavour: Bad ☐ Indifferent ☐ Good ☐
Texture: Bad ☐ Indifferent ☐ Good ☐

2) How did you cook it? Boiled ☐ Grilled ☐ Roasted ☐ Fried ☐ Other ☐ __________

3) Would you buy this fish?

____

4) How much would you pay for it?

____

5) What price would you consider fair, considering that this is a fish captured at sea?

____

6) Do you prefer this fish to gilthead seabream, seabass and meagre caught in the sea?

____

7) Do you prefer this fish to gilthead seabream, seabass and meagre produced in aquaculture?

____

8) For the same price, would you rather buy this fish (caught in the sea) instead of gilthead seabream, seabass or meagre produced in aquaculture?

____

9) Order according to your preference: (1 to 7, being preferred is 1 and least appreciated 7).

☐ Salmon
☐ Sardine
☐ Horse mackerel
☐ Gilthead seabream
☐ Sole
☐ Meagre
☐ Weakfish

10) Why did you put weakfish in this position?

____

11) Do you prefer fish from the sea or aquaculture?

____

Thank you for your collaboration!

Figure 3.1. Questionnaire survey delivered to a panel of 30 weakfish consumers.

Table 3.1. Hypotheses formulated in the consumers' questionnaire survey.

Hypotheses
H1: Consumer's evaluation of weakfish traits, i.e., appearance, flavor, and texture, is good.
H2: Cooking method influences consumer evaluation of weakfish' appearance, flavor, and texture.
H3: Consumers would buy the fish if they saw it for sale in the market.
H4: Consumers would pay a higher price than the current sale price (5 € Kg^{-1}).
H5: Considering weakfish origin, the price consumers consider fair is higher than the current sale price (5 € Kg^{-1}).
H6: Consumers prefer wild native fish species (meagre, gilthead seabream and seabass) to weakfish.
H7: Consumers prefer weakfish to native fish species produced in aquaculture.
H8: For the same price, consumers prefer weakfish to farmed native fish species.
H9: Consumers prefer native species to weakfish.
H10: Lack of awareness about weakfish is translated in a lower evaluation when comparing to native fish species.
H11: Consumers prefer wild fish to reared fish in aquaculture.

2.2. Awareness events

A series of awareness events were created to share information with the public about weakfish (i.e., introduction pathways, impacts, control, culinary use) and promote weakfish as a valuable resource and assess the public response to the idea of consuming weakfish to help controlling this invasive species. For this purpose, we partnered up with two Chefs, who prepare weakfish in their kitchens and published social media publications and two press releases trough the Centre of Marine Sciences (CCMAR) communication department to attract the curiosity from the public and press.

Partnership with local Chefs

The first created event consisted in a tasting session that took place on September 19^{th}, 2017, in Loulé (Algarve) at the canteen of ASMAL (Algarve Mental Health Association) – a non-profit organization working with people struggling with mental issues – where Chef Avelino Falé prepared weakfish for people that eat daily at the canteen. The goal was to obtain people's evaluation through a tasting survey, adapted from the one listed on Table 3.1 since fish were not prepared by the consumers.

The second event resulted from the collaboration with Chef Leonel Pereira, owner of the Michelin star restaurant *São Gabriel* in Almancil (Algarve), who was enthusiastic in testing weakfish recipes in his experimental kitchen, along with other edible species that are not consumed in Portugal, such as the non-indigenous *Blackfordia virginica* Mayer, 1910 (Hydrozoa) and the native jelly blubber *Catostylus tagi* (Haeckel, 1869) (Scyphozoa).

Press releases

The CCMAR's communication department published two press releases in September 2017, and on our request, that announced the tasting session that occurred at ASMAL's canteen in tandem with detailed information about the two newest – at the time – non-indigenous species present in the Guadiana estuary, weakfish and the Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda) (Figures 3.2 and 3.3). The Atlantic blue crab is native from the western Atlantic and has a great potential to become a commercial fishery (Morais *et al.* (2019). These press releases were published on CCMAR's website and Facebook (CCMAR 2017a, b) and sent to the press using a list with more than two hundred contacts, including LUSA (largest news agency in Portugal) and the main Portuguese newspapers, TVs, and radio stations.

CORVINA AMERICANA É DADA A CONHECER EM PROVA DE DEGUSTAÇÃO

18/09/2017

A corvina americana, ou corvinata, não é natural de águas nacionais, mas mesmo tratando-se de uma espécie invasora já está instalada nos estuários dos nossos rios. Por isso, os investigadores do CCMAR vão dar a conhecer numa prova de degustação, de que forma se pode consumir este peixe e assim tentar aliviar a pressão que esta espécie faz nos nossos recursos pesqueiros.

A iniciativa está marcada para esta terça-feira, dia 19 de Setembro, pelas 13:00 horas, altura em que na cantina da ASMAL (Associação de Solidariedade Social do Algarve), em Loulé, a corvina americana vai fazer parte do menú de almoço.

O objetivo dos investigadores do CCMAR que estão à frente da iniciativa é dar a conhecer melhor o potencial de uma espécie invasora, promovendo o consumo da mesma.

A corvinata, ou corvina americana, é uma espécie proveniente da costa americana, mas que já se instalou nos estuários portugueses. Para além de ser uma espécie invasora não tem também predadores naturais no mar português, o que faz com que consiga sobreviver sem grandes dificuldades e afete, inevitavelmente, as restantes espécies nativas.

A sustentabilidade das espécies nativas e tradicionalmente exploradas, como a sardinha, carapau, entre outras, passa também pela alteração de consumo, utilizando outros recursos atualmente disponíveis na nossa costa e ainda não explorados.

Desta forma, no futuro, a pesca poderá reduzir a densidade das espécies invasoras, como a corvina americana, e aliviar a pressão sobre os nossos recursos pesqueiros tradicionais.

A prova de degustação tem em vista sensibilizar e alertar para todas estas questões e a possibilidade de alteração de hábitos de consumo, começando desde já pelos alunos e monitores da ASMAL, em Loulé.

Figure 3.2. Press release published at CCMAR’s website about the weakfish taste test done at the Algarve Mental Health Association on September 2017 (CCMAR 2017a).

CARANGUEJO-AZUL DESCOBERTO NO RIO GUADIANA

28/09/2017

A espécie é nativa da costa leste da América, mas foi encontrada pela primeira vez no rio Guadiana no passado mês de Junho. A estreita colaboração entre pescadores do Guadiana e investigadores do CCMAR permitiu identificar neste estuário, o siri ou caranguejo azul (Callinectes sapidus) que é nativa da costa leste americana.
O facto surpreendeu os pescadores locais, que se mostraram intrigados com a descoberta de uma espécie originária de um local tão distante, e pelo fato de ser um recurso pesqueiro com muito valor na costa americana.
Há registo de outros exemplares da mesma espécie capturados anteriormente no Estuário do Sado, o que indicia que a espécie estará numa fase de expansão na nossa costa, depois de provavelmente ter navegado, enquanto larva, nas águas de lastro de um navio que cruzou o Atlântico.

A introdução de espécies não-nativas no estuário do rio Guadiana tem vindo a aumentar nos ultimos anos, com mais de uma dezena de espécies registadas, incluindo peixes, amêijoas, alforrecas, camarões, e mais recentemente este caranguejo, tal pode consequências nefastas para as espécies nativas. No entanto, as espécies invasoras com valor comercial, como é o caso do caranguejo azul, ou da corvinata real, registada no ano anterior, podem ser um exemplo de como uma ameaça se pode transformar numa oportunidade de exploração.
Este aspecto assume especial relevância, face à inexistência de predadores naturais destas espécies invasoras, e a sua pesca contribuirá para o controlo da sua densidade, ao mesmo mesmo que aliviará a pressão de exploração em muitos dos nossos recursos pesqueiros tradicionais, como por exemplo a sardinha. Novas alternativas de consumo deste tipo de espécies estão também a ser estudadas com chefs de restaurantes algarvios.
Os investigadores do CCMAR estão sempre dispostos a colaborar com a sociedade na resposta às suas questões ecológicas, e também no desenvolvimento de parcerias tecnológicas e científicas com qualquer sector da indústria pesqueira.

Figure 3.3. Press release published at CCMAR's website about the appearance of the Atlantic blue crab in the Guadiana estuary, Algarve (CCMAR 2017b).

We conducted an extensive web search to evaluate the impact of these two press releases by assessing the number of articles published. The online search was performed on Google on March 28, 2018. A combination of keywords was used, in English and Portuguese, singular and plural, referring to the non-indigenous species described in the press release (i.e., espécie, invasora, corvina, corvinata, americana, rainha, weakfish, Cynoscion regalis, caranguejo, azul, caranguejo-azul, blue crab), to the research centre (CCMAR), university (UALG, Universidade do Algarve), and location (Algarve, Portugal), and to the research leader of this project (Maria Alexandra Teodósio).

The Portuguese news agency LUSA expressed interest in interviewing us, along with Chef Leonel Pereira, to explain the reasons behind the appearance of invasive species in Portuguese estuaries, their potential for commercial exploitation, and their application to cuisine. The interview was held on December 30, 2017, and was published as a digital article with an audio file with a total duration of 6’40’’(Lusa 2017). A similar online search was conducted applying the previously used method, i.e., using an association of keywords related to the addressed species (e.g., corvina, corvinata real, caranguejo-azul, siri, medusas, alforrecas), to the research centre, university, and locations (Universidade, UALG, CCMAR, Algarve, Restaurant São Gabriel, Portugal), and to the researcher and Chef (professor Maria Alexandra Teodósio, Chef Leonel Pereira).

3. Results

3.1. Questionnaire survey

A total of 27 people replied to the questionnaire, and the average age was 45 ± 18 years (Figure 3.4), of which 70% were females. The youngest consumer was 22 and the oldest 78 years old.

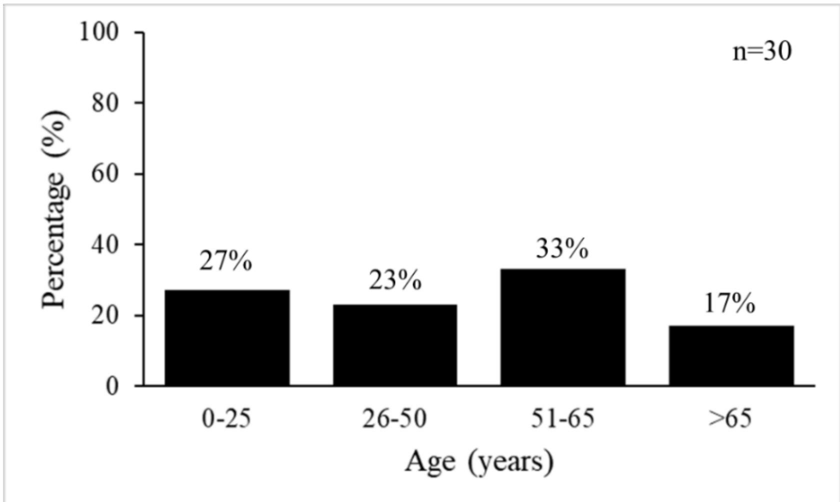


Figure 3.4. Age-distribution (%) of the consumers that replied to the questionnaire survey on weakfish.

When evaluating weakfish general traits, a large majority of consumers rated the fish's appearance as good (97%, χ^2= 180.85, df= 2, p< 0.05), as well as it's flavor (90%, χ^2= 144.67, df= 2, p< 0.05), and texture (83%, χ^2=34.2, df=2, p<0.05) (Figure 3.5). Regardless of the evaluation that consumers gave to texture, the flesh was pointed as ideal for recipes that use the fish shredded or sliced.

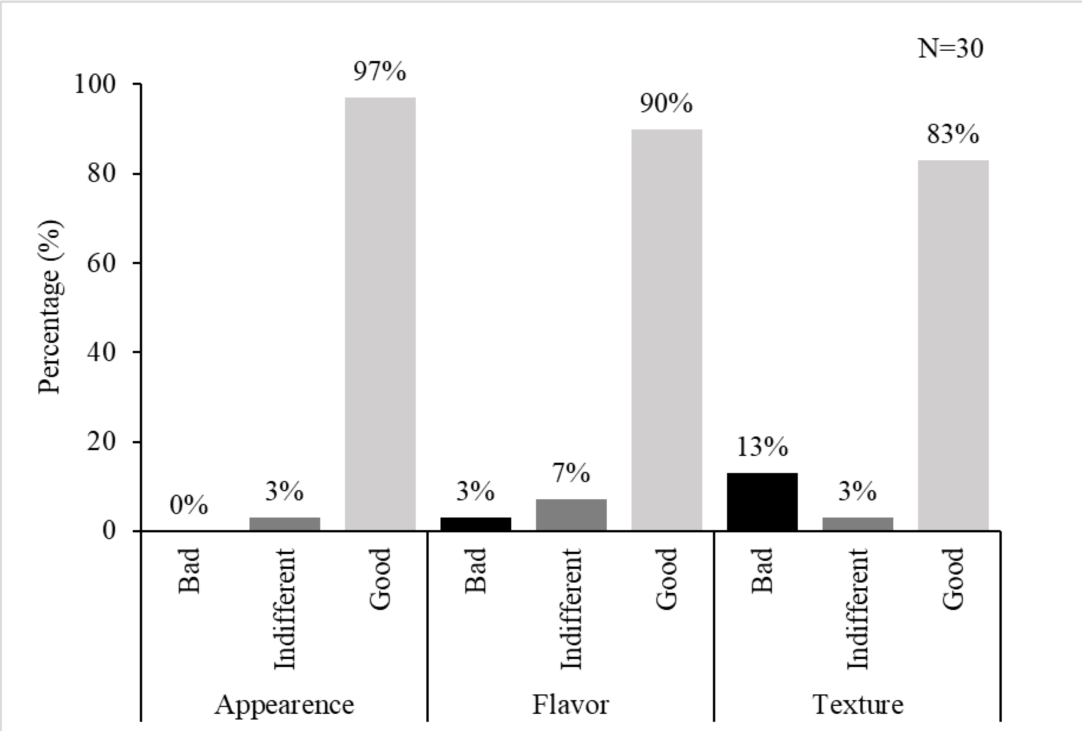


Figure 3.5. Percentage of consumers evaluating on the general traits of weakfish (appearance, flavor, and texture) as bad, indifferent, or good.

