ASSESSMENT OF THE STATE OF THE GLASS EEL POPULATION IN THE BOJANA RIVER

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EXCERPT

After more than twenty years, the first research of the glass eel in the area of the river Bojana was conducted. During the two-year, 2021 (period March-June) and 2022 (February-mid-March), sampling, fifty specimens of glass eel were registered in the area of the river Bojana. The individuals were caught in the period February-March. Biometric characteristics of registered individuals indicate that one migration wave was registered. During this research, four pigment stages were recorded: VI A0, VI A1, VI A2 and VII. In the recorded migration wave, the VI A1 stage dominated. Stages VI A2 and VII correspond to the final stages of pigmentation and physical metamorphosis of glass eels. Although in a small percentage, the finding of the VIA2 stage is significant since it is considered the limit of metamorphosis at which the animal begins to feed. The number of registered pigment stages and their good condition indicate good conditions for the survival of the glass eel in the river Bojana. The overall global situation influenced the fact that in the first year of the research, the work started in March, and in the second year of the research, the sampling lasted only a month and a half. Nevertheless, significant results have been obtained which represent a good basis for further research and the original idea of a two-year project duration is justified. As global climate change is likely to lead to minor disturbances in periods of mass occurrence of juvenile glass eels, which are completely dependent on sea currents, and their mass occurrences are associated with periods of lunar darkness, for a finer assessment of glass eel would be necessary to begin research calendar much earlier (December / January) in order to determine with greater reliability the main periods of migration of the glass eel into the river system.

1. Introduction

The European eel is a catadromous, panmictic and semelparic species. Its biological life cycle is characterized by five phases: leptocephalus, glass eel, elver, yellow and silver stage. Despite its exceptional ability to adapt to different types of environments, the renewal of European eel livestock was declining for the period 1980-2011. The International Council for the Exploration of the Sea (ICES) eel working group has confirmed that the amount of eels is outside safe biological limits (Durif et al., 2011). Recent research for 2011-2019 shows an increase in the recovery trend of livestock, however, given the overall declining trend in the previous period, the impact on adult livestock is likely to continue for one generation (ICES, 2020).Since 2008, the eel has been placed on the Red List of Endangered Species of the International Union for Conservation of Nature and Natural Resources (IUCN) as critically endangered (Durif et al., 2011). It is included in Appendix II of the CITES Red List of Endangered Species, implying drastic restrictions on trade (Maes and Volckaert, 2007), and is also listed in Annex III of the Barcelona Convention. There are also recommendations from the European Union, i.e. the EU Commission (General Fisheries Commission for the Mediterranean) for its constant monitoring. As an additional mechanism in the conservation of this species, the EU Eel Directive EC No. 1100/2007.

The first data on eels in Montenegro mainly represent information given in the lists of species from certain localities, where eels are listed as present and economically important species (Franetović, 1960; Drecun and Miranović, 1962). The first extensive study dealing with the biology of this species, its distribution, physiological characteristics, diseases and migrations was published by Morović (1976), entitled "The Wonderful World of the Eel".For Montenegrin waters, the author states that significant quantities of young eels can be found at delta of the Bojana River. A more detailed study of eels in Montenegrin waters was presented in the doctoral dissertation Hegediš (2007). The research period in the dissertation was 1997-1998. The research continued at the end of 1999 and in the spring months of 2000 and 2002. The dissertation presents the results that describe the characteristics of migration and the basic characteristics of glass eels in the river Bojana. In this work, on about 3,300 glass eels that were sampled during three successive migration waves, an analysis of body length and mass, length-weight ratio, pigment stages, biometric characteristics, phenological aspects of migration, dynamics and intensity of migration, and a mathematical model of migration is proposed.

Despite the global biodiversity and commercial importance, there are very few published papers dealing with the ecology of European eels in Montenegro (Milošević and Mrdak, 2016; Piria et al., 2016; Rakočević et al., 2018) as well as two papers related to morphometric and microchemical analysis of eel sagittal otoliths (Kanjuh et al., 2018, Milošević et al., 2020). Apart from the above, in the area of our country, very little detailed and comprehensive research has been conducted on eels. Unfortunately, precise data on the state of the population of this important species in Montenegro do not exist. It should be noted that the European eel is an important commercial species, but there is no continuous and reliable data on its catch in Montenegro. For the Skadar Lake area, there is data to catch 50 tons each year (legally and illegally) (Simon, 2020).

The coastal waters of Montenegro are suitable habitats for European eels and are a significant source of its juveniles (glass eel stage). It has already been pointed out that for the area of Montenegro there is no precise data on the state of the European eel, nor have researches been conducted in the previous 20 years that would assess the state of the glass eel.

This fact imposes the need for detailed and continuous research of glass eels in coastal waters, especially the river Bojana, which, in addition to a number of specifics, is also characterized by great potential when it comes to young European eels. The importance of this research is reflected primarily at the national level because the results create the basis for the development of management plans, and also globally because it is a species with a status of international importance and protection.

1.1. Description of the studied species

Sistematyc affiliation:

Phylum: Chordata

Subphylum:Vertebrata

Superclassis:Gnathostomata

Classis: Osteichthyes

Subclassis: Actinopterygii

Infraclassis: Teleostei

Ordo: Anguilliformes, Goodrich, 1909

Familia: Anguillidae Rafinesque, 1815

Genus: Anguilla Schrank, 1798

Species: *Anguilla anguilla* Linnaeus, 1758

Synonyms:

Anguilla anguilla Drecun, 1957: 37; Drecun, 1962: 4; Ivanović, 1973: 112, fig. 54; Knežević, 1981: 313; Knežević, 1984: 115; Drecun et al., 1985: 30; Marić i Krivokapić, 1997:218; Kottelat and Freyhof, 2007: 51 (Marić i Milošević, 2011).

