AGE AND GROWTH OF THE LESSEPSIAN MIGRANT *LAGOCEPHALUS SPADICEUS* (ACTINOPTERYGII: TETRAODONTIFORMES: TETRAODONTIDAE) FROM THE GULF OF ISKENDERUN, NORTH-EASTERN MEDITERRANEAN, TURKEY

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Background. The population of *Lagocephalus spadiceus* (Richardson, 1845), one of the Lessepsian species that migrated from the Red Sea to the Mediterranean Sea through the Suez Canal, has been growing rapidly in Turkey in recent years. This situation poses a serious danger to fisheries and human health. There is little information about the biology of this species. The presently reported study permits a greater understanding of the age and growth characteristics of the pufferfish *L. spadiceus* from the Gulf of Iskenderun, north-eastern Mediterranean.

Materials and methods. Samples were captured by commercial bottom trawlers at depths of 24–50 m during 9 trawling operations conducted during the fishing seasons between 2012 and 2013 in the Gulf of Iskenderun, north-eastern Mediterranean. Age determinations were carried out using vertebrae. Images of vertebrae suitable for age determination were obtained with a dissecting microscope. The index of the mean percentage error (IMPE) was calculated to assess the precision of the age determinations between two independent readers. Growth parameters for all individuals were then determined by fitting observed and length-at-age data using the von Bertalanffy growth equation. In addition, the condition factor (CF) was determined.

Results. A total of 1116 specimens (529 females and 587 males) were sampled, ranging from 6.7 to 34.0 cm in total length and from 4.30 to 557.64 g in weight. The female-to-male ratio was 1:1.1. Age determination was conducted using vertebral band counts. The age of the examined specimens ranged from 0 to 10 years. One female individual was determined to be 17 years of age (45.6 cm and 1269.0 g). The von Bertalanffy growth equations were $L_t = 47.21[1 - e^{-0.116(t + 2.04)}]$ and $W_t = 1296.18\ [1 - e^{-0.116(t + 2.04)}]^{2.846}$ for females, $L_t = 46.36\ [1 - e^{-0.133(t + 1.76)}]$ and $W_t = 1381.86\ [1 - e^{-0.133(t + 1.76)}]^{2.817}$ for males and $L_t = 46.90\ [1 - e^{-0.120(t + 1.97)}]$ and $W_t = 1345.67\ [1 - e^{-0.120(t + 1.97)}]^{2.831}$ for all individuals. The growth performance index (Φ') value was calculated as 2.421 for all individuals. The condition factor ranged from 0.890 to 2.768 for females and from 0.899 to 2.767 for males.

Conclusions. It is very important to investigate *L. spadiceus*, an invasive species, in order to prevent its harmful effects on fisheries and human health. As this study is the first study on the age and growth of *L. spadiceus* in the north-eastern Mediterranean, it will significantly contribute to future ecosystem conservation and management strategies.

Keywords: half-smooth golden pufferfish, growth parameters, vertebrae, condition factor, north-eastern Mediterranean

INTRODUCTION

There are approximately 192 valid species in the family Tetraodontidae worldwide (Fricke et al. 2020). *Lagocephalus spadiceus* (Richardson, 1845) is one of the oldest Lessepsian fishes and the first of the four *Lagocephalus* species to date that have entered the Mediterranean via the Suez Canal (Kiparissis et al. 2018). Lessepsian species were so named by Por (1978) after the founder of the canal engineer and diplomat, Ferdinand de Lesseps (Mater et al. 1995, Başusta and Erdem 2000, Mavruk and Avsar 2008). In the Mediterranean Sea, *L. spadiceus* was first recorded in the Gulf of Iskenderun (Turkey) by Kosswig (1950) (Tuncer et al. 2008). *Lagocephalus spadiceus* is a benthopelagic species; it swims in mid-water but descends to the substrate to feed on benthic organisms, which it crushes with its massive teeth. It is occasionally caught by trawl or purse seines and originally had wide Indo-Pacific distribution (Golani et al. 2006). Many studies

