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**The diet and the feeding habits of *Lagocephalus  
sceleratus* in the Eastern Mediterranean**



**UNIVERSIDADE DO ALGARVE**

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**The diet and the feeding habits of *Lagocephalus  
sceleratus* in the Eastern Mediterranean**

**Mestrado em Biologia Marinha**

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**“The diet and the feeding habits of *Lagocephalus sceleratus* in the Eastern Mediterranean”**

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Ana Rita Vieira de Carvalho Gomes

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## **Abstract**

This study focused on the diet and feeding habits of the invasive species *Lagocephalus sceleratus* off the coast of Crete (southeast Mediterranean). We found a low number of specimens with totally empty tracts. Its diet is composed of a wide range of organisms, but mainly fish and complemented by crustaceans and molluscs (mostly gastropods and cephalopods). Spatiotemporal differences were identified. Based on frequency of occurrence and weight contribution, there seems to be some variability in the general diet of *L. sceleratus* amongst season, region, fishery type, maturity stage and sex. The employment of generalised additive models suggested that depth at which the specimens were captured, season, region, total length and feeding intensity are significant predictors for the occurrence of its most frequent prey. Fish occurrence was more probable in autumn and summer and at circa 20-25 m deep. In contrast, that of crustaceans was higher in spring and winter and in mid-sized specimens and low at around 20-25 m deep whilst increasing until a maximum at 40 m. Non-cephalopod molluscs probability was highest in winter, in the Libyan Sea and increased from the minimum at 20-25 m to a maximum at 40 m as well. TL influenced the likelihood of algae and seagrass and cephalopod consumption, which increases as the fish grow. As for feeding intensity (FI), it shows a different effect on each group, but overall seems to have a positive effect on probability of occurrence.

**Keywords:** *Lagocephalus sceleratus*; Mediterranean Sea; Lessepsian migration; diet; feeding biology

## Resumo

Espécies invasoras constituem atualmente uma das maiores ameaças para os meios marinhos, representando um risco significativo para a biodiversidade e tendo profundas consequências tanto a nível ecológico como económico. O mar Mediterrâneo é uma das regiões mais afetadas por espécies não-indígenas, sendo que estes impactos são exacerbados pelas mudanças climáticas, poluição e outras pressões antropogénicas. Com a construção do Canal Suez em 1869, desencadeou-se a dispersão de várias espécies do Mar Vermelho em direção ao Mediterrâneo, comumente designado como *migração Lessepsiana*, tornando-se esta a via mais importante para a entrada de biota invasora nestes ecossistemas. Entre os invasores Lessepsianos mais bem-sucedidos encontra-se a espécie *Lagocephalus sceleratus*, identificada no Mediterrâneo (Turquia) pela primeira vez em 2003. Na Grécia, o primeiro registo ocorreu apenas dois anos mais tarde, nas ilhas de Creta e Rodes. Este peixe rapidamente colonizou o resto desta bacia, trazendo consigo uma panóplia de efeitos nefastos. *L. sceleratus* afeta pradarias marinhas e os serviços que oferecem, e compete com carnívoros nativos. Esta espécie é também considerada uma enorme ameaça para a pesca artesanal, devido aos danos que causa tanto nas artes e utensílios de pesca como nas capturas, uma vez que se alimenta de presas capturadas em redes e palangres. A intensidade da sua predação pode contribuir para o declínio de populações nativas, particularmente de cefalópodes, o que potencialmente causa uma disrupção nos ecossistemas e adicionalmente afeta a indústria da pesca e o sector económico. Representa ainda um enorme risco à saúde e ao bem-estar dos seres humanos. Há relatos de ataques a banhistas e, além disso, esta espécie contém uma das toxinas marinhas mais potentes, a tetrodotoxina (TTX). A TTX é encontrada principalmente nas gónadas, fígado e trato gastrointestinal, mas também nos músculos e pele, o que se torna especialmente problemático quando o peixe é capturado e usado para alimentação humana. Apesar da interdição do consumo desta espécie por parte de vários governos, vários casos de intoxicação continuam a ocorrer, inclusive com desfechos fatais.

O presente trabalho focou-se nos hábitos alimentares desta espécie nas zonas costeiras da ilha de Creta, Grécia (sudoeste mediterrânico). Um total de 236 espécimes foram obtidos através de pesca comercial e experimental de Junho de 2017 a Outubro de 2022. O comprimento total (*TL*, mm), peso total (*TW*, g) e peso eviscerado (*EW*, g) foram medidos, e o sexo e estado de maturidade foram determinados. Os tratos gastrointestinais foram dissecados e os seus conteúdos analisados. A importância relativa de cada tipo de item foi quantificada com base na frequência de ocorrência

(*FO*, %) e contribuição média em peso (*WC*, %). Adicionalmente, *Generalized Additive Models* (*GAMs*) foram usados para testar o efeito de vários fatores na probabilidade de ocorrência das presas mais frequentes (peixe, crustáceos, cefalópodes e outros moluscos, ervas marinhas e algas). Apenas 14.4% dos tractos analisados estavam vazios, o que demonstra que esta espécie se encontra bem-alimentada na região estudada. A análise dos conteúdos mostrou que a dieta de *L. sceleratus* é maioritariamente piscívora e complementada por crustáceos e moluscos (em grande parte gastrópodes e cefalópodes). Adicionalmente, esta espécie alimenta-se de outros invertebrados, tais como equinodermes, e ainda de algas e ervas-marinhas. Contudo, há a possibilidade de que a presença destes últimos seja acidental e esteja relacionada com a sua alimentação bêntica, sendo que em 11.5% dos tractos partes de substrato (como areia e seixos) foram encontrados. Neste estudo foram encontradas diversas evidências de depredação. 18% dos tractos continham partes de equipamentos de pesca, designadamente linhas, anzóis e pedaços de redes, por vezes com partes de peixe emaranhadas. Tendo em conta a *FO* e contribuição em peso, parece haver na dieta de *L. sceleratus* variações sazonais, regionais e entre o sexo e maturidade dos indivíduos.

Estação do ano, a profundidade a que os espécimes foram capturados, região, intensidade alimentar (*FI*), e *TL* foram identificados como fatores explicativos da ocorrência de diferentes presas. No entanto, as variabilidades explicadas por todos os *GAMs* foram relativamente pequenas. Os modelos sugeriram que o consumo de peixe é significativamente mais provável durante o verão e outono, mas a probabilidade de consumo de crustáceos é menor nestes meses, sendo maior durante a primavera e inverno, e o consumo de moluscos não-cefalópodes mais provável no inverno. A probabilidade de consumo deste grupo é também maior em peixes oriundos do sul de Creta, o que pode estar relacionado com a maior toxicidade nesta região. Relativamente à profundidade, há um pico na probabilidade de consumo de peixes dos 20 aos 25 m, seguido de um decréscimo a profundidades mais elevadas. Por outro lado, a probabilidade de consumo de crustáceos e moluscos parece ser mínima dos 20 aos 25 m, aumentando a maiores profundidades. Parece haver uma relação inversamente proporcional na probabilidade desta espécie consumir peixes e consumir crustáceos e moluscos, que poderá estar relacionada com a mobilidade destes grupos. *TL* influencia a probabilidade de consumo de algas, ervas marinhas e cefalópodes, que aumenta com peixes de maior tamanho, sendo máxima a cerca de 550 mm. Quanto a *FI*, mostra um efeito diferente em cada grupo de presas, mas no geral parece ter um efeito positivo nas probabilidades de ocorrência. As diferenças encontradas entre estações do ano, maturidade do

peixe (relacionada com tamanho) e região geográfica estão potencialmente associadas a diferenças de habitat. Esta espécie altera o seu habitat consoante o seu estado de maturidade e, consequentemente, altura do ano. Mudanças morfológicas ontogénicas, flutuações sazonais na temperatura da água e o efeito do tipo de arte de pesca com que os espécimes foram obtidos poderão também desempenhar um papel.

Em suma, os nossos resultados demonstram que esta espécie bem-adaptada é generalista e capaz de se alimentar de uma enorme variedade de itens, com a sua dieta a variar de região. Este invasor causa danos nas artes de pesca e alimenta-se de espécies já capturadas, muitas de elevado valor. Devido aos seus impactes, tomamos como importante que haja um estudo mais aprofundadas dos hábitos alimentares nas inúmeras populações estabelecidas no mar Mediterrâneo, e que este se foque na potencial relação entre a dieta dos espécimes e a concentração de TTX, e, se possível, recorra a métodos de análise genética para identificação das presas, de modo a obter resultados menos enviesados.

**Palavras-chave:** *Lagocephalus sceleratus*; mar Mediterrâneo; migração Lessepsiana; hábitos alimentares

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### The diet and the feeding habits of *Lagocephalus sceleratus* in the Eastern Mediterranean

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## **List of Abbreviations**

**EW** - Eviscerated Weight

**F** - female

**FI** - feeding intensity

**FO** - Frequency of Occurrence

**GAM** – General Additive Model

**k** – knots

**M** – male

**N** – number

**NIS** - Non-indigenous species

**TL** - Total Length

**TTX** - Tetrodotoxin

**TW** - Total Weight

**W** - Weight

**WC** - average weight contribution

## General Introduction

Pollution, overfishing, and invasive species are greatly impacting global diversity (Ulman, Harris, et al., 2021; Ulman, Yildiz, et al., 2021). In particular, coastal ecosystems are changing under the action of connected factors, from climate change to direct human pressures (Bianchi et al., 2014), which often act in synergy (Tsirintanis et al., 2022). Invasive species are among the most serious threats to the marine milieu, posing a significant risk to biodiversity and having both ecological and economic consequences (Chartosia et al., 2021; Galanidi, Zenetos, & Bacher, 2018). Non-indigenous species (NIS) are linked to the displacement of native species or even local extinctions, alteration of trophic interactions, habitat degradation, and disruption of ecosystem functioning and services, besides potential harm to human health and well-being (Galanidi, Zenetos, & Bacher, 2018; Karachle et al., 2022; Tsirintanis et al., 2022). Nevertheless, having a complete picture of the impact an organism might have on its new home is challenging, since it is difficult to eliminate the influence of other factors (Çiçek, 2019).

This is exacerbated in the Mediterranean, which is severely affected by climate change and pollution, as well as other heavy human-related pressures (Bianchi et al., 2014; Cramer et al., 2020). This poses a problem, as the region is a biodiversity hotspot with over 17000 native species and a high level of endemism (Tsirintanis et al., 2022; Ulman, Harris, et al., 2021; Ulman, Yildiz, et al., 2021), but also one of the main hotspots for bioinvasions (Carbonara et al., 2017; El-Haweet et al., 2016; Kalogirou, 2013). The Mediterranean had a total of 751 established NIS as of December 2021, in addition to 242 casual alien taxa (Zenetos et al., 2022). Introductions in the Mediterranean are caused by both natural and anthropogenic stressors (Karachle et al., 2022). Ship ballast water, aquarium releases, aquaculture accidental entries, and corridors such as the Gibraltar Strait or the Suez Canal are the primary routes of invasion (El-Haweet et al., 2016; Farrag et al., 2015; Galanidi, Zenetos, & Bacher, 2018; Karachle et al., 2022).

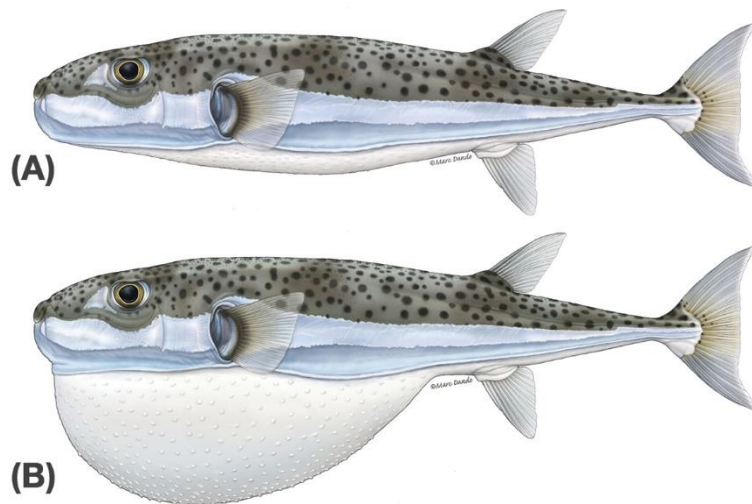
The natural opening in Gibraltar resulted in the Atlanto-Mediterranean biota (Karachle et al., 2022). However, on the opposite side of the basin, the recent anthropogenic opening of the Suez Canal created the current primary vector for the introduction of alien species into the Mediterranean (Carpentieri et al., 2009; Kalogirou et al., 2012; Ulman, Harris, et al., 2021). This structure was built in 1869 to shorten commercial navigation routes and facilitate trade (Gabel et al., 2022; Zakaria, 2015). Although it has its advantages, such as cutting the sailing distance between the edges of Europe and Asia and reducing the costs of goods delivery (Bal & Khan, 2022) it has led

to dramatic consequences. This connection brought down millions of years of geographical isolation (Abd Rabou, 2019; Nader et al., 2012), thus joining two greatly distinct biogeographical areas: the subtropical Mediterranean and the tropical Red Sea, which is the northernmost region of the Indian Ocean (Katikou et al., 2009). Since then, Indo-Pacific biota encompassing nearly all marine taxonomic groups have vastly invaded Mediterranean ecosystems (Ali et al., 2015; Azzurro et al., 2020; Nader et al., 2012). While the canal permits bidirectional passage, many more species have successfully migrated toward the Mediterranean compared to the opposite direction (Bentur et al., 2008; Katikou et al., 2009). This biogeographical phenomenon has been termed Lessepsian migration (Por, 1978), after the engineer Ferdinand de Lesseps who planned the construction of the canal (Bentur et al., 2008). It is an ongoing process (Carpentieri et al., 2009) that might represent the most important maritime biogeographic phenomenon of our times, according to Por (1978). Many of these Red Sea species have established self-sustaining reproducing populations and are often associated with devastating impacts (Carpentieri et al., 2009; Rousou et al., 2014). For instance, 63% of the non-indigenous ichthyofauna in the Mediterranean are Red Sea species that arrived through this pathway, totaling 127 fish species (Ünal & Göncüoğlu Bodur, 2017), of which 97 were considered established (Zenetos et al., 2022).

