DETERMINATION OF POPULATION STATUS AND MIGRATION PERIOD OF EUROPEAN EEL (*Anguilla anguilla* L.) IN GULLUK LAGOON, TURKİYE

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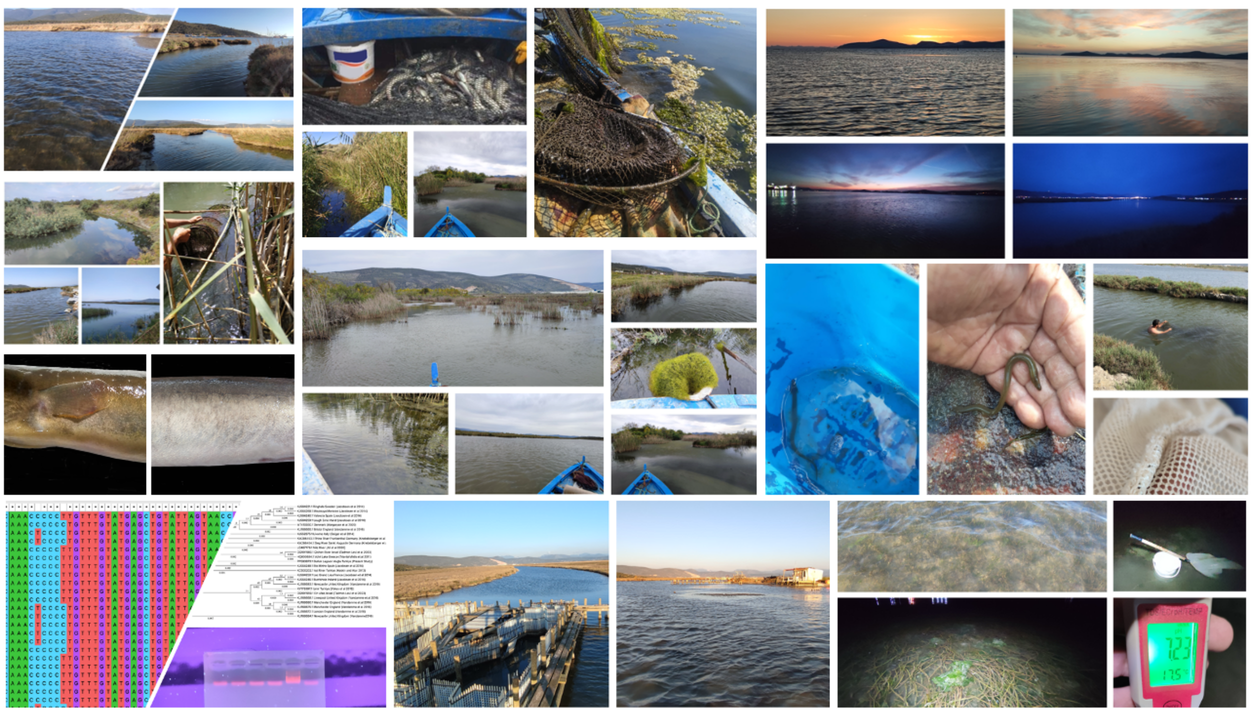
2024



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ABSTRACT

In this study, the spawning migration period, glass eel recruitment, length-weight relationship, catch per unit effort (CPUE) and cytochrome oxidase gene sequence of the European eel population in the Güllük Lagoon (Muğla, Türkiye) were investigated. A total of 224 European eels were captured using 6 mm (24 individuals) and 15 mm (180 individuals) mesh-sized fyke nets. The live weight and total length were measured after each sampling. Ten individuals were kept at first sampling for DNA barcoding and laboratory analysis while the remaining eels were released to their habitat. The glass eel recruitment surveys were carried out at the lagoon's entrance covering approximately 160 hm^2 area during the study. The surveys were done using a hand scoop for 2-4 days (5 pm to 10 pm) each week including the new moon phase from the beginning of February 2024 to the end of May 2024. Elvers were caught in the upper parts of the lagoon at the same sampling period using 6 mm mesh-sized fyke-nets. The CPUE analysis was done using the weekly catchment data provided by fishermen between September 2023 and February 2024. The monthly proportion of yellow and silver European eels was recorded by observing the skin colorations. Biological investigations were also carried out on the ten specimens collected for DNA barcoding. The project results have been shared with students, academics, local authorities, and with a few fishermen through the meetings held during the study period. The mean total length and live weights were 52.37 ±15.62 cm and 387.97 ±265.64 g, respectively. The length-weight relationship ($W=0.0027 \times L^{2.9418}$, $R^2 = 0.9029$) and condition factor ($K=0.285 \pm 0.095$) of the population were calculated. The results of glass eel survey and elver catchments suggest that glass eel recruitment still occurs, however, the abundance of glass eels is too low to use the hand scooping method for sampling. There was a 6-fold peak for the silver eel proportion while the yellow eel proportion decreased 3-fold between November and December 2023. The average $CPUE_{kg}$ was 0.29 kg/gear/day and average $CPUE_{\#}$ was 0.63 ind./gear/day during the study. These data suggest that the silver eel migration in the studied lagoon mostly occurs between November and December. DNA barcoding of European eels revealed that our samples were closely related to other European eel populations distributed around the continental than the Türkiye's population. These results support that the glass eel and leptocephali distribution occurs randomly.

Keywords: Glass eel, Recruitment, Migration, DNA barcoding, Phylogenetic analysis

1. INTRODUCTION

Eels (Anguillidae) are distributed across a vast area, including the northern Atlantic Ocean, the Indian and Pacific Oceans, and the coasts of Korea and Japan. The most well-known species within the Anguillidae family are *Anguilla anguilla* (European eel), *A. rostrata* (American eel), A. japonica (Japanese eel), and *A. australis* (Australian and New Zealand eel). Of these, two species, *A. anguilla* and *A. rostrata*, are found in the Atlantic Ocean (Geldiay & Balık, 1996; Moriarty & Dekker, 1997; Verhelst et al., 2022; Wright et al., 2022). These two species well studied mostly in Europe and America continents. However, there is insufficient long-term data regarding the fisheries, biology, population dynamics and habitats of the European eel in Turkish coastal waters.

The European eel is a panmictic and catadromous species distributed in many countries along the coasts of North Africa and Europe (Moriarty & Dekker, 1997). *A. anguilla*, being a commercially fished species, is reported to have a distribution encompassing the waters from Mauritania and the Barents Sea to the entire Mediterranean basin (ICES, 2018). The European eel, a member of the Teleostei, belongs to the class Actinopterygii, the order Anguilliformes, and the family Anguillidae (freshwater eels). There are reported to be 19 eel species registered in the Anguilla genus (Fishbase, 2023). Among these 19 species, *A. anguilla* (European eel), its close relatives *A. rostrata* (American eel) and *A. australis* (Australian eel), and the more distantly related *A. japonica* (Japanese eel) are currently the most fished and consumed eel species (Pérez et al., 2004; Lago et al., 2012).

A. anguilla and *A. rostrata* partially share breeding grounds in the Sargasso Sea, located in the western North Atlantic Ocean (Ringuet et al., 2002; Van Ginneken & Maes, 2005). Additionally, *A. rostrata* has been found naturally in Germany and other European inland water systems, likely due to inadvertent introduction during the stocking of glass eels or juvenile fish. One possible reason for incorrect stocking is that *A. rostrata* is less expensive than *A. anguilla* and is thus widely used in aquaculture for stocking purposes (Trautner, 2006). *A. rostrata* is classified as an endangered species (EN) on the IUCN Red List, whereas *A. anguilla* is currently classified as critically endangered (CR). Consequently, the European Council has issued a regulation outlining the measures needed to improve *A. anguilla* stocks (European Council, 2007). In Türkiye, the production quantities of European eels have been declining over the years. According to FAO (2022) and TUIK (2023) data, eel fishing in Turkey has remained below the average of 42 countries worldwide from 1980 to 2018. However, since 2018, Turkey’s total European eel catch amounts have been above the global average (Figure 1).

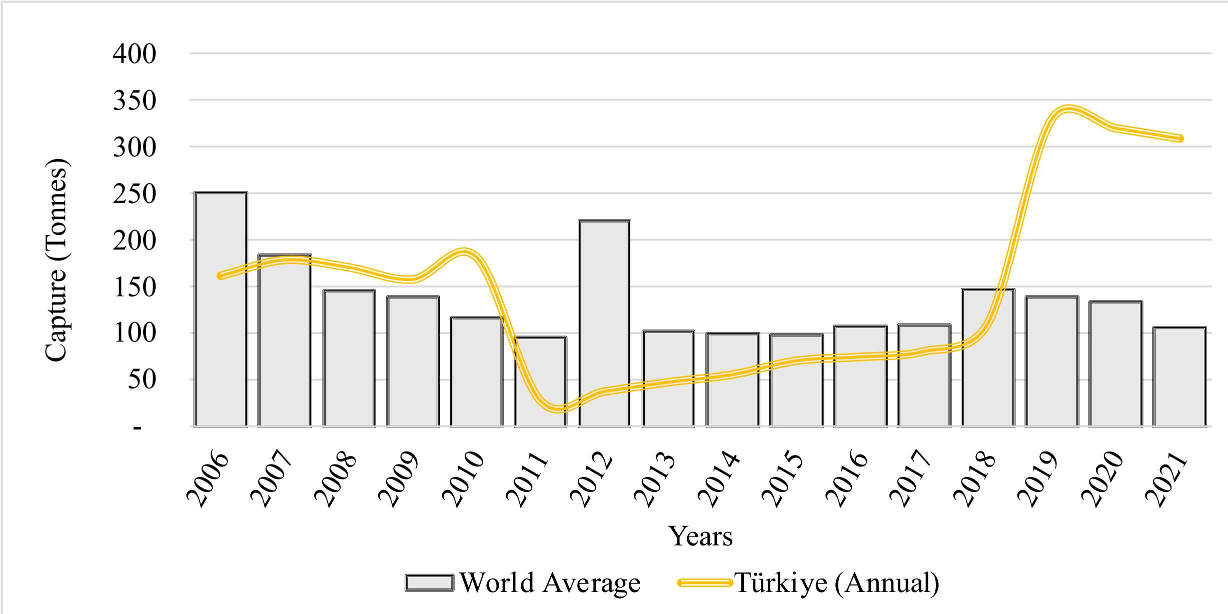

Figure 1. FAO (2022) data, shows the average global European eel fishing and the total European eel fishing in Turkey between 1980 and 2021 (Bahrioğlu, 2023).

