Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.38444

The diet and feeding habits of *Lagocephalus sceleratus* in the eastern Mediterranean: A case study carried out off the island of Crete (southern Greece)

Rita V.C. GOMES[1,3], Georgios CHRISTIDIS[2,3], Panagiota PERISTERAKI[3], Stylianos SOMARAKIS[3], and George TSERPES[3]

[1] University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[2] Department of Biology, University of Crete, 70013 Heraklion, Greece
[3] Hellenic Centre for Marine Research (HCMR), Institute of Marine Biological Resources and Inland Waters, 71500 Heraklion, Greece

Corresponding author: Rita V. C. GOMES; ritavcgomes@gmail.com, rita.gomes@ipma.pt

Contributing Editor: Konstantinos TSAGARAKIS

Received: 16 June 2024; Accepted: 12 December 2024; Published online: 11 February 2025

Abstract

This study focused on the diet and feeding habits of the invasive pufferfish *Lagocephalus sceleratus* in the coastal waters of Crete (Cretan and Libyan Sea, eastern Mediterranean). The diet was composed of a wide variety of organisms, while the number of specimens with empty gastrointestinal tracts was low (14.41%). In total we were able to identify 38 different species of fish and invertebrates within the tracts analysed. Fish were the main diet component, followed by crustaceans and mollusks (mostly gastropods and cephalopods). At family level, the most significant preys were Sparidae and Mullidae teleosts, along with Cavoliniidae gastropods. The trophic level of *L. sceleratus* was estimated to be 4.13. The trophic niche breadth was intermediate, whereas the *L. sceleratus* population exhibits a mixed feeding strategy. Spatiotemporal differences in diet composition were also identified. The application of generalized additive models (GAMs) revealed that depth at which the fish were captured, season, region and total length, were significant predictors of the probability of occurrence of its most frequent prey groups. Fish prey occurrence was more probable in autumn and summer and at circa 20-25 m depth. In contrast, the probability of consuming crustaceans was higher in spring and winter and in specimens measuring 450-500 mm in length, and lower at 20-25 m, whilst increasing until a maximum at 40 m. The probability of non-cephalopod mollusks consumption was higher in winter and in the Libyan Sea, as well as at greater depths. Fish size had a significant effect on the likelihood of consuming photosynthetic organisms (algae and seagrass) and cephalopods, which was higher in larger fish. This study presents further evidence of the negative impact of *L. sceleratus* on the fisheries sector, revealing both predation on commercial species and the ingestion of various fishing gear parts.

Keywords: *Lagocephalus sceleratus*; diet; trophic level; binomial GAMs.

Introduction

Human-mediated species introductions are accelerating all over the world, and the Mediterranean Sea is especially at risk, as a major hotspot of marine bioinvasions (Kalogirou *et al.*, 2012; Farrag *et al.*, 2015). The Suez Canal is the primary entry point of non-indigenous species into this region (Galil, 2023). The opening of this canal in 1869 set off an ongoing process of colonization (Carpentieri *et al.*, 2009; Bal & Khan, 2022). 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 a certain extent, the entire Mediterranean (Papaconstantinou, 1990; Albano *et al.*, 2021; Gabel *et al.*, 2022).

Lagocephalus sceleratus (Gmelin, 1789), commonly known as the silverstripe blaasop or silver-cheeked toadfish (Jribi & Bradai, 2012; Özbek *et al.*, 2017), is among the rapidly expanding Lessepsian invaders. Originating from the Red Sea, the Indian and the Pacific Ocean (Smith & Heemstra, 1986), this pufferfish was first recorded in the Mediterranean Sea in 2003 (Akyol *et al.*, 2005). Since then, it has significantly extended its range, now spanning the Black Sea and most Mediterranean subregions, recently reaching the Strait of Gibraltar (Azzurro *et al.*, 2020). Its expansion and establishment are aided by its unique biological and ecological aspects, such as reproduction at an early age, the ability to compete with native top predators and the lack of predatory control (Ulman *et al.*, 2021a). It is likely that rising sea temperatures are likewise enhancing its further dispersal (Jribi & Bradai, 2012; Coro *et al.*, 2018).

L. sceleratus is one of the largest members of the family Tetraodontidae (Kasapidis *et al.*, 2007; Michailidis,

2010) and is found mainly at depths from 18 to 100 m, in a wide range of habitats, including sandy and muddy bottoms, rocky substrates, and seagrass meadows (Yaglioglu *et al.*, 2011; Ulman *et al.*, 2021a; Ulman *et al.*, 2021b). It is regarded as a generalist carnivore with a broad and diversified diet (Boustany *et al.*, 2015), mainly consisting of crustaceans, fish, cephalopods, and other mollusksmolluscs (Sabrah *et al.*, 2006; Rousou *et al.*, 2014; Boustany *et al.*, 2015).

The silver-cheeked toadfish is considered as one of the Mediterranean's worst 100 invasive species (Streftaris & Zenetos, 2006), as it negatively affects native biodiversity, local fisheries, and human health (Ünal *et al.*, 2015; Ulman *et al.*, 2021a; Ulman *et al.*, 2022; Christidis *et al.*, 2024; Ulman *et al.*, 2024). Most importantly, the invasion of *L. sceleratus* has raised public health awareness as its consumption has led to many cases of food-poisoning, some of which were lethal (Kalogirou, 2013; Ben Souissi *et al.*, 2014; Ünal *et al.*, 2015; Özbek *et al.*, 2017; Galanidi *et al.*, 2018; Kleitou *et al.*, 2018; Abd Rabou, 2019). These poisoning cases are attributed to the ingestion of tetrodotoxin (TTX), a potent highly stable neurotoxin contained in the tissues of pufferfish species, which has no known antidote (Moczydlowski, 2013). Moreover, some rare incidents of attacks and bites on bathers by this species pose another safety threat (Galanidi *et al.*, 2018; Ulman *et al.*, 2024). Additionally, fishers operating in the eastern Mediterranean have reported that the silver-cheeked toadfish has major socio-economic impacts on small-scale fisheries (Ünal & Bodur, 2017; Abd Rabou, 2019; Christidis *et al.*, 2024). Pufferfish can damage nets and longlines with their strong beak-like teeth, during feeding on prey caught by fishing gears (Nader *et al.*, 2012; Boustany *et al.*, 2015). Such pufferfish-fisheries interactions lead to catch losses and extra costs for gear replacement (Ünal *et al.*, 2015; Christidis *et al.*, 2022; Christidis *et al.*, 2024). Lastly, fishers presume that this species is a major contributor to the reduction of local stocks of commercial cephalopods through predation (Nader *et al.*, 2012).

Several studies have investigated the diet composition of the silver-cheeked toadfish in the eastern Mediterranean (e.g., Michailidis, 2010; Aydin, 2011; Kalogirou, 2013; Boustany *et al.*, 2015; Hammoud & Salama, 2016; Hussain *et al.*, 2020; Ulman *et al.*, 2021b; Ersönmez *et al.*, 2023). Nonetheless, only few of them have managed to identify preys to family and species level (Hussain *et al.*, 2020; Ulman *et al.*, 2021b; Gabel *et al.*, 2022; Ersönmez *et al.*, 2023). In Crete (southern Greece), this species was first recorded in 2005 (Kasapidis *et al.*, 2007), and today it is widespread throughout the waters surrounding the island (Christidis *et al.*, 2022), where it is a recurrent bycatch and often reported to practice depredation (Christidis *et al.*, 2024). However, so far, no research has focused on its feeding habits in this area. Generally, invasive species can disrupt native food webs (Gallardo *et al.*, 2016), and since *L. sceleratus* might be a top predator in the Mediterranean (Ulman *et al.*, 2021b), the investigation of predator-prey interactions is essential in order to elucidate the ecological and economic impacts of this species. Moreover, gut content analysis and diet studies allow us to document a fish's prey spectrum and the trophic relationships in which they are involved (Devi & Sivan, 2017). This research attempts to investigate aspects of *L. sceleratus* feeding ecology in the coastal waters of Crete, i.e., diet composition, trophic level and feeding strategy. For the first time, binomial generalized additive models were also utilized for this species in order to investigate the factors influencing the probability of predation on different prey groups.

Materials and Methods

Sample collection

The collection of an adequate number of fish to carry out an analysis of diet composition proved to be a difficult task, mainly due to the recent alternations in fishing tactics of the Cretan fishers in order to avoid *L. sceleratus* and the associated gear and catch damages caused by this species (Christidis *et al.*, 2024). During our extended sampling period (June 2017 to October 2022), the number of specimens collected each year was low, especially during the winter months (Table S1). Consequently, the analysis of *L. sceleratus* diet was performed after pooling all data irrespective of year. Pooling data over broad temporal scales (years, months) integrates temporal heterogeneity and the results of the analysis present average conditions.

In total, 236 specimens of *L. sceleratus* were analysed. Out of these, 204 specimens were collected onboard the commercial fishing fleet and 32 from samplings carried out with experimental fishing gears (handlines, fishing rods) in the seas around Crete (107 and 129 specimens from the Cretan Sea and Libyan Sea, respectively) (Fig. 1). Fishing depth ranged from 1.5 to 60 m.

Collected specimens were 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 from 36.7 to 4910 g. The sex of the specimens was identified macroscopically (Christidis *et al.*, 2021), resulting in a sample of 112 males and 124 females.