The prevalence of unsaturated niches in the eastern Mediterranean explains in part the success of Lessepsian migrants (Carpentieri et al., 2009). Additionally, the more recent salinity reduction of the Bitter Lakes and the construction of the Aswan Dam near the canal have also altered the Levantine environment, favouring these species (Kiparissis et al., 2018). It is beyond doubt that Lessepsian migration has already altered coastal communities throughout much of the Mediterranean eastern basin (Michailidis, 2010; Tsirintanis et al., 2022), with Greece in particular being severely affected, as it was the country that received the biggest number of alien species from 1988 to 2017 (Zenetos, 2019). This spread of thermophilic Indo-Pacific species into Mediterranean ecoregions is not only expected to continue but also increase due to the rise in seawater temperature (Abd Rabou, 2019; Nader et al., 2012; Ünal et al., 2015). It will further impact native species and disturb ecological equilibria, not to mention negatively influencing the fisheries sector (Beköz et al., 2013; Boustany et al., 2015).

Amongst the most successful and unwanted Lessepsian invaders is the silver-cheeked toadfish or silverstripe blaasop *Lagocephalus sceleratus* (Fig. 1) (Azzurro et al., 2020; Özbek et al., 2017; Rambla-Alegre et al., 2017). *L. sceleratus* is one of the largest members of the

Tetraodontidae family (Akyol & Ünal, 2017; Boustany et al., 2015). This family comprises the so-called pufferfish (Chartosia et al., 2021) and contains 197 species globally (Ulman, Yildiz, et al., 2021). This unique group of fish inhabits freshwater, marine, and estuarine environments, both in temperate and tropical regions (Devi & Sivan, 2017). In the Mediterranean, there are currently 11 established pufferfishes (Ulman, Yildiz, et al., 2021), and *L. sceleratus* is the one that reaches the greatest lengths and weights (Akyol & Ünal, 2017; Ulman, Yildiz, et al., 2021). Previously recorded maximum sizes include weights up to 7 kg (South Africa) and a maximal length of 110 cm (Japan and Turkey) (Ulman et al., 2022). However, specimens with around 8 to 9 kg have been fished in Greece, in Rhodes, Zakynthos, and Kos (Ulman et al., 2022), and several Turkish fishers claim to have caught specimens between 10 and 12 kg (Ulman, Yildiz, et al., 2021).



**Figure 1.** *Lagocephalus sceleratus* (A – lateral view; B – puffed lateral view). (In Ulman, Yildiz et al. 2021, original drawing by Marc Dando).

The silver-cheeked toadfish was originally found throughout the Indian and West Pacific oceans, as well as in the Red Sea (Akyol & Ünal, 2017; Azzurro et al., 2014). Its native geographical distribution goes from Japan, Australia, and Hong Kong to Mozambique and South African shores (Akyol et al., 2005; Kleitou et al., 2018). Recently, its natural range has drastically expanded (Başusta et al., 2013). This pufferfish was first documented in the Mediterranean Sea in 2003, with a confirmed record obtained in Gökova Bay, Turkey (Akyol et al., 2005). A previous 1977 record in Lebanon was a misidentification of the similar congeneric *Lagocephalus suezensis* (Carbonara et al., 2017; Nader et al., 2012). It was first identified in Greece in the year of 2005,

both in Rhodes (Corsini-Foka et al., 2006) and Crete (Kasapidis et al., 2007). It has since then established significant populations in Greece and Turkey, as well as in other countries along the Mediterranean, including Egypt, Syria, Israel, Palestine, Lebanon, Cyprus, Libya, Tunisia, Malta, and Algeria (Akyol & Ünal, 2017; Ben Souissi et al., 2014; Boustany et al., 2015; Deidun et al., 2015; Galanidi, Zenetos, & S. Chapman, 2018; Hammoud & Salama, 2016; Kasapidis et al., 2007; Özbek et al., 2017; Rambla-Alegre et al., 2017). It is equally present in Spain, Croatia, Montenegro, and Italy (Azzurro et al., 2014; Carbonara et al., 2017; Deidun et al., 2015; Özbek et al., 2017; Rambla-Alegre et al., 2017; Sulić Šprem et al., 2014). Additionally, *L. sceleratus* has also been found in the Sea of Marmara (Bal & Khan, 2022) and the Black Sea (Ulman, Harris, et al., 2021). Curiously, it has not yet been reported off France (Ulman, Yildiz, et al., 2021). In just two decades, this Indo-Pacific species quickly colonized the whole eastern Mediterranean basin and made its way westward (Coro et al., 2018). In 2017, it was caught in the Gibraltar Strait, and such a record might conjure up images of a future in which this Lessepsian invader expands beyond the Mediterranean and spills into the Atlantic Ocean (Azzurro et al., 2020). Even though it is only moderately likely to establish, at best, in southern Spain and Portugal (Galanidi, Zenetos, & S. Chapman, 2018), this possibility needs to be taken seriously due to climate change (Azzurro et al., 2020; Coro et al., 2018).

*L. sceleratus* owes its success to its high growth and reproduction rates, the ability to exploit many food resources, its broad thermal tolerance, a lack of commercial interest and the absence of serious competitors and predators in its invaded range (Coro et al., 2018; Deidun et al., 2015; Nader et al., 2012; Torcu-Koç & Erdoğan, 2022). In the Mediterranean the only reported predators of juveniles are the common dolphinfish *Coryphaena hippuris*, garfish *Belone belone*, white grouper *Epinephelus aeneus*, and larger *L. sceleratus* specimens, while the loggerhead turtle *Caretta caretta* is the sole species that has been found to predate on adults (Kleitou et al., 2018; Ulman, Harris, et al., 2021). As pufferfish are slow swimmers (Nader et al., 2012), they instead use the ability to inflate themselves with water when threatened and their toxicity to deter predation (Aydin et al., 2013; Nader et al., 2012; Ulman, Yildiz, et al., 2021).

This species is most commonly found at 10-50 m, but often also occurs in depths up to 100 m (Akyol & Ünal, 2017), with a record of a maximum depth of 350-400 m in Spain (Galanidi, Zenetos, & S. Chapman, 2018). It mostly inhabits sandy or muddy substrate areas (El-Haweet et al., 2016; Nader et al., 2012). Juveniles tend to prefer sandy bottoms, but adults move to *Posidonia*

*oceanica* seagrass meadows (Hussain et al., 2020; Kalogirou et al., 2012), with large specimens being found there during summer and autumn (Kalogirou, 2013).

The spawning season in the Gulf of Suez and the Mediterranean lasts from spring to summer (Ali et al., 2015; Boustany et al., 2015; Peristeraki et al., 2010; Rousou et al., 2014). *L. sceleratus* is most active during the day and at night with full moons (Michailidis, 2010; Ünal & Göncüoğlu Bodur, 2017), which might indicate this species depends greatly on its sight to hunt (Michailidis, 2010). It has a diversified diet (Boustany et al., 2015), with main prey changing between geographical regions. Overall, it is classified as a generalist predator, feeding mostly on a carnivorous diet of a combination of invertebrates such as crustaceans (shrimp and crabs) and small molluscs (gastropods and bivalves), fish, and cephalopods (Aydin, 2011; Christidis et al., 2021; Hussain et al., 2020; Sabrah et al., 2006; Ulman et al., 2022). Although not as abundantly, photosynthetic organisms have also been reported (Christidis et al., 2021; Michailidis, 2010). This species shows great adaptability regarding its feeding, as it has been found to feed on all types of food at all ages (Michailidis, 2010). Possessing such a wide range of prey significantly impacts the ecosystem and the fisheries sector (Boustany et al., 2015). Nevertheless, this can represent an associated positive trait, as the pufferfish appears to feed also on a wide range of invasive species, including its own (Kondylatos et al., 2023; Ulman, Yildiz, et al., 2021). The diet of this species can change with age and, consequently, also size, which has been related to a shift in habitat and morphology (Hussain et al., 2020; Kalogirou, 2013; Ulman et al., 2022). Additionally, differences in feeding intensity and food items between seasons have also been found (Hussain et al., 2020; Sabrah et al., 2006; Torcu-Koç et al., 2020).

*L. sceleratus* is considered one of the 100 worst invasive species of the Mediterranean Sea (Ben Souissi et al., 2014; Kleitou et al., 2018), being the one with highest socio-economic impact and overall highest number of impact reports (Galanidi, Zenetos, & Bacher, 2018). It has a panoply of negative effects on native diversity, local fisheries and human health (Christidis et al., 2021; Ulman et al., 2022; Ulman, Harris, et al., 2021; Ünal et al., 2015). Invasive species negatively compete for resources with native biota and can act as ecosystem engineers, altering habitat structures (Tsirintanis et al., 2022). Additionally, their intensive feeding activities can lead to the decline of native populations and disrupt food chains (Bal & Khan, 2022; Tsirintanis et al., 2022). The impacts of *L. sceleratus* on biodiversity and ecosystem services are moderate-to-high (Tsirintanis et al., 2022). Through high predation on juvenile fishes, this species might be affecting

the services provided by *Posidonia oceanica* meadows, reducing the importance of these ecosystems as nursery grounds (Galanidi, Zenetos, & S. Chapman, 2018). More importantly, *L. sceleratus* is a prominent potential health risk to humans, because it contains tetrodotoxin (TTX) and its analogues, which can cause food poisoning (Rodríguez et al., 2012). It is the second most toxic pufferfish in the Mediterranean basin, after *Torquigener flavimaculosus* (Ulman, Harris, et al., 2021). TTX, commonly associated with the Tetraodontidae family, is a heat-stable neurotoxin that is thus resistant to cooking (El-Sayed et al., 2003; Nader et al., 2012). This toxin has also been identified in gastropods in Portugal, as well as in bivalves in England, the Netherlands, and Greece (Rambla-Alegre et al., 2017). It is one of the most potent marine toxins (Carbonara et al., 2017), far worse than cyanide (Boustany et al., 2015). The source of this compound is unknown, but it is more likely that TTX is exogenous and bioaccumulates through the food chain, having been produced by free-living marine bacteria rather than bacteria within the pufferfish (Christidis et al., 2021; Kheifets et al., 2012; Nader et al., 2012). TTX serves many functions, including in defence and predation, as a male-attracting pheromone and as an egg- and larvae-protecting agent (Christidis et al., 2021; El-Sayed et al., 2003; Galanidi, Zenetos, & S. Chapman, 2018). It is primarily found in the gonads, liver, gastrointestinal tract and also skin of these fish, but it can be detected in any tissue, including the musculature (Aydin, 2011; Aydin et al., 2013; Ben Souissi et al., 2014; Christidis et al., 2021; Rambla-Alegre et al., 2017). Toxin levels are difficult to predict, but tissue type, area, sex, maturity, size, and season all play a role (Christidis et al., 2021; El-Sayed et al., 2003; Nader et al., 2012; Sabrah et al., 2006). Differences in TTX concentrations between populations and seasons may be explained by the diet of the fish (Christidis et al., 2021; El-Sayed et al., 2003). Toxicity is generally higher in fish of larger size, and small specimens often present no toxicity at all, which is encouraging, since smaller *L. sceleratus* are frequently caught and confused with edible species such as *Spicara smaris*, *Boops boops*, and *Atherina hepsetus* (Kalogirou, 2013; Katikou et al., 2009). Poisoning symptoms range from mild gastrointestinal disturbance and paraesthesia to severe muscular paralysis, respiratory failure, and fatal arrhythmias (Akyol et al., 2005; Beköz et al., 2013). TTX works by inhibiting nerve and muscle action potentials through the blocking of voltage-gated sodium channels (Azzurro et al., 2014; Bentur et al., 2008; Kheifets et al., 2012). There are no antidotes, with treatment being supportive only (Ben Souissi et al., 2014; Nader et al., 2012). Many pufferfish of this family (also known as *fugu*) are traditionally consumed in a number of Asian countries, including Japan, China, Taiwan, and Korea

(Aydin et al., 2013; Nader et al., 2012). Nonetheless, due to its high toxicity and great mortality risk, this specific species is not amongst the widely consumed (Nader et al., 2012), with consumption being banned in Japan (Ünal et al., 2015) and Malaysia (Ben Souissi et al., 2014). Since the arrival of this species, many Mediterranean countries have conducted public awareness campaigns (Azzurro et al., 2020; Galanidi, Zenetos, & S. Chapman, 2018). Furthermore, its marketing is illegal in various countries (Galanidi, Zenetos, & S. Chapman, 2018). Egypt (El-Haweet et al., 2016), Turkey (Özbek et al., 2017), and Lebanon (Ben Souissi et al., 2014) have prohibited the fishing and consumption of this fish. Similarly, according to European Union legislation (853/2004/EC, 854/2004/EC), poisonous fish belonging to the Tetraodontidae and their derived products cannot enter markets (Christidis et al., 2021). Nevertheless, intoxication cases continue to occur, mainly due to consumer ignorance, misidentification of species, or a flawed understanding of detoxification methods (Nader et al., 2012). The overexploitation of this stock in Egypt demonstrates its rampant illegal trade (Farrag et al., 2015), and in Suez City, this fish is considered a delicacy (Jribi & Bradai, 2012; Sabrah et al., 2006). *L. sceleratus* is likewise illegally sold in Lebanon, Turkey, Cyprus, Greece, Tunisia, Syria, Libya, Israel, and Palestine, resulting in numerous cases of poisoning and even fatalities in these countries (Abd Rabou, 2019; Ben Souissi et al., 2014; Galanidi, Zenetos, & S. Chapman, 2018; Kalogirou, 2013; Kleitou et al., 2018; Özbek et al., 2017; Ünal et al., 2015). As an example, off the Greek island of Crete the crewmembers of a tanker suffered mass hospitalization after consuming the fish (Ben Souissi et al., 2014).

