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A Preliminary Study on Gonadal Development in the European Eel (*Anguilla anguilla*, Linnaeus, 1758) within the Gediz Delta, Izmir Bay (Aegean Sea, Türkiye)

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ABSTRACT

Anguilla anguilla, a catadromous species, is believed to undergo significant morphological and physiological changes in the final stage of its freshwater life. These changes are closely associated with gonad development and enable adaptation for long migration. This study examined 89 eels from the Gediz Delta, caught by commercial fishermen between 2020-2021. The eels' total lengths and weights were measured in the laboratory before they were dissected to examine gonad development. The total length and weight values of the specimens ranged from 19.2-72.5 cm (average: 46.77 cm) and 10.1-1002.4 g (average: 254.69 g) respectively. The ratio of female to male eels was 1:1.28. The chi-square test revealed no significant difference between female and male individuals (χ^2 calculation: 0.625 < χ^2 table: 3.841). The Gonadosomatic Index (GSI) value of the species was found to be between 0.22-0.47, peaking in the fall (0.47). Upon histological examination of the females' ovaries, two phases were identified - immature and maturing. Oocytes in the ovary consisted of pre-vitellogenic and early vitellogenic phases. The pre-vitellogenic phase featured a large nucleus situated in the centre of dense cytoplasm. The diameter of oocytes in this stage was measured between 90-200 µm. The early vitellogenic stage was characterized by larger oocytes located primarily in the peripheral zone of the ovarian stroma, containing a high amount of adipose tissue. The oocytes in this stage had a diameter ranging from 210-280 µm.

Gediz Deltası, İzmir Körfezi (Ege Denizi, Türkiye)'ndeki Avrupa Yılan Balığının (*Anguilla anguilla*, Linnaeus, 1758) Gonad Gelişimi Üzerine Bir Ön Çalışma

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ÖZ

Katadrom bir tür olan *Anguilla anguilla*'nın tatlı sudaki yaşamlarının son aşamasında uzun bir göçe uyum sağlamak adına morfolojik ve fizyolojik olarak birçok değişime uğradığı, bu değişikliklerin de gonad gelişimi ile yakından ilişkili olduğu düşünülmektedir. Bu amaçla Gediz Deltası'nda dağılım gösteren yılan balığı popülasyonunda gonad gelişimini incelemek amacıyla 2020-2021 yılları arasında ticari balıkçılardan 89 adet ölü yılan balığı alınarak incelenmiştir. Laboratuvarda total boy ve ağırlıkları ölçülen balıklar disekte edilmiştir. Örneklerin total boy ve ağırlık değerleri sırasıyla; 19,2-72,5 (ort: 46,77) cm, 10,1-1002,4 (ort: 254,69) g'dır. Dişi:erkek oranı 1:1,28 olarak saptanmış olup ki kare testi sonucu dişi ve erkek bireyler arasında belirgin bir fark tespit edilmemiştir ($\chi^2_{hesap0.625}<\chi^2_{tablo3.841}$). Türün GSI değerinin 0,22-0,47 arasında olduğu ve maksimum değere sonbaharda (0,47) ulaştığı saptanmıştır. Dişilere ait ovaryumlar histolojik olarak incelendiğinde olgunlaşmamış ve olgunlaşmakta olmak üzere 2 safha tespit edilmiş olup ovaryum içerisindeki oositlerin pre-vitellogenik safha ve erken vitellogenik safhalardan oluştuğu gözlenmiştir. Pre-vitellogenik safhadaki oositlerde yoğun sitoplazma ile merkezde konumlanmış büyük bir çekirdek saptanmıştır. Bu safhadaki oositlerde çap; 90-200 µm olarak tespit edilmiştir. Erken vitellogenik evre, periferik bölgede bulunan daha büyük oositler ve yüksek miktarda yağ dokusu ile karakterize edilmiştir. Bu safhadaki oositlerde çap; 210-280 µm olarak tespit edilmiştir.