Laboratory analysis

The gastrointestinal tracts of the specimens were weighed and dissected. This species lacks a stomach that is well-separated from the intestine, given that pufferfish stomachs do not have a digestive function, being primarily used for inflation for defence purposes (Wilson & Castro, 2010). The contents were extracted from the tract and preys were identified macroscopically, or using a stereoscope, to the lowest taxonomic level possible. They were then counted and weighed with a precision of 0.01 g. Fish otoliths, scales and cephalopod beaks aided in the identification. The contents were categorized into non-food items (fishing gears, substrate, metal), food items (prey and baits) and unidentified items. Preys were further divided into eight groups [cephalopods, crustaceans, fish, echi-

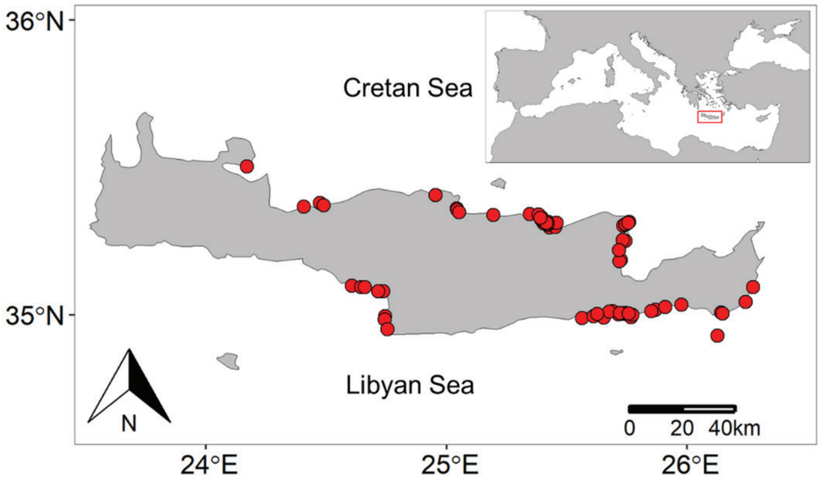

Fig. 1: Sampling locations of *L. sceleratus* specimens.

noderms, non-cephalopod mollusks (herein “mollusks”), photosynthetic organisms (herein “plants”), polychaetes, and coral fragments].

Data analysis

The proportion of empty stomachs was calculated for the entire sample and per season, using the vacuity index (Vi):

$$Vi\% = \frac{number\ of\ empty\ stomachs}{number\ of\ total\ stomachs} * 100$$

For each prey, the frequency of occurrence (FO%) and its contribution in numbers (N%) and weight (W%) were quantified using the equations proposed by Berg (1979), Hyslop (1980) and Cortés (1997), respectively:

$$FO\% = \frac{number\ of\ stomachs\ with\ prey\ i}{number\ of\ total\ tracts} * 100$$

$$N\% = \frac{total\ number\ of\ prey\ i}{total\ number\ of\ all\ preys} * 100$$

$$W\% = \frac{total\ weight\ of\ prey\ i}{total\ weight\ of\ all\ preys} * 100$$

The significance of each prey in the diet of *L. sceleratus* was expressed through the index of relative importance IRI and the IRI%, calculated by the equations proposed by Hacunda (1981):

$$IRI = (\mathrm{N\%} + \mathrm{W\%}) * \mathrm{FO\%}$$

$$IRI\% = \frac{\mathrm{IRI}}{\sum IRI} * 100$$

The adequacy of the collected sample size for describing the diet of the species was verified by computing a prey accumulation curve for the whole sample based on random addition of individuals and 100 permutations. This computation was performed using the *vegan* package in R (Oksanen *et al.*, 2022). Prey accumulation curves indicate a sufficient sample size when an asymptote is reached, demonstrating that the cumulative number of identified prey taxa (y-axis) stabilizes as the cumulative number of examined stomachs increases.

The trophic niche breadth of the population was calculated using Levin’s standardized index (B_A), as proposed by Hurlbert (1978):

$$B = \frac{1}{\sum p_j^2}$$

$$B_A = \frac{B-1}{n-1}$$

where *B* is the Levin’s measure for niche breadth, p_j is the proportion of individuals using resource *j* or fraction of items in the diet that belong to food category *j*, and *n* is the number of food categories. The B_A index ranges from 0 to 1, where values 0-0.39, 0.4-0.6 and 0.61-0.9 indicate a narrow, intermediate and broad trophic niche, respectively (Grossman, 1986). The niche breadth calculation was based on the numerical abundance of all identified prey groups.

The feeding strategy of *L. sceleratus* was explored graphically by plotting the prey-specific abundance (A%) against the frequency of occurrence (FO) (Amundsen *et al.*, 1996). Prey-specific abundance was calculated for each prey group using the following equation, proposed by Amundsen *et al.* (1996):

$$A\% = \frac{\Sigma S_i}{\Sigma S_t} * 100$$

where S_i is the tract content (number) composed by prey group *i*, and S_t is the total tract content only in predators with prey group *i* in their tracts. The feeding strategy is represented on the vertical axis of the plot, where a predator can be characterized as specialist if points are located in the upper part, or as a generalist if points are located in the lower part of the plot. Anally, the diagonals of the plot provide information on prey importance and phenotypic contribution to niche width. Specifically, points in the lower left corner indicate rare or unimportant prey, while points in the upper right corner indicate dominant prey. Points in the upper left corner suggest that some individuals of the predator population specialize in specific prey, and points in the upper right corner indicate specialization of the whole population in specific prey.

The trophic level for *L. sceleratus* was estimated using the *dietr* package in R, with the DietTroph function (Borstein, 2020). This estimation was based on the weight contribution of each prey group, in 190 of our specimens with non-bait food items. We used the default trophic levels of our broad taxonomic prey groups (fish, cephalopods, mollusks, crustaceans, corals, echinoderms, polychaetes, and plants) available in *dietr* through the *FishBasePreyVals* dataset. Food items identified as baits of hooked gears were excluded from all calculations.

The probabilities of occurrence of the major prey groups (fish, crustaceans, "mollusks", "plants" and cephalopods) in *L. sceleratus* tracts were investigated by means of generalized 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') and total fish length ('TL'). In addition, geographical region ('REGION'), season ('SEASON'), sex ('SEX') and fishery type ('CR') from which the specimens had been collected – either commercial or recreational/experimental – were entered as factors into the models. The smoother function used for the non-linear predictors was a penalized cubic regression spline and model fitting was accomplished using the *mgcv* library (Wood, 2017) in the R language environment. The procedure automatically selects the degree of smoothing based on the generalized cross-validation score, which is a proxy of 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).

Results

Prey accumulation curve

The prey accumulation curve presented in Figure 2 shows that no asymptote is reached, indicating that the collected sample size is inadequate to describe the full range of the *L. sceleratus* diet. However, when considering only prey with IRI > 0.1%, an asymptote is reached at approximately 110 specimens.

Diet composition and feeding habits

From the 236 tracts examined, the vacuity index was 14.41%, while it varied throughout the year (4.48% in spring, 23.53% in summer, 16.18% in autumn and 17.91% in winter). In total, 1090 items (total weight 3377.22 g) were found, consisting mainly of food (990 items weighting 3273.30 g), whereas non-food (81 items, total weight 88.04 g) and unidentified (19 items, total weight 15.96 g) items were also recorded. Moreover, in two tracts only the baits were found, eight tracts

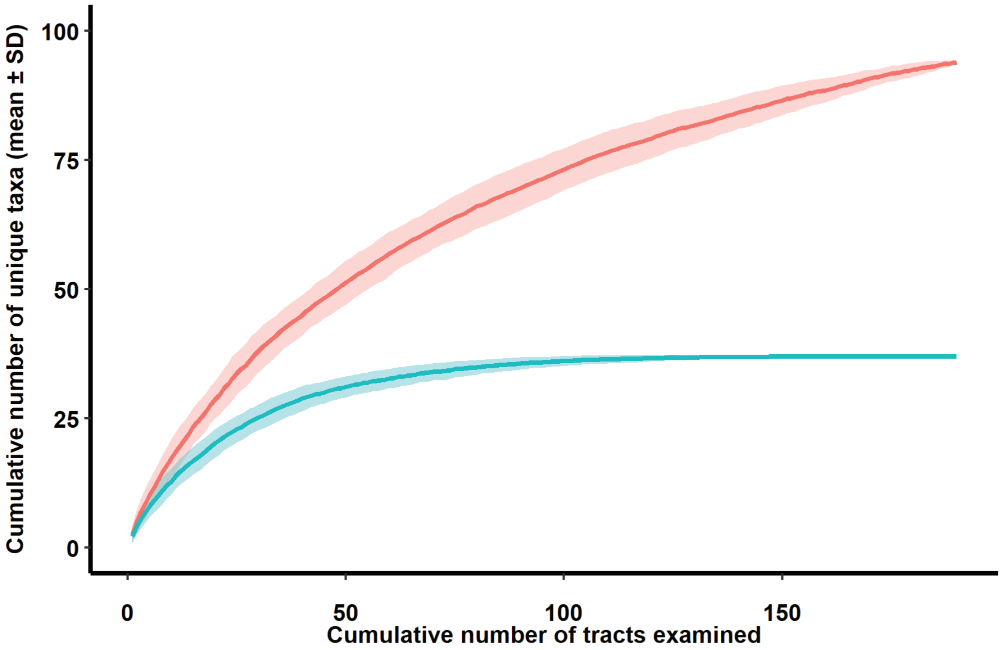

Fig. 2: Cumulative prey curves of *L. sceleratus* in Crete coastal waters. Red line represents the curve using all prey found within the tracts, whereas the blue line represents the curve using only prey with IRI >0.1%.

contained only non-food items, such as parts of substrate and a small square piece of metal (one tract), and in another two, only unidentified items were found. Regarding the non-food items, identified parts of substrate were mainly grains of sand (43.20% of total substrate weight), followed by stones (5.08%), pebbles (1.74%) and fragments of Foraminifera tests (external shells) (0.04%). Fishing gears found were mainly pieces of net (84.62% of the total number of fishing gears recorded), hooks (12.82%) and lines (2.56%) (Table 1). The highest percentage of fishing gear was recorded in the tracts of specimens caught by purse seines (25.00% of the total purse seine specimens), followed by specimens caught by longlines (22.22%) and static nets (19.62%) (Table 2).