Muraena anguilla Linnaeus, 1758: 245 (Locus typicus: Europa; maxima in lacu Cornachio Ferrariensi).

Anguilla vulgaris Shaw, 1803: 15, pl. 1.

Anguilla acutirostris Risso, 1826: 198.

Anguilla canariensis Valenciennes, 1843: 8, pl. 20 fig. 1.

Anguilla migratoria, Kröyer, 1849: 616.

Anguilla morena Kaup. 1857: 35, pl. 3 fig. 18.

Anguilla eurystoma Heckel and Kner, 1858: 325, fig. 168.

Main characteristics:

The body of the eel is serpentine and elongated, laterally flattened. The head is relatively small, the mouth is terminal. The lower jaw is longer than the upper. The eyes are small and are located at the very top of the head, just above the mouth. The gill openings are small. The odd fins (dorsal, caudal, and anal) are joined together to form a frame around the body. There are no ventral fins. There are 245-275 spokes in the dorsal fin and 205-255 spokes in the anal fin. The tail is flattened laterally. The scales are deeply embedded in the skin, so the skin looks smooth. The color of the body is variable, it depends on the habitat (locality) and it is mostly dark olive - greenish or grayish brown, and the lower part is yellow. In the larval stage, they are transparent, laterally flattened and have the shape of a leaf. It reaches a length of 50 cm (males), up to 133 cm (females). The maximum published weight is 6.6 kg (Froese and Pauly, 2014). The average life expectancy is 15–20, while the maximum recorded age is 88 years (Froese and Pauly, 2014).

Local name in Montenegro: eel

Distribution:

They are found in all European rivers belonging to the basins: the Mediterranean, the North and Baltic Seas, the South Atlantic and the Canary Islands. They very rarely reach the White and Barents Seas, and they have also been recorded east of Pechora (Russia, near the northern Urals). A small number reach the Black Sea and migrate east to the Kuban River Basin. Occasionally some specimens reach the Volga basin through canals. A large part of the population remains in the seas (Northwest Atlantic and Mediterranean) (Kottelat and Freyhof, 2007).

In Montenegro, it inhabits the Bojana River, Skadar and Šasko Lakes. It was found in the lower reaches of Cijevna, Morača and Zeta, but in small numbers (Drecun et al., 1985). It was found throughout the Zeta River through Bjelopavlići, then in Koštanica (a tributary of the Morača), as well as in all small tributaries that flow directly into the Adriatic Sea (Hegediš et al., 1997; Marić and Milošević, 2011).

Biology and ecology:

Eels are catadromous fish. Life begins in the Sargasso Sea area of the western North Atlantic, but continental distribution (as resident yellow eels) encompasses the coastal and inland waters of Europe and North Africa (Dekker, 2003; Tesch, 2003). Towards the end of their life cycle, yellow eels transform into migratory silver eels that return to the Sargasso Sea where they leave offspring and die (Tesch, 2003). The peak of spawning begins in March and lasts until July. There are no specific data on spawning locations, but they are assumed to be at depths of 100–200 m and at temperatures of about 20 ° C (Kottelat and Freyhof, 2007).

Females lay up to 1000 pieces of eggs, about 1 mm in diameter. After embryonic a transparent larva leptocephalus is formed, which does not have the characteristics of an individual, but they are laterally flattened, transparent and have the shape of a leaf. The mechanisms by which larvae reach European shores have not been fully resolved. There are indications that the larvae are actively swimming and that their floating has only a small role. Leptocephalus larvae reach the continental basins and metamorphose into glass eels (which look like adults, except that their body is transparent), 5-10 cm long, which enter the mouth. It takes 1-3 years from spawning to the glass eel stage. During upstream migration, pigmentation increases and young eels in this phase are called elvers. The feeding phase in males lasts 5-8 years, and in females 12 years or more. Males have rarely been recorded more than 200km upstream. Downstream migration begins in late summer or fall, and adult eels reach the hatchery the following spring. It has been observed that the diameter of the male eye increases significantly before migration (Kottelat and Freyhof, 2007). In fresh water they feed on bottomless invertebrates (mollusks, crustaceans), fish and caviar of other fish. In the sea, on the way to the hatchery, the diet is interrupted (Tesch, 2003). Infection with the parasitic nematode *Anguillicola crassus* Kuwahara, Niimi and Itagaki, 1974, which feeds on blood from the blood vessels of the air bladder, is becoming more common. It is assumed that the parasite was introduced from Japan with experimental stocks of Japanese eel *Anguilla japonica* Temminck and Schlegel, 1846 (Kottelat and Freyhof, 2007).

Conservation status:

Since 2008, the European eel has been included in the Red List of Endangered Species of the International Union for Conservation of Nature and Natural Resources (IUCN) as critically endangered (Durif et al., 2011), and since 2010 it has been included in the IUCN Red List of Endangered Species critically endangered species (CR) (IUCN, 2010).Recent publications indicate that it still remains in the same category (Pikke et al., 2020). It is included in Appendix II of the CITES Red List of Endangered Species, implying drastic restrictions on trade (Maes and Volckaert, 2007), and is also listed in Annex III of the Barcelona Convention. There are also recommendations from the European Union, ie the EU Commission (General Fisheries Commission for the Mediterranean) for its constant monitoring. As an additional mechanism in the conservation of this species, the EU Eel Directive EC No. 1100/2007. The International Council for the Exploration of the Sea (ICES) working group on eels has confirmed that the amount of eels is outside safe biological limits (Durif et al., 2011).

Economic significance:

The European eel is an economically important species in Montenegro and other countries where it occurs. Adults are hunted throughout the year, and in Western Europe, especially in Great Britain and Ireland, in addition to adults, glass eels are also considered, which are consider an exceptional delicacy. Therefore, the price of this species reaches 15-20 euros per kilogram and is an important resource for local fishermen. The largest caught individual in Skadar Lake was 107.3 cm long and weighed 2,650 g.

