



Maturity of the pufferfish *Lagocephalus sceleratus* in the southeastern Mediterranean Sea

Maria Rousou^{1,2}, Konstantinos Gantias^{2,*}, Demetris Kletou^{1,3}, Alexandros Loucaides¹, Markos Tsinganis²

¹Marine and Environmental Research (MER) Lab Ltd, Nicosia, Cyprus

²School of Biology, Aristotle University of Thessaloniki, 54 124 Thessaloniki, Greece

³School of Marine Science and Engineering, University of Plymouth, Plymouth, UK

ABSTRACT: The present study assesses maturity and reproductive dynamics of pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) off Cyprus (southeastern Mediterranean Sea), in relation to regional differences in oceanographic conditions, habitat characteristics and fishing activity. In addition, the allometric pattern of gonadal growth was studied to validate the use of the gonadosomatic index (GSI) in assessments of the reproductive cycle. To do so, 4743 pufferfish were sampled between May and October 2010 so as to cover the reproductive period of the population, which peaks from late spring to middle summer. Analysis of GSI data showed a clear differentiation between 2 pufferfish populations residing in the southwestern (SWA) and the south-east (SEA) coastal areas of Cyprus. Specifically, the SWA population was mainly dominated by immature individuals and exhibited a more delayed and narrow reproductive period. Moreover, generalized linear model analysis (GLM) showed that the SWA population exhibited significantly smaller size at 50% maturity, L_{50} (41.9 cm), compared to the SEA population (48.8 cm). These differences in reproductive seasonality and L_{50} were not found to be related to differences in sea surface temperature (SST) between the 2 regions, whilst mature individuals almost entirely disappeared from both areas outside the reproductive period. The latter could not be attributed to overfishing of the larger size classes since the fishery was not shown to be selective to pufferfish size. These results indicate that *L. sceleratus* may be a spawning migratory species that visits Cyprus southeastern coasts to reproduce and then migrates to other warm areas of the eastern Levantine basin.

KEY WORDS: *Lagocephalus sceleratus* · Maturity · Lessepsian migrant · Invasive species · Eastern Mediterranean

INTRODUCTION

The opening of the Suez Canal in 1869, which connected the Red Sea with the less salty Mediterranean Sea, resulted in the migration of more than 600 tropical Indo-Pacific species into the Mediterranean Sea, establishing reproducing populations and often associated with adverse economic and ecological impacts (Coll et al. 2010). One of these Lessepsian migrants, also considered among the 100 worst invasive species in the Mediterranean Sea (Streftaris & Zenetos

2006), is the pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) (Tetraodontidae). The pufferfish is geographically distributed in the tropical and subtropical marine waters such as the Indian and Pacific Ocean, Red Sea, and southern African shores (Jianying et al. 2003, Cappo et al. 2007, Al Jufaili et al. 2010, Veeruraj et al. 2011). In the Mediterranean Sea, it was first recorded in Gökova Bay (southern Aegean Sea, Turkey) in 2003 (Feliz & Er 2004, Akyol et al. 2005), followed by a second record in 2004 in Jaffa (Israel) (Golani & Levy 2005) and later on near

*Corresponding author: kgantias@bio.auth.gr

the Greek islands of Rhodes and Crete (Corsini et al. 2006, Kasapidis et al. 2007). Since then, *L. sceleratus* has formed reproducing populations and is now a dominant non-indigenous species in many locations of the eastern Mediterranean Sea, including Cyprus, Greece, Egypt, Libya, Lebanon and Turkey (Carpentieri et al. 2009, Katsanevakis et al. 2009, Michailidis 2010, Aydin 2011, Halim & Rizkalla 2011, Keskin et al. 2011, Zenetos et al. 2011, Milazzo et al. 2012). Recently, a new record of the species in the central Mediterranean Sea (Tunisia) indicates a westerly immigration of the species (Jribi & Bradai 2012).

L. sceleratus can be found in a variety of habitats including sandy, rocky substrates and seagrass meadows (Heithaus 2004, Kalogirou et al. 2010, 2012, Michailidis 2010), in depths ranging from 0 to 100 m, and has a preference for shallow and intermediate waters (0 to 50 m) (Cinar et al. 2011). In the Mediterranean, adults of *L. sceleratus* tend to inhabit *Posidonia oceanica* meadows, while juveniles seem to prefer sandy habitats, perhaps due to the lower level of competition for available resources (Kalogirou et al. 2012).

The pufferfish is a generalist carnivore with a broad and variable diet composed of crustaceans (shrimps, crabs), molluscs (squids, octopus) and fish, that is subject to variations indicating an adaptive feeding behaviour to available resources (Kulbicki et al. 2005, Sabrah et al. 2006, Romanov et al. 2009, Michailidis 2010, Aydin 2011, Kalogirou 2013). Biological studies of *L. sceleratus* in the Mediterranean Sea and the Suez Canal are rather scarce. Biometrical analysis of pufferfish captured by fishermen estimate the maximum total length and weight in the Mediterranean Sea to be 77 cm and 5600 g, respectively (Michailidis 2010), and 78.5 cm and 5100 g, respectively, in the Suez Canal (Sabrah et al. 2006), without any significant size differences detected between male and female individuals. Furthermore, in both areas, reproduction peaks in late spring to middle summer at a minimum body length size of around 30 cm (Sabrah et al. 2006, Michailidis 2010, Peristeraki et al. 2010, Aydin 2011, Kalogirou 2013).