The most chosen cooking method was a roasted (40%), followed by boiled (37%), grilled (37%), and lastly the other ways of preparation which included fish stew ("caldeirada") and fish pasta ("massada de peixe") (Figure 3.6). Six consumers prepared the fish in three different ways – grilled, roasted, and stewed ("caldeirada"). We decided not to evaluate if the cooking method influenced consumers evaluation on weakfish' flavor, and texture, since it was necessary that all consumers had prepared the fish in one way.

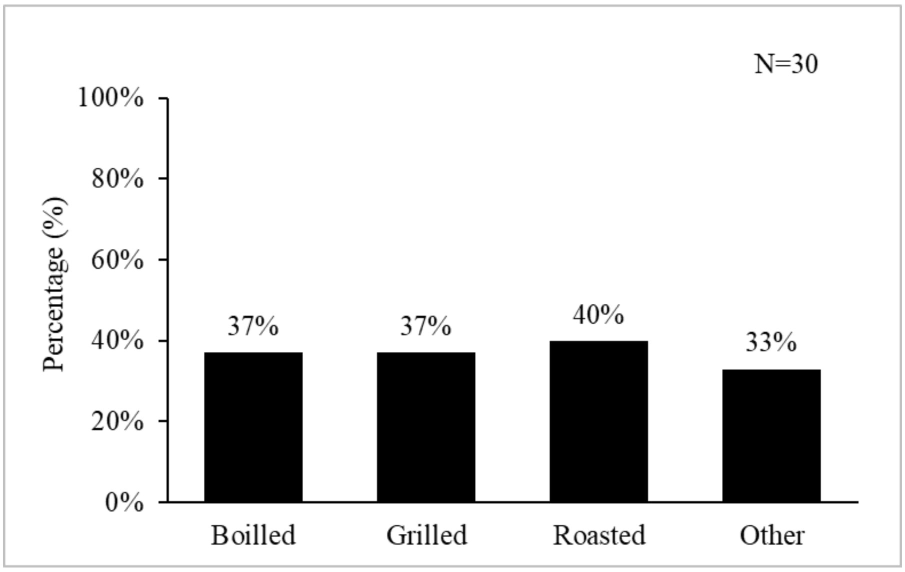


Figure 3.6. Cooking methods chosen by consumers to cook weakfish at their home.

When asked if consumers would buy the fish in the future, 90% of consumers responded yes, and there are significant differences between the two answers (χ^2= 64, df= 1, p-value< 0.05). The average price that consumers are willing to pay for weakfish is 1.20 € less (8.3 ± 6.2 € kg^{-1}) than the one that they consider to be the fair price (9.5 ± 6.4 € kg^{-1}). The difference between these two values is significant (T= 13.5, p< 0.05) (Figure 3.7). The majority of consumers would prefer buying wild native fish (87%) over weakfish (10%) (χ^2= 38.6, df= 2, p-value< 0.05) (Figure 3.8A). However, consumers prefer buying weakfish (63%) if the native fish available at the market would be farmed fish (33%) (χ^2= 16.2, df= 2, p-value< 0.05) (Figure 3.8B). Consumers would still prefer buying weakfish (57%) over farmed fish (33%) even if their price would be the same (χ^2= 9.8, df= 2, p-value< 0.05) (Figure 3.8C). Consumers were asked to rank seven fish species according to their preferences. Sardine was the most preferred fish, with 30% of consumers ranking it as their favorite fish (Figure 3.9F), while sole was the least favorite fish (Figure 3.9G). Weakfish was rated in different proportions (χ^2= 13.867, df= 6, p< 0.05), but overall it was never the consumers' favorite fish (0%) and most consumers ranked it in 5th place in their preference list (37%) (Figure 3.9A).

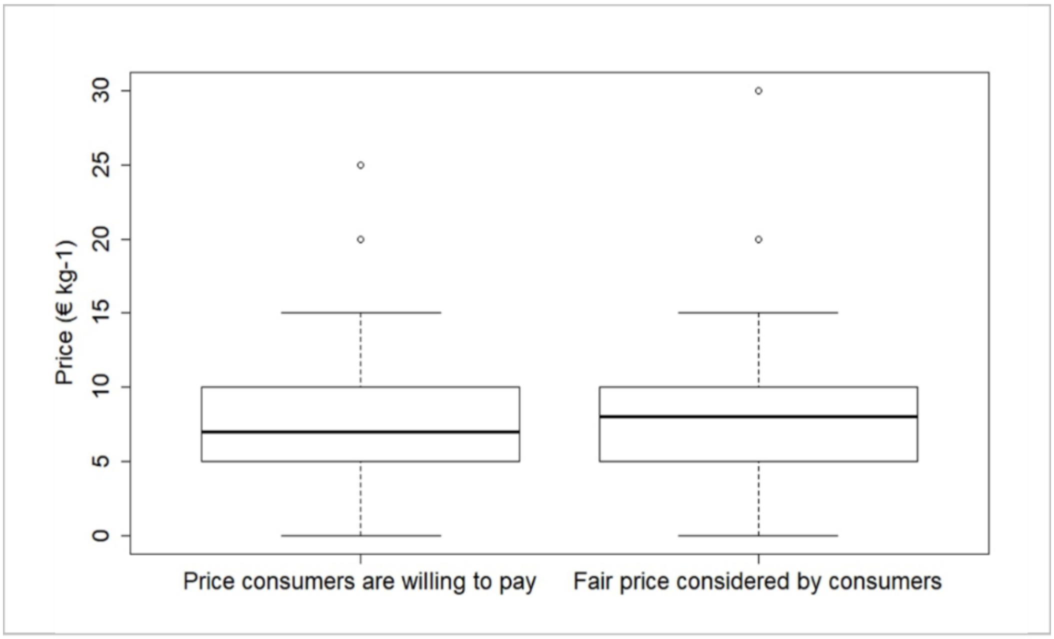


Figure 3.7. Comparison between the average price consumers are willing to pay for weakfish and the price that they consider to be the fair price.

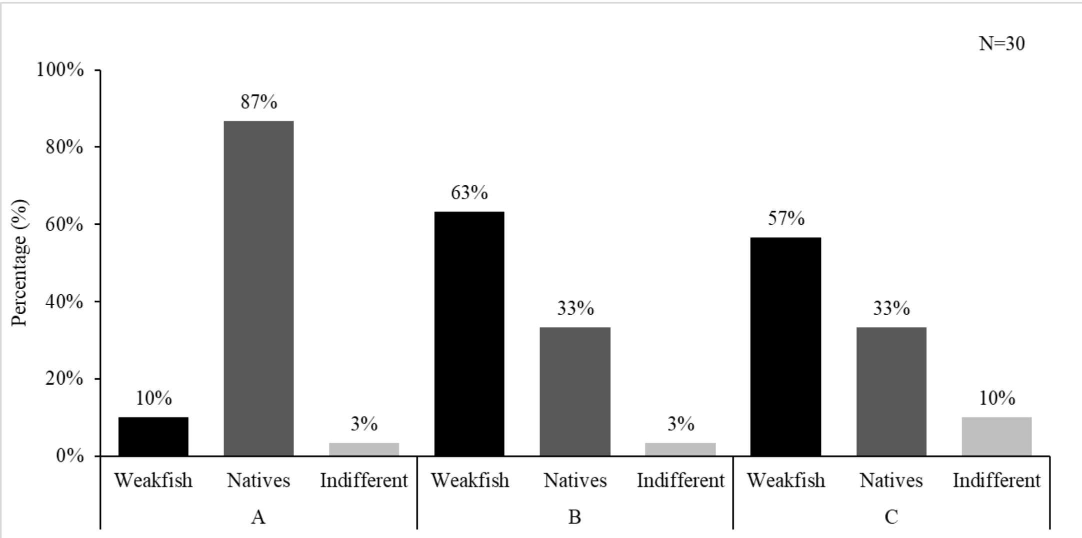


Figure 3.8. The consumers' preference between weakfish and other three native species (meagre, gilthead seabream, and seabass) when these natives are wild (A), farmed (B), and if the price of farmed fish is the same as weakfish (C).

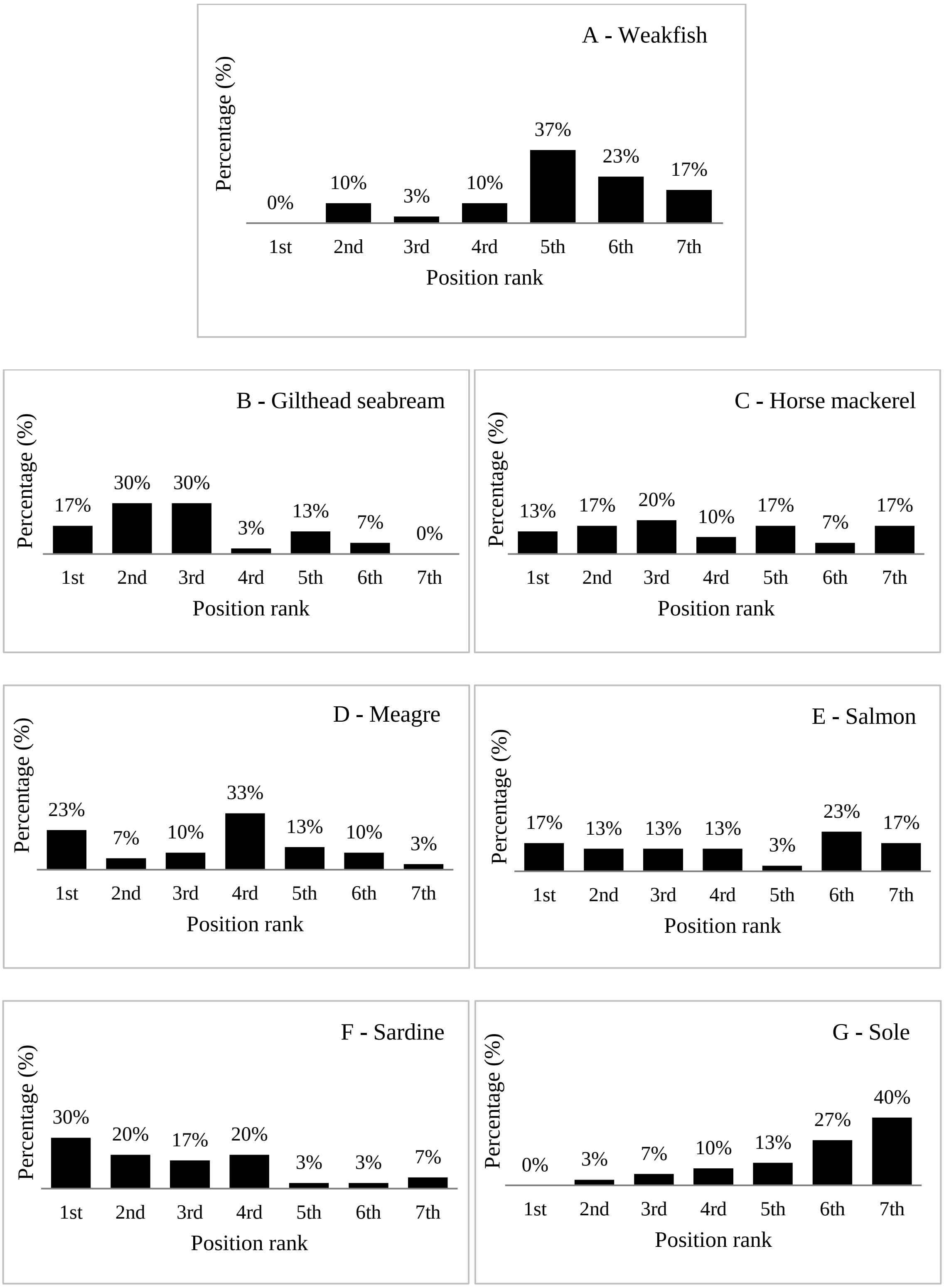


Figure 3.9. Preference order by which consumers ranked weakfish (A), gilthead seabream (B), horse mackerel (C), meagre (D), salmon (E), sardine (F) and sole (G). Consumers placed their preferred species in 1st place and the least favourite in 7th place.

Regarding the origin of fish, consumers significantly prefer wild (83%) over farmed fish (0%) (χ^2= 35, df= 2, p-value< 0.05), while 17% of consumers had no preference (Figure 3.10).

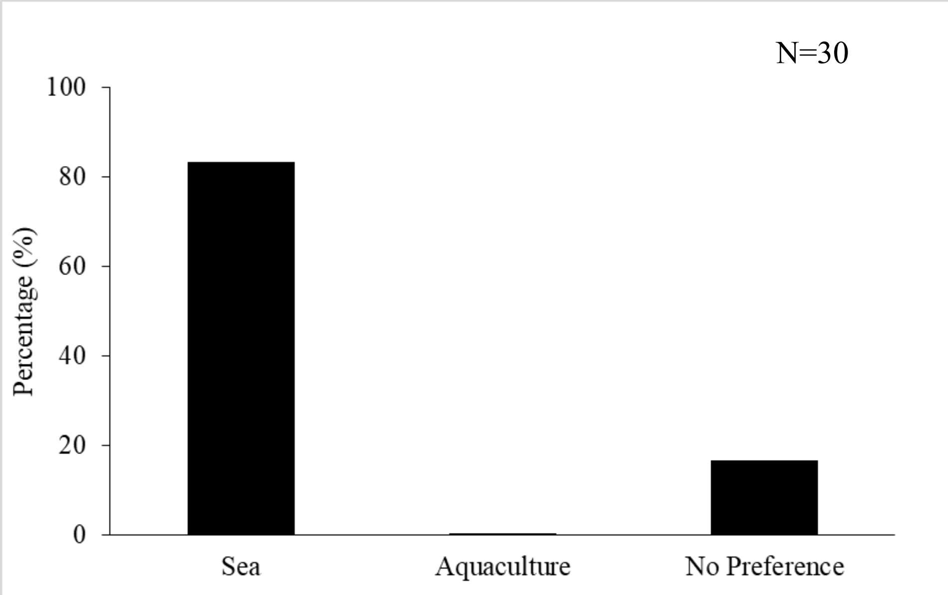


Figure 3.10. Consumers' preference regarding fish origin: sea, aquaculture, and no preference.

