# Mediterranean Marine Science

Vol 14, No 2 (2013)


Vol 14, No 2 (2013)


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doi: 10.12681/mms.364

Research Article

*Mediterranean Marine Science*
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.364

# Ecological characteristics of the invasive pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) in Rhodes, Eastern Mediterranean Sea. A case study

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Handling Editor: Argyro Zenetos

Received: 6 July 2012; Accepted: 13 February 2013; Published on line 25 April 2013

## Abstract

In this study, the ecological and social impact of the invasive pufferfish *Lagocephalus sceleratus* on the coastal habitats of an area in the eastern Mediterranean Sea (Rhodes Island) was investigated. Seasonal quantitative sampling in two common coastal habitats were used to investigate habitat use by different life-stages. Sandy areas were found to be highly important for the early life stages of *L. sceleratus*. In contrast, *Posidonia oceanica* habitats were mainly preferred by larger (>29 cm) reproductive adults, not exceeding 64 cm. *Lagocephalus sceleratus* was found to feed on invertebrates and fish, while size classification revealed a tendency for a diet shift with increased size. During the early life stages, *L. sceleratus* inhabits sandy bottoms where it feeds on various invertebrates. The predominant molluscan species found in the diet of larger (> 20 cm) *L. sceleratus* individuals were the economically important *Sepia officinalis* and *Octopus vulgaris*. The size at which 50% of individuals have reached maturity was estimated at 36 cm. With increased size, habitat shift to seagrass meadows most possibly occurs to meet both the increased demand in prey availability and requirement for appropriate spawning ground. The condition factor for *L. sceleratus* showed significantly higher values during summer than all other seasons and this was attributed to the spawning season and increased feeding. Social impacts were alarming due to increased public attention concerning its lethal effects (presence of tetrodotoxin), if consumed. Its high abundance in coastal fish communities of the studied area combined with ecological and social impacts, clearly classify *L. sceleratus* as a pest for fisheries and a potential threat for biodiversity.

**Keywords:** *Lagocephalus sceleratus*, Mediterranean, impact, ecology, feeding, reproduction.

## Introduction

The man-made break down of geographical barriers, combined with climate change and increased sea water temperatures, are reshuffling the geographical distributions of plant and animal species (Galil *et al.*, 2007), thus enhancing the pole-ward spread and success of species in new environments. The acceleration and magnitude of this phenomenon is well illustrated in Mediterranean Sea systems (Galil *et al.*, 2007) and especially in fish communities (Quignard & Tomasini, 2000; Ben Rais Lasram & Mouillot, 2009).

The Mediterranean Sea is considered to be one of the main hotspots of marine bioinvasions on earth, with increasing rates of introductions being reported (Rilov & Galil, 2009; Edelist *et al.*, 2012, Zenetos *et al.*, 2012; Edelist In Press). Since the opening of the Suez Canal in 1869, the coastal ecosystems of the eastern Mediterranean Sea have been subject to the establishment of non-indigenous species (NIS), predominantly of Indo-Pacific origin. Concerning fish, 85 species have already been reported in the eastern Mediterranean Sea (EastMed, 2010; Golan, 2010; Bariche, 2011; Bariche & Heemstra, 2012; Edelist *et al*., 2012, Fricke *et al*., 2012), and immigration via the shallow Suez Canal explains the dominance of coastal fish species. The rate of fish immigration has increased in recent decades (Edelist *et al*., 2012) and is predicted to have profound ecological and social impacts. Until now, research has focused on the origin of invaders, the mode of transport and also biotic and abiotic descriptions of the invaded areas (Corsini-Foka & Economidis, 2007; Golani, 2010). The limited information on ecological and social consequences attributed to the establishment of NIS, but also on ecological traits and environmental affinity of NIS (Belmaker *et al.*, 2013), signifies the importance of studying such processes.

Puffers are marine fish species that are distributed in tropical and subtropical areas of the Atlantic, Indian and Pacific Ocean. Puffers include 19 genera and approximately 130 species within the Tetraodontidae family (Matsuura, 2001; Nelson, 2006), among which six are found in the eastern Mediterranean (Golani *et al*., 2006). The family name, literally meaning four teeth in Greek, refers to their fused jaw teeth which are very sharp. Some puffers carry the strongest paralytic toxin known today i.e. tetrodotoxin (Sabrah *et al*., 2006). European legislation (854/2004/EC) states that toxic fish of the Tetraodontidae family should not enter the European markets. In Egyptian Mediterranean markets, *L. sceleratus* is sold beheaded and eviscerated (Halim & Rizkalla, 2011).

*Lagocephalus sceleratus* received considerable public attention shortly after its first reports in 2003 from Gökova bay along the south-eastern coastline of the Aegean Sea due to the presence of tetrodotoxin (Feliz & Er, 2004; Akyol *et al*., 2005). It has since then showed a rapid expansion throughout the eastern Mediterranean Sea reaching the northernmost parts of the Aegean Sea and south-west to Tunis but has to date not been observed in the western Mediterranean or reached Italy (Fig. 1). In a global perspective, occasional accidental poisonings have lead to numerous human deaths, the majority of which have been documented in south-eastern Asia, including Malaysia, Taiwan, Hong Kong, and Korea (Kan *et al*., 1987; Yang *et al*., 1996). Transportation of puffer fish from Japan to other areas has also led to poisonings (Tanner *et al*., 1996). In the eastern Mediterranean, many cases of tetrodotoxin poisoning have been reported (Bentur *et al*., 2008). The high numbers of *L. sceleratus* that have been caught by coastal fishermen in the eastern Mediterranean Sea has initiated major national efforts to alert fishermen and the public about the toxicity of this fish. These efforts have included posters warning the public about the lethal effects if consumed. Moreover, fishermen have been warned by the Greek ministry of health that small individuals could be misidentified with other small commercial edible species such as *Spicara smaris*, *Boops boops* and *Atherina hepsetus* (Kalogirou, pers. obs.). *Lagocephalus sceleratus* affects the local fish market in three ways: deterring customers from buying this fish (Katikou *et al*., 2009), creating additional work consisting in discarding unwanted fish (including reinforcement using steel lines) and reducing local stocks of commercial squid and octopus through predation. The most important aspect from a social point of view is undoubtedly the risks involved with consuming the fish, due to its toxicity. Studies from the Mediterranean Sea show that there is a significant positive correlation between toxicity levels and size of fish (Katikou *et al*., 2009). According to the results of Katikou *et al.* (2009), the toxicity levels of individuals smaller than 16 cm in length are not lethal. This reduces the risks connected