L. sceleratus was officially recorded in the coastal waters of Cyprus in 2006, although local fishermen were already aware of its presence. The species displays an extremely invasive character and is gradually becoming a dominant species in many areas around the island, especially at the southern and eastern coasts of Cyprus (Katsanevakis et al. 2009, Michailidis 2010). The impacts of the growing pufferfish population to the coastal ecosystems of Cyprus are yet to be explored, but it is thought to be out-

competing native fishes and exhausting invertebrates such as the *Octopus vulgaris* and squids (D. Kletou pers. obs.). Currently, the most obvious impact is to the small-scale local fishery sector, as the pufferfish feeds on commercial species caught in nets, leading to significant losses of income and damage to fishing gears. *L. sceleratus* is treated as discarded bycatch because of concentrated tetrodotoxin (TTX) in its liver, gonads, gastrointestinal tract and skin (Sabrah et al. 2006, Katikou et al. 2009). Poisoning incidents following consumption of *L. sceleratus* have been reported in some countries and, consequently, the toxic fish is associated with health hazards (Streftaris & Zenetos 2006, Bentur et al. 2008, Katikou et al. 2009). So far, no poisoning incidents have been reported in Cyprus, which could be attributed to the effective publicity to stakeholders carried out by national authorities.

The present study is the first to investigate the maturity and reproductive dynamics of a large sample of *L. sceleratus* individuals caught by commercial fisheries off Cyprus and tests whether these are affected by regional differences in oceanographic conditions, habitat characteristics and fishing activity.

MATERIALS AND METHODS

Sampling

Samples of *Lagocephalus sceleratus* were collected off Cyprus between May and October 2010 to meet the requirements of a tender initiated by the Department of Fisheries and Marine Research (DFMR). Pufferfish captured during regular commercial fishing activities were provided by fishermen to 6 regional offices of the DFMR located at Paralimni, Larnaca, Zygi, Limassol, Pafos and Latsi (Fig. 1). The study area was stratified into 2 sub-regions: the southwestern area (SWA), which extended from Pyrgos Tillirias to the Akrotiri Peninsula, and the southeastern area (SEA), which extended from Cape Akrotiri to Paralimni (Fig. 1). Individuals caught on a daily fishing trip represented a single sample. For each sample, a data form was completed by DFMR officers with the following information: (1) fishermen contact details, (2) fishing trip details (date, area and depth of fishing, starting and ending time of fishing), (3) fishing gear information (type of fishing gear used—type of nets, number of nets, net mesh size, type of bait if used), (4) total number of pufferfish caught, and (5) other information (type of substrate,

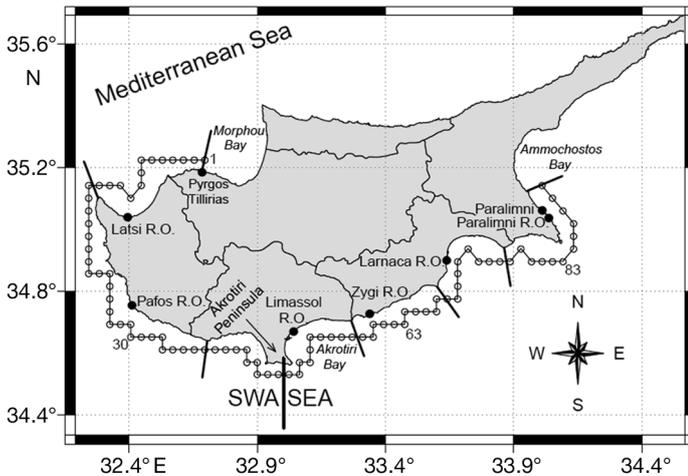


Fig. 1. Study area in Cyprus extending from Pyrgos Tillias to Paralimni. The sea surface temperature (SST) transect is shown along the coast (●●●); numbers represent transect points. Solid lines perpendicular to the coast correspond to the limits of the regional offices (R.O.) of the Department of Fisheries and Marine Research (DFMR). SWA: southwestern area, SEA: southeastern area

current and wind magnitude). During the study period, DFMR officers registered a total of 1686 hauls (each haul being a single fishing trip) resulting in the capture of 44 036 ind. (3767 ind. in the SWA and 40 269 ind. in the SEA).

Laboratory analysis

Samples of pufferfish were transferred on a weekly basis from each office to the laboratory of 'Marine and Environmental Research Lab' (MER) for further analysis. This analysis was performed using individuals from 447 randomly selected hauls (i.e. 27% of total hauls). The mean number of captured individuals per haul was 10.6 (SE = 3.1); 185 of these hauls contained 1 to 10 ind., while only 26 contained >80 ind.. When the number of individuals in the haul was <10, almost all were subjected to laboratory analysis, whilst for larger hauls, the mean number of individuals that were processed was 19.2 (SE = 2.3). A total of 4743 pufferfish were analysed during the study period (Table 1). Individuals were measured for total length (TL; cm) and total weight (W_t ; g); sex was identified macroscopically, as was gonadal maturity, using the 5 point scale proposed by Brown-Peterson et al. (2011) (I: immature; II: developing; III: spawning capable; IV: regressing; V: regenerating). Our study did not include any histological analysis of gonadal material. However, as will be discussed in the 'Data

Table 1. *Lagocephalus sceleratus*. Sampling table. SWA: southwestern area, SEA: southeastern area, n_t : number of total individuals recorded, n_s : number of individuals sampled; TL: mean total length (in cm); standard deviation in brackets