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have reported a lack of toxicity of *L. spadiceus* (Yu and Yu 1997, Brilliantes et al. 2003, Ngy et al. 2008, Simon et al. 2009, Chulanetra et al. 2011, Kosker et al. 2019), and the species is consumed in some countries (Berry and bin Hassan 1973, Yu and Yu 1997, Brillantes et al. 2003, Kaewnern et al. 2013, Yamaguchi et al. 2013, Kosker et al. 2019). To date, there have been no studies on the age and growth of *L. spadiceus* in Turkish waters. The knowledge of the age and growth of fish populations is important for the control of fish stocks. However, many studies have been conducted on the length–weight relation of *L. spadiceus* (see Taskavak and Bilecenoglu 2001, Erguden et al. 2009, Wang et al. 2011, Başusta et al. 2013, Aydın et al. 2017, Bilge et al. 2017). Although the first detailed study on the biology of *Lagocephalus sceleratus* (Gmelin, 1789) in the Mediterranean was conducted by Aydın (2011), there has been no study on the biology of *Lagocephalus spadiceus* in Turkish waters. Tuncer et al. (2008) provided the first record of *L. spadiceus* in the Sea of Marmara. Tuney (2016) conducted molecular identification of *L. spadiceus*. Kiparissis et al. (2018) studied the range expansion of restricted Lessepsian taxa, including the westbound expansion of *L. spadiceu*s. Kosker et al. (2019) investigated the tetrodotoxin levels of three pufferfish species. Bilge et al. (2019) found that for both basic risk assessment and basic risk assessment + climate change assessment, the second-highest scoring invasive fish species was the half-smooth golden pufferfish, *L. spadiceus*, along the south-western coasts of Turkey.

In recent years, *L. spadiceus* has rapidly reproduced in the Mediterranean, posing a risk to the ecosystem as well as to human activities (Katsanevakis et al. 2014, Streftaris and Zenetos 2006, Mavruk et al. 2017). However, there is very little research on the biology and ecology of this species. The purpose of this study was to determine, for the first time, some growth parameters of *L. spadiceus* in the north-eastern Mediterranean Sea.

MATERIAL AND METHODS

The samples were collected by commercial bottom trawlers at depths of 24–50 m over 9 trawling operations conducted between the 2012 and 2013 fishing seasons in the Gulf of Iskenderun, north-eastern Mediterranean (Fig. 1). The sampled fish were transported to a laboratory of the Fisheries Faculty of the Firat University. The fish were measured for total length [cm] and weight [g]. Sex was determined by macroscopic examination of the gonads, and deviation from a 1:1 sex ratio was tested with a chi-square test.

Age determinations were carried out using vertebrae. Eight to ten vertebrae were removed from the anteroposterior of the body of each specimen. The remaining soft tissue in the center of each vertebra was removed with the help of a knife. The vertebrae were then left immersed in 5% sodium hydrochloride for one day to remove excess connective tissue and then rinsed with purified water. The vertebrae were then stored in 70% ethanol until processing.

A total of 5 vertebrae from each specimen were read for age at 2× magnification independently by two readers. One opaque zone and one adjacent transparent zone were together counted as one year. Images of all vertebrae ready for age reading were taken with high-resolution Leica IM1000 image analysis software and a Leica M40 dissecting microscope (Fig. 2). Adobe Photoshop CS2 was then used to improve the visibility of the vertebral rings.

The index of the mean percentage error (IMPE) (Beamish and Fournier 1981)* was calculated to assess the precision of the age determinations between two independent readers using the following equation

$$\mathrm{IMPE} = \frac{1}{n}\sum_{j=1}^{N}\left(\frac{1}{N}\sum_{i=1}^{R}\frac{\left|X_{ij}-X_{j}\right|}{X_{j}}\right)\times 100\%$$

where n is the number of fish aged, N = number of times each fish was aged, X_{ij} is the ith age determination of the jth fish, X_j is the mean age calculated for the jth fish.