Apart from poisonings, another public safety risk comes in the form of erratic attacks on bathers, with possible implications for tourism (Ace et al., 2022; Sümen & Bilecenoğlu, 2019; Ulman, Yildiz, et al., 2021; Ünal & Göncüoğlu Bodur, 2017). Moreover, the possibility that *L. sceleratus* contains paralytic shellfish poisoning toxins is another associated risk (Katikou et al., 2009). This species is also a major menace to small-scale fisheries, as it causes catch losses and damage to gears (Christidis et al., 2022; Galanidi & Zenetos, 2019). It exhibits highly opportunistic tendencies, as evidenced by its depredation, which involves feeding on specimens entangled in nets and hooked on longlines (Abd Rabou, 2019; Coro et al., 2018; Ulman et al., 2022). This phenomenon is reported in many Mediterranean regions (Abd Rabou, 2019; Michailidis, 2010; Özbek et al., 2017; Ünal et al., 2015; Ünal & Göncüoğlu Bodur, 2017), even though such a problem has yet to be noticed in its native range (Hussain et al., 2020; Ulman et al., 2022). Like others in its family, this species has a highly mineralized beak composed of four

strong teeth (Thiery et al., 2017). These cause severe damage to fishing nets, longlines, and even handline hooks, as the fish bite and cut off parts of these gear (Ulman et al., 2022; Ulman, Harris, et al., 2021). Moreover, there are reports of spearfishing catches being attacked (Galanidi, Zenetos, & S. Chapman, 2018). The result is increased labour and equipment costs for the fishing industry, as fishers must spend more time discarding this fish and mending equipment (Kalogirou, 2013; Kleitou et al., 2018; Ulman et al., 2022). Additionally, the pufferfish is probably leading to a marked reduction in stocks of important commercial cephalopods, due to predation (Katikou et al., 2009; Michailidis, 2010; Panagopoulou et al., 2017; Ulman, Yildiz, et al., 2021). In Crete, the species is becoming increasingly more prevalent, and the frequency of occurrence in fisheries watches reached 57% in 2017 (Galanidi, Zenetos, & S. Chapman, 2018). The fish is seen by 22% of Cretan fishers as the leading species in terms of damage (Panagopoulou et al., 2017), and it is the most common alien species caught in longlines in the South (Peristeraki et al., 2015). Its spread has been accompanied by frequent complaints from fishers (Christidis et al., 2022), and even led to their withdrawal from the small-scale local fishery, which constitutes a major impact for the island (Galanidi & Zenetos, 2019). Fishing in deeper waters, reinforcing hooks with steel lines, and manufacturing nets with resistant material are possible mitigation measures (Abd Rabou, 2019; Kalogirou, 2013), and government-subsidized bounty systems might also reduce economic impacts (Akyol & Ünal, 2017; Kleitou et al., 2018). However, physical removal of specimens solely as a means to control populations is often inefficient and costly (Rousou et al., 2014). A better option would be to establish a fishery oriented toward commercial uses, focusing on the many biomedical and pharmaceutical applications the species and TTX have (Nader et al., 2012; Rousou et al., 2014; Ulman, Yildiz, et al., 2021).

The silver-cheeked toadfish can be considered a top predator, and understanding predator-prey interactions is critical to fully comprehend the role of this fish in the Mediterranean (Ulman, Yildiz, et al., 2021). Gut content analysis allows us to document the prey spectrum of a fish and the trophic relations of which they are a part of (Devi & Sivan, 2017; Herrán, 1988). Some studies have mentioned the subject of its feeding biology and diet in the region (Akboru et al., 2020; Aydin, 2011; Boustany et al., 2015; Christidis et al., 2021; Gabel et al., 2022; Hammoud & Salama, 2016; Hussain et al., 2020; Kalogirou, 2013; Michailidis, 2010; Torcu-Koç et al., 2020; Ulman, Yildiz, et al., 2021). Of those, only two were set in Greece (Christidis et al., 2021; Kalogirou, 2013). However, these studies present some gaps regarding the analysis of seasonal variations, sex, and

life stages. This work attempts to fill in those and investigate the feeding habits of the pufferfish *Lagocephalus sceleratus* off the coast of Crete, Greece, and to add further information to our understanding of this NIS's food web.

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# The diet and the feeding habits of *Lagocephalus sceleratus* in the Eastern Mediterranean

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## Abstract

This study focused on the diet and feeding habits of the invasive species *Lagocephalus sceleratus* off the coast of Crete (southeast Mediterranean). We found a low number of specimens with totally empty tracts. Its diet is composed of a wide range of organisms, but mainly fish and complemented by crustaceans and molluscs (mostly gastropods and cephalopods). Spatiotemporal differences were identified. Based on frequency of occurrence and weight contribution, there seems to be some variability in the general diet of *L. sceleratus* amongst season, region, fishery type, maturity stage and sex. The employment of generalised additive models suggested that depth at which the specimens were captured, season, region, total length and feeding intensity are significant predictors for the occurrence of its most frequent prey. Fish occurrence was more probable in autumn and summer and at circa 20-25 m deep. In contrast, that of crustaceans was higher in spring and winter and in mid-sized specimens and low at around 20-25 m deep whilst increasing until a maximum at 40 m. Non-cephalopod molluscs probability was highest in winter, in the Libyan Sea and increased from the minimum at 20-25 m to a maximum at 40 m as well. TL influenced the likelihood of algae and seagrass and cephalopod consumption, which increases as the fish grow. As for feeding intensity (FI), it shows a different effect on each group, but overall seems to have a positive effect on probability of occurrence.

## 1. Introduction

Human-mediated species introductions are accelerating all over the world, and it represents a global environmental change so profound that it might even surpass natural drivers of selection and dispersal (Tsirintanis et al., 2022). The Mediterranean Sea is especially at risk. Not only is this basin one of the most overfished regions on the planet (Ulman, Harris, et al., 2021), but it is also a major hotspot of marine bioinvasions (Farrag et al., 2015; Kalogirou et al., 2012). The Suez Canal is the primary entry point into this region (Chartosia et al., 2021; Kalogirou et al., 2012). The opening of this structure in 1869 permitted the merging of two previously very distinct zoogeographical areas, the Atlantic-Mediterranean and the Indo-Pacific (Bentur et al., 2008; Papaconstantinou, 1990), which set off an ongoing process of colonization (Bal & Khan, 2022; Carpentieri et al., 2009). Although dispersal in the opposite direction does occur, the majority of alien species arrive into the Mediterranean from the Red Sea (Nader et al., 2012). Commonly called

Lessepsian migration, a term coined by Por (1978), this phenomenon has caused a dramatic change in the fauna of the Levantine basin, and to some extent, the entire Mediterranean (Albano et al., 2021; Gabel et al., 2022; Papaconstantinou, 1990).

One of the faster expanding Lessepsian invaders is *Lagocephalus sceleratus*, known as the silverstripe blaasop or silver-cheeked toadfish (Jribi & Bradai, 2012; Katikou et al., 2009; Özbek et al., 2017). This species is native to the Red Sea and the Indian and Pacific oceans (Michailidis, 2010). Having first been documented in the Mediterranean Sea in 2003 (Akyol et al., 2005), its range has expanded to include the Black Sea and the majority of the Mediterranean, having recently reached the Strait of Gibraltar (Azzurro et al., 2020). Frequent sightings occur in most of the Levantine region, as well as in Tunisia, Malta, and Algeria (Akyol & Ünal, 2017; Ben Souissi et al., 2014; Boustany et al., 2015; Deidun et al., 2015; Galanidi et al., 2018; Hammoud & Salama, 2016; Kasapidis et al., 2007; Özbek et al., 2017; Rambla-Alegre et al., 2017). The rapid spread of this fish across the Mediterranean shows it is well-adapted to new environments (Kalogirou, 2013).

Its expansion and establishment are aided by reproduction at an early age, the ability to compete with native top predators, the rampant overfishing that characterizes the Mediterranean, the lack of serious predatory control, and by not being a targeted species (Beköz et al., 2013; Michailidis, 2010; Ulman, Yildiz, et al., 2021). It is likely that rising sea temperatures are likewise enhancing the further dispersal of this fish (Coro et al., 2018; Jribi & Bradai, 2012). This species is one of the largest members of the Tetraodontidae pufferfish family (Kasapidis et al., 2007; Michailidis, 2010). *L. sceleratus* is found mainly at depths from 18 to 100 m (Torcu-Koç & Erdoğan, 2022), in a wide range of habitats, including sandy or muddy bottoms, rocky substrates, and seagrass meadows (Ulman, Harris, et al., 2021; Ulman, Yildiz, et al., 2021; Yaglioglu et al., 2011). It has a broad and diversified diet (Boustany et al., 2015). Overall, it is regarded as a generalist carnivore, with its main prey consisting of crustaceans, fish, cephalopods, and other molluscs (Boustany et al., 2015; Rousou et al., 2014; Sabrah et al., 2006). Such a wide range of prey brings about a significant impact on the ecosystem and fisheries sector (Boustany et al., 2015).

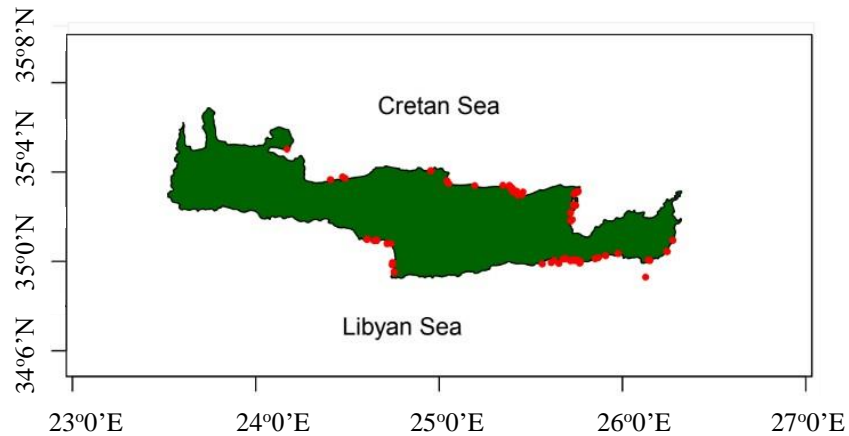
*L. sceleratus* is seen as one of the Mediterranean's worst 100 invasive species (Ben Souissi et al., 2014; Kleitou et al., 2018). It affects native biodiversity, the economy sector, and human health (Christidis et al., 2021; Ulman et al., 2022; Ulman, Harris, et al., 2021; Ünal et al., 2015). *L. sceleratus* contains tetrodotoxin (TTX), a potent neurotoxin of high stability with no known antidote (Christidis et al., 2021; Zhang et al., 2020) that has a defensive, offensive, and

communicative function (Kalogirou, 2013). It is concentrated in the gonads, liver, and gastrointestinal tract, but it is also found in the skin and muscles of the fish (Katikou et al., 2009). Since this species is illegally sold across Lebanon, Turkey, Cyprus, Greece, Tunisia, Syria, Libya, Israel, and Palestine, many severe and lethal cases of poisoning have occurred (Abd Rabou, 2019; Ben Souissi et al., 2014; Galanidi et al., 2018; Kalogirou, 2013; Kleitou et al., 2018; Özbek et al., 2017; Ünal et al., 2015), and raising awareness about the risks associated with its consumption is crucial to mitigate any impacts (Ben Souissi et al., 2014). Attacks and bites on bathers, which have been increasing, pose another safety threat (Galanidi et al., 2018). In addition to these, the silver-cheeked toadfish exhibits extremely invasive behaviour that results in major socio-economic impacts for small-scale fisheries (Rousou et al., 2014). It is an abundant by-catch in many Mediterranean countries, leading to frequent complaints from local fishers (Abd Rabou, 2019; Boustany et al., 2015; El-Haweet et al., 2016; Michailidis, 2010; Torcu-Koç et al., 2020). This tetraodontid is thought to be a major contributor to the reduction of local stocks of commercially targeted cephalopods due to predation (Nader et al., 2012), and it often feeds on fish that had already been caught in fishing gear (Boustany et al., 2015; Nader et al., 2012). This species possesses strong beak-like teeth capable of ripping and damaging nets, longlines and hooks, which simultaneously leads to catch losses and destructed gear (Christidis et al., 2022; Nader et al., 2012; Ünal et al., 2015).

Some studies conducted in the Mediterranean have investigated the feeding biology and diet of the silver-cheeked toadfish (Akbora et al., 2020; Aydin, 2011; Boustany et al., 2015; Christidis et al., 2021; Gabel et al., 2022; Hammoud & Salama, 2016; Hussain et al., 2020; Kalogirou, 2013; Michailidis, 2010; Torcu-Koç et al., 2020; Ulman, Yildiz, et al., 2021). In Crete, Greece, it was first recorded in 2005 (Kasapidis et al., 2007), and today it is widespread throughout the island (Christidis et al., 2022). However, so far, no research has focused solely on its feeding habits in this region. The goal of this work is to fill this knowledge gap regarding the food preferences of *L. sceleratus* in Cretan waters, which is critical to fully comprehend the impacts of this species on these coastal ecosystems.

## 2. Materials and Methods:

236 specimens of *Lagocephalus sceleratus* were collected from commercial fisheries (204 specimens) and samplings with experimental fishing gears (32 specimens) off the island of Crete, Greece (107 and 129 specimens from the Cretan Sea and Libyan Sea, respectively), from June 2017 to October 2022 (Fig. 1). Fishing depth ranged from 1.5 to 60 m.



**Figure 1.** Sampling locations.

Sea surface temperatures (SST) in the study area present minimum values in winter, an increase during spring, maximum values during summer and a progressive decrease towards lower values during autumn (Pastor et al., 2019). The Libyan Sea has warmer waters than the Cretan Sea, but is less productive. The abundance of Lessepsian migrants, including that of *L. sceleratus*, is higher in this region, as this species is often caught by local fishers (Christidis et al., 2021 and references therein).

Each fish was dissected after recording their total length (TL, mm), total weight (TW, g) and eviscerated weight (EW, g). TL ranged from 145 to 787 mm, and TW 36.7 to 4910 g. The specimens consisted of 112 males and 124 females. Following Christidis et al. (2021), the maturity stage of each specimen was identified macroscopically based on gonads, using a simplified maturity scale (0: virgin, 1: resting/early developing, 2: maturing, 3: spawning, 4: spent). Stage 0 specimens were considered as ‘immature’ and all others as ‘mature’ (Table 1).