Month	SWA			SEA		
	n_t	n_s	TL	n_t	n_s	TL
May	641	144	36.1 (11.3)	10 058	446	38.6 (14.3)
June	374	155	30.0 (15.2)	11 477	607	43.1 (12.1)
July	615	240	29.8 (10.8)	3956	587	36.2 (13.3)
August	493	119	27.2 (6.5)	5717	754	29.1 (7.6)
September	665	209	31.5 (9.0)	4722	686	30.3 (7.5)
October	979	247	30.6 (8.8)	4339	540	32.4 (8.8)

analysis' section and the 'Results' section, we managed to overcome this weakness and make necessary distinctions between mature and immature individuals through a series of data analyses (application of temporal filter, gonad allometry plots, etc.). Gonadal weight (W_g) was measured to the nearest 0.01 g. The gonadosomatic index (GSI) was estimated as the percentage of gonad weight to gonad-free somatic weight (W_{gf}) ($GSI = W_g/W_{gf} \times 100$) (McPherson et al. 2011).

Data analysis

Since macroscopic maturity scorings are often subjective and inaccurate and can lead to biased estimations of the reproductive condition (Hunter & Macewicz 2001), a validation was performed by examining the pattern of gonadal allometry for each maturity stage, separately for males and females. More specifically, log-transformed values of W_g were plotted against log-transformed values of W_{gf} and the plots were visually examined to check whether the scatter points corresponding to values of maturity stage were satisfactorily separated (McPherson et al. 2011). The gonadal allometry plots not only serve to discern the different maturity stages but also offer a good indication of which stages correspond to mature and immature fish. Specifically, by applying a temporal filter to the reproductive peak of the population (Hunter & Macewicz 2001, Lowerre-Barbieri et al. 2011), the prevalence of mature inactive individuals is eliminated and the population mostly comprises

of immature and spawning-capable fish. In this case, the shift in gonad weight from Stages I and II to Stage III in the gonadal allometry plot would occur at a narrow body size range. In addition, the degree of separation of the scatters between these stages would allow some validation of macroscopic maturity scorings.

Following validation, individuals were classified as immature or mature. Dichotomous maturity data (0: immature; 1: mature) were modelled as a function of TL, sex and area (SWA, SEA) using generalised linear models (GLM), with a binomial error distribution and a logit link. TL was treated as a continuous variable, and sex and area as factorial variables. Variable selection was performed through a stepwise backward Akaike's information criterion (AIC)-based process. The analysis led to estimates of length at maturity (L_{50}), i.e. the length at which 50% of the population becomes mature. Standard errors for the L_{50} estimates were calculated using the delta method. GLM analysis was also used to test the preference of individuals for sandy substrates in relation to body length and area (SWA, SEA).

To test for differences in the catches of pufferfish between the 2 areas, the number of pufferfish caught at each haul was analysed as a function of the area using a GLM with Gamma error distribution and an identity link. The duration of the haul was used as a covariate in order to standardise its effect on the fishing effort. Moreover, the reproductive season (inside/outside) was used as an extra binary factorial variable to test for possible effects of reproductive behaviour (e.g. formation of spawning aggregations) on the catchability of the pufferfish. All data were analysed using R v.2.14.1 (R Development Core Team 2012).

Sea surface temperature data

The potential existence of distinct temperature regimes in the 2 sub-regions of the study area was investigated to examine whether it contributes to the observed differences in the demographic and reproductive characteristics of the SWA and SEA pufferfish populations (see 'Results' section). A sea surface temperature (SST) transect along the sampled coast of Cyprus, extending

from the northern limit of Latsi to the eastern limit of Paralimni (Fig. 1), was extracted separately from monthly satellite images (May to October 2010) using the SeaDAS 6.2 image analysis package (seadas.gsfc.nasa.gov/seadas/) and Aqua MODIS satellite imagery (level-3 standard mapped image products, oceancolor.gsfc.nasa.gov/). Daily proxies of SST by NOAA Advanced Very High Resolution Radiometer (NOAA-AVHRR) imagery were extracted from CYCOFOS Bulletin (www.oceanography.ucy.ac.cy/cycfos/remote-sensing.php).

RESULTS

As shown in Fig. 2, the reproductive period of pufferfish off Cyprus was satisfactorily covered for both sexes by our study. GSI values were particularly elevated in June, suggesting that this month coincides with the seasonal reproductive peak of the population. Sampling at reproductive peak was important for applying a temporal filter in our data both for analysing gonadal allometry and for the assessment of size at maturity (see next paragraphs). For better resolution, seasonality in GSI was analysed separately for populations in SEA and SWA by also break-