1.2. Description of the research area

Bojana River

The Bojana River, together with the neighboring Porta Milena wetland complex, the Bajo Sekulić saltworks in Ulcinj and Šasko Lake, is hydrologically and biologically the largest and most complex aquatic unit on the entire Montenegrin coast (Hegediš, 2007). It is characterized by an unusually diverse complex of unique and threatened natural and cultural landscapes, habitats and species. It is an area with various plant communities, the most important of which are very rare associations of psamo-halophytes, mixed deciduous forests with endemic Skadar oak, meadows with daffodils and orchids that are strictly protected by Montenegrin law. Bojana is a partially navigable international river. It is 43 km long. It flows from Lake Skadar 18km through Albanian territory, and the remaining 25km represents the border between Montenegro and Albania.

As a distributary of Skadar Lake, Bojana is under its strong influence in terms of water inflow and water level regime. On the other hand, the Adriatic Sea is characterized by relatively small variations of tides, so that the influence of the sea is weak and it is possible to register it only in the last 2-3 km before the mouth. The influence of the sea is reflected in the changing salinity regime, primarily in the bottom layer of the water column, while this influence in the surface layer is almost negligible. The temperature regime is also strongly influenced by the inflow of water, while the influence of the sea is quite weak in this regard. Bojana flows into the Adriatic Sea with two main branches, ie about 2 km in front of the mouth of the river it forks into left and right branches ("eastern" and "western" mouth). The left branch represents the border part of the stream with Albania, while Ada Bojana and the right branch of the mouth are located on the territory of Montenegro. The left arm is significantly larger and carries more water, about 2/3 of the total flow, which averages about $350m^3$ / sec (Hegediš, 2007).

The water regime of the Bojana is determined by its tributaries. The flow is slowed down, due to the small slope of 1.2 m / km. The average depth of Bojana is about 3-5 m, and in some parts it exceeds 8 m. At the delta of the river, sea waves have created a reef, which is visible when the water level is low.

The range of water temperature variations in Bojana during the year is between 4.8 ° and 25 ° C. Salinity during the year ranges between 0.1 and 2.6 ‰, although under certain conditions (strong south wind), in the area of about 0.6-0.7 km upstream from the delta, in the bottom layers can reach close to 20‰. The pH of the water reaction is slightly alkaline and ranges between 7.2 and 7.8. The amount of dissolved oxygen in the water is within the limits of saturation (92-105%). The value for BPK5 is usually about 0.5mg / l O_2. The differences in water levels during high tide and low tide range between 40 and 60 cm.

1.3. Ichthyofauna of the Bojana River

The ichthyofauna of the river Bojana has the characteristics of lake fauna, while in its lower part it has fauna of estuarine character. Most of the species listed for Skadar and Šasko lakes are also found in Bojana. Since it connects Skadar Lake with the sea, it is also the main migration route for anadromous and catadromous species. The Bojana estuary is characterized by ichthyofauna typical of brackish waters, but also by the presence of species from the primarily freshwater family Cyprinidae, which are characteristic of Skadar Lake: *Rutilus prespensis*, *Alburnus scoranza*, etc. Five species of mullet have been registered at the mouth of this river, followed by sea bass *Dicentrarhus labrax*, *Atherina boyeri*, *Syngnathus acus* and *S. abaster*, as well as *Aphanius fasciatus* (Marić, 2019). The presence of typically freshwater fish species, constant freshwater character and large water mass clearly separate the Bojana estuary from other coastal watercourses in Montenegro. The constant freshwater character is the result of a large inflow of fresh water throughout the year and relatively low tides (up to 0.5 m), which are not sufficient to give the Bojana estuary typical estuary features (Hegediš et al., 1997). The composition of ichthyocenoses in the Bojana River is largely determined by the diversity and variation of abiotic conditions (water level, water temperature, salinity, oxygen content, pH) and biotic conditions in habitats (complex inter- and intra-species interactions, dynamic trophic relationships conditioned by constant migrations of juveniles and adults of different fish species).

Of the total number of fish species inhabiting the Bojana River, two species are protected by national legislation, while six have international protection status (Table 1). It should be emphasized that these species of sturgeon, which have the status of both national and international protection, have not been registered in the previous 35 years.The absence of these two species of fish is a consequence of the existence of insurmountable obstacles for fishing located on the river Bojana (Albania), immediately after its emergence from Skadar Lake. Such barriers and their insufficiently clear way of functioning (periods of the year when fishing is done, as well as periods of the day) are the basic problem and the cause of the extinction of these two species from our watercourses. According to Marić, 2019 *Allosa fallax* has VU endangered status in Montenegro. The river Bojana is also inhabited by *Pelasgus minutus* (Karaman, S., 1924) , which is endemic to Montenegro, Albania and Macedonia. This species inhabits the river Bojana as well as a number of smaller Adriatic tributaries (Marić, 2019).

