

# Post-Mucilage Distribution, Daily Growth, Mortality, and Hatch Date Timing of Sand steenbras *Lithognathus mormyrus* (Linnaeus, 1758) Juveniles in the Sea of Marmara

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## ABSTRACT

Between 2021-2022, a dense mucilage disaster occurred in the Sea of Marmara in Türkiye. Mucilage mostly covered coastal areas, which juvenile fish use for nurseries, growth, and protection. Due to Sand steenbras having been suggested as an environmental bioindicator species for Mediterranean coastal waters, this study attempts to reveal post-mucilage distribution and potential differences on the timing of hatching, daily age, growth, and mortality as revealed by the otolith microstructure. Individuals were sampled using beach seine nets from 12 equally spaced stations along the Sea of Marmara. Its presence and significant abundance in 10 out of 12 stations showed the Sand steenbras to be a common juvenile species for the Sea of Marmara. Hatching occurs between May-January, with peak hatching occurring in October. When considering that the mucilage had completely disappeared from the environment in August, the first set of hatching months can be seen to coincide with the dense mucilage. Thus, Sand steenbras can be considered as able to reproduce under these environmental conditions. The daily age of the Sand steenbras juveniles ranged between 38-235 days, with a mean of  $120.3 \pm 1.8$  days. The daily growth and mortality rates were also calculated respectively as 0.226 mm/day and 4.11%.

**Keywords:** Hatch date distribution, daily growth, juvenile fish, spatial distribution, mucilage

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## INTRODUCTION

The Sea of Marmara is a semi-enclosed basin located between the Black Sea and the Aegean Sea that connects them through the respective Istanbul and Dardanelles Straits. The variable physicochemical conditions of Marmara Sea such as global warming-related changes (Savun-Hekimoğlu & Gazioğlu, 2021), fluxiation (Altıok & Kayışoğlu, 2015), and pollution (Bilgili et al., 2022) cause undesirable environmental problems such as mucilage. Mucilage is defined as massive gelatinous aggregates that are produced by marine organisms under these afore-

mentioned undesirable conditions (Mecozzi et al., 2001). A dense mucilage event occurred in the Sea of Marmara between April-July, 2021. In the first months, the mucilage covered relatively all surface areas before becoming a submersion. The coastal areas later were completely covered by mucilage in May and June.

Mucilage has direct and indirect effects on aquatic living organisms. Mucilage directly affects sessile animals such as corals, sponges, mussels, and anemones by covering them. These animals are suspension-feeders, and water flow is crucial to their survival. In addition,



marine algae and plants need light for photosynthesis. Thus, the adverse effects of mucilage on these organisms are fatal and inevitable. The indirect effects of mucilage may occur on other animals such as gastropods, decapods, cephalopods, and fish. However, relatively few studies have been conducted on this. Ertürk-Gürkan et al. (2022) found the mucilage to be consumed as a food source by benthic species *Eriphia verrucosa*. Dalyan et al. (2021) studied the effects of mucilage on cryptobenthic adult fish assemblages in the North Aegean Sea where mucilage was less impactful and stated these species to have had to change their habitats due to mucilage. Even worse, Karadurmuş and Sarı (2022) revealed mass deaths for 12 teleost, two cartilaginous, and four decapod species in the Sea of Marmara.

Due to mucilage occurring mostly along coastal areas which are known as nursery areas for new settlers and juveniles, understanding the mucilage-juvenile fish relationship may be important. Because fish in their early life stages have only been alive for a short time, retrospectively monitoring the effects of sudden environmental conditions may be more logical as these carry more noticeable signs regarding adult fish. Micro-increment studies of the sagittal otolith allows one to determine the daily age and the related time when hatched. Some pollution- and environmental change-related variations on these parameters have been previously examined (Campana, 1984; Meier et al., 2010; Isnard et al., 2015; Sardi et al., 2021) for some fish larvae and juveniles. Due to most teleost fish laying their eggs on surface areas and the fertilization and development of eggs occurring in a pelagic environment, the success of these development process is questionable in the environments where mucilage occurs. This study hypothesizes that eggs may not be fertilized as a result of the dense mucilage aggregates and the need for external fertilization. In addition, hatching larvae may get stuck in the mucilage and fail to move. Larvae may also not be able to find prey after their mouth gap opens. All these possibilities may result in high mortalities. To understand these effects, the species should be selected as a reference as their spawning season overlapped with the occurrence of dense mucilage. Thus, the study has selected Sand steenbras both for having been suggested as an environmental bioindicator species for Mediterranean coastal waters (Tom et al., 2003; Funkenstein et al., 2004) as well as for the overlap their reproduction cycle had with the dense mucilage event.