3.2. Awareness events

Partnership with local Chefs

The feedback obtained from people present in the tasting session at ASMAL on September 19th, 2017, was positive. However, only six people responded to the survey, since few fish were available at the moment and were not enough to serve all the people present at the canteen. Therefore, we decided not to include the results of these surveys in this work (Figure 3.11).

Regarding the collaboration with Chef Leonel Pereira, the results obtained from his experiments were so positive that he decided to introduce weakfish in the seasonal menu of São Gabriel restaurant. He also published an image on social media while preparing weakfish to share the development of the experience (Figure 3.12). Chef Leonel Pereira was also subsequently invited by the Centre for Marine Sciences to present a seminar to researchers and members of the university, about the application of science to culinary, where he spoke about his experience with invasive species while giving the audience some of the delicacies we prepared with invasive species (Figure 3.13).

Figure 3.11. Tasting session at ASMAL canteen. Chef Avelino Falé with his staff (left) and the weakfish dish that was served – roasted weakfish with roast potatoes and red bell peppers (right).

Figure 3.12. Image posted by Chef Leonel Pereira on Instagram about his experiments with weakfish at his experimental kitchen in the São Gabriel restaurant (CreativeCookGarage 2018).

Figure 3.13. Seminar presented by chef Leonel Pereira at the University of Algarve showing some of the results and techniques applied to prepare weakfish and other invasive species.

Press releases

The two press releases resulted in thirty-eight online articles, and three news pieces broadcasted on national television. The online articles were published in Portuguese along two months, between September 28 and November 28, 2017. Twenty-two news pieces mentioned weakfish, while sixteen mentioned the Atlantic blue crab. Of the twenty-two items referring weakfish, fourteen were published in online news websites, that include some of the leading Portuguese daily (e.g., *Público*, *Diário de Notícias*, *Correio da Manhã*) and weekly (e.g., Expresso) newspapers, TV stations websites (*RTP*, *SIC Notícias*, *Porto Canal*), as well as local newspapers (e.g., *Diário Online Região Sul*, *Jornal do Algarve*) (Figure 3.14). Eight online publications were made in blogs. All references are listed in Table 3.2.

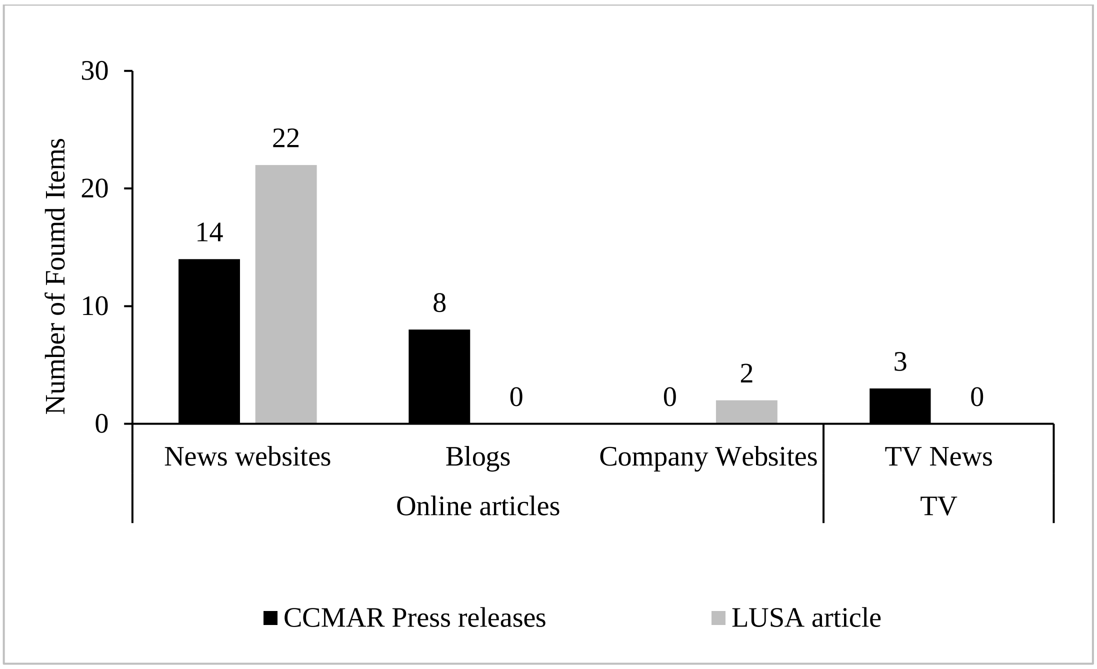
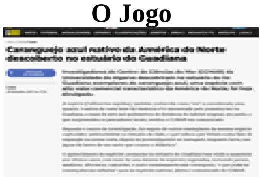


Figure 3.14. Number of news published and broadcasted mentioning weakfish as an invasive species in Portugal, as a consequence of the press releases made by the communication department of CCMAR on September 2017, and the article published by LUSA on December 30, 2017.

Table 3.2. Links to twenty-two national media news mentioning weakfish, as a result of the two press releases published in September 2017 by CCMAR. Articles were published online between September 28 and October 21, 2017. They are listed in chronological order.

Correio da Manhã	**Diário de Notícias**	**Diário Online Região Sul**	**O Jogo**
			
September 28, 2017	September 28, 2017	September 28, 2017	September 28, 2017
Link	Link	Link	Link
País ao Minuto	**Portugal Notícias**	**Público**	**RTP Notícias**
			
September 28, 2017	September 28, 2017	September 28, 2017	September 28, 2017
Link	Link	Link	Link

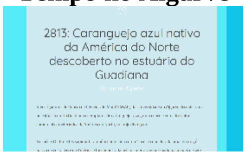
SIC Noticias

September 28, 2017
Link

Sul Informação

September 28, 2017
Link

Tempo no Algarve

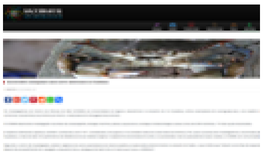


September 28, 2017
Link

TVI 24

September 28, 2017
Link

Ultimate Science



September 28, 2017
Link

A Roda

September 29, 2017
Link

Diário de Notícias

September 29, 2017
Link

Net Madeira

September 29, 2017
Link

Últimas Curiosidades

September 29, 2017
Link

Ambiente Ondas3

September 30, 2017
Link

Helder Barros

September 30, 2017
Link

Cidadania

September 30, 2017
Link

Jornal do Algarve

October 8, 2017
Link

Portal do Mar

October 21, 2017
Link

Regarding the TV broadcasts, two national TV channels reported the presence of weakfish and other invasive species in Portuguese estuaries. This coverage resulted in three appearances on TV with a total duration of 19’57”. The first news piece was transmitted by *TVI* in *Jornal das 8* (TV newscast) on October 8th, 2017 and lasted for 2’17’’ (TVI 2017) (Figure 3.15). In the following days, two appearances were broadcasted by *RTP 1*, featuring three researchers involved in this project and a fisherman from the Guadiana estuary that collaborates with our research group since 1999. The first broadcast was aired on October 9th, 2017, in the show *Portugal em Direto* with a duration of 14’15’’ (RTP1 2017a) (Figure 3.16). A shortened version, with 2’40”, was broadcasted on October 30th, 2017, during the evening’s newscast (RTP1 2017b).

Figure 3.15. Interview given to *Jornal das 8* on TVI aired on October 8th, 2017.

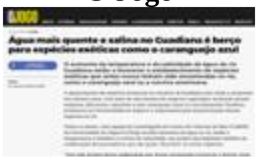
Figure 3.16. Interview given to *Portugal em Direto* of RTP 1 on October 9th, 2017.

A few weeks later, the Portuguese news agency LUSA interviewed us and Chef Leonel Pereira, to talk about the appearance of invasive species in Portuguese estuaries, their potential for commercial exploitation, and their application to cuisine. The interview was held on December 30, 2017 and published as a digital article with an audio file (Lusa 2017), which resulted in twenty-four news published between December 30, 2017, and February 2, 2018. Twenty-four news pieces were published on news websites including some of the most relevant online newspapers in Portugal (e.g., *Expresso*, *Jornal de Notícias*, *Destak*, *Observador*, *O Jogo*), local newspapers (e.g., *A Voz do Algarve*, *JM Madeira*) (Figure 3.17), and two in company websites. References are listed on table 3.3.

Figure 3.17. Some of the online news headlines retrieved from Portuguese websites, as result of the LUSA article published in December 2017.

Table 3.3. Links to twenty-four national media news pieces about invasive species published after the article published by LUSA on December 30, 2017 (Lusa 2017). These articles were published online between December 30, 2017, and February 2, 2018. News are listed chronologically.

Destak	Diário de Notícias	Expresso	Impala News
			
December 30, 2017	December 30, 2017	December 30, 2017	December 30, 2017
Link	Link	Link	Link
JM Madeira	**Jornal de Notícias**	**O Jogo**	**Observador**
			
December 30, 2017	December 30, 2017	December 30, 2017	December 30, 2017
Link	Link	Link	Link

Porto Canal	RTP Notícias	Sapo 24	TSF Rádio Notícias
			
December 30, 2017	December 30, 2017	December 30, 2017	December 30, 2017
Link	Link	Link	Link
A Voz do Algarve	**Antena 1**	**Diário de Notícias**	**Impala News**
			
January 1, 2018	January 1, 2018	January 1, 2018	January 1, 2018
Link	Link	Link	Link
Lusa	**Observador**	**O Jogo**	**RTP Notícias**
			
January 1, 2018	January 1, 2018	January 1, 2018	January 1, 2018
Link	Link	Link	Link
Somanjar	**Diário Campanário**	**Folha do Domingo**	**O Instalador**
			
January 1, 2018	January 2, 2018	January 3, 2018	February 2, 2018
Link	Link	Link	Link

4. Discussion

Weakfish has great potential to be accepted by the Portuguese consumers and be a targeted fishery that may help minimize the species putative ecological impacts. Overall, the evaluation of weakfish characteristics was good, and a large part of consumers showed an interest in buying the species after the experiment. Through the awareness events, we managed to reach a large audience due to the broad media coverage. Therefore, considering the good evaluation of weakfish as a fishing resource, the attention received by the public, and the current information available about weakfish population (see chapter 2), we believe that a harvest program can be used as an approach to control the density of its population. In the following sub-chapters (4.1. to 4.3), we address the arguments that support these interpretations and discuss the reasons why creating a control plan for this species trough harvest could be implemented.

Table 3.4. Summary table of the results obtained in response to the hypotheses formulated in this work.

Hypotheses	**Results**
H1: Consumer's evaluation of weakfish appearance, flavor and texture is good.	Supported
H2: Cooking method influences consumer evaluation on weakfish' appearance, flavor and texture.	Not tested
H3: Consumers would buy the fish if they saw it for sale in the market.	Supported
H4: Consumers would pay a higher price than the current mean sale price (5 € Kg^{-1}).	Supported
H5: Considering weakfish origin, the price consumers consider fair is higher than current mean sale price (5 € Kg^{-1}).	Supported
H6: Consumers prefer wild native fish species (meagre, gilthead seabream and seabass) to weakfish.	Supported
H7: Consumers prefer weakfish to native fish species produced in aquaculture.	Supported
H8: For the same price, consumers prefer weakfish to farmed native fish species.	Supported
H9: According to preference, consumers prefer native species to weakfish.	Partially supported
H10: Lack of awareness about weakfish is translated in a lower evaluation when comparing to native fish species.	Supported
H11: Consumers prefer wild fish to reared fish in aquaculture.	Supported

4.1. Questionnaire survey

This survey provided some insights about the possible acceptability of weakfish by Portuguese consumers. It gave us a better perception of which qualities should be highlighted when promoting weakfish, and which of the less appreciated characteristics should be softened. The evaluation of the general traits of the weakfish (i.e., appearance, flavour, texture) was positive, and most consumers would buy the fish if they saw it for sale. The test to evaluate if the cooking method influenced consumers' evaluation on weakfish' appearance, flavour and texture was not performed, since six consumers prepared the fish in three different ways and their answers could not be compared to the rest of the respondents. However, this should certainly be addressed in future studies or outreach actions. Many consumers suggested that weakfish texture is ideal for recipes that use shredded or sliced fish, as it is commonly used in several traditional dishes in

Portugal (Modesto 1983). We believe linking weakfish with traditional recipes is an important aspect to consider in the future when promoting weakfish.

The price consumers are willing to pay for weakfish and the price they consider fair are higher than 5 € kg^{-1}, meaning that weakfish could be sold for a higher price. Consumers are willing to pay at least 8 € kg^{-1} (60% higher than current mean price), supporting our hypothesis that the current average selling price is underestimated. The difference between both values (1.20 € Kg^{-1}) could be an indicator that consumers may have a personal preference for other species, although they find great value in weakfish as a wild species.

The fish origin appears to be an important indicator of fish quality for consumers, so it should be highlighted when promoting this species. Although wild native fish species continue to be preferred, most respondents would opt to buy weakfish instead of farmed native species, even if they were sold for the same price. However, preference based on origin could be related to sociodemographic aspects of consumers (not assessed in this work), like age (Cardoso *et al.* 2013), education, or economic condition (Myrland *et al.* 2000; Cardoso *et al.* 2013). For example, older Portuguese consumers prefer wild species and are less willing to experiment seafood from aquaculture (Cardoso *et al.* (2013).