The general frequency of occurrence and relative importance of each prey category are presented in Table 3. The most important preys were fish, crustaceans, cephalopods, “mollusks”, and “plants” such as seagrasses and algae (Table 3). Overall, preys that were identified to lower taxonomic levels belonged to 44 families, 46 genera and 38 species (Table S2). Based on IRI% the most important families were Sparidae (5.30%) and Mullidae (0.94%) teleosts, the Cavoliniidae gastropods (1.88%) and Octopodidae, specifically *Octopus vulgaris* Cuvier, 1797 (0.44%). A notable finding was the occurrence of non-indigenous species (NIS) in the diet of *L. sceleratus* such as the lionfish *Pterois miles* (Bennett, 1828), the yellow-spotted puffer *Torquigener flavimaculosus* Hardy & Randall, 1983*,* the dusky spinefoot *Siganus luridus* (Rüp-

Table 1. 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	80.50	975	3124.07
	Baits		13	5.51	15	149.23
Non-food	Fishing gears		36	15.25	39	65.26
		Net	33	13.98	33	57.84
		Line	1	0.42	1	0.11
		Hook	4	1.69	5	7.31
	Substrate		23	9.75	41	22.66
		Sand	14	5.93	-	9.79
		Pebbles	4	1.69	-	0.39
		Stones	3	1.27	-	1.15
		Others*	3	1.27	-	11.33
	Metal		1	0.42	1	0.04
Unidentified			17	7.20	19	15.96
Empty			34	14.41	-	-
Total					1090	3377.22

*Foraminifera tests, unspecified

Table 2. 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	**Gear**	**N of individuals**	**% of tracts with:**		
			Net	**Line**	**Hook**
Commercial	Boat seine	29	0.00	0.00	0.00
	Purse seine	8	25.00	0.00	0.00
	Nets	158	19.62	0.00	1.27
	Longlines	9	0.00	0.00	22.22
Recreational	Handlines	14	0.00	7.14	0.00
	Fishing rod	16	0.00	0.00	0.00
	Speargun	2	0.00	0.00	0.00

Table 3. Frequency of occurrence (FO%), number (N), numeric percentage (N%), weight (W in g), weight percentage (W%), index of relative importance (IRI) and index of relative importance percentage (IRI%) of items found in *L. sceleratus* tracts by prey group. Only tracts with non-bait prey items were considered (n = 190). Trophic level (Troph) is presented for each group.

Group	Number of Tracts	FO%	N	N%	W	W%	IRI	IRI%	Troph*
Cephalopods	22	11.57	27	2.77	431.66	13.81	191.8	1.82	3.2
Coral	1	0.53	1	0.10	0.14	<0.10	0.05	<0.00	2.3
Crustaceans	64	33.68	157	16.10	273.59	8.76	837.28	7.92	2.5
Echinoderms	16	8.42	21	2.15	54.71	1.75	32.84	0.31	2.4
Fish	148	77.89	322	33.03	2319.27	74.24	8355.26	79.07	3.5
Mollusks	47	24.73	398	40.82	22.09	0.71	1027.04	9.72	2.6
Plants	42	22.11	47	4.82	22.44	0.72	122.49	1.16	1.0
Polychaetes	2	1.05	2	0.21	0.17	<0.1	0.22	0.00	2.1

*Values from FishBasePreyVals data frame following FishBase and TrophLab.

pell, 1829), the Golani round herring *Etrumeus golanii* DiBattista, Randall & Bowen, 2012, the African blue swimming crab *Portunus segnis* (Forskål, 1775) and the bigfin reef squid *Sepioteuthis lessoniana* Lesson, 1831. The contribution of identified NIS to the IRI index was 1.88%. Moreover, unidentified remains of pufferfish species (teeth of tetraodontids) were found in six tracts (Table S2).

The Levin's standardized index was found to be 0.4, indicating an intermediate niche breadth. *L. sceleratus* was found to exhibit a mixed feeding strategy with some individuals specializing in invertebrate preys; overall, however, the entire sampled population exhibited a generalized feeding on fish preys (Fig. 3). The feeding strategy diagram (Fig. 3) reveals that the majority of *L. sceleratus* specimens in our study fed on fish, indicating that the Cretan population specialises in this prey (point located above the diagonal from the upper left to the lower right of the figure). Small proportions of some prey (e.g., coral parts) were occasionally included in the

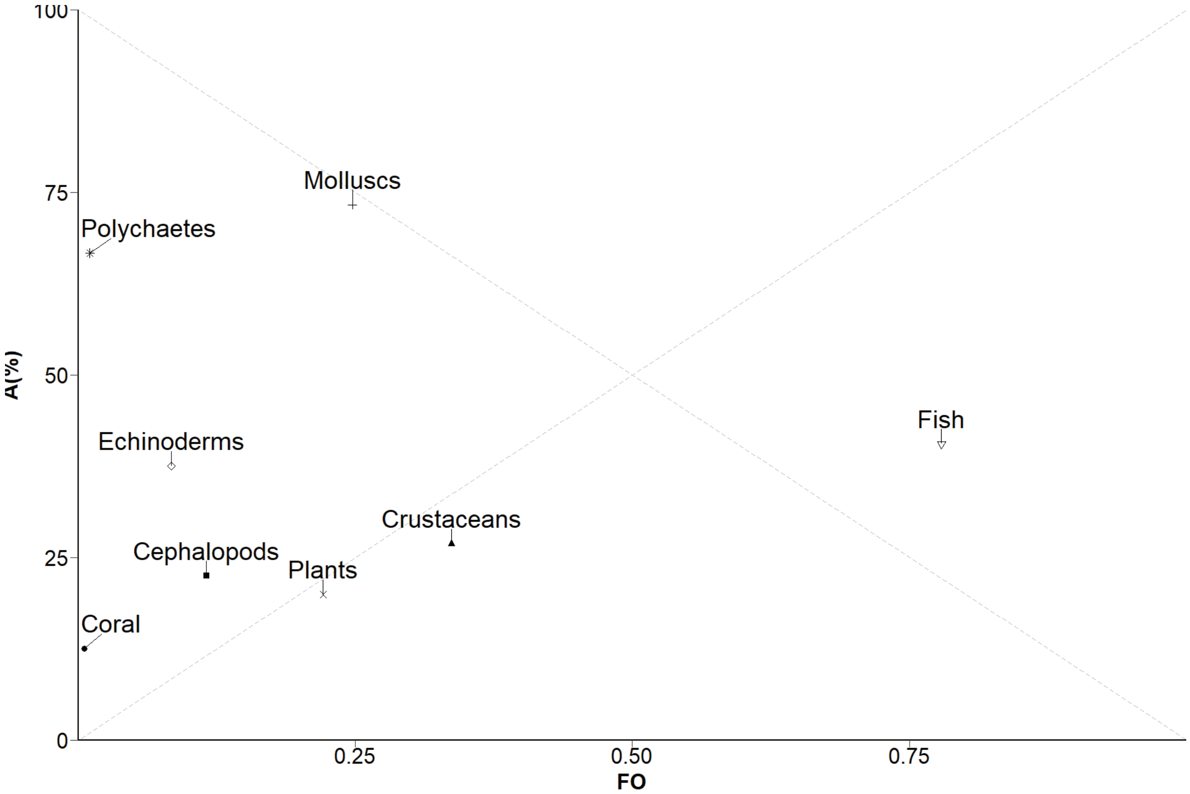

Fig. 3: Feeding strategy plot of *L. sceleratus*. Points represent different prey types. FO: frequency of occurrence; A(%): prey-specific abundance.

diet of some specimens, and are therefore characterized as rare and unimportant (points in the lower left part). Finally, some individuals specialized mainly in benthic invertebrates, such as polychaetes and mollusksmolluscs, primarily gastropods and bivalves (points positioned towards the upper part).

Across all specimens, trophic level ranged from 2.00 ± 0.00 to 4.50 ± 0.80, with the first representing the specimens that were found to feed only on seagrasses and algae, and the latter ones found to feed only on fish. The mean trophic level of *L. sceleratus* in our study was estimated to be 4.13 ± 0.69.

GAMs for the probability of occurrence of preys

The GAM for the probability of finding fish prey explained 13.90% of total deviance and revealed that the effects of SEASON and DEPTH were significant (Table 4). The probability of fish consumption was higher during summer and autumn. With regard to DEPTH, the probability decreased after 20-25 m. (Fig. 4). Meanwhile, the GAM fitted for crustaceans explained 14.00% of the total deviance and included SEASON, DEPTH and, marginally, TL as significant predictors (Table 4). The probability of occurrence of crustaceans decreased at around 20-25 m and increased thereafter, peaking at 40 m. It was

Table 4. Analysis of deviance for the GAM models for fish, crustacean, non-cephalopod mollusk (‘Mollusk’), photosynthetic organisms (‘Plant’) and cephalopod preys of *L. sceleratus*. The statistical significance of the explanatory variables of each model is also presented.

Explanatory variable	**Residual d.f**	**Residual deviance**	**Cumulative variance explained (%)**	***p-value***
Fish prey probability				
MEAN	186.00	196.71		
SEASON	183.00	182.27	7.34	0.02
REGION	182.00	182.21	7.37	0.11
SEX	181.00	181.37	7.80	0.88
CR	180.00	179.66	8.67	0.23
DEPTH	176.92	169.31	13.90	0.02
TL	176.92	169.31	13.90	0.73
Crustacean prey probability				
MEAN	186.00	237.59		
SEASON	183.00	225.04	5.28	0.00
REGION	182.00	225.03	5.29	0.87
SEX	181.00	217.70	8.37	0.09
CR	180.00	216.19	9.01	0.07
DEPTH	177.65	207.79	12.50	0.02
TL	176.46	204.28	14.00	0.05
Mollusk prey probability				
MEAN	186.00	208.65		
SEASON	183.00	198.00	5.10	0.03
REGION	182.00	185.35	11.20	0.03
SEX	181.00	182.27	12.60	0.36
CR	180.00	181.86	12.80	0.48
DEPTH	176.56	172.24	17.40	0.04
TL	176.56	172.24	17.40	0.54
Plant prey probability				
MEAN	186.00	196.71		
SEASON	183.00	186.81	5.03	0.25
REGION	182.00	183.06	6.94	0.07
SEX	181.00	182.46	7.24	0.07
CR	180.00	180.69	8.14	0.70
DEPTH	179.09	178.53	9.24	0.09
TL	174.03	165.09	16.10	0.02
Cephalopod prey probability				
MEAN	186.00	135.47		
SEASON	183.00	132.62	2.10	0.39
REGION	182.00	126.42	6.68	0.12
SEX	181.00	126.38	6.71	0.92
CR	180.00	125.44	7.40	0.29
DEPTH	180.00	125.44	7.40	0.87
TL	178.13	118.36	12.60	0.02

significantly higher in winter and spring. Additionally, it was slightly higher in mid-sized specimens (circa 450 mm) (Fig. 4). The model for non-cephalopod mollusks ('Mollusks') had DEPTH, SEASON and REGION as significant predictors and explained 17.40% of total deviance (Table 4). The relationship between DEPTH and probability of mollusk consumption was similar to that of crustaceans, with minimum probability at around 22 m and maximum at 40 m. The probability was also higher in winter and in the Libyan Sea (Fig. 4). The model for algae and seagrass ('Plant') explained 16.10% of deviance (Table 4), with plant consumption being higher in medium-sized fish as well as those measuring about 520 mm TL (Fig. 4). Similarly, only TL was significant in the model for cephalopods, which explained 12.60% of total deviance (Table 4). The probability of cephalopod consumption was positively related to size, being maximum at 550 mm TL (Fig. 4).

