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RESEARCH ARTICLE

Growth and Reproductive Capacity of the Endemic Fish Species *Egirdira nigra* (Teleostei: Leuciscidae)

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Abstract

Objective: In this study, various characteristics of the Eğirdir minnow, an endemic species of Anatolia that inhabits specific/narrow areas, were determined from 142 individuals sampled between 2021 and 2022. These characteristics include length-weight relationship, Von Bertalanffy growth equation, condition factors, spawning season, first spawning size, and fecundity attributes.

Materials and Methods: A monthly sampling of 142 specimens of *Egirdira nigra* was conducted using multi-mesh gill nets between March 2021 and April 2022.

Results: The sampled *E. nigra* population consists of individuals aged II-V, with 57.75% female and 42.25% male. The length-weight relationships were as follows: $W = 0.0059\ L^{3.5445}$, $R^2 = 0.9312$ (female + male), $W = 0.009\ L^{3.3077}$, $R^2 = 0.9353$ (male), and $W = 0.0058\ L^{3.5679}$, $R^2 = 0.9233$ (female). The condition factors were calculated as 1.67 (male) and 1.87 (female). The gonadosomatic index was highest in March (13.95) and lowest in August (0.5073). The population's mean fecundity was calculated as 4787 individuals/egg.

Conclusion: Although the population exhibits a wide age range and positive growth values, it is believed to be under significant threat from invasive/exotic species sharing the same environment, such as *Atherina boyeri, Gambusia holbrooki, Pseudorasbora parva*, and *Carassius gibelio*. Therefore, the conservation of species and their habitats is of paramount importance.

Keywords: Conservation, Eğirdir minnow, endemic, growth, reproduction

Introduction

Spring minnows of the cyprinid genus *Pseudophoxinus* inhabit regions spanning Central Anatolia to Azerbaijan and south to Israel. Their distribution is often confined to limited streams or springs, making them one of the most challenging fish groups for conservation within the heavily impacted Middle Eastern landscape (Güçlü & Küçük, 2017; Hrbek *et al.,* 2004). Recent phylogenetic analyses have delineated *Pseudophoxinus* into two distinct lineages, each corresponding to a specific geographic region. Past isolation within Anatolia's inland systems has been identified as a significant factor shaping the speciation process of this genus, with central and southwestern Anatolia's lake basins proposed as potential centres of origin (Küçük *et al.,* 2012). Taxonomic investigations conducted in Anatolia have identified 22 species, among which *P. handlirschi*, endemic to Lake Eğirdir, became extinct in the early 1970s, purportedly because of the introduction of pikeperch (*Sander lucioperca*). With the exception of *P. libani* and *P. zeregi*, all *Pseudophoxinus* taxa, which are crucial components of Anatolia's inland water fish fauna, are endemic to the region. Major threats to *Pseudophoxinus* species include water abstraction, dam-induced flow alterations, heightened drought occurrences linked to climate change, invasive alien species, agricultural pollution, and habitat degradation (Güçlü & Küçük, 2017).

Egirdira nigra, which is endemic to Eğirdir Lake and its basin in Central Anatolia, has undergone taxonomic revision over the years. Initially described as *Pararhodeus niger* by Kosswig and Geldiay in 1952, it was later reclassified as *Phoxinellus egridiri, Pseudophoxinus egridiri* (Bogutskaya, 1997). In the latest morphology-based taxonomic study by Freyhof (2022), the species was redefined as *E. nigra* (Fig. 1). The species is known to inhabit not only Lake Eğirdir and certain streams (Çayköy and Yalvaç streams) that feed into the lake but also karstic spring water (Yeşilyurt village-Sütçüler) located approximately 35-40 km south of Lake Eğirdir, which is currently disconnected from the lake (Küçük & Gülle, 2016). An increase in the population of this species has been observed in the lake over the last 20 years. However, recent field studies and observations over the past five years indicate that many of the conservation criteria set by the IUCN (2013-2014) have changed adversely. In fact, it has been determined that some taxa (due to factors such as climate change, drought, habitat loss, pollution, predation, agricultural irrigation, etc.) could completely disappear from nature. It is also believed that *E. nigra* will be under significant medium-term threat for similar reasons (Güçlü, 2022; Küçük *et al*., 2021).