The growth in length was determined by the length-based von Bertalanffy (1938) growth equation as follows

$$\mathrm{TL}_t = \mathrm{TL}_\infty\,[1 - e^{-k(t-t0)}]$$

where TL_t is the expected total length at age t years, TL_∞ is the asymptotic average maximum total length, k is

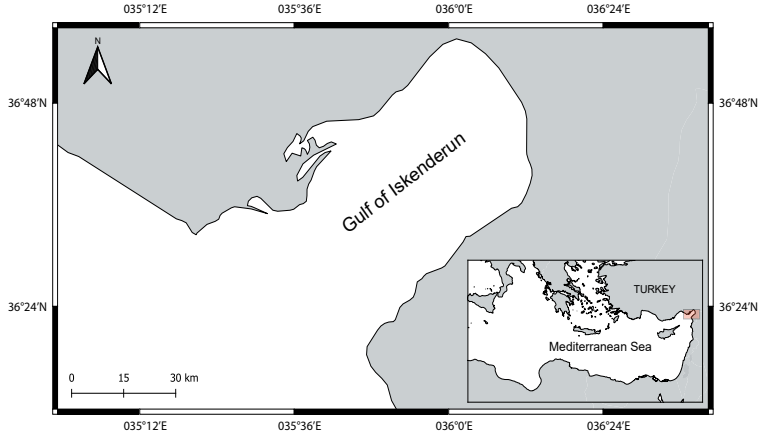

Fig. 1. The sampling area in the Gulf of Iskenderun, north-eastern Mediterranean within 2012–2013

* The original authors Beamish and Fournier (1981) and many subsequent ones referred to this index imprecisely as the "index of the average percentage error" (IAPE).

the growth coefficient, and t_0 is the theoretical age at zero length.

The growth in weight was described by the weight-based von Bertalanffy growth equation

$$W_t = W_\infty [1 - e^{-k(t-t0)}]^b$$

where W_∞ is the asymptotic average maximum weight and b is the constant in the length–weight relation)

The accuracy of the growth parameters (Gayanilo and Pauly 1997) was tested using von Bertalanffy growth performance

$$\Phi' = log_{10}(k) + 2log_{10}(L_\infty)$$

The condition factor values of fish were acquired with the equation of Le Cren (1951)

$CF = 100W \cdot TL^{-b}$

All data were statistically analyzed using Excel 2013 and SPSS version 24 for Windows.

RESULTS

A total of 1116 specimens of *Lagocephalus spadiceus* (including 529 females and 587 males) were caught during the study. Females varied from 7.3 to 34.0 cm in TL and from 6.07 to 557.64 g in *W*. Males varied from 6.7 to 33.8 cm in TL and from 4.30 to 516.00 g in *W*. One female individual was 45.6 cm in length. The sex composition was 47.40% females and 52.60% males. The sex ratio was 1:1.1 for *L. spadiceus*; the chi-square test showed that this ratio was significantly different from the theoretical ratio of 1:1 (chi-square, $P < 0.05$). The 16–20 cm length category was the most dominant category in the population (Fig. 3).

The index of the mean percentage error (IMPE) of age estimation by the 2 independent readers was 7.59. The IMPE confidence interval of 5% to 15% indicated that the ageing method we used for age estimation is reliable (Campana 2001).

The age of the captured specimens of *L. spadiceus* ranged from 0 to 10 years for all individuals. The most dominant age group in the population was the age group of 2 years (856 specimens) (Table 1). One female individual was determined to be 17 years of age (45.6 cm and 1269.0 g). The age-frequency distribution by sex is provided in Fig. 4.