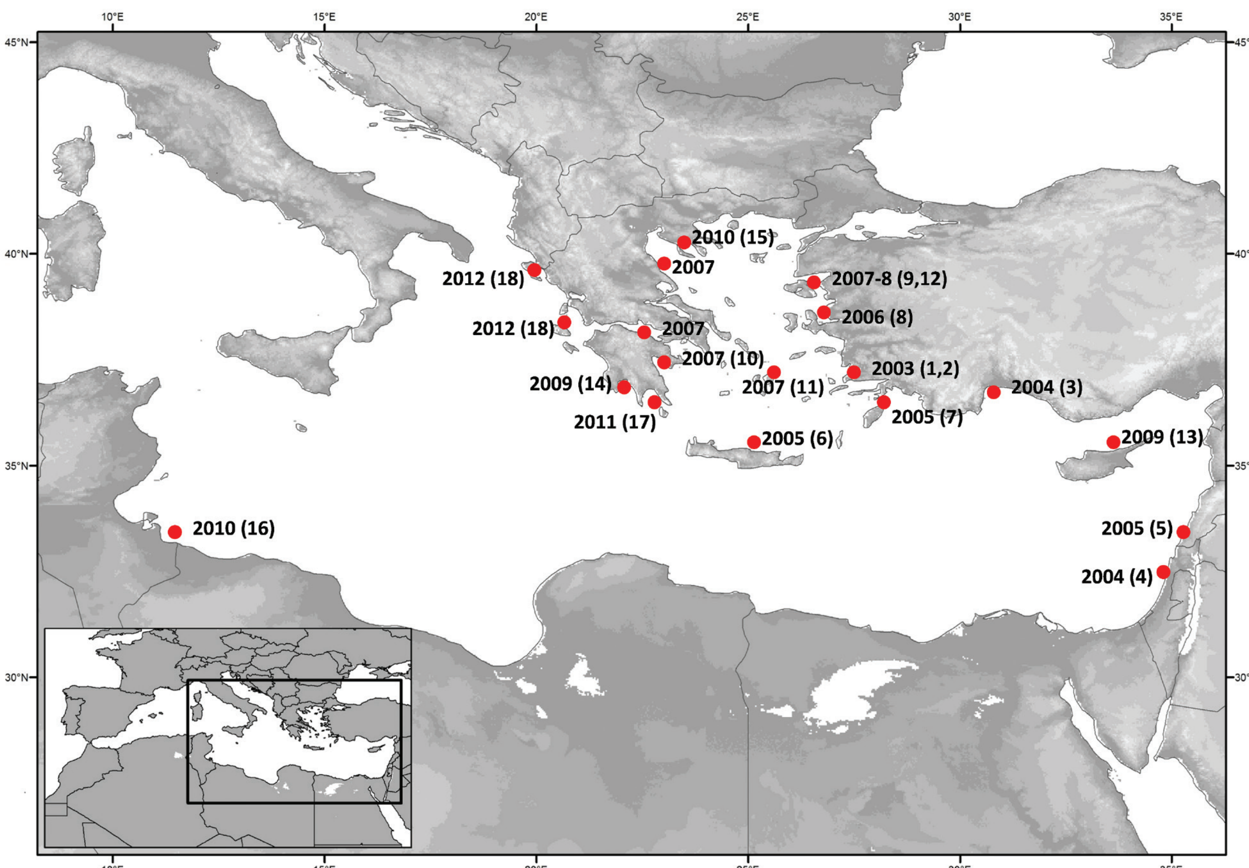


***Fig. 1:*** Spatial and temporal distribution of *Lagocephalus sceleratus* in the eastern Mediterranean Sea with numbers (1-18) representing the order of reports: 1, 2 (Feliz & Er, 2004, Akyol *et al.,* 2005); 3, (Bilecenoglu *et al.,* 2006); 4, (Golani & Levy, 2005); 5, (Carpentieri *et al.,* 2009); 6, (Kasapidis *et al.,* 2007); 7, (Corsini *et al.,* 2006); 8, (Bilecenoglu *et al.,* 2006); 9, (Peristeraki *et al.,* 2006); 10, (Peristeraki *et al.,* 2006); 11, (Kasapidis *et al.,* 2007), 12; (Türker-Çakır *et al.,* 2009); 13, (Katsanevakis *et al.,* 2009); 14, (ELNAIS 2012); 15, (Minos *et al.,* 2010); 16, (Jribi & Bradai, 2012); 17, (ELNAIS 2012) and 18, (Zenetos *et al.,* 2013).

with misidentification, since commercial *S. smaris*, *B. boops* and *A. hepsetus* rarely exceed this size.

*Lagocephalus sceleratus* is listed among the 100 Invasive Alien Species (IAS) in the Mediterranean Sea with profound social and ecological impacts due to the presence of tetrodotoxin, a source of food poisoning (Streftaris & Zenetos, 2006). The social impacts are obvious due to toxicity but the ecological impacts can only be supported with quantitative data. In the study area (Rhodes), *L. sceleratus* has become well established since its first record in 2005 (Corsini *et al*., 2006), but its ecology has only been touched recently (Kalogirou *et al*., 2010; Minos *et al*., 2010; Aydin, 2011; Kalogirou *et al*., 2012).