There are not sufficient research available regarding the biology of *E. nigra*. Thus far, studies have been limited to length-weight relationship analyses (Apaydın Yağcı *et al*., 2022; Saç & Özuluğ, 2018). The only detailed study conducted on the species' growth characteristics is by Yeğen & Sarı (2021). There are no studies on the reproductive features of this species. Therefore, our study emphasises the necessity of investigating the reproductive biology and growth characteristics of the *Egirdira nigra* population, an endemic species of Anatolia, using samples collected between 2021 and 2022 from 142 individuals. Recommendations for the conservation of the species are also proposed.

Materials and Methods

The samples were collected from Eğirdir Lake in Akkeçili Village (38°08'27.28"N 30°47'40.49"E) between March 2021 and April 2022. For fish sampling, sampling equipment and beach seine nets (5 and 15 mm mesh size) were used in accordance with the European Standard methodology EN 14757 (Water quality - Sampling of fish with multi-mesh gillnets) determined by the European Union for pelagic and demersal sampling. According to EN 14757 standards, these multi-mesh gillnets are designed to catch freshwater fish of all sizes and species. Each net consists of eight panels with mesh sizes ranging from 10 mm to 100 mm (10, 15, 20, 40, 55, 70, 80, and 100 mm mesh size). The length of the nets was 35 m, and their depths ranged from 1.5 to 6 m (35×1.5 m and 35×6 m). Individual specimens underwent precise measurements of total length (TL) to the nearest 0.01 cm and total weight (W) to the nearest 0.01 g. Age was determined by examining scales extracted from the left lateral side of each specimen, specifically from the region between termination of the pectoral fin and commencement of the dorsal fin. Observations were conducted using a stereoscope equipped with transmitted light, ensuring optimal visualisation and accurate data acquisition.

Figure 1. *Egirdira nigra*, 83.21 mm SL, male (+3 age).

The distribution of female and male participants was assessed according to age. The male to female ratio was evaluated using chi-square tests (with a significance level of 0.05) following the methodology described by Düzgüneş *et al.* (1995). The frequency distributions of total length and weight in centimetres were computed for all specimens. An exponential regression equation of the form $W = a \times L^b$ was employed to establish the relationship between weight (W) and length (TL), where *W* is body weight in grammes and *TL* denotes total length in centimetres. In this equation, *a* represents the intercept, and *b* denotes the regression coefficient. Additionally, the coefficient of determination (R^2) was calculated to assess the goodness of fit of the regression model, as outlined by Ricker (1975). The growth of the *E. nigra* population was estimated using the following Von Bertalanffy growth equations: $L_t = L\infty\ (1 - e^{-k(t - to)})$, where *Lt* is the total length in cm at age *t*, *L*∞ the average asymptotic length in mm, and *k* is the body growth coefficient, *to* the hypothetical age and *a* and *b* constants (Le Cren, 1951). The statistical significance level of the coefficient of determination (R^2) and the 95% confidence intervals (95% CI) of the regression coefficient *b* were calculated following the methodology outlined by Zar (1999). To compare the deviation of the slope value from the expected value of *b* = 3, which represents isometric growth, Pauly's *t*-test was conducted for all species, as described by Pauly (1984). Pauly's *t*-test was calculated as follows:

$$t = \frac{Sd_{\log TL}}{Sd_{\log W}} \frac{|b-3|}{\sqrt{1-r^2}} \sqrt{n-2},$$

where Sd_{logTL} is the standard deviation of the *logTL* values, Sd_{logW} is the standard deviation of the *logW* values, and *n* is the number of fish species used in the computation. If the calculated *t* value exceeds the critical *t* value from the *t*-distribution table for degrees of freedom (n-2), as outlined by Pauly (1984), then slope parameter (*b*) is deemed significantly different from 3, indicating non-isometric growth. Furthermore, a *t*-test with a significance level of 0.05 was employed to assess the agreement between the measured total length and total length calculated using the Von Bertalanffy growth equation. The average growth performance (Ø ', phi prime) was calculated with the formula $Ø' = \text{Log } k + 2 \times \text{Log } L_\infty$ (Gayanilo *et al*., 1988). Fulton's coefficient of condition factor was calculated using $CF = (W/TL^3) \times 100$ (Sparre & Venema, 1989). The gonadosomatic index (GSI) was calculated as: GSI= GW / W × 100, where *GW* was the gonad weight and *W*, the total body weight of the fish (Gibson & Ezzi, 1980). The spawning period was determined from the monthly evaluation of the GSI. Fecundity was determined gravimetrically based on the number of mature oocytes in 82 females at the spawning stage. The diameter of each egg was measured using a microscopic micrometre, following the protocol outlined by Nikolsky (1980). The maturity level of the female participants was assessed using the sigmoid logistic curve fitting method. The estimation of length at first sexual maturity was conducted according to the approach described by De Martini *et al*. (2000): Firstly, it was plotted against $\ln [(1-P_x)/P_x]$ using simple linear regression to estimate values for *a* and *b*. Here, *Px* is the observed proportion of mature at length *x*. Second, the mean length at 50% maturity was calculated using $L_m = a/b$. Subsequently, the proportions of maturity at various lengths (L) were estimated using the formula $PL = 100 / [1+e^{b(L-Lm)}]$, allowing for a graphical representation of the onset of first sexual maturity. Here, *PL* denotes the estimated proportion of maturity at length *L*, as defined by De Martini *et al*. (2000).

Ethics committee approval was obtained for this study from the ethics committee of Isparta University of Applied Sciences Local Ethics Committee for Animal Experiments (Date: 12.03.2020, No: 001). The experimental conditions did not disturb the fish involved in our experiments.

Results

The sampled population of *E. nigra* consisted of 57.75% females and 42.25% males within the age range of II-V (Table 1).

Table 1. Age and sex distributions of the *E. nigra* population in Lake Eğirdir.

Age group	F		M		F+M		F:M
	N	%N	N	%N	N	%N	
II	6	4.22	17	11.97	23	16.19	0.35:1.00 (p<0.05)
III	38	26.76	31	21.83	69	48.59	1.22:1.00 (p<0.05)
IV	34	23.95	11	7.74	45	31.69	3.09:1.00 (p<0.05)
V	4	2.81	1	0.7	5	3.52	4.00:1.00 (p<0.05)
∑	**82**	**57.75**	**60**	**42.25**	**142**	**100**	**1.36:1.00 (p<0.05)**

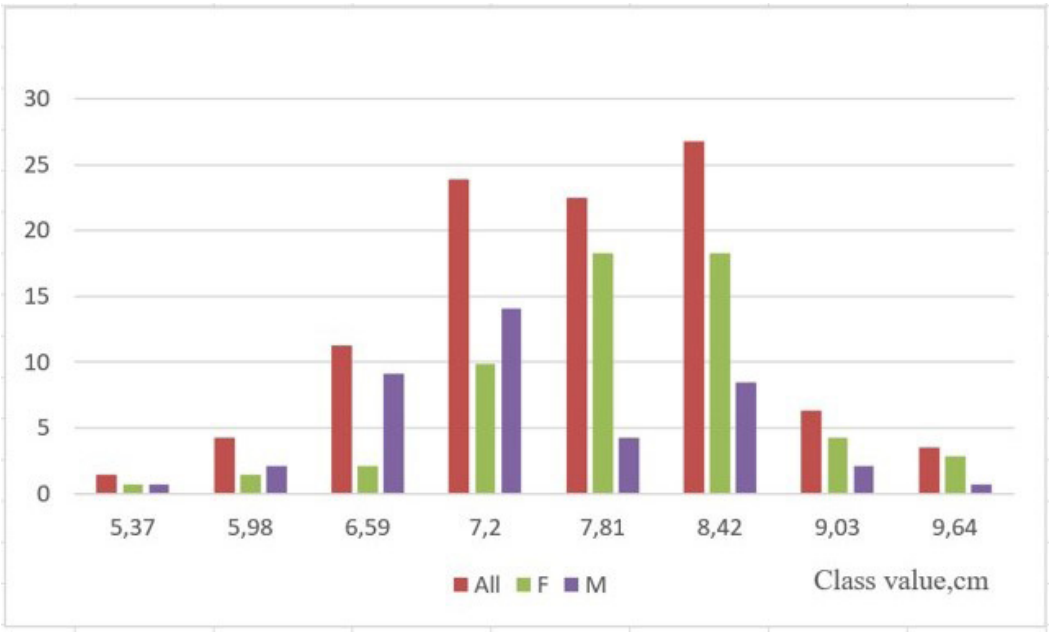

Figure 2. The size (cm) distribution (%) of the *E. nigra* population in Lake Eğirdir.