Table 1. Overview of Bojana river fish species protected by national and international legislation

Species name	**Local name**	**National protection status**	**International vulnerability and protection status**	**Note**
Acipenser naccarii Bonaparte, 1836	Jadranska jesetra	+	Annex II and IV Habitat Directive, Appendix II of the Berne Convention, CITES	This species has not been registered in the last 35 years. It lives in the Adriatic Sea from when it enters the brackish and fresh waters. Šasko Lake (Knežević,1984)
Acipenser sturio Linnaeus, 1758	Atlanska jesetra	+	Annex II and IV Habitat Directive, Appendix II of the Berne Convention, CITES	This species has not been registered in the last 35 years.Registered in the river Bojana, which leads to Skadar (Vuković and Ivanović, 1971) and Šasko Lake (Knežević, 1984)
Alosa falax (La Cepede, 1803)	Kubla, fraga		Habitat of the Directive, Appendix III of the Berne Convention	It lives in the Adriatic Sea from where it flows into the Bojana River as well as smaller tributaries such as the Railway near Bar (Hegediš et al., 1997).
Aphanius fasciatus (Valenciennes, 1821)			Habitat of the Directive, Appendix III of the Berne Convention	Inhabits the river Bojana (Hegediš et al., 1997)

Ninnigobius canestrinii (Ninni,1883)	Kanestrinijev glavoč		Habitat of the Directive, Appendix III of the Berne Convention	Synonym: *Pomatoschistus canestrinii*. Morača River, Skadar Lake, Bojana (Stelbrink and Freyhof, 2006)
Anguilla anguilla (Linnaeus, 1758)	jegulja		EU Eel Directive EC No. 1100/2007, CITES	It lives in the river Bojana, Skadar, Šasko lakes as well as small Adriatic tributaries. In the lower reaches of the Ciijevna, Morača and Zeta. (Hegediš et al., 1997; Marić and Milošević, 2011)

2. THE GOAL

This project has the following goals:

1. Determine the length-weight ratio of the glass eel stage in the area of the river Bojana
2. To determine the coding state of the glass eel in the area of the river Bojana
3. Analyze the stage of pigmentation of registered individuals
4. Provide a preliminary assessment of the state of the glass eel in the river Bojana area
5. Register the presence of endangering factors on the glass eel in the area of the river Bojana

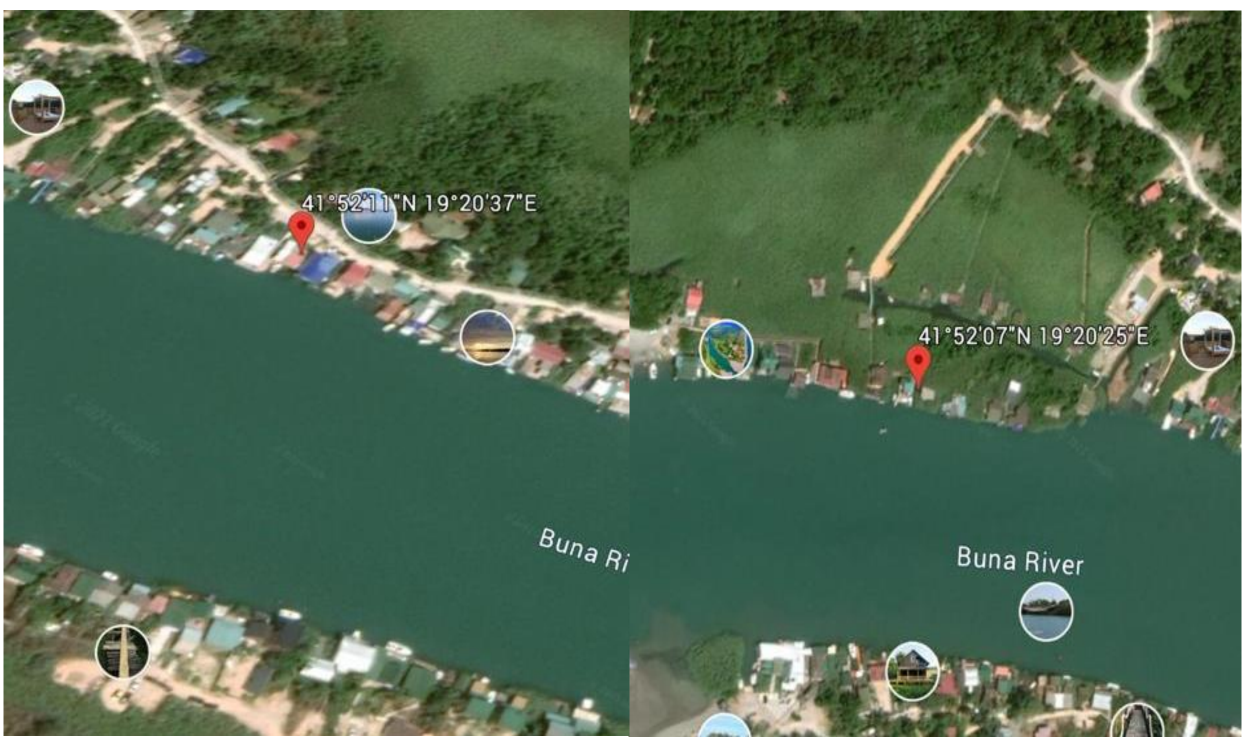
3. Material and methods

3.1. Research area and period

Exploration of the glass eel on the Bojana River was carried out in 2021 and 2022. The research plan foresaw that sampling would begin on February 1, 2021. year, but due to certain technical reasons as well as the fact that the weather conditions were unfavorable, the sampling started with a delay of one month. Therefore, sampling in 2021 was performed in the period from March 1 to June 1, 2021.

In the second year, sampling was performed from January 28 to March 17, 2022. In the first year of the research, sampling was performed on the right branch of Bojana at locations whose selection was made on the basis of position as well as the possibility of good placement and provision of this type of network (Figure 1).

Figure 1. Positions where glass eel nets were set up on the Bojana River during the spring sampling in 2021.



As no significant number of specimens were collected in the first year of sampling, in the second year of research the nets were placed on the river flowing into the Bojana River because during sampling in 2021 it was noticed that it is a more favorable place for sampling (Figure 2).