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1. Introduction

The family Anguillidae, part of the order Anguilliformes, encompasses 20 species across two genera worldwide (Fricke et al., 2020). It hides under rocks or in muddy areas during the day and emerges to feed at twilight, exhibiting increased activity at night (Reshetnikov, 2003; Froese and Pauly, 2005). This species inhabits areas from the Atlantic coast from Scandinavia to Morocco and extends to the Baltic, Black, and Mediterranean Seas, as well as the rivers of the North Atlantic, Baltic, and Mediterranean seas (Deelder, 1984; Rochard and Elie, 1994).

The European eel, *A. anguilla*, is categorized as "critically endangered (CR)" by the International Union for Conservation of Nature (IUCN, 2023). To protect this species, several conservation action plans have been implemented. In 2007, the European Union (EU) introduced a management plan for the eel's recovery (EU, 1100/2007). By 2010, the trade of glass eels outside the EU was prohibited, and a 3-month fishing ban within the EU has been enforced since 2018 (Gentile et al., 2022).

Numerous studies have focused on eels. Vincent and Maes (2005) examined the life cycle, evolution, and reproduction of the European eel. The breeding performance of eels was evaluated by Guarniero et al. (2020) and Capoccioni et al. (2014). Di Biase et al. (2016) compared the eels' reproductive performance in natural and controlled environments. The reproductive biology of eels living on the southern coast of Brittany, France, was investigated by Sbaihi et al. (2001). Dębowska et al. (2016) gauged the fecundity of the European Eel in Polish waters. Denis et al. (2022) studied the growth and abundance of European eels in small estuarine habitats in the English Channel. Lastly, Macnamara et al. (2014) identified the reproductive potential of Silver European eels migrating from Lake Vistonis in the North Aegean Sea.

Freshwater eels of the Anguillidae family are characterized as facultative catadromous. Adults spawn in the ocean, and their larvae journey to coastal and inland waters for growth before returning to the ocean to spawn (Schmidt, 1922). The hatchlings, referred to as leptocephalus, drift as plankton for approximately 7-11 months, though this stage can extend up to 3 years (Maitland, 1977).

The leptocephalus transitions into the "glass eel" stage before reaching coastal waters, resulting in a shorter, more cylindrical body shape (Sinha and Jones, 1975). As they gain pigmentation and transform into the elver stage, they move into freshwaters and become known as "yellow eels" (Sinha and Jones, 1975).

After migrating to either brackish or fresh waters, eels feed and grow before returning to the sea to breed (males 6-12 years; females 9-20 years). Eels that migrate from freshwater to the sea for maturation are referred to as "silver" eels (Sinha and Jones, 1975). During their migration to the sea for reproduction, eels undergo significant morphological and physiological changes. These transformations are thought to be closely linked to gonad development (Pankhurst, 1982; Fontaine et al., 1995; Waring, 1963; Rohr et al., 2001). Boëtius and Boëtius (1985) believe eels to build up substantial amounts of lipid in their bodies for long-distance migration. Optimal environmental conditions promote this lipid accumulation, which is vital for gonad development, helping eels achieve sexual maturity and ensuring a successful migration (Svedäng and Wickström, 1997).

Eel maturity can be determined through several methods, including color, eye index, length, weight (Pankhurst, 1982; Durif et al., 2005), and the gonadosomatic index (GSI) measurement, which we used in our study (Devlaming et al., 1982). This technique distinguishes between mature and immature eels. However, the relationship between morphological changes and gonad development is still not fully understood, and the maturation degree can be defined using both morphological and histological analyses.

In this study, the changes in the gonads of the eel (*Anguilla anguilla*) population distribution in the Gediz Delta were investigated in order to adapt to migration This research is the first study conducted to determine the reproductive characteristics of this species in Turkey.