The aim of this study was to investigate the establishment of *Lagocephalus sceleratus* in coastal habitats where commercial boat seine fisheries exists. A quantitative study of size distribution and habitat use during the life cycle was carried out that focused on the identification of potential ontogenetic habitat shifts and indications of correlations between feeding and reproduction. In addition, investigation of feeding habits aimed to assess possible food web interactions and potential impacts on commercial fisheries.

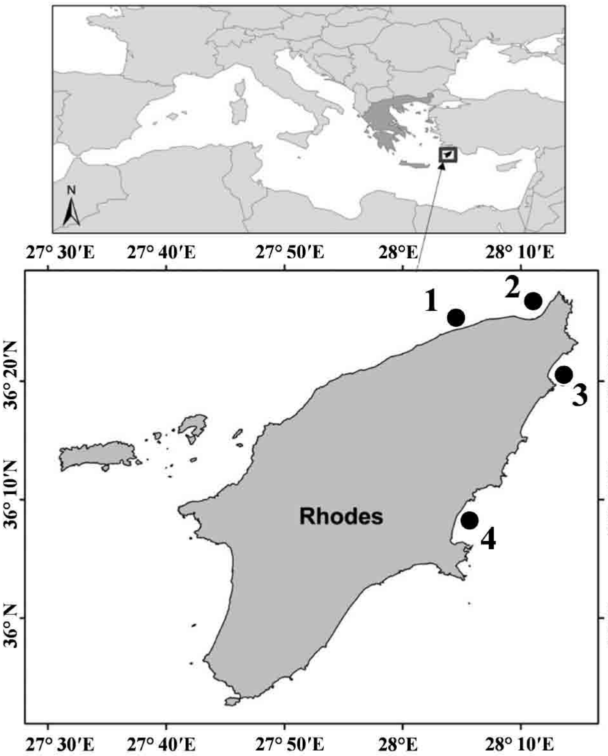


***Fig. 2:*** Map of Rhodes Island showing *Lagocephalus sceleratus* sampling sites.

## Materials and Methods

### *Study Area*

This study was performed in coastal habitats of Rhodes Island, located in the straddle between the Aegean and Levantine Sea, eastern Mediterranean Sea. The coastal continuum between Rhodes and the Suez Canal is > 1500 km, but the studied coastal habitats are similar to those found along the coasts between the two areas.

Mean surface water temperature in the study area ranges between 16°C and 18°C in winter, 21°C and 23°C in autumn and spring respectively, reaching 28°C in summer. Salinity is constant throughout the year and ranges between 39.3 and 39.7.

### *Sampling*

The investigation was carried out at four locations around Rhodes Island during the years 2008-2009, two representing *Posidonia oceanica* meadows (1 and 2), and two sandy habitats (3 and 4) (Fig. 2). In order to study temporal variations in the occurrence of *Lagocephalus sceleratus* in the two habitats, daylight samples were taken on four occasions over a year; December 2008 representing autumn and March, May and August 2009 representing winter, spring and summer respectively.

The boat-seine method was used to sample *L. sceleratus*, from a local commercial fishing boat. The procedure consisted in placing a start warp with an anchor and a buoy near the shore. The boat heads away from the shore and forms a triangle back to the start warp while the net (350 m in length) is set parallel to the shore at 35 m depth. This method enables sampling of each habitat, from 5 to 35 m in depth. The mesh size of the gear decreases from the outer end of the wing towards the centre with the sequence 500, 180, 32-34, 12, and 11 mm, with minimum mesh size of 5-8 mm at its cod-end. For further details, see Kalogirou *et al.* (2010, 2012). Three samples using the seine were taken randomly at each location and sampling occasion. The total time elapsing from deployment of the start line with an anchor to the time the seine was taken onboard was *c.* 35 min.

All samples were placed in labelled plastic bags and immediately stored in a freezer at -17°C, to prevent further digestion of the prey items.

### *Feeding ecology and reproduction*

A total of 290 *Lagocephalus sceleratus* individuals were analyzed. Each specimen was thawed, measured (total length: TL accuracy of 0.1 cm) and wet weighed (accuracy 0.01 g). All length measurements given throughout the manuscript are in TL.

Identification of prey items was generally limited to higher taxonomic levels since the beak-like jaws of *L. sceleratus* crush food items to the extent that prey could rarely be identified to species. Dietary estimations were limited to number (N), percent number (%N), occurrence (O) and percent occurrence (%O) of each prey taxa (Hyslop, 1980). Quantitative measurements of prey biomass were not possible due to the advanced stage of digestion. However, cephalopod beaks found in the stomachs could easily be identified to species level according to Clarke (1986). Due to differences in the level of taxonomic classification, prey items were divided into three major groups, namely, Mollusca, Crustacea and Fish for the presentation of diets.

Additionally, in order to investigate ontogenetic and diet shift with increased fish size, *L. sceleratus* individuals were categorized into seven size classes accordingly: 0-10 cm, class 1; 10.1-20 cm, class 2; 20.1-30 cm, class 3; 30.1-40 cm, class 4; 40.1-50 cm, class 5; 50.1-60 cm, class 6 and 60.1-70 cm to class 7.

Indications of maturity stages were obtained through macroscopic examination. Stages of maturity were classified, for combined sexes, as I, immature; II, developing; III, spawning capable; IV, regressing; and V, regenerating, following Brown-Peterson (2011). Length at first maturity was estimated as the length at which 50% of fish individuals had matured (stage III) using logistic regression model fitting.