According to the data obtained from samples comprising 142 individuals, the size distribution of the population ranged from 5.07 cm to 9.90 cm, with individuals between 6.90 cm and 8.72 cm constituting 73.23% of the population (Fig. 2, Table 2). Regarding weight distribution, in the population ranging from 1.91 g to 19.89 g, individuals between 6.43 and 13.20 g accounted for the majority of the population (77.46%) (Table 3).

When examining the age-size distribution of the sample population, it is evident that the majority of specimens (114 individuals) belonged to the age group III-IV, reaching a size range of 7.51-8.72 cm (Table 2). A similar pattern was observed in the age-weight distribution, where the majority of the population (114 individuals) fell within the III-IV age group, ranging between 6.43 and 13.20 g in weight (Table 3).

Table 2. Distribution of total length and age among female (F) and male (M) *E. nigra* specimens.

Age class	**II**		**III**		**IV**		**V**		**Total**
Total Length cm)	♀	♂	♀	♂	♀	♂	♀	♂	
5.07-5.67	1	1	-	-	-	-	-	-	**2**
5.68-6.28	2	3	-	1	-	-	-	-	**6**
6.29-6.89	2	11	1	2	-	-	-	-	**16**
6.90-7.50	1	2	12	18	1	-	-	-	**34**
7.51-8.11	-	-	19	6	7	-	-	-	**32**
8.12-8.72	-	-	6	4	20	8	-	-	**38**
8.73-9.33	-	-	-	-	6	2	-	1	**9**
9.34-9.94	-	-	-	-	-	1	4	-	**5**
∑	**6**	**17**	**38**	**31**	**34**	**11**	**4**	**1**	**142**
	TL ± SD(min-max)								
♀+♂	6.40±1.00 (5.07-7.09)		7.53±0.57 (6.07-8.54)		8.41±0.61 (7.41-9.52)		9.44±1.17 (9.08-9.90)		7.94±0.80 (5.07-9.90)

Table 3. Distribution of weight and age among female (F) and male (M) *E. nigra* specimens.

Age class	II		III		IV		V		Total
Weight (g)	♀	♂	♀	♂	♀	♂	♀	♂	
1.91-4.16	4	10	-	-	-	-	-	-	**14**
4.17-6.42	1	6	-	1	-	-	-	-	**8**
6.43-8.68	1	1	21	26	2	-	-	-	**51**
8.69-10.94	-	-	12	3	12	3	-	-	**30**
10.95-13.20	-	-	5	1	15	7	-	1	**29**
13.21-15.46	-	-	-	-	5	1	-	-	**6**
15.47-17.72	-	-	-	-	-	-	3	-	**3**
17.73-19.98	-	-	-	-	-	-	1	-	**1**
∑	**6**	**17**	**38**	**31**	**34**	**11**	**4**	**1**	**142**
				W ± SD (min-max)					
♀+♂	4.26±0.27 (1.91-6.80)		7.79±0.23 (4.36-12.73)		11.31±0.23 (7.71-14.90)		16.44±1.00 (12.95-19.89)		9.95±0.43 (1.91-19.89)