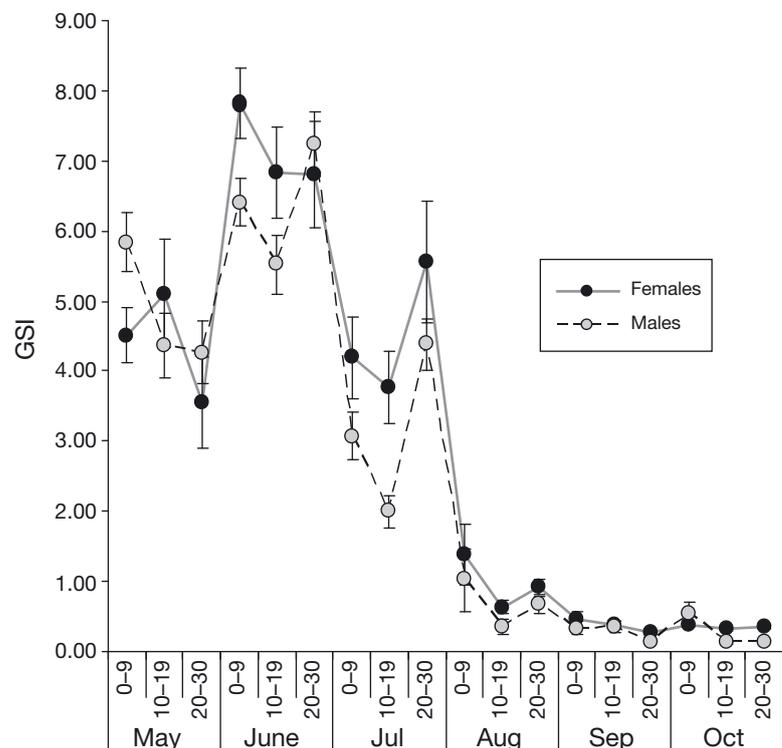


Fig. 2. *Lagocephalus sceleratus*. Monthly evolution of the gonadosomatic index (GSI) for female and male pufferfish in the whole study area. Vertical error bars correspond to standard error

ing down data into 2 length classes (<40 and \geq 40 cm). The latter treatment mostly filtered immature fish from the analysis. It was shown that the larger size class (\geq 40 cm) in the SWA displayed both a delayed and a narrower reproductive season (Fig. 3). Therefore, the temporal filter for the subsequent assessment of size at maturity was set from late May to late July.

A large number of Stage I individuals ($n = 1259$) had very tiny gonads, and their sex could not be determined. The remaining Stage I and the Stage II individuals discriminated well from Stage III individuals in both male and female pufferfish gonadal allometry plots (Fig. 4), confirming the accuracy of the macroscopic stage classification. In addition, the slopes of the gonad over body weight relationships did not differ significantly between Stages I and II combined and Stage III in both males and females (ANCOVA: $p > 0.1$). This further suggested that there was no shift in the allometric growth pattern of both testes and ovaries during gonadal development. No Stage V individuals were recorded in our study.

As shown in Fig. 4, there is very little overlap of body sizes between Stages I and II combined and Stage III. Specifically, there is a clear absence of larger individuals at Stages I and II and of smaller individuals at Stage III. This suggests that almost all of the Stage II pufferfish examined were first time spawners and were thus grouped as immature in subsequent analyses. The above is reinforced by the fact that sampling started very close to the seasonal peak of pufferfish reproduction and thus mature, repetitive spawners had already entered the spawning-capable stage.

Backward stepwise entry in the GLM analysis showed that only length and region affected maturity, whilst sex and its interaction with length were not significant predictors (Table 2). Since the interaction between length and region was also non-significant (Table 2), the final model had region-specific intercepts and common slopes (Fig. 5). Therefore, L_{50} in each region was calculated using the formula:

$$L_{50} = -(\mu + \alpha_s)/\gamma \quad (1)$$

where μ is the intercept, α_s is the coefficient for region and γ is the coefficient for length. L_{50} in the SWA was estimated to be 41.9 cm (SE = 0.2) whilst in SEA, L_{50} was 48.8 cm (SE = 0.6) (Fig. 5).

As shown in Fig. 6A, differences in L_{50} between the 2 areas were accompanied by significant demographic differences inside the reproductive period, with SEA being mainly inhabited by mature individuals (i.e.