Discussion

The analysis of tract contents showed that the diet of *L. sceleratus* is mostly composed of fish and supplemented by crustaceans and mollusks. Comparisons with results of previous studies carried out in the Mediterranean are presented in Table 5. No studies on the diet of this species in its native range have been found. Past studies indicate that the diet of *L. sceleratus* is highly variable, but mostly carnivorous, as reported for several other Tetraodontidae species (e.g., Tortonese, 1986; Denadai *et al.*, 2012; Mohamad & Fadhilah, 2013; Devi & Sivan, 2017; Chartosia *et al.*, 2021; Seetha *et al.*, 2023; Ulman *et al.*, 2023).

Our study estimated that the trophic level of *L. sceleratus* is circa 4.13. A similar estimate (4.15) has been reported by Ulman *et al.* (2021b), but a more recent study by Ersönmez *et al.* (2023) calculated an even higher trophic level (4.41). The silver-cheeked toadfish can thus be classified as a top predator in its invaded range.

The proportion of empty tracts was overall low, peak-

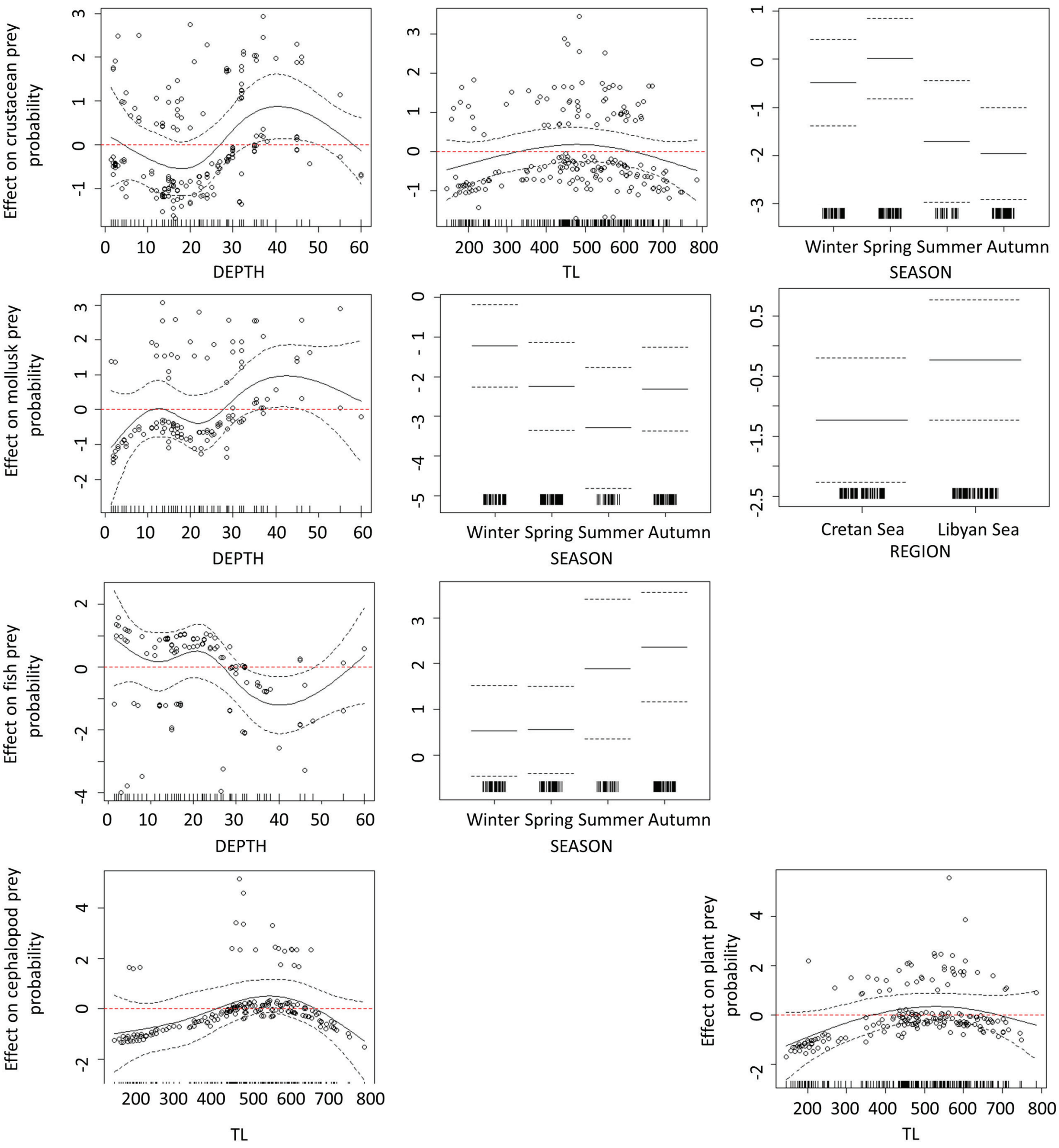

Fig. 4: Plots showing the results of the GAMs performed. Only significant predictors for each model are shown. TL: total length.

Table 5. Contribution of the main prey categories to the diet of *L. sceleratus* from various locations in the Mediterranean Sea (FO= frequency of occurrence, W= weight contribution, V= volume contribution, IRI= index of relative importance).

Reference	Sample size	Region	Fish (%)	Crustacea (%)	Mollusks (%)	Cephalopods (%)	Echinoderms (%)	Fishing Gear (%)
Akbora *et al*., 2020 (FO%)	24	Cyprus (North)	43	22		14		21
Aydin *et al*., 2011 (W%)	656	Turkey (Levantine)	14	71		4		
Boustany *et al*., 2015 (FO%)	132	Lebanon	38	15		14		12.6
Christidis *et al*., 2021 (FO%)	83	Crete, Greece	76.0	29.6	8.4	16.9	5.6	
Ersönmez *et al*., 2023 (IRI%)	287	Turkey (Levantine)	44	54	negligible	negligible	negligible	10.0
Hussain *et al*., 2020 (V%)	146	Ain El-Ghazala, Libya	10.2	17.4		72.5		
Hussain *et al*., 2020 (V%)	22	Derna, Libya	100					
Kalogirou 2013 (FO%)	290	Rhodes, Greece	5.2	18.6	14.5	28.6		
Michailidis 2010 (FO%)	6656	Cyprus	27	7.3		4.5	0.2	8.6
Sabrah *et al*., 2006 (FO%)	174	Suez Canal	5	25		70		
Torcu-Koç *et al*., 2020 (FO%)	208	Turkey (Levantine)	41.2	12.3		18.7		
Ulman *et al*., 2021b (FO%)	563	Turkey (Aegean and Levantine)	24	26		11		<2% 48 hooks, 9 nets, 2 wires
Hammoud & Salama 2016 (FO%)	192	Syria	46.5	5.9		12.8	17.7	
This study (FO%)	236	Crete, Greece	74.00	32.00	23.50	11.00	8.00	18.00
This study (IRI%)	236	Crete, Greece	79.07	7.92	9.72	1.82	0.31	-
This study (N%)	236	Crete, Greece	33.03	16.10	40.82	2.77	2.15	-
This study (W%)	236	Crete, Greece	74.24	8.76	0.71	13.81	1.75	-

ing in summer and gradually decreasing until spring when it was extremely low. The high frequency of empty tracts indicates low feeding intensity during this season. Other studies have reported similar findings (Hussain *et al.*, 2020; Ulman *et al.*, 2021b; Ersönmez *et al.*, 2023). This seasonal pattern could be related to the spawning period, which occurs from late spring to early summer (Peristeraki *et al.*, 2010, unpublished data). The lower feeding intensity during the spawning period could be due to reproductive behaviour, as pufferfishes are known to make benthic nests as well as guarding and caring for their eggs (Kawase *et al.*, 2014; Santhanam, 2017).

The feeding strategy plot (Fig. 3) suggests that *L. sceleratus* exhibits a mixed feeding strategy in Crete, with varying degrees of specialization and generalization on different prey types (*sensu* Amundsen *et al.*, 1996). Although a wide variety of preys is consumed and the majority of the sampled population is overwhelmingly piscivorous, some individuals seem to specialize in mollusks or polychaetes (i.e., the prey groups in the upper left corner of Figure 3). A closer look at the dataset revealed that mollusks or polychaetes dominated (> 60%) the diet of a subgroup of 23 fish, ranging from 157 to 709 mm TL and caught during the cold months of the year (74% in winter, 26% in spring/autumn). Specialization in such low mobility preys can be attributed to a temperature effect on *L. sceleratus* feeding behaviour (see below).