Weakfish is clearly not the preferred fish in comparison with native wild fish, except the sole that was the least favorite species. Reasons that led to the response were personal taste and consumption habits. Therefore, being an unfamiliar species to the Portuguese consumers could give a competitive advantage to native species, but that could reverse with time.

In the future, we intend to enlarge our sample size and include descriptors of the consumers that are known to influence their choices, like income, education level, and location (Cardoso *et al.* 2013; Almeida *et al.* 2015). This approach allows targeting groups of consumers willing to buy and eat weakfish as a way to control an invasive species while turning future efforts to manage the invasion more effective.

4.2. Awareness events

Partnership with local Chefs

Chef Leonel Pereira found a great potential in weakfish and decided to include weakfish in the menu of his restaurant. So, *São Gabriel* clients tasted weakfish and were pleasantly surprised, which made them curious to buy weakfish in local fish markets. In locations where the species is being sold, we expect a raising curiosity from consumers and an increasing number of gastronomic events promoting ways to cook weakfish. However, this can only be achieved through the implementation of a program that promotes the harvesting of weakfish and sets a marketing strategy around this new product. We also hope that people from other parts of the country become interested in buying weakfish, and that information shared by Chef Leonel Pereira in social networks inspire other chefs to incorporate invasive species on their menus.

Press releases

Trough the awareness events created, we realized that weakfish has a great potential of being accepted by Portuguese consumers and being introduced in a commercial harvest plan. We consider that our goal of attracting press and media attention to weakfish and invasive species subject was achieved. The response and interest of the media on this subject was very positive and, despite being difficult to estimate the precise number of people who read the online news pieces and watched the reports broadcasted on television, we estimate that we certainly reached thousands of people in Portugal, and abroad since all these media outlets are available online – including the TV shows. Hopefully, a continued outreach work will result in higher awareness of the Portuguese population about weakfish and the non-indigenous species thematic, consequently resulting in higher curiosity and willingness to try.

4.3. Commercial harvest program to control population density

Conservation management plans aiming at controlling invasive species need to consider population dynamics, impacts, the ecology of the invaded community, available financial and material resources, risks, and unintended outcomes of the approaches in question (Pasko *et al.* 2014). Harvesting programs dedicated to aquatic invasive species are being implemented (Hauton *et al.* 2007; Holbrook *et al.* 2016; Závorka *et al.* 2018), and some were successful in reducing population size, especially in confined areas, like lakes (Weidel *et al.* 2007; Wittmann *et al.* 2012). For example, the invasive rusty crayfish

Orconectes rusticus (Girard, 1852) was experimentally removed via intensive trapping in a lake in Wisconsin, United States (Hein *et al.* 2006). The abundance of rusty crayfish declined 99% in 8 years (2001-2008) and did not increase significantly in the first four years postharvest (Hansen *et al.* 2013).

One case that we can analyze and use as a model for weakfish is the Asian carp, imported to the US from China around 1970s and established feral populations in the Mississippi River and Ohio River Basins (ACRCC 2016). A control plan is underway since 2010 and focuses on three areas: (1) prevention (e.g., construction of an electric barrier that reduces threat of dispersal to other areas); (2) detection, fishery management, and control (e.g., mass removal via fishing, improving gear to increase capture rates, monitoring trough eDNA, food web modelling); and (3) program management (e.g., outreach actions through an website www.asiancarp.us (AsianCarp.us 2019)) and communication work to reach audiences about the program goals (ACRCC 2016)). During three years of sampling, densities of Asian carp decreased in the Upper Illinois River through commercial harvest and remained at lower levels (Love *et al.* 2018). Certain hydrological factors are also likely to determine the population response, so understanding population dynamics and ecology in introduced areas is essential (ACMRWG 2016; MacNamara *et al.* 2016).

Although there is not much information about weakfish ecology in the introduced range, the eradication of aquatic organisms has proven to be difficult, especially when they are not confined to small and enclosed areas, as lakes (Simberloff 2014). Thus, and considering all the data available (see chapter 2), we believe that eradicating the species will be difficult considering its broad distribution in the Iberian Peninsula (Morais *et al.* 2017), however, a harvesting program dedicated to the commercialization of weakfish would at least mitigate putative ecological impacts. Therefore, a commercial harvest program could be developed to reduce the population to a level where it becomes manageable and that would need to focus in three areas:

1) **Detection**
    a. increase early detection efforts in adjacent areas to where the species is present (assessment of eggs, larvae, juvenile and adult fish, population dynamics, movements);
    b. inform and timely assess response needs.

2) **Monitoring**

   a. enhance monitoring protocols to increase our knowledge about population dynamics using conventional techniques (e.g., fishing gears) or more innovative approaches as eDNA;
   b. population dynamics (stock assessment) and food web modelling using updated data to better assess ecological and economic impacts.
   c. improve our understanding of how weakfish may threat invaded ecosystems by implementing sampling surveys in the Sado estuary and in the other locations where weakfish is present.
   d. develop a citizen science online platform where anyone can report species occurrences and a photo to confirm identification;

3) **Fishery management**

   a. decrease population density trough commercial fishing;
   b. test fishing gears to increase the efficiency of capture rates without harming native species (consider the need to use multiple gears targeting each life stage);

4) **Program management**

   a. explore new ways to process and sell weakfish (e.g. cut and freeze, shred, canning, smoking, salting).
   b. outreach activities to increase curiosity about weakfish and awareness about the purpose, function, actions, and results of the management plan;
   c. awareness campaigns to encourage the harvest of weakfish instead of native species, during gastronomical sessions supported by local Chefs, lectures, and workshops, publications on social media and online forums (e.g., angling forums), specialized magazines, (e.g., fishing magazines), or even during fishing competitions events.

The benefits of adopting a control management program based on weakfish consumption are multiple, but there are risks to consider. So, solutions must be found to avoid the risks and find the best decisions to minimize ecological impacts (Table 3.4). In terms of ecological benefits, reducing population size trough harvest would likely minimize weakfish impacts, reduce predation pressure on native species, the chances of reintroduction from surrounding areas would be low since density is higher in certain areas, and citizens would become more aware of the negative impacts of invasive species.

The risks are, respectively, not consuming the sufficient individuals to affect population density, harming native species while targeting weakfish (Pasko *et al.* 2014), unpredictable ecological consequences by altering trophic interactions (Zavaleta *et al.* 2001), and the need of a stricter control measures that prevents the introduction of new specimens (Wasson *et al.* 2001; Burgiel *et al.* 2006; Boothe 2007). Therefore, solutions for these problems must increase our understanding of weakfish life history in the new range, including modeling to assess which lifecycle stages are more likely to be affected by harvest, or whether the capture of adult fish (as it is usually sold in markets) has any effect on species demographics; testing fishing gear that only targets weakfish; evaluating species interactions and the effects of removing weakfish; increasing surveillance on non-indigenous species entry routes; and establishing monitoring plans dedicated to coastal invasive species.

In terms of economic benefits, weakfish is a species with high potential of commercialization (Table 3.5), that would contribute to improving the local economy. The risks of creating an economically valuable fishery are the discouragement of weakfish eradication (Nuñez *et al.* 2012), possibly in detriment of native species, and promoting introductions in new areas (Varble & Secchi 2013). Possible solutions go from raising people awareness about the main goal of the program – reducing weakfish population size to minimize its putative impacts – and educate the public about the negative effects of invasive species, that introducing non-indigenous species is illegal, and that the economic benefits that these invasive species may generate are smaller than the long-term costs associated with a program that controls the population of an invasive species (Lambertucci & Speziale 2011). Complementarily, it should be encouraged the conservation of native populations, fisheries restoration, and comercialization of native species that are not being valued could be useful strategies to keep the interest on fishig invasive species while aiming at not promoting introductions elsewhere.

A harvest program to reduce an invasive population requires a great commitment and persistence over time that entails fixed costs (Pasko *et al.* 2014). In such a program, scientific research provides crucial information to assess its feasibility, so funds are essential to keep research on non-indigenous species. A conceptual framework of how this research can be sustained by invasive species with potential of commercial exploitation is summarized in Figure 3.18. The acquisition of information and samples of non-indigenous species by fishers, scientists will be able to deepen existing information about the species in the non-indigenous range and elaborate a management plan that assesses risks and benefits of introducing a certain species in the market. Simultaneously, scientists will be able to share updated information to the public through media and social networks, inciting society's interest in these species, so that its introduction in the market becomes possible (McKinley *et al.* 2017). By increasing interest, sales are also enhanced, which will consequently bring more money to the fishers who harvest them, resulting in greater motivation both for fishers to contribute to with more information and specimens to scientists, and for funding agencies to contribute with more funds for the research of invasive species.

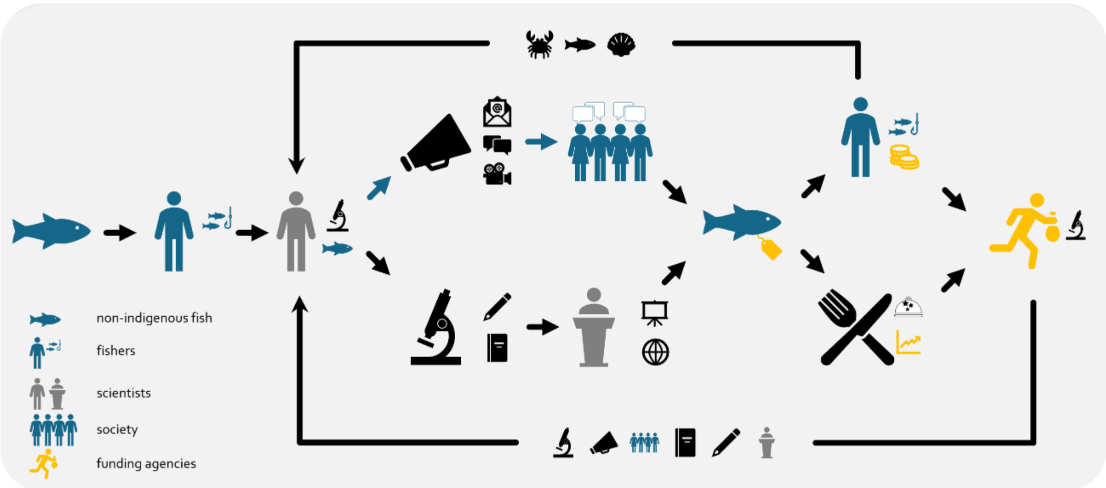


Figure 3.18. Conceptual framework on non-indigenous fish research with the potential to be commercially exploited.

Table 3.5. Benefits, risks, and solutions to consider when adopting a program based on the consumption of weakfish.

	Benefits	Risks	Solutions
Ecological	Minimizing weakfish impacts on the ecosystem through reduction of population size.	To reduce population size, it is necessary to consume more individuals than those affected by the natural mortality rate (Pasko *et al.* 2014), otherwise there will be no significant changes.	Understand the life history characteristics of the target species, like density-dependent processes, demographic structure, specific vital rates, and minimum viable population size.
	Reduce fishing pressure on native species.	Harming native species while targeting weakfish (e.g. by-catch) (Pasko *et al.* 2014).	Nets used to catch native fishes also capture weakfish (e.g., trammel net), indicating that targeting weakfish is difficult without more selective techniques. Fishing gear used to catch weakfish should be tested and controlled.
	Reduce the risk of introduction and dispersal to surrounding ecosystems.	Unpredictable ecological consequences by altering trophic interactions (e.g., biological overcompensation, open of ecological niches for other species) (Zavaleta *et al.* 2001; Zipkin *et al.* 2009). The removal method could lead to changes in population size distribution and size at maturity, altering ecosystem processes (Evangelista *et al.* 2017; Závorka *et al.* 2018).	Species interactions and effects of removing an invasive species from the ecosystem should be evaluated before the start of a program. After implementing the initial plan, it might be necessary to adapt the strategy.
	People become aware about the negative impacts of invasive species and may help detecting future invasions (Scyphers *et al.* 2015).	Without strict laws and control that prevent the introduction of new specimens the applied effort could be in vain and at greater costs (Wasson *et al.* 2001; Burgiel *et al.* 2006; Boothe 2007).	Increasing surveillance on possible entry routes for non-indigenous species, as ships (ballast water discharges and biofouling) and aquarium trade, for example, and establishing more monitoring plans dedicated to invasive species in the coastal areas through techniques such as eDNA.
Economical	Species with high potential of commercialization: - high potential of acceptance by consumers; - weakfish selling price could support catches, avoiding greater costs; - easy to harvest; - no risks to human health when handling or consuming; - it can be sold away from the capture region without the risk of introduction into those areas.	Risk of creating an economically profitable fishery, discouraging the eradication of the target species (Nuñez *et al.* 2012). Overpricing invasive species in relation to native species could lead to its protection in detriment of natives (Lambertucci & Speziale 2011).	Citizens should be informed about the main goal of the program- reduce weakfish population size to minimize its putative impacts on the ecosystem – and about the negative impacts of invasive species, especially about impairing native species. Encouraging the conservation of native populations, fisheries restoration, and exploitation of native species that are not being valued.
	Improve the local economy.	Promoting new introductions, either from weakfish in new areas or new species, in the hope of creating a new business opportunity (e.g., illegal fish stocking) (Varble & Secchi 2013).	Citizens must know that it is illegal to introduce any non-indigenous species, and that the benefits that these species generate are smaller than the long-term benefits of controlling them.

5. Conclusion

Weakfish has great potential to be well accepted by Portuguese consumers since most respondents showed interest in buying the weakfish in fish markets. We also found that weakfish mean price is underestimated, with a margin to increase of 3.30 € kg^{-1} up to 8 € kg^{-1} as based on the price consumers are willing to pay. Consumers recognize its high potential since it is a wild species because they prefer wild fish over farmed native fish species. Therefore, we recommend highlighting the origin of the fish when promoting this new resource, as well as its use in dishes that use sliced or shredded fish. Based on the feedback demonstrated by the media and chefs, we consider that the invasive species subject attracts enough interest to promote weakfish successfully as a new fishing resource and increase society's awareness on this matter.