### *Length-weight relationship and condition of fish*

The length-weight relationship was calculated for each season according to the following equation:

$$W=aTL^b,$$

where W is the wet weight (g), *a* the intercept of the relationship, TL the total length (cm), and *b* the slope. In addition, *a* and *b* values, given from the length-weight relationship, are used to estimate the condition factor of the fish.

Seasonal variations in the condition factor of *Lagocephalus sceleratus* were calculated according to Le Cren (1951) and recommendations given by Froese (2006) using the following equation:

$$K_{rel}= W/aTL^b,$$

where $K_{rel}$ is the relative condition factor, W the total weight (g), TL the total length (cm) and *a* and *b* are the constants resulting from the length-weight relationship. Tukey's honest significant difference test (HSD) was used to test for condition factor seasonality.

## Results

### *Habitat preference*

In total, higher abundances of *L. sceleratus* occurred on sandy habitats (168 individuals) compared to *P. oceanica* meadows (122 individuals). During samplings in summer, juvenile fish with a length of five to six cm were caught on sandy bottoms, whereas larger individuals (30 to 40 cm) were found in *P. oceanica* meadows (Fig. 3a, Table 1). In autumn, the density of fish increased on sandy bottoms and fish size varied between 5 and 32 cm (Fig. 3b, Table 1). At this time, two size classes (9 to 33 cm and 42 to 62 cm) of *L. sceleratus* occurred in the *P. oceanica* habitat (Fig. 3b, Table 1). Later in the season, during winter and spring, *L. sceleratus* were only found on sandy bottoms and fish size ranged from 15 to 27 cm (Fig. 3c, d, Table 1).

### *Feeding ecology and reproduction*

Out of 290 individuals examined, 59 (20.3%) had empty stomachs, among which 41 (69 %) in winter and 18 (31%) in autumn. The diet of *L. sceleratus* consisted of various prey species within the taxa of mollusca, crustacea and fish. Numerically, mollusca represented approximately 76% of the diet (N = 324) and 56% by occurrence. The two most frequent classes of molluscs were gastropoda (%N = 46.84; %O = 14.14) and cephalopoda (%N= 28.57; %O = 28.57) (Table 2). Even though the largest part of mollusca was represented by gastropods (%N= 46.84; %O = 14.14), only a fraction could be identified to a lower level of classification, among which the genus *Nassarius* and the family Dentaliidae were present (Table 2). On the other hand, all cephalopod species could be identified to species level and were represented by *Sepia officinalis* and *Octopus vulgaris* (Table 2). *Sepia officinalis* was both numerically (%N = 7.73) and by occurrence (%O =30.69) more abundant in the stomachs than *O. vulgaris* (%N = 7.73; %O = 11.38). Crustacea made up a large part of the diet, both by number (%N = 18.74) and by occurrence (%O = 18.62). Among Crustacea, *Calappa granulata* was the only species identified to a lower level of classification (Table 2). Fish did not contribute significantly to the diet, whether by number (%N = 5.39%) or occurrence (%O = 5.17). None of the fish found in the stomachs could be identified to species level and the four prey fish families were Synodontidae, Trachinidae, Syngnathidae and Tetraodontidae (Table 2).

Stomach content analyses show a high tendency for *L. sceleratus* to be an invertebrate feeder (Table 2). With increased body size, *L. sceleratus* was shown to shift its diet to molluscivore feeding (Fig. 4). According to size classification, none of the individuals found during this study exceeded size class 7 (64 cm) (Table 1). The length at which 50 % of *L. sceleratus* individuals had reached maturity was 36 cm (for combined sexes) (Fig. 5). All 40 individuals in size class one were immature (Table 3). Size class two was represented by 15 (25 %) immature, 42 (70 %) developing and 3 (5 %) spawning capable individuals (stages III-V) (Table 3). Size class three was represented by 73 individuals, among which 66 (90.4 %) were classified as developing and 7 (9.6 %) as spawning capable (stages III-V) (Table 3). Size class four was rep-

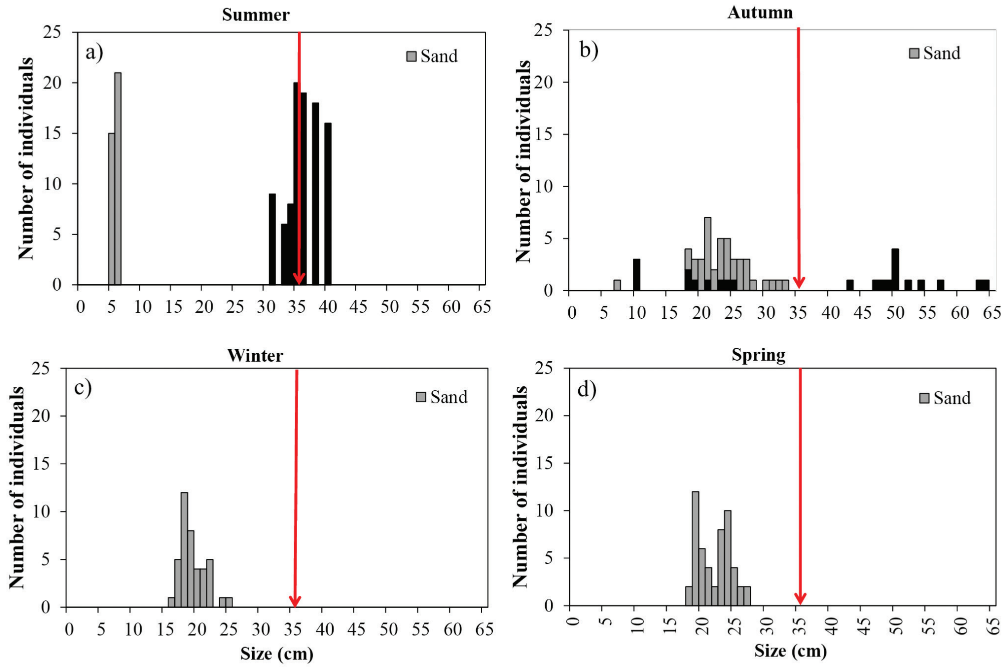


***Fig. 3:*** Length and number of *Lagocephalus sceleratus* individuals found over *Posidonia oceanica* and sandy habitats in summer (a), autumn (b), winter (c) and spring (d). Red arrows indicate the minimum length of mature individuals (36 cm).

