

The biology and ecology of the invasive silver-cheeked toadfish (*Lagocephalus sceleratus*), with emphasis on the Eastern Mediterranean

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Abstract

Invasive species pose threats to either human health or inflict ecological and/or economic damage. The silver-cheeked toadfish (*Lagocephalus sceleratus*), a Lessepsian species, is one of the most harmful species in the Mediterranean Sea, because of its potent neurotoxin, impacts on marine biodiversity, and the increased costs and labor they inflict on fishers. Since the catch and consumption of this pufferfish is prohibited by almost all countries bordering the Mediterranean, they have now expanded into the entire Mediterranean and Black Sea. We performed a comprehensive study of *L. sceleratus* covering ecological aspects, growth, reproduction, diet and trophic level based on samples from southwestern coasts of Turkey. The estimated growth parameters were $L_{\infty} = 88.7$ cm, $K = 0.27$ year⁻¹, $C = 0.6$ and $WP = 0.1$. Their sex-ratio was $M/F = 1:0.69$. *Lagocephalus sceleratus* appears to be a batch spawner with discontinuous oocyte recruitment and has different spawning seasons in the Eastern Mediterranean which seem to be based on temperature cues which get shorter in duration as one moves north from the Suez. We also report their first positive ecological trait, that they are controlling some other invasive species through their diets, such as lionfish, Red Sea goatfish, rabbitfish and longspine sea urchins, in addition to controlling themselves through cannibalism, which appears to be density-dependent. They are indeed a top predator in the region with a trophic level of 4.1. We suggest that targeted fishing using improved gear-types to reduce fishing gear damages are initiated, and that finding commercial markets for pufferfish could help to naturally fund ongoing control efforts.

Keywords

Cannibalism, growth, Invasive Alien Species (IAS), pufferfish, reproduction, Tetraodontidae

Introduction

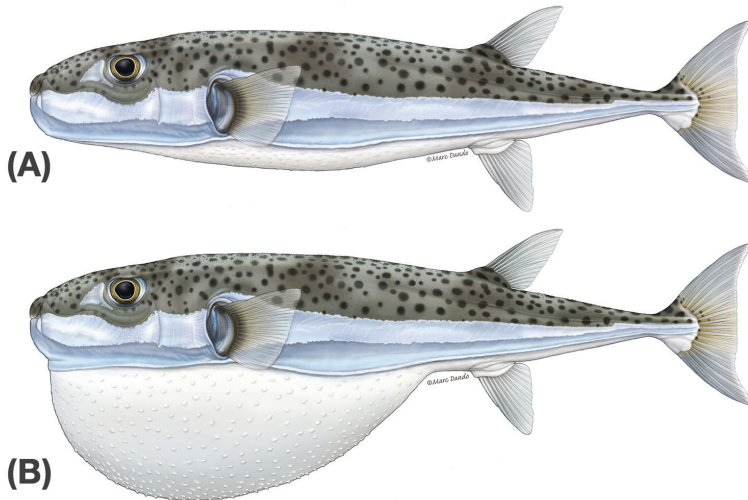
Global biodiversity is currently being threatened by overfishing, pollution and invasive species (Costello et al. 2010). The Mediterranean Sea is both a biodiversity hotspot and the most invaded sea of the planet, with currently about 800 marine non-indigenous species (Galil et al. 2015; Zenetos et al. 2017; Zenetos and Galanidi 2020), approximately 500 of which are Lessepsian species that invaded from the Red Sea after the creation of the Suez Canal (Por 1978; Galil et al. 2018). Due to the combination of overfishing (resulting in a loss of large predators), globalization (increased marine traffic), and the 2015 widening of the Suez Canal, the fauna of the Eastern Mediterranean is becoming more and more akin to that of the Red Sea. In fact, closer proximity to the Suez Canal correlates to a higher incidence of Lessepsian species (Ulman et al. 2019a). Some fish can swim through the canal unassisted, while their larvae may be stowed-away in the ballast tanks of ships, with sessile species (mostly macro-invertebrates) hitchhiking to new destinations on boat-hulls as part of their biofouling communities (Ulman et al. 2019a, 2019b). Given that the connection between the Red Sea and the Mediterranean is anthropogenic, all biota which arrive via the Suez Canal into the Mediterranean are considered to be non-indigenous species, rather than being the result of natural range expansions (European Environment Agency 2012). Lessepsian migrations may represent the ‘most important biogeographic phenomenon witnessed in the contemporary oceans’ (Por 1978), and also present ongoing opportunities to better understand general biological processes such as species interactions, physiological and ecological adaptations, and evolutionary processes (Ruiz et al. 2000; Bernardi et al. 2010). The Eastern Mediterranean is oligotrophic (Longhurst 2010), which limits the productivity of the subregion, which also suffers from severe overfishing (Demirel et al. 2020; Tsikliras et al. 2021). Because of the ‘fishing down marine food webs’ phenomenon (Pauly et al. 1998), which also occurs in the Mediterranean, including its eastern basin (Stergiou 2005; Keskin and Pauly 2018), high-trophic level, large fish (such as sharks) are now rare, leaving room for new non-indigenous top-level predators to establish themselves.

Non-indigenous species (NIS) are called invasive when they cause either ecological, economical damage, or pose a threat to human health. Marine invasive species can pose major threats to biodiversity by altering community structure and function, and by modifying ecosystem processes, which can have long-lasting ecological and economic consequences (Molnar et al. 2008). Once a marine species establishes itself in a new area, its eradication becomes near impossible, due to the three-dimensional nature of marine ecosystems, and their interconnectedness. Thus, managers commonly decide to wait and hope that the situation naturally resolves itself in time, although when the risks are too great, they may need to implement control measures.

Table 1. Pufferfish species found in the Mediterranean, their native region, first year of introduction, first locality introduced, established status in the Mediterranean, and reported in Turkey (Y= yes, N= no).

Common name	Scientific name	Native region	Year	Locality	Established	In Turkey
Prickly puffer	<i>Ephippion guttiferum</i>	E. Atlantic & W. Med.	NA	NA	Y	N
Oceanic puffer	<i>Lagocephalus lagocephalus</i>	Subtropical	NA	NA	Y	Y
Diamondback puffer	<i>Lagocephalus guentheri</i>	Indo-Pacific	1950 ¹	Egypt	Y	Y
Suez puffer	<i>Lagocephalus suezensis</i>	W. Indian, Red Sea	1977	Lebanon	Y	Y
Silver-cheeked toadfish	<i>Lagocephalus scleratus</i>	Indo-Pacific	2003	Turkey	Y	Y
Guinean puffer	<i>Sphoeroides marmoratus</i>	E. Atlantic	1977	Italy	Y	N
Blunthead puffer	<i>Sphoeroides pachygaster</i>	Subtropical	1979	Spain	Y	Y
Bandtail puffer	<i>Sphoeroides spengleri</i>	W. Atlantic	2000	Spain	Y	N
Yellowspotted puffer	<i>Torquigener flavimaculosus</i>	W. Indian	1987	Israel	Y	Y
Spiny blaasop	<i>Tylerius spinosissimus</i>	Indo-Pacific	2004	Greece	Y	Y
Spotbase burrfish	<i>Cylichthys spilostylus</i>	Indo-Pacific	1993	Israel	Y	Y
Spotfin burrfish	<i>Chilomycterus reticulatus</i>	Subtropical	2009	Sardinia	N	N
Spotfin porcupinefish	<i>Diodon hystrix</i>	Circumtropical	1956, 2016	Italy, Spain	N	N

¹ Formerly misidentified as *Lagocephalus spadiceus*.

**Figure 1.** The silver-cheeked toadfish *Lagocephalus scleratus* (Gmelin, 1789) **A** lateral view **B** 'puffed' lateral view (original drawings by Marc Dando).