The organization of a management plan using commercial harvest that has already begun can be effective if it includes other components, such as monitoring and outreach actions, and considers the risks mentioned above. Such a program can also be complemented with different approaches, like recreational harvest or volunteer actions, to increment efficiency. Finally, there is research that needs to be done to assess the pros and cons of this approach; however, the problems posed by invasive species forces scientists and managers to act simultaneously since securing funding and obtaining scientific results can take many years, and invasive species wait for no one.

Chapter IV: Final considerations

In Sado estuary, weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) is feeding mainly on crustaceans, fish, and cephalopods, in similar proportions to that of the native range. Its feeding strategy appears to be generalist which might have helped in the establishment and persistence in the estuary, since survival is likely independent on the abundance of a particular species. However, the Sado population seems composed by specialized individuals, i.e., with narrow niche widths, indicating a low prey overlap among them. The feeding overlap (Pianka index) between weakfish and the three native prize species – meagre *Argyrosomus regius* (Asso, 1801), European bass *Dicentrarchus labrax* (Linnaeus, 1758), and white seabream *Diplodus sargus* (Linnaeus, 1758) – was not significant. However, it was higher for meagre, indicating that the intensity of exploitative competition (indirect competition by using the same resources) is increased between these two sciaenids.

Based on stable isotope analysis, weakfish, meagre and seabass seem to be feeding at the same trophic level, while seabream is a trophic level below, feeding on different prey types. There was no significant isotopic niche overlap between weakfish and the native fish, but it was also higher between weakfish and meagre.

Weakfish migration pattern in the Sado estuary is similar to that in the native range: a repeated pattern of inshore migrations in spring, spending the summer in estuarine waters (up to the upper estuary) and offshore migrations in the winter. Therefore, the probability of competition for space and resources is higher for species that use the estuary in the period between spring and autumn. This information is critical to ascertain potential impacts that weakfish might exert on other fisheries resources in Sado estuary, namely direct impacts on its preys and indirect impacts on other species by competing for food and space. The preys with higher frequency of occurrence like the sand smelt *Atherina* sp. Linnaeus, 1758, the European anchovy *Engraulis encrasicolus* (Linnaeus, 1758), Caridea Dana, 1852, and speckled sea louse *Eurydice pulchra* Leach, 1815 are the most likely to be affected. Having a broad niche width, the potential feeding overlap with native species is higher, thus, species with overlapping feeding habits and habitat use are potentially indirectly affected by weakfish. Some examples are the gilthead seabream *Sparus aurata* Linnaeus, 1758, Senegal seabream *Diplodus bellottii* (Steindachner, 1882), European anchovy *Engraulis encrasicolus* (Linnaeus, 1758), horse mackerel *Trachurus trachurus* (Linnaeus, 1758), European common cuttlefish *Sepia* *officinalis* Linnaeus, 1758, common squid *Loligo vulgaris* Lamarck, 1798, common octopus *Octopus vulgaris* Cuvier, 1797, and even the Atlantic bottlenose dolphin *Tursiops truncatus* (Montagu, 1821).

Weakfish has a vast potential to be used as a new fishing resource. It is common to see weakfish being sold for 5 € kg^{-1} but selling price can be increased since consumers would be willing to pay at least 8 € kg^{-1}. Nonetheless, weakfish is less appreciated than other fish species traditionally bought by Portuguese consumers. Therefore, we advocate for an integrated marketing strategy (e.g., awareness events, cooking recipes, social media outreach) when promoting the consumption of this species to minimize the impacts of weakfish invasion. Finally, we recommend that future studies would benefit from comprehensive population dynamic surveys and genetic studies to assess the extension of the invasion and identify its origin, as well as to continue with the citizen science approach started in this study.

References

A Voz do Algarve (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas como o caranguejo azul. Available at: http://www.avozdoalgarve.pt/detalhe.php?id=28808

Abecasis, D., Bentes, L. & Erzini, K. (2009). Home range, residency and movements of *Diplodus sargus* and *Diplodus vulgaris* in a coastal lagoon: connectivity between nursery and adult habitats. *Estuarine, Coastal and Shelf Science,* 85, pp. 525-529.

Able, K.W. & Fahay, M.P. (2010). *Ecology of estuarine fishes.* Johns Hopkins University Press.

Abreu, C. (2017). Mundo da Pesca, Lisboa, Portugal. Personal Communication.

ACMRWG (2016). 2015 Asian Carp Monitoring and Response Plan. Asian Carp Monitoring and Response Working Group Illinois, Chicago.

ACRCC (2016). Asian Carp Action Plan for Fiscal Year 2017. (ed. Committee, A.C.R.C.).

Almeida, C., Karadzic, V. & Vaz, S. (2015). The seafood market in Portugal: Driving forces and consequences. *Marine Policy*, 61, pp. 87-94.

Alvarez, R.Z. (1968). Decápodos ibéricos. *Inv. pesq*, 32, p. 510.

Amarasekare, P. (2002). Interference competition and species coexistence. *Proceedings of the Royal Society of London B: Biological Sciences*, 269, pp. 2541-2550.

Ambiente Ondas 3 (2017). O caranguejo azul promete oportunidades de exploração. Available at: https://onda7.blogspot.pt/2017/09/o-carangueijo-azul-promete.html

Amundsen, P.A., Gabler, H.M. & Staldvik, F. (1996). A new approach to graphical analysis of feeding strategy from stomach contents data—modification of the Costello (1990) method. *Journal of fish biology*, 48, pp. 607-614.

ANB (2011). DNA ontmaskert de verdachte. In: *Vislijn - Infoblad voor de openbare visserij in Vlaanderen*. Agentschap Voor Natuuren Bos, p. 28.

Andvik, R., VanDeHey, J., Fincel, M., French, W.E., Bertrand, K., Chipps, S.R. *et al.* (2010). Application of non-lethal stable isotope analysis to assess feeding patterns of juvenile pallid sturgeon *Scaphirhynchus albus*: a comparison of tissue types and sample preservation methods. *Journal of Applied Ichthyology*, 26, pp. 831-835.

Antena1 (2018). Rio Guadiana. Available at: http://www.pt.cision.com/cp2013/ClippingDetails.aspx?id=3c29ffd0-ed9d-42b3-8a09-8e5524c21ad2&userId=3db2776e-5856-466f-bde6-b4b8b9e853f9

APPA (2018). American Association of Port Authorities. Available at: http://www.aapa-ports.org Last accessed: September 2018.

APSS (2018). Porto de Setúbal - Terminais Portuários. Available at: http://www.portodesetubal.pt/terminais_portuarios.htm. Last accessed: October 2018.

ARoda (2017). Caranguejos-azuis Nativos Norte-americanos Aparecem No Guadiana E Sado. Available at: http://mfm-a-roda.blogspot.pt/2017/09/

AsianCarp.us (2019). The Asian Carp Problem. Available at: www.asiancarp.us/

ASMFC (2016). 2016 Review of the Atlantic States Marine Fisheries Comission: Fishery Management Plan for Weakfish (*Cynoscion regalis*). Available at: http://www.asmfc.org/uploads/file/599dc7242016WeakfishFMPreview.pdf

ASMFC (2017). Atlantic States Marine Fisheries Commission - weakfish. Available at: http://www.asmfc.org/species/weakfish Last accessed: 18 January 2017.

Azzurro, E., Maynou, F., Belmaker, J., Golani, D. & Crooks, J. (2016). Lag times in Lessepsian fish invasion. *Biological Invasions*, 18, pp. 2761-2772.

Baack, E.J. & Rieseberg, L.H. (2007). A genomic view of introgression and hybrid speciation. *Current opinion in genetics & development*, 17, pp. 513-518.

Bailey, S.A. (2015). An overview of thirty years of research on ballast water as a vector for aquatic invasive species to freshwater and marine environments. *Aquatic ecosystem health & management*, 18, pp. 261-268.

Bañón, R., Arias, A., Arana, D. & 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, pp. 19-26.

Bartley, D.M. (2011). Aquaculture. In: *Encyclopedia of Biological Invasions* (eds. Simberloff, D. & Rejmánek, M.). University of California Press, Berkeley and Los Angeles, USA, pp. 27–32.

Bax, N., Williamson, A., Aguero, M., Gonzalez, E. & Geeves, W. (2003). Marine invasive alien species: a threat to global biodiversity. *Marine policy*, 27, pp. 313-323.

Béarez, P., GaBriel, S. & Dettai, A. (2016). Unambiguous identification of the non-indigenous species *Cynoscion regalis* (Sciaenidae) from Portugal. *Cybium*, 40, pp. 245-248.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & MacLeod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of animal ecology*, 73, pp. 1007-1012.

Bengtsson, J. (2002). Disturbance and resilience in soil animal communities. *European Journal of Soil Biology*, 38, pp. 119-125.

Bernardo, J. (1996). The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *American zoologist*, 36, pp. 216-236.

Bigelow, H.B. & Schroeder, W.C. (1953). *Fishes of the Gulf of Maine*. US Government Printing Office, Washington, DC.

Bij de Vaate, A., Jazdzewski, K., Ketelaars, H.A., Gollasch, S. & Van der Velde, G. (2002). Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, pp. 1159-1174.

Blackburn, T.M., Essl, F., Evans, T., Hulme, P.E., Jeschke, J.M., Kühn, I. *et al.* (2014). A unified classification of alien species based on the magnitude of their environmental impacts. *PLoS Biol*, 12, pp. 1-11.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., de Bruyn M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in ecology & evolution*, 29, pp. 358-367.

Boltovskoy, D., Sylvester, F. & Paolucci, E.M. (2018). Invasive species denialism: Sorting out facts, beliefs, and definitions. *Ecology and evolution*, 8, pp. 11190-11198.

Boothe, J.A. (2007). Defending the Homeland: A Call to Action in the War Against Aquatic Invasive Species. *Tul. Envtl. LJ*, 21, p. 407.

Bowman, R.E., Stillwell, C.E., Michaels, W.L. & Grosslein, M.D. (2000). Food of northwest Atlantic fishes and two common species of squid. *NOAA Tech. Memo. NMFS-NE*, 155, p. 138.

Britton, J.R., Ruiz-Navarro, A., Verreycken, H. & Amat-Trigo, F. (2018). Trophic consequences of introduced species: Comparative impacts of increased interspecific versus intraspecific competitive interactions. *Functional ecology*, 32, pp. 486-495.

Burgiel, S., Foote, G., Orellana, M. & Perrault, A. (2006). Invasive alien species and trade: integrating prevention measures and international trade rules. *The Center for International Environmental Law and Defenders of Wildlife, Washington, DC*, pp. 66-74.

Byers, J.E., Gribben, P.E., Yeager, C. & Sotka, E.E. (2012). Impacts of an abundant introduced ecosystem engineer within mudflats of the southeastern US coast. *Biological Invasions*, 14, pp. 2587-2600.

Cabral, H. & Costa, M.J. (2001). Abundance, feeding ecology and growth of 0-group sea bass, *Dicentrarchus labrax*, within the nursery areas of the Tagus estuary. *Journal of the Marine Biological Association of the United Kingdom*, 81, pp. 679-682.

Cano-Rocabayera, O., Maceda-Veiga, A. & de Sostoa, A. (2015). Fish fins and scales as non-lethally sampled tissues for stable isotope analysis in five fish species of north–eastern Spain. *Environmental biology of fishes*, 98, pp. 925-932.

Cardoso, C., Lourenço, H., Costa, S., Gonçalves, S. & Nunes, M.L. (2013). Survey into the seafood consumption preferences and patterns in the Portuguese population. Gender and regional variability. *Appetite*, 64, pp. 20-31.

Carlton, J.T. & Ruiz, G.M. (2005). Vector science and integrated vector management in bioinvasion ecology: conceptual frameworks. In: *Invasive Alien Species: A New Synthesis* (eds. Mooney, H.A., Mack, R., McNeely, J.A., Neville, L.E., Schei, P.J. & Waage, J.K.). IslandPress, p. 36.

Carlton, J.T. (1996). Pattern, process, and prediction in marine invasion ecology. *Biological conservation*, 78, pp. 97-106.

Carlton, J.T., Chapman, J.W., Geller, J.B., Miller, J.A., Carlton, D.A., McCuller, M.I. *et al.* (2017). Tsunami-driven rafting: Transoceanic species dispersal and implications for marine biogeography. *Science*, 357, pp. 1402-1406.

Caut, S., Angulo, E. & Courchamp, F. (2009). Avoiding surprise effects on Surprise Island: alien species control in a multitrophic level perspective. *Biological Invasions*, 11, pp. 1689-1703.

CBD (2017). Convention on Biological Diversity: What are Invasive Alien Species? Available at: https://www.cbd.int/invasive/WhatareIAS.shtml Last accessed: January 2017.

CCMAR (2017a). Corvina americana é dada a conhecer em prova de degustação. Available at: https://www.ccmar.ualg.pt/news/corvina-americana-e-dada-conhecer-em-prova-de-degustacao.

CCMAR (2017b). Caranguejo-azul descoberto no Rio Guadiana Available at: https://www.ccmar.ualg.pt/news/caranguejo-azul-descoberto-no-rio-guadiana.

Chainho, P., Fernandes, A., Amorim, A., Ávila, S.P., Canning-Clode, J., Castro, J.J. *et al.* (2015). Non-indigenous species in Portuguese coastal areas, coastal lagoons, estuaries and islands. *Estuarine, Coastal and Shelf Science*, 167, pp. 199-211.

Chao, L.N. (1977). Life history, feeding habits, and functional morphology of juvenile sciaenid fishes, in the York River estuary, Virginia. *Fish. Bull. US*, 7, pp. 487-496.

Cidadania (2017). Há caranguejos-azuis norte-americanos no Guadiana. Available at: http://armacaodepera.blogspot.pt/2017/09/

Cohen, J., Mirotchnick, N. & Leung, B. (2007). Thousands introduced annually: the aquarium pathway for non-indigenous plants to the St Lawrence Seaway. *Frontiers in Ecology and the Environment*, 5, pp. 528-532.