Thus, this study aims to reveal evidence for the spawning that has occurred using the spatial distribution of biomass and hatch date distributions. In addition, the study attempts to observe potential differences regarding the daily age, growth, and mortality rates compared to previous studies.

## MATERIALS AND METHODS

*L. mormyrus* juveniles were collected using beach seine sampling from 12 equally spaced stations located along the coast of the Sea of Marmara (Figure 1). Monthly beach seine sampling hauls were carried out with two replications from each station between December 2021-March 2022. The length of wings, the height of the wings, and the length, width, and height of the bag of the beach seine were rigged as 30 m, 1.8 m and 2x2x2 m, respectively. The 4 mm nominal bar length was used in the bag net, whereas a 6.5 mm nominal bar length was used in the wing net (Figure 2). The beach seine hauls were conducted on seagrass beds and sandy habitats. Specimens were kept on ice packs and transported immediately to the laboratory. The individuals were then measured to the nearest 0.1 cm regarding total length (TL) and weighed to the nearest 0.01 g regarding total weight (W). The mean abundance at each location was given with the catch per unit effort (CPUE) based on the number of fish per haul. The CPUE was calculated with the following equation:

$$CPUE (n / haul) = N_i / H_i \quad (1)$$

where  $N_i$  is the total individual number of Sand steenbras obtained from station  $i$  in the study period, and  $H_i$  is the total number of hauls from station  $i$ . The total number of hauls was calculated by multiplying the replication number (equal to 2) with the total monthly survey number (equal to 4).

The length-weight relationship parameters are calculated using Le Cren's (1951) formula:

$$W = a * TL^b \quad (2)$$

where  $W$  is the total weight (g),  $TL$  is the total length (cm), and  $a$  and  $b$  are regression parameters. The growth type was identified using Student's t-test in accordance with the following equation (Sokal & Rohlf, 1987):

$$ts = (b - 3) / SE_b \quad (3)$$

where  $ts$  is the t-test value,  $b$  is the slope, and  $SE_b$  is the standard error of the slope. Student's t-test examines the significance of the difference of  $b - 3$ , which represents isometric growth (Pauly, 1984).

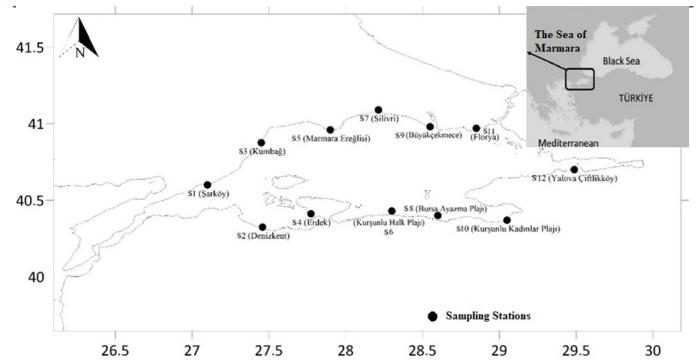


Figure 1. Study area and sampling stations.

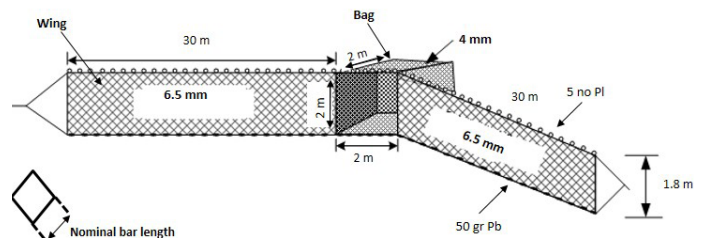


Figure 2. Technical plan of beach seine net.

A linear regression analysis ( $y = bx + a$ ) was used to determine the relationships between  $TL$  (total length) and  $FL$  (fork length), as well as  $TL$ -otolith length ( $OL$ ), total weight ( $TW$ )-otolith weight ( $OW$ ),  $TL$ -otolith width ( $OW_i$ ),  $TL$ - $OW$ ,  $OL$ - $OW_i$ , and  $OL$ - $OW$ .