2. Materials and Methods

In this study, 45 yellow eels, 44 the pre-silver were obtained seasonally from a local fisherman in Izmir Bay between 2020 and 2021 (Figure 1). The eel samples were brought to the laboratory and stored in containers with a 10% formalin solution. The length and weight of the yellow eels brought to the laboratory were first measured. In the next stage, the fish were dissected, their sexes were determined, and the gonad development stages were determined according to the methodology of Holden and Raitt (1974) (Table 1).

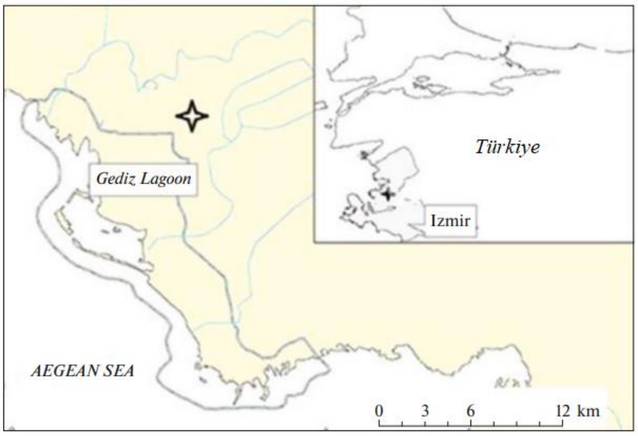

Figure 1. Sampling area of European eel, *Anguilla anguilla* (Linnaeus, 1758) samples

Table 1. A five-point maturity scale for partial spawners (Holden and Raitt, 1974).

Stage	State	Description
I	Immature	Ovary and testis about 1/3rd length of body cavity. Ovaries pinkish, translucent; testis whitish. Ova not visible to naked eye.
II	Maturing virgin and recovering spent	Ovary and testis about 1/2 length of body cavity. Ovary pinkish, translucent; testis whitish, more or less symmetrical. Ova not visible to naked eye.
III	Ripening	Ovary and testis is about 2/3rds length of body cavity. Ovary pinkish-yellow colour with granular appearance, testis whitish to creamy. No trans- parent or translucent ova visible.
IV	Ripe	Ovary and testis from 2/3rds to full length of body cavity. Ovary orange-pink in colour with conspicuous superficial blood vessels. Large transparent, ripe ova visible. Testis whitish- creamy, soft.
V	Spent	Ovary and testis shrunken to about 1/2 length of body cavity. Walls loose. Ovary may contain remnants of disintegrating opaque and ripe ova, darkened or translucent. Testis bloodshot and flabby.

The gonadosomatic index values were calculated using the GSI= [gw/(W-gw)]*100 formula (Barber and Blake, 2006) to identify the breeding season of the species. However, the precise breeding period could not be fully determined due to the lack of specimens that completed vitellogenesis during the full breeding period. Consequently, only general observations could be made.

For the histological demonstration of gonad development, 40 ovary samples from four seasons were used. These samples, which went through an alcohol series, were embedded in paraffin, and 7 µm thick sections were extracted (Dufour et al., 1988). The samples were then stained using the hematoxylin-eosin staining method and photographed with Olympus SZ60. Oocyte diameters were measured using a micrometre.

3. Results

In the study, a total of 89 eel samples were evaluated. The total length and weight values were between 19.2-72.5 cm (average: 46.77) and 10.1-1002.4 g (average: 254.69) respectively. Out of the obtained specimens, 35 were female (39.33%), 45 were male (50.56%), and 9 were indeterminate (10.11%). Among the 80 individuals whose sex was identified, there were 35 females and 45 males, resulting in a female-to-male ratio of 1:1.28. The chi-square test results showed no statistically significant difference between male and female specimens (χ^2 calculated 0.625 < χ^2 table 3.841).

The gonadosomatic index value of the species was examined and it was found that the GSI value ranged from 0.22 to 0.47, peaking in the fall (0.47) (Figure 2). However, as no individual in the full reproductive period, having completed vitellogenesis, was found, the reproductive period of the species could not be determined based on the gonadosomatic index values.