The von Bertalanffy growth parameters were estimated as $L_t = 47.21[1 - e^{-0.116(t + 2.04)}]$ and $W_t = 1296.18\ [1 - e^{-0.116(t + 2.04)}]^{2.846}$ for females, $L_t = 46.36\ [1 - e^{-0.133(t + 1.76)}]$ and $W_t = 1381.86\ [1 - e^{-0.133(t + 1.76)}]^{2.817}$ for males and $L_t = 46.90\ [1 - e^{-0.120(t + 1.97)}]$ and $W_t = 1345.67\ [1 - e^{-0.120(t + 1.97)}]^{2.831}$ for all individuals. The growth performance index (Φ') value was calculated as 2.421 for all individuals (Table 2). Figure 5 presents the age–total length relation according to age group and sex.

The condition factor was calculated for both sexes (Table 1) and all age groups. The difference between sexes by age group was not significant ($P > 0.05$). Across all individuals, the highest average condition factor was obtained for age group 1 (1.732), and the lowest was obtained for age group 9 (1.404) (Fig. 6).

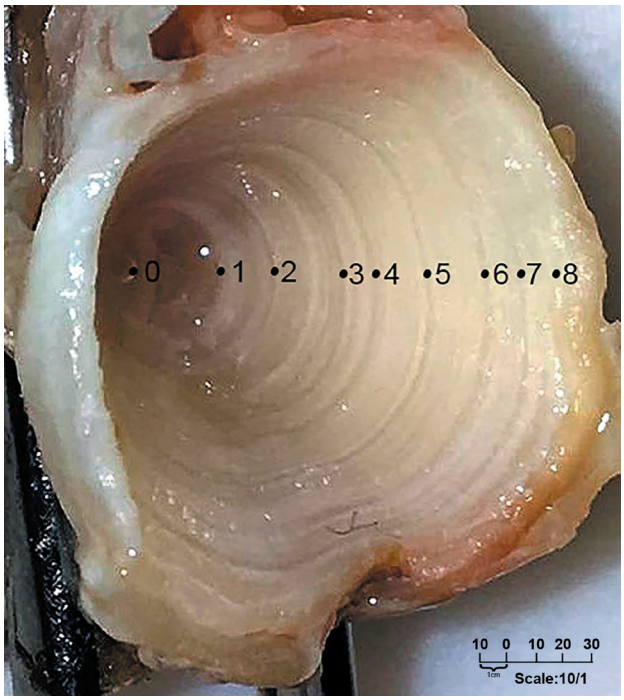

Fig. 2. Vertebrae centrum from a 31.5 cm TL and estimated to be 8 years for *Lagocephalus spadiceus* captured in the Gulf of Iskenderun, north-eastern Mediterranean within 2012–2013

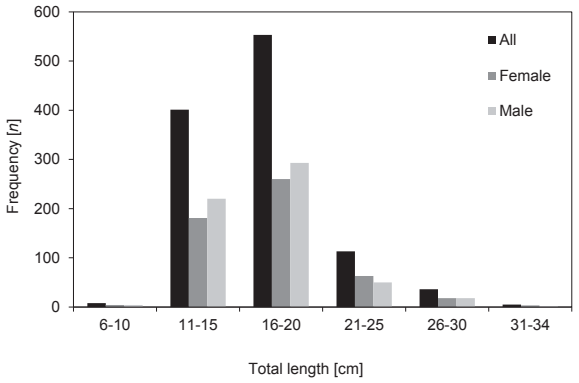

Fig. 3. Total length-frequency distribution of *Lagocephalus spadiceus* from the Gulf of Iskenderun, north-eastern Mediterranean (2012–2013)

DISCUSSION

In this study, the length range (minimum–maximum) for *Lagocephalus spadiceus* was found to be 6.7 to 34.0 cm. The maximum total lengths reported for this species in other regions included 16.1 cm for the coasts of the Mediterranean and the southern Aegean Sea of Turkey, reported by Torcu and Mater (2000); 19.9 cm for the eastern Mediterranean coast of Turkey, reported by Taskavak and Bilecenoglu (2001); 12.6 cm in the Sea of Marmara, reported by Tuncer et al. (2008); 26.9 cm on the coast of the Gulf of Iskenderun, reported by Erguden