For the otolith microstructure analyses, the sagittal otoliths of *L. mormyrus* juveniles were grounded and polished with abrasive papers. The sagittal otoliths of *L. mormyrus* were removed using forceps. The right otoliths were placed on a glass slide with thermoplastic glue for viewing under a microscope. After grounding and polishing, increment rings were counted from the first visible check mark succeeding the primordium to the outer edge along the maximum diameter axis, as described by Brothers (1984; Figure 3).

The daily growth rate ( $GR$ ) of *L. mormyrus* was calculated based on simple linear regressions using the least-squares method between the larval lengths ( $SL$ ) and juvenile lengths ( $TL$ ) with respect to age in days (Leonarduzzi et al., 2010) according to the following formula:

$$L = a + b(t) \quad (4)$$

where  $L$  is the larval and juvenile lengths,  $a$  is a constant,  $t$  is a function of age in days, and  $b$  is the daily growth rate (mm). The daily mortality rates were estimated using the slope coefficient in the regression relationship of the natural log values of the abundance-per-length groups (Pauly, 1984). Hatching time was determined by subtracting the age in days of the individual from the sampling date, and the hatch peak and hatch interval were determined in order to apply the calculation over all sampling months. Dissolved oxygen, salinity, and temperature were measured using a YSI 6600 (6-series multiparameter water quality sondes).



**Figure 3.** A 36 mm total length juvenile Sand steenbras individual and sagittal otolith at 90 days old.

## RESULTS AND DISCUSSION

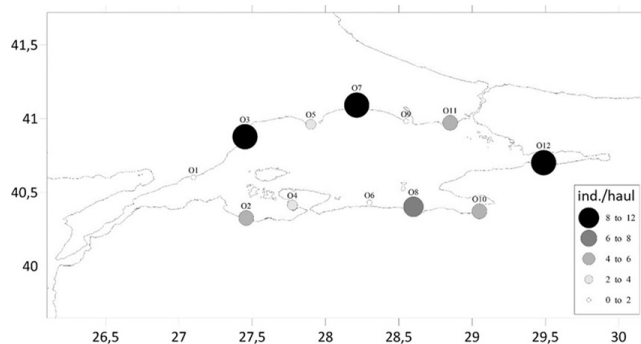
In terms of the spatial variation of abundance, *L. mormyrus* juveniles were detected in 10 of 12 stations in the Marmara Sea. *L. mormyrus* juveniles were not found in Stations S9 (Büyükçekmece) or S6 (Bandırma Kurşunlu Shores). Stations S3 (Kumbağ) and S12 (Yalova Tıgем) had the highest abundance with a mean of 11.6 ind./haul. Station S7 (Silivri) also had a high abundance (9.1 ind./haul). As can be seen in Figure 4, *L. mormyrus* juveniles were well distributed throughout all of the Marmara Sea.

Table 1 summarizes the minimum (min.), maximum (max.) and mean ( $M$ ) lengths for  $TL$  and  $FL$ , as well as the weight values of the 479 individuals. The highest individual number, mean  $TL$ , and mean  $FL$  were detected in January, whereas the max. weight was found in February. The length-frequency distribution showed 27% of the individuals to be found in the 53-57 mm  $TL$  group. The lowest size (28-32 mm) and highest size (73-77 mm)  $TL$  groups were represented with low individual numbers due to selectivity and recruitment patterns.

The length frequency distribution shows two different peaks, accordingly two cohorts in one spawning season (Figure 5). The length-weight relationship curve can be seen in Figure 6. The  $b$  value was found to significant vary from 3 ( $t = 2.41$ ;  $t$ -table = 1.96;  $t > t$ -table;  $p < 0.05$ ) and to exhibit negative allometric growth (Table 2).

The measurements related to sagittal otolith length ( $OL$ ), otolith width ( $OW_i$ ), and otolith weight ( $OW$ ) of the *L. mormyrus* juveniles are shown in Table 3. A linear relationship has been detected between  $TL$ - $FL$ ,  $TL$ - $OL$ ,  $TW$ - $OW$ ,  $TL$ - $OW_i$ ,  $TL$ - $OW$ ,  $OL$ - $OW_i$ , and  $OL$ - $OW$ . The equations and related parameters are summarized in Table 4.