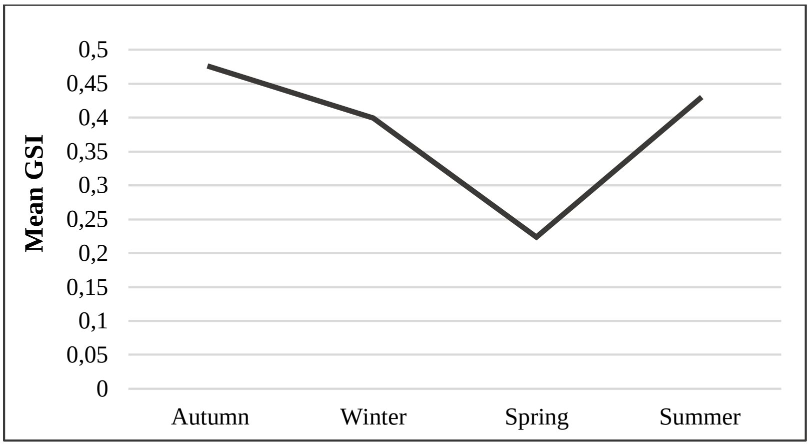

Figure 2. Gonadosomatic index values (GSI) for *Anguilla anguilla*

Upon examining the colors of 89 individuals grouped by size, it was found that 45 were yellow eels, while 44 were in the pre-silver stage. The size of yellow eels ranged from 19.2-45 cm, whereas pre-silver eels measured between 45.2-72.5 cm (Figure 3).

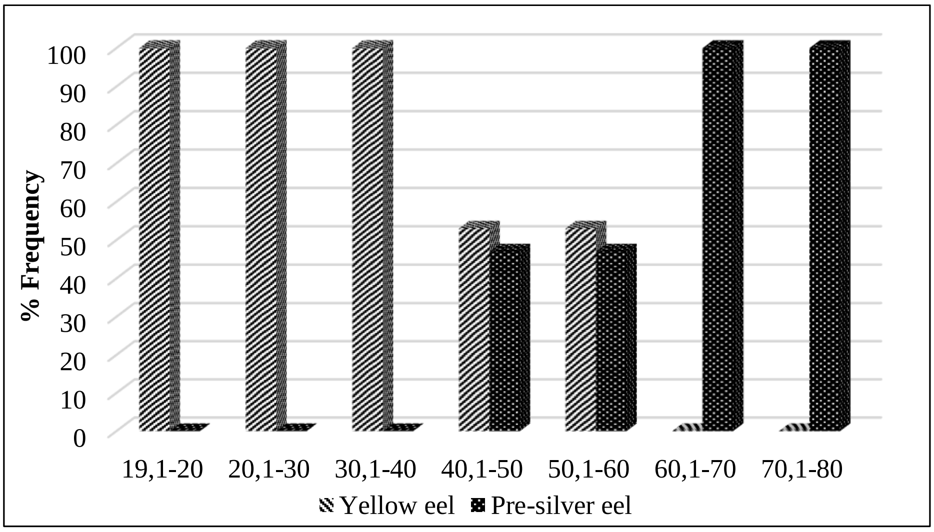

Figure 3. Size distribution in yellow and pre-silver eels

The distribution of gonad stages in female and male individuals was also examined according to seasons (Figure 4).