The quantities of European eel fishing in Turkey from 2013 to 2022 are given in Figure 2. The data shows that European eel fishing showed an increasing trend until 2018, and it tripled from previous years starting in 2019, but the export quota remained around 100 tons. It is understood that fishing, which reached its highest quantity in 2019, is currently on a downward trend (Figure 2). The reason for the sudden increase in 2018 and 2019 is related to the GFCM (General Fisheries Commission for the Mediterranean) multi-annual management plan. As a result of the study conducted within the framework of GFCM/42/2018/1 recommendation (ICES, 2018) for the European eel multi-annual management plan in the Mediterranean, habitat-based data collection has been initiated throughout Turkey. During this process, comprehensive data collection, including recreational fishing, has been carried out, and the increase in fishing also includes recreational fishing quantities that were not previously recorded in previous years (Ciccotti & Morello, 2023).

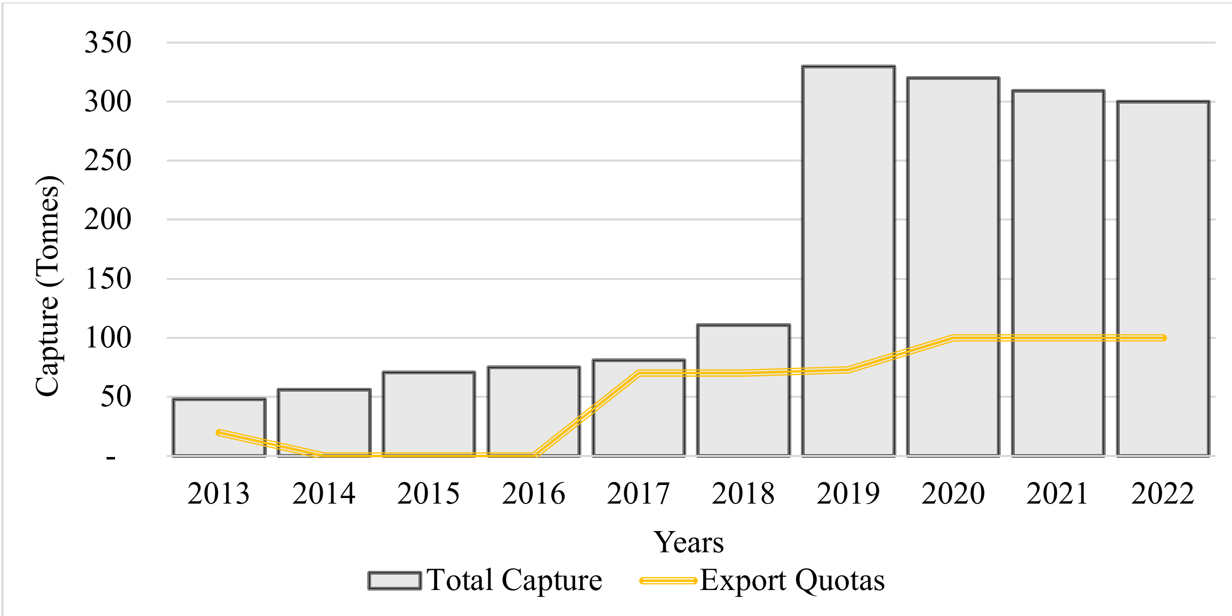

Figure 2. The quantity of European eel fishing in Turkey from 2013 to 2022 and the changes in the annual export quota set by CITES (2023) (Bahrioğlu, 2023).

European eels have a highly complex life cycle involving long-distance migration activities (Figure 3). This species is characterized as semelparous and facultative catadromous (Verhelst et al., 2018a; Küçük et al., 2018; Kroes et al., 2020). The facultative catadromous feature is defined by the discovery that European eels do not only reside in freshwater habitats throughout their continental lives, but also enter and exit marine habitats multiple times before beginning their spawning migration. Semelparous species die after reproducing once. Since individuals of newly spawned European eels with gonads in post-reproductive stage are not caught, it is considered that this species is semelparous. The facultative catadromous feature is based on the examination of otolith microchemistry (Sr/Ca ratio) in various studies (Tzeng, 2000).

As is known, the term "catadromous" is used for fish that migrate to marine habitat for breeding purposes. Feeding migration of the larvae to the freshwater habitats takes place after hatching in oceans and seas. Facultative catadromous feature is based on the knowledge that these fish reside in freshwater habitats, occasionally transitioning between freshwater and saltwater sources (Arai et al., 2006; Martin et al., 2010; Lin et al., 2011). In recent years, the facultative catadromous life cycle feature has become a widely accepted view for eels. Additionally, this feature provides significant benefits for those studying the biology, conservation, and management of eels (Verhelst et al., 2018a; Küçük et al., 2018; Kroes et al., 2020). There are also studies classifying this species as panmictic (Als et al., 2011). Species classified as panmictic are those that exhibit random mating and breeding characteristics. The migration of European eels begins in the Atlantic Ocean after hatching, with larvae entering the Mediterranean Sea through ocean currents, reaching the coasts of Europe (Lecomte-Finger, 1992) and extending to the coasts of Türkiye, and starting to migrate to inland waters as the sea waters warm up (Küçük et al., 2005;

O'Leary et al., 2022). European eels are distributed over a wide area, including all European continental coastlines, the coastlines of Great Britain, the western coastlines of Russia, the coastlines of North Africa, the Aegean Sea, and the Mediterranean coastlines (Fishbase, 2023). After entering inland waters, European eels feed and grow, spending the majority of their adult lives in these areas before migrating back to the Atlantic Ocean for breeding (Tesch, 2003).

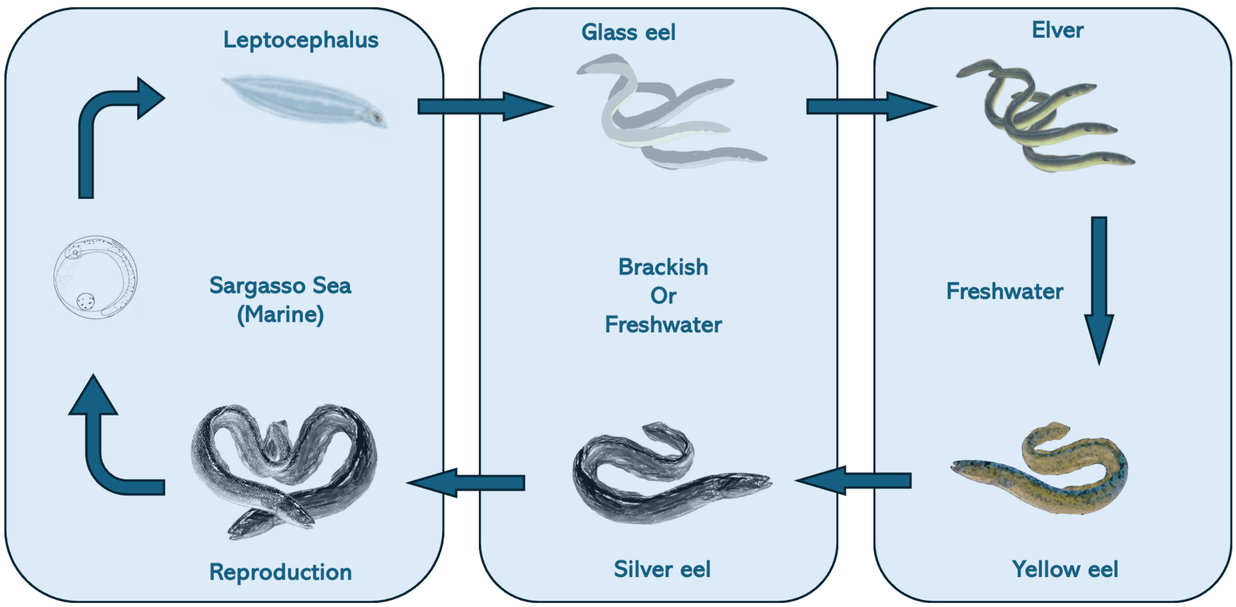

Figure 3. Life cycle of the European eel. Generally, elvers and yellow eels found in Freshwater habitats, glass and silver eels usually move between Marine, Brackish and Fresh water habitats. Migrating silver eel usually stays in marine habitats and spawn in Sargasso Sea.