Colautti, R.I. & MacIsaac, H.J. (2004). A neutral terminology to define 'invasive'species. *Diversity and distributions*, 10, pp. 135-141.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, pp. 721-733.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, pp. 721-733.

Colwell, R.K. & Futuyma, D.J. (1971). On the measurement of niche breadth and overlap. *Ecology*, 52, pp. 567-576.

Correio da Manhã (2017). Caranguejo azul da América do Norte descoberto no Guadiana. Available at: http://www.cmjornal.pt/sociedade/detalhe/caranguejo-azul-nativo-da-america-do-norte-descoberto-no-estuario-do-guadiana

Costello, M. (1990). Predator feeding strategy and prey importance: a new graphical analysis. *Journal of Fish Biology*, 36, pp. 261-263.

Cote, J., Clobert, J., Brodin, T., Fogarty, S. & Sih, A. (2010). Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. *Philos Trans R Soc Lond B Biol Sci*, 365, pp. 4065-4076.

CreativeCookGarage (2018). Creative Cook Garage. Available at: https://www.instagram.com/creativecookgarage/. Last accessed: April 2018.

Crooks, J.A. (1998). Habitat alteration and community-level effects of an exotic mussel, *Musculista senhousia*. *Marine ecology Progress series*, 162, pp. 137-152.

Crooks, J.A. (2002a). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, 97, pp. 153-166.

Crooks, J.A. (2002b). Predators of the invasive mussel *Musculista senhousia* (Mollusca: Mytilidae). *Pacific Science*, 56, pp. 49-56.

Crooks, J.A. (2005). Lag times and exotic species: The ecology and management of biological invasions in slow-motion1. *Ecoscience*, 12, pp. 316-329.

Curtis, J.S., Wall, K.R., Albins, M.A. & Stallings, C.D. (2017). Diet shifts in a native mesopredator across a range of invasive lionfish biomass. *Marine Ecology Progress Series*, 573, pp. 215-228.

Destak (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: http://www.destak.pt/artigo/323258

Diário Campanário (2018). Espécies exóticas nas águas do Guadiana devido a aumento de temperatura e salinidade. Available at: http://www.radiocampanario.com/ultimas/regional/especies-exoticas-nas-aguas-do-guadiana-devido-a-aumento-de-temperatura-e-salinidade

Diário de Notícias (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: https://www.dn.pt/lusa/interior/chef-algarvio-testa-pratos-com-medusas-e-outras-especies-em-parceria-com-universidade-9015790.html

Diário de notícias (2017). Há caranguejos-azuis norte-americanos no Guadiana. Available at: https://www.dn.pt/sociedade/interior/caranguejo-azul-nativo-da-america-do-norte-descoberto-no-estuario-do-guadiana-8805575.html

Diário de Notícias (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas como o caranguejo azul. Available at: https://www.dn.pt/lusa/interior/agua-mais-quente-e-salina-no-guadiana-e-berco-para-especies-exoticas-como-o-caranguejo-azul-9017949.html

Diário Online Região Sul (2017). Caranguejo-azul descoberto no estuário do Rio Guadiana. Available at: https://regiao-sul.pt/2017/09/28/ambiente/caranguejo-azul-descoberto-no-estuario-do-rio-guadiana/395711

DNoticias (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: http://www.dnoticias.pt/pais/caranguejo-azul-nativo-da-america-do-norte-descoberto-no-estuario-do-guadiana-FM2097106

Docapesca (2018). Estatísticas Diárias. Available at: http://www.docapesca.pt/pt/estatisticas/diarias.html. Last accessed: October 2018.

Duggan, I.C. (2010). The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biological Invasions*, 12, pp. 3757-3770.

Duncan, N., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C., Roo, J., Schuchardt, D., Vallés, R. (2013). Aquaculture production of meagre (*Argyrosomus regius*): hatchery techniques, ongrowing and market. In: *Advances in aquaculture hatchery technology* (ed. Burnell, G.A.a.G.). Woodhead Publishing, pp. 519-541.

Elvira, B. & Almodóvar, A. (2001). Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st century. *Journal of fish Biology*, 59, pp. 323-331.

EUMOFA (2016). EU Fish Market Report 2016. In: *Maritime Affairs and Fisheries*. European Market Observatory for Fisheries and Aquaculture Products.

Evangelista, C., Lecerf, A., Britton, J.R. & Cucherousset, J. (2017). Resource composition mediates the effects of intraspecific variability in nutrient recycling on ecosystem processes. *Oikos*, 126, pp. 1439-1450.

Expresso (2017). Chef do restaurante São Gabriel experimenta pratos com alforrecas. Available at: http://expresso.sapo.pt/cultura/ocio/2017-12-30-Chef-do-restaurante-Sao-Gabriel-experimenta-pratos-com-alforrecas

FAO (2018). Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/home/en/ Last accessed: 17 January 2017.

Ferguson, T. & Akins, L. (2010). *The Lionfish Cookbook: The Caribbean's New Delicacy*. REEF Environmental Education Foundation.

Fernandes, C.P. (2017). The consumption of seafood in Portugal: preferences, knowledge and public perception. Faculdade de Ciências, Universidade de Lisboa.

Fissues (2019). News & Opinion By & For Conservation-Minded Anglers. Available at: http://fissues.org/weakfish/ Last accessed: March 2019.

Folha do Domingo (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas como o caranguejo azul. Available at:https://folhadodomingo.pt/agua-mais-quente-e-salina-no-guadiana-e-berco-para-especies-exoticas-como-o-caranguejo-azul/

França, S., Costa, M.J. & Cabral, H.N. (2011). Inter-and intra-estuarine fish assemblage variability patterns along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, 91, pp. 262-271.

Frey, M.A., Simard, N., Robichaud, D.D., Martin, J.L. & Therriault, T.W. (2014). Fouling around: vessel sea-chests as a vector for the introduction and spread of aquatic invasive species. *Management of Biological Invasions*, 5, pp. 21-30.

Froese & Pauly (2018). FishBase. Available at: http://www.fishbase.org Last accessed: September 2018.

Fry, B. (2006). *Stable isotope ecology*. Vol. 521, Springer, New York.

Fultonfishmarket.com (2019). Weakfish - Sea Trout, Fresh, Wild, USA, Whole (3lb avg). Available at: https://shop.fultonfishmarket.com/fresh-weakfish.html Last accessed: June 2019.

FWC (2014). Weakfish, *Cynoscion regalis* (Bloch & Schneider, 1801). Fish and Wildlife Research Institute, pp. 177-180.

Gaertner, M., Fisher, J., Sharma, G. & Esler, K. (2012). Insights into invasion and restoration ecology: time to collaborate towards a holistic approach to tackle biological invasions. *NeoBiota*, 12, p. 57.

Gallardo, B., Clavero, M., Sánchez, M.I. & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global change biology*, 22, pp. 151-163.

Gallardo, B., Clavero, M., Sánchez, M.I. & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global change biology*, 22, pp. 151-163.

Gendron, A.D., Marcogliese, D.J. & Thomas, M. (2011). Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes-St. Lawrence Basin. *Biological Invasions*, 14, pp. 367-384.

Gertzen, E., Familiar, O. & Leung, B. (2008). Quantifying invasion pathways: fish introductions from the aquarium trade. *Canadian Journal of Fisheries and Aquatic Sciences*, 65, pp. 1265-1273.

Gibbs, M., Saastamoinen, M., Coulon, A. & Stevens, V.M. (2010). Organisms on the move: ecology and evolution of dispersal. *Biology Letters*, 6, pp. 146-148.

Gil, M.D.M., Grau, A., Basilone, G., Ferreri, R. & Palmer, M. (2013). Reproductive strategy and fecundity of meagre *Argyrosomus regius* Asso, 1801 (Pisces: Sciaenidae): implications for restocking programs. *Scientia Marina*, 77, pp. 105-118.

Giller, P. (2012). *Community structure and the niche*. Springer Science & Business Media.

Gomes, P., Vieira, A., Oliveira, R., Silva, H., Martins, R. & Carneiro, M. (2017). First record of *Cynoscion regalis* (Pisces, Sciaenidae) in Portuguese continental waters. *Journal of fish biology*, 90, pp. 2470-2474.

Green, B.S. & Fisher, R. (2004). Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. *Journal of Experimental Marine Biology and Ecology*, 299, pp. 115-132.

Grosholz, E. (2002). Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology & Evolution*, 17, pp. 22-27.

Guzzo, M.M., Haffner, G.D., Legler, N.D., Rush, S.A. & Fisk, A.T. (2013). Fifty years later: trophic ecology and niche overlap of a native and non-indigenous fish species in the western basin of Lake Erie. *Biological invasions*, 15, pp. 1695-1711.

Hansen, G.J., Hein, C.L., Roth, B.M., Vander Zanden, M.J., Gaeta, J.W., Latzka, A.W. *et al.* (2013). Food web consequences of long-term invasive crayfish control. *Canadian Journal of Fisheries and Aquatic Sciences*, 70, pp. 1109-1122.

Hardin, G. (1960). The competitive exclusion principle. *science*, 131, pp. 1292-1297.

Hartman, K.J. & Brandt, S.B. (1995). Trophic resource partitioning, diets, and growth of sympatric estuarine predators. *Transactions of the American Fisheries Society*, 124, pp. 520-537.

Harzen, S. (1998). Habitat use by the bottlenosed dolphin (*Tursiops truncatus*) in the Sado Estuary, Portugal. *Aquatic Mammals*, 24, pp. 117-128.

Hauton, C., Howell, T., Atkinson, R. & Moore, P. (2007). Measures of hydraulic dredge efficiency and razor clam production, two aspects governing sustainability within the Scottish commercial fishery. *Journal of the Marine Biological Association of the United Kingdom*, 87, pp. 869-877.

Hayden, B., Tongnunui, S., Beamish, F., Nithirojpakdee, P. & Cunjak, R. (2017). Variation in stable-isotope ratios between fin and muscle tissues can alter assessment of resource use in tropical river fishes. *Journal of fish biology*, 91, pp. 574-586.

Hein, L., Van Koppen, K., De Groot, R.S. & Van Ierland, E.C. (2006). Spatial scales, stakeholders and the valuation of ecosystem services. *Ecological economics*, 57, pp. 209-228.

Helder Barros (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: https://informaticahb.blogspot.pt/2017/09/zoologia-investigadores-do-centro-de.html

Holbrook, C.M., Bergstedt, R.A., Barber, J., Bravener, G.A., Jones, M.L. & Krueger, C.C. (2016). Evaluating harvest-based control of invasive fish with telemetry: performance of sea lamprey traps in the Great Lakes. *Ecological applications*, 26, pp. 1595-1609.

Hyslop, L. (2015). 'Like rabbit, but sweeter': the verdict on eating squirrel. In: *The Telegraph*.

IMO (2017). List of Certeficates and documents required to be carried on board ships, 2017. (ed. Organization, I.M.). Available at: http://www.imo.org/en/OurWork/Facilitation/docs/FAL%20related%20nonmandatory%20instruments/FAL.2-Circ.131-MEPC.1-Circ.873-MSC.1-Circ.1586-LEG.2-Circ.3.pdf

Impala News (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: http://www.impala.pt/noticias/atualidade/chef-algarvio-testa-pratos-com-medusas-e-outras-especies-em-parceria-com-universidade/

Impala News (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas como o caranguejo azul. Available at: http://www.impala.pt/noticias/atualidade/agua-mais-quente-e-salina-no-guadiana-e-berco-para-especies-exoticas-como-o-caranguejo-azul/

Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80, pp. 595-602.

Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J. (2012). Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PloS one*, 7, p. e31757.

Jacob, U., Mintenbeck, K., Brey, T., Knust, R. & Beyer, K. (2005). Stable isotope food web studies: a case for standardized sample treatment. *Marine Ecology Progress Series*, 287, pp. 251-253.

Jesus, D. (2017). Associação de Moradores da Ilha da Culatra, Culatra, Portugal. Personal communication.

JM Madeira (2017). Chef algarvio testa pratos com medusas e outras espécies. Available at: https://www.jm-madeira.pt/nacional/ver/23523/Chef_algarvio_testa_pratos_com_medusas_e_outras_especies

Johnston, E.L., Piola, R.F. & Clark, G.F. (2009). The Role of Propagule Pressure in Invasion Success. 204, pp. 133-151.

Jornal de Notícias (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: https://www.jn.pt/local/noticias/faro/vila-real-de-santo-antonio/interior/chef-algarvio-testa-pratos-com-medusas-e-outras-especies-em-parceria-com-universidade-9015967.html

Jornal do Algarve (2017). Cada vez há mais espécies "invasoras" no rio Guadiana. Available at: http://www.jornaldoalgarve.pt/cada-vez-ha-mais-especies-invasoras-no-rio-guadiana/

Juncos, R., Milano, D., Macchi, P.J. & Vigliano, P.H. (2015). Niche segregation facilitates coexistence between native and introduced fishes in a deep Patagonian lake. *Hydrobiologia*, 747, pp. 53-67.

Keller, R.P., Drake, J.M., Drew, M.B. & Lodge, D.M. (2011). Linking environmental conditions and ship movements to estimate invasive species transport across the global shipping network. *Diversity and Distributions*, 17, pp. 93-102.

Kelly, J., Tosh, D., Dale, K. & Jackson, A. (2013). The economic cost of invasive and non-native species in Ireland and Northern Ireland. *Ireland Environment Agency and National Parks and Wildlife Service*, 86.

Kirk, R. (2003). The impact of Anguillicola crassus on European eels. *Fisheries Management and Ecology*, 10, pp. 385-394.

Knop, E. & Reusser, N. (2012). Jack-of-all-trades: phenotypic plasticity facilitates the invasion of an alien slug species. *Proc Biol Sci*, 279, pp. 4668-4676.

Krakau, M., Thieltges, D. & Reise, K. (2006). Native parasites adopt introduced bivalves of the North Sea. *Biological Invasions*, 8, pp. 919-925.