**Table 1.** Number of collected *L. sceleratus* specimens, range and mean value of total length (TL, mm) by region, season (Winter: January-March. Spring: April-June. Summer: July-September. Autumn: October-December), sex (F: Female, M: Male) and maturity status.

<b>Region</b>	<b>Season</b>	<b>Sex</b>	<b>Maturity stage</b>	<b>N</b>	<b>mean TL (mm)</b>	<b>range TL (mm)</b>	
Cretan sea	Autumn	F	Immature	0			
			Mature	18	554	433-787	
	Spring	M	Immature	6	350	145-384	
			Mature	9	543	405-702	
		F	Immature	1	226		
			Mature	17	551	402-748	
	Summer	M	Immature	0			
			Mature	16	581	483-716	
		F	Immature	0			
			Mature	1	534		
	Winter	M	Immature	0			
			Mature	1	599		
		F	Immature	10	204	179-289	
			Mature	10	562	479-677	
	Libyan sea	Autumn	F	Immature	7	217	168-279
				Mature	9	484	361-577
M			Immature	9	290	179-354	
			Mature	10	527	418-582	
Spring		F	Immature	1	210		
			Mature	19	548	368-745	
		M	Immature	1	240		
			Mature	12	474	395-571	
Summer		F	Immature	2	236	189-251	
			Mature	14	479	396-590	
	M	Immature	6	329	225-378		
		Mature	10	525	436-702		

Gastrointestinal tracts were then weighed and dissected, and their contents examined. Feeding intensity (FI) was calculated for each specimen as the ratio between total intestine content weight and eviscerated weight. After removal of the food items from the tract, these were identified macroscopically, or with the help of a stereoscope, to the lowest taxonomic level possible. Fish otoliths, scales and cephalopod beaks aided in the identification. This process was hindered by advanced digested states and by the potent teeth of *Lagocephalus sceleratus*, which can easily crush food items (Kalogirou, 2013). Various textbooks (Bräger & Moritz, 2016; Clarke, 1986; Golani et al., 2006) and an otolith database (Parisi-Baradad et al., 2010) were used. The items were grouped into broad categories of non-food items (fishing gears, substrate, metal), food items (prey, baits, and discards from fisheries) and unidentified items. Prey items were further divided into eight general categories (cephalopods, crustaceans, fish, echinoderms, non-cephalopod molluscs, photosynthetic organisms, polychaetes, and coral fragments). Each item was weighted to the nearest 0.01 g. Their numerical amount was not used for analysis due to the nature of some of these (e.g. parts of substrate) and due to the feeding behaviour of the fish (e.g. cutting prey in many pieces), since it could bias the true number of ingested items.

The relative importance of each group category for the diet was quantified using two standard indices: frequency of occurrence (FO%) and average weight contribution (W%). The first was estimated as the percentage of non-empty tracts containing each category (Hyslop, 1980), as follows:

$$FO (\%) = \sum \frac{N_i}{N} * 100$$

with  $N_i$  representing the number of tracts containing the category  $i$  and  $N$  the total number of tracts found with items inside.

Average weight contribution of each category was calculated as follows:

$$Wc (\%) = \bar{x} \left( \frac{W_i}{W_t} * 100 \right)$$

with  $W_i$  representing the weight of items from category  $i$  in a tract,  $W_t$  the total item weight in the same tract, and  $\bar{x}$  mean value.

The probabilities of occurrence of the major prey categories (fish, crustaceans, non-cephalopod molluscs, photosynthetic organisms and cephalopods) in *L. sceleratus* tracts were investigated by means of Generalised Additive Model (GAM) techniques (Hastie & Tibshirani, 1990). Binomial GAMs assuming a logit link function were used. The main advantage of GAM

over traditional regression methods is its capability to model non-linear relationships, which is a common feature of many ecological datasets (Darbyson et al., 2003; Fleming et al., 2016), between a response variable and multiple explanatory variables using non-parametric smoothers. In the present case, the non-linear predictors included sampling depth ('DEPTH'), total fish length ('TL') and feeding intensity ('FI'). In addition, geographical region ('REGION'), season ('SEASON'), sex ('SEX') and fishery type ('CR') were entered as factors into the models. The smoother function used for the non-linear predictors was a penalised cubic regression spline and model fitting was accomplished using the *mgcv* library (Wood, 2006) under the R language environment. The procedure automatically selects the degree of smoothing based on the generalised cross-validation score, which is a proxy for the model's predictive performance. Moreover, the "select = TRUE" option was used, which shrinks to 0 any spurious model terms, indicating that they should not be included in the model. In order to avoid dubious relationships regarding depth and total length, the model was constrained to be at maximum a quartic relationship for those variables. Hence, the maximum degrees of freedom for the relevant smoothing terms, measured as the number of knots (k), was set to 4 (i.e. k=5 in the GAM formulation).

### **3. Results**

#### **3.1. General diet analysis**

Of the 236 tracts examined, 202 had at least one item and 34 were completely empty (14.4% of our total samples). The proportion of empty stomachs varied throughout the year (4.5% in spring, 23.5% in summer, 16.2% in autumn and 17.9% in winter). In total, 648 items (total weight 3377 g) were found, consisting mainly of food (560 items weighting 3273 g), whereas non-food (69 items, total weight 88.04 g) and unidentified (19 items, total weight 15.96 g) items were also recorded (Table 2).

Moreover, in two tracts only the baits/discards were found, eight tracts only contained non-food items, such as parts of substrate and a small square piece of metal (one tract), and in other two only unidentified items were found. Discards had been collected from fishers and then thrown to the sea during sampling to attract pufferfish. Regarding the non-food items, parts of substrate were mainly grains of sand (57% of the total substrate items), followed by pebbles (27%), stones (13%) and fragments of Foraminifera exoskeletons (3%). Fishing gears found were mainly pieces of net (88% of the total number of fishing gears recorded), hooks (11%) and lines (1%) (Fig. 1). The

highest percentage of fishing gear was recorded in the tracts of specimens caught by purse seines (25% of the total purse seine specimens), followed by specimens caught by longlines (22.2%) and static nets (19.6%) (Table 3).

**Table 2.** Frequency of occurrence (FO, %), number (N) and weight (W, g) of items found in *L. sceleratus* tracts by general category.

Categories		N of tracts	FO	N	W
Food	Preys	190	80.5	545	3124
	Baits	11	4.7	12	68
	Discards from fisheries	2	0.8	3	81
Non-food	Fishing gears*	36	15.3	38	65
	Substrate**	23	9.7	30	23
	Metal	1	0.4	1	0.04
Unidentified		17	7.2	19	16
Empty		34	14.4	0	0
Total				648	3377

\* Pieces of net, hooks and lines

\*\* Sand, stones, pebbles, exoskeleton of Foraminifera

**Table 3.** Number of collected specimens and percentage of tracts in which fishing gears were found, according to the gear by which the specimens were caught.

Fisheries		N of individuals	% of tracts with:		
			Net	Line	Hook
Commercial	Boat seine	29	0.0	0.0	0.0
	Purse seine	8	25.0	0.0	0.0
	Gillnets and Trammel nets	158	19.6	0.0	1.3
	Longlines	9	0.0	0.0	22.2
Recreational	Handlines	14	0.0	7.1	0.0
	Fishing rod	16	0.0	0.0	0.0
	Speargun	2	0.0	0.0	0.0
Total		236	14.0	0.4	1.7



**Figure 2.** Fishing gear (left: hooks; right. Fishing net) found within *L. sceleratus* tracts.

For an overall analysis of the item composition in the tracts of *L. sceleratus*, a total of 200 tracts were considered, with those that were empty or contained only baits and discards (not considered for our analysis) being excluded. Prey items were identified to the lowest taxonomic level possible, and their frequency of occurrence (FO, %) and weight percentage (W, %) are presented in Table 4.

**Table 4.** Frequency of occurrence (FO%) and weight percentage (W%) of prey items found in 190 *L. sceleratus* tracts.

Prey Category	Number of Tracts	FO%	Total Weight	W%
<b>Annelida</b>	<b>2</b>	<b>1.1</b>	<b>0.2</b>	<b>0.0</b>
Polychaeta	2	1.1	0.2	0.0
Sabellidae	1	0.5	0.1	0.0
<b>Crustacea</b>	<b>64</b>	<b>33.7</b>	<b>273.6</b>	<b>8.8</b>
Malacostraca	59	31.1	268.9	8.6
Amphipoda	3	1.6	0.6	0.02
Decapoda	50	26.3	263.7	8.4
Pleocyemata	48	25.3	263.5	8.4
Brachyura	42	22.1	220.9	7.1
Calappidae	1	0.5	7.9	0.3
<i>Calappa granulata</i>	1	0.5	7.9	0.3
Dromiidae	1	0.5	0.4	0.01
Inachidae	1	0.5	0.2	0.0
Inachus spp.	1	0.5	0.2	0.0
Parthenopidae	2	1.1	8.0	0.3
<i>Parthenopoides massena</i>	2	1.1	8.0	0.3
Polybiidae	1	0.5	9.5	0.3
<i>Liocarcinus</i> spp.	1	0.5	9.5	0.3
Portunidae	10	5.3	33.7	1.1
<i>Portunus segnis</i>	1	0.5	3.2	0.1
Anomura	9	4.7	40.1	1.3
Paguridae	3	1.6	22.5	0.7
Diogenidae	2	1.1	11.9	0.4

Isopoda	7	3.7	3.7	0.1
Cirolanidae	1	0.5	0.1	0.0
<i>Eurydice</i> spp.	1	0.5	0.1	0.0
<b>Chlorophyta</b>	<b>2</b>	<b>1.1</b>	<b>0.8</b>	<b>0.03</b>
Caulerpaceae	2	1.1	0.8	0.03
<i>Caulerpa prolifera</i>	2	1.1	0.8	0.03
<b>Chordata</b>	<b>148</b>	<b>77.9</b>	<b>2319.3</b>	<b>74.2</b>
Elasmobranchii	1	0.5	5.8	0.2
Batoidea	1	0.5	5.8	0.2
Teleostei	147	77.4	2313.5	74.1
Acanthuriformes	2	1.1	4.2	0.1
Siganidae	2	1.1	4.2	0.1
<i>Siganus luridus</i>	2	1.1	4.2	0.1
Atheriniformes	2	1.1	1.8	0.1
Atherinidae	2	1.1	1.8	0.1
<i>Atherina</i> spp.	1	0.5	0.4	0.01
Aulopiformes	6	3.2	141.6	4.5
Synodontidae	6	3.2	141.6	4.5
<i>Synodus saurus</i>	6	3.2	141.6	4.5
Carangiformes	5	2.6	10.2	0.3
Carangidae	5	2.6	10.2	0.3
<i>Trachurus</i> spp.	5	2.6	10.2	0.3
Clupeiformes	12	6.3	232.0	7.4
Alosidae	2	1.1	34.9	1.1
<i>Sardina pilchardus</i>	2	1.1	34.9	1.1
Clupeidae	8	4.2	109.4	3.5
<i>Sardinella</i> spp.	3	1.6	52.7	1.7
<i>Sardinella aurita</i>	2	1.1	47.3	1.5
Dussumieriidae	2	1.1	87.7	2.8
<i>Etrumeus golanii</i>	2	1.1	87.7	2.8
Eupercaria <i>incertae sedis</i>	41	21.1	753.8	24.1
Scaridae	3	1.6	51.7	1.7
<i>Sparisoma cretense</i>	3	1.6	51.7	1.7

Sparidae	38	20.0	702.0	22.5
<i>Boops boops</i>	11	5.8	132.1	4.2
<i>Dentex dentex</i>	2	1.1	18.6	0.6
<i>Pagellus erythrinus</i>	2	1.1	22.4	0.7
<i>Pagrus pagrus</i>	4	2.1	104.5	3.3
<i>Sarpa salpa</i>	3	1.6	146.8	4.7
<i>Diplodus</i> spp.	4	2.1	52.1	1.7
<i>Diplodus annularis</i>	2	1.1	41.6	1.3
<i>Spicara</i> spp.	10	5.3	107.1	3.4
<i>Spicara maena</i>	3	1.6	55.3	1.8
<i>Spicara smaris</i>	7	3.7	48.5	1.6
Kurtiformes	1	0.5	2.3	0.1
Apogonidae	1	0.5	2.3	0.1
<i>Apogon imberbis</i>	1	0.5	2.3	0.1
Mulliformes	17	8.9	286.3	9.2
Mullidae	17	8.9	286.3	9.2
<i>Mullus</i> spp.	12	6.3	220.7	7.1
<i>Mullus barbatus</i>	3	1.6	61.4	2.0
<i>Mullus surmuletus</i>	6	3.2	130.2	4.2
Ovalentaria incertae sedis	3	1.6	91.8	2.9
Pomacentridae	3	1.6	91.8	2.9
<i>Chromis chromis</i>	3	1.6	91.8	2.9
Perciformes	9	4.7	316.3	10.1
Scorpaenidae	4	2.1	159.9	5.1
<i>Pterois miles</i>	1	0.5	44.0	1.4
<i>Scorpaena</i> spp.	3	1.6	116.0	3.7
<i>Scorpaena porcus</i>	2	1.1	25.0	0.8
<i>Scorpaena scrofa</i>	1	0.5	35.5	1.1
Serranidae	1	0.5	2.1	0.1
Trachinidae	2	1.1	48.9	1.7
<i>Trachinus</i> spp.	2	1.1	48.9	1.7
Triglidae	1	0.5	14.0	0.4
<i>Chelidonichthys lastoviza</i>	1	0.5	14.0	0.4