Historical data on populations spread across the European continent indicate that European eel stocks in these regions were quite high and considered a primary food source among freshwater catches in many European countries (Ringuet et al., 2002). However, it has been observed that European eel populations have been declining across their distribution areas since the 1980s (Dekker, 2003; Dekker & Casselman, 2014), and it is currently estimated to be only about 1% of the levels seen in those years (Correia et al., 2018; ICES, 2023). Changes in climate and ocean currents, pollution, fishing pressure, the presence of migration barriers (such as dams and hydroelectric power plants), habitat loss, deteriorating water quality, diseases, and overfishing are the most known reasons for this decline (Dekker, 2003; Dekker & Casselman, 2014). Particularly, many European eel populations have suffered greatly due to potential dam constructions and hydroelectric power plants. Sharp declines in recruitment to stocks and escapement to the seas in the last thirty years have accelerated scientists' efforts to assess their long-term conservation status. With the acquisition of scientific data on the decline in stocks, it has been recognized that the species has been critically endangered since 2008 (Jacoby & Gollock, 2014, Ammar et al., 2021).

European eels must navigate through numerous obstacles commonly found on waterways across many regions of Europe, such as flood control dams, spillways, weirs, hydroelectric power plants (HEPs), sluices, and pumping stations. These barriers restrict the free movement of European eels and access to suitable habitats during their growth, hindering or delaying their migration for breeding and feeding (Ammar et al., 2021). Therefore, identifying these obstacles in eel habitats and making necessary adjustments (such as constructing fish passages, conducting stocking efforts) when needed is considered crucial for the future of populations. It is believed that the growth and feeding migrations of eels are directly related to water temperatures and are influenced by environmental variables. For example, eels that have completed their first metamorphosis (leptocephalus to glass eels) migrate to estuarine areas and freshwater sources as water levels rise and currents increase, doing so with minimal energy expenditure. Hence, monitoring water level fluctuations and tidal cycles is necessary in population dynamics studies to detect recruitment to stocks (Weihs, 1978; Metcalfe et al., 1990). Determining the migration periods of silver eels is crucial for conservation efforts. Studies on European eels have shown that these organisms cease feeding during migration, leading to changes in body condition (length-weight) (Tabeta & Mochioka, 2003). Therefore, research on European eels should investigate the distribution, condition, and feeding habits of migrating silver eels, resident silver eels, yellow eels, elvers, and glass eels through biometric and morphometric measurements. In addition to biological characteristics, revealing environmental and ecological relationships contributes significantly to regional conservation efforts.

In this study, the current status of the European eel population in Güllük Lagoon, located in Milas District of Muğla Province, has been aimed to be determined. The study investigated length-weight relationships, Catch Per Unit Effort (CPUE), and migration periods. Additionally, DNA barcoding of Cytochrome c oxidase subunit 1 (mt-CO1 or COX1) gene was applied, and phylogenetic analysis were done.

2. MATERIALS AND METHODS

2.1. Project site

This study carried out in Güllük Lagoon located in Milas-Muğla, Türkiye. Güllük Lagoon is located southwest of Türkiye, on the Aegean Sea coast. Güllük Lagoon, fed by Sarıçay stream. Sarıçay stream arises from the Aydın – Muğla border and feeds the Geyik Dam (37.385493, 27.884185) and then pours into the Akgedik Dam (37.3287227, 27.8252733). After this dam, it passes through Kırcağız-Dibekdere (37.3376387, 27.7887524 and 37.3448541, 27.7303478) and Avşar (37.2772527, 27.6514822) districts, then connects to Güllük Lagoon (37.2633634, 27.6279897). The European eel sampling was carried out at the Güllük Lagoon and its upper locations (Figure 4).

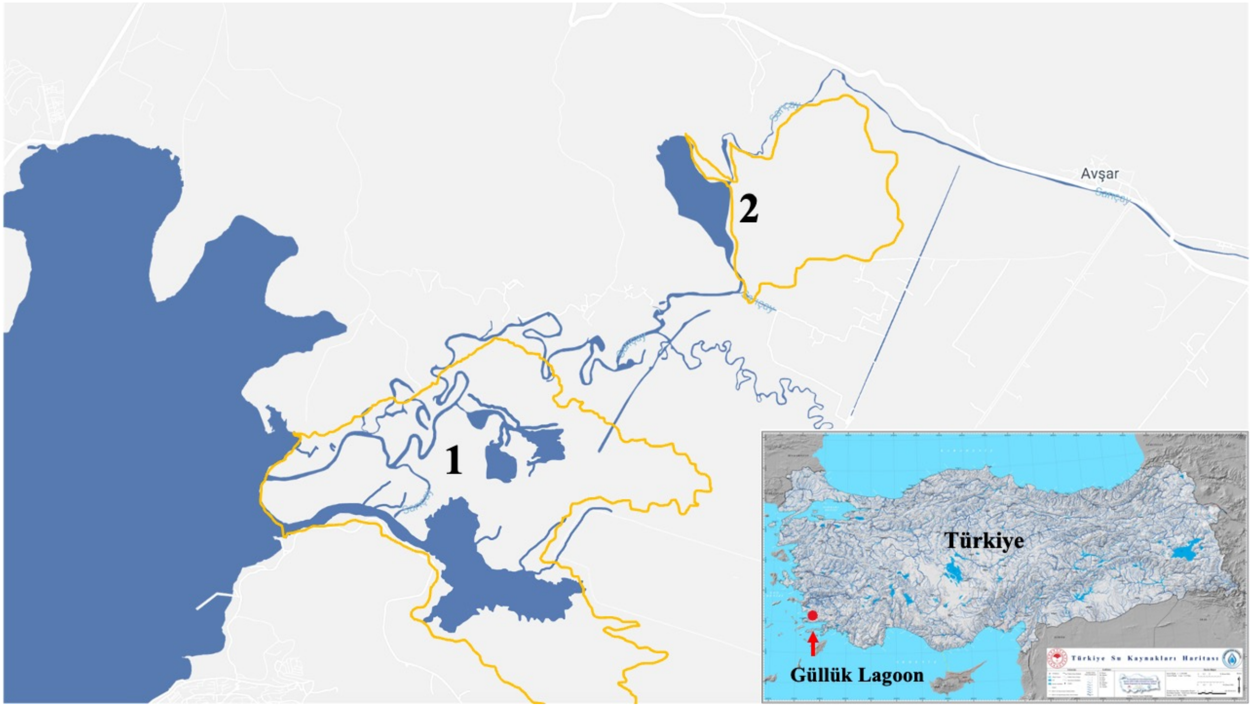

Figure 4. Research area and two sampling locations. First location (37°15'43"N, 27°37'47"E) is directly opening to the Aegean Sea and there has been un-operational sein net fishery for last two years. Second location (37°16'42"N, 27°39'09"E) is more closed and shallow area which has similar fishing activities with first location. Areas covered with yellow lines shows the wetlands mostly occupied with *Phragmites spp*

2.2. DNA barcoding

Ten individuals of European eels were sub-sampled and sacrificed for DNA barcoding processes at the beginning of the study. At the same time, internal examinations were done immediately on the sacrificed eels. Fin specimens were collected from the caught eels, preserved in %96 Ethanol, and frozen at -80 ^{o}C until the DNA extraction. Extraction was carried out with a DNAzol DNA isolation kit following the manufacturer's protocols. Cytochrome c oxidase subunit I (COI or COX1) gene was amplified in the Polymerase Chain Reaction (PCR) using conventional methods (Bahrioğlu et al., 2024) by adjusting the annealing temperature for the below primers. The following primer set were used for the analysis.

FishF1-5′ TCAACCAACCACAAAGACATTGGCAC 3′
FishR1-5′ TAGACTTCTGGGTGGCCAAAGAATCA 3′

PCR products were controlled using horizontal gel electrophoresis (Figure 5). Sequencing analyses were done by sanger sequencing method (BMLabosis, Turkey). Determined sequence were trimmed and an open reading frame (ORF) submitted to NCBI Genebank (Accession Number: PP593873.1). Obtained sequence has also been submitted to NCBI Blast Nucleotide

Search to find similar sequences. Found sequences were chosen to represent various European eel habitats and populations across the distribution range (Table 1).