Table 1

The principal biometric parameters of females and males of *Lagocephalus spadiceus* from the Gulf of Iskenderun, north-eastern Mediterranean (2012–2013)

Age group	Sex	N	%*N*	Total length	Total weight	Condition factor
0	♀	3	0.267	7.3–9.6	6.07–14.99	1.481–1.694
	♂	1	0.089	6.7	4.30	1.429
1	♀	13	1.158	10.6–12.4	21.0–32.23	1.234–2.029
	♂	12	1.069	10.0–12.0	17.4–37.66	1.537–2.542
2	♀	394	35.085	12.1–19.9	21.96–160.0	0.890–2.768
	♂	462	41.140	12.2–19.5	22.14–164.64	0.899–2.767
3	♀	66	5.877	19.6–22.6	89.18–189.74	1.098–2.279
	♂	73	6.500	19.6–22.7	87.7–194.63	1.033–1.820
4	♀	27	2.404	22.7–25.0	157.41–242.51	1.287–1.922
	♂	11	0.980	22.9–25.5	175.00–265.93	1.204–1.673
5	♀	12	1.069	25.2–26.5	236.31–273.40	1.181–1.555
	♂	12	1.069	25.3–26.9	210.00–334.00	1.276–1.735
6	♀	9	0.801	27.0–29.3	270.00–360.00	1.317–1.540
	♂	10	0.890	27.0–29.5	264.92–366.00	1.316–1.514
7	♀	2	0.178	29.6–30.2	395.10–430.00	1.523–1.561
	♂	4	0.356	29.6–30.4	380.80–445.46	1.468–1.605
8	♀	1	0.089	31.5	440.00	1.408
	♂	1	0.089	31.0	444.00	1.490
9	♀	1	0.089	32.5	505.00	1.471
	♂	1	0.089	33.8	516.00	1.336
10	♀	1	0.089	34.0	557.64	1.412
	♂	0	0.000	0.00	0.00	0.00
All groups	♀	529	47.401	7.3–34.0	6.07–557.64	0.890–2.768
	♂	587	52.599	6.7–33.8	4.30–516.00	0.899–2.767

N = number of individuals studied, %*N* = percentage of individuals studied.

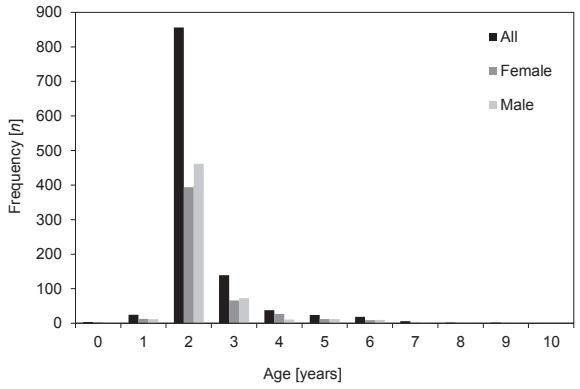

Fig. 4. Age-frequency distribution of *Lagocephalus spadiceus* the Gulf of Iskenderun, north-eastern Mediterranean (2012–2013)

et al. (2009); 43.1 cm in the Gulf of Iskenderun, north-eastern Mediterranean, Turkey, reported by Başusta et al. (2013); 45 cm in Antalya Bay, reported by Aydın et al. (2017); 27.9 cm on the Muğla coasts of Turkey, reported by Bilge et al. (2017); and 37.6 cm in the north-eastern Mediterranean Sea, reported by Kosker et al. (2019). The presently reported study provides a new maximum length of 45.6 cm (one female individual) for *L. spadiceus* from the north-eastern Mediterranean. The differences in maximum length among the studies could be attributable to study differences in sampling areas, food densities, fishing gear, and age and sex differences of fish samples. The sex ratio (female-to-male) was 1:1.1 and was significantly different from 1:1. The sex ratio is very important for understanding the relation between population status and the environment (Oliveira et al. 2012).