**Table 1.** Mean ± s.d. (cm) and range (cm) of *L. sceleratus* total length ($L_T$) per habitat (*Posidonia oceanica* and sandy) and size class[a].

| Size class | Posidonia: Number of individuals | Posidonia: Mean ± s.d. | Posidonia: Range | Sand: Number of individuals | Sand: Mean ± s.d. | Sand: Range |
|---|---|---|---|---|---|---|
| 1 | 4 | 9.05 ± 1.58 | 6.7-10.0 | 39 | 5.38 ± 0.38 | 5.3-7.0 |
| 2 | 3 | 17.73 ± 0.59 | 17.3-18.4 | 57 | 18.24 ± 1.04 | 15.3-19.6 |
| 3 | 4 | 22.68 ± 1.75 | 20.3-24.3 | 69 | 23.09 ± 2.03 | 20.1-29.3 |
| 4 | 97 | 35.58 ± 2.83 | 30.2-40.0 | 3 | 31.63 ± 0.78 | 31.0-32.5 |
| 5 | 8 | 47.89 ± 2.43 | 42.5-49.9 | | | |
| 6 | 4 | 52.98 ± 2.43 | 50.9-56.2 | | | |
| 7 | 2 | 62.80 ± 0.42 | 62.5-63.1 | | | |

[a]Size class 1: 0-10 cm $L_T$; 2: 10.1-20 cm $L_T$; 3: 20.1-30 cm $L_T$; 4: 30.1-40 cm $L_T$; 5: 40.1-50 cm $L_T$; 6: 50.1-60 cm $L_T$; and 7: 60.1-70 cm $L_T$

resented by 54 (54 %) developing and 46 (46 %) spawning capable. All individuals, except one classified as developing at size class five, in size classes five to seven had reached maturity (stages III-V) (Table 3).

### *Length-weight relationship and condition of fish*

Length-weight relationships are important in fisheries biology because they allow estimation of the average weight of the fish at a given length, by establishing a mathematical relationship between weight and length. The overall length-weight relationship for *L.sceleratus* showed a good fit to the exponential curve ($R^2 > 0.99$); the value of $a$ was 0.0164 and for $b$ 2.8932 (Fig. 6).

The condition factor showed small variations throughout the year, except summer (Fig. 7; Table 4). The condition factor was available for all size classes during autumn only and values varied from 0.87 to 1.09. During autumn, the lowest condition factor values were observed for the smaller size classes i.e. one to four, while the highest were observed for size classes five to seven. In winter, condition factor values were calculated for size classes two and three only, with values close to 0.99. In spring, condition factor values were calculated for size classes two (0.91), three (0.92) and six (1.15) only. During summer, both size class one (found over sandy bottoms) and size class four (found over *P. oceanica* meadows) showed a good condition factor value of more than 1 (Table 4). *Lagocephalus sceleratus* was found to have a similar average condition factor close to 1 throughout the year with only summer possessing significantly higher values among the seasons (Tukey's HSD; $P < 0.05$; Fig. 7; Table 4).

**Table 2.** Overall number (N), percent number (%N), occurrence (O) and percent occurrence (%O) of prey items found in the stomachs of 290 *Lagocephalus sceleratus* sampled in December 2008 and March, May and August 2009.

| Prey category | N | % N | O | % O |
|---|---|---|---|---|
| Total prey | **427** | | 231 | |
| Total prey identified to genus | 147 | 34.43 | | |
| Total prey identified to species | 93 | 21.78 | | |
| **Mollusca** | **324** | **75.88** | **162** | **55.86** |
| Unidentified Mollusca | 2 | 0.47 | 1 | 0.34 |
| Gastropoda | 200 | 46.84 | 41 | 14.14 |
| Nassariidae | 5 | 1.17 | 3 | 1.03 |
| *Nassarius* sp. | 3 | 0.70 | 2 | 0.69 |
| Dentaliidae | 2 | 0.47 | 1 | 0.34 |
| Cephalopoda | 122 | 28.57 | 122 | 42.07 |
| Octopodidae | 33 | 7.73 | 33 | 11.38 |
| *Octopus vulgaris* | 33 | 7.73 | 33 | 11.38 |
| Sepiidae | 89 | 20.84 | 89 | 30.69 |
| *Sepia officinalis* | 89 | 20.84 | 89 | 30.69 |
| **Crustacea** | **80** | **18.74** | **54** | **18.62** |
| Unidentified Crustacea | 79 | 18.50 | 53 | 18.28 |
| Brachyura | 1 | 0.23 | 1 | 0.34 |
| Calappidae | 1 | 0.23 | 1 | 0.34 |
| *Calappa granulata* | 1 | 0.23 | 1 | 0.34 |
| **Teleostei** | **23** | **5.39** | **15** | **5.17** |
| Unidentified fishes | 16 | 3.75 | 10 | 3.45 |
| Identified fishes to genus | 7 | 1.64 | 6 | 2.07 |
| Identified fishes to species | 0 | 0.00 | 0 | 0.00 |
| Synodontidae | 2 | 0.47 | 2 | 0.69 |
| Trachinidae | 3 | 0.70 | 3 | 1.03 |
| Syngnathidae | 1 | 0.23 | 1 | 0.34 |
| Tetraodontidae | 1 | 0.23 | 1 | 0.34 |
| **Empty** | 59 | | | |