Table 1. Information of sequences used for phylogenetic analysis from various habitats of European eels

Accesion Number	Region	Country	Reference
PP593873.1	Muğla	Türkiye	Present Study
HQ600684.1	Volvi Lake	Greece	Triantafyllidis et al., 2011
KC500202.1	Asi River	Türkiye	Keskin & Atar, 2013
KJ552675.1	Livorno	Italy	Geiger et al., 2014
KJ564221.1	Ringhals	Sweden	Jacobsen et al., 2014
KJ564240.1	Valencia	Spain	Jacobsen et al., 2014
KJ564246.1	Burrishole	Ireland	Jacobsen et al., 2014
KJ564248.1	Rio Minho	Spain	Jacobsen et al., 2014
KJ564250.1	Lac Grand-Lieu	France	Jacobsen et al., 2014
KJ564259.1	Lough Erne	Ireland	Jacobsen et al., 2014
KJ564268.1	Moulouya	Morocco	Jacobsen et al., 2014
KM286453.1	Rhine River	Germany	Knebelsberger et al., 2014
KM286454.1	Sieg River	Germany	Knebelsberger et al., 2014
KU168665.1	Bristol	United Kingdom	Vandamme et al., 2016
KU168668.1	Liverpool	United Kingdom	Vandamme et al., 2016
KU168672.1	London	United Kingdom	Vandamme et al., 2016
KU168675.1	Manchester	United Kingdom	Vandamme et al., 2016
KU168680.1	Manchester	United Kingdom	Vandamme et al., 2016
KU168683.1	Newcastle	United Kingdom	Vandamme et al., 2016
KU168684.1	Newcastle	United Kingdom	Vandamme et al., 2016
KY176387.1	Izmir	Türkiye	Salman, 2016
MT410930.1	-	Denmark	Margaryan et al., 2020
LC487179.1	Nile River	Egypt	Ali et al., 2020
OQ991959.1	Ein Afeg	Israel	Tadmor-Levi et al., 2023
OQ991960.1	Qishon River	Israel	Tadmor-Levi et al., 2023

The multiple sequence alignment was conducted using the gene sequences obtained from the search results. Multiple sequence alignments were performed with the ClustalW algorithm. The aligned sequences were also trimmed to obtain equal lengths at each sequence to build phylogenetic tree. BIC scores (Bayesian Information Criterion) were calculated for the Maximum-Likelihood phylogenetic tree using MEGA software. Kimura-2 parameter (K2p) model had the smallest BIC value and considered to describe substitution pattern the best. Therefore, Maximum-

Likelihood (K2p) model was used to build phylogenetic tree of *Anguilla anguilla* individuals which were reported from different European habitats.

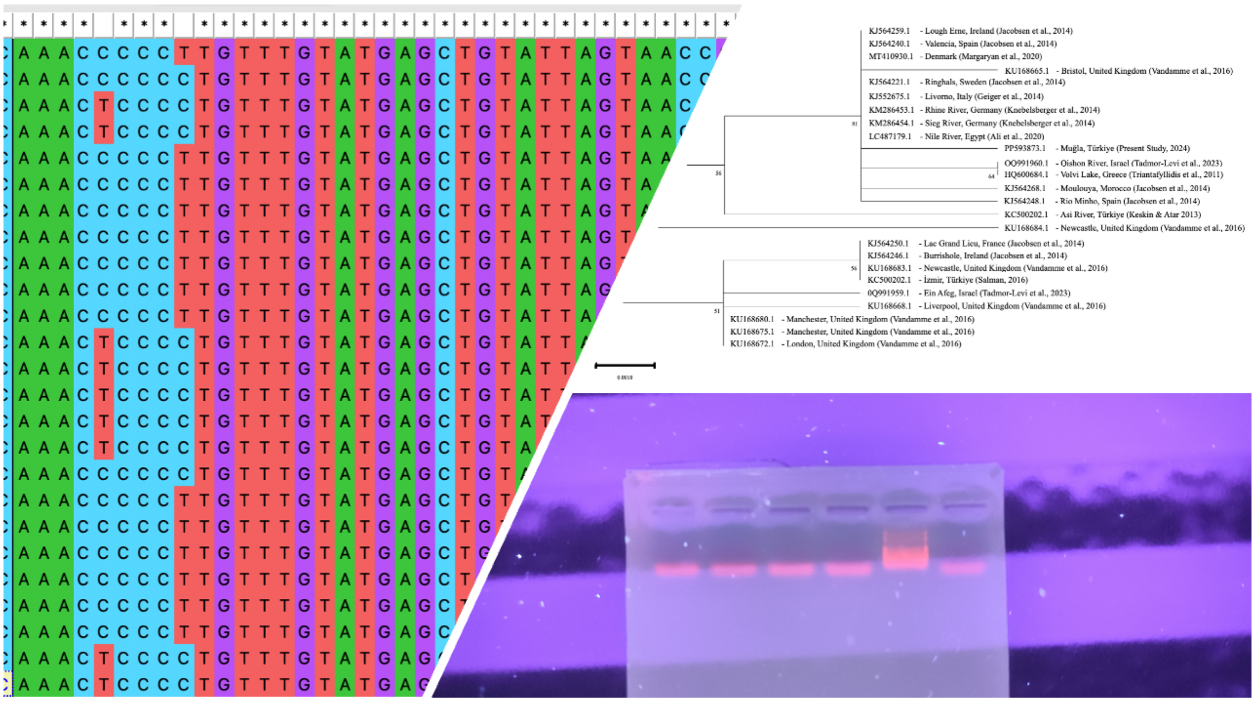

Figure 5. Sample images from the aligned sequences, constructed phylogenetic tree, and gel electrophoresis results.

2.3. Determination of spawning migration period

We have monitored the eel catchments on various locations using fyke-nets (15 mm mesh size) in Güllük Lagoon, Muğla, Türkiye with a help of few fishermen. Sampling activities were carried out in September, October, November, December, January and February with the commercial gear (15 mm mesh sized fyke-nets). Deployed fyke-nets were kept in water for 2 to 4 days and controlled daily. Eel specimens were collected, counted and total live weights were recorded by fishermen. Fishermen taught to be able to differentiate the yellow and silver eels basically from their skin coloration (Figure 6). In this way we have been able to observe silver eel and yellow eel ratios in the catchments to determine the spawning migration. Additionally, we have collected random sub samples from catchments (n=30), every month in new moon period to record individual live weights and total lengths. Eel specimens were measured for their length using a ruler with ±1 mm precision and for their weight using an electronic scale with ±1 g accuracy. European eels were released to their habitat after each sampling activity.

Catch Per Unit Effort (CPUE) represents the amount of European eel (weight, kg) that can be caught with one unit of fishing gear (fyke net, hand scoop) in a unit of time (minute, hour, day) (MacNamara and McCarthy, 2014). In the calculation, the total fish weight (W, kg) and total fish number (N, number) caught during the sampling period (2-4 days) were divided by the sampling duration (t, days) to determine the number of fish caught per day with all fyke nets. The obtained result was then divided by the number of fyke nets (P) to determine the number of fish caught per day with one fyke net (kg or number (#)) (Equations 1 and 2).

$$CPUE\ (kg) = \frac{\frac{W}{t}}{P} \qquad \text{(Eq. 1)}$$

$$CPUE\ (\#) = \frac{\frac{N}{t}}{P} \qquad \text{(Eq. 2)}$$

Figure 6. Skin coloration of European eels (Yellow and Silver)

The relationship between length and weight is calculated using the nonlinear exponential Equation (Eq. 3).

$$W = a\,.L^{b} \qquad \text{(Eq. 3)}$$

To calculate the values of *a* and *b* in the exponential equation, a regression analysis was performed using logarithmic transformation (Eq. 4).

$$Log(W) = Log(a) + b.Log(L) \qquad \text{(Eq. 4)}$$

In the equation, “W” represents body weight (g), “L” represents total length (cm), “*a*” is the intercept, and “*b*” is the slope of the regression curve. These values are particularly important for calculating the condition factor (body condition). For eel species, the value of *b* is expected to be between 2.5 and 4 (Küçük et al., 2005; Piria et al., 2014; Verhelst et al., 2018b). The *b* value provides information about the weight growth of the fish (Denis et al., 2022). If this value is equal to 3, the weight increase is isometric; if it differs from 3, the growth is allometric. When $b > 3$, the growth is positively allometric, and when $b < 3$, the growth is negatively allometric (Verhelst et al., 2018b). To statistically determine whether the growth of European eels caught from the study area is allometric or isometric, a t-test was used based on the obtained data (Eq. 5).

$$t = \frac{b-3}{Sb} \qquad \text{(Eq. 5)}$$

In the equation, Sb represents the standard error of the slope. Aside from the importance of the *b* value, it is also known that the *a* coefficient observed in the Anguilliform body is significant and very close to zero (Beverton & Holt, 1996; Bayhan et al., 2020).

The condition factor for European eels caught during the study was calculated allometrically (Eq. 6). In the calculation, total length (L, cm), live weight (W, g), and the coefficient (b) obtained from the length-weight relationship were used.

$$K = \frac{W}{L^b} \qquad \text{(Eq. 6)}$$

2.4. Glass eel survey

Küçük et al. (2005) reported that the elvers migrate inland waters between February and July in Fethiye-Muğla, Türkiye. Glass eel and elver migrations were monitored weekly from February 1st to May 31st in 2024. Küçük et al. (2005) were able to use hand scoop for juvenile eel sampling and they reported that the hand scoop was most efficient sampling gear for Gözlen creek in Fethiye. Specially designed semicircular scoops with a 1 mm mesh size were used at the shoreline for the collection of glass eels (FAO, 1980; Küçük et al., 2005). Vertical and horizontal dragging were made with the hand scoop. Additionally, we have deployed 1 set of fyke net (6 mm mesh size) to sample elvers.

2.5. Project meeting

During the project, we collaborated with a couple of fishermen in the field. At the same time, communications were established with the personnel of the Muğla Provincial Directorate of Agriculture and Forestry and its Fisheries and Aquaculture Branch. A successful meeting was held with the attendance of a few fishermen and local authorities. At the meeting, the project results were shared with the authorities, and opinions were exchanged regarding future applications and conservation efforts.