In the family Tetraodontidae, otoliths have an hourglass shape and are asymmetric, with a fan-shaped, well developed ventral area (Tuset et al. 2012). The otoliths are small and difficult to remove from the body. Despite many attempts at age reading, it has not been possible to use otoliths for efficient age determination in this family. Therefore, the vertebrae are preferred for this purpose; in this study, the vertebral method and length frequency distribution analysis for this species were compared. Zengin and Türker (2020) reported the age of *L. sceleratus* were determined by vertebrae. Our study investigated the age and growth characteristics of *L. spadiceus* from the Gulf of Iskenderun, north-eastern Mediterranean. The age determination revealed that *L. spadiceus* was found to vary between age groups 0 and 10. One individual was determined to be 17 years of age (45.6 cm and 1269.0 g). Since no other age study concerning this species is available, comparisons were made with *L. sceleratus*, for which Aydın (2011) conducted age determination by applying the Bhattacharya method from a length-frequency distribution obtained from pooling monthly samplings. This researcher determined the maximum length and age of *L. sceleratus* from Turkey's Mediterranean coasts to be 65 cm and 6

Table 2

The age–length parameters of *Lagocephalus spadiceus* from the Gulf of Iskenderun, north-eastern Mediterranean (2012–2013)

Sex	N	L_∞ [cm]	W_∞ [g]	k [year^{-1}]	t_0 [year]	Φ'
♀	529	47.21	1296.18	0.116	–2.04	2.413
♂	587	46.36	1381.86	0.133	–1.76	2.456
♀ + ♂	1116	46.90	1345.67	0.120	–1.97	2.421

N = number of individuals studied, L_∞ = asymptotic length, W_∞ = asymptotic weight, t_0 = theoretical age, k = body growth coefficient, Φ' = growth performance index.

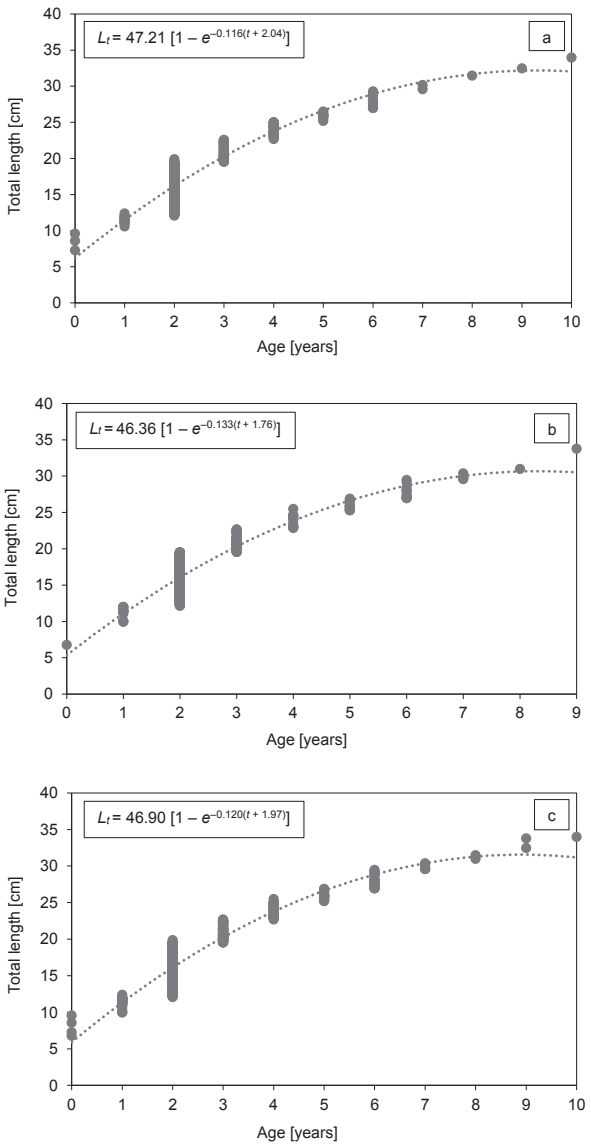

Fig. 5. Age–total length relation due to age groups for female (a), male (b) and all individuals of *Lagocephalus spadiceus* from the Gulf of Iskenderun, north-eastern Mediterranean (2012–2013)