Krebs, C. (1998). Niche measures and resource preferences. *Ecological methodology*, pp. 455-495.

Lagardère, J. & Mariani, A. (2006). Spawning sounds in meagre *Argyrosomus regius* recorded in the Gironde estuary, France. *Journal of fish biology*, 69, pp. 1697-1708.

Lai, B. (2015). How (and why) to eat invasive species. In: *Scientific American*

Lambertucci, S.A. & Speziale, K.L. (2011). Protecting invaders for profit. *Science*, 332, pp. 35-35.

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

Lee, C.E. (2002). Evolutionary genetics of invasive species. *Trends in ecology & evolution*, 17, pp. 386-391.

Lee, C.E. (2002). Evolutionary genetics of invasive species. *Trends in ecology & evolution*, 17, pp. 386-391.

Leppäkoski, E., Gollasch, S. & Olenin, S. (2002). Alien Species in European Waters. In: *Invasive Aquatic Species of Europe. Distribution, Impacts and Management* (eds. Leppäkoski, E., Gollasch, S. & Olenin, S.). Springer. Netherlands Dordrecht, pp. 1-6.

Love, S.A., Lederman, N.J., Anderson, R.L., DeBoer, J.A. & Casper, A.F. (2018). Does aquatic invasive species removal benefit native fish? The response of gizzard shad (*Dorosoma cepedianum*) to commercial harvest of bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*). *Hydrobiologia*, 817, pp. 403-412.

Lowerre-Barbieri, S. (1994). Life history and fisheries ecology of weakfish, *Cynoscion regalis*, in the Chesapeake Bay region. PhD Thesis, College of William and Mary, Williamsburg, Virginia, USA.

Lowerre-Barbieri, S.K., Chittenden Jr, M.E. & Barbieri, L.R. (1996). The multiple spawning pattern of weakfish in the Chesapeake Bay and Middle Atlantic Bight. *Journal of Fish Biology*, 48, pp. 1139-1163.

Lusa (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: https://www.lusa.pt/article/23463790/lusa%C3%A1udio-chef-algarvio-testa-pratos-com-medusas-e-outras-esp%C3%A9cies-em-parceria-com-universidade

Lusa (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas como o caranguejo azul. Available at: https://www.lusa.pt/article/23467236/%C3%A1gua-mais-quente-e-salina-no-guadiana-%C3%A9-ber%C3%A7o-para-esp%C3%A9cies-ex%C3%B3ticas-como-o-caranguejo-azul

MacArthur, R. & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *The american naturalist*, 101, pp. 377-385.

Macedo, M.C.C., Macedo, M.I.C.M., Borges, J.P., Davies, G. & Sacchetti, A. (1999). *Conchas marinhas de Portugal*. Verbo, p. 516.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000). Biotic invasions - causes, epidemiology, global consequences, and control. *Ecological Applications*, 10, pp. 689-710.

MacNamara, R., Glover, D., Garvey, J., Bouska, W. & Irons, K. (2016). Bigheaded carps (*Hypophthalmichthys* spp.) at the edge of their invaded range: using hydroacoustics to assess population parameters and the efficacy of harvest as a control strategy in a large North American river. *Biological invasions*, 18, pp. 3293-3307.

Martins, F., Leitão, P., Silva, A. & Neves, R. (2001). 3D modelling in the Sado estuary using a new generic vertical discretization approach. *Oceanologica Acta*, 24, pp. 51-62.

McCloud, C. (2011). Target Hunger Now! Program features Asian Carp. Available at: https://www.dnr.illinois.gov/news/Pages/TargetHungerNow!ProgramFeaturesAsianCarp.aspx Last accessed: March 2018.

McKinley, D.C., Miller-Rushing, A.J., Ballard, H.L., Bonney, R., Brown, H., Cook-Patton, S.C. *et al.* (2017). Citizen science can improve conservation science, natural resource management, and environmental protection. *Biological Conservation*, 208, pp. 15-28.

Mercer, L.P. (1989). Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (mid-Atlantic): weakfish. North Carolina Division of Marine Fisheries, Morehead City, NC.

Merriner, J.V. (1975). Food habits of the weakfish, *Cynoscion regalis*, in North Carolina waters. *Chesapeake Science*, 16, pp. 74-76.

Minagawa, M. & Wada, E. (1984). Stepwise enrichment of 15 N along food chains: further evidence and the relation between δ 15 N and animal age. *Geochimica et cosmochimica acta*, 48, pp. 1135-1140.

Minchin, D. & Gollasch, S. (2002). Vectors - How Exotics get around. In: *Invasive aquatic species of Europe. Distributions, Impact and Management* (eds. Leppakoski, E., Gollasch, S. & Olenin, S.). Kluwer Academic Publishers, pp. 183-192.

Modesto, M.L. (1983). *Cozinha Tradicional Portuguesa.* Editorial Verbo, Lisbon.

Montgomery, W.I., Lundy, M.G. & Reid, N. (2011). 'Invasional meltdown': evidence for unexpected consequences and cumulative impacts of multispecies invasions. *Biological Invasions*, 14, pp. 1111-1125.

Mooney, H.A. & Cleland, E.E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, 98, pp. 5446-5451.

Morais, P. & Teodósio, M.A. (2016). The transatlantic introduction of weakfish *Cynoscion regalis* (Bloch & Schneider, 1801)(Sciaenidae, Pisces) into Europe. *BioInvasions Record*, 5.

Morais, P., Cerveira, I. & Teodósio, M.A. (2017). An Update on the Invasion of Weakfish *Cynoscion regalis* (Bloch & Schneider, 1801)(Actinopterygii: Sciaenidae) into Europe. *Diversity*, 9, p. 47.

Morais, P., Gaspar, M., Garel, E., Baptista, V., Cruz, J., Cerveira, I. *et al.* (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, pp. 123-133.

Morales-Nin, B. (1992). Determination of growth in bony fishes from otolith microstructure. In: *FAO Fisheries technical paper 322*. Food & Agriculture Organization Rome, pp. 1-51.

MundoDaPesca (2014). À descoberta do Rio Sado: Pesca fundeada de kayak. In: *Mundo da Pesca*, pp. 16–19.

Myrland, Ø., Trondsen, T., Johnston, R.S. & Lund, E. (2000). Determinants of seafood consumption in Norway: lifestyle, revealed preferences, and barriers to consumption. *Food quality and Preference*, 11, pp. 169-188.

Naylor, R.L., Williams, S.L. & Strong, D.R. (2001). Aquaculture - A gateway for exotic species. American Association for the Advancement of Science.

Nemerson, D.M. & Able, K.W. (2004). Spatial patterns in diet and distribution of juveniles of four fish species in Delaware Bay marsh creeks: factors influencing fish abundance. *Marine Ecology Progress Series*, 276, pp. 249-262.

Net Madeira (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: http://www.netmadeira.com/noticias/pais/artigo/231818-caranguejo-azul-nativo-da-amrica-do-norte-descoberto-no-esturio-do-guadiana

Neves, A., Cabral, H., Figueiredo, I., Sequeira, V., Moura, T. & Gordo, L.S. (2008). Fish assemblage dynamics in the Tagus and Sado estuaries (Portugal). *Cahiers de biologie marine*, 49, pp. 23-35.

Niu, H., Zhao, L. & Sun, J. (2012). Phenotypic plasticity of reproductive traits in response to food availability in invasive and native species of nematode. *Biological Invasions*, 15, pp. 1407-1415.

NOAA (2011). Filleting the Lion. Available at: https://oceanservice.noaa.gov/news/weeklynews/june10/eatlionfish.html Last accessed: March 2018.

Novak, S.J. (2007). The role of evolution in the invasion process. *Proceedings of the National Academy of Sciences*, 104, pp. 3671-3672.

Nuñez, M.A., Kuebbing, S., Dimarco, R.D. & Simberloff, D. (2012). Invasive species: to eat or not to eat, that is the question. *Conservation Letters*, 5, pp. 334-341.

O Instalador (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas. Available at: https://oinstalador.com/noticia/id/557/Agua-mais-quente-e-salina-no-Guadiana-e-berco-para-especies-exoticas

O jogo (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: https://www.ojogo.pt/extra/lusa/interior/caranguejo-azul-nativo-da-america-do-norte-descoberto-no-estuario-do-guadiana-8805578.html?utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+OJ-Ultimas+%28OJ+-+Ultimas%29

O jogo (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: https://www.ojogo.pt/extra/lusa/interior/chef-algarvio-testa-pratos-com-medusas-e-outras-especies-em-parceria-com-universidade-9015793.html?utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+OJ-Ultimas+%28OJ+-+Ultimas%29

O Jogo (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas como o caranguejo azul. Available at: https://www.ojogo.pt/extra/lusa/interior/agua-mais-quente-e-salina-no-guadiana-e-berco-para-especies-exoticas-como-o-caranguejo-azul-9017952.html?utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+OJ-Ultimas+%28OJ+-+Ultimas%29

Observador (2017). Este chef algarvio quer fazer pratos com alforrecas. Available at: http://observador.pt/2017/12/30/este-chef-algarvio-quer-fazer-pratos-com-alforrecas/

Observador (2018). Água mais quente e salina no Guadiana é berço para espécies exóticas como o caranguejo azul. Available at: http://observador.pt/2018/01/01/agua-mais-quente-e-salina-no-guadiana-e-berco-para-especies-exoticas-como-o-caranguejo-azul/

Økland, B., Erbilgin, N., Skarpaas, O., Christiansen, E. & Långström, B. (2011). Inter-species interactions and ecosystem effects of non-indigenous invasive and native tree-killing bark beetles. *Biological Invasions*, 13, pp. 1151-1164.

Orth, D. & Schmitt, J. (2018). Hyperbole, Simile, Metaphor, Invasivore: Messaging around Blue Catfish. *Department of Fish and Wildlife Conservation.* Virginia Polytechnic Institute and State, University Blacksburg.

Padilla, D.K. & Williams, S.L. (2004). Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Frontiers in Ecology and the Environment*, 2, pp. 131-138.

Padilla, D.K. & Williams, S.L. (2004). Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Frontiers in Ecology and the Environment*, 2, pp. 131-138.

País ao Minuto (2017). Caranguejo azul da América do Norte descoberto no estuário do Guadiana. Available at: https://www.noticiasaominuto.com/pais/872754/caranguejo-azul-da-america-do-norte-descoberto-no-estuario-do-guadiana

Panfili, J., De Pontual, H., Troadec, H. & Wrigh, P.J. (2002). *Manual of fish sclerochronology*. Ifremer-lRD coedition edn., Brest, France.

Parker, I.M., Simberloff, D., Lonsdale, W., Goodell, K., Wonham, M., Kareiva, P. *et al.* (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological invasions*, 1, pp. 3-19.

Pasko, S., Goldberg, J., MacNeil, C. & Campbell, M. (2014). Review of harvest incentives to control invasive species. *Management of Biological Invasions*, 5, pp. 263-277.

Pasquaud, S., Elie, P., Jeantet, C., Billy, I., Martinez, P. & Girardin, M. (2008). A preliminary investigation of the fish food web in the Gironde estuary, France, using dietary and stable isotope analyses. *Estuarine, Coastal and Shelf Science*, 78, pp. 267-279.

Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-Perret-Gentil, L., Beja, P., Boggero, A. *et al.* (2018). The future of biotic indices in the ecogenomic era: Integrating (e) DNA metabarcoding in biological assessment of aquatic ecosystems. *Science of the Total Environment*, 637, pp. 1295-1310.

Pedersen, M.W., Overballe-Petersen, S., Ermini, L., Der Sarkissian, C., Haile, J., Hellstrom, M. *et al.* (2015). Ancient and modern environmental DNA. *Phil. Trans. R. Soc. B*, 370, p. 20130383.

Perlmutter, A., Miller, W.S. & Poole, J.C. (1956). The weakfish (*Cynoscion regalis*) in New York waters. *NY Fish Game J*, 3, pp. 1-43.

Peterson, B.J. & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*, 18, pp. 293-320.

Phillips, D.L. & Eldridge, P.M. (2006). Estimating the timing of diet shifts using stable isotopes. *Oecologia*, 147, pp. 195-203.

Phillis, C.C., Sturrock, A.M., Johnson, R.C. & Weber, P.K. (2018). Endangered winter-run Chinook salmon rely on diverse rearing habitats in a highly altered landscape. *Biological Conservation*, 217, pp. 358-362.

Pianka, E.R. (1973). The structure of lizard communities. *Annual review of ecology and systematics*, 4, pp. 53-74.

Portal do Mar (2017). Caranguejo-azul descoberto no estuário do Rio Guadiana. Available at: http://portaldomar.blogspot.pt/2017/10/o-caranguejo-azul-uma-especie.html

Porto Canal (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: http://portocanal.sapo.pt/noticia/142948

Portugal Notícias (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: http://www.portugalnoticias.net/caranguejo-azul-nativo-da-america-do-norte-descoberto-no-estuario-do-guadiana/

Prista, N., Costa, J.L., Costa, M.J. & Jones, C.M. (2009). Age determination in meagre *Argyrosomus regius*. In: *Relatórios Cientíificos e Técnicos: Série Digital 49*. IPMA, pp. 1-54.

Público (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: https://www.publico.pt/2017/09/28/ciencia/noticia/caranguejo-azul-nativo-da-america-do-norte-descoberto-no-estuario-do-guadiana-1787052/amp

Quignard, J.-P. (1966). *Recherches sur les" Labridae"(poissons téléostéens perciformes) des côtes européennes: systématique et biologie.* Montpellier : Institut botanique, 1, p. 236.

Ramalho, A. & Teresa Dinis, M. (2011). Portuguese aquaculture: Current status and future perspectives. *World Aquaculture*, 42, p. 26.

Ramsey, G. (2009). Gordon Ramsay's F Word. Available at: https://www.youtube.com/watch?v=5yp9rZBhrVs Last accessed: March 2018.