Uranoscopidae	1	0.5	17.1	0.5
<i>Uranoscopus scaber</i>	1	0.5	17.1	0.5
Pleuronectiformes	2	1.1	6.8	0.2
Bothidae	1	0.5	2.4	0.1
Syngnathiformes	2	1.1	0.6	0.02
Syngnathidae	2	1.1	0.6	0.02
Tetraodontiformes	10	5.3	91.9	2.9
Tetraodontidae	10	5.3	91.9	2.9
<i>Torquigener flavimaculosus</i>	4	2.1	55.2	1.8
<b>Cnidaria</b>	<b>1</b>	<b>0.5</b>	<b>0.1</b>	<b>0.0</b>
Anthozoa	1	0.5	0.1	0.0
<b>Echinodermata</b>	<b>16</b>	<b>8.4</b>	<b>54.7</b>	<b>1.8</b>
Echinozoa	15	7.9	21.9	0.7
Echinoidea	8	4.2	21.9	0.7
Parachenidae	1	0.5	0.1	0.0
<i>Paracentrotus lividus</i>	1	0.5	0.1	0.0
Loveniidae	2	1.1	11.5	0.4
<i>Echinocardium</i> spp.	2	1.1	11.5	0.4
Asterozoa	7	3.7	32.8	1.1
Ophiuroidea	7	3.7	32.8	1.1
Ophiurida	6	3.2	4.8	0.2
<b>Mollusca</b>	<b>65</b>	<b>34.2</b>	<b>453.8</b>	<b>14.5</b>
Bivalvia	8	4.2	1.4	0.04
Cephalopoda	22	11.6	431.7	13.8
Sepiidae	6	3.2	54.4	1.7
<i>Sepia</i> spp.	6	3.2	54.4	1.7
<i>Sepia officinalis</i>	4	2.1	42.7	1.4
Octopodidae	7	3.7	368.1	11.8
<i>Octopus vulgaris</i>	7	3.7	368.1	11.8
Loliginidae	3	1.6	1.4	0.04
<i>Loligo vulgaris</i>	1	0.5	0.01	0
<i>Sepioteuthis lessoniana</i>	1	0.5	0.5	0.02

Gastropoda	39	20.5	20.6	0.7
Cavoliniidae	18	9.5	6.0	0.2
<i>Cavolinia</i> spp.	11	5.8	3.4	0.1
<i>Diacria</i> spp.	14	7.4	2.7	0.1
Creseidae	3	1.6	1.9	0.1
<i>Creseis</i> spp.	3	1.6	1.9	0.1
Cerithiidae	1	0.5	0.1	0.0
<i>Bittium</i> spp.	1	0.5	0.1	0.0
Hyalocyliidae	5	2.6	0.9	0.03
<i>Hyalocyliis</i> spp.	5	2.6	0.9	0.03
Muricidae	2	1.1	9.4	0.3
<i>Hexaplex trunculus</i>	2	1.1	9.4	0.3
Nassariidae	3	1.6	1.7	0.05
<i>Tritia</i> spp.	3	1.6	1.7	0.05
<i>Tritia mutabilis</i>	2	1.1	1.5	0.05
<i>Tritia turulosa</i>	1	0.5	0.2	0.0
Scaphopoda	3	1.6	0.2	0.0
<b>Ochrophyta</b>	<b>1</b>	<b>0.5</b>	<b>0.01</b>	<b>0</b>
<b>Thraceophyta</b>	<b>28</b>	<b>14.7</b>	<b>15.4</b>	<b>0.5</b>
Cymodoceaceae	7	3.7	0.1	0.0
<i>Cymodocea nodosa</i>	7	3.7	0.1	0.0
Posidoniaceae	7	3.7	0.8	0.03
<i>Posidonia oceanica</i>	7	3.7	0.8	0.03

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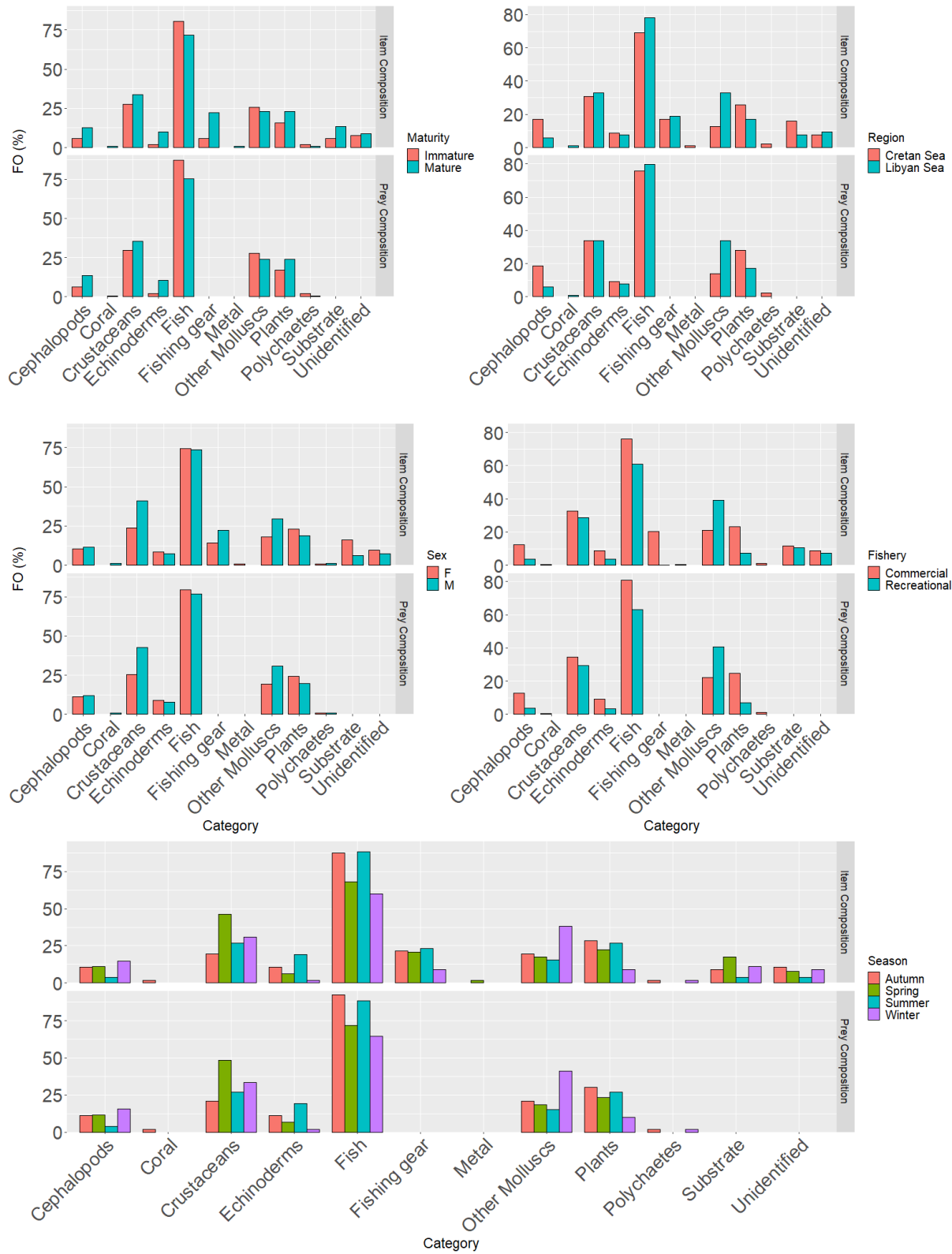
### 3. 1. 1. Frequencies of occurrence of each group

The general frequency of occurrence of each prey and item category are presented in Table 5. The frequency of occurrence according to maturity stage, sex, geographical region and fishery type for total items and prey only are presented in Table A1 and A2, respectively, of Appendix A. The most common items found in *L. sceleratus* tracts were fish, crustaceans, non- cephalopod molluscs, and parts of photosynthetic organisms. Fishing gear, substrate parts and cephalopods were also frequent (Table 5).

**Table 5.** Frequency of occurrence (FO) of each category of prey and other items in 190 *L. sceleratus* tracts containing only food items and 200 tracts containing both food and non-food.

	Group	Number of tracts	FO% (190 tracts)	FO% (200 tracts)
Prey	Cephalopods	22	11.6	11.0
	Coral	1	0.5	0.5
	Crustaceans	64	33.7	32.0
	Echinoderms	16	8.4	8.0
	Fish	148	77.9	74.0
	Non-cephalopod molluscs	47	24.7	23.5
	Photosynthetic organisms	42	22.1	21.0
	Polychaetes	2	1.1	1.0
Other Items	Fishing Gear	36	-	18.0
	Metal	1	-	0.5
	Substrate	23	-	11.5
	Unidentified	17	-	8.5

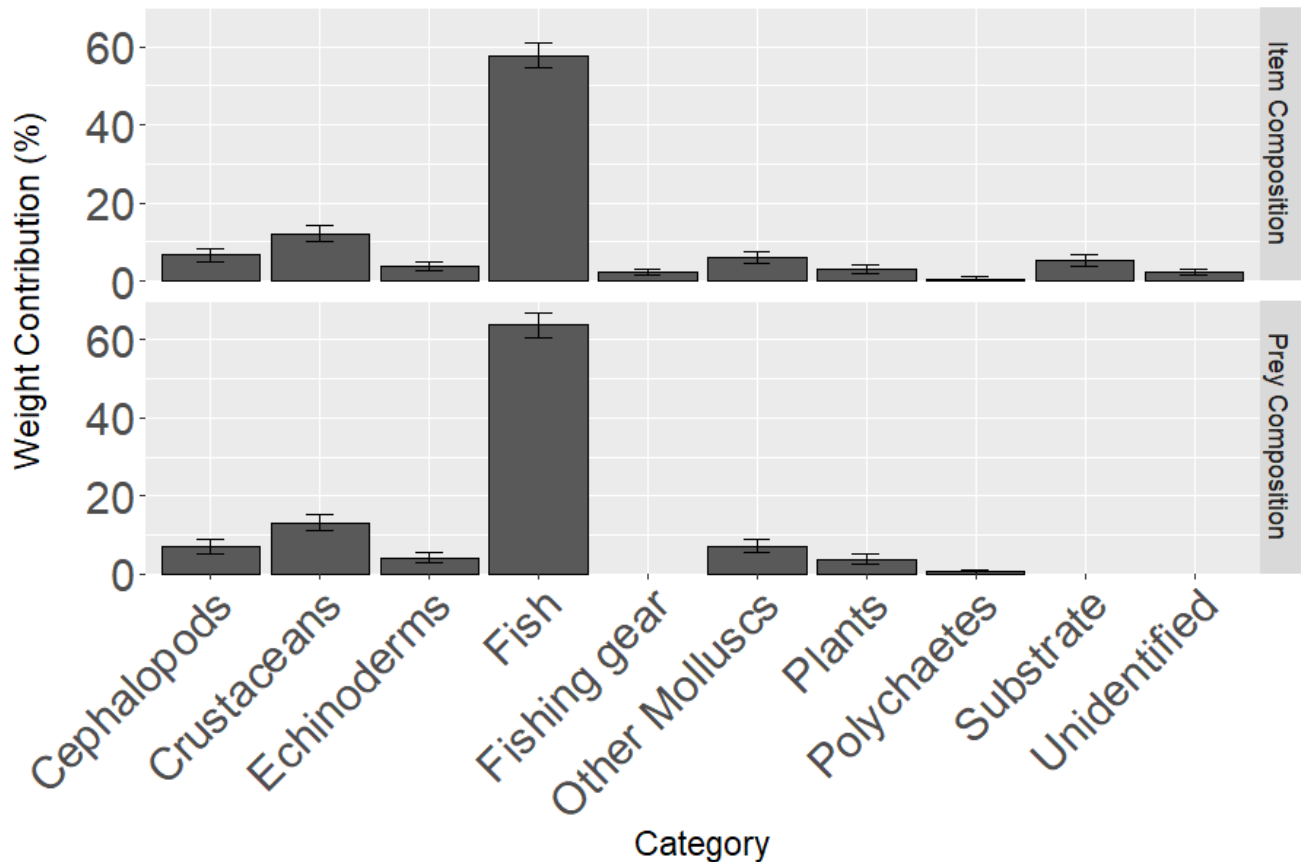
Regarding the maturity stage, we found a higher frequency of fish within tracts belonging to immature specimens. On the other hand, cephalopods, echinoderms, fishing gear and substrate were at least two times more frequent in mature specimens. Still, prey composition is overall very similar between the two (Fig. 3). When comparing the area from which the fish were sampled, differences in frequency were most remarkable for molluscs, as cephalopods occurred more in the fish caught in the north of Crete, and other molluscs were more frequent in those caught in the south. Additionally, while fish and crustaceans were still the most frequent prey in both regions, the third most frequent differed, with other molluscs being as important as crustaceans in the Libyan Sea whilst in the Cretan Sea it was algae and seagrass (Fig. 3). With regard to sex, males seem to feed more often on non-cephalopod molluscs and crustaceans, whilst substrate parts occurred almost three times more frequently in females. While fish and crustaceans are the most frequent prey for both, the third was non-cephalopod molluscs in the case of males and photosynthetic organisms in the case of females (Fig. 3). Fish, cephalopods, gear and photosynthetic organisms were found more frequently in those specimens caught by commercial fisheries, while other molluscs were more frequent in the ones caught by recreational types. In this latter case, non-cephalopod molluscs were the second most common prey, while in commercial-type specimens, it was the crustaceans (Fig. 3). Finally, we found differences in FOs throughout the year. While in wintertime fish and molluscs were the most frequent prey, in springtime it was fish and crustaceans, in summertime fish and photosynthetic organisms plus crustaceans, and in autumn fish and photosynthetic organisms. Comparing each category, fish were more frequent in the second part of the year, non-cephalopod molluscs specially in winter and crustaceans during spring. On the other hand, cephalopods were barely found during summer, and echinoderms during winter (Fig. 3).



**Figure 3.** Frequency of occurrence of each category, by maturity stage, geographical region, sex, fishery type and season. ‘Other Molluscs’: non-cephalopod molluscs; ‘Plant’: photosynthetic organisms.