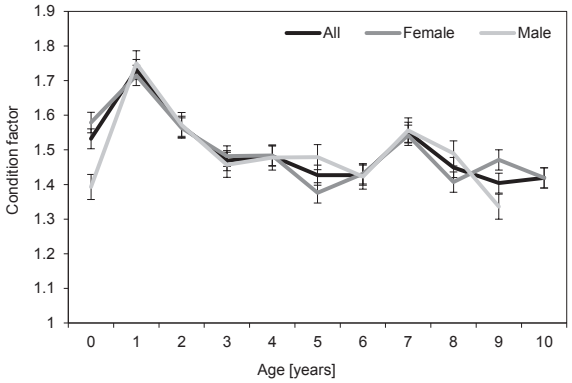

Fig. 6. According to age–condition factor for *Lagocephalus spadiceus* from the Gulf of Iskenderun, north-eastern Mediterranean (2012–2013)

years. Yıldırım (unpublished*) calculated a maximum length and age of 63.5 cm and 6 years for *L. sceleratus* sampled in the Mediterranean Sea. Tüzün (unpublished**) obtained values of 46.36 cm and 6 years for *L. sceleratus* in Antalya Bay, whereas Özbay (unpublished***) reported 67.6 cm (FL) and 6 years for the same species in the Gulf of Mersin. Başusta et al. (2017) reported a maximum length and age of 78.4 cm and 10+ years, determined via the Alcian blue staining technique, for *L. sceleratus* in the Gulf of Mersin, north-eastern Mediterranean. Zengin and Türker (2020) reported a maximum length and age of 57.6 cm and 5 years for *L. sceleratus* sampled from the Mediterranean Coast of Turkey. These differences among studies might be due to differences in regions and min–max lengths. Age determination in fish is a fundamental procedure for understanding fish biology and population status (Beamish and McFarlane 1983) and is therefore important for fisheries management.

The von Bertalanffy growth parameters of *L. spadiceus* in this study were determined to be L_∞ = 46.90 cm, W_∞ = 1345.67 g, k = 0.120 y, and t_0 = –1.97year^{-1} for all individuals from the Gulf of Iskenderun, north-eastern Mediterranean. The L_∞ value was higher for females (47.21) than for males (46.36). The reason for this difference may

* Yıldırım U.G. 2011. Akdeniz'deki balon balığı, *Lagocephalus sceleratus* (Gmelin, 1789)' un bazı biyolojik özelliklerinin tespiti. [Determination of some biological features of silverstripe blaasop, *Lagocephalus sceleratus* (Gmelin,1789) in the Mediterranean Sea.] MSc thesis, Department of Fishing and Fish Processing Technology, Graduate School of Applied and Natural Sciences, Süleyman Demirel University, Isparta, Turkey. [In Turkish.]

** Tüzün S. 2012. Benekli balon balığı'nın (*Lagocephalus sceleratus* Gmelin, 1789) Antalya Körfezi'ndeki büyüme özellikleri. [Growth characteristics of the silverstripe blaasop (*Lagocephalus sceleratus* Gmelin, 1789) in Antalya Bay.] MSc thesis, Department of Biology, Institute of Science, Adnan Menderes University, Aydın, Turkey. [In Turkish.]