Ricciardi, A. & Atkinson, S.K. (2004). Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems. *Ecology Letters*, 7, pp. 781-784.

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology letters*, 9, pp. 981-993.

Richardson, D.M. & Pyšek, P. (2006). Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography*, 30, pp. 409-431.

Rocha, C. (1998). Rhythmic ammonium regeneration and flushing in intertidal sediments of the Sado estuary. *Limnology and Oceanography*, 43, pp. 823-831.

Roman, J. (2006). Bon Appétit. *Conservation in practice*, 7(1), pp. 23-27.

RTP Notícias (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: https://www.rtp.pt/noticias/pais/caranguejo-azul-nativo-da-america-do-norte-descoberto-no-estuario-do-guadiana_n1030198

RTP Notícias (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: https://www.rtp.pt/noticias/pais/chef-algarvio-testa-pratos-com-medusas-e-outras-especies-em-parceria-com-universidade_n1049112

RTP Notícias (2018). Espécies invasoras exóticas surgem no Guadiana devido a alterações ambientais. Available at: https://www.rtp.pt/noticias/ambiente/especies-invasoras-exoticas-surgem-no-guadiana-devido-a-alteracoes-ambientais_a1049448

RTP1 (2017a). Novas Espécies no Rio Guadiana. In: *Portugal em Direto*.

RTP1 (2017b). Espécies Invasoras no Rio Guadiana. In: *Telejornal*.

Ruiz, G.M., Fofonoff, P.W., Steves, B.P. & Carlton, J.T. (2015). Invasion history and vector dynamics in coastal marine ecosystems: A North American perspective. *Aquatic Ecosystem Health & Management*, 18, pp. 299-311.

Russell, J.C. & Blackburn, T.M. (2017). The rise of invasive species denialism. *Trends in ecology & evolution*, 32, pp. 3-6.

Sanjuán-López, A.I., Philippidis, G. & Resano-Ezcaray, H. (2011). How useful is acceptability to explain economic value? An application on the introduction of innovative saffron products into commercial markets. *Food quality and preference*, 22, pp. 255-263.

Santos, M.E., Coniglione, C. & 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.

Sapo 24 (2017). Ceviche de alforreca? Chef algarvio quer transformar espécies invasoras do Guadiana em pratos "gourmet". Available at: http://24.sapo.pt/vida/artigos/ceviche-de-alforreca-chef-algarvio-quer-transformar-especies-invasoras-do-guadiana-em-pratos-gourmet

Sasal, P., Morand, S. & Guégan, J.-F. (1997). Determinants of parasite species richness in Mediterranean marine fishes. *Marine Ecology Progress Series*, 149, pp. 61-71.

Savini, D., Occhipinti–Ambrogi, A., Marchini, A., Tricarico, E., Gherardi, F., Olenin, S. *et al.* (2010). The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology*, 26, pp. 1-7.

Sax, D.F. & Brown, J.H. (2000). The paradox of invasion. *Global Ecology and Biogeography*, 9, pp. 363-371.

Schierenbeck, K.A. (2011). Hybridization and introgression. In: *Encyclopedia of biological invasions* (eds. Simberloff, D. & Rejmánek, M.). Univ of California Press, pp. 342-346.

Schoener, T.W. (1968). The Anolis lizards of Bimini: resource partitioning in a complex fauna. *Ecology*, 49, pp. 704-726.

Scyphers, S.B., Powers, S.P., Akins, J.L., Drymon, J.M., Martin, C.W., Schobernd, Z.H. *et al.* (2015). The role of citizens in detecting and responding to a rapid marine invasion. *Conservation Letters*, 8, pp. 242-250.

SeaRates (2018). Searates. Available at: https://www.searates.com/ Last accessed: October 2018.

SeaTemperature (2018). SeaTemperature.org. Available at: http://www.seatemperature.org Last accessed: October 2018.

Seebens, H., Gastner, M. & Blasius, B. (2013). The risk of marine bioinvasion caused by global shipping. *Ecology letters*, 16, pp. 782-790.

Severinghaus, L.L. & Chi, L. (1999). Prayer animal release in Taiwan. *Biological Conservation*, 89, pp. 301-304.

Shabana, N.M.A., El Rahman, S.H.A., Al Absawy, M.A. & Assem, S.S. (2012). Reproductive biology of *Argyrosomus regius* (Asso, 1801) inhabiting the south eastern Mediterranean Sea, Egypt. *The Egyptian Journal of Aquatic Research*, 38, pp. 147-156.

Shannon, C.E. & Weaver, W. (1963). The mathematical theory of communication. 1949. *Urbana, IL: University of Illinois Press*.

Shepherd, G.R. & Brunswick, N. (1984). Reproduction of Weakfish, *Cynoscion regalis*, in the New York Bight and Evidence for Geographically Specific Life History Characteristics. *Fish. Bull*, 82, pp. 501-511.

SIC Noticias (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at: http://sicnoticias.sapo.pt/pais/2017-09-28-Caranguejo-azul-nativo-da-America-do-Norte-descoberto-no-estuario-do-Guadiana

Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M. *et al.* (2010). Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, 119, pp. 610-621.

Simberloff, D. & Stiling, P. (1996). How risky is biological control? *Ecology*, 77, pp. 1965-1974.

Simberloff, D. & Von Holle, B. (1999). Positive interactions of nonindigenous species: invasional meltdown? *Biological invasions*, 1, pp. 21-32.

Simberloff, D. (2009). The Role of Propagule Pressure in Biological Invasions. *The Annual Review ofEcology, Evolution, and Systematics*, 40.

Simberloff, D. (2014). Biological invasions: What's worth fighting and what can be won? *Ecological Engineering*, 65, pp. 112-121.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J. *et al.* (2013). Impacts of biological invasions: what's what and the way forward. *Trends Ecol Evol*, 28, pp. 58-66.

Só Manjar (2018). Chef algarvio quer fazer pratos com alforrecas. Available at: https://www.somanjar.com/mobile/noticias_detalhe.php?id=569

Sousa, R., Morais, P., Dias, E. & Antunes, C. (2011). Biological invasions and ecosystem functioning: time to merge. *Biological Invasions*, 13, pp. 1055-1058.

Stickney, R., Taylor, G. & White, D. (1975). Food habits of five species of young southeastern United States estuarine Sciaenidae. *Chesapeake Science,* 16, pp. 104-114.

StopAquaticHitchhikers (2019). Protect Your Waters! Available at: http://stopaquatichitchhikers.org/ Last accessed: May 2019.

Strauss, S.Y., Lau, J.A. & Carroll, S.P. (2006). Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecol Lett*, 9, pp. 357-374.

Strayer, D.L. (2012). Eight questions about invasions and ecosystem functioning. *Ecol Lett*, 15, pp. 1199-1210.

Strecker, A.L., Campbell, P.M. & Olden, J.D. (2011). The aquarium trade as an invasion pathway in the Pacific Northwest. *Fisheries*, 36, pp. 74-85.

Sul Informação (2017). Caranguejo-azul descoberto no Rio Guadiana pode ser oportunidade ou ameaça. Available at: http://www.sulinformacao.pt/2017/09/caranguejo-azul-descoberto-no-rio-guadiana-pode-ser-oportunidade-ou-ameaca/

Tempo no Algarve (2017). Caranguejo azul nativo da América do Norte descoberto no estuário do Guadiana. Available at:http://temponoalgarve.blogs.sapo.pt/2017/09/

Thomas, D.L. (1971). Early life history and ecology of six species of drums (Sciaenidae) in the lower Delaware River a brackish tidal estuary. *Food and Agricultural Organization of the United Nations.*

Thomsen, P.F. & Willerslev, E. (2015). Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, 183, pp. 4-18.

Torchin, M.E., Lafferty, K.D. & Kuris, A.M. (2001). A Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions,* 3, pp. 333-345.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J. & Kuris, A.M. (2003). Introduced species and their missing parasites. *Nature,* 421, pp. 628-630.

TSF Rádio Notícias (2017). Chef algarvio testa pratos com medusas e outras espécies em parceria com universidade. Available at: https://www.tsf.pt/lusa/interior/chef-algarvio-testa-pratos-com-medusas-e-outras-especies-em-parceria-com-universidade-9015791.html

TVI (2017). Caranguejo Azul no Guadiana. In: *Jornal das 8*. Available at: https://tviplayer.iol.pt/programa/jornal-das-8/53c6b3903004dc006243d0cf/video/59d9272c0cf2d0f9b7f401a4

TVI24 (2017). Iguaria americana invade Guadiana. Available at: http://www.tvi24.iol.pt/tecnologia/caranguejo-azul/iguaria-norte-americana-descoberta-no-guadiana

Últimas curiosidades (2017). Há caranguejos-azuis norte-americanos no Guadiana. Available at: https://ultimascuriosidades.wordpress.com/2017/09/29/ha-caranguejos-azuis-norte-americanos-no-guadiana/

Ultimate Science (2017). Encontrados caranguejos-azuis norte-americanos no Guadiana. Available at: http://www.ultimatescience.org/encontrados-caranguejos-azuis-norte-americanos-no-guadiana/

Vander Zanden, M.J., Cabana, G. & Rasmussen, J.B. (1997). Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ15N) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, pp. 1142-1158.

Vander Zanden, M.J., Casselman, J.M. & Rasmussen, J.B. (1999). Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature*, 401, p. 464.

Vander Zanden, M.J., Clayton, M.K., Moody, E.K., Solomon, C.T. & Weidel, B.C. (2015). Stable isotope turnover and half-life in animal tissues: a literature synthesis. *PloS one*, 10, p. e0116182.

Varble, S. & Secchi, S. (2013). Human consumption as an invasive species management strategy. A preliminary assessment of the marketing potential of invasive Asian carp in the US. *Appetite*, 65, pp. 58-67.

Vigoe (2016). Descubiertos en la Ría ejemplares de un pez del este de Norteamérica. Available at: https://www.vigoe.es/medioambiente/item/11522-descubiertos Last accessed: January 2017.

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L. *et al*. (2011). Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology letters*, 14, pp. 702-708.

Wassef, E. & El Emary, H. (1989). Contribution to the biology of bass, *Dicentrarchus labrax* L. in the Egyptian Mediterranean waters off Alexandria. *Cybium*, 13, pp. 327-345.

Wasson, K., Zabin, C.J., Bedinger, L., Diaz, M.C. & Pearse, J.S. (2001). Biological invasions of estuaries without international shipping: the importance of intraregional transport. *Biological conservation*, 102, pp. 143-153.

Waterkeyn, A., Vanschoenwinkel, B., Elsen, S., Anton-Pardo, M., Grillas, P. & Brendonck, L. (2010). Unintentional dispersal of aquatic invertebrates via footwear and motor vehicles in a Mediterranean wetland area. *Aquatic conservation: marine and freshwater ecosystems*, 20, pp. 580-587.

Weidel, B.C., Josephson, D.C. & Kraft, C.E. (2007). Littoral fish community response to smallmouth bass removal from an Adirondack lake. *Transactions of the American Fisheries Society*, 136, pp. 778-789.

Weir, S.M. & Salice, C.J. (2012). High tolerance to abiotic stressors and invasion success of the slow growing freshwater snail, *Melanoides tuberculatus*. *Biological Invasions*, 14, pp. 385-394.

Welcomme, R. (1991). International introductions of freshwater fish species into Europe. *Finnish Fisheries Research (Finland) eng (no. 9) p. 11-18*.

Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J. & Tortonese, E. (1985). *Fishes of the north-eastern Atlantic and the Mediterranean*. Volume 1, Unesco, Paris.

Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J. & Tortonese, E. (1986a). *Fishes of the North-eastern Atlantic and the Mediterranean*. Volume 2, Unesco, Paris.

Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J. & Tortonese, E. (1986b). *Fishes of the North eastern Atlantic and the Mediterranean*. Volume 3, Unesco, Paris.

Wilk, S.J. (1978). Biology and ecology of the weakfish, *Cynoscion regalis* (Bloch and Schneider). In: *Proceedings of the Red Drum and Seatrout Colloquium*, pp. 19-31.

Willis, C.M., Richardson, J., Smart, T., Cowan, J. & Biondo, P. (2015). Diet composition, feeding strategy, and diet overlap of 3 sciaenids along the southeastern United States. *Fishery Bulletin*, 113.

Wittmann, M.E., Chandra, S., Reuter, J.E., Caires, A., Schladow, S.G. & Denton, M. (2012). Harvesting an invasive bivalve in a large natural lake: species recovery and impacts on native benthic macroinvertebrate community structure in Lake Tahoe, USA. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 22, pp. 588-597.

Wonham, M.J., Carlton, J.T., Ruiz, G.M. & Smith, L.D. (2000). Fish and ships: relating dispersal frequency to success in biological invasions. *Marine Biology*, 136, pp. 1111-1121.

WWF-Mediterranean (2017). Seafood and the Mediterranean: local tastes, global markets. *World Wide Fund For Nature*. Galnd, Switzerland.

Young, T.P., Chase, J.M. & Huddleston, R.T. (2001). Community succession and assembly comparing, contrasting and combining paradigms in the context of ecological restoration. *Ecological restoration*, 19, pp. 5-18.

Zavaleta, E.S., Hobbs, R.J. & Mooney, H.A. (2001). Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology & Evolution*, 16, pp. 454-459.

Závorka, L., Lang, I., Raffard, A., Evangelista, C., Britton, J.R., Olden, J.D. *et al.* (2018). Importance of harvest-driven trait changes for invasive species management. *Frontiers in Ecology and the Environment*, 16, pp. 317-318.

Zipkin, E.F., Kraft, C.E., Cooch, E.G. & Sullivan, P.J. (2009). When can efforts to control nuisance and invasive species backfire? *Ecological Applications*, 19, pp. 1585-1595.

Zuberi, A., Ali, S. & Brown, C. (2011). A Non-Invasive Assay for Monitoring Stress Responses: A Comparison Between Wild and Captive-Reared Rainbowfish (*Melanoteania duboulayi*). *Aquaculture*, 321, pp. 267-272.