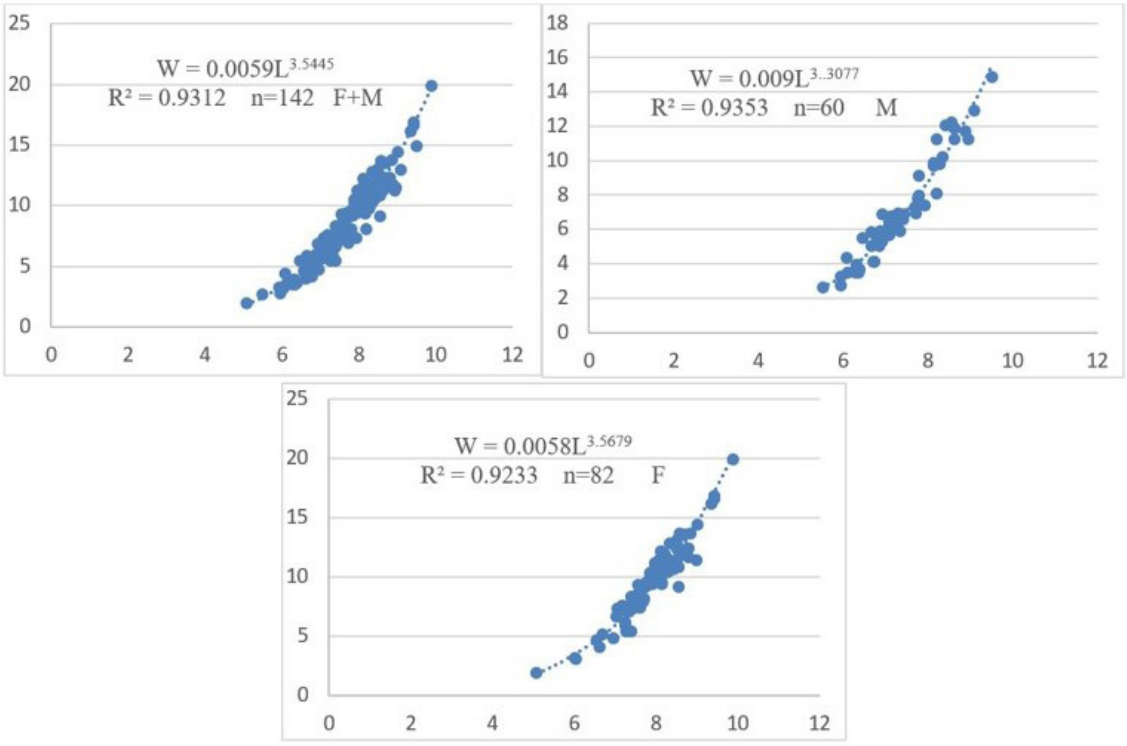

Figure 3. The length-weight correlation values for the population of *E. nigra* (F: Female, M: Male, n: Number of individuals).

The length-weight relationships; $W = 0.0059\ L^{3.5445}$, $R^2 = 0.9312$ (male + female), $W = 0.009\ L^{3.3077}$, $R^2 = 0.9353$ (male), and $W = 0.0058\ L^{3.5679}$, $R^2 = 0.9233$ (female) (Fig. 3, Table 4); the condition factor is calculated as 1.67 (male) and 1.87 (female).

The following Von Bertalanffy growth equation was obtained for all $L_t=11.76\ (1\text{-}e^{-0.2357(t\text{-}1.329)})$ and $W_t=38.24\ (1\text{-}e^{-0.2357(t\text{-}1.329)})^{3.5679}$ (Fig. 4). The differences between the observed and expected total lengths were not statistically significant in all age groups (*t*-test, $p>0.05$) (Table 5). The average growth performance (Ø ‘, phi prime) was calculated as 1.51.

Table 4. Growth characteristics of the *E. nigra* population.

	a	b	R^2	95% Confidence interval *b* (±SH)	Pauly's *t*-test	P	CF	Growth type
Female	0.0058	3.5679	0.9233	3.4214-3.6247 (±0.0012)	4.526	<0.001	1.87	+ Allometry
Male	0.009	3.3077	0.9353	3.2957-3.3201 (±0.0125)	8.259	<0.001	1.67	+ Allometry
All	0.0059	3.5445	0.9312	3.5403-3.5526 (±0.0542)	5.361	<0.001	1.74	+ Allometry