There are 197 species of pufferfish globally, 112 of which live in marine environments, 48 in brackish environments and 37 in freshwater (Santharam 2018). In the Mediterranean, there are currently eleven established pufferfish species present, eight of which are found in Turkey; and six of those in Turkey being Lessepsian migrants (Table 1). However, before management can design applicable solutions, at the very least, their biology, and in particular the specific nature of their invasiveness, needs to be assessed. In Turkey, a risk assessment on the invasiveness of five pufferfish species from its southwestern coast, revealed the silver cheeked toadfish *Lagocephalus scleratus* (Gmelin 1789) (Fig. 1) to be the most invasive based on its diet and life-history traits

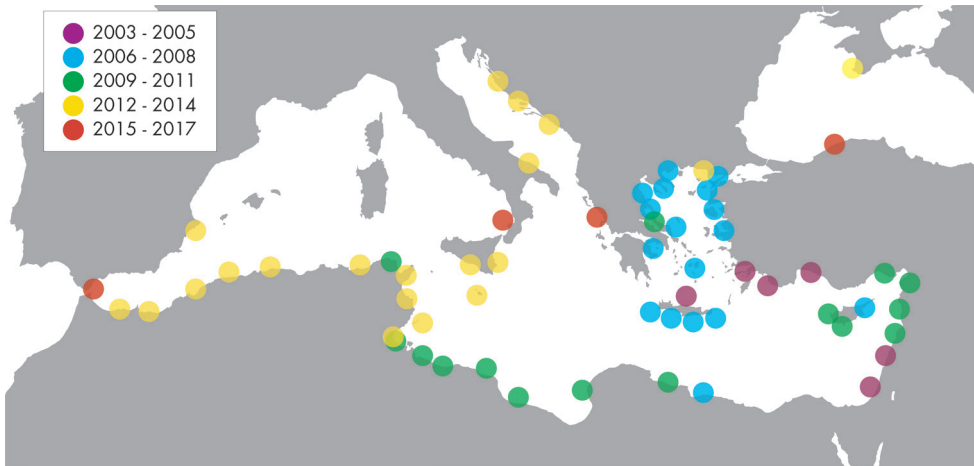


Figure 2. Distribution of *Lagocephalus sceleratus* in the Mediterranean, as documented by records in Bilecenoğlu and Öztürk (2018), Galanidi and Zenetos (2019), Azzurro (2020), and Gücü et al. (2021).

(Filiz et al. 2017). *Lagocephalus sceleratus* causes negative impacts to human health through its high poison content, native ecology from its high abundances and generalist diet, and to the economy from both depredation and fishing gear losses to fishers.

Lagocephalus sceleratus (Fig. 1) is one of the largest and most abundant invasive predatory fish species established in the Eastern Mediterranean. It is a generalist predatory carnivore feeding on crustaceans, fish and cephalopods. Its maximum published weight is 7 kg (Smith and Heemstra 1986; Froese and Pauly 2020), although several Turkish fishers claim to have caught individuals between 10 to 12 kg (pers. comm. to A.U).

An important part of the ecological success of *L. sceleratus* is due to their having one of the most advanced forms of teeth in the animal kingdom. The ‘first generation teeth’ are coated with recurring toothbands which are continuously regenerated by stem cells (Thiery et al. 2017). These toothbands fuse to form upper and lower plates, which jointly forms a beak. This strong beak and accompanying plates enable them to crush and slice very tough prey organisms such as decapods and bivalves (Turingan 1994).

The first record of *Lagocephalus sceleratus* in the Mediterranean was from Gökova Bay, southwestern Turkey in 2003 (Filiz and Er 2004; Akyol et al. 2005). *Lagocephalus sceleratus* is normally shy of humans, and thus not readily encountered by snorkelers and divers. This species rapidly and successfully established itself in Turkey and in the waters of Eastern Mediterranean countries such as Egypt, Lebanon, Syria, Cyprus and Greece. Lately, it has now expanded its range to the Black Sea (Bilecenoğlu and Öztürk 2018) and most of the Mediterranean, all the way to the Strait of Gibraltar (Azzurro et al. 2020), implying that it may soon spill into the Atlantic Ocean. Strangely, it has not yet been reported from mainland France, or Corsica, its largest island (Fig. 2). The success of *L. sceleratus* implies a high phenotypic plasticity and an ability to cope with a range of environmental conditions (Golani et al. 2010). For example, the average salinity along the coast of Ukraine in the Black Sea, where *L. sceleratus* has its northernmost

occurrence, is only 10–12 psu, much lower than the Mediterranean average of 34 psu. As a result, *L. sceleratus* is found in a variety of benthic habitats, including sandy bottoms, rocky substrates and seagrass meadows (Rousou et al. 2014).

Strong defenses against predation

In *Lagocephalus sceleratus*'s 18-year presence in the Mediterranean, only loggerhead turtles (*Caretta caretta*) have been documented preying on adult *L. sceleratus*, while garfish (*Belone belone*), common dolphinfish (*Coryphaena hippurus*), and cannibalism has been documented in juveniles (Ulman et al. 2021). Potential common predators require large throats which can accommodate a puffed up fish, which is the case in loggerhead turtles, and also in predators of *Lagocephalus inermis* from India, which included cobia (*Rachycentron canadum*), and catfish (*Arius* spp.) (Mohamed 2013).

Lagocephalus sceleratus combine two exceptional defense mechanisms which benefit them in evading predators, i.e., the ability to 'puff' themselves up, and their highly toxic tissues. The combination of these two factors contribute, in the Mediterranean, to a scarcity of predators.

Puffing

The peculiar head, buccal cavity and pectoral girdle structures of these fishes facilitate their unique ability to 'puff' themselves up by rhythmic buccal pumping, swallowing and forcing water (or air if they are outside of water) into their stomach. While their 'stomach' can increase its size 50–100-fold depending on the species (Brainerd 1994), it is not a true stomach, having lost its digestive function to allow for puffing. In addition to stretchable skin and a distensible stomach, pufferfish lack pleural ribs and pelvis which would be impediments to 'puffing' (Amores et al. 2004). It has been demonstrated that some predators perished due to the puffing of ingested pufferfish, notably a lemon shark due to blocked gills resulting in asphyxiation (Ulman et al. 2021). Their second major deterrent to potential predators are high levels of tetrodotoxin neurotoxin throughout the body.

Tetrodotoxin

Lagocephalus sceleratus is the second most poisonous Mediterranean pufferfish species after *Torquigener flavimaculosus* Hardy & Randall, 1983 (Ayas 2017; Kosker et al. 2018), and pufferfish are the second most poisonous family after the Synanceiidae family of stonefishes. This is one of the reasons why *L. sceleratus* is viewed as one of the worst invasive species (Streftaris and Zenetos 2006; Otero et al. 2013).

Tetrodotoxin (TTX) is an extremely potent neurotoxin found in *L. sceleratus* and other pufferfish (Amano et al. 2019). TTX inhibits voltage-gated sodium channels, blocking the propagation of nerve impulses (Geffeney and Ruben 2006). Animals that contain tetrodotoxin are resistant to the neurological effects of the toxin themselves

(Kotipoyina et al. 2020). Also, pufferfish containing TTX were found to grow at faster rates, exhibited fewer signs of aggression, and lower stress hormones than pufferfish without TTX (Amano et al. 2019). TTX testing of two very small juvenile *L. sceleratus* (5 cm) found TTX from the muscle of one, and in nearly all tissues from the other, to be above the lethal human 2 mg·kg⁻¹ dose (Leonardo et al. 2019), which is a new important finding as juveniles were previously considered to be non-toxic.

Out of the 197 pufferfish species, only 55 (28%) are considered toxic (Santhanam 2018). The high TTX content of *L. sceleratus* preclude this species from being a food fish, even as Japanese *fugu*, which only incorporates pufferfish with TTX values of <2 mg TTX/kg (Noguchi and Ebesu 2001). Generally, the ovaries and male gonads leading up to spawning season have the highest TTX content (Sabrah et al. 2006). High TTX concentrations are also found in the liver, skin, eyes and muscle, but they vary according to season, locality and fish size (Kosker et al. 2016; Rambla-Alegre et al. 2017).

Impacts on fishers and other persons

Lagocephalus sceleratus has strong negative impacts on the livelihoods of small-scale fishers of the Eastern Mediterranean, most of whom are already marginalized due to declining catches and revenues (Ünal et al. 2015). These impacts of *L. sceleratus* are caused by damaging fishing nets, consuming caught fish within the nets (depredation) and eating the bait and hooks from set longlines (Ünal and Göncüoğlu 2017). Thus, losses to fishers occur through fishing gear losses, time losses and losses of catches. Ninety seven percent of surveyed fishers from Turkey's southern Aegean and Mediterranean coasts suffered an average of US\$ 183 in fishing gear losses during 2011–2012, which increased to US\$ 325 during 2013–2014, and US\$ 370 during 2015–2016; note that US\$ 370 represents 4.3% of the per capita GDP in Turkey in 2020, equivalent to US\$ 8548 in the US (Ünal et al. 2015; Ünal and Göncüoğlu 2017; Öndes et al. 2018). Economic losses due to foregone catches are comparable to fishing gear losses, and were evaluated to be an additional of US\$ 353 in 2015–2016 (Ünal and Göncüoğlu 2017). Fishers from southern Turkey are more affected than those in western Turkey due to the higher abundance of *L. sceleratus*, with losses calculated at about US\$ 538 per longline vessel in 2016 and US\$ 616 per vessels with set nets (Öndes et al. 2018). Gillnets normally used to last between two to five years, but currently, many small-scale fishers are unable to afford replacing their nets in just months after suffering irreparable pufferfish damage; a new two km long gillnet now costs over US\$ 2000 in Turkey, which recently increased by 40% due to ongoing currency devaluations.