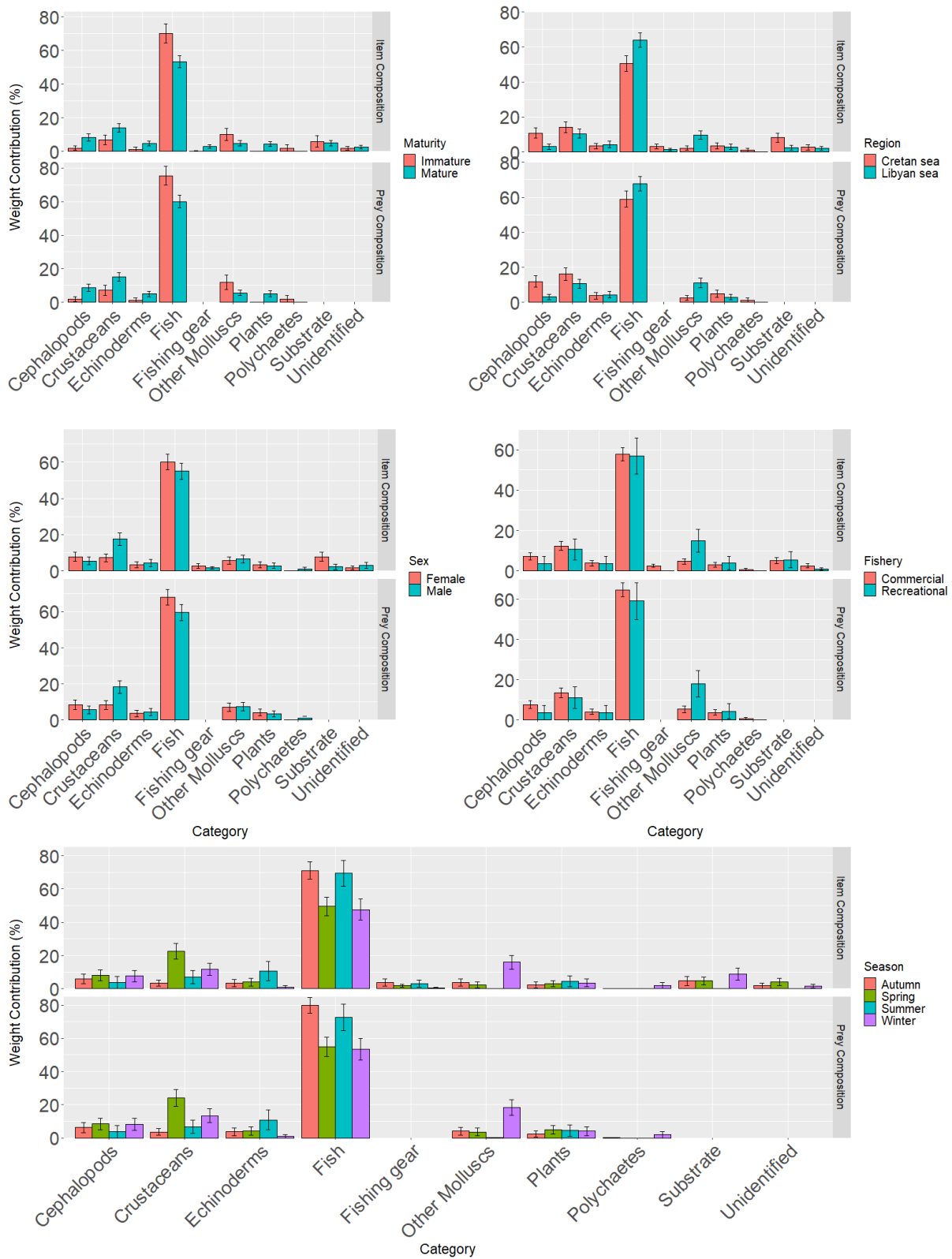
### 3. 1. 2. Average individual weight contribution of each group

The average weight contribution in a tract for each category (according to maturity stage, sex, season, geographical region and fishery type) for total items and prey only are presented in Table B1 and B2, respectively, of Appendix B. Similarly to what was found in regards to FO, when we take into consideration the average weight contribution per tract fish were the most abundant category, both when concerning all items found in the gastrointestinal tracts ( $57.7 \pm 3.1\%$ ) or only food prey ( $63.8 \pm 3.2\%$ ). In both of these circumstances, crustaceans were the second most important ( $12.1 \pm 2.0\%$  and  $13.2 \pm 2.2\%$ , respectively). Substrate parts were also abundant, averaging  $5.2 \pm 1.5\%$ . Average coral and metal weight contributions were insignificant (Fig. 4).



**Figure 4.** Average weight contribution (%) of each group in 190 *L. sceleratus* tracts containing food items (Prey Composition) and 200 tracts containing both food and non-food items (Item Composition). ‘Other Molluscs’: non-cephalopod molluscs; ‘Plant’: photosynthetic organisms. Coral and metal were excluded due to extremely small values. Error bars represent standard errors.

Average contribution of ingested fish and non-cephalopod molluscs was higher in immature *L. sceleratus*. In mature individuals, crustaceans were the second most abundant, and, along with cephalopods, their weight was on average greater. Only in mature specimens were photosynthetic organisms and fishing gear a considerable percentage of the weight (Fig. 5). Regarding region, substrate and cephalopods made up on average a bigger part of the weight found in the tracts of specimens caught in the Cretan Sea when compared to the Libyan Sea. However, other molluscs and fish appear to contribute more to Libyan Sea individuals (Fig. 5). When comparing sex, average substrate weight contribution was higher in the tracts of female specimens, while that of crustaceans was substantially larger in males, being the most important group along with fish (Fig. 5). Average weight contribution of each category was similar for specimens caught by recreational and commercial fisheries, except for non-cephalopod molluscs (higher in those caught by recreational methods) and fishing gear (higher in those by commercial ones) (Fig. 5). Although fish were always the most important weight-wise throughout the year, their weight contribution was highest in autumn and summer. During the first, this group was by far the most abundant, with other groups contributing very little. Meanwhile, during summer the contribution of substrate and non-cephalopod molluscs was negligible. Fishing gear and echinoderms were least important in wintertime, but non-cephalopod molluscs contributed more in this season. During spring, crustaceans were more abundant compared to other seasons, accounting on average for more than one fifth of the diet weight and being the most important prey after fish (Fig. 5).



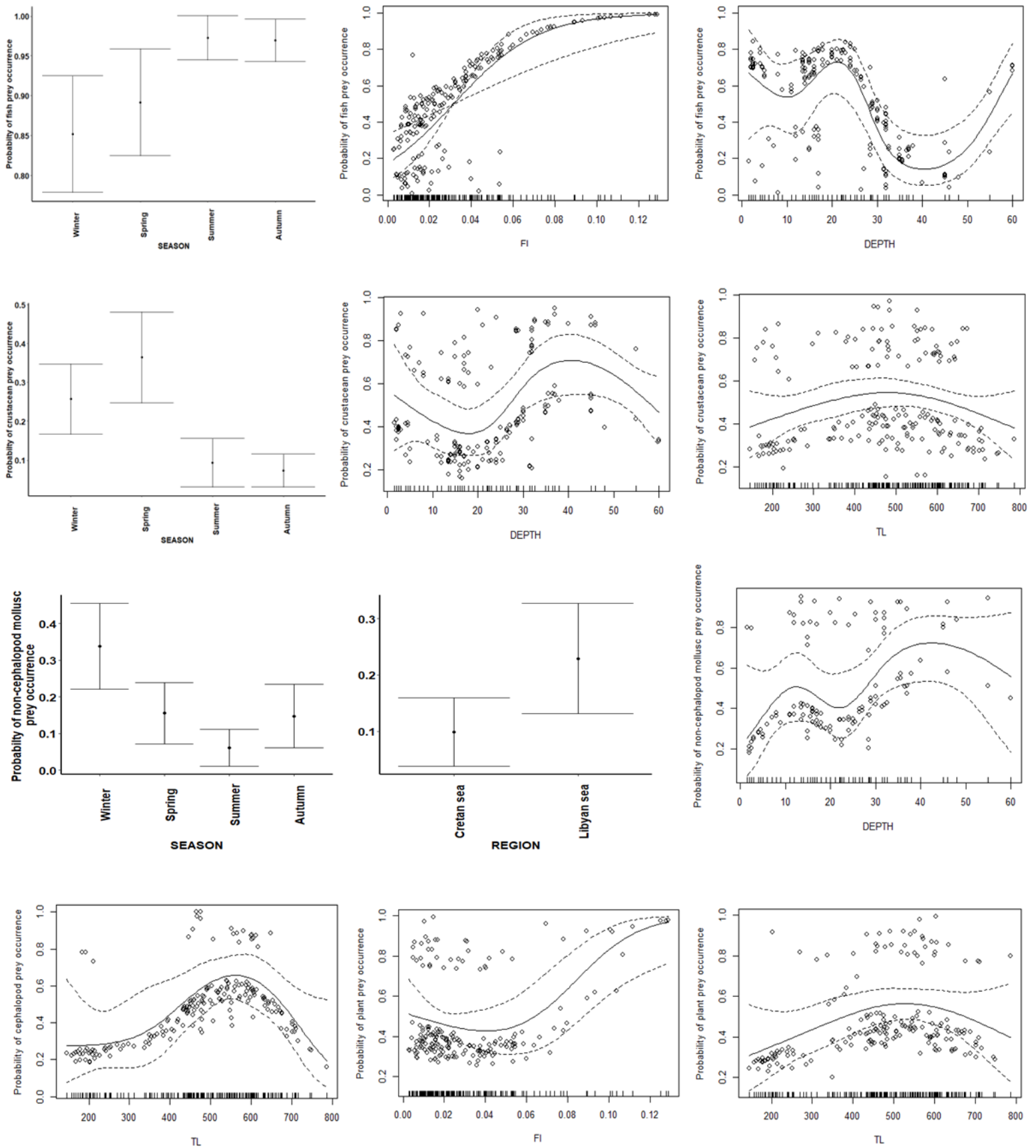
**Figure 5.** Average weight contribution of each group in 190 *L. scleratus* tracts containing food items (Prey Composition) and 200 tracts containing both food and non-food items (Item Composition) by maturity stage, geographical region, sex, fishery type and season. ‘Other Molluscs’: non-cephalopod molluscs; ‘Plant’: photosynthetic organisms. Coral and metal were excluded due to extremely small values. Error bars represent standard errors.

### **3. 2. General additive models (GAMs) for the probability of occurrence of the main prey groups**

The final GAM for fish probability of occurrence revealed that the effects of season, FI and depth at which fish were caught were significant ( $p \leq 0.05$ ), and the model explained 24.18% of the total deviance (Table 6). With regards to depth, it suggested a peak in probability at 20-25 m, followed by a decline in deeper zones, with a minimum probability around 40 m. Probability increased with FI. The effect of season revealed a higher probability of fish consumption during summer and autumn (Fig. 6). Meanwhile, the final GAM fitted for crustacean probability of occurrence explained only 14% of the total deviance, and included the significant variables season, TL and depth ( $p \leq 0.05$ ) (Table 6). Probability of occurrence decreases around 20 to 25 m and increases afterwards, peaking at 40 m. It is significantly higher in winter and spring. Additionally, it seems to be slightly higher when fish are mid-sized (circa 450 mm) (Fig. 6). The model for non- cephalopod molluscs ('Molluscs') had as significant ( $p < 0.05$ ) variables depth, season, and geographical region, which together explained 17.4% of the total deviance (Table 6). The relationship between depth and probability of mollusc consumption seemed similar to that in the model fitted for crustaceans, with a minimum around 22 m and a maximum around 40 m. Probability was higher in winter and in the Libyan Sea (Fig. 6). In the model fitted for algae and seagrass ('Plant'), both FI and TL had a significant effect ( $p \leq 0.05$ ), although explaining only 17% of the probability of occurrence of photosynthetic organisms (Table 6). Plant consumption seems to be higher in medium-sized fishes as well (around 520 mm TL), and when FI is high (Fig. 6). The model for cephalopod probability included the significant variable TL ( $p = 0.02$ ), which explained 21.9% of the total deviance (Table 6). The probability of occurrence of cephalopods is positively influenced by size, being maximal at 550 mm TL (Fig. 6).

**Table 6.** Results of the GAMs performed for the probability of occurrence of the main prey of *L. sceleratus* (fish, crustaceans, non-cephalopod molluscs ('Molluscs'), photosynthetic organisms ('Plant'), and cephalopods). The statistical significance of the explanatory variables of each model is shown.

	$\chi^2$	<i>p</i> -value
<b>Fish (deviance explained: 24.8%)</b>		
DEPTH	11.91	0.00
CR	0.48	0.49
FI	12.71	0.00
SEASON	7.78	0.05
SEX	0.00	0.98
REGION	3.27	0.07
<b>Crustaceans (deviance explained: 14%)</b>		
DEPTH	6.77	0.02
CR	3.28	0.07
SEASON	17.06	0.00
SEX	2.83	0.09
REGION	0.03	0.87
TL	2.09	0.05
<b>Molluscs (deviance explained: 17.4%)</b>		
DEPTH	7.32	0.04
CR	0.50	0.48
SEASON	8.93	0.03
SEX	0.85	0.36
REGION	4.40	0.04
<b>Plant (deviance explained: 17%)</b>		
CR	2.20	0.14
FI	9.67	0.00
SEASON	4.54	0.21
SEX	3.15	0.08
REGION	0.64	0.42
TL	3.01	0.04
<b>Cephalopod (deviance explained: 21.9%)</b>		
CR	1.52	0.22
FI	8.56	0.34
SEASON	3.55	0.31
SEX	0.01	0.91
REGION	1.95	0.16
TL	6.02	0.02



**Figure 6.** Plots showing the results of the GAMs performed. Only significant variables for each model are shown.

## 4. Discussion

Analysis of the tract contents show that the *L. sceleratus*' diet is generalist, mostly composed of fish and supplemented by crustaceans and molluscs. A comparison of other studies with the present one is found in table 7. Our results are in agreement with previous findings for Crete, Cyprus, Lebanon, Turkey and Syria (Akboru et al., 2020; Boustany et al., 2015; Christidis et al., 2021; Hammoud & Salama, 2016; Michailidis, 2010; Torcu-Koç et al., 2020). On the other hand, cephalopods were the main prey in the Suez Canal (Sabrah et al., 2006) and Ain El- Ghazala coast, Libya (Hussain et al., 2020), shrimps and crabs in other studies from Turkey (Aydin, 2011; Ulman, Yildiz, et al., 2021) and molluscs in Rhodes, Greece (Kalogirou, 2013). No study on the diet of this species in its native range was found. However, a swimming crab has been discovered in a tract of *L. sceleratus* from the Indian Ocean (Romanov et al., 2009).