The most significant fish prey families were Sparidae (namely *Diplodus* spp., *Spicara* spp. and *Boops boops* (Linnaeus, 1758)) and Mullidae (*Mullus* spp.) (Table S1). A previous study carried out in Crete showed that the main species depredated in nets and longlines were *Pagrus pagrus* (Linnaeus, 1758), *Diplodus sargus* (Linnaeus, 1758) and *Mullus surmuletus* Linnaeus, 1758 (Christidis *et al.*, 2022). As Akbora *et al.* (2020) have reported, the type of fishery from which the specimens are collected for diet analysis could influence the observed importance of fish prey, since this species often depredates on fishing gears. In the current study, fishing nets were frequently found in the tracts, with fish wrapped within them, demonstrating that this tetraodontid is capable of attacking the catch on the nets and ripping them apart. Lines and hooks were also found yet less frequently, possibly due to the ease with which hooked fish can be eaten (Boustany *et al.*, 2015). Still, hooks were found not only in specimens caught with longlines but also in those collected by nets, indicating that *L. sceleratus* has the ability to swallow hooks without causing lethal harm. The fishing gear parts found in the tracts confirm that *L. sceleratus* may seriously affect coastal fisheries, depredating on fishing gears and damaging commercial catches and fishing gears. This has also been evidenced in previous studies (Michailidis, 2010; Boustany *et al.*, 2015; Akbora *et al.*, 2020; Ulman *et al.*, 2021b; Ersönmez *et al.*, 2023; Christidis *et al.*, 2024). Small-scale fisheries in Crete seem to be particularly affected (Christidis *et al.*, 2024). It is interesting to note that in our study the probability of fish ingestion was lower at greater depths, in accordance with the findings of Christidis *et al.* (2024), who reported that the probability of depredation decreases with increasing depth. Nevertheless, in our study, fishery type did not have a significant effect on the probability of fish occurrence in the tracts (Table 4) and, therefore, we may conclude that, even without depredation, fish would remain the primary prey of *L. sceleratus*. More in depth analysis of species depredation on fishery catches in the Mediterranean Sea is required.

Algae and seagrass were frequently found among the prey items of *L. sceleratus*, while similar results have been reported from Cyprus (Michailidis *et al.*, 2010). Seagrass and specific algal substrates often serve as habitats for small fish and benthic crustaceans which are potential preys of *L. sceleratus*. Therefore, it remains unclear whether the silver-cheeked toadfish is intentionally assimilating plant material or if this is simply an unintentional act during prey capture. In this study, approximately 10% of the tracts contained parts of substrate (pebbles, sand, stones), indicating that this species ingests a variety of non-food items, likely acquired while digging on the bottom to feed on benthic organisms (Ulman *et al.*, 2021b).

With regard to overall mollusks, gastropods were the most frequent preys, with the family Cavoliniidae being the most important. However, cephalopods were also found, with the main prey being *Octopus vulgaris* (Table S1). In Crete, this species is often depredated by *L. sceleratus* from nets (Christidis *et al.*, 2022). In some other regions of the eastern Mediterranean, fishers claim that the expansion of the silver-cheeked toadfish has been causing a decline in squid and octopus populations (Michailidis, 2010; Kalogirou, 2013; Ünal & Bodur, 2017). Although not as high as in previous studies (e.g., Sabrah *et al.*, 2006; Kalogirou, 2013; Hussain *et al.*, 2020), the FO of cephalopods (11.57%) in this study may support this allegation. However, multiple factors can influence cephalopod stocks. For instance, there is evidence that the increase in sea temperature due to climate change may be provoking a decline in octopus populations (Vargas-Yáñez *et al.*, 2009). A direct contribution of *L. sceleratus* predation to the decline of cephalopod populations should be further investigated.

It is worth noting that prey items of *L. sceleratus* rapidly transform into digested items and lose their visually identifiable characteristics (Ulman *et al.*, 2021b), making it difficult to identify preys to low taxonomic levels unless they have been eaten recently. This potentially introduces a bias in diet composition with taxa having hard parts, such as bones, otoliths, cephalopod beaks, or shells being overrepresented. Still, the results confirm the opportunistic predator profile of *L. sceleratus*, given that 38 species, 46 genera and 44 families were identified during the tract content analysis.

In this study, various NIS were identified in the diet of *L. sceleratus* i.e., the teleost species *P. miles*, *T. flavimaculosus*, *E. golanii* and *S. luridus*, as well as the decapod *P. segnis* and the squid *S. lessoniana*. These preys occurred in 4.66% of the tracts analysed and made up 6.23% of total prey weight. Previous studies conducted in the eastern Mediterranean found that NIS are a significant component of the diet of *L. sceleratus* and concluded that this pufferfish may control other NIS populations through predation

(Chaikin *et al.*, 2023; Ulman *et al.*, 2021b). However, in our study, the occurrence and quantity of NIS prey were substantially lower compared to those of indigenous prey, suggesting that predation pressure from *L. sceleratus* is mostly exerted on indigenous preys, and thus may not be sufficient to control other NIS populations through top-down regulation. Although cannibalistic tendencies have been observed in the native range of the species and the Mediterranean Sea (Ulman *et al.*, 2021b; Ersönmez *et al.*, 2023), this study does not provide any direct confirmation of cannibalism. However, cannibalism incidents cannot be excluded, as the pufferfish teeth found in six tracts, unidentified to lower taxonomic level, might belong to *L. sceleratus* specimens. Furthermore, Cretan fishers mention that the species predate on dead discarded specimens of *L. sceleratus* (personal communication).

Although Ulman *et al.* (2021b) found that prey composition did not differ significantly between juvenile and adult specimens, our models show that fish size, which is directly related to maturity attainment, has a significant effect on the consumption of crustaceans, plants and cephalopods, with these groups being preferred by larger individuals. Similarly, Hussain *et al.* (2020) found an increasing dependence on a crustacean-based diet with fish growth, and Kalogirou (2013) showed that large-sized *L. sceleratus* shift to molluscivorous feeding, heavily preying on *Sepia officinalis* Linnaeus, 1758 and *Octopus vulgaris*. The latter authors suggested that such an ontogenetic diet shift is possibly related to a habitat change, which happens when fish reach first maturity, i.e., early-life stages tend to concentrate on sandy bottoms, whereas mature specimens switch to seagrass meadows. Additionally, the suggested diet shift to large-sized preys, such as cephalopods and crabs, could be attributed to morphological changes, as older fish have bigger mouths, and are better equipped for crushing and ingesting large prey (Hussain *et al.*, 2020).

In the GAMs models, sampling area (i.e., Cretan or Libyan Sea) was a significant predictor only of the probability of mollusks consumption. Specifically, mollusks were consumed more frequently in the Libyan Sea compared to the Cretan Sea (Fig. 4). A previous work focusing on the toxicity of *L. sceleratus* in Crete showed that the TTX concentration in pufferfish tissues was substantially higher in the Libyan Sea compared to the Cretan Sea (Christidis *et al.*, 2021). It is noteworthy that some mollusk taxa, such as gastropods and bivalves, contain TTX (Rambla-Alegre *et al.*, 2017). Among these, members of the families Muricidae and Nassaridae, both of which were found during this study, are known TTX-bearers (Biessy *et al.*, 2019). In conclusion, the previously reported regional difference in TTX concentration in Crete could be explained by higher mollusks consumption in the Libyan Sea.

In this study, fish contributed more to the diet of the silver-cheeked toadfish during summer and autumn. On the other hand, crustacean consumption was more prevalent in both spring and winter, with mollusks being consumed more frequently during winter. Our findings agree with those of Ersönmez *et al.* (2023), who observed a higher importance of crustaceans from March to May and of fish prey from June to September in southern Turkey. The abovementioned seasonal differences in prey consumption could be related to the effect of temperature on the metabolic rates of the species and, consequently, its hunting behaviour. It has been demonstrated that a change of just 3ºC in water temperature significantly affects oxygen consumption rates in the pufferfish *Takifugu flavidus* (Li, Wang & Wang, 1975) (Shi *et al*., 2010). In recent years, sea surface temperature in Crete exhibits minimum values in winter (16-17ºC), increases from April to June (18-24ºC), peaks in summer (25-27ºC) and progressively decreases from October to December (24-19ºC) (Fig. 8 in Somarakis *et al.*, 2021). Consequently, during winter and spring, when water temperature and the metabolic rates of the species are lower, *L. sceleratus* possibly opts to consume low-mobility prey such as benthic crustaceans and bivalves, while in summer it prefers to prey on fish.

Our models show that depth also influences the probability of occurrence of fish, crustaceans, and mollusksmolluscs in the *L. sceleratus* diet. It appears that *L. sceleratus* ingests fish more often in shallow waters, and barely consumes fish at depths greater than 30 m. On the other hand, depth had the opposite effect on the occurrence of crustaceans and other mollusk preys, which was maximal at circa 40 m. This may also be attributed to water temperature, as deeper waters are cooler, rendering *L. sceleratus* less mobile and reducing its swimming speed (Wardle, 1980). It appears that, due to temperature differences amongst seasons and/or depth, the probability of consuming fish is inversely related with that of consuming crustaceans and non-cephalopod mollusks. Interestingly, an increase in temperature can positively influence depredation by this species on fishing gears, which mostly relates to preying on fish and cephalopod catches (Christidis *et al.*, 2024).

The findings of this study enrich our knowledge on the feeding biology of *L. sceleratus* in the eastern Mediterranean. Identifying such a large quantity of prey to family or even species level is a notable accomplishment, and it contributed to providing a more thorough picture of the *L. sceleratus* diet. Our data further demonstrate that this species severely impacts small-scale fisheries by simultaneously consuming targeted catch and damaging fishing gears. Given the potential ecological threat posed by *L. sceleratus*, which competes with native or even other invasive carnivores, as well as the health threat resulting from its high toxicity, additional research is vital to minimize the current outcomes of its invasion and to predict upcoming impacts. Due to the potential effect of the diet of this species on TTX concentration in its flesh, future studies should focus on the diet-TTX relationship. Furthermore, considering the difficulty in identifying digested items, DNA analysis tools could be employed in order to obtain more detailed results. In addition, the analysis of stable isotopes in the soft tissue and otoliths of *L. sceleratus* could also improve our knowledge on the trophic ecology of this species and, in particular, gain a better understanding of its relationship with TTX-bearing organisms.

Funding: This research is part of the LIONHARE project (https://lionhare.hcmr.gr/, accessed on 13 February 2024) funded by the Fisheries and Maritime Operational Program 2014–2020 of the Greek Ministry of Agricultural Development and Food and the European Maritime and Fisheries Fund.

Acknowledgements

We would like to thank all fishers and the HCMR scientific personnel who helped in the collection of the samples, and especially Ioannis Kosoglou and Kyriakos Tampakakis, who collected the samples by experimental fishing. Many thanks are also due to Dr Nikos Lampadariou for his help in identifying benthos prey items.