Interestingly, around Turkey, this species is normally shy of humans and is not commonly encountered while snorkeling or scuba diving. In August 2019, a first human attack by *L. sceleratus* occurred in Kaledran, Turkey where *L. sceleratus* bit a child three times on the left hand, resulting in the amputation of her ring finger (Sümen and Bilecenoglu 2019). In September 2020, a snorkeler was bitten in his calf in Mersin, Turkey (Melih Görkem Bilgin, pers. comm.), and in May 2021, in Antalya, Turkey,

there were some snorkelers who had large portions of their fins attacked and eaten by *L. sceleratus*. Aggressive behavior has also been reported by divers in Cyprus (Hasan Deniz Akbora, pers. comm.), and there are growing concerns for safety especially in highly touristic areas of high *L. sceleratus* abundances. For example, during the COVID pandemic, there were several months where locals were restricted from going to the beach, but tourists (very few in number at the time) were permitted, and alarmingly they recorded small aggregations of very large *L. sceleratus* specimens (each between 2–5 kg) in shallow popular beach areas in both Muğla and Antalya provinces, making national news headlines. As Turkey is a popular tourist destination primarily for its beaches and clear waters, an aggressive predatory fish has the potential to negatively impact the tourism sector if interactions with people increase.

Lagocephalus sceleratus poisoning has caused dozens of human fatalities in the Mediterranean region, which is a severe underestimate given that most of these fatalities are not officially recorded (Ben Souissi et al. 2014). In Turkey, from October 2020 to March 2021, five deaths have occurred from consuming *L. sceleratus*.

Despite the multiple negative impacts of *L. sceleratus*, most Mediterranean studies have been dedicated to the high content of tetrodotoxin (TTX) in its tissues, with only a handful investigating its biology (Sabrah 2006; Aydin 2011; Nader et al. 2012; Kalogirou 2013; Farrag 2014; Khalaf et al. 2014; Rousou et al. 2014; Ersönmez et al. 2017; Zengin and Türker 2020). As well, two studies reported on the abundance of *L. sceleratus* in Egypt (Farrag et al. 2015; Elhaweet et al. 2016) and another from Antalya Bay, Turkey (Özbek et al. 2017).

This contribution is an attempt to correct this imbalance. Due to nearly a complete lack of control in the region, its negative impacts to marine biodiversity, human health and fishers' livelihoods continue to worsen. This study presents new data on the species behaviour (eg., spawning, ecology and feeding) based on fishers' knowledge, and from biological studies, presenting new data on their distribution, size, growth, spawning season and reproductive status, reproductive morphology and fecundity, and the taxonomic composition of their prey. This contribution aims to improve current knowledge about this invasive species, to help direct further research needs and management options.

Materials and methods

Study area and sample collection

Pufferfish samples were purchased from small-scale commercial fishers in southwestern Turkey primarily from Datça where they were targeted (36.726°N, 27.685°E) and about 15% of samples were caught as by-catch from Fethiye (36.659°N, 29.126°E), both Muğla Province, Turkey, from June 2019 to November 2020. This stock has not yet been studied and is understood to be a different stock from the neighbouring Antalya province, which has been somewhat studied. This area is very close to Gökova

Bay, where the first Mediterranean *L. sceleratus* occurrence was reported (Filiz and Er 2004; Akyol et al. 2005). The majority of pufferfish were caught by one fisher in Datça, who initially tried using reinforced steel lines with three separate hooks to deter fishing gear losses. However, many of these steel lines were severed by *L. sceleratus* the first day, so the fisher continued both with hook and line, continually replacing lost hooks, and then by trammel net. In the first six months of the study, chicken flesh was used as bait and for the next six months, strips of flesh of adult *L. sceleratus* were used as bait, with similar success (S. Taşkıran, personal observation).

A total of 1013 fish: 456 males, 270 females and 287 juveniles (where juveniles were generally < 25 cm and could not have their sex determined due a lack of gonadal development) were collected for this study from June 2019 to November 2020. Fishers were paid 10 Turkish Lira (\approx US\$ 1.20; April 23/2021) per kg for *L. sceleratus* from June 2019 until mid-April 2020, and 20 Turkish Lira per kg from mid-April 2020 onwards. The fish were purchased from approximately 20 fishers from Fethiye and Datça, Muğla province, who all had special permissions to collect them for this study. Permission to collect pufferfish for the specified designated fishers for scientific research purposes was granted from the Turkish Ministry of Agriculture and Forestry and General Directorate of Water Products under Permission #67852565-140.03.03-E.1354602 & #6987137-663.08.

Fisher's knowledge of behaviour

We formally surveyed 45 small-scale fishers face-to-face from the Muğla province (Fethiye to Bodrum) in April 2019 to help understand some of the behavior of this species and to inform them of this study. An initial structured survey consisting of 18 questions pertaining to their contact details, fisher characteristics, vessel and gear types, average days fished, *L. sceleratus* catches, catch areas, caught depths, average sizes, maximum sizes, fishing gear losses in nets and longline hooks, and interest in catching pufferfish for this study was initially undertaken at the beginning of the study in April 2019. Twelve of those initially interviewed supplied fish afterwards for this study all using trammel nets, after permissions were granted for them to catch pufferfish. Any new information learnt as the study progressed was written down and transferred to the spreadsheets containing the other data. These data were then summarized for each topic. Their responses, aside from the new maximum depth record, should be viewed as anecdotal evidence.

Morphometrics and growth

Information on such basic biological parameters of species, such as growth, reproduction and fecundity are essential in understanding the basic life history traits of a species and are prerequisites needed to develop scientifically sound fisheries management policies. For all 1013 samples, the total length (L) and body weight (W) of fish were measured to the nearest 0.1 cm and the nearest 1 g, respectively, and gonads and livers

were weighed to the nearest 0.01 g. The length-weight relationship yields authentic biological information about a species in a particular region and is of great importance in fishery assessments. The parameter of length-weight relationships (LWRs) of the form $W = a \cdot L^b$ were estimated through re-expression of the LWR equations in linearized form, i.e., $\log(W) = \log(a) + b \cdot \log(L)$, where a is a scaling coefficient for the weight at length and b is a shape parameter; note that if $b < 3$, a fish become thinner as it grows, and plumper if $b > 3$.

The growth of water-breathing ectotherms such as fish can be conceived as the net result of two processes with opposing tendencies (Bertalanffy 1938):

$$dW/dt = HW^d - kW \quad (1)$$

where dW/dt is the growth rate, W is body weight (or mass), H and k are the coefficients of anabolism and catabolism, and d is the scaling exponents of anabolism, which depend on oxygen, and hence of the growth of gill surface area (Pauly 1984, 2021). Assuming that $d = 2/3$ and integrating, i.e., re-expressing the differential Equation 1 as a growth curve leads to the von Bertalanffy growth function (VBGF), which is commonly used to describe the growth of fish and which has the form:

$$L_t = L_\infty (1 - e^{-K(t-t_0)}) \quad (2)$$

where L_t is the length at age t , L_∞ is the asymptotic length, i.e. the mean length the individuals of a given population would reach if they grew indefinitely, K is rate, or dimension time⁻¹ (here: year⁻¹) at which L_∞ is approached, and t_0 is the age at $L = 0$.

The mutual compatibility of the growth parameters L_∞ and K can be evaluated by $\emptyset' = \log(K) + 2\log(L_\infty)$ which should be roughly similar between populations of the same species (Longhurst and Pauly 1987; Pauly 1998).