The diet of this teleost is highly variable, but mostly carnivorous, similar to what has been reported for many other Tetraodontidae species. For instance, *L. spadiceus* in the Arabian Sea feeds mainly on benthic fishes, crustaceans and cephalopods (Devi & Sivan, 2017), while *L. inermis* mostly preys on fish and squid (Seetha et al., 2023). *L. lagocephalus* is reported to feed mostly on crustaceans and cephalopods (Tortonesi, 1986). In Brazil, *L. laevigatus* frequently feeds on fish, crustaceans and, additionally, cnidarians (Denadai et al., 2012). *L. lunaris* in South China Sea was found to consume mostly crabs and prawns, and to some extent also small fish and squids (Mohamad & Fadhilah, 2013). In the Mediterranean, *Torquigener flavimaculosus* was found to feed mostly on benthic invertebrates, as the species feeds on a high amount of crustaceans, gastropods and echinoderms, but few fish (Chartosia et al., 2021; Ulman et al., 2023).

Pufferfish have been establishing further into the Mediterranean, indicating that conditions for the growth and survival of this NIS are improving (Farrag et al., 2015). Particularly for the silver-cheeked toadfish, this expansion leads to major environmental impacts through predation (Galanidi et al., 2018). The silver-cheeked toadfish can be classified as a top predator, especially when taking into account the prevalent overfishing in the eastern Mediterranean, which has possibly led to important losses of native predators (Ulman, Yildiz, et al., 2021). The fact that inside the tracts we found pebbles, sand, numerous pieces of fishing gear, and even a piece of metal in addition to food shows that this species is capable of ingesting a wide variety of items, largely affecting the fishing industry and the ecosystems they have invaded (Boustany et al., 2015)

**Table 7.** Comparison of tract content findings.

<b>Reference</b>	<b>Region</b>	<b>Fish (%)</b>	<b>Crustacea (%)</b>	<b>Other Molluscs (%)</b>	<b>Cephalopods (%)</b>	<b>Echinoderms (%)</b>	<b>Fishing Gear (%)</b>
<b>Akbora et al. 2020 (FO%)</b>	Cyprus (North)	43	22		14		21
<b>Aydin et al. 2011 (W%)</b>	Turkey (Levantine)	14	71		4		
<b>Boustany et al. 2015 (FO%)</b>	Lebanon	38	15		14		12.6
<b>Christidis et al. 2021 (FO%)</b>	Crete, Greece	76	29.6	8.4	16.9	5.6	
<b>Hussain et al. 2020 (V%)</b>	Ain El-Ghazala, Libya	10.2	17.4		72.5		
<b>Hussain et al. 2020 (V%)</b>	Derna, Libya	100					
<b>Kalogirou 2013 (FO%)</b>	Rhodes, Greece	5.2	18.6	14.5	28.6		
<b>Michailidis 2010 (FO%)</b>	Cyprus	27	7.3		4.5	0.2	8.6
<b>Sabrah et al. 2006 (FO%)</b>	Suez Canal	5	25		70		
<b>Torcu-Koç et al. 2020 (FO%)</b>	Turkey (Levantine)	41.2	12.3		18.7		<2%
<b>Ulman et al. 2021 (FO%)</b>	Turkey (Aegean and Levantine)	24	26		11		48 hooks, 9 nets, 2 wires
<b>Hammoud and Salama 2016 (FO%)</b>	Syria	46.5	5.9		12.8	17.7	
<b>This study (FO%)</b>	Crete, Greece	74	32	23.5	11	8	18

The number of empty stomachs is overall low, most likely due to its generalist character and strong teeth (Ulman, Yildiz, et al., 2021). The proportion of empty stomachs was greatest in the summer and then gradually declined until spring, when it is extremely low. This could be related to its spawning period, which occurs from spring until summer (Peristeraki et al., 2010). Lower feeding intensity during the spawning period is common in teleosts, since gonads take up more space (Chartosia et al., 2021; Hussain et al., 2020). Another explaining factor can be the greater sea surface temperatures during summer, which lead to increased digestion and gastrointestinal evacuation rates (Volkoff & Rønnestad, 2020). In Libya, Hussain et al. (2020) also observed lower feeding intensity during summer months and high intensity during spring. Studies in the Suez Canal (Sabrah et al., 2006) and Lebanon (Boustany et al., 2015) likewise found the percentage of empty stomachs to be lower during spring, but the overall amount was higher in comparison to our study. Meanwhile in Cyprus, feeding intensity was found to be lower but stable throughout the year (Michailidis, 2010), while in Rhodes feeding decreased during winter and autumn (Kalogirou, 2013).

We found *L. sceleratus* to be overwhelmingly piscivorous. Fish were found in the majority of the tracts, which is consistent with earlier results for Crete (Christidis et al., 2021). The most significant families were Sparidae (namely *Diplodus spp.*, *Spicara spp.* and the species *Boops boops*) and Mullidae (*Mullus spp.*). Along with Derna, Libya, where all the specimens fed solely on fish (Hussain et al., 2020), Crete appears to be the area with the highest consumption of this group. As Akbora et al. (2020) mention, these findings could be influenced by the type of fishery from which the specimens were collected, since this species often practices depredation on nets. Indeed, specimens caught by commercial fisheries had a greater frequency of fish in their tracts. In addition to that, fishing nets were frequently found (mostly in mature specimens) and many times had fish wrapped within them, demonstrating that this tetraodontid is capable of attacking the catch in the nets and ripping them apart. Lines and hooks occurred at a lower frequency, possibly due to the ease with which hooked fish can be eaten (Boustany et al., 2015). Still, hooks were discovered not only in longline specimens but also in those collected by nets, indicating *L. sceleratus* has the ability to ingest them without lethal harm. It is undeniable that this species is a nuisance, regularly damaging gears and feeding on catch targets. According to Michailidis (2010), this slow-swimming fish uses the nets to prey on faster fish that have been entangled, which can either imply high intelligence or simply opportunistic behaviour. In Crete, the main species damaged in nets and

longlines were *P. pagrus*, *D. sargus* and *M. surmuletus* (Christidis et al., 2022) and in Gökova bay, Turkey, fishers also mentioned a big decrease in *M. surmuletus* catches (Galanidi et al., 2018), which we can associate with the invasive behaviour of this pufferfish. Nevertheless, even without depredation fish would remain the primary prey of *L. sceleratus*, since the average weight contribution of fish in specimens captured by commercial and recreational gear was similar and fishing type had no significant effect in our model for fish occurrence. For crustaceans, crabs were the major taxon we found, which can be expected, as their chitinous exoskeletons and Anomura shells are harder to break down (Ulman, Yildiz, et al., 2021). Echinoderms seem also to play a somewhat important role in Crete, especially when compared to other regions in the Mediterranean, where they were so far seldom reported. An exception is the study by Hammoud and Salama (2016), in which this taxon was found in 17.7% of the tracts. Another interesting finding is the presence of polychaetes in two tracts, as it seems to be the first time the silver-cheeked toadfish is reported to feed on this group. Photosynthetic organisms (algae and seagrasses), although not paramount in terms of weight, were found to occur very frequently, akin to no other study bar the one by Christidis et al. (2021). Whether the silver-cheeked toadfish is truly assimilating plant material or this is simply the result of benthic feeding and prey capture should be investigated. In this study, parts of substrate occurred on circa 11% of tracts, which can further suggest some specimens dig on the bottom to capture their prey (Ulman, Yildiz, et al., 2021). In terms of overall molluscs, gastropods occurred most frequently, with the family Cavoliniidae being the most significant. However, cephalopods had the biggest contribution in terms of weight. The major prey was *Octopus vulgaris*, which in Crete is often found damaged within nets (Christidis et al., 2022). In some regions of the eastern Mediterranean, fishers complain that the expansion of the silver-cheeked toadfish has been causing a decline in squid and octopus stocks (Kalogirou, 2013; Michailidis, 2010; Ünal & Göncüoğlu Bodur, 2017). A high feeding rate on cephalopods would mean *L. sceleratus* is thus, once more, directly yet also indirectly affecting fishers, by also impacting other commercial species that rely on cephalopods for food (Ünal & Göncüoğlu Bodur, 2017). As Ulman et al. (2021) suggests, a wide decline in cephalopod populations might be one of the reasons why their occurrence in this study is lower when compared to older ones (e.g. Hussain et al., 2020; Kalogirou, 2013; Sabrah et al., 2006), but it is challenging to truly quantify predation impacts, thanks to the lack of assessments on local invertebrates stocks and advanced digestion states (Kalogirou, 2013).

It is worth noting that prey items of *L. sceleratus* rapidly transform into digested items and lose their visually identifiable characteristics (Ulman, Harris, et al., 2021), making it difficult to identify prey to lower taxonomic levels unless they have been recently eaten, which potentially skews results towards taxa with hard parts, such as bones, otoliths, cephalopod beaks, or shells.

Despite all the reported threats this species brings about to the Mediterranean, *L. sceleratus* might have one useful effect, as it potentially helps to control invasive populations through predation, causing great ecological impacts on other NIS (Chaikin et al., 2022; Ulman, Yildiz, et al., 2021). In this study, non-indigenous prey were found within ten tracts, making up 6.2% of prey weight. Along with the decapod *Portunus segnis* and the teleosts *Siganus luridus*, *Pterois miles*, *Torquigener flavimaculosus* and *Etrumeus golanii* that we identified, other reported NIS prey in the Mediterranean include *L. suezensis*, *Fistularia commersonii*, *Upeneus moluccensis*, *Equulites klunzingeri*, *Stephanolepis diaspros*, *Parupeneus forsskali*, *Plotosus lineatus*, *Saurida lessepsianus*, *Stephanolepis diaspros*, *Siganus rivulatus*, gastropods such as *Cerithium scabridum*, the sea urchin *Diadema setosum*, the cephalopod *Sepia prashadi* and other crustaceans such as *Erugosquilla massavensis*, *Thalamita poissonii*, *Charybdis feriata*, *C. hellerii*, *C. longicollis*, *Myra subgranulata*, *Macrophthalmus (Macrophthalmus) indicus*, *Dorippe quadridans* and *Arcania brevifrons* (Chaikin et al., 2022; Chartosia et al., 2021; Gabel et al., 2022; Hussain et al., 2020; Kondylatos et al., 2023; Ulman, Yildiz, et al., 2021). Although in this study cannibalistic tendencies were not found, this behaviour has been described both in this species' native range and the Mediterranean (Ulman, Yildiz, et al., 2021). Curiously, off the Israeli coast the majority of species-level prey identified are Lessepsian migrants (Chaikin et al., 2022; Gabel et al., 2022), which is probably related to this region's closeness to the Suez Canal.

This study found that overall, the main prey of immature and mature individuals were the same, and this species feeds on all types of prey throughout its life, suggesting that it is adaptable in its feeding behaviour (Michailidis, 2010). Based on frequency and weight contribution, immature specimens appear to consume more fish and non-cephalopod molluscs than mature specimens, which in comparison might feed more on crustaceans and cephalopods. However, our models show size (directly correlated to maturity stage) to have an effect only in the probability of occurrence of photosynthetic organisms and cephalopods, which both play a bigger role in the diet of larger specimens. Ulman, Yildiz, et al. (2021) found prey composition to not differ significantly between juvenile and non-juvenile specimens, but our results are in partial agreement with Hussain et al.

(2020), as it showed dependence on crustaceans increased as specimens grew, and with Kalogirou (2013), which showed that with higher body sizes, *L. sceleratus* shifted to a molluscivore feeding, heavily preying on *Sepia officinalis* and *Octopus vulgaris*. An ontogenetic diet shift is possibly related to habitat change, which happens when fish reach maturity (Kalogirou, 2013). While early-life stages tend to cluster in sandy bottoms, older specimens move over to seagrass meadows (Kalogirou, 2013). Plus, morphological changes may be a factor, as older fishes have bigger mouths better equipped to ingest large prey (Hussain et al., 2020), such as cephalopods and crabs.

Similarly, differences in diet amongst geographical areas can also be attributed to habitat differences between both sites, and these variations in prey selectivity indicate the ability of a species to adapt to a variety of environmental conditions (Aydin, 2011; Torcu-Koç et al., 2020). Previous preliminary results for Crete had shown the diet of *L. sceleratus* to differ significantly between the Libyan and Cretan seas (Christidis et al., 2021). In addition to habitat and biodiversity differences, it is possible some of the disparities in the diet composition we found between south and north Crete are explained by the different fishing gears used for these areas, since approximately 98% of the Cretan Sea specimens were caught using commercial gears, whereas in the Libyan Sea only 77%. Cephalopods were more important for specimens obtained by this fishery type, while other molluscs were less. The same was found for Cretan Sea specimens. The fact that non-cephalopod molluscs are more prone to be consumed by individuals from the Libyan Sea is interesting, considering that mean TTX is higher in fishes from this area compared to the Cretan Sea (Christidis et al., 2021) and that many gastropods and bivalves contain this toxin (Lobo de Sousa, 2011; Rambla-Alegre et al., 2017), amongst them the family Muricidae and the genus *Nassarius* (Biessy et al., 2019), both preyed by *L. sceleratus* (Kalogirou, 2013). Besides, some pufferfish species become non-toxic when they inhabit an environment where TTX-bearing organisms are absent from their food web (Nader et al., 2012). Diet is probably related to TTX accumulation in tissues and might be largely responsible for the individual variations in toxin concentration found between specimens (Christidis et al., 2021).