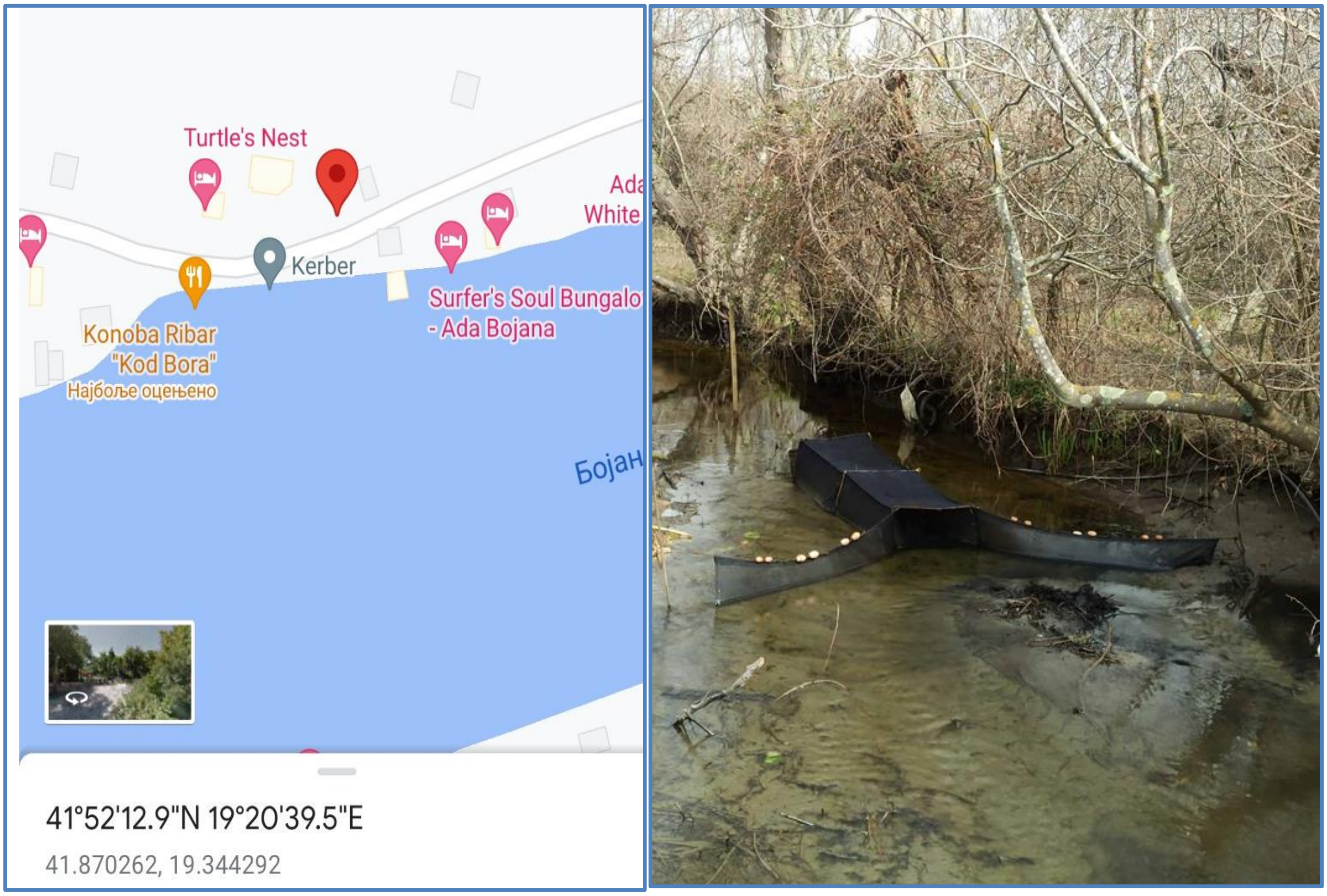


Figure 2. Position at which the nets were placed during sampling 2022 (figure left). Installed nets in the water (picture right)

3.2. Sample collection

Glass eels were collected using two hand-made wing tips and two funnels (three chambers) (Figure 3). The tops are made of aluminum profiles and tulle. The recommendation to use this type of pond as the best tool for catching glass eels was taken from Hegedis, 2007. The pots were placed one by one along the right and left banks of the river, at a depth of about 60 cm (Figure 4).They were placed so that their end was closed and firmly tied to a wooden holder, and then the top was spread to the front opening, which was also fixed with two holders. The total area covered by the front of the bench is 1.2 m2. The pots are emptied twice a week. Some of the caught glass eels were transferred to separate vials, labeled and transported to the laboratory, while some were returned to the water.

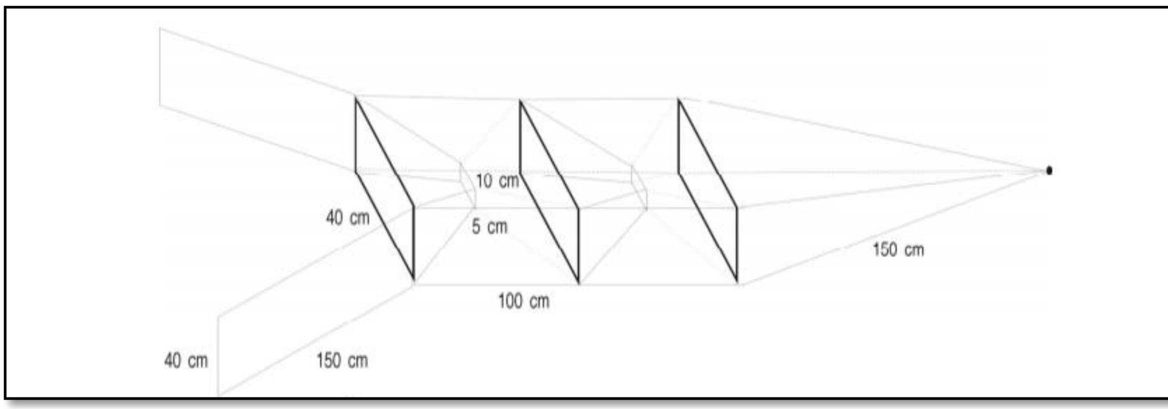


Figure 3. Schematic representation of a glass for eel sampling (according to Hegediš, 2007)

Figure 4. Setting the nets in the field (March 2021)

3.3. Laboratory analyzes:

The following measurements were performed on the sampled individuals: total body length (TL), body weight (W), horizontal and vertical diameters of both eyes. Morphometric measurements were performed with a movable beak "nonius" with an accuracy of 0.02 mm. Body weight (gr), total body length and maximum body height (H) were measured on freshly caught units.