3. RESULTS

3.1. Project site

The length of Sarıçay is approximately 45 km, and the lagoon area was reported to be about 2500 decare in 1993. However, the site is getting smaller by being covered by vegetation (mostly *Phragmites* sp.). Aquacultural activities are settled in the study area. Chronically, fish farms discharge antibiotics, formaldehyde, and other chemicals into the habitat. In sampling site 2 (Figures 4, 7, 8), characterized by wetland conditions and brackish water, we found species including *Mugil cephalus* (Flathead grey mullet), *Dicentrarchus labrax* (European seabass), *Gambusia holbrooki* (Mosquitofish), and *Syngnathidae* (Pipefish), along with the European eel. Similarly, in sampling site 1 (Figures 4, 9, 10), another wetland area with brackish water, we identified species such as *Mugil cephalus* (Flathead grey mullet), *Dicentrarchus labrax* (European seabass), *Solea solea* (Common sole), and *Sparus aurata* (Gilthead seabream), in addition to the *Anguilla anguilla* (European eel). Moreover, we observed crustacean species like *Gammarus* sp., *Microdeutopus* sp., *Idotea* sp., *Leptocheirus* sp., *Chironomus* sp., *Callinectes sapidus* (Blue crab), and *Carcinus aestuarii* (Mediterranean green crab) in the research area. Additionally, aquatic plant species found in the region include *Enteromorpha cladrata*, *Ulva lactuca*, *Chaetomorpha linum*, *Codium adhaerens*, *Cystoseira amentacea*, *Phragmites australis*, *Juncus hybridus*, *Nasturtium officinale*, *Salicornia europaea*, and *Eleocharis palustris*. We have also observed various fish species in glass eel survey area (Figures 11, 12, 13, 14) such as *Solea solea* (Common sole), *Mugil cephalus* (Flathead grey mullet), various species of *Syngnathidae* (Pipefish) and *Rhinobatos* sp.

Figure 7. Images of catchment in November (taken by Fishermen), collecting Fyke-nets, *Ulva* sp. and *Phragmites* sp covering the area (Sampling site 2)

Figure 8. Images of locations between sampling site 1 and 2, shallow areas, submerged plants, hand scooping.

Figure 9. Images of river tributaries and controlling fyke-nets at the sampling site 1.

Figure 10. Images of caught elvers and controlling fyke-nets at the sampling site 1.

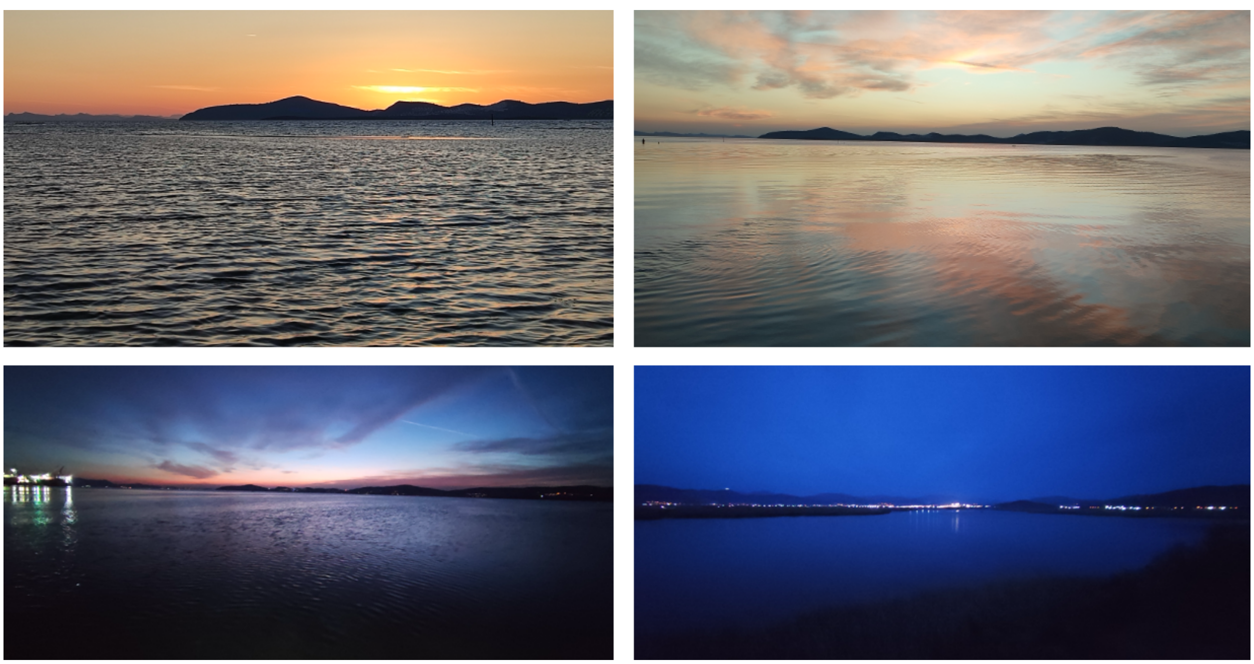

Figure 11. Landscape images from the glass eel survey area at the various times.

Figure 12. Images from the glass eel survey area presenting Estuary and river tributaries

Figure 13. Images from the glass eel survey area presenting estuary, river tributaries and stake net operational structures.

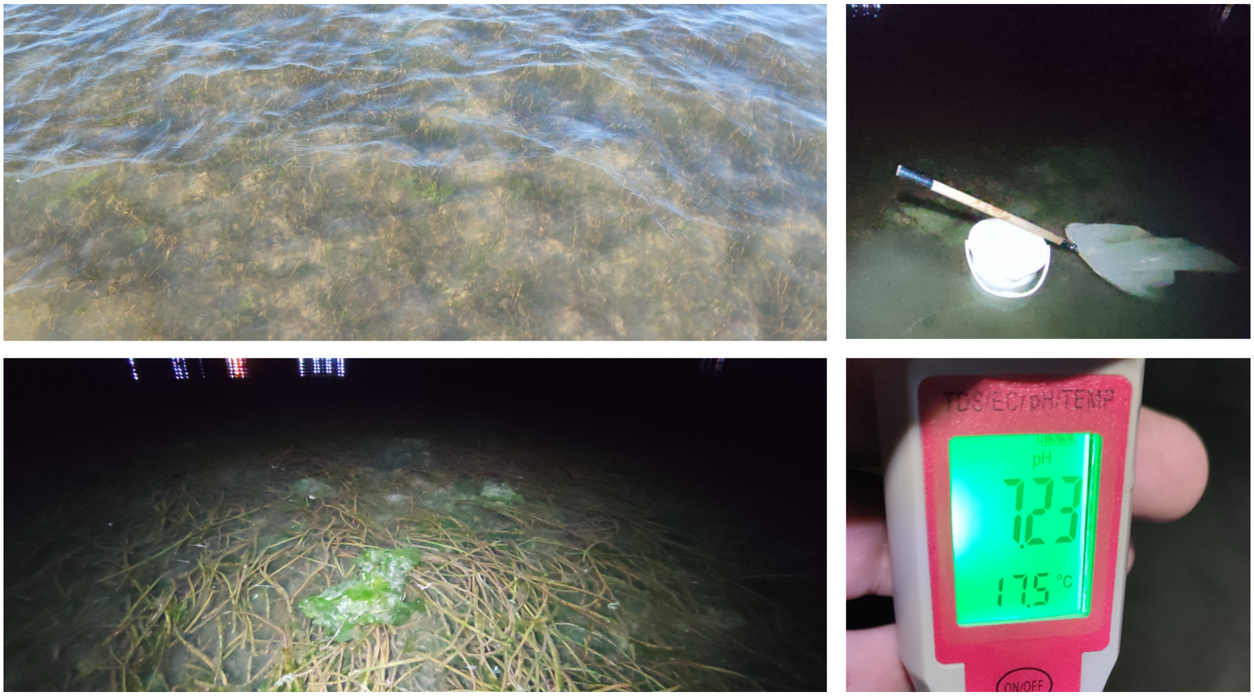

Figure 14. Images from the glass eel survey area presenting vegetation in day and night, equipment used for glass eel sampling and sea water temperature on March 11, 2024.

3.2. DNA barcoding and Phylogenetic analyses

The phylogenetic analysis of European eels (*Anguilla anguilla*) based on the MT-CO1 gene locus provides significant insights into the evolutionary relationships among various populations across Europe, including the Güllük Lagoon in Türkiye. The phylogenetic tree (Figure 15), constructed using the Maximum Likelihood method with the Kimura-2 parameter (K2p) model, comprises 25 nucleotide sequences and a total of 431 positions in the final dataset. The model selection criteria indicated that the K2p model, with a Bayesian Information Criterion (BIC) score of 1887.06 and an Akaike Information Criterion corrected (AICc) value of 1527.82, was the most appropriate model. The transition/transversion ratio (R) was estimated to be 4.35.

The phylogenetic tree is characterized by several well-supported clades, as indicated by the bootstrap values. Notably, a clade with a bootstrap value of 81 includes samples from Lough Erne (Ireland), Valencia (Spain), Denmark, Bristol (UK), Ringhals (Sweden), Livorno (Italy), and the Rhine River (Germany), indicating a close evolutionary relationship among these populations. Another distinct cluster, supported by a bootstrap value of 64, comprises samples from the Güllük Lagoon (Turkey), Qishon River (Israel), Lake Volvi (Greece), and other locations in Turkey. This grouping suggests a closer genetic relationship among the eel populations in these regions compared to others in the dataset. Additionally, another clade with a bootstrap value of 56 includes European eels from Lac Grand Lieu (France), Burrishoole (Ireland), Newcastle (UK), and Izmir (Turkey), highlighting shared genetic characteristics among these geographically dispersed populations.