Here, a seasonally oscillating variant of the von Bertalanffy growth function (VBGF) was used to estimate growth parameters from the length-frequency data available for *L. sceleratus*; this version of the VBGF has the form:

$$L_t = L_\infty \{1 - e^{-[K(t-t_0) + S(t) - S(t_0)]}\} \quad (3)$$

where $S(t) = (CK/2\pi) \cdot \sin(2\pi(t - t_s))$, $S(t_0) = (CK/2\pi) \cdot \sin(2\pi(t_0 - t_s))$, and L_∞ , K and t_0 are defined as above; see Pauly (1991) for a first application to a pufferfish.

Equation (3) involves two parameters more than the standard VBGF: C and t_s . Of these, the former is easiest to visualize, as it expresses the amplitude of the growth oscillations. When $C = 0$, Equation (3) reverts to Equation (2). When $C = 0.5$, the seasonal growth oscillations are such that growth rate increases by 50% at the peak of the 'growth season' (i.e., in 'summer'), and, briefly, declines by 50% in 'winter'. When $C = 1$, growth increases by 100%, doubling during 'summer', and becoming zero in the depth of 'winter'. The other new parameter, t_s expresses the time elapsed between $t = 0$ and the start of a sinusoid growth oscillation. However, visualization is facilitated

if we define $t_j + 0.5 = WP$ ('Winter Point'), which expresses, as a fraction of the year, the period when growth is slowest. WP is often close to 0.1 (i.e., early February) in the Northern Hemisphere and 0.6 (early August) in the Southern Hemisphere.

The parameters of Equation 3 were estimated through the ELEFAN method, which fits growth to the peaks of length-frequency (L/F) samples arranged in time (represented by black, positive histograms, and deemed to represent age classes) while avoiding the trough between peaks (represented by white, negative histograms). Peaks and troughs are identified by a simple high-pass filter, i.e., a running average which leads to definition of peaks as those parts of a length-frequency distribution that are above the corresponding running average and conversely for the troughs separating peaks. Then, hundreds of growth curves, each with a different set of growth parameters, are traced, and the growth curve (i.e., parameter set) is retained which has the highest score in linking the peaks of L/F distributions, whose 'point' values are positive, while avoiding troughs, whose point values are negative (Pauly 1991, 1998). The software used here to implement the ELEFAN method was FiSAT, documented in Gayanilo et al. (2005).

Reproduction

Variations in fish gonadal morphology explain important behavioral and ecological adaptations during reproduction. Particularly knowledge about the reproductive period is considered a major life-history trait and evaluating the changes in gonadal development, liver size and body weight can help to understand energy trade-offs in the development of reproductive strategies, notably in the inverse relationship between the gonadosomatic index (GSI) and the hepato-somatic index (HSI), while condition factor (CF) shows the relative health of the fish.

To estimate fecundity, the gonads were removed, weighed and preserved in formalin. To identify the reproductive season, temporal changes in the gonadosomatic index were assessed using the relation: $GSI = 100 \times [G_w / (TW - G_w)]$ where G_w is the gonad weight and TW is the total weight. Also, the hepato-somatic index analyses was computed as an indicator of reserves in the liver, i.e., $HSI = 100 \times [H_w / (TW - H_w)]$ where H_w and TW represent liver weight and total weight, respectively. Understanding changes in liver reserves, helps to better understand how energy is transferred from storage to reproduction. Finally, the overall plumpness of individuals was determined from their condition factor $CF = 100 \cdot W/L^3$.

The size at first maturity (and spawning) was estimated by plotting the fraction of mature individual females and males against their lengths, and fitting a logistic curve. Mean length at first maturity (L_m) was the length at which, in a given population, 50% of individuals were mature. This was evaluated separately for fish sampled during the main spawning season (i.e., in June) and outside, to test if *L. sceleratus* reach maturity at smaller sizes within than outside the spawning season.

We also used the lengths of first maturity and maximum lengths in several population of *L. sceleratus* to indirectly estimate their ratios of metabolic rate at first maturity

(Q_m) to maintenance rates (Q_{maint}). These ratios were then used to test whether their mean value is compatible with earlier estimate ranging from 1.22 to 1.53 and suggesting that it is a declining relative oxygen supply which triggers maturation and spawning (Pauly 2021); see ‘Gill-Oxygen Limitation Theory’ in Suppl. material 1: Appendix 1.

Knowledge on fecundity is used to calculate the reproductive potential of a stock and is another important factor for effective fish stock management. Ovary samples were collected in May and June 2020, to capture the peak GSI values. The oocyte size–frequency method (Murua et al. 2003) was applied to females with migratory nucleus or early hydrated oocytes to assess the fecundity. Murua et al. (2003) explained that if highly advanced oocytes (≥ 500 μm) were used for batch fecundity estimation, the results become typically similar to the hydrated-oocyte method. Given these considerations, three subsamples, weighing between 20–40 mg, were taken from the anterior, middle and posterior parts of the ovaries. The relationships between number of eggs per batch, length, and ovary free weight were determined by (log)linear regression. The diameters of the oocytes were measured using the Zeiss Labscope App (version 1.3.1) for iPad.

Examination of oocyte development is evaluated to help identify reproductive strategies of species such as ovary organization, fecundity type and spawning patterns (Murua et al. 2003). In order to examine spawning strategy of this species, histological analyses were performed on 70 ovaries. Tissues were removed from the center of each ovary, fixed in 10% formalin solution, dehydrated in an increasing series of ethanol and embedded in paraffin. Tissue sections of 5 μm were stained with Mayer’s hematoxylin and eosin and examined with an Olympus BX51 light microscope equipped with an Olympus DP72 digital camera (Roberts et al. 2012). The diameters of oocytes were validated by a second person using Leica image analysis software.

Prey

Knowledge on predator-prey interactions for species are essential to understanding their role in the ecosystem, impacts on biodiversity, and are essential in building accurate ecosystem models for a region. Two complementary studies were conducted on the diet of *L. sceleratus*. The prey/diet preferences were examined by a visual taxonomic examination of stomach content for 563 samples from Fethiye and Datça, Muğla province in Turkey. Food items were removed from the esophagus, stomach and intestine and identified to the lowest taxonomic level possible; fishing hooks and pieces of fishing net were also accounted for, as were sand and algae. The prey taxa were then grouped into three main categories: crustaceans, fish and cephalopods, and also identified as either indigenous or non-indigenous taxa where possible. A t-test was performed on the ratios of the three prey groups for juvenile (< 45 cm) and adult fish (> 45 cm) to determine if they target different taxonomic groups as they grow.

To better understand the role of *L. sceleratus* in the ecosystem, and to estimate their trophic level (TL), their mean fractional level of their prey for 34 stomachs, where the contribution of prey items in numbers (%N), weight (%W) and frequency of oc-

currence (%F) was recorded. These values were then used for calculating the Index of Relative Importance (IRI) of prey item ($IRI = \%F \times (\%N + \%W)$), which was then re-expressed using $\%IRI = (IRI / SIRI) \times 100$ (Cortes 1997). SIRI is the percentage which a discrete prey taxon contributes to the sum of all IRI values in the prey spectrum. Based on the dietary composition (expressed as W%), the mean fractional trophic level (TP) of the *L. sceleratus* was estimated using the method of Pauly et al. (2000), as implemented in their TrophLab software and the equation: $TL_i = \sum_j TL_j \times DC_{ij}$, where TL_j is the fractional trophic levels of prey j , and DC_{ij} represents the fraction of j in the diet of i . Trophic levels range from 1 for primary producers to 5 for apex predators such as marine mammals and sharks. Stomach fullness was evaluated using a 5-point scale, where 0 = empty, 1 = food residues, 2 = less than half full, 3 = more than half full, and 4 = full (Gaykov and Bokhanov 2008).

Results

Fisher's knowledge of behavior

The fishers who informed this study consisted of 12 using trammel nets, 12 using longlines, 21 using both trammel nets and longlines, and five occasionally using rods. The fishers who provided fish for this study used trammel nets, with three sometimes using fishing rods.