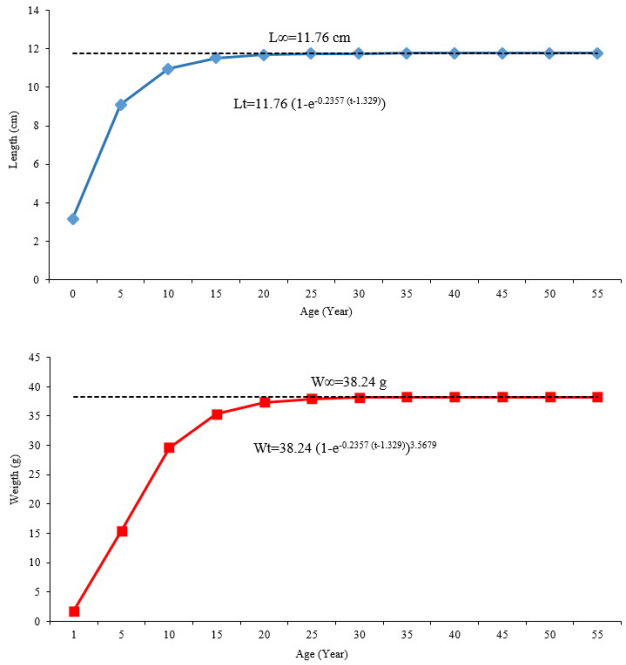

Figure 4. L_∞ and W_∞ value according to Von Bertalanffy growth equation of *E. nigra.*

Table 5. Measured total average length and calculated total average length values by age groups in the Von Bertalanffy growth equation of *E. nigra.*

Age groups	Measured average length (cm)	Calculated average length (cm)	*t*-test
II	6.40	6.39	p>0.05
III	7.53	7.52	p>0.05
IV	8.41	8.41	p>0.05
V	9.12	9.11	p>0.05

The Gonadosomatic Index (GSI) exhibits its highest value in March (13.95) and lowest in August (0.5073) (Fig. 5). Through observation of gonad development and monthly fluctuations in the GSI, intermittent spawning of the population has been identified, occurring between March and August. The population's fecundity was determined to be 4787.112 ± 202.52 individuals/eggs (Table 6). The size at first spawning (L_{mat}) was calculated to be 6.11, representing 50% maturity (Fig. 6). Additionally, the mean diameter of eggs was measured at 0.88 ± 0.02 mm.

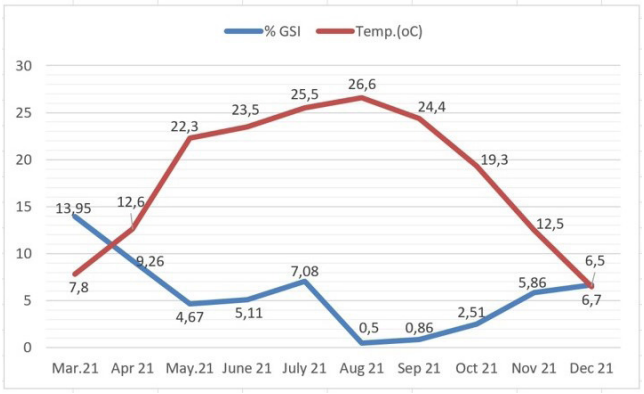

Figure 5. GSI (%) and water temperature (°C) values of *E. nigra* population.

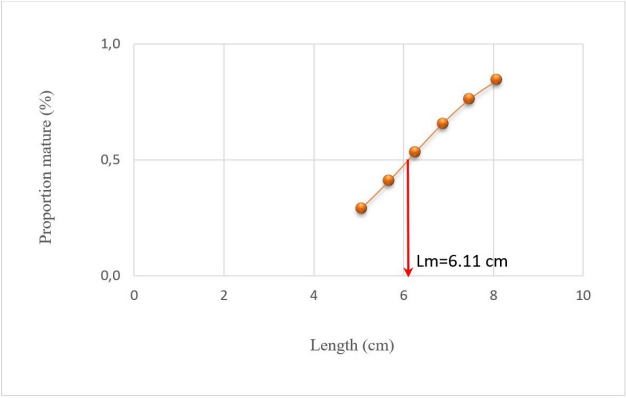

Figure 6. Length at first maturity of male and female *E. nigra* in Lake Eğirdir.

Discussion

Numerous investigations have been undertaken to elucidate the molecular taxonomic and population attributes of *E. nigra*. Nevertheless, the majority of efforts aimed at delineating its ecological traits have remained confined to the analysis of length-weight correlations. Only 1-2 studies have comprehensively disclosed all bio-ecological characteristics pertinent to the species.