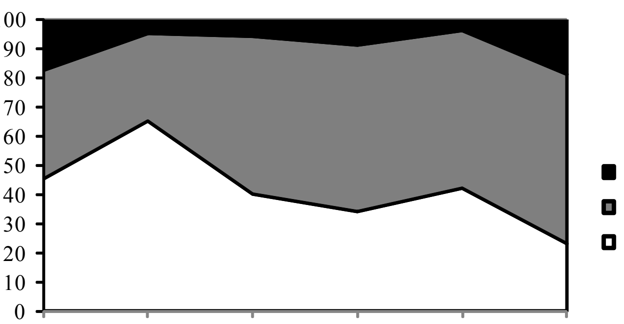


*Fig. 4:* Frequency of occurrence for each prey taxa (Fish, Crustacea and Mollusca) in the diet of *Lagocephalus sceleratus* related to size-class where 1: 0-10 cm $L_T$; 2: 10.1-20 cm $L_T$; 3: 20.1-30 cm $L_T$; 4: 30.1-40 cm $L_T$; 5: 40.1-50 cm $L_T$; 6: 50.1-60 cm $L_T$; and 7: 60.1-70 cm $L_T$.

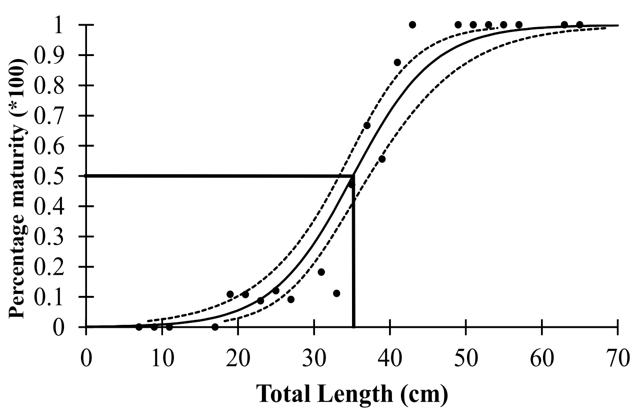


*Fig. 5:* Logistic regression on the percentage of mature individuals at different total lengths (cm) for *Lagocephalus sceleratus*. Dashed lines indicate upper and lower 95% confidence intervals. Length at which 50% of individuals reached maturity stage III was 36 cm.

## Discussion

In this study, the status of establishment of one of the most invasive fish species for the Mediterranean Sea, *L. sceleratus*, was investigated only six years after its first record in the studied area. Quantitative sampling allowed measurements of the population size-structure of *L. sceleratus* in two important coastal habitats. Along with information on size at maturity and feeding habits, it provides important life-cycle characteristics of this species in its new environment.

In sandy habitats, *L. sceleratus* were present throughout the year and most of the individuals did not exceed size class 3 (30 cm) (Fig. 3) while larger fish (size classes 3 to 6) were found to inhabit *Posidonia oceanica* meadows mainly (Fig. 3) indicating a possible habitat shift with increased body size. Higher abundances of *L. sceleratus* were generally found in sandy habitats (168 individuals) compared to *P. oceanica* meadows (122 individuals), due to high concentrations of small-sized indi-

**Table 3.** Number of *L. sceleratus* individuals per maturity stage[a] according to Brown-Peterson (2011) and size class[b].

| | | Maturity Stage | | |
|---|---|---|---|---|
| | | I | II | III-V |
| Size Class | Class 1 | 43 | 0 | 0 |
| | Class 2 | 15 | 42 | 3 |
| | Class 3 | 0 | 66 | 7 |
| | Class 4 | 0 | 54 | 46 |
| | Class 5 | 0 | 1 | 7 |
| | Class 6 | 0 | 0 | 4 |
| | Class 7 | 0 | 0 | 2 |

[a] Maturity stage I, immature; II, developing; III, spawning capable; IV, regressing and V, regenerating.
[b] Size class 1: 0-10 cm $L_T$; 2: 10.1-20 cm $L_T$; 3: 20.1-30 cm $L_T$; 4: 30.1-40 cm $L_T$; 5: 40.1-50 cm $L_T$; 6: 50.1-60 cm $L_T$; 7: 60.1-70 cm $L_T$.

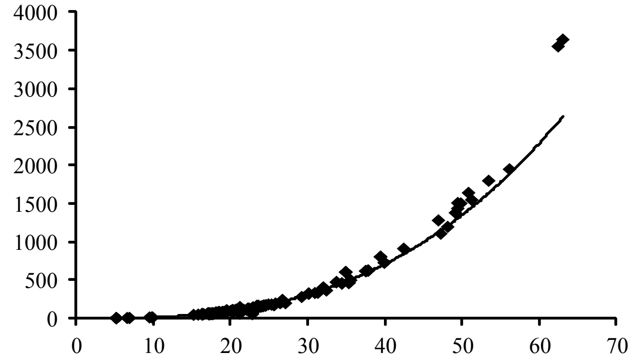


***Fig. 6:*** Length-weight relationship for 290 individuals of *Lagocephalus sceleratus* around the coasts of Rhodes Island.