In our study, fish contributed more to the diet of the silver-cheeked toadfish during summer and autumn. On the other hand, crustacean consumption peaked in spring and winter. Molluscs were vastly more important during winter, barely being consumed in summertime. Except for fish, the findings are similar to those of Torcu-Koç et al. (2020), but they contrast with the results of Hussain et al. (2020), which showed molluscs importance steadily increased throughout the year,

whilst crustaceans and fish observed the opposite trend. Differences in diet amongst seasons can be related to a change in habitat and maturity as populations evolve throughout the year (Kalogirou, 2013). For instance, abundance is high over seagrass patches over summer, while reproductive cohorts leave these spawning sites to move to deeper waters or rocky bottoms during autumn and winter (El-Haweeet et al., 2016; Galanidi et al., 2018; Kalogirou, 2013). Our models showed depth also influenced the probability of occurrence of fish, crustaceans, and non- cephalopod molluscs. It seems *L. sceleratus* ingests fish more often at shallower depths, and barely consumes this group at depths higher than 30m. On the other hand, depth had the opposite effect on the probability of occurrence of crustaceans and molluscs, which was maximal at circa 40 m. It appears that the probability of consuming fish is inversely correlated to that of consuming these two groups. This might be related to water temperature, as colder waters render fish less mobile and reduce their swimming speed (Wardle, 1980). It is possible that during winter and spring months, when SST is lower, this species opts to consume low-mobility prey such as benthic crustaceans and bivalves, while in summer it prefers to prey on fish. The same reason might explain why the probability of fish occurrence is lower at higher depths.

## 5. Conclusions

This study showed *Lagocephalus sceleratus* is a generalist species with a preference for fish. The diet of this invasive pufferfish seems to be influenced by area, season and size, whilst sex did not seem to greatly affect their feeding habits. These findings enrich our knowledge of the feeding biology of this Lessepsian migrant in the eastern Mediterranean. The data further demonstrate that this species severely impacts small-scale fisheries by simultaneously consuming targeted catch and damaging fishing gear, particularly nets. Furthermore, it adds credibility to the notion that this expansion can lead to the depletion of cephalopod populations. Given the potential ecological threat posed by *L. sceleratus*, which competes with native or even other invasive carnivores, as well as the sociological threat resulting from its high toxicity and pest-like behaviour, additional research is vital to minimise the current effects of its invasion, and to predict upcoming impacts. Due to the effect their diet possibly has on TTX concentrations, future studies should focus on this relationship. For more accurate results, we suggest DNA analysis tools could be employed. The possible effect of the gear from which these fish are captured should also be taken into account, due to the many evidence of depredation.

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## Appendix A

**Table A1.** FO (%) of each item category, per maturity stage, sex, region, fishery type and season.

Group	Maturity		Sex		Region		Fishery Type			Season		
	Immature	Mature	F	M	Cretan Sea	Libyan Sea	Recreational	Commercial	Winter	Summer	Spring	Autumn
Cephalopods	5.9	12.8	10.5	11.6	17.0	5.7	3.6	12.2	14.6	3.9	11.1	10.7
Coral	0	0.7	0	1.1	0.0	0.9	0.0	0.6	0.0	0.0	0.0	1.8
Crustaceans	27.5	33.6	23.8	41.1	30.9	33.0	28.6	32.6	30.9	26.9	46.0	19.6
Echinoderms	2.0	10.1	8.6	7.4	8.5	7.6	3.6	8.7	1.8	19.2	6.4	10.7
Fish	80.4	71.8	74.3	73.7	69.2	78.3	60.7	76.2	60.0	88.5	68.3	87.5
Fishing gear	5.9	22.2	14.3	22.1	17.0	18.9	0.0	20.4	9.1	23.1	20.6	21.4
Metal	0	0.7	1.0	0	1.1	0.0	0.0	0.6	0.0	0.0	1.6	0.0
Non-cephalopod molluscs	25.5	22.8	18.1	29.5	12.8	33.0	39.3	20.	38.2	15.4	17.5	19.6
Photosynthetic organisms	15.7	22.8	22.9	19.0	25.5	17.0	7.1	23.3	9.1	26.9	22.2	28.6
Polychaetes	2.0	0.7	1.0	2.0	2.1	0.0	0.0	1.2	1.8	0.0	0.0	1.8
Substrate	5.9	13.4	16.2	6.3	16.0	7.6	10.7	11.6	10.9	3.9	17.5	8.9
Unidentified	7.8	8.7	9.5	7.4	7.5	9.4	7.1	8.7	9.1	3.9	7.9	10.7

**Table A2.** FO (%) of each prey category, per maturity stage, sex, region, fishery type and season.

Group	Maturity		Sex		Region		Fishery Type			Season		
	Immature	Mature	F	M	Cretan Sea	Libyan Sea	Recreational	Commercial	Winter	Summer	Spring	Autumn
Cephalopods	6.4	13.4	11.2	12.1	18.6	5.8	3.7	13.0	15.7	3.9	11.7	11.3
Coral	0.0	0.7	0.0	1.1	0.0	1.0	0	0.6	0.0	0.0	0.0	1.9
Crustaceans	29.8	35.2	25.5	42.9	33.7	33.7	29.6	34.6	33.3	26.9	48.3	20.8
Echinoderms	2.1	10.6	9.2	7.7	9.3	7.7	3.6	9.3	2.0	19.2	6.7	11.3
Fish	87.2	75.4	79.6	76.9	75.6	79.8	63.0	80.9	64.7	88.5	71.7	92.5
Non-cephalopod molluscs	27.7	23.9	19.4	30.8	14.0	33.7	40.7	22.2	41.2	15.4	18.3	20.8
Photosynthetic organisms	17.0	23.9	24.5	19.8	27.9	17.3	7.1	24.7	9.8	26.9	23.3	30.2
Polychaetes	2.1	0.7	1.0	1.1	2.3	0	0.0	1.2	2.0	0.0	0.0	1.9

## Appendix B

**Table B1.** Weight contribution of each item category (mean  $\pm$  SE %) in each tract, per maturity stage, sex, region, fishery type and season. Coral and metal were not included in the table due to insignificant values.

Group	General	Maturity		Sex		Region		Fishery Type			Season		
		Immature	Mature	F	M	Creatn Sea	Libyan Sea	Commercial	Recreational	Winter	Summer	Spring	Autumn
Cephalopods	6.7 $\pm$ 1.6	1.9 $\pm$ 1.3	8.3 $\pm$ 2.1	7.8 $\pm$ 2.5	5.5 $\pm$ 2.1	10.8 $\pm$ 2.9	3.0 $\pm$ 1.6	7.2 $\pm$ 1.8	3.6 $\pm$ 3.6	7.5 $\pm$ 3.3	3.7 $\pm$ 3.7	7.9 $\pm$ 3.2	5.8 $\pm$ 2.8
	12.1 $\pm$ 2.0	6.7 $\pm$ 2.9	14.0 $\pm$ 2.5	7.2 $\pm$ 2.2	17.6 $\pm$ 3.4	14.1 $\pm$ 3.2	10.4 $\pm$ 2.5	12.4 $\pm$ 2.2	10.7 $\pm$ 5.2	11.7 $\pm$ 3.8	6.8 $\pm$ 3.9	22.5 $\pm$ 4.7	3.4 $\pm$ 2.0
Echinoderms	3.8 $\pm$ 1.2	1.3 $\pm$ 1.3	4.7 $\pm$ 1.6	3.3 $\pm$ 1.6	4.4 $\pm$ 2.0	3.3 $\pm$ 1.6	4.3 $\pm$ 1.8	3.9 $\pm$ 1.2	3.6 $\pm$ 3.6	0.9 $\pm$ 0.9	10.7 $\pm$ 5.9	3.9 $\pm$ 2.3	3.4 $\pm$ 2.1
	57.7 $\pm$ 3.1	70.2 $\pm$ 5.8	53.5 $\pm$ 3.6	60.1 $\pm$ 4.4	55.1 $\pm$ 4.4	50.5 $\pm$ 4.6	64.1 $\pm$ 4.1	57.8 $\pm$ 3.3	56.9 $\pm$ 9.0	47.7 $\pm$ 6.2	69.4 $\pm$ 7.9	49.6 $\pm$ 5.6	71.1 $\pm$ 5.2
Fishing gear	2.2 $\pm$ 0.7	0.2 $\pm$ 0.2	2.9 $\pm$ 1.0	2.6 $\pm$ 1.3	1.8 $\pm$ 0.7	3.2 $\pm$ 1.4	1.4 $\pm$ 0.6	2.6 $\pm$ 0.9	0.01 $\pm$ 0.01	0.6 $\pm$ 0.4	3.2 $\pm$ 2.2	1.9 $\pm$ 0.9	3.8 $\pm$ 2.2
	6.2 $\pm$ 1.4	10.0 $\pm$ 3.5	4.8 $\pm$ 1.5	5.8 $\pm$ 2.0	6.6 $\pm$ 2.1	2.2 $\pm$ 1.2	9.6 $\pm$ 2.5	4.7 $\pm$ 1.4	14.9 $\pm$ 5.7	15.9 $\pm$ 4.1	0.07 $\pm$ 0.04	2.3 $\pm$ 1.7	3.7 $\pm$ 2.1
Photosynthetic organisms	3.1 $\pm$ 1.1	0.02 $\pm$ 0.01	4.2 $\pm$ 1.5	3.4 $\pm$ 1.6	2.9 $\pm$ 1.5	3.5 $\pm$ 1.6	2.8 $\pm$ 1.5	3.0 $\pm$ 1.2	4.0 $\pm$ 3.3	3.5 $\pm$ 2.4	4.4 $\pm$ 3.4	3.1 $\pm$ 1.8	2.2 $\pm$ 1.8
	0.5 $\pm$ 0.5	2.0 $\pm$ 2.0	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	1.1 $\pm$ 1.1	1.1 $\pm$ 1.1	0.0	0.6 $\pm$ 0.6	0.0	1.8 $\pm$ 1.8	0.0	0.0	0.04 $\pm$ 0.04
Substrate	5.2 $\pm$ 1.5	5.9 $\pm$ 3.3	4.9 $\pm$ 1.6	7.8 $\pm$ 2.4	2.3 $\pm$ 1.5	10.8 $\pm$ 2.9	2.5 $\pm$ 1.4	5.1 $\pm$ 1.6	5.5 $\pm$ 4.0	8.8 $\pm$ 3.6	0.05 $\pm$ 0.05	4.6 $\pm$ 2.3	4.7 $\pm$ 2.7
	2.3 $\pm$ 0.9	1.8 $\pm$ 1.2	2.5 $\pm$ 1.2	1.7 $\pm$ 1.0	3.0 $\pm$ 1.6	14.1 $\pm$ 3.2	2.0 $\pm$ 1.0	2.5 $\pm$ 1.1	0.8 $\pm$ 0.6	1.7 $\pm$ 1.1	0.1 $\pm$ 0.1	4.1 $\pm$ 2.3	1.9 $\pm$ 1.6

**Table B2.** Weight contribution of each prey category (mean  $\pm$  SE %) in each tract, per maturity stage, sex, region, fishery type and season. Coral and metal were not included in the table due to insignificant values.

Group	Maturity		Sex		Region		Fishery Type			Season				
	General		Immature	Mature	F	M	Creatn Sea	Libyan Sea	Commercial	Recreational	Winter	Summer	Spring	Autumn
Cephalopods	7.1 $\pm$ 1.7	2.1 $\pm$ 1.4	8.8 $\pm$ 2.2	8.3 $\pm$ 2.7	5.8 $\pm$ 2.2	11.9 $\pm$ 3.2	3.1 $\pm$ 1.6	7.6 $\pm$ 1.9	3.7 $\pm$ 3.7	8.11 $\pm$ 3.5	3.7 $\pm$ 3.7	8.5 $\pm$ 3.4	6.2 $\pm$ 3.0	
	13.2 $\pm$ 2.2	7.2 $\pm$ 3.1	15.2 $\pm$ 2.7	8.4 $\pm$ 2.5	18.3 $\pm$ 3.5	16.2 $\pm$ 3.6	10.6 $\pm$ 2.6	13.5 $\pm$ 2.4	11.1 $\pm$ 5.4	13.4 $\pm$ 4.2	6.9 $\pm$ 3.9	24.1 $\pm$ 4.9	3.7 $\pm$ 2.1	
Echinoderms	4.1 $\pm$ 1.3	1.3 $\pm$ 1.3	5.0 $\pm$ 1.7	3.7 $\pm$ 1.7	4.5 $\pm$ 2.0	3.8 $\pm$ 1.8	4.3 $\pm$ 1.9	4.2 $\pm$ 1.4	3.7 $\pm$ 3.7	1.0 $\pm$ 1.0	10.8 $\pm$ 5.9	4.2 $\pm$ 2.4	3.7 $\pm$ 2.2	
	63.8 $\pm$ 3.2	75.4 $\pm$ 5.6	59.9 $\pm$ 3.7	67.9 $\pm$ 4.3	59.6 $\pm$ 4.6	59.0 $\pm$ 4.8	67.8 $\pm$ 4.3	64.6 $\pm$ 3.4	59.2 $\pm$ 9.1	53.3 $\pm$ 6.6	73.6 $\pm$ 8.0	54.9 $\pm$ 5.8	79.8 $\pm$ 4.7	
Non-cephalopod molluscs	7.2 $\pm$ 1.7	11.9 $\pm$ 4.2	5.6 $\pm$ 1.7	7.0 $\pm$ 2.4	7.3 $\pm$ 2.3	2.5 $\pm$ 1.3	11.1 $\pm$ 2.8	5.4 $\pm$ 1.6	18.0 $\pm$ 6.6	18.3 $\pm$ 4.6	0.1 $\pm$ 0.04	3.6 $\pm$ 2.3	4.1 $\pm$ 2.3	
	3.9 $\pm$ 1.3	0.02 $\pm$ 0.01	5.2 $\pm$ 1.7	4.4 $\pm$ 4.2	3.5 $\pm$ 1.8	5.0 $\pm$ 2.2	3.0 $\pm$ 1.6	3.8 $\pm$ 1.4	4.4 $\pm$ 3.7	4.0 $\pm$ 2.8	4.4 $\pm$ 3.4	4.8 $\pm$ 2.5	2.4 $\pm$ 1.9	
Polychaetes	0.6 $\pm$ 0.5	2.1 $\pm$ 2.1	0.06 $\pm$ 0.06	0.1 $\pm$ 0.1	1.1 $\pm$ 1.1	1.3 $\pm$ 1.2	0.0	0.7 $\pm$ 0.6	0.0	2.0 $\pm$ 2.0	0.0	0	0.2 $\pm$ 0.2	