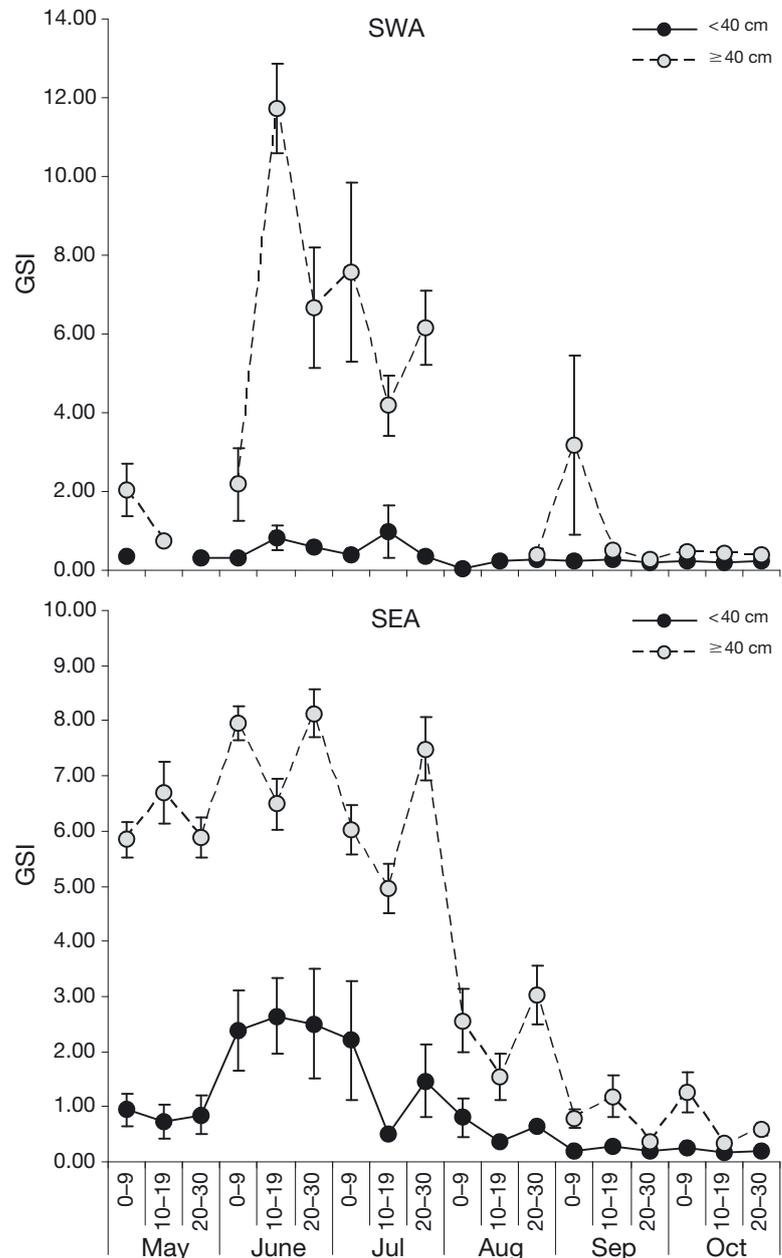


Fig. 3. *Lagocephalus sceleratus*. Seasonal evolution of the gonadosomatic index (GSI) of small (<40 cm) and large (\geq 40 cm) female pufferfish in the 2 sub-regions (SWA: southwestern area, SEA: southeastern area). Vertical error bars correspond to standard error

Table 2. *Lagocephalus sceleratus*. Coefficients of the generalized linear models (GLMs) used to analyse the effect of body length (L), sex, area (southwestern area: SWA; southeastern area: SEA), duration of catch (DUR) and reproductive season (RS; inside vs. outside) on the percentage of maturity (%MAT), occurrence over sandy substrates (%SS), and number of individuals in each sample (CATCH). Only coefficients for explanatory variables are provided in the table. ns: non-significant; *005 > p > 001; **p < 001

Source of variation	%MAT	%SS	CATCH
Null	-2035**	062**	3353**
L	042**	-0023**	
SEX	ns		
AREA	279**	-139**	4012*
$L \times \text{SEX}$	ns		
$L \times \text{AREA}$	ns		
DUR			ns
RS			299*

>45 cm) and the SWA by immature individuals. This pattern changed during the following non-reproductive period, since the cohort of mature fish had almost entirely disappeared and both areas were mainly inhabited by smaller, immature fish (Fig. 6B).

GLM analysis showed that, for both regions, pufferfish catches were significantly higher during the reproductive season (Table 2). Moreover, the probability of occurrence in sandy substrate was significantly related both to body length and the study area (Table 2). Indeed, 47.6% of SWA samples were collected from regions with sandy substrate, while this percentage was considerably smaller (16.2%) in the SEA. Using only length as an explanatory variable, the GLM predicted that a 20 cm individual had

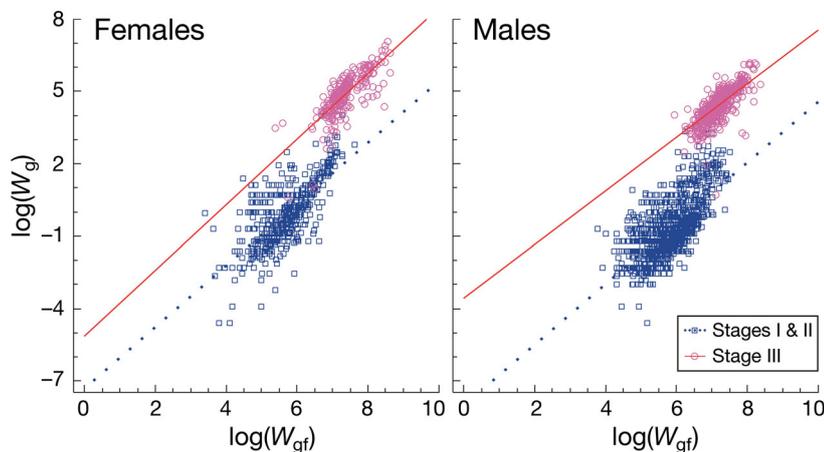


Fig. 4. *Lagocephalus sceleratus*. Gonadal allometry plots for female and male pufferfish. The logarithms of gonadal weights (W_g) were plotted against the logarithms of gonad-free body weights ($W_{gf} = W_t - W_g$) for immature (combined Stages I and II) and mature (Stage III) individuals separately. Least squares trend lines are superimposed. W_t : total weight