According to these fishers, when *L. sceleratus* first appeared along the southwestern Turkish coast, it was found mostly in rocky areas from depths of about 10 m, and never deeper than 100 m. However, over time *L. sceleratus* were increasingly found in deeper locations to a maximum of 220 m depth (recorded in April 2021 from Fethiye Bay). In June, i.e., during their spawning season, they aggregate in the shallows of bays, between 5–10 m depths; however, a few individuals have also been caught at the surface. Based on the accounts of 45 fishers in Muğla Province, Turkey, *L. sceleratus* regularly consumes bait from rods and longlines, severing many of the hooks and even steel lines in the process. Some fishers reported hook losses from 50–90% of their longlines in extreme cases, but the majority of long-line fishers claimed an average of about 10–20% of hooks lost. Hooks were found in 8% of *L. sceleratus* stomachs; nearly all samples were collected by net. *Lagocephalus sceleratus* uses its fused parrot-like teeth to bite holes in set trammel nets and consume the fish caught in the nets, as evidenced by nine pieces of fishing net between 3–20 cm in diameter in their stomachs, weighing up to 10 g. All fishers in the region are regularly affected by this and try to cast their nets away from *L. sceleratus* hotspots to minimize damages. One fisher from Fethiye (Mehmet Taniş, pers. comm.) explained that on several occasions, *L. sceleratus* bit through his trammel nets, and consumed the stingrays caught inside, leaving only the needle tail portion behind as evidence.

As one fisher, S. Taşkıran, was the main fisher in Datça that targeted *L. sceleratus* for this study, and thus has the most experience with this species, his observations are

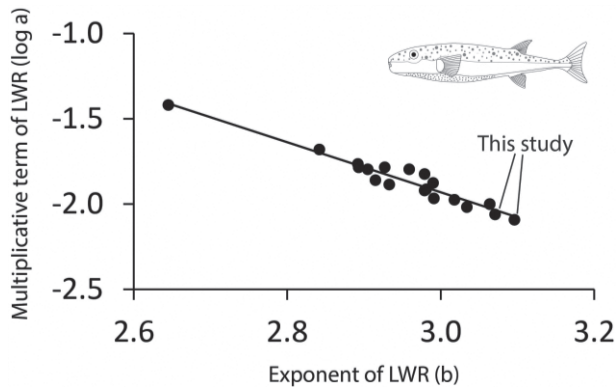


Figure 3. Illustrating the relationship between the multiplicative term (a) and the exponent (b) of length-weight relationship in *Lagocephalus sceleratus*. (Based on the results of 13 studies from data in Suppl. material 1: Table S3 which based their measurements on TL).

separately noted here. He estimated that in June 2020, there were approximately 10 tonnes of *L. sceleratus* spawning in InçiBurnu Bay near Datça. At this locality, during their spawning period, the fish were inactive at night, and actively fed at dusk and dawn. In July and August, they fed very little, but in September onwards for a few months, they again fed very aggressively.

Morphometrics and growth

In total, 1013 fish were examined, and of those, 456 were male, 270 were female and 287 were juveniles generally below 25 cm whose sex could not be determined. The overall sex-ratio was calculated as M:F = 1.0:0.69. Total length ranged from 13 to 77.2 cm. The mean lengths of females and males were not statistically different ($p > 0.05$, $p = 0.71$) but males were more abundant throughout the entire year. Suppl. material 1: Table S1 compares sex ratios from different localities.

Suppl. material 1: Fig. S2 illustrates the LWRs that we obtained; the slopes (b) of the LWRs for females, males, and unidentified individuals were compared and were found to be statistically different ($p < 0.05$, $p = 0.001$; see also Suppl. material 1: Table S2). Notably, females were plumper than males of the same length. Figure 3 compares the LWR results of this study compared to other published studies from Suppl. material 1: Table S2.

The close inverse relationship of $\log(a)$ vs b in Fig. 3 implies that the LWRs in Suppl. material 1: Table S3 (i.e., including those in Figure S2) are all mutually compatible, and predict similar weight for a given length. Pauly (2019), p. 94–95 shows that the different locations of LWRs along gradients $\log(a)$ vs b such as illustrated in Fig. 3 are largely due to different sampling periods of the L-W data pairs used to establish each LWR; such gradients are also well documented in FishBase (Froese and Pauly 2020), for example for the well-studied Atlantic cod (*Gadus morhua*).

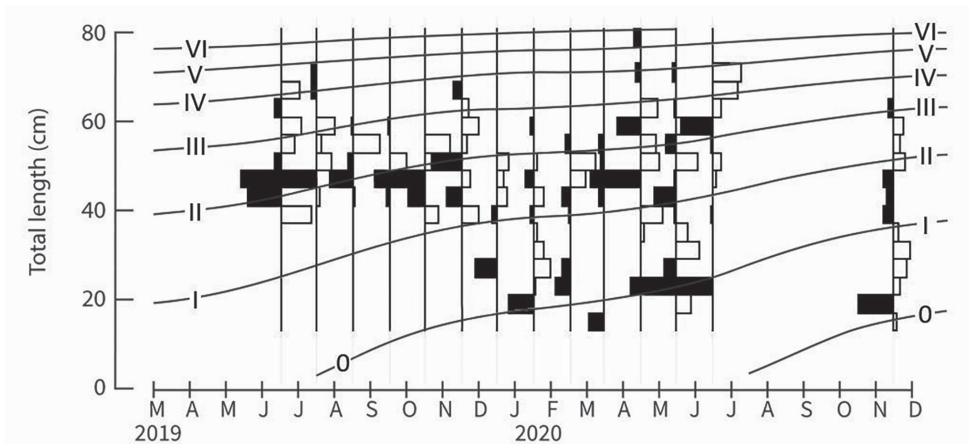


Figure 4. Seasonally oscillating growth curve fitted using ELEFAN to 14 length-frequency samples of *Lagocephalus sceleratus* ($n = 1013$) collected from June 2019 to November 2020; the estimated growth parameters were $L_{\infty} = 88.7$ cm (TL), $K = 0.27$ year⁻¹, $C = 0.6$ and $WP = 0.1$. The Roman numerals (& 0) refer to the 7 cohorts (= sequences of peak, i.e., black histograms) that were identified by ELEFAN (Based on data in Suppl. material 1: Table S4).

The best fit to the length-frequency data that we gathered (see Fig. 4, Suppl. material 1: Table S4) was obtained for the growth parameter $L_{\infty} = 88.7$, $K = 0.32$ year⁻¹, $C = 0.6$ and $WP = 0.1$. The estimates for C and WP imply that the seasonal changes in water temperature in the sampling area impact the growth of *L. sceleratus*, which the estimate of WP implies is most reduced in early February 2020. The growth parameters that we estimated are compatible with those estimated by other authors from other parts of the Mediterranean Sea (see values of \emptyset' in Suppl. material 1: Table S5).

Reproduction

The ovarian organization of *L. sceleratus* appears to be based on synchronous development of groups of oocytes. Two concurrent populations of oocytes were found during spawning, i.e., larger oocytes and a more heterogeneous group of smaller oocytes (Fig. 5). Post-ovulatory follicles were not observed in our samples, but atresia (the degeneration of ovary follicles which do not ovulate) occurred in both the previtellogenetic (before formation of the yolk) and vitellogenetic (yolk formation process in the oocyte) phases (Fig. 5). Overall, the spawning pattern thus appears to be batch spawning with discontinuous oocyte recruitment.

Oocyte diameter during vitellogenesis were found to range between 0.42–0.58 mm, with an average oocyte size of 0.50 mm for the migratory nucleus stages. Oocyte counts were performed on 23 female ovaries from the peak reproductive period. Average fecundity was calculated as 134,000 oocytes for females of 55 cm and

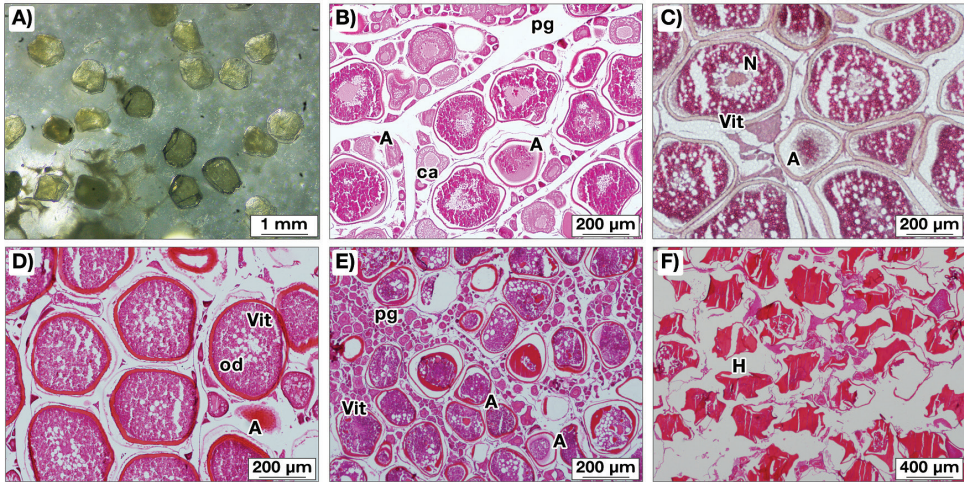


Figure 5. Stages of oocyte development in *Lagocephalus scleratus*. Whole oocytes on the slide **A** and histological sections **B–E** from nucleolus to vitellogenesis; and **F** hydration: Primary growth (pg), cortical alveoli (ca), nucleus (N), vitellogenic oocytes (Vit), oil droplets (od), atretic oocyte (A), and hydrated oocytes (H). Scale bars 1 mm (**A**); 200 μ m (**B–E**); 400 μ m (**F**).