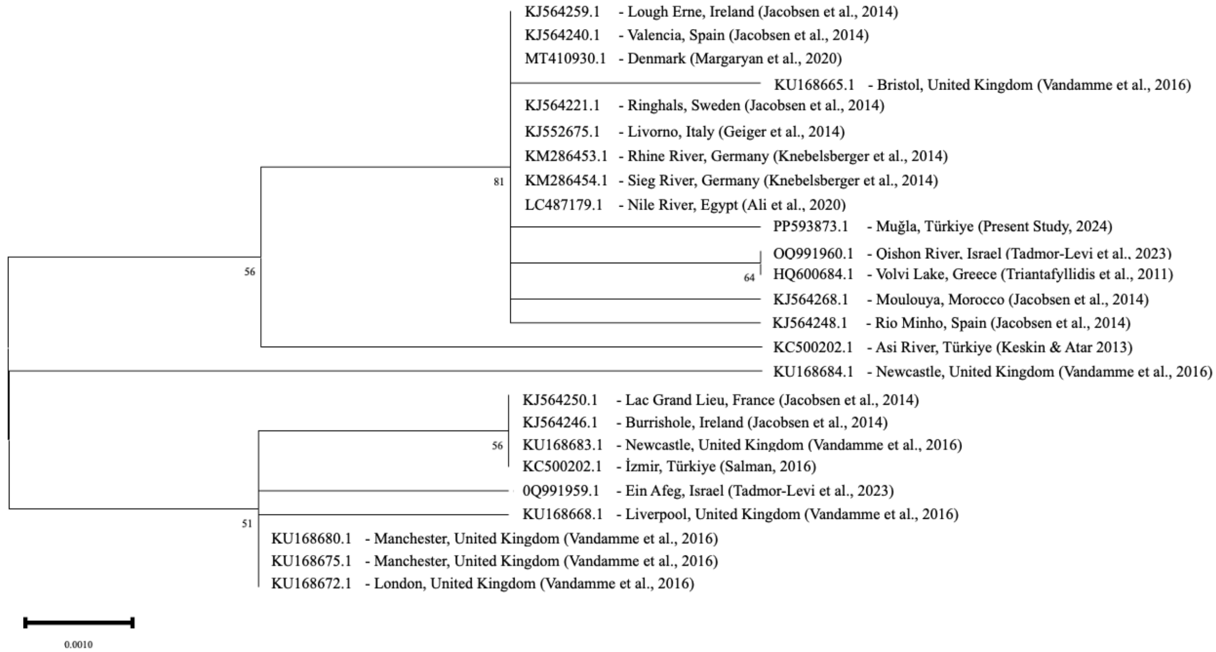

Figure 15. The phylogenetic tree and evolutionary relationship of European eels (*A. anguilla*) caught from the Güllük Lagoon (Turkey) and other European populations, based on the MT-CO1 gene locus using the ClustalW algorithm for pairwise and multiple sequence alignment; the tree was constructed using Maximum-Likelihood Kimura-2-parameter model (ML-K2p).

3.3. Determination of spawning migration period

European eel adults were caught using fyke nets between the September 2023 and February 2024. The spawning migration of eels was properly identified using the catch per unit effort (CPUE) method in the region. The migration of silver European eels occurs between November and December. From September 2023 onwards, a total of 204 European eels were caught and examined over a six-month sampling period. Of these, 180 were caught using fyke nets with a 15 mm mesh size, and 24 were caught using fyke nets with a 6 mm mesh size. In the field, the life stages of the samples were estimated based on their body coloration. Based on proportional distribution of the caught eels in the population, silver individuals were 7%, 17%, 77%, 63%, 37% and 13% while the yellows were 93%, 83%, 23%, 37%, 63% and 87% in September, October, November, December, January and February, respectively. The most abundant silver eel proportion was observed in November and at the end of December, 19 out of 30 European eels (63%) was silver eels. In January, the proportion was found to be 37% of silver eels. Based on this data, it is inferred that the majority of the European eel's spawning migration in the study area occurs between November and December, continuing until the February. For the calculation of catch per unit effort (CPUE), data were collected on the number, weight, and stages of the fish caught by the fishermen. The phenotypic characteristics of the fish (skin coloration), reported by the fishermen, were used to differentiate silver and yellow individuals. The monthly CPUE calculation was analyzed in terms of number (#) and weight (kg). The $CPUE_{kg}$ fluctuated between 0.10 kg/gear/day and 0.75 kg/gear/day while the average was 0.29 kg/gear/day during the study. The $CPUE_{\#}$ was changed between 0.19 ind./gear/day and 1.50 ind./gear/day while the average was 0.63 ind./gear/day during the study. The CPUE values, differentiated into silver and yellow eels, showed that the catch of silver individuals in November was statistically higher than in other months ($p<0.05$). Due to multiple fishing activities within monthly periods, the CPUE values were standardized by evaluating the data separately for each fishing activity, and the monthly averages are presented in Figure 16. The number of silver individuals increased from the end of October, peaked by the end of the November, and then declined from the December onwards. Data analyzed by weight revealed similar results. Statistically significant differences were observed between silver and yellow individuals throughout the study. While this statistical difference in November supported the dominance of silver eels in the sample, yellow eels were dominant in the remaining months.

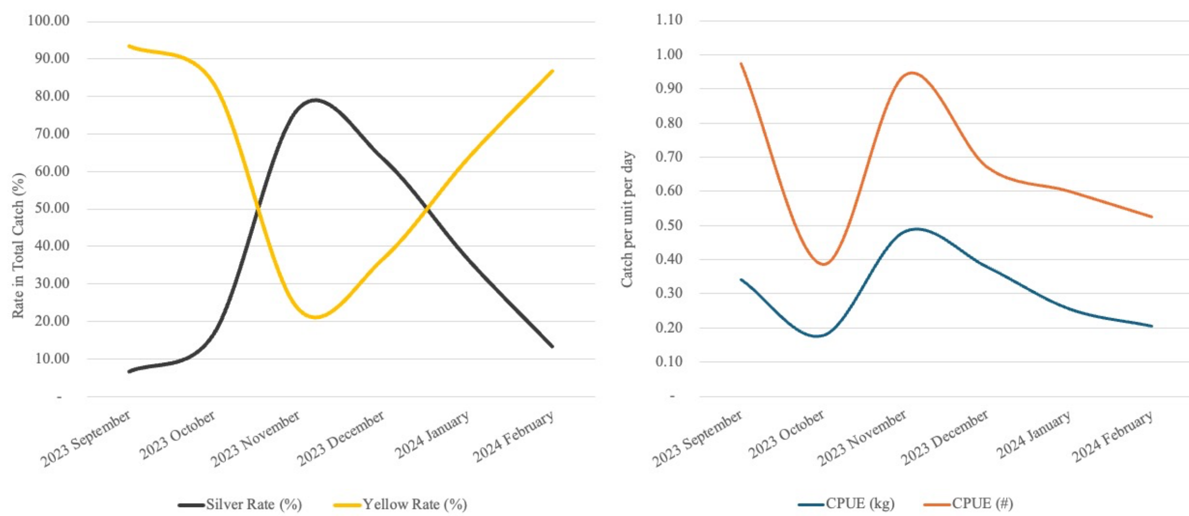

Figure 16. Monthly changes in the ratios of yellow and silver European eels in the sample, and monthly changes in CPUE (#) and CPUE (weight).

The exponential length-weight relationship of the 204 European eels caught during the study indicated that the "*a*" value was 0.0059 and the "b" value was 2.7583. Although the determined "*b*" value suggested negative allometric growth, a t-test showed that the *b* value was not statistically different from 3 ($p>0.05$), indicating that the population exhibited isometric growth (Figure 17).

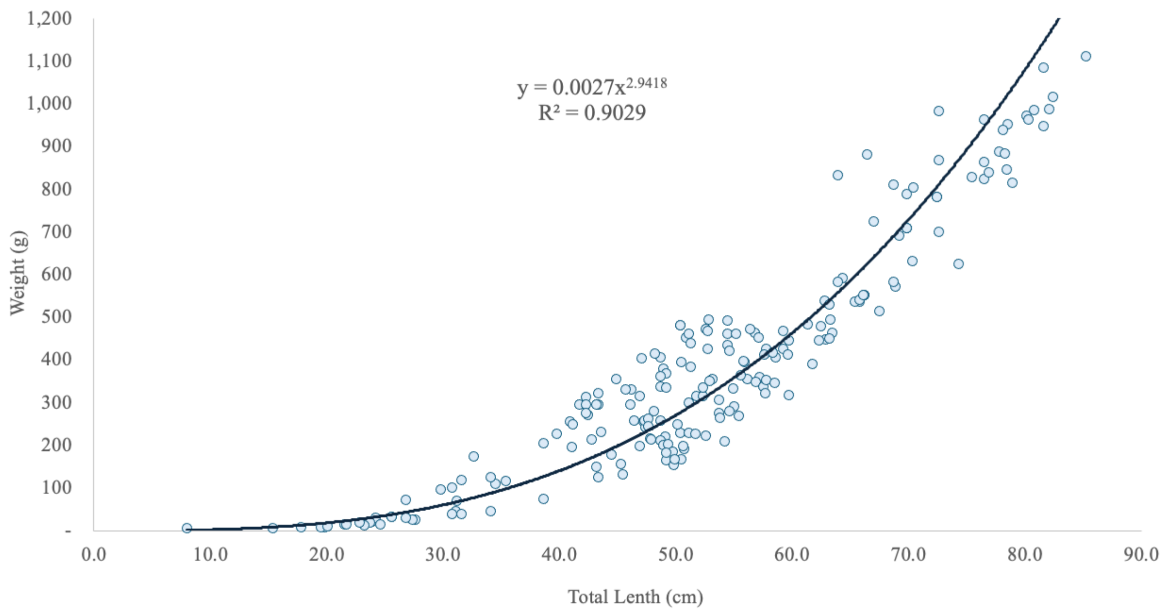

Figure 17. Length-Weight relationship of caught European eels in the Güllük Lagoon, Muğla, Türkiye