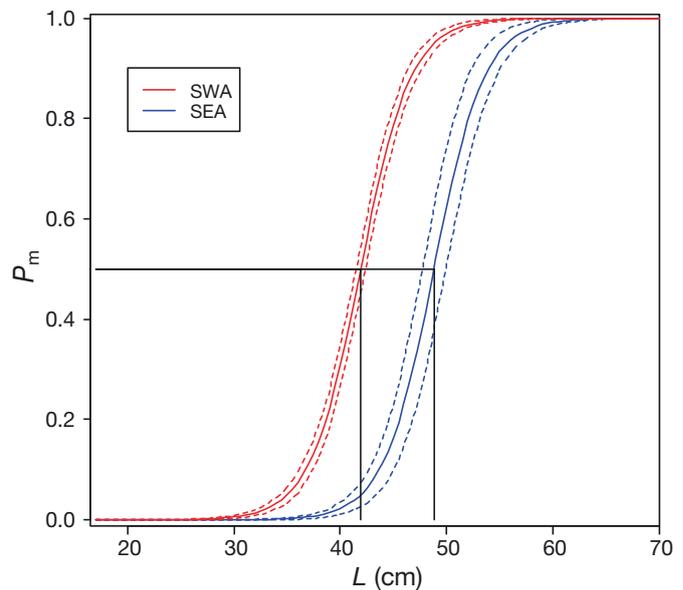


Fig. 5. *Lagocephalus sceleratus*. Probability of being mature (P_m) versus body length (L) relationships for the pufferfish populations of the southwestern (SWA) and the southeastern (SEA) areas. Dotted lines correspond to 95% confidence intervals. Rectangular segments indicate length at 50% maturity for the 2 areas

a 32% probability of being found over sandy substrate, while the probability of a 70 cm individual was only 10% (Fig. 7).

Analysis of monthly mean SST data did not reveal any particular differences between the 2 regions that could explain the aforementioned differences in demography and reproductive dynamics (Fig. 8). It should be mentioned that there was a small decline of ca. 1°C at the border between the 2 areas during July and August, but this could not explain demographic or reproductive patterns. Differences in SST during the reproductive period (8°C between May and August) over-exceeded these small regional differences, suggesting that mean monthly temperature difference itself could not justify the aforementioned differences between the 2 populations. However, daily proxies of SST from NOAA-AVHRR imagery reveal frequent, short-lived upwelling phenomena during summer when north-westerly winds prevail. These cause the off-shore transport of warm water and the upwelling of cold deep water off-shore from Cape Akrotiri with an SST up to 5°C cooler than in other coastal areas of Cyprus (Fig. 9).

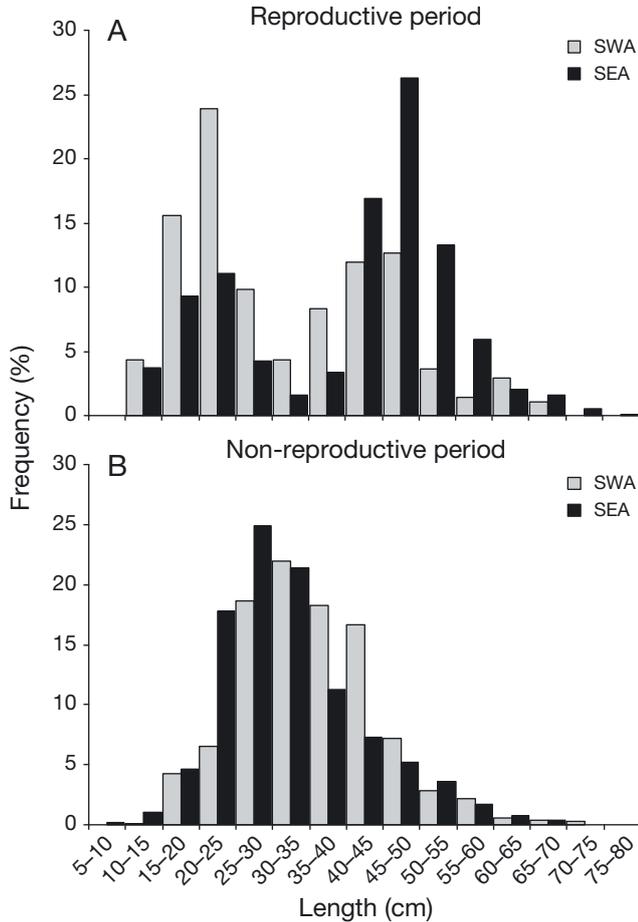


Fig. 6. *Lagocephalus sceleratus*. Length frequency distributions of pufferfish during the (A) reproductive and (B) non-reproductive period for the 2 sub-regions (SWA: south-western area, SEA: southeastern area)

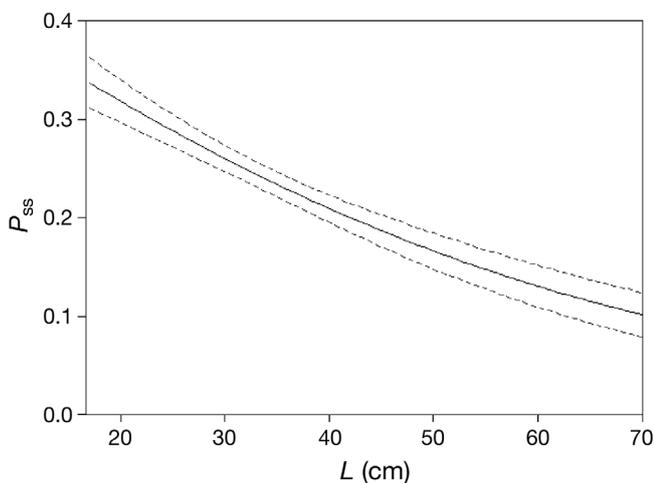


Fig. 7. *Lagocephalus sceleratus*. Fitted probability of occurrence in sandy substrate (P_{ss}) with body length (L) in the 2 sub-regions (SWA: south-western area, SEA: southeastern area). Mean fitted probability (solid line) and 95% confidence intervals (dotted lines)