2,000 g. The relationships between fecundity vs. length, and oocyte number vs. body weight are provided in Suppl. material 1: Fig. S3.

GSI starts to increase in April and May, peaks in June (9%), then declines sharply in July (see Fig. 6, the top panel showing the GSI results of this study- Fethiye), suggesting that the main reproductive season of *L. scleratus* in southwestern Turkey is late spring-early summer (May-June). This is confirmed by fishers' observations that spawning aggregations of *L. scleratus* occur from the last days of May and span the month of June. Near Datça, Muğla, in 2020, the highest GSI values occurred in mid-June (10.2%) with numerous individuals caught while spawning. A second, minor spawning season is suggested by a small increase in GSI in September for both sexes. Fig. 6 illustrates that there is a tendency for the spawning season (i.e., the high GSI season) of *L. scleratus* to become shorter, the further one gets from the Suez Canal; this graph assimilated the spawning seasons of *L. scleratus* from the Mediterranean and other nearby regions with the results of this study at the top of the graph (Fethiye).

Condition factors were similar between sexes, and its monthly variability (not shown) was not very pronounced; it exhibited a weak peak in June (during peak spawning season) and another in November. The baseline of the HSI index was around 3–4%. The HSI index started to increase in November to peak at 8% in April, thus suggesting that reserves were taken from the liver to be used for gonadal development.

As in other fish species, observed maturity stages in *L. scleratus* were a function of size (Figure 7), and the mean size at maturity, or L_m (i.e., the size at which 50% of the examined fish were mature, or L_{50}) for females and males are presented in Fig. 7. Here, the two features of interest are that there appears no clear pattern of one of the

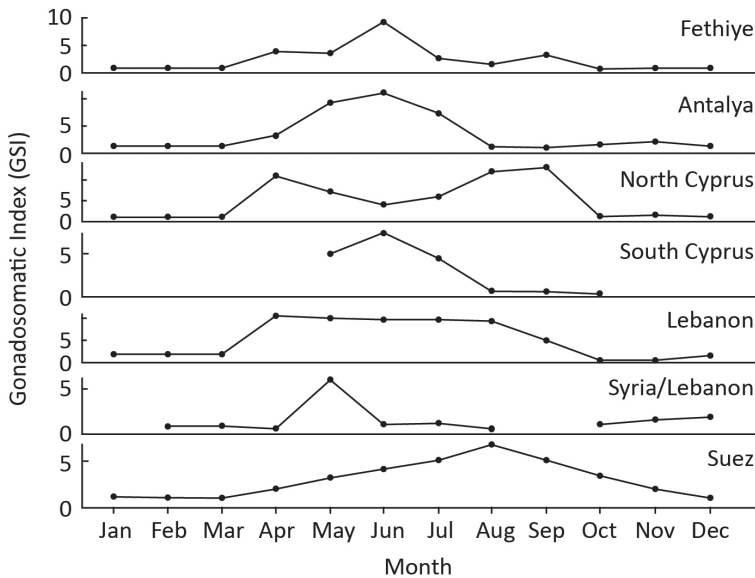


Figure 6. Seasonal variation of the Gonadosomatic Index (GSI) of *Lagocephalus sceleratus* in the Mediterranean and the Suez region, based on data by Sabrah 2006, (1st location at the bottom of the figure), Syria-Leb/Khalaf 2014 (2nd location), Lebanon/Boustany 2015 (3rd location), S. Cyprus/Rousou, 2014 (4th location), N. Cyprus/Akbora 2020 (5th location), Antalya Bay/Aydin 2011 (6th location), and Datça and Fethiye (top trend) from this study (values are averages of n = 14–340 fish per month, Suppl. material 1: Table S4). Note the trend toward a shorter spawning season as one moves North (upward from Suez).

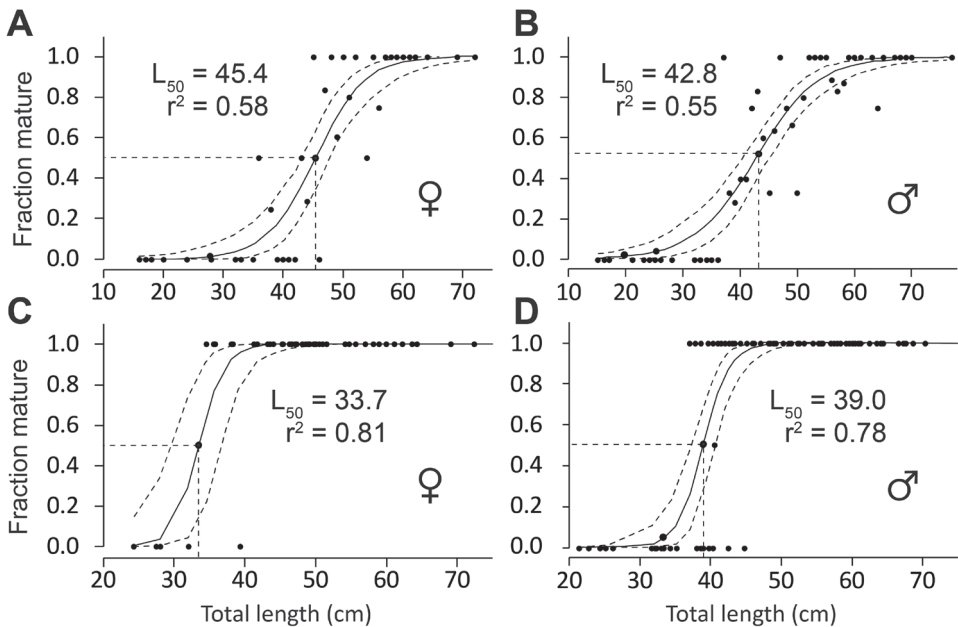


Figure 7. Maturity as a function of length in for *Lagocephalus sceleratus*. Note that mean length at first maturity is higher outside the spawning season (A, B) than inside (C, D) the spawning season, for both sexes.

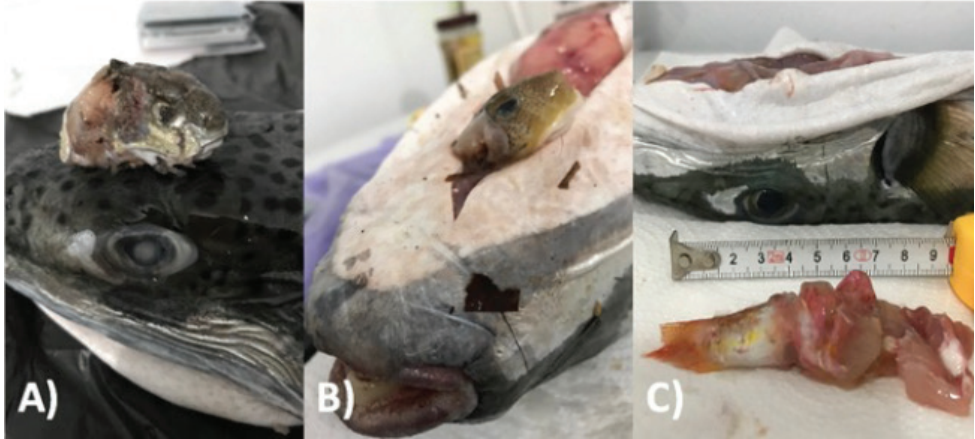


Figure 8. Stomach contents of *Lagocephalus scleratus* presenting evidence of cannibalism (A) and predation on other invasive species, i.e., *Torquigener flavimaculosus* (B); and *Parapeneus forsskali* (C).

sexes reaching maturity earlier than the other, and perhaps more interestingly that in both sexes, Fig. 7 sexual maturity (L_m) is reached earlier during the spawning season than outside.