References

Abd Rabou, A.F.N., 2019. On the occurrence and health risks of the Silver-cheeked Toadfish (*Lagocephalus sceleratus* Gmelin, 1789) in the marine ecosystem of the Gaza Strip, Palestine. *Biodiversitas*, 20 (9), 2620-2627.

Akbora, H.D., Kunter, İ., Erçetin, T., Elagöz, A.M., Çiçek, B.A., 2020. Determination of tetrodotoxin (TTX) levels in various tissues of the silver cheeked puffer fish (*Lagocephalus sceleratus* (Gmelin, 1789)) in Northern Cyprus Sea (Eastern Mediterranean). *Toxicon*, 175, 1-6.

Akyol, O., Ünal, V., Ceyhan, T., Bilecenoglu, M., 2005. First confirmed record of *Lagocephalus sceleratus* (Gmelin, 1789) in the Mediterranean Sea. *Journal of Fish Biology*, 66, 1183-1186.

Albano, P.G., Steger, J., Bošnjak, M., Dunne, B., Guifarro, Z. *et al*., 2021. Native biodiversity collapse in the eastern Mediterranean. *Proceedings of the Royal Society B: Biological Sciences*, 288 (1942).

Amundsen, P.A., Gabler, H.M., Staldvik, F.J., 1996. A new approach to graphical analysis of feeding strategy from stomach contents data – modification of the Costello (1990) method. *Journal of fish biology*, 48 (4), 607-614.

Aydin, M., 2011. Growth, reproduction and diet of pufferfish (*Lagocephalus sceleratus* Gmelin, 1789) from Turkey's Mediterranean Sea coast. *Turkish Journal of Fisheries and Aquatic Sciences*, 11 (4), 589-596.

Azzurro, E., Bariche, M., Cerri, J., Garrabou, J., 2020. The long reach of the Suez Canal: *Lagocephalus sceleratus* (Gmelin, 1789) an unwanted Indo-pacific pest at the Atlantic gate. *BioInvasions Records*, 9 (2), 204-208.

Bal, H., Khan, U., 2022. The invasive silver-cheeked toadfish, *Lagocephalus sceleratus* (Gmelin, 1789) appeared in the Sea of Marmara after more than a decade: A call for awareness. *Ege Journal of Fisheries and Aquatic Sciences*, 39 (3), 261-266.

Ben Souissi, J., Rifi, M., Ghanem, R., Ghozzi, L., Boughedir, W. *et al*., 2014. *Lagocephalus sceleratus* (Gmelin, 1789) expands through the African coasts towards the Western Mediterranean Sea: A call for awareness. *Management of Biological Invasions*, 5 (4), 357-362.

Berg, J., 1979. Discussion of methods of investigating the food of fishes, with reference to a preliminary study of the prey of *Gobiusculus flavescens* (Gobiidae). *Marine Biology*, 50, 263-273.

Biessy, L., Boundy, M.J., Smith, K.F., Harwood, D.T., Hawes, I. *et al*., 2019. Tetrodotoxin in marine bivalves and edible gastropods: A mini-review. *Chemosphere*, 236, 124004.

Borstein, S.R., 2020. dietr: an R package for calculating fractional trophic levels from quantitative and qualitative diet data. *Hydrobiologia*, 847 (20), 4285-4294.

Boustany, L., El Indary, S., Nader, M., 2015. Biological characteristics of the Lessepsian pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) off Lebanon. *Cahiers de Biologie Marine*, 56, 137-142.

Carpentieri, P., Lelli, S., Colloca, F., Mohanna, C., Bartolino, V. *et al.*, 2009. Incidence of lessepsian migrants on landings of the artisanal fishery of south Lebanon. *Marine Biodiversity Records*, 2, e71.

Chaikin, S., De-Beer, G., Yitzhak, N., Stern, N., Belmaker, J., 2023. The invasive silver-cheeked toadfish (*Lagocephalus sceleratus*) predominantly impacts the behavior of other non-indigenous species in the Eastern Mediterranean. *Biological Invasions*, 25 (4), 983-990.

Chartosia, N., Michailidis, N., Constantinou, A., Karachle, P.K., 2021. Shedding light on the diet of the Lessepsian yellowspotted puffer *Torquigener flavimaculosus* Hardy and Randall, 1983 in the Eastern Mediterranean. *Acta Adriatica*, 62 (2), 199-208.

Christidis, G., Mandalakis, M., Anastasiou, T.I., Tserpes, G., Peristeraki, P. *et al.*, 2021. Keeping *Lagocephalus sceleratus* off the table: Sources of variation in the quantity of TTX, TTX analogues, and risk of tetrodotoxication. *Toxins*, 13(12).

Christidis, G., Batziakas E, Peristeraki P, Kosoglou I, Tampakakis K. *et al.*, 2022. The Impact of *Lagocephalus sceleratus* (Gmelin, 1789) on Small-Scale Fisheries in Crete: Preliminary Results. p. 16-19. In: *Marine and Inland Waters Research Symposium, Porto Heli, Argolida, Greece, 16-20 September 2022.* HCMR, Heraklion.

Christidis G., Batziakas S., Peristeraki P., Tzanatos E., Somarakis S. *et al.*, 2024. Another One Bites the Net: Assessing the Economic Impacts of *Lagocephalus sceleratus* on Small-Scale Fisheries in Greece. *Fishes*, 9 (3), 104.

Coro, G., Vilas, L. G., Magliozzi, C., Ellenbroek, A., Scarponi, P. *et al.*, 2018. Forecasting the ongoing invasion of *Lagocephalus sceleratus* in the Mediterranean Sea. *Ecological Modelling*, 371, 37-49.

Cortés, E., 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Canadian journal of fisheries and aquatic sciences*, 54 (3), 726-738.

Darbyson, E., Swain, D.P., Chabot, D., Castonguay, M., 2003. Diel variation in feeding rate and prey composition of herring and mackerel in the southern Gulf of St Lawrence. *Journal of Fish Biology*, 63 (5), 1235-1257.

Denadai, M.R., Santos, F.B., Bessa, E., Bernardes, L.P., Turra, A., 2012. Population biology and diet of the puffer fish *Lagocephalus laevigatus* (Tetraodontiformes: Tetraodontidae) in Caraguatatuba Bay, south-eastern Brazil. *Journal of the Marine Biological Association of the United Kingdom*, 92 (2), 407-412.

Devi, S.S., Sivan, A., 2017. Diet Diversity in Tetraodontid Fish, *Lagocephalus spadiceus* (RICHARDSON) Using Conventional And DNA Barcoding Approaches. *Journal of Aquatic Biology & Fisheries*, 5, 203-209.

Ersönmez, H., Özyurt, C.E., Mavruk, S., Yıldız, T., Ulman, A., 2023. An in-depth study of the biology, trophic ecology and catchability of the invasive pufferfish *Lagocephalus sceleratus* from southern Turkey, eastern Mediterranean Sea. *Scientia Marina*, 87 (4).

Farrag, M.M.S., El-Haweet, A.E.A.K., Akel, E.-S Kh. A., Moustafa, M.A., 2015. Stock Status of Pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) Along the Egyptian Coast, Eastern Mediterranean Sea. *American Journal of Life Sciences*, 3 (6-1), 83-93.

Fleming, A.H., Clark, C.T., Calambokidis, J., Barlow, J., 2016. Humpback whale diets respond to variance in ocean climate and ecosystem conditions in the California Current. *Global Change Biology*, 22 (3), 1214-1224.

Gabel, M., Unger, P., Theisen, S., Palm, H.W., Rothman, S.B.S. *et al.*, 2022. Parasites of pufferfish, *Lagocephalus* spp. and *Torquigener flavimaculosus* of the Israeli Mediterranean: A new case of Lessepsian endoparasites. *International Journal for Parasitology: Parasites and Wildlife*, 19, 211-221.

Galanidi, M., Zenetos, A., S. Chapman, D., 2018. *Study on Invasive Alien Species-Development of Risk Assessments: Final Report (year 2) Risk assessment template developed under the "Study on Invasive Alien Species-Development of risk assessments to tackle priority species and enhance prevention".*

Galil S.B., 2023. A Sea, a Canal, a Disaster: The Suez Canal and the Transformation of the Mediterranean Biota. In: *The Suez Canal: Past Lessons and Future Challenges*. Lutmar, C., Rubinovitz, Z. (Eds). Palgrave Macmillan, Cham, London.

Gallardo, B., Clavero, M., Sánchez, M.I., Vilà, M., 2016. Global ecological impacts of invasive species in aquatic ecosystems. *Global change biology*, 22 (1), 151-163.

Grossman, G.D., 1986. Food resource partitioning in a rocky intertidal fish assemblage. *Journal of Zoology*, 1 (2), 317-355.

Hacunda, J.S., 1981. Trophic relationships among demersal fishes in a coastal area of the Gulf of Maine. *Fishery Bulletin*, 79 (4), 775.

Hammoud, V., Salama, L., 2016. Food and feeding habits of the invasive puffer fish *Lagocephalus sceleratus* (Tetraodontidae) in the Syrian marine waters. *Journal of King Abdulaziz University-Science*, 28 (2).

Hastie, T.J., Tibshirani, R.J., 1990. *Generalized additive models*. Chapman and Hall/CRC, London, 352 pp.

Hurlbert, S.H., 1978. The measurement of niche overlap and some relatives. *Ecology*, 59 (1), 67-77.

Hussain, N.S., El-Maremie, H.A., Ali, R.A.S., Ali, S.M., El-Sayed El-Mor, M., 2020. Food And Feeding Habits of *Lagocephalus sceleratus* (Gmelin, 1789) in some areas of the eastern coast of Libya. *International Journal of Fisheries and Aquaculture Research*, 6 (2), 22-28.

Hyslop, E.J., 1980. Stomach contents analysis – A review of methods and their application. *Journal of Fish Biology*, 17, 411-429.

Jribi, I., Bradai, M.N., 2012. First record of the lessepsian migrant species *Lagocephalus sceleratus* (Gmelin, 1789) (Actinopterygii: Tetraodontidae) in the central Mediterranean. *BioInvasions Records*, 1 (1), 49-52.