Table 6. Average total length (TL, cm), weight (W, g), gonad weight (GW, g), and fecundity (F, number/individual/year) values of female individuals with eggs according to age of *E. nigra* (SE= Standart error, n= number of samples)

Age	n	TL± SE	W ± SE	GW± SE	F ± SE
II	6	6.23±1.30	3.91±1.01	0.18±0.05	2696.15±593.67
III	38	8.58±0.64	8.58±0.94	0.70±0.08	3423.12±592.16
IV	34	8.34±0.98	11.24±0.86	1.16±0.04	6616.21±412.23
V	4	9.53±0.28	17.33±0.63	2.41±0.11	6413.02±284.02
Σ	82	8.17±0.80	10.26±0.86	1.11±0.07	4787.11±202.52

The age range of the species was consistent with the limited possible age range of small fish species (Nikolsky, 1980). The reason why there are no 0 and I age groups is due to the selective nature of the fishing equipment used. If the eye openings used in fishing were smaller, it would be possible to identify age groups 0 and I. The ability of individuals of a species capable of reaching a maximum size of 11-12 cm can grow up to 5 years old is considered a significant achievement in terms of the current population and future of the species. In the only growth study conducted on the species between 2010 and 2011, the 0-V age range was determined, and approximately 80% of the population consisted of individuals in the II and III age groups (Yeğen & Sarı, 2021). Nikolsky (1980) proposed that a population with a diverse age distribution could serve as an indicator of adequate nutrient levels within aquatic ecosystems. The decline in older age cohorts within the population is anticipated to result in a corresponding rise in younger cohorts, thereby mitigating food competition. Such dynamics also signify successful reproductive endeavours, as evidenced by the influx of new individuals into the population. However, in our study, the opposite situation was observed. Small individuals could not be caught due to the selective nature of the hunting equipment or the lack of fishing with small mesh nets. Thus, most population consisted of individuals in the III-IV age group.

In their research, Yeğen & Sarı (2021) observed that the *E. nigra* population exhibits a length ranging from 2.1 to 10.0 cm, with weights ranging from 0.11 to 17.38 g, and attains maturity within a 5-year timeframe. Furthermore, the study revealed that a significant proportion (79.7%) of the population comprised individuals aged between two and three years. Apaydın Yağcı *et al*. (2022) determined, based on 551 samples, that the population had a total length of 4.7-10 cm and a weight of 2.4-17.4 g and showed isometric growth ($b = 2.9425$). Our study is similar to other studies.

The sex ratio of females to males in *E. nigra* was 1.36:1.00 (χ^2, $p<0.05$). Nikolsky (1980) asserted that sex ratios vary significantly among species, although most species tend to exhibit ratios close to unity. Discrepancies in sex ratios may stem from various factors, such as differences in lifespan and early maturation rates between sexes, differential growth patterns, mortality rates, and reproductive energy expenditure. Additionally, the dominance of one sex over the other could be attributed to divergent behaviours facilitating the preferential capture of one sex and discrepancies in mortality rates between sexes (Ghafouri *et al*., 2019). The deviation from the expected male-to-female (M:F) ratio observed in our study may be ascribed to the sampling approach used.

Variations in growth parameters could arise from ecological disparities among research regions, including differences in water temperature, water quality, and nutrient levels within the environment. The disparity between observed and anticipated total lengths of *E. nigra* was not statistically significant (*t* test, $p > 0.05$). The population reached a length of 11.76 cm, denoted as L_∞, at approximately 20 years of age. Variations in L_∞ are hypothesised to be influenced by species-specific factors, water temperature, environmental conditions, and nutritional availability. The infinite weight has been determined as 38.24 g, and it is thought that it will reach infinite weight in the next 25 s. The data of our study are compatible with those of another study conducted ($L\infty$=11.22 cm) in 2010-2011 (Yeğen & Sarı, 2021).