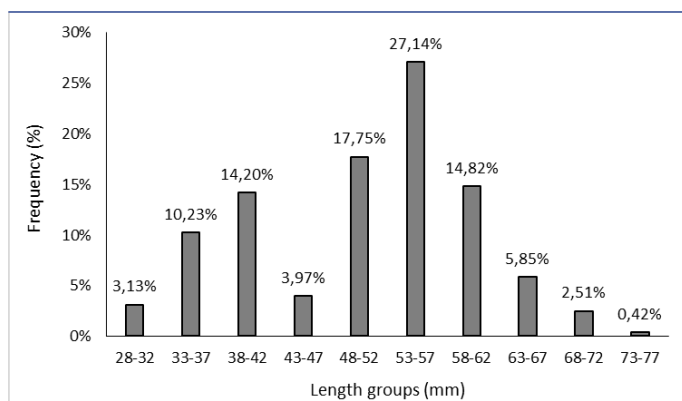
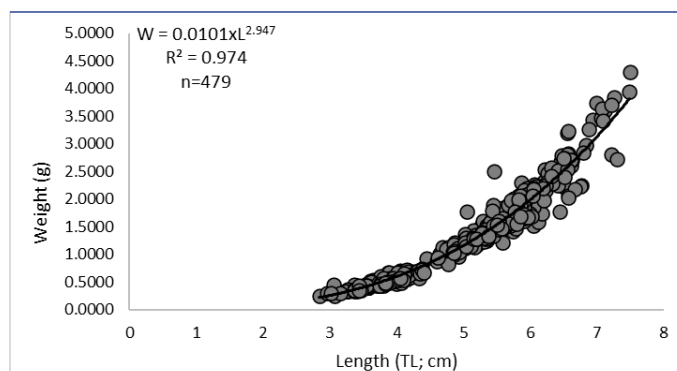
The pattern for age in days as estimated by the sagittal otoliths of *L. mormyrus* juveniles ranged between 38-235 days. The mean age in days of *L. mormyrus* was determined as  $120.3 \pm 1.8$ . The youngest individual was sampled on December 16, 2021, whereas the oldest one was sampled on February 18, 2022. The 120-139 (22.8%) age-in-days grouping was determined to be the dominant age group, followed by the 140-159 (17%) age-in-days grouping. The age in days-length key for juvenile *L. mormyrus* is shown in Table 5.



**Figure 4.** Spatial variation of mean CPUE of the *Lithognathus mormyrus* in the Sea of Marmara.

**Table 1.** The length and weight values and temporal variations of juvenile Sand steenbras (*Lithognathus mormyrus*).

		December	January	February	March	Total
Total Length (TL; mm)	Individual	146	149	127	57	479
	Min.	28.42	30.73	30.13	34.39	28.42
	Max.	72.61	74.88	74.84	65.04	74.88
	M	47.38	53.24	52.36	50.99	50.95
	SE	0.96	0.69	0.79	0.94	0.45
	SD	11.65	8.36	8.85	7.07	9.79
	CI	47.38 ± 1.58	53.24 ± 1.13	52.36 ± 1.30	50.99 ± 1.58	50.95 ± 0.74
Fork Length (FL; mm)	Individual	146	149	127	57	479
	Min.	26.45	28.52	28.1	32.25	26.45
	Max.	69.09	71.01	68.88	59.95	71.01
	M	43.81	50.02	48.7	46.98	47.46
	SE	0.95	0.64	0.73	0.89	0.43
	SD	11.49	7.77	8.22	6.76	9.42
	CI	43.81 ± 1.56	50.02 ± 1.05	48.7 ± 1.20	46.98 ± 1.50	47.46 ± 0.71
Weight (g)	Individual	146	149	127	57	479
	Min.	0.2439	0.2445	0.2967	0.3479	0.2439
	Max.	3.8538	4.2984	3.9433	2.4182	4.2984
	M	1.23	1.46	1.47	1.37	1.37
	SE	0.075	0.049	0.063	0.064	0.033
	SD	0.91	0.6	0.71	0.48	0.73
	CI	1.23 ± 0.12	1.46 ± 0.08	1.47 ± 0.10	1.37 ± 0.11	1.37 ± 0.05

**Figure 5.** Length-frequency distribution of juvenile Sand steenbras (*Lithognathus mormyrus*).**Figure 6.** Length-weight relation of juvenile Sand steenbras (*Lithognathus mormyrus*).**Table 2.** The length-weight relationship parameters of Sand steenbras *Lithognathus mormyrus* juveniles.