Prey

Of the 563 fish that had their stomach contents examined, 48 (9%) of the stomachs were empty, 58 (10%) had food residues, 253 (45%) had stomachs less than half full, 170 (30%) were over half full, and 34 (6%) were full. A total of 34 specimens (Suppl. material 1: Table S7) were found as prey items from 8 non-indigenous species (NIS); Of these, 23 were Tetraodontidae species: 10 juvenile *L. scleratus*, 10 *Torquigener flavimaculosus*, two *Lagocephalus* spp., and one *L. suezensis* (Fig. 8). Other NIS were three *Pterois miles*, three *Parapeneus forsskali*, two *Siganus* spp., dozens of small gastropods (*Cerithium scabridum*) and one long-spine sea urchin (*Diadema setosum*). A total of 6% of *L. scleratus* had consumed non-indigenous species.

Crustaceans and fish made up the majority of diets being found in 26% and 24% of stomachs, respectively, with cephalopod remains in 11%. There was no statistical difference between the taxonomic prey composition between juvenile and adult *L. scleratus* ratios of crustaceans, fish and cephalopods ($p = 0.225$). For crustaceans, small crabs were the major taxon, with only a few stomachs containing shrimp remains, as expected, since crab shells take longer to be digested and/or evacuated. Of the crabs, *Carappa granulata* was found in two stomachs, one *Carcinus aestuarii*, one *Charybdis* sp., and one *Scylliarides latus*. For fish, those that could be identified were three *Pterois miles*, *Scorpaena* spp., *Epinephelus* spp., Mugilidae spp., *Atherina* spp., *Diplodus* sp., *Sparus aurata* and *Siganus* spp. Of cephalopods, there were about a dozen cases each of common squid (*Loligo vulgaris*), common octopus (*Octopus vulgaris*), one violet blanket octopus (*Tremoctopus violaceus*) and unidentified cephalopod beaks and ink (Suppl. material 1: Fig. S4). Three of the stomachs examined contained a lot of sand

(between 8 to 10 g) suggesting that some individuals dig in the seafloor looking for food items. One stomach contained some seagrass *Posidonia oceanica*. In addition to food, a total of 48 fishing hooks were found, 9 pieces of fishing net (weighing between 4–7 grams, with one very large 20 cm × 15 cm net sample), and 2 pieces of metal wires.

From the IRI examination, 34 additional *L. sceleratus* stomachs were analysed, and 91% (31) of those had food in their stomachs (coefficient of vacuity: 23.5). The IRI results are presented in Suppl. material 1: Table S8, and prey fish taxa were identified as *Lagocephalus* sp. and *Mullus* sp., and one *Octopus vulgaris*. The remaining shrimp, crab and cephalopod species could not be clearly identified to lower taxonomic groups. The trophic level of *L. sceleratus* was estimated as 4.15, which is the level assigned to tertiary consumers or carnivorous fish.

Discussion

L. sceleratus offers a trifecta of highly negative impacts due to its high toxicity, economic losses to fishers, and negative effects on native marine biodiversity. Their unique ability to puff and high toxicity likely contribute to their invasive success in the Mediterranean. Due to a nearly complete lack of population control, this species has expanded to all corners of the basin, putting people, fishers, fisheries and the native ecology at risk. Its conquest of the Mediterranean is one of the most successful marine invasions in modern history, comparable with that of the invasive Western Atlantic lionfish *Pterois volitans* and *Pterois miles*, the latter having also established itself as a Lessepsian species in the Mediterranean in 2012 (Bariche et al. 2013; Côté and Smith 2018).

Their marked expansion benefits from both a lack of human control (as fishing and sales of pufferfish are prohibited in most countries, including all countries of the EU), and limited predatory control (due to their ability to puff, and high TTX content). Their success is also likely enhanced by the overfishing that characterizes the Mediterranean basin, which has lost its top predators (Halouani et al. 2015). Indeed, with a Mediterranean trophic level estimate of 4.15, *L. sceleratus* can be considered a top predator, and may even be an apex predator due to a very pronounced regional loss of top predators due to overfishing (Demirel et al. 2020). Despite the overfished nature of the Eastern Mediterranean, *L. sceleratus* appears to be well-fed due to its generalist nature and sharp beak, with over 80% of samples having consumed a recent meal. It should also be emphasized here that *L. sceleratus* prey items very rapidly disintegrate into a ‘soup’ in their stomachs, and the species which were identified (other than tough shelled organisms) were very freshly swallowed and hence found in their esophagus.

Here we reveal the results of biological studies on their morphometrics and growth, reproduction, and diet before presenting some management advice and ideas for further directed research. The morphometric (LWRs) and growth studies conducted here produced results that were comparable to those of other authors. This also included the ratio L_{max}^D / L_m^D , which was statistically undistinguishable from estimates of this ratio in other teleosts (Pauly 1984; Amarasinghe and Pauly 2021; Meyer and Schill 2021). This

implies that although *L. sceleratus* is unique in its invasive abilities and some biological features, its life-history is still constrained in the manner predicted by the Gill-Oxygen Limitation Theory (GOLT; Pauly 2019, 2021). However, one interesting finding that apparently has not been highlighted by other authors, or in other fish species that we are aware of, is that mean length at first maturity (L_m) of *L. sceleratus* is lower during the peak spawning season than outside of it (Fig. 7). This may occur in other teleosts, but to our knowledge, this feature has not been previously reported. Clearly, this should be examined further, notably when comparing non-indigenous and native fish, to test if perhaps this is an adaptation of successful non-indigenous species.

Using traditional biological sampling combined with fishers' knowledge improved the biological understanding of *L. sceleratus*, e.g. their spawning periodicity. We also found the HSI and GSI patterns to be asynchronous, which explains how its energy is stored and utilized (Torcu-Koç et al. 2020): peak HSI occurred in April, suggesting that the liver stores reserves up to that month; after which the reserves are used for gonadal development in the two months following. The June peak in condition factor, on the other hand, corresponds to the period prior to the major annual spawning event.

From its ovarian organization, *L. sceleratus* was identified as a group synchronous batch spawner from the presence of both previtellogenic oocytes (in a range of sizes) and larger vitellogenic oocytes (of larger similar sizes) in the ovaries during the peak spawning period. The presence of these two clearly different size groups of oocytes is defined as group asynchronous ovarian organization, with a heterogeneous population of oocytes in their primary growth stage together with a synchronous population of larger oocytes in the yolked stage, indicating further recruitment into the oocyte stock at any time during the spawning season (Hunter and Goldberg 1980; Murua et al. 2003). It is well identified that fecundity types of fish species exhibiting group synchronous ovarian organization have determinate fecundity, meaning the species has a fixed potential annual fecundity at the onset of spawning (Murua et al. 2003). The most important indication of fecundity type is the observation of atresia in different ovarian stages, but our histological samples consisted of mature ovaries from peak spawning season and did not exhibit atresia to clearly evaluate fecundity type. Therein, our findings slightly differ from the only other study on the reproduction of *L. sceleratus* in the Mediterranean coast, which reported their fecundity type as determinate with clear presence of atresia (Farrag et al. 2019). One possible explanation could be that our study was limited to the peak spawning season, which implied a lack of oocyte size changes before and after the spawning season. Also, Ganiyas et al. (2015) noted that the indeterminate pattern of the oocyte size-frequency distribution was continuous until almost the end of the reproductive period. It is commonly accepted that fecundity type is strongly related to environmental impacts, such as temperature and food availability, and is thus flexible (van Damme 2010). Considering this flexibility, it can be speculated that *L. sceleratus* is still adapting to its new habitats in the Mediterranean. Indeed, its various Mediterranean subpopulations have different spawning seasons, likely connected to different temperature cues, and their spawning seasons may change with continuous sea warming. The spawning season reported here for Muğla

province, Turkey, which is restricted to late May and June, is the shortest spawning season reported from the Mediterranean (Fig. 6), and it supports the hypothesis of environmental factors determining fecundity types. We are confident that the fish we collected from Fethiye and Datça in this study are representative of the fish from the Muğla province, due to their slightly different reproductive season than the neighbouring Antalya province. As a recent invasive species, the *L. sceleratus* populations in the Mediterranean can be assumed to be relatively homogenous, as not enough time has occurred for much genetic differentiation to have evolved. Additionally, this study offers new insights into the dynamics of a different stock in Turkey. Clearly, further research should be directed at detailed spawning studies spanning an annual cycle, and by collecting samples daily, which would improve on the fecundity values reported here and allow for testing of this hypothesis.