Kalogirou, S., Wennhage, H., Pihl, L., 2012. Non-indigenous species in Mediterranean fish assemblages: Contrasting feeding guilds of *Posidonia oceanica* meadows and sandy habitats. *Estuarine, Coastal and Shelf Science*, 96 (1), 209-218.

Kalogirou, S., 2013. Ecological characteristics of the invasive pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) in Rhodes, Eastern Mediterranean Sea. A case study. *Mediterranean Marine Science*, 14 (2), 251-260.

Kasapidis, P., Peristeraki, P., Tserpes, G., Magoulas, A., 2007. First record of the Lessepsian migrant *Lagocephalus sceleratus* (Gmelin 1789) (Osteichthyes: Tetraodontidae) in the Cretan Sea (Aegean, Greece). *Aquatic Invasions*, 2 (1), 71-73.

Kawase, H., Okata, Y., Ito, K., Ida, A., 2014. Spawning behavior and paternal egg care in a circular structure constructed by pufferfish, *Torquigener albomaculosus* (Pisces: Tetraodontidae). *Bulletin of Marine Science*, 91 (1), 33-43.

Kleitou, P., Kalogirou, S., Marmara, D., Giovos, I., 2018. *Coryphaena hippurus:* A potential predator of *Lagocephalus sceleratus. Mediterranean Sea International Journal of Fisheries and Aquatic Studies,* 6 (3), 93-95

Michailidis, N., 2010. Study on the lessepsian migrant *Lagocephalus sceleratus* in Cyprus. p. 74-87. In: *Report of the Sub-Regional Technical Meeting on the Lessepsian Migration and Its Impact on Eastern Mediterranean Fishery, Nicosia, Cyprus, 7-9 December 2010.* Fao-EastMed Publisher, Nicosia.

Moczydlowski, E.G., 2013. *The molecular mystique of tetrodotoxin. Toxicon,* 63, 165-183.

Mohamad, S., Fadhilah, I., 2013. Morphology and Stomach Content Analysis of Green Rough-backed Puffer Fish, *Lagocephalus lunaris* from Kuching, Sarawak. *Borneo Journal of Resource Science and Technology*, 2 (2), 67-70.

Nader, M.R., Indary, S., Boustany, L.E., 2012. The puffer fish *Lagocephalus sceleratus* (Gmelin, 1789) in the Eastern Mediterranean. *FAO EastMed*. GCP/INT/041/EC – GRE – ITA/TD-10

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt R., Legendre, P. *et al.*, 2022. vegan: Community Ecology Package. R package version 2.6-2. https://CRAN.R-project.org/package=vegan

Özbek, E.Ö., Çardak, M., Kebapçioğlu, T., 2017. Spatio-temporal patterns of abundance, biomass and length of the silver-cheeked toadfish *Lagocephalus sceleratus* in the gulf of Antalya, Turkey (Eastern Mediterranean sea). *Turkish Journal of Fisheries and Aquatic Sciences*, 17 (4), 725-733.

Papaconstantinou, C., 1990. Spreading of Lessepsian fish into the Aegean Sea (Greece). *Scientia Marina*, 54 (4), 313-316.

Rambla-Alegre, M., Reverté, L., del Río, V., de la Iglesia, P., Palacios, O. *et al.*, 2017. Evaluation of tetrodotoxins in puffer fish caught along the Mediterranean coast of Spain. Toxin profile of *Lagocephalus sceleratus*. *Environmental Research*, 158, 1-6.

Rousou, M., Ganias, K., Kletou, D., Loucaides, A., Tsinganis, M., 2014. Maturity of the pufferfish *Lagocephalus sceleratus* in the southeastern Mediterranean Sea. *Sexuality and Early Development in Aquatic Organisms*, 1, 35-44.

Sabrah, M., El-Ganainy, A.A., Zaky, M.A., 2006. Biology and toxicity of the pufferfish *Lagocephalus sceleratus* (GMELIN, 1789) from the Gulf of Suez. *Egyptian Journal of Aquatic Research*, 32 (1), 283-297.

Santhanam, R., 2017. *Biology and ecology of toxic pufferfish*. CRC Press, New York, pp. 448.

Seetha, P.K., Balachandran, R., Zacharia, P.U., 2023. Pufferfish fishery along the Kerala coast and biology of the smooth-backed puffer, *Lagocephalus inermis* (Temminck & Schlegel 1850). *Marine Biological Association of India*, 65 (1), 97-101.

Smith, M.M., Heemstra, P.C., 1986. Tetraodontidae. p. 894-903. In: *Smith's sea fishes*. Smith, M.M, Heemstra, P.C. (Eds). Springer-Verlag, Berlin.

Somarakis, S., Giannoulaki, M., Markakis, K., Tsiaras, K., Schismenou, E. *et al.*, 2021. Ovarian dynamics, batch fecundity and spawning phenology of the lessepsian migrant *Etrumeus golanii* DiBattista, Randall & Bowen, 2012 (Clupeidae: Dussumieriinae). *Mediterranean Marine Science*, 22 (3), 466-479.

Streftaris, N., Zenetos, A., 2006. Alien Marine Species in the Mediterranean - the 100 'Worst Invasives' and their Impact. *Mediterranean Marine Science*, 7 (1), 87-118.

Torcu-Koç, H., Erdoğan, Z., Özbay Adigüzel, T., 2020. Some biological parameters of silverstripe blaasop, *Lagocephalus sceleratus* (Gmelin, 1789) from the Mersin Bay, the Eastern Mediterranean of Turkey. *Acta Biologica Turcica*, 33 (4), 180-192.

Tortonese, E., 1986. Tetraodontidae. p. 1341-1347. In: *Fishes of the north-eastern Atlantic and the Mediterranean.* P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, E. Tortonese (Eds). UNESCO, Paris.

Ulman, A., Kalogirou, S., Pauly, D., 2022. The Dynamics of Maximum Lengths for the Invasive Silver-Cheeked Toadfish (*Lagocephalus sceleratus*) in the Eastern Mediterranean Sea. *Journal of Marine Science and Engineering*, 10 (3), 387.

Ulman, A., Harris, H.E., Doumpas, N., Akbora, H.D., Al Mabruk, S.A.A. *et al.*, 2021a. Low Pufferfish and Lionfish Predation in Their Native and Invaded Ranges Suggests Human Control Mechanisms May Be Necessary to Control Their Mediterranean Abundances. *Frontiers in Marine Science*, 8, 670413.

Ulman, A., Yildiz, T., Demirel, N., Canak, O., Yemişken, E. *et al.*, 2021b. The biology and ecology of the invasive silver-cheeked toadfish *(Lagocephalus sceleratus*), with emphasis on the Eastern Mediterranean. *NeoBiota*, 68, 145-175.

Ulman, A., Akbora, H.D., Çanak, O., Chu, E., Çiçek, B.A. *et al.*, 2023. A biological and ecological study of the invasive pufferfish *Torquigener hypselogeneion* (Bleeker 1852) [conspecific *Torquigener flavimaculosus* Hardy & Randall, 1983] in the Eastern Mediterranean. *Aquatic Invasions*, 18 (1), 59-81.

Ulman, A., Abd Rabou, A.F.N., Al Mabruk, S., Bariche, M., Bilecenoğlu, M. *et al.*, 2024. Assessment of Human Health Impacts from Invasive Pufferfish (Attacks, Poisonings and Fatalities) across the Eastern Mediterranean. *Biology*, 13 (4), 208.

Ünal, V., Göncüoğlu Bodur, H., 2017. The socio-economic impacts of the silver-cheeked toadfish on small-scale fishers: A comparative study from the Turkish coast. *Ege Journal of Fisheries and Aquatic Sciences*, 34 (2), 119-127.

Ünal, V., Göncüoğlu, H., Durgun, D., Tosunoğlu, Z., Deval, M.C. *et al.*, 2015. Silver-cheeked toadfish, *Lagocephalus sceleratus* (Actinopterygii: Tetraodontiformes: Tetraodontidae), causes a substantial economic losses in the Turkish Mediterranean coast: A call for decision makers. *Acta Ichthyologica et Piscatoria*, 45 (3), 231-237.

Vargas-Yáñez, M., Moya, F., García-Martínez, M., Rey, J., González, M. *et al.*, 2009. Relationships between *Octopus vulgaris* landings and environmental factors in the northern Alboran Sea (Southwestern Mediterranean). *Fisheries Research*, 99, 159-167.

Wardle, C.S., 1980. Effects of Temperature on the Maximum Swimming Speed of Fishes. In: *Environmental Physiology of Fishes.* M. A. Ali (Ed). Springer, Boston.

Wilson, J.M., Castro, L.F.C., 2010. Morphological diversity of the gastrointestinal tract in fishes. p. 1-55. In: *Fish Physiology.* M. Grossell, A. P. Farrell, C. J. Brauner (Eds). Academic Press, New York

Wood, S.N., 2017. *Generalized Additive Models: An Introduction with R*. Chapman and Hall/CRC, London, 496 pp.

Yaglioglu, D., Turan, C., Erguden, D., Gurlek, M., 2011. Range expansion of silverstripe blaasop, *Lagocephalus sceleratus* (Gmelin, 1789), to the northeastern Mediterranean Sea. *Biharean Biologist*, 5 (2), 159-161.

Appendix

Table S1. Number of *L. sceleratus* specimens (N) collected per year and season (Winter: January-March, Spring: April-June, Summer: July-September, Autumn: October-December).

Year	Season	N
2017	Autumn	6
	Spring	1
	Summer	4
2018	Autumn	23
	Winter	1
	Spring	8
	Summer	12
2019	Autumn	2
	Winter	4
	Spring	19
2020	Autumn	26
	Winter	41
	Spring	10
	Summer	13
2021	Autumn	8
	Winter	21
	Spring	15
	Summer	5
2022	Autumn	3
	Spring	14

Table S2. Frequency of occurrence (FO%), number (N), numeric percentage (N%), weight (W, g), weight percentage (W%), index of relative importance (IRI) and index of relative importance percentage (IRI%) of prey items found in 190 *L. sceleratus* tracts.