a*	b*	SE(b)*	R <sup>2</sup> *	Growth Type	SD <sub>a</sub>	SD <sub>b</sub>
0.0101	2.947	0.02194	0.98	Negative allometric	0.00945 - 0.01087	2.9043 – 2.9906

\*a and b are regression parameters; SE = standard error of the slope (95% CI); R<sup>2</sup> = coefficient of determination; SD<sub>a</sub> = standard deviation of a (95% CI); SD<sub>b</sub> = standard deviation of b (95% CI).

The daily growth rate of the species was found as 0.226 mm/day according to the linear regression between the age in days and juvenile lengths (Figure 7). The instantaneous and mean daily mortality rates of juvenile *L. mormyrus* were calculated as 0.0403 and 4.11%, respectively (Figure 8).

By subtracting the age in days from the time of sampling, the hatching of *L. mormyrus* was determined to have occurred between May 2021-January 2022, with an increase occurring between August-October and peaking in October (Figure 9).

**Table 3.** Otolith length (OL), otolith width (OWi), and otolith weight (OW) of Sand steenbras (*Lithognathus mormyrus*) juveniles.

	Individual	Min.	Max.	Mean	SE	SD
Otolith Length (OL, mm)	200	1.282	3.387	2.331	0.032	2.331 ± 0.053
Otolith Width (OWi, mm)	200	0.885	1.982	1.475	0.018	1.475 ± 0.030
Otolith Weight (OW, g)	200	0.0001	0.0042	0.0024	0.0001	0.0024 ± 0.0001

\*OL = otolith length; OW = otolith weight; OWi = otolith width; SE = standard error; SD = standard deviation of b (95% CI).

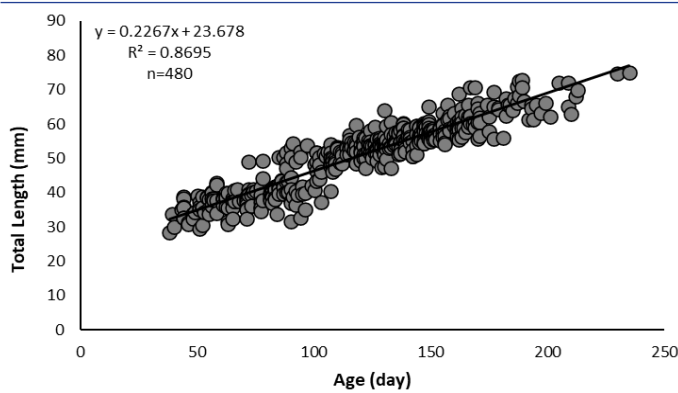
**Table 4.** Linear Regression parameters of body measurements and sagittal otoliths of Sand steenbras (*Lithognathus mormyrus*) juveniles.

Relationship	Equation	SE <sub>b</sub> *	R <sup>2</sup> *	95% SD <sub>b</sub> *	
TL*-FL*	FL = 0.9468TL - 0.9367	0.0073	0.99	0.9322	0.9610
TL-OL*	OL = 0.0398TL + 0.3117	0.154	0.88	0.0377	0.0419
TW*-OW*	OW = 0.0011TW + 0.0008	0.0006	0.66	0.00101	0.00123
TL-OWi*	OWi = 0.0219TL + 0.3687	0.091	0.87	0.0207	0.023
TL-OW	OW = 9E-05TL - 0.0023	0.0004	0.83	8.63E-05	9.82E-05
OL-OWi	OWi = 0.5376OL + 0.2366	0.073	0.92	0.5133	0.558
OL-OW	OW = 0.0022OL - 0.0029	0.0004	0.86	0.002112	0.002365