DISCUSSION

The reproductive season of *Lagocephalus sceleratus* off Cyprus was found to extend from late spring to middle summer (reproductive peak recorded in June), which is in agreement with the observations of Aydin (2011) in Antalya Bay, Kalogirou (2013) and Peristeraki et al. (2010) in Greece, and Sabrah et al. (2006) in the Suez Canal. The statistical analysis of data revealed some significant, small scale regional differences between the 2 neighbouring populations of *L. sceleratus*. In particular, the SWA population presented a more delayed and narrow reproductive period than the SEA population. The length at which 50% of the individuals reached first maturity (L_{50}) was estimated to be 41.9 cm for the SWA and 48.8 cm for the SEA populations. Kalogirou (2013) estimated the L_{50} for both sexes to be 36 cm, which is considerably lower than the L_{50} found in Cyprus. Sabrah et al. (2006) estimated the L_{50} of pufferfish in the Suez Canal to be around 42 to 43 cm, which is similar to the L_{50} estimated for the SWA but lower than that recorded for the SEA.

Spatial differences in L_{50} were followed by demographic differences during the peak of spawning months. During the study period, the SEA was found to be mainly inhabited by mature individuals while the SWA by immature individuals.

We tried to associate the aforementioned differences in reproductive and demographic patterns to potential regional differences in mean monthly SST proxies; however, apart from a small decline in SST at the border of the 2 areas during the peak of the spawning months, we could not find any substantial monthly SST differences between SWA and SEA that could justify the time lags in the gonadal development of the 2 populations. However, high resolution remote-sensing SST data, obtained almost daily over a decade, confirm a short-lived upwelling of cold water (about 4 to 5°C lower) on the southern coasts of Cyprus. This phenomenon is observed frequently during the summer months, which coincides with the reproductive period of *L. sceleratus*. According to Belmaker et al. (2013), Lessepsian migrants present an increased introduction success in areas with high water temperature, productivity range and SST. Therefore, in this case, the temperature gradient induced by the advected cold water, even though short-lived, may act as a physical barrier to the reproductively active tropical pufferfish of the SEA and could justify the large aggregations of mature individuals in the SEA and their near absence from the SWA.

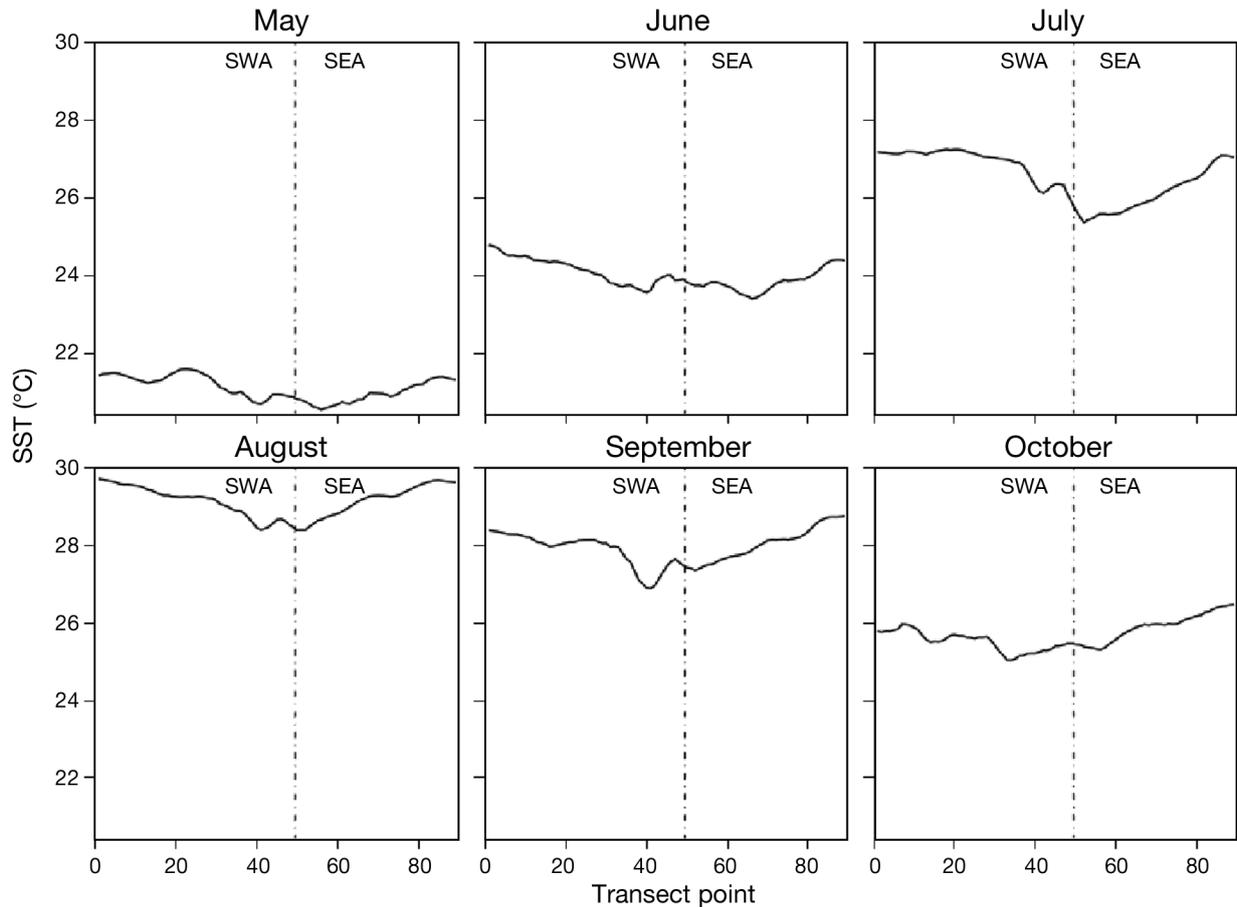


Fig. 8. Monthly mean sea surface temperature (SST) along the transect for the whole study period (May to October 2010). Solid lines represent a 5-point moving average. SWA: southwestern area; SEA: southeastern area