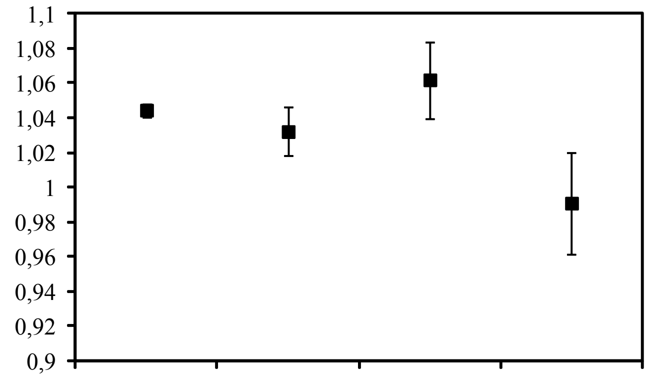


***Fig. 7:*** Seasonal variation in mean ± S.E. of relative condition factor according to Le Cren (1951) for *Lagocephalus sceleratus.*

viduals over sandy bottoms during the summer. On sandy bottoms, the small size range (5-6 cm) of *L. sceleratus* individuals observed during the summer suggests that the fish recruit into this habitat. During the summer, larger reproductive individuals (30 to 40 cm) were caught in *P. oceanica* meadows, thus confirming that summer is the main reproductive season for *L. sceleratus*, as shown in the Gulf of Suez by Sabrah *et al*. (2006). In the autumn, fish abundance increased on sandy bottoms and the mean fish size of 22 cm suggests that small individuals are affiliated to sandy bottoms. The small number of small-sized specimens (9 to 26 cm) caught during the same season in *P. oceanica* meadows could possibly be attributed to a patchy seagrass habitat interrupted by sand. During the autumn, few adult *L. sceleratus* individuals (42 to 62 cm) were present in the *P. oceanica* habitat, indicating that this species might be in the process of further ontogenetic shift to deeper or other coastal habitats (rocky bottoms). Later in the season, during the winter and spring, *L. sceleratus* were only found on sandy bottoms where fish size ranged from 15 to 27 cm, thus underlining the importance of this habitat for the early life stages of *L. sceleratus*. In an earlier study, *L. sceleratus* was classified as a seagrass resident with intermediate affinity to the *Posidonia oceanica* habitats of the same area (Kalogirou *et al*., 2010). This study adds to the knowledge of habitats used during the life cycle by proposing a habitat shift from sand to *P. oceanica* with increased size. Its high, year-round abundance underlines that *L. sceleratus* is well established in the area and the lack of larger (> 65 cm) individuals in the data could most probably be attributed to its recent establishment in the area (only

**Table 4.** Mean ± s.e. (cm) and range (cm) of *L. sceleratus* condition factor per season (winter, spring, summer and autumn) and size class[a].

| | | Winter | | Spring | | Summer | | Autumn | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± s.e. | Range | Mean ± s.e. | Range | Mean ± s.e. | Range | Mean ± s.e. | Range |
| Size Class | 1 | - | - | - | - | 1.09 ± 0.00 | 1.09 | 0.92 ± 0.03 | 0.91 - 0.96 |
| | 2 | 0.99 ± 0.01 | 0.98 – 1.00 | 0.91 ± 0.01 | 0.90 - 0.92 | - | - | 0.91 ± 0.04 | 0.87 -0.94 |
| | 3 | 0.99 ± 0.02 | 0.97 – 1.01 | 0.92 ± 0.03 | 0.89 - 0.95 | - | - | 0.93 ± 0.01 | 0.91 - 0.95 |
| | 4 | - | - | - | - | 1.05 ± 0.01 | 1.03 - 1.06 | 0.95 ± 0.01 | 0.94 - 0.96 |
| | 5 | - | - | - | - | - | - | 1.07 ± 0.02 | 1.05 - 1.09 |
| | 6 | - | - | 1.15 ± 0.00 | 1.15 | - | - | 1.06 ± 0.02 | 1.04 - 1.06 |
| | 7 | - | - | - | - | - | - | 1.38 ± 0.00 | 1.38 |
| | Total | 0.99 ± 0.02 | | 0.99 ± 0.02 | | 1.07 ± 0.01 | 1.03 - 1.09 | 1.02 ± 0.03 | 0.87 - 1.38 |

[a]Size class 1: 0-10 cm $L_T$; 2: 10.1-20 cm $L_T$; 3: 20.1-30 cm $L_T$; 4: 30.1-40 cm $L_T$; 5: 40.1-50 cm $L_T$; 6: 50.1-60 cm $L_T$; and 7: 60.1-70 cm $L_T$.

5 years). Long-line fishery reports (pers. obs.) of larger individuals reaching 78 cm in total length, caught over rocky bottoms, indicate further habitat shift to deeper or rocky grounds.

According to the results of this study, habitat shift from sand to *P. oceanica* meadows was related to maturity (Figs. 3 and 5) and shift in feeding preferences to increased body size (Fig. 5).

Diet descriptors of L. *sceleratus* in the Mediterranean are limited (Golani *et al*., 2006,; Minos *et al*., 2010; Aydin, 2011; Froese & Pauly, 2011). This study increases the knowledge on feeding preferences by revealing that L. *sceleratus* is an invertebrate and fish feeder, in accordance with recent findings by Aydin (2011), thus confirming its recent classification in the studied area (Kalogirou *et al*., 2012). This study suggests a diet shift with increased body size (to a molluscivore feeding), possibly explaining habitat shift due to changed prey availability/ demands or preferences (Fig. 4). In the study area, it is commonly believed by fishermen that the rapid expansion of *L. sceleratus* might have adversely affected commercial squid and octopus populations. However, it is difficult to quantify the predation impact of the puffer fish due to the lack of quantitative studies on local invertebrate communities but also to the digested state of prey items. Interestingly, the indication of fish prey found in this study were burrowing and venomous species (Table 2), thus showing the ability of *L. sceleratus* to actively search for prey and possible resistance to venom.

Habitat shift was shown to occur when *L. sceleratus* reach approximately 27-32 cm in length, showing its high correlation with maturity. In this study, the size at which 50% of *L. sceleratus* individuals reach maturity was 36 cm, indicating that size at maturity and habitat shift are correlated (Fig. 3, 5). In the Gulf of Suez, Sabrah *et al.* (2006) showed that *L. sceleratus* reach maturity during the third year of life, at a size of 42-43 cm (size class 5), which agrees with this study.