The length frequency distributions of all individuals caught during the study were determined (Figure 18). The smallest individual measured 8.0 cm, while the largest was 85.2 cm from all specimens. There were two different mesh sized fyke-net sets used to collect European eel samples. Minimum length (8 cm) and weight (6.26 g) was observed in the specimens collected using 6 mm mesh sized fyke-nets while the maximum length and weight recorded as 38.6 cm and 74.79 cm, respectively. Lowest length was 26.8 cm and lowest weight was 45.09 gr for the specimens collected with 15 mm mesh sized fyke-nets. The maximum length and weight were also observed in 15 mm fyke-net as 85.2 cm and 1111.92 g, respectively. The histogram shown in Figure 18 has revealed that the abundant size class of the samples was 50-59 cm total length range, and majority was clustered between 40-79 cm size range. It can be also observed that the 15 mm fyke-nets somehow caught the eels that were under the minimum catch size (50 cm).

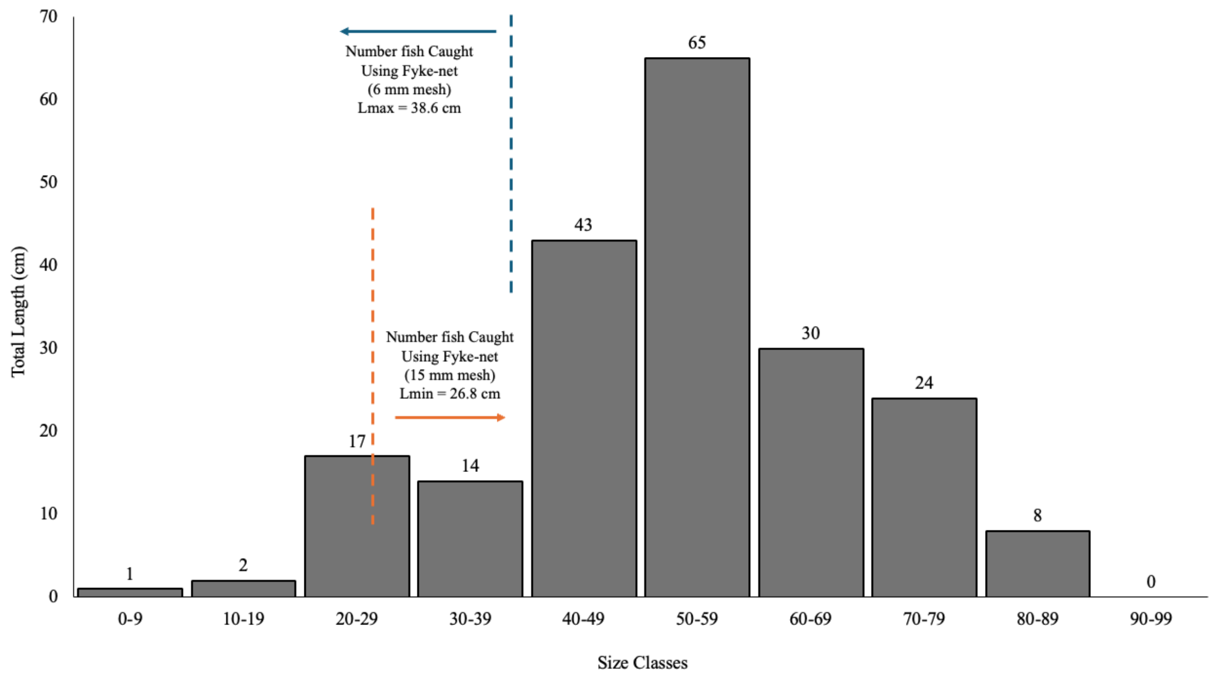

Figure 18. Size distribution of *Anguilla anguilla* catchment from Güllük Lagoon. Size classes were determined to show how many eels caught under the size limit. Orange line and arrow represents the catchment size limits of 15 mm mesh sized fyke-net, and the blue ones represents catchment of 6 mm mesh sized fyke-net

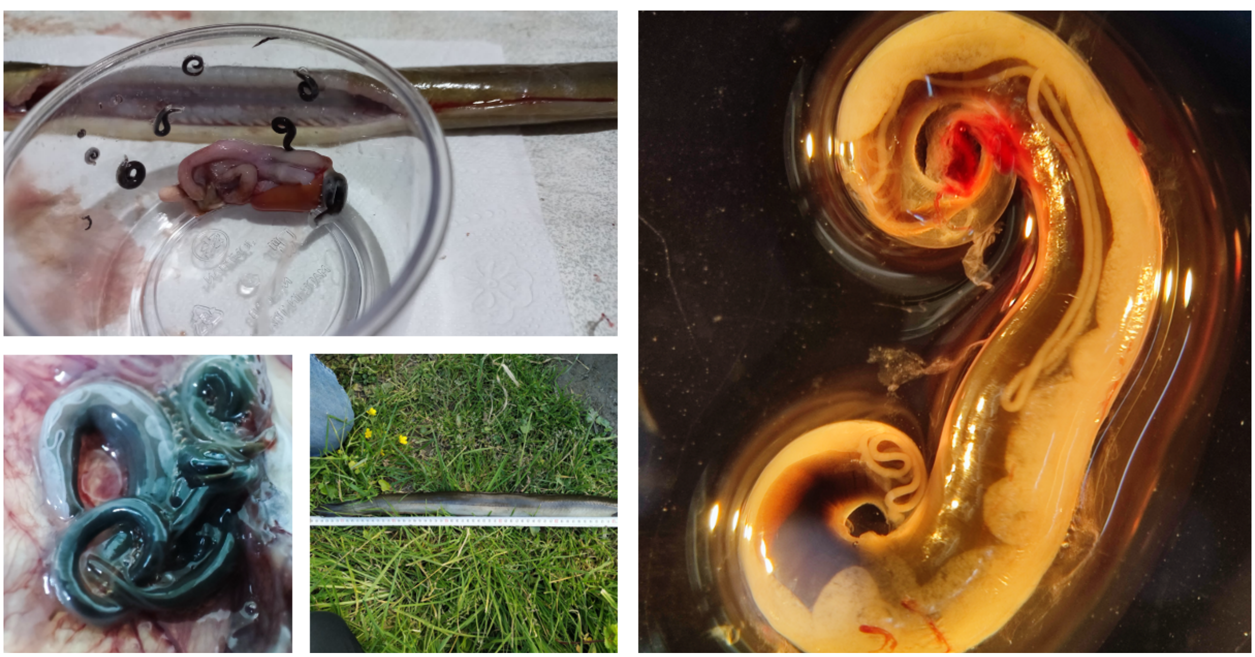

Figure 19. Nematode parasites, *Anguillicoloides crassus* individuals were also detected in the air bladder of sub-sampled European eels in Güllük Lagoon. Forty-seven individuals of *A. crassus* were found in 3 out of 10 sub-sampled eels.

3.4. Glass eel survey

We could not catch any glass eels or elvers in the locations present in Figure 20. We were able to catch a few individuals with the fyke-nets (6 mm mesh size) deployed in the upper parts of the lagoon (mostly wetlands). Glass eel surveys were done in an area covered approximately 13.16 hm^2 at the entrance of Güllük Lagoon in Muğla (Türkiye) (Figure 20).

Figure 20. Glass eel survey area. Hand scoop was used for survey. All survey activities were carried out at new moon periods and started 2 hours before sunset and extended to midnight. Additionally, extra surveys were made at each weekend for 2 days from February to end of the May.

3.5. Project Meeting

During the project, we collaborated with a couple of fishermen in the field. At the same time, communications were established with the personnel of the Muğla Provincial Directorate of Agriculture and Forestry and its Fisheries and Aquaculture Branch. The fishermen were eager to learn about European eel biology, feeding behaviour, and migration periods. Moreover, their interest in eels was primarily related to how they could catch more eels rather than conservation efforts. A successful meeting was held with the attendance of a few fishermen and local authorities. Brochures were distributed, and it is also reported that the eel stock in the region was decreasing, and that illegal fishing should be stopped. During the discussion with the authorities, it was learned that all the fishermen in the region were fishing illegally. At the meeting, the project results were shared with the authorities, and opinions were exchanged regarding future applications and conservation efforts. It was learned that the primary reason for illegal fishing is bureaucratic deficiencies, and no organization, including the fisheries cooperative, has not been officially operating for fishing in the region for the last three years. In conclusion, we can report that there is a positive development regarding this issue that the necessary permits will be given to the fisheries cooperative in the Güllük region to protect the eel and prevent illegal fishing.