	Number of Tracts	FO%	W	W%	N	N%	IRI	IRI%
'Plants'								
'Plants' unid.	14	7.37	6.28	0.20	14	1.44	12.09	0.49
Chlorophyta								
Caulerpaceae								
Caulerpa prolifera	2	1.05	0.79	0.03	2	0.21	0.25	0.01
Thracheophyta								
Alismatales unid.	14	7.37	14.49	0.46	16	1.64	15.48	0.63
Cymodoceaceae								
Cymodocea nodosa	7	3.68	0.10	0.00	7	0.72	2.65	0.11
Posidoniaceae								
Posidonia oceanica	7	3.68	0.79	0.03	7	0.72	2.76	0.11
Cnidaria								
Anthozoa								
Anthozoa unid.	1	0.52	0.14	0.00	1	0.10	0.05	0.00
Annelida								
Polychaeta								
Polychaeta unid.	1	0.52	0.09	0.00	1	0.10	0.05	0.00
Sabellidae unid.	1	0.52	0.08	0.00	1	0.10	0.05	0.00

Continued

Table S2 continued

	Number of Tracts	FO%	W	W%	N	N%	IRI	IRI%
Crustacea								
Crustacea unid.	7	3.68	4.72	0.15	8	0.82	3.57	0.15
Malacostraca								
Malacostraca unid.	3	1.58	0.93	0.03	3	0.31	0.54	0.02
Amphipoda unid.	3	1.58	0.56	0.02	6	0.62	1.01	0.04
Decapoda								
Decapoda unid.	4	2.11	0.14	0.00	4	0.41	0.87	0.04
Pleocyemata								
Pleocyemata unid.	1	0.52	0.01	0.00	1	0.10	0.05	0.0
Brachyura								
Brachyura unid.	32	16.84	161.24	5.16	72	7.38	211.17	8.61
Calappidae								
Calappa granulata	1	0.52	7.95	0.25	1	0.10	0.18	0.01
Parthenopidae								
*Parthenopoides massen*a	2	1.05	7.96	0.25	2	0.21	0.48	0.02
Inachidae								
Inachus spp.	1	0.52	0.19	0.00	2	0.21	0.11	0.00
Polybiidae								
Liocarcinus spp.	1	0.52	9.50	0.30	3	0.31	0.32	0.01
Dromiidae unid.	1	0.52	0.37	0.01	1	0.10	0.06	0.00
Portunidae								
Portunidae unid.	9	4.74	30.48	0.98	11	1.13	10.00	0.41
Portunus segnis	1	0.52	3.17	0.10	1	0.10	0.10	0.00
Anomura								
Anomura unid.	4	2.11	5.67	0.2	8	0.82	2.15	0.09
Paguridae unid.	3	1.58	22.54	0.72	7	0.72	2.28	0.09
Diogenidae unid.	2	1.05	11.89	0.38	5	0.51	0.93	0.04
Caridea								
Caridea unid.	3	1.58	2.54	0.08	3	0.31	0.62	0.03
Isopoda								
Isopoda unid.	6	3.16	3.62	0.12	18	1.85	6.23	0.25
Cirolanidae								
Eurydice sp.	1	0.52	0.11	0.00	1	0.10	0.05	0.00
Mollusca								
Cephalopoda								
Cephalopoda unid.	7	3.68	7.81	0.25	8	0.82	3.94	0.16
Octopodidae								
Octopus vulgaris	7	3.68	368.08	11.78	8	0.82	46.37	1.89
Sepiidae								
Sepia spp.	2	1.05	11.73	0.38	2	0.21	0.62	0.03
Sepia officinalis	4	2.11	42.66	1.37	6	0.62	4.20	0.17
Loliginidae								
Loliginidae unid.	1	0.52	0.86	0.03	1	0.10	0.07	0.00
Loligo vulgaris	1	0.52	0.01	0.00	1	0.10	0.05	0.00
Sepioteuthis lessoniana	1	0.52	0.51	0.02	1	0.10	0.06	0.00
Gastropoda								
Gastropoda unid.	16	8.42	0.55	0.02	21	2.15	18.27	0.75

Continued

Table S2 continued

	Number of Tracts	FO%	W	W%	N	N%	IRI	IRI%
Muricidae								
Hexaplex trunculus	2	1.05	9.39	0.30	2	0.21	0.54	0.02
Nassaridae								
Tritia mutabilis	2	1.05	1.52	0.05	3	0.31	0.38	0.02
Tritia turulosa	1	0.52	0.17	0.00	1	0.10	0.05	0.00
Cerithiidae								
Bittium sp.	1	0.52	0.10	0.00	3	0.31	0.16	0.01
Hyalocylidae								
Hyalocylis spp.	5	2.63	0.88	0.03	9	0.92	2.50	0.10
Creseidae								
Creseis spp.	3	1.58	1.93	0.06	20	2.05	3.33	0.14
Cavoliniidae								
Cavolinia spp.	11	5.79	3.37	0.11	86	8.82	51.70	2.11
Diacria spp.	14	7.37	2.66	0.09	117	12.00	89.10	3.63
Bivalvia unid.	8	4.21	1.38	0.04	132	13.54	57.17	2.33
Scaphopoda unid.	3	1.58	0.15	0.00	4	0.41	0.65	0.03
Echinodermata								
Echinodermata unid.	1	0.52	0.01	0.00	1	0.10	0.05	0.00
Echinoidea								
Echinoidea unid.	6	3.16	10.23	0.33	9	0.92	3.95	0.16
Parachenidae								
Paracentrotus lividus	1	0.52	0.14	0.00	1	0.10	0.05	0.00
Loveniidae								
Echinocardium spp.	2	1.05	11.52	0.37	2	0.21	0.61	0.02
Ophiuroidea								
Ophiuroidea unid.	1	0.52	28.00	0.90	2	0.21	0.58	0.02
Ophiurida unid.	6	3.16	4.81	0.15	6	0.62	2.43	0.10
Chordata								
Elasmobranchii								
Batoidea	1	0.52	5.79	0.19	1	0.10	0.15	0.01
Teleostei								
Teleostei unid.	105	55.26	439.71	16.82	164	14.07	1706.98	69.61
Siganidae								
Siganus luridus	2	1.05	4.16	0.13	3	0.31	0.46	0.02
Pomacentridae								
Chromis chromis	3	1.58	91.83	2.94	8	0.82	5.94	0.24
Apogonidae								
Apogon imberbis	1	0.52	2.33	0.07	1	0.10	0.09	0.00
Synodontidae								
Synodus saurus	6	3.16	141.55	4.53	7	0.72	16.59	0.68
Carangidae								
Trachurus spp.	5	2.63	10.23	0.33	7	0.72	2.76	0.11
Alosidae								
Sardina pilchardus	2	1.05	34.92	1.12	6	0.62	1.83	0.07
Dussumieriidae								
Etrumeus golanii	2	1.05	87.71	2.81	6	0.62	3.60	0.15
Clupeidae								
Clupeidae unid.	6	3.16	56.62	1.81	7	0.72	7.99	0.33

Continued

Table S2 continued

	Number of Tracts	FO%	W	W%	N	N%	IRI	IRI%
Sardinella spp.	1	0.52	5.46	0.17	2	0.21	0.20	0.01
Sardinella aurita	2	1.05	47.27	1.51	4	0.41	2.02	0.08
Scaridae								
Sparisoma cretense	3	1.58	51.74	1.66	3	0.31	3.11	0.13
Sparidae								
Sparidae unid.	9	4.74	126.93	4.06	10	1.03	24.13	0.98
Boops boops	11	5.79	132.08	4.23	14	1.44	32.83	1.34
Dentex dentex	2	1.05	18.64	0.60	2	0.21	0.85	0.03
Pagellus erythrinus	2	1.05	22.43	0.72	3	0.31	1.08	0.04
Pagrus pagrus	4	2.11	104.52	3.35	4	0.41	7.93	0.32
Sarpa salpa	3	1.58	146.83	4.70	3	0.31	7.92	0.32
Diplodus spp.	2	1.05	10.50	0.34	2	0.21	0.58	0.02
Diplodus annularis	2	1.05	41.64	1.33	2	0.21	1.62	0.07
Spicara sp.	1	0.52	3.24	0.10	1	0.10	0.10	0.00
Spicara maena	3	1.58	55.34	1.77	3	0.31	3.29	0.13
Spicara smaris	7	3.68	48.47	1.55	10	1.03	9.49	0.39
Mullidae								
Mullidae unid.	5	2.63	65.62	2.10	5	0.51	6.86	0.28
Mullus spp.	4	2.11	29.15	0.93	4	0.41	2.83	0.12
Mullus barbatus	3	1.58	61.37	1.96	3	0.31	3.59	0.15
Mullus surmuletus	6	3.16	130.17	4.17	7	0.72	15.45	0.63
Scorpaenidae								
Pterois miles	1	0.52	43.97	1.41	1	0.10	0.79	0.03
Scorpaena spp.	3	1.58	55.43	1.77	3	0.31	3.29	0.13
Scorpaena porcus	2	1.05	25.02	0.80	2	0.21	1.06	0.04
Scorpaena scrofa	1	0.52	35.45	1.13	1	0.10	0.64	0.03
Triglidae								
Chelidonichthys lastoviza	1	0.52	14.02	0.45	1	0.10	0.29	0.01
Uranoscopidae								
Uranoscopus scaber	1	0.52	17.06	0.55	1	0.10	0.34	0.01
Trachinidae								
Trachinus spp.	2	1.05	48.91	1.57	2	0.21	1.87	0.08
Serranidae unid.	1	0.52	2.05	0.07	1	0.10	0.09	0.00
Tetraodontidae								
Tetraodontidae unid.	7	3.68	36.73	1.18	7	0.72	6.99	0.29
Torquigener flavimaculosus	4	2.11	55.21	1.77	5	0.51	4.81	0.20
Atherinidae								
Atherinidae unid.	1	0.52	1.49	0.05	1	0.10	0.08	0.00
Atherina spp.	1	0.52	0.35	0.01	2	0.21	0.11	0.00
Syngnathidae unid.	2	1.05	0.58	0.02	2	0.21	0.24	0.01
Pleuronectiformes								
Pleuronectiformes unid.	1	0.52	4.35	0.14	1	0.10	0.12	0.00
Bothidae unid.	1	0.52	2.40	0.08	1	0.10	0.09	0.00