The rapid expansion of *L. sceleratus* indicates that the species is well adapted to its new environment. *Lagocephalus sceleratus* was generally found to be in good condition in the area under study with low seasonal fluctuations, given the single year of sampling. Even though the condition factor used during this study is believed to be a good indicator of the physiological state of the species, it should be mentioned that the condition factor is also related to other factors such as reproductive period and fat storage. The $a$ (0.0164) and $b$ (2.8932) values resulting from the length-weight relationship during this study correspond to the values obtained by Sabrah (2006) in the Attaka fishing harbour, Suez Canal; Edelist (In Press) from the Israeli coast and Aydin (2011) from conspecific populations of Antalya Bay, Turkey, revealing similar growth patterns. The a and b values found for this study comply with values given by Edelist (In Press) who found these values to be 0.022 and 2.82 along the Israeli coastline, respectively, thus revealing negative allometric growth.

*Lagocephalus sceleratus* possess one of the strongest paralytic toxin known today, namely, tetrodotoxin (Sabrah *et al*., 2006). It has been regarded as one of the most invasive species of the Mediterranean Sea (Streftaris & Zenetos, 2006) and as harmful to human health, fishing gears (Katsanevakis *et al*., 2009) and biodiversity (Bilecenoglu, 2010). The behavioural and chemical ecology of tetrodotoxin-bearing organisms has been reviewed extensively by Williams (2010) who discusses the defensive, offensive and communicative function of TTX in pufferfishes. In Turkey, *L. sceleratus* is considered to be the most dominant pufferfish in terms of biomass (Bilecenoglu *et al*., 2006; Torcu Koç *et al*., 2011). In the study area, *L. sceleratus* was found to rank among the 10 most dominant fish species in terms of biomass in *Posidonia oceanica* habitats (Kalogirou *et al*., 2010) and among the ten most dominant species, both in terms of biomass and number of individuals, on sandy bottoms (Kalogirou *et al.*, 2012). This undoubtedly shows its significant ability to colonize new areas rapidly and establish populations. Its large size was considered the main reason for this species to be marketed in some fishing ports and several cases of poisoning have been reported (Bentur *et al*., 2008; Bilecenoglu, 2010; Golani, 2010). The Turkish (Bilecenoglu, 2010), Greek and Cypriot (EastMed, 2010) ministries of Agriculture have recently banned fishing and marketing of *L. sceleratus*. Complaints of local fishermen in newspapers about its presence have been recorded from Turkey (Bilecenoglu, 2010), Cyprus, Greece, Egypt, Israel and Lebanon (Kalogirou, pers. obs.). Complaints of fishermen included destruction of gill nets due to entangling or predation on already captured fish, reduction of local commercial catches of *Sepia officinalis* and *Octopus vulgaris*, cut-off of long-line hooks and worrying fish consumers due to false alerts regarding the difficulties in distinguishing small-sized individuals of *L. sceleratus* from other important commercial fishes of the same size (e.g. *Spicara smaris*, *Atherina hepsetus* and *Boops boops*) (Katikou *et al*., 2009). As an indication of the possible economic impacts in Fethiye Bay, Turkey, five minutes of recreational fishing resulted in 3 broken fishing lines, ten missing hooks and the capture of one *L. sceleratus* weighing 1 kg (Bilecenoglu, 2010). Bilecenoglu's observations correspond to the results of this study where 52 long-line hooks were found in 33 stomachs, thus confirming the pest status of *L. scleratus* for commercial long-line fishermen. The time spent on cleaning the gear from discarded fish is also considered as economically undesirable; however, this was not within the scope of this study. Adaptation of long- and handline fisheries, included fishing in deeper areas (> 60 m), where *L. sceleratus* was considered to be absent, and reinforcement of fishing lines using steel.The potential effects following the introduction of *L. sceleratus* are hard to predict, and the fundamental knowledge provided here on life-cycle characteristics is important for our understanding of environmental responses and invasion biology. An invading species might sometimes reach a peak in density and then decline, a development often referred to as "boom and bust" (Reise *et al*., 2006). This dynamic leads to a significant reduction of the invading species population. According to unpublished data, the invasive NIS *Fistularia commersonii* (Kalogirou *et al*., 2007) clearly followed this boom and bust development in the studied area, but the future will reveal whether *L. sceleratus* will follow the same pattern. When a NIS becomes established in an area where its preferred food is under-utilized by indigenous species the resulting population explosion can later return to equilibrium with available resources (Wellcome, 1988). There is no doubt that biodiversity in the Mediterranean Sea is changing but to what extent forthcoming warm-water species will affect the trophic web and the functioning of marine ecosystems remains to be seen and requires continuous monitoring (Reise *et al*. , 2006; Bianchi, 2007; Kalogirou *et al*., 2010; 2012). Most importantly, investigation of ecological applications on how to manage unwanted NIS must be pursued and will surely constitute a future challenge for all stakeholders in the Mediterranean Sea.

In conclusion, this is the first study in the Mediterranean Sea that focuses on the ecology of this invasive pufferfish, showing the importance of sandy habitats for the early life stages of *L. sceleratus* and *P. oceanica* meadows for its adult stages. Additionally, shifts in habitat related to maturity and feeding preferences were suggested. The ecological and social impacts of *L. sceleratus* in the studied area are significant. Future studies will reveal how *L. sceleratus* will affect the food web structure and function of its new ecosystem, but most importantly they should also focus on the social and economic benefits for humans.


## Acknowledgements

This work is part of my PhD thesis written at the University of Gothenburg and I kindly thank my supervisors Prof. Leif Pihl and Assoc. Prof. Håkan Wennhage for lending their support during the years of study, and also Prof. Alf Norrko for manuscript reviewing during the writing process. I am also thankful to Maria Corsini-Foka and Dr. Andreas Sioulas from the Hydrobiological Station of Rhodes where all the samplings were performed, and H. Kyriakidou for providing maps.


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