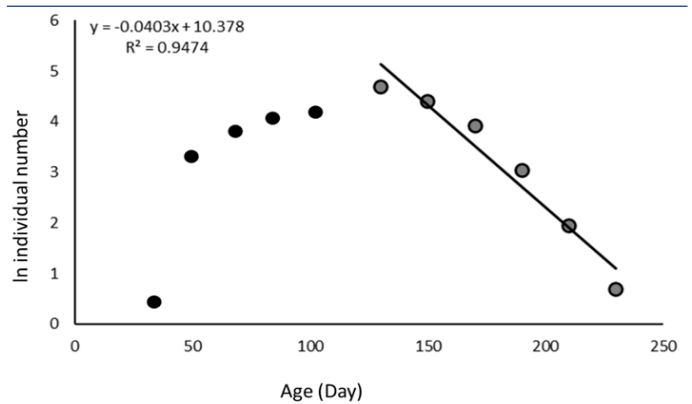
\*TL = total length; TW = total weight; FL = fork length; OL = otolith length; OW = otolith weight; OWi = otolith width; SE<sub>b</sub> = standard error of the slope (95% CI); R<sup>2</sup> = coefficient of determination; SD<sub>b</sub> = standard deviation of b (95% CI).

**Table 5.** The age-length key of juvenile *Lithognathus mormyrus* in the Marmara Sea, Türkiye.

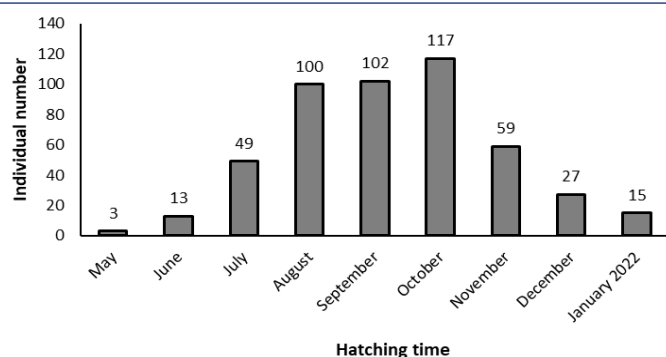
Length (mm)	Age in days										
	20-39	40-59	60-79	80-99	100-119	120-139	140-159	160-179	180-199	200-219	220-239
25-29	1	1									
30-34	1	14	5	4							
35-39		21	33	19	1						
40-44		4	9	23	4						
45-49			2	3	19	8					
50-54				9	31	73	12				
55-59					4	24	61	21	1		
60-64						4	7	21	6	2	
65-69							2	6	10	3	
70-74								2	4	2	2



**Figure 7.** Age-length relationship of juvenile Sand steenbras (*Lithognathus mormyrus*).



**Figure 8.** Linearized catch curve of juvenile Sand steenbras (*Lithognathus mormyrus*).



**Figure 9.** Hatching time of juvenile Sand steenbras (*Lithognathus mormyrus*) in the Marmara Sea.

The detection of juvenile individuals for each age-in-days class with a significance abundance from each location in the present study may be an indication that spawning might have occurred in the area and the individuals had not been sampled accidentally. According to the microstructure of the sagittal otoliths, the time of hatching had occurred between May-January, with peak hatching having occurred in October. When considered that the mucilage had completely disappeared from the environment in August, the first hatching months can be seen to have coincided with the dense mucilage. Thus, Sand steenbras can be considered as able to reproduce under these environmental conditions. Thus, the hypothesis related to "spawning being prevented due to unsuccessful external fertilization and being stuck in mucilage" is rejected.

Recruitment lengths of Sand steenbras have been stated as 60-70 mm TL (Matic-Skoko et al., 2007) and 72 mm TL (Lasiak, 1986). Ayyıldız et al. (2014) also only found 14 of 416 individual specimens to be longer than 75 mm TL. Ayyıldız and Altın (2021) additionally measured the largest juvenile individual they found as 74 mm TL. The largest individual found in the present study measured was 75 mm TL, which coincides with the findings of previous studies and confirms the relative recruitment length of *L. mormyrus*. Also, the well-distributed age and size class under 75 mm TL in the present study may be an indication that recruitment is able to occur. The spawning frequency of adult *L. mormyrus* identified has been identified as one clear seasonal peak per year (Bauchot & Hureau, 1986). Hereby, age 0 cohorts with different ages in days within a certain period of this single reproductive period need to be observed as juvenile individuals. The hatch date distribution and age-length data set presented in this study have revealed a single cohort. This may be proof of the spawning success of adult *L. mormyrus* under mucilage conditions.