The most important finding of this study is that *L. sceleratus* appears to prey on a wide range of other invasive species, and its control of them is its first positive documented ecological trait. The spines of lionfish (*P. miles*) found inside three *L. sceleratus* suggest that pufferfish are preying on lionfish. *L. sceleratus* also provide some control on other invasive species such as Red Sea goatfish, rabbitfish, other pufferfish species, their own species, and even the longspine sea urchin (*Diadema setosum*). The finding that their target prey composition is nearly equally comprised of fish and crustaceans, and a lesser extent of cephalopods, did not differ between juveniles and adults which contrasts the earlier findings of Kalogirou (2013) who found a dietary shift in prey from crustaceans to cephalopods to occur as *L. sceleratus* grows. This contrast may be due to density-dependent factors such as the increased abundances of pufferfish resulting in a marked reduction in cephalopods, which is commonly noted by fishers. Note also that their prey included toxic and venomous species, such as lionfish mentioned previously, scorpionfish and other pufferfish species. It was known that *L. sceleratus* is cannibalistic in its native range (EastMed 2010; Aydin 2011), and this study provides the first evidence of cannibalism in the Mediterranean. Their toxicity is also likely heightened by their newer cannibalistic trend, which may cause higher TTX concentrations due to bioaccumulation (Zhang et al. 2020). Cannibalism was not reported in earlier studies (Kalogirou 2013; Rousou et al. 2014), suggesting it may be a density-dependent factor which developed more recently, possibly induced by hunger. Thus, even juveniles should be considered as potentially toxic, as was recently reported.

Lagocephalus sceleratus are now in direct competition with small-scale fishers in the Eastern Mediterranean, consuming their catches, revenue, time and thus much of their livelihoods. Their increasing damage to fishing gear also negatively impacts their livelihoods. In Cyprus, fishers often use newspaper articles mentioning pufferfish damage to lobby for financial support. In the Muğla province of Turkey, Ünal (2013) found over 90% of small-scale fishers were no longer generating a net income from their work. Small-scale fishers from southern Turkey, already highly marginalized, with many being forced out of the profession due to declining catches and incomes, have to completely replace their fishing nets every few months at an added cost of over \$2000 US due to pufferfish damage,

which previously lasted them several years. As both an incentive with the benefit of aiding fishers offset the increasing costs from pufferfish, a bounty program was recently initiated in Turkey.

The Turkish government recently completed a pilot bounty project collecting *L. sceleratus* tails from the Turkish Mediterranean coast in December 2020. A total of 46,000 tails were collected for a reward price of US \$0.60 each. A second bounty program was established on June 27, 2021 for a duration of three years, during which *L. sceleratus* will be, this time, collected in its entirety (@ US \$0.60 each) so that proper disposal can be ensured (Mahir Kanyılmaz, Fisheries Directorate, Ankara, Turkey, pers. comm.). Even if this initiative is not effective at reducing abundances, it will still add some positive economic benefit to some fishers. To predict how much the population of *L. sceleratus* should be reduced to negate its impacts to native biodiversity through predation, its biomass, the biomass or abundance of its prey and its feeding rates must first be known. However, only one stock assessment from a small area has been completed in Turkey (Özbek et al. 2017). One study that modeled the required reduction of invasive lionfish densities to improve native fish communities found the reduction threshold to vary by site from 25–92% (Green et al. 2014).

After discussing the bounty program with twenty small-scale fishers from the Muğla province of Turkey, we strongly believe that this new bounty will not be effective at reducing their population enough to negate their effects. At present, small-scale fishers refuse to target this species due to the low reward and high costs of fishing gear damage; however, large-scale fishers may return specimens for reward if many are caught in a net at once. Alternatively, to control this species, we suggest a better solution would be to hire select commercial fishers, equip them with more resistant fishing nets, and have them specifically target *L. sceleratus* in their spawning season where they tend to aggregate. One Turkish fishing gear technology expert, Dr. Zafer Tosunoğlu, Ege University, who was contacted for advice on the most applicable net material to specifically target *L. sceleratus* suggested using Dyneema netting (used for catamaran trampoline netting), which is the strongest netting fibre currently available, 15 times stronger than steel, and should minimize fishing gear damage. Also, emerging ‘genetic biocontrol’ may be applied (Teem et al. 2020) to control *L. sceleratus* and other invasives.

The development of commercial applications for invasive species such as *L. sceleratus* may financially support their ongoing removal (Giakoumi et al. 2019). Applications in the bio-medical industry include using their skin as antimicrobial wound dressing (Iswariya et al. 2016), their teeth as regenerative dental implants (Thiery et al. 2017), and TTX for extreme pain relief (Hagen et al. 2008, 2017). *L. sceleratus* skin is also being trialed as a type of exotic fish leather (Alla et al. 2017). Since each surveyed fisher despises this species as it causes them a great deal of stress from fishing gear and fish losses, the possible development of a commercial market for pufferfish would not likely encourage any fishers to undertake fishing this species over the long-term to secure their new stream of income, as sometimes occurs after commercialization. Regardless, this invader appears to be here to stay in the Mediterranean.

This NIS top predator in the Mediterranean threatens local biodiversity, human health, fishing communities and potentially even tourism. Since it is currently lacking control on its population in most of the Mediterranean Sea, we suggest that removal through targeted fishing during its spawning period is the best control recommendation for decision-makers. Removals need to be prioritized but can be expensive to fund, which is why commercialization of this species could help financially sustain their long-term control. The current commercial solutions, which would use a highly invasive species to benefit our teeth and skin, heal our wounds, alleviate our pain and protect our feet, if successful, could represent the largest turnaround in the history of marine invasions.

Prior to this study, most Mediterranean research on this species either studied its growth, or toxicity. This study added to this body of knowledge by determining the spawning strategy and reproductive ecology of *L. sceleratus*, factors relating to its growth, and its position within the trophic web, its density-dependent cannibalistic nature, and its potential ability to help control subsequent invasions. The diversity of their prey can be used as inputs for ecosystem modeling efforts, which, along with improved biomass estimates, can help to understand how much should be removed to help improve the state of native biodiversity. Further directed research needed to better understand and hence manage this invasion should involve mapping its various spawning habitats and seasons, its larval ecology and growth, its feeding rates, DNA stomach content analysis, a forum to update on their interactions with humans, and baseline stock assessments along the entire Levantine coast.

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Supplementary material I

Appendix 1, Figures S1–S4, Tables S1–S8

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Data type: docx. file

Explanation note: **Appendix 1.** The Gill-Oxygen Limitation Theory. **Figure S1.** Plot of L_{\max}^D vs. L_m^D (for $D = 0.6$) in different populations of *L. sceleratus*. **Figure S2.** Length-weight relationships for A) female and B) male *Lagocephalus sceleratus*. **Figure S3.** Relationships, in *Lagocephalus sceleratus* between fecundity and total length, total weight and gonad weight during peak spawning in June 2020. **Figure S4.** Stomach contents of *Lagocephalus sceleratus* showing freshly ingested cephalopods: *Loligo vulgaris* (A), *Octopus vulgaris* (B); and parts of *Tremoctopus violaceus* (C). **Table S1.** Sex ratios for *L. sceleratus* from Mediterranean and Suez Canal studies. **Table S2.** Length-weight relationships of *Lagocephalus sceleratus* from this study in southwestern Turkey. **Table S3.** Some length-weight parameters with sex, length range, length type and sample size (N) for *L. sceleratus* from Mediterranean and other studies. **Table S4.** Length-frequency data of *Lagocephalus sceleratus* collected in Southwestern Turkey from June 2019 to November 2020. **Table S5.** Growth parameters estimates for *Lagocephalus sceleratus* in the Mediterranean Sea (L in cm). **Table S6.** Mean length at first maturity (L_m) and maximum length (L_{\max}) for *L. sceleratus* at various locations of the Suez Canal and Mediterranean Sea. **Table S7.** Frequency of non-indigenous species (NIS) preyed upon by *L. sceleratus* in this study. **Table S8.** IRI Results of prey items for 34 sampled *L. sceleratus*.

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