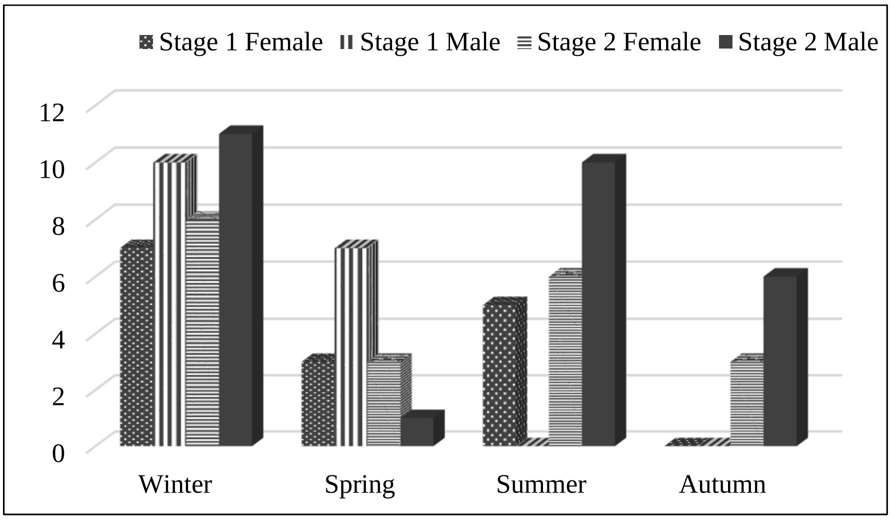

Figure 4. Distribution of gonad stages of female and male eels according to seasons

The specimens obtained from fishermen included deceased eels, hence, for various reasons, the gonads of 9 eels couldn't be determined. A morphological examination revealed a fringed structure forming in the females' ovaries as their size increased (Figure 5).

Figure 5. Morphological structure of the ovary

Upon examining the ovaries of females histologically, two phases were detected: immature and mature. In this study was determined that the oocytes in the ovary were in the pre-vitellogenic and early vitellogenic phases. In the pre-vitellogenic phase, the one-nucleoli stage, a large nucleus with dense cytoplasm in the center, was detected. This stage was characterized by only one nucleolus inside the germinal vesicle and the absence of lipid droplets. The total length and ovary weights of individuals in this phase range from 29.1-44 cm and 0.05-0.6 g respectively, with a GSI value of <0.40. The diameter of the oocytes in this phase was 90-200 µm (Figure 6A).

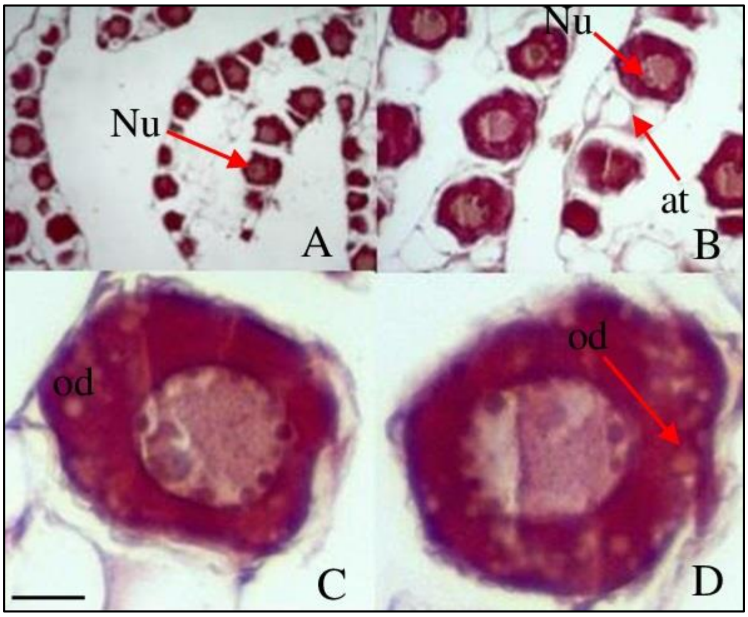

Figure 6. Histological structure Follicles of Female Gonad (Ovaries) of European eel *Anguilla anguilla* A: One-nucleoli stage; B: Multiple nucleoli stage; C-D: Perinucleolar stage. Nucleoli (Nu). Oil droplet (od). Adipose tissue (at). Scale bar: 20 μm.