Figure 21. Images from PhD seminar, PhD dissertation and Project meeting. Status of local and global European eel populations, problems and efforts in conservation actions were discussed in all meetings.

4. DISCUSSION

At the basin scale, the distribution and abundance of eels in river ecosystems are influenced by various factors (Ogden, 1970; Degerman et al., 1986; Jessop, 2000; Feunteun et al., 2002; Laffaille et al., 2009). These factors include the number of juvenile eels (elvers) entering the river (Feunteun et al., 2002), temperature (Ogden, 1970), water quality (Degerman et al., 1986), river flow rate and velocity (Jessop, 2000), and the accessibility of habitats via water resources (Laffaille et al., 2009). At the macro-habitat scale, young eels prefer riverbank habitats with suitable water depth, substrate, plant roots, and both in-stream and riparian vegetation (Lamouroux et al., 1999; Laffaille et al., 2003; Domingos et al., 2006; Graynoth et al., 2008; Acou et al., 2011; Ovidio et al., 2013). Additionally, there are differences in habitat use related to the size of the individuals, with smaller eels preferring shallower and smaller areas such as river branches (Lamouroux et al., 1999; Laffaille et al., 2003; Graynoth et al., 2008; Benchetrit et al., 2017).

In the present project, European eels were sampled up to a maximum distance of 8 km from the sea. In general, the sampling locations were identified as areas with a muddy substrate, moderate macrophyte density, and typically surrounded by reeds. These areas were characterized by relatively still and shallow (0.5-1.5 m) brackish water. Experimental studies on yellow eels have shown that these eels spend the winter in areas with muddy, soft substrate at low temperatures. Nyman (1972) reported that yellow eels exhibit hiding behaviour in areas with coarse substrate (sand, gravel, stones, or rocks) when water temperatures rise above 8-9°C. This indicates that eels display substrate selectivity based on temperature. Other studies have also demonstrated that soft substrates can be used as shelters by Anguillids (Jellyman & Chisnall, 1999; Aoyama et al., 2005; Acou et al., 2011).

In the study area of Güllük Lagoon and its surroundings, the general substrate structure has been observed to be muddy-marshy and filled with alluvium. In previous research, it was found that during the summer months, when high temperatures are observed, the CPUE (catch per unit effort) in fishing with standard fyke nets on muddy ground was quite low in terms of both quantity and weight (Bahrioğlu, 2023). In the same study, during the autumn and spring months, CPUE values were high at sampling sites with muddy substrates when water temperatures were relatively cooler. In the winter months, contrary to yellow eels, the proportion of silver eels in the catch composition increased. Our study did not conduct any research to correlate this phenomenon with differences in substrate type or water temperature. Thus, we have only evaluated the changes of silver eel proportion in the catchment. Studies on eel populations in Northern Europe (NE), Southern Europe (SE), and individuals reared under laboratory conditions indicate that the age and size values of populations exhibit regional differences (Durif et al., 2009). Although very different size ranges have been observed regionally in these studies, the smallest sizes of female European eels classified as silver were found to be 40 cm in both NE and SE regions, with maximum sizes around 110 cm for NE and 100 cm for SE. In a study by Durif and Ellie (2008), the smallest size for silver female individuals was reported to be 50 cm. In our previous study, completed in January 2023 (Bahrioğlu, 2023), the smallest total length for female individuals was found to be 24.9 cm, while for silver female individuals, it was 46.2 cm. However, the maximum total lengths for female individuals were recorded as 81.1 cm for yellow eels and 88.7 cm for silver eels. In the present study, the smallest individual was found to be 8 cm, and the largest was 85.2 cm.

In the length-weight relationship, a *b* value of 3 indicates isometric growth (Ricker, 1975; Froese & Law, 2006). A *b* value less than 3 signifies negative allometric growth, suggesting that as fish increase in length, their body form becomes slenderer. Conversely, a *b* value greater than 3 indicates positive allometric growth, implying the presence of larger fish in the population, reflecting optimal growth conditions. In present study, the measured *b* values for eels in Güllük Lagoon were 2.93, indicating negative allometric growth. Despite this, the lack of statistical significance in the differences between the calculated b values suggests that the population generally exhibits isometric growth. Castaldelli et al. (2014) reported positive allometric growth for yellow individuals and negative allometric growth for silver individuals, yet the population (Comacchio Lagoon, Italy) overall exhibited isometric growth. In a recent study by Denis et al. (2022), male individuals were reported to have b values between 2.7 and 3.0, while female individuals had b values between 2.9 and 3.7. Piria et al. (2014) found that the population they studied (English Channel coast, France) exhibited negative allometric growth (b = 2.7), Verreycken et al. (2011) reported isometric growth (b = 3.1) in the population they studied (Flanders, Belgium), and Maric et al. (2022) indicated that the population in their study could exhibit either negative or positive allometric growth (b = 2.54 – 3.25) depending on size groups.

Temperature (Tongiorgi et al., 1986) and salinity (Tosi et al., 1990; Cresci et al., 2020) changes have been reported to influence the migratory behaviour of glass eels. In a study determining the direct effects of temperature on glass eels, it was observed that swimming activity against the current (stream) increased at higher water temperatures (18°C), while there was a decrease in swimming activity against the current at lower temperatures (9-10°C) (Vøllestad et al., 1986; Edeline et al., 2006). In our sampling conducted in Güllük Lagoon, few individuals classified as elvers, or sexually undifferentiated eels were encountered during the study period in upper parts of lagoon. In case for glass eel survey, we could not catch any glass eels in Güllük Lagoon during the study period. These results can be related to various reasons. In terms of the effects of sea water temperature on the glass eel migration, the lowest sea water temperature (14.2°C) was observed in February which is starting month of glass eel survey and increased to 17.5°C in mid-March. This increasing temperature in the entrance of Güllük lagoon continued until the glass eel survey ends by reaching to 24.8°C in May. These temperature data suggest that our sampling period for glass eels was appropriate for their feeding migration. It can be also discussed that the sampling gear was not proper. However, Küçük et al. (2005) were able to catch glass eels with the hand scoop in Fethiye-Muğla, Türkiye. Additionally, during the study conducted by Sağlam (2020), a total of 1873 fish from 13 species belonging to 8 families were caught with Seine nets from June 2014 to

December 2016 (mesh size given as tulle net, < 1 mm). Among the identified fish families, the most caught species were 5 species of the Mugilidae family, and they do not report any individuals belonging to *Anguilla* genus. Therefore, we suspect that the glass eel recruitment has decreased to an undetectable level in Güllük Lagoon, Türkiye.

In this study, gene sequences from various European eel populations with specific sampling locations were used to create the phylogenetic tree. Although there was a clear geographical separation between some selected populations, significant gene flow and genetic admixture was observed between these geographically distant populations. The close genetic relationship between the European eel population sampled from Güllük Lagoon (Muğla, Turkey) and the European eel population in Morocco (Jacobsen et al., 2014), Spain (Jacobsen et al., 2014), Greece (Triantafyllidis et al., 2011) and Israel (Tadmor-Levi et al 2023) also indicates greater genetic similarity between these regions. Similarly, the clustering of samples from France (Jacobsen et al., 2014), Ireland (Jacobsen et al., 2014), the United Kingdom (Vandamme et al., 2016) and Türkiye (Keskin & Atar, 2013; Salman, 2017) supports the panmixia hypothesis by suggesting that all individuals within the species are part of a single global population. In general, populations in Turkey, especially those in the Asi River (Keskin & Atar, 2013) and Güllük Lagoon (Present Study), show genetic similarity to Mediterranean and Northern European eel populations. This model also shows that populations distributed in coasts of Türkiye are integral components of the global genetic network of European eels and participate in extensive gene flow across Europe. Phylogenetic analysis of the MT-CO1 gene locus in European eels reveals extensive genetic connectivity among populations across Europe, supporting the concept of panmixia. Additionally, the genetic clusters observed in the phylogenetic tree are not perfectly aligned with geographical boundaries, highlighting complex migration patterns and reproductive behaviors that facilitate gene flow over long distances. The closeness of Türkiye’s European eel populations to other European eel populations in different geographical areas may be related to the ages of the fish included in the genetic analysis, and therefore to which year their parents participated in global breeding activities.

5. CONCLUSION

Project results revealed that traditional traps used in European eel fishing, catches the individuals smaller than the legal catch size limits. Therefore, this situation showed that it could lead the studied population to a decreasing trend. Additionally, it can be said that the future of European eel in Güllük Lagoon is in the hands of the fishermen. A few individuals classified as elvers, or sexually undifferentiated eels, were encountered in the upper reaches of the lagoon during the study period. In the glass eel survey, no glass eel was caught on the seashore of Güllük Lagoon during the study period. Captured elvers suggest that European eel recruitment still occurs to some extent in the region, but glass eel abundance is too low to be sampled. Phylogenetic analysis of the MT-CO1 gene locus in European eels reveals extensive genetic connectivity among European eel populations, supporting the concept of panmixia. Despite the geographical distance between them, the genetic similarity was seen in phylogenetic analysis of European eel populations distributed in Turkey and the other populations in different geographical regions of Europe. It may be related to the age of the fish used, which depends on the year their parents participated in global breeding activities. Finally, we could say that the low glass eel recruitment and high fishing pressure will probably lead the studied population to an extinction at Güllük Lagoon in the future.

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