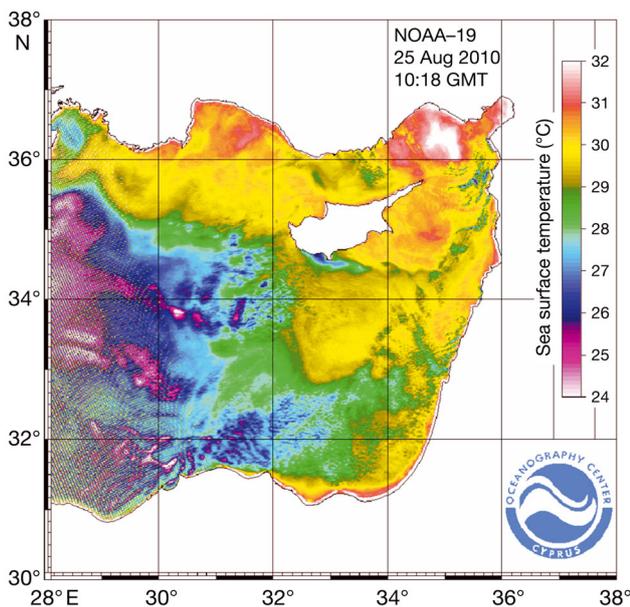


Fig. 9. NOAA-AVHRR image taken on 25 August 2010 from the CYCOFOS ground satellite receiving station at the Cyprus Oceanography Centre, showing the frequent summer upwelling phenomena offshore from Cape Akrotiri

Furthermore, in both areas, young individuals seem to prefer sandy substrate, which is in agreement with the observations of Kalogirou et al. (2012). Likewise, Denadai et al. (2012) noted that small individuals of the pufferfish *L. laevigatus* preferred to inhabit shallower areas close to sandy beaches or rocky shores in Brazil. Therefore, demographic and consequently maturity differences between the 2 populations could possibly be explained by regional differences in the substrate composition between the 2 areas: 47.6% of SWA samples were collected from regions with sandy substrate while this percentage was considerably smaller (16.2%) in the SEA. Sections of the SEA with high pufferfish abundance are dominated by calcareous sediment while most sections of the SWA with low pufferfish abundance are dominated by grey terrigenous sediment, which may offer less suitable habitat conditions for the large mature pufferfish or their offspring (D. Kletou pers. obs.).

Throughout the non-reproductive period, mature fish had almost entirely disappeared from both areas

and were replaced by younger immature individuals. This change in population demography could not be attributed to overfishing of larger size classes since fishery was not shown to be size selective. On the other hand, the disappearance of the larger mature fish could be attributed to migration. Thus, the absence of mature individuals during the non-reproductive period could indicate that *L. sceleratus* may be a spawning migratory species that regularly visits Cyprus southeastern coasts to reproduce and then migrates to other areas, possibly in the Levantine basin.

As mentioned above, the toxic pufferfish displays an extremely invasive character with major implications to the local fishery sector (causing significant damages to fishing gear as the pufferfish tries to consume commercial species caught on nets). The findings of this study suggesting that *L. sceleratus* may be a migratory species could advocate that mitigation strategies taken in Cyprus may mitigate problems associated with the invasive pufferfish elsewhere in the eastern Mediterranean, such as in Greece (Kalogirou 2011) and Turkey. Mitigation actions taken so far by the National Authorities of Cyprus to control the *L. sceleratus* population have focused on the physical removal of pufferfish. A compensation of € 1 and € 3 per pufferfish were provided to local fisherman by the DFMR in 2010 (data of this study) and later on in 2012 (June to July, no biological data were collected), which resulted to the removal of massive amounts of pufferfish catches that were later combusted at a local authorised incineration unit. In several cases, this financial reward acted as a stimulus to some fishermen of the eastern coasts, who adapted their fishing techniques and bait to target pufferfish specimens, with catches during the peak period exceeding 500 ind. d⁻¹. However, even though the physical removal of individuals is a common technique used for controlling alien species, there are many limitations, as it is an energy inefficient and expensive procedure and is often ineffective in controlling non-indigenous species populations which tend to have a spatially broad ecological biotope (Byers et al. 2002, Britton et al. 2011), such as in the case of *L. sceleratus*. Perhaps the exploration of TTX for medical use may hold the solution for mitigating its invasive impacts in the Mediterranean.

In conclusion, the current study indicates that *L. sceleratus* may be a spawning migratory species that visits Cyprus southeastern coasts to reproduce and then migrates to other warmer areas of the eastern Levantine basin. The spawning area selection could be due to temperature gradients induced by short-

lived summer upwelling phenomena and substrate differences or perhaps food availability. Nonetheless, more thorough large-scale studies of the behaviour and reproductive strategy of the pufferfish need to be carried out before drawing any valid conclusions.

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