The early vitellogenic stage was distinguished by larger oocytes, primarily located in the outer zone of the ovarian stroma, surrounded by a significant amount of fatty tissue, which sometimes constitutes more than 50% of the stroma (Figure 6B). The Perinucleolar stage, also known as the Multiple nucleoli stage, is also found in this phase. It was characterized by the presence of the first lipid droplets and an increase in oocyte diameter (Figure 6 C-D). The total length and ovary weights of individuals in this phase range from 45-72.5 cm and 0.62-16.53 g respectively, with ovaries covering half the body cavity. An increase in the width of the ovaries and a pleated appearance is observed (Figure 5). The GSI value in these individuals is >0.40. Many irregularly distributed nucleoli within the germinal vesicle are observed. The phase is characterized by larger oocytes containing lipid vesicles in their cytoplasm (Figure 6D) and an increased density of fat cells. The diameter of the detected oocytes is 210-280 μm.

4.Discussion

In their study, Sbaihi et al. (2001) researched the reproductive biology of *Conger conger* and *Anguilla anguilla* species on the southern coasts of Brittany, France. Sbaihi et al. (2001) were detected that the gonadosomatic index (GSI) value ranged from 0.06 to 2.08. In our study was determined a similar GSI value range of 0.22 to 0.47, although it did not encounter any individuals who had fully completed vitellogenesis. Additionally, this study was observed fat vesicles in the cytoplasm at the early vitellogenic stage. This observation aligns with the findings from studies conducted by Dufour et al. (1988) and Colombo and Grandi (1997). Ohta et al. (1997) also noted the potential for fat droplets inside the oocytes at the beginning of vitellogenesis in the silver phase of *A. anguilla* and *A. japonica* species, which further supports our study.

In a recent study, Gentile et al. (2022) explored the gonad development of eels in the North Adriatic lagoons, concluding that the eels were mature silver eels ready to migrate. Our study, which examined oocyte development in the ovaries up to the pre-silver stage, bears significant resemblance to their findings. Both studies identified oocyte maturation, a stage considered pre-migration in eels. Evidence of the species' readiness for reproductive migration includes both the morphological silver colour and the increase in oocyte diameter and lipid content, as observed in histological studies (Durif et al., 2005). Thus, histological studies play a crucial role. The lipid increase detected in the silver eels in our study further confirms that they are preparing for migration.

The *A. anguilla* species has seen significant declines in its populations in recent years. This alarming reduction can be attributed to a variety of factors including overfishing, loss of natural habitats, the presence of parasites, pollution and diseases (Feunteun, 2002; Dekker, 2003), as well as changes in the oceanic environment that affect the growth and migration patterns of the eels' larvae (Knights, 2003; Friedland et al., 2007). Problems created by humans are also a significant factor in this decline (Lobón-Cervía, 1999; Dekker, 2003; Allen et al., 2006). Despite the pressing issue of declining populations, and despite the International Union for Conservation of Nature (IUCN) categorizing the *A. anguilla* species as "critically endangered CR", there is an apparent dearth of reproductive studies on these species. This lack of information is one of the major hindrances to conserving and managing these species effectively. Achieving a detailed and comprehensive understanding of the reproductive behaviours of these catadromous species is not just important, but crucial, for the creation of strategies aimed at preserving their populations and managing their habitats. This research caried out the gonad development of A. anguilla in the Gediz Delta, marking the first study of its kind in our country. It is thought that this research will form the basis for eel studies to be carried out in Turkey in the following years and will also contribute to sustainable population plans.

5.Conclusion

The species *Anguilla anguilla*, which is a catadromous species, is classified as "critically endangered (CR)" according to the International Union for Conservation of Nature (IUCN), and its sustainability is of great importance. There are very few studies on the reproductive biology of this species. This study, which is unique in our country, will form the basis for future studies.

Conflict of interest

No conflict of interest has been declared by the authors.

Authorship contributions

The contribution of the authors is equal.

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