*** Özbay T. 2015. Mersin Körfezi'nde dağılım gösteren balon balığı, *Lagocephalus sceleratus* (Gmelin, 1789)'un biyolojik özelliklerinin araştırılması. [Investigating of biological features of puffer fish *Lagocephalus sceleratus* (Gmelin, 1789) distributed in Mersin Bay.] MSc thesis, Balıkesir University Institute of Science Biology. [In Turkish.]

be that females grow faster and live longer than males (Weatherley 1972, Türkmen et al. 2002) due to growth differences between the sexes, according to Froese and Binohlan (2000). The values of k were found to be 0.116 and 0.133 in females and males, respectively. Fish with high k values are short lived (Sparre and Venema 1998). Since no other growth study concerning this species was available, comparisons were made with other pufferfish species. Sabrah et al. (2006) obtained an L_∞ of 81.1 cm and a k of 0.26 for *L. sceleratus* (maximum length of 78.5 cm) from the Gulf of Suez. Aydın (2011) reported 126.11 cm for L_∞, 0.099 for k, and –1.4349 for t_0 as obtained based on Ford Walford for *L. sceleratus* from Antalya Bay; the growth performance index was estimated as 3.197. In our study, the growth performance index was lower ($\Phi' = 2.421$). Tüzün (unpublished*) reported $L_\infty = 48.2$ cm, $k = 0.520$, and $t_0 = –0.270$ for *L. sceleratus* in Antalya Bay, and Özbay (unpublished*) reported $L_\infty = 118.71$ cm, $k = 0.115$, and $t_0 = –0.178$ for the same species in the Gulf of Mersin, and Zengin and Türker (2020) reported $L_\infty = 79.48$ cm, $k = 0.18537$, and $t_0 = – 0.61791$ for the same species from the Mediterranean Coast of Turkey. By developing species-specific von Bertalanffy growth function equations, the history and trend of growth of a target fish population can be studied. Information on such basic biological parameters is essential to develop scientifically sound fisheries management policies (Khan and Khan 2014).

The mean condition factor (CF), estimated for each age group in this study, was found to be lowest for age group 9 (1.404) and highest for age group 1 (1.732) for all individuals. Yıldırım (unpublished*) obtained the lowest CF value for age group 4+ (1.312) and the highest for age group 5+ (1.506) in *Lagocephalus suezensis* Clark et Gohar, 1953. Özbay (unpublished*) obtained the lowest CF value for age group 1 (0.987) and the highest for age group 3 (1.58) in *L. suezensis*. The differences in the condition factor values reported in the literature might be due to differences in biological conditions, such as environmental adaptation and nutrient availability (Le Cren 1951). The condition factor calculated from length and weight reflects the growth characteristics of the fish population (Macun 2014).

Successfully established Lessepsian migrant species, such as Lessepsian pufferfish species, are associated with serious adverse economic and ecological impacts (Coll et al. 2010). Pufferfish are considered among the worst invasive species in the Mediterranean Sea (Streftaris and Zenetos 2006). They can be found in a variety of habitats, including sandy, rocky substrates, and seagrass meadows and are currently among the dominant non-indigenous species along the coast of the eastern Mediterranean Sea, including the south-western coast of Turkey (Rousou et al. 2014). Pufferfish have no commercial value due to their toxic flesh, and they damage fishing gears. Effective fisheries management strategies to regulate and control the pufferfish population in the Mediterranean Sea are urgently needed. To date, attempts have mainly focused on the physical removal of pufferfish in Cyprus and Turkey (Rousou et al. 2014), but this method has many limitations, being energy inefficient, expensive, and often ineffective (Byers et al. 2002, Britton et al. 2011). Therefore, to find viable solutions to decrease population growth, thorough, large-scale studies of the behavior and reproductive strategy of the pufferfish are needed (Rousou et al. 2014).

It should be emphasized that this is the first study on the age and growth of *L. spadiceus* from the Gulf of Iskenderun, of the north-eastern Mediterranean of Turkey. Fishery regulations in Turkey forbid the landing of any pufferfish species captured in fisheries operations except in the case of officially permitted scientific surveys. However, we believe that it of importance to study this species in detail to prevent their negative effects on fisheries and human health in Turkey.

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