- The pigmentation stage was determined according to Elie et al., 1982.
- Length-to-weight ratio (LWR) is calculated by the formula:

$$W = aTLb$$

where *W* is the total body weight expressed in grams (g), *TL* is the total body length expressed in centimeters (cm), *a* is a constant and *b* is the regression coefficient (allometry factor) (Ricker, 1975).

- To assess the condition of the population, the condition factor (K) was calculated, ie the coefficient of nutrition according to Fulton, using the equation:

$$K = 100(W/TL3)$$

where *W* is the total body weight expressed in grams (g) and *TL* is the total body length expressed in centimeters (cm). The ratio of the conditioning factor and the total body length is calculated by the formula:

$$K = a + bTL$$

4. RESULTS

4.1. Recorded individuals (2021-2022)

During a quarterly survey in 2021, 10 glass eels were recorded. The first individuals were registered on March 12, 2021. and the last ones were caught on April 5, 2021. During April and May (until the beginning of June), no glass eels were registered in the catch. In the second year of the study, 40 glass eels were recorded (Figure 6). The first individuals were registered in the first week of February, while from the end of February to the middle of March, the largest number of individuals was registered (Figure 5). The only intensive research conducted so far on the Bojana River, which was conducted during 1997 and 1998, and then continued at the end of 1999, lists three eel migration waves, namely February-March, March-April and April-May (Hegediš, 2007). From the above, it can be concluded that the period of our research coincides with the above literature data and that this study recorded one migration wave. Although the first year of the research covered the period April and May, our research did not record the other two migration waves.It should not be neglected that in the cited literature, only sampling was performed for a period of five years (cumulatively observed). In addition, we must not ignore the fact that the conditions on the river Bojana in the previous 24 years since the last research have changed significantly, which certainly makes it difficult to catch these valuable individuals.