Some population parameters related to stock status, such as growth type, condition factors, and daily growth and mortality rates were estimated and compared with previous studies. Matic-Skoko et al. (2007) examined the growth of juvenile *L. mormyrus* ranging from 8 mm-103 mm in length around the Adriatic Sea. They found the  $b$  exponent estimated from the length-weight relationship to be 3.141 and calculated the condition factor ( $CF$ ) as 1.245. Ayyıldız and Altın (2021) calculated the  $b$  value as 3.106 in the Gökçeada Island of the North Aegean Sea. Reis and Ateş (2020) also found the  $b$  value for immature individuals to be 3.276

in the Köyceğiz Lagoon. Meanwhile, the current study detected  $b$  and  $CF$  as 2.947 and 0.94 ( $SD \pm 0.04$ ), respectively. The higher  $b$  and  $CF$  values in the Adriatic Sea, North Aegean Sea, and South Aegean Sea indicate those stocks to perhaps have better conditions compared to the Marmara Sea. Ayyıldız et al. (2014) evaluated the growth parameters of juvenile *L. mormyrus* around the Dardanelles Strait in Türkiye. The distribution for age in days was seen to closely correspond to length. Conversely, they found a higher mean daily growth rate (0.325 mm/d) compared to the current study's finding (0.226 mm/d). In addition, they found relatively lower daily mortality rates (2.16%) compared to the current study's result (4.11%). Ayyıldız and Altın (2021) also detected a higher mean daily growth rate (0.317 mm<sup>-1</sup>) and relatively comparable mortality rate (4.61%) around Gökçeada Island of the North Aegean Sea. Some negative variations related to stock health such as conditions, growth and mortality were also detected. These differences may be a result of the species' feeding style, which feed by scratching the seabed. Reaching food may have been difficult due to the bottom being covered by mucilage. Also, the differences may be related to many other variables such as variations regarding the physicochemical parameters stemming from temporal and geographical differences, food availability, and pollutants along coastal areas. Thus, making a definitive judgement is difficult due to the studies not have been conducted simultaneously.

The timing of hatchings first occurred in May and peaked in October in the present study. The peak hatching period had been determined as June-July in the Adriatic Sea (Matic-Skoko et al., 2007), as August in the Dardanelles Strait (Ayyıldız et al., 2014), and between July-December in the North Aegean Sea (Ayyıldız & Altın, 2021). The main parameter controlling the spawning activity of Sand steenbras has been stated as sea surface temperature (SST; Vitale et al., 2011). Mandić et al. (2014) studied an ichthyoplankton community in Boka Kotorska Bay (South Adriatic Sea) and stated Sand steenbras to dominate the ichthyoplankton biomass in April, when their measured SST values ranged between 14.6°C-16.4°C ( $M = 15.5^\circ\text{C}$ ). According to Karadurmuş and Sarı (2022), the SST ranged between 14.5°C and 15.6°C in the Marmara Sea during the dense mucilage on April 25, 2021. The estimated onset of hatching occurred in May 2021. When additionally examining the fertilization period, the onset of hatching in May overlaps exactly with the species SST preferences for spawning. The onset of hatching, which seems to have been delayed, may actually have been caused not by the mucilage but by the factors that had caused the mucilage. In addition, hypoxia was reported (dissolved oxygen [DO] ranging between 2.3 mg/l-3.6 mg/l) in the Marmara Sea in April 2021 (Karadurmuş & Sarı, 2022), just before the hatching. Meanwhile, this study measured DO as ranging between 6.5 mg/l-9.4 mg/l ( $M = 8.4$  mg/l) between December 2021-March 2022. As the mucilage density decreased, the oxygen values were observed to have increased. Thus, another possibility for the slightly delayed hatching and/or spawning times may have been caused by low oxygen levels.

## CONCLUSION

Whether the differences mentioned in this study are directly related to the mucilage effect or the environmental conditions that

caused the mucilage to appear remains unclear. To better understand the direct and indirect effects of environmental factors such as mucilage on fish and their early life stages, factors such as distribution, feeding, reproduction, behavior, and pollution should be monitored more closely during and immediately after a mucilage event. Using back-calculated estimations based on the micro-increments of juvenile otoliths becomes more accurate when considering the obtained data alongside observations that were made during an incident. Simultaneously taking samples from areas affected by mucilage and unaffected areas should also increase the accuracy of back-calculation estimations. The world may encounter similar environmental disasters more frequently due to pollution and global warming, which are expected to increase in the near future. More detailed studies should be performed for understanding the direct and indirect effects environmental disasters such as mucilage events have on the early life stages of teleost fishes.

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