The exponents derived from the total length-weight relationship of *E. nigra* exhibit positive allometry (Table 4). Typically, a narrower length range and lower abundance of smaller fish may lead to a higher b value, as smaller juvenile fish tend to possess a more rounded body shape, gradually elongating as they mature (Froese, 2006). In contrast, other studies by Yeğen & Sarı (2021) reported positive allometric growth, whereas Apaydın Yağcı *et al*. (2022) observed negative allometric growth (Table

7). The regression coefficient (R^2) for the total length-weight relationship across all samples is 0.93, indicating a negligible deviation from the expected increase in regulation within total length-weight relations. Although the R^2 value aligns closely with findings from prior studies (Saç & Özuluğ, 2018; Yeğen & Sarı, 2021), it surpasses the value reported in the study by Apaydın Yağcı *et al*. (2022) (Table 7).

In the reproductive strategies of fish, there is considerable diversity in characteristics such as reproductive system, mating system, sex, spawning habitat, spawning season, and fecundity (Helfman *et al.,* 1997). Spawning typically occurs from March to August, with the first maturation observed at age II. The initial maturity length (L_m) was estimated as 6.11 cm. In addition, the phenomenon of repeated spawning over an extended reproduction period may serve as an adaptation to mitigate food competition between juvenile and adult individuals. Early attainment of sexual maturity and reproductive strategies among fish inhabiting dynamic ecosystems (Nikolsky, 1980). The way to terrestrial environments. The numerous narrowly distributed endemic aquatic organisms in the region have been significantly affected by these changes, both due to sharing specialised habitats and limited living spaces. According to a study on "The Effects of Climate Change on Endemic Fish Species in Central Anatolia," similar to other regional endemics, *E. nigra* is expected to be affected by the prevailing climate change in our country, with a projected decline in its population during the period 2041-2060 (Güçlü, 2022). *Egirdira nigra,* which is endemic to the Lake Eğirdir basin, thrives particularly in areas with abundant underground water sources, especially in the northern region of the lake. The decrease in the piscivorous pressure exerted by *S. lucioperca* over the past 20-30 years in the lake indicates a positive development for *E. nigra* and A*natolichthys iconii* populations. On the other hand, as is the case worldwide, the disappearance of aquatic habitats due to prevailing drought conditions in most parts of our country pose a threat to the future of specialised species like *E. nigra*.

Table 7. Research findings on the growth characteristics of the *E. nigra* population in Lake Eğirdir.

Ref.	N	Age	TL (cm)	M:F	L∞	k	to	Ø'	*a*	*b*	R^2	CF	Growth Type
1	85	-	2.3-6	-	-	-	-	-	0.019	3.10	0.97	-	-
2	544	0-V	2.1-10	0.82:1.00	11.22	0.30	-0.64	1.57	0.009	3.27	0.95	1.48	+A
3	551	-	4.7-10	-	-	-	-	-	0.018	2.94	0.88	-	-A
This Study	**142**	**II-V**	**5.07-9.9**	**1.36:1.00**	**11.76**	**0.23**	**-1.32**	**1.51**	**0.005**	**3.54**	**0.93**	**1.79**	+A

Ref: 1- Saç & Özuluğ, 2018; 2-Yeğen & Sarı, 2021; 3- Apaydın Yağcı *et al*., 2022

data obtained regarding the reproductive characteristics of *E. nigra* possess the attributes of being initial data. Reproductive potential is a measure of a population's capacity to produce viable eggs and larvae and can be considered the primary outcome of a reproductive strategy. Reproductive potential is defined by various factors, including spawning stock biomass (Bagenal, 1973; Myers & Barrowman, 1996), age distribution and diversity of adults (Alheit *et al*., 1983; Cardinale & Arrhenius, 2000), proportion of first-time spawners and repeat spawners within the population (Evans *et al*., 1998; Trippel, 1998), nutritional status (Brooks *et al*., 1997; Hislop *et al*., 1978), as well as size at age and sexual maturity (Morgan & Hoening, 1997; Roff, 1981).

The aquatic habitats of Central Anatolia have been steadily shrinking since the Pleistocene period, giving

According to these results, despite the wide age range of the population and positive growth values, it is believed that the species may be under significant threat from invasive/exotic species such as *A. boyeri*, *G. holbrooki*, *P. parva*, and *C. gibelio*, which share the same environment. Therefore, the conservation of species and their habitats is necessary.

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