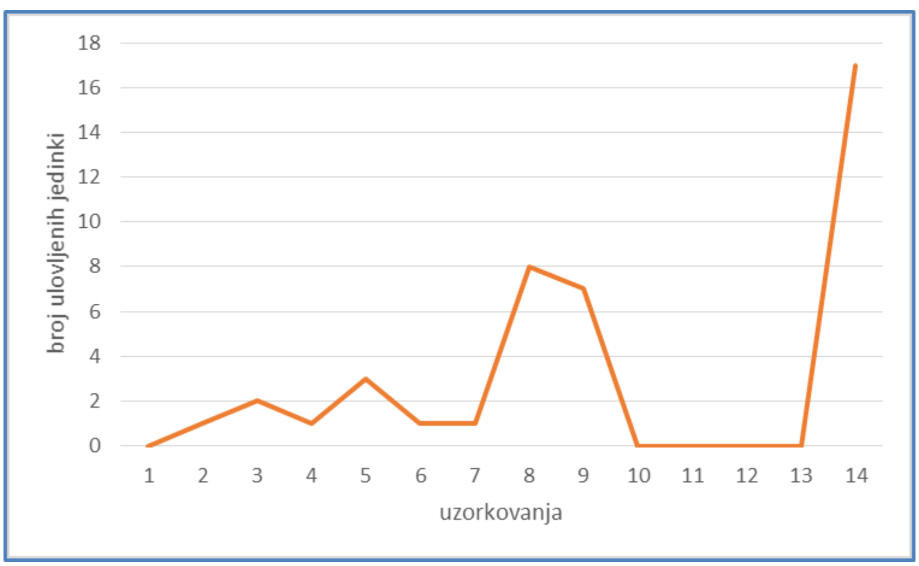


Figure 5. Display of the number of caught individuals during sampling in 2022

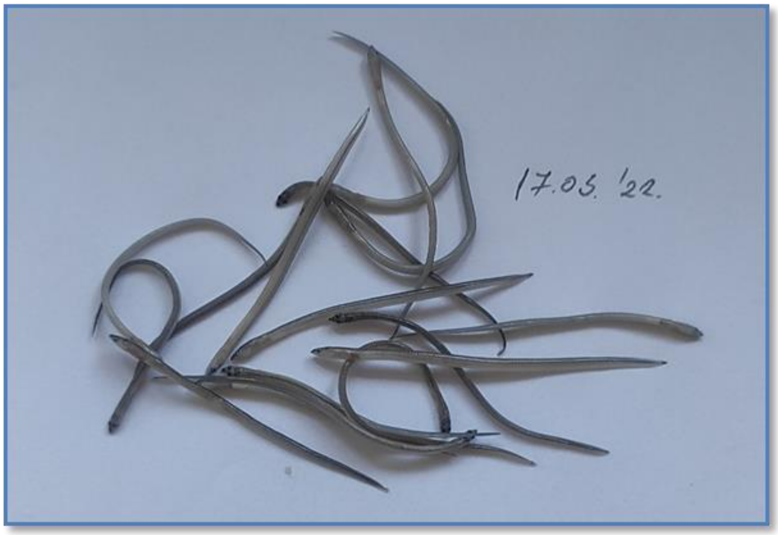


Figure 6. Part of the registered glass eel specimens

4.2. Analysis of body length, body mass and length-weight ratios

As the number of registered individuals during 2021 did not meet the statistically valid number of individuals to make relevant conclusions about their condition and quality, their basic biometric characteristics will be presented below, since these are the first findings and descriptions of these individuals after 24 years; a more detailed analysis was done on a sample from 2022. The average TL of individuals registered during 2021 was 8.8 cm ± 2.52.
The minimum recorded value of TL was 5.9 cm while the maximum registered TL was 11.3 cm. The mean W was 1.12 gr ± 1.01. The minimum recorded value of W was 0.1 gr while the maximum registered W was 2.1 gr. The average TL of individuals registered during 2022 was 2.28 ± 1.25. The minimum recorded value of TL was 5.5 cm while the maximum registered TL was 9.1 cm. The average W was 10.17 gr ± 1.01. The minimum recorded value of W was 0.1 gr while the maximum registered TL was 1.5 gr. Data on biometric characteristics of registered pigment stages during the 2022 survey are shown in Table 2.

Table 2. Biometric characteristics of pigment stages (N-number of individuals, minimum, maximum and mean value of total length (TL) and body weight (W)

Pigment stage	Number of individuals	TL (min-max; average value)	W (min-max; average value)
VI $_{A0}$	15	5.5-6.6; 6.02	0.1-0.2; 0.12
VI $_{A1}$	18	5.8-6.9; 6.22	0.1-0.2; 0.12
VI $_{A2}$	5	5.8-6.6; 6.2	0.1-0.2; 0.12
VII	2	8.1-9.1; 8.6	0.8-1.5; 1.15

As the largest number of individuals was registered in the period from the end of February to the middle of March, an analysis of their average lengths was done. The Hegedish study, 2007, states that the average body length of glass eels during a migratory wave has been successively declining since its onset. Although a small number of individuals of their average length were registered during 2021, they fit into this phenomenon. Namely, the longest specimens were caught in the first days of sampling, while only smaller specimens were caught later. However, sampling results during 2022 did not confirm these results. Analysis of the average body lengths of registered individuals showed that longer individuals were hunted in mid-March than mid-late February when most individuals were registered (Figure 7). Although this study found a statistically valid number of individuals to draw conclusions about the beginning and end of a migration wave, it is necessary to conduct research over a longer period of time, both one year and many years.It should be emphasized that the basic characteristics of juvenile eels, body length and weight, condition coefficient as well as pigment status are of great importance in assessing their condition and quality.

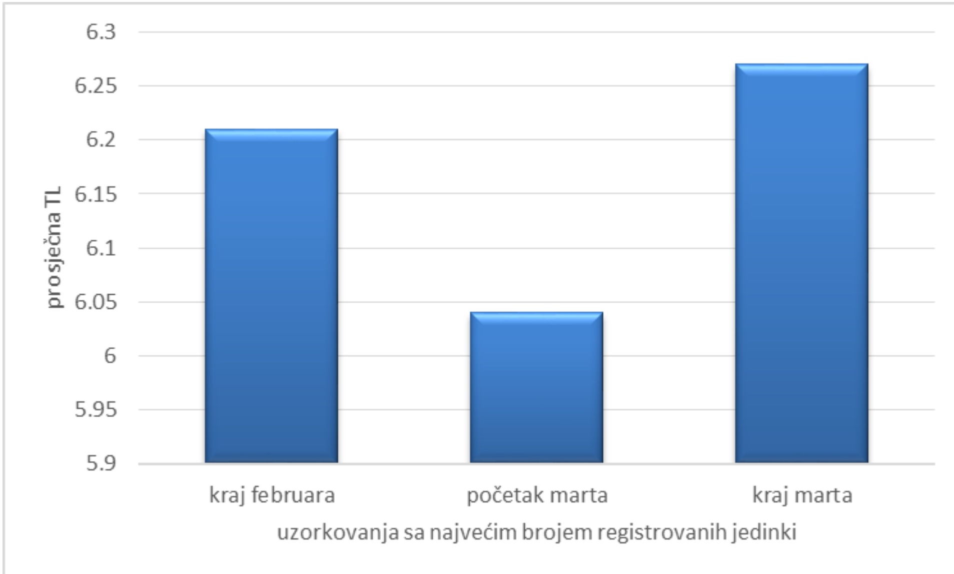


Figure 7. Average body length of individuals during the most numerous sampling

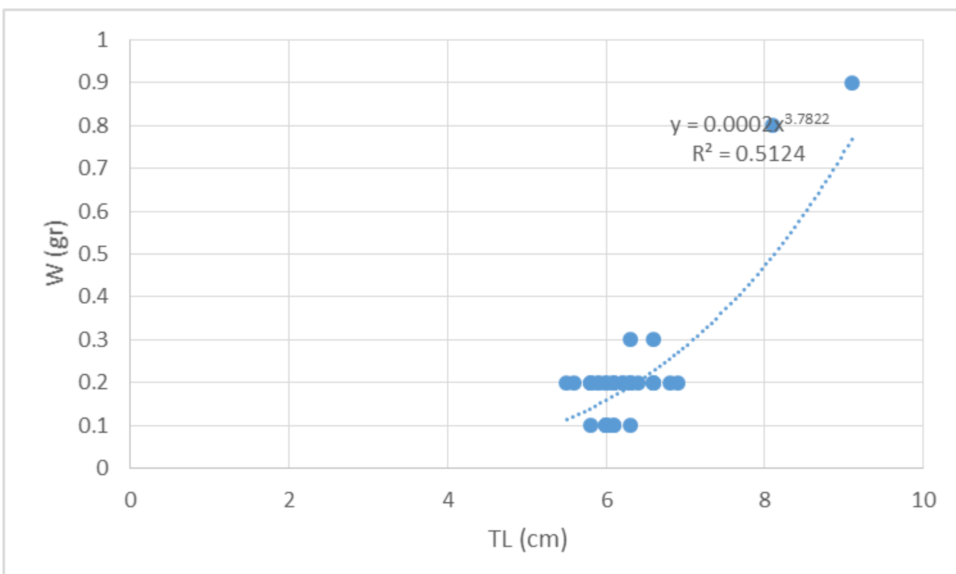


Figure 8.The length-weight ratio of glass eels collected during the period February-March 2022.

As in the case of *TL* in the Hegedish study, in 2007 it was stated that during the first two migration waves, there was a decrease in body weight in successive catches. Unfortunately, at this moment, it is impossible to confirm or refute this phenomenon, since the registered individuals obviously originate from a single migration wave, which can be concluded on the basis of their uniform body weights.

It has been determined that, within each period of life, the weight of an individual fish changes as a degree of function of its length. Therefore, an analysis of the length-weight ratio of glass eels collected during 2022 was performed (Figure 8). The registered value of the coefficient *b* shows that the glass eel is characterized by allometric growth, which is in accordance with the literature data, while the value of R-0.512 indicates a moderate dependence of the variables of length and body weight.

4.3. Analysis of pigment stages

During the research conducted in 2021, two pigment stages were recorded in registered individuals, namely VI A1 and VII (Figures 9 and 10). In the second year of research, four pigment stages were registered: VIA0, VIA1, VIA2 and VII (Figure 11). The following are the basic characteristics of the registered stages:

Stage VI AO

Rostral surface pigment exceeds the heart-shaped spot and is characterized by a more or less intense melanin depot (1 to 3 melanophores behind the heart-shaped spot). Pigmentation of the last gill arch occurs. The dorsal edge shows a narrow pigmented band that reaches the anal region; dorso-lateral pigmentation is always limited to the posterior half of the caudal region, the pigment is clearly distributed along the myoseptum; medio-lateral pigmentation is developed in almost all individuals in the caudal region. The combination of surface rostral and caudal pigmentation has not yet been realized.

Stage VI A1

The head of the individuals of this stage is with rostral surface pigmentation that exceeds the cerebral spot by joining the pigment of the posterior dorsal edge. This dorsal joint has the shape of a narrow strip. The cerebral spot is fully developed and heart-shaped but lacks an anterior semicircle of large stellar melanophores. Dorsolateral pigmentation of the body is developed all the way to the postanal region.

Stage VI A2

The cerebral spot is complete and present in all cases; all gill ports are melanized; dorsal pigmented bands thicker laterally. Dorso-lateral pigmentation developed in the area covered by the beginning of D and the anus; at that point there is a very clear deep pigmentation in the form of multiple bands along the heart, liver, stomach and intestines, all the way to the anus.

Stage VII

Individuals of this stage are characterized by generalized development of cells with yellow pigment and loss of transparency, which in rare cases still survives in the tail region. The abdominal cavity is silvery, the digestive tract is no longer recognizable.

Figure 9: Glass eel (stage VIA1) from the river Bojana

Figure 10: Glass eel (stage VII) from the river Bojana

Figure 11. Pigment stages registered during the 2022 survey.

The analysis of the prevalence of registered pigment stages during 2021 showed that the dominant stage was VI A1 with 70%, while stage VII was represented with 30%. Thus, in the second year of the research, the largest number of registered individuals belonged to stages VI A1 and VIAo (45% and 37.5%), while the other two stages (VIA2 and VII) were significantly less represented (Figure 12).

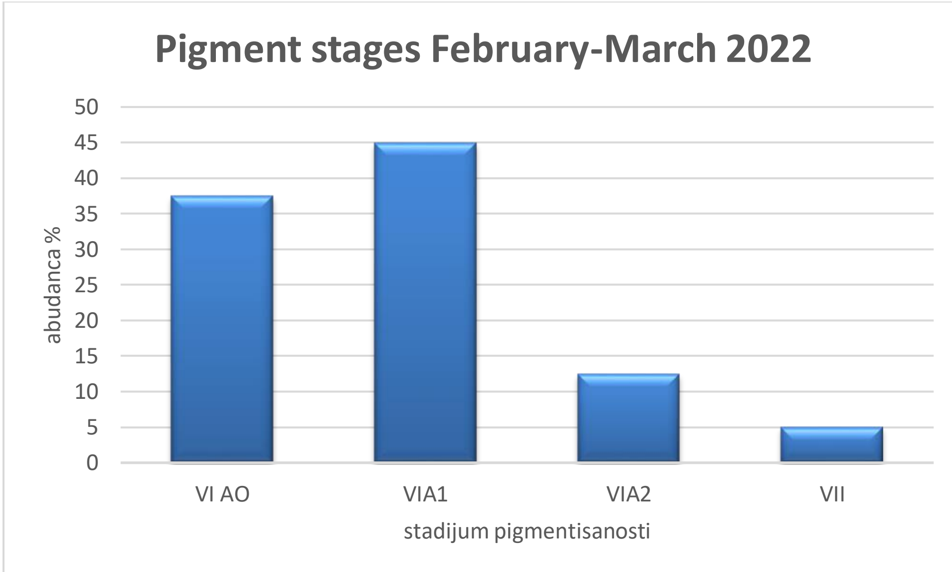


Figure 12. Relative representation of individual pigment stages during the 2022 survey.

The obtained results of this research somewhat coincide with the research of Hegedish, 2007. Namely, in the mentioned research during the first migration wave (February - March), stage VI A1 was dominant, which is in accordance with our results. In addition to this stage, stage VIA0 was represented in a significant percentage. It is interesting that stage VII was not registered in the Hegedish research, 2007, while during our research it was registered on two occasions, on March 12. 2021 and 14-22.02.2022. years.Although the appearance of the final stage of pigmentation (VII) is mentioned in the literature for the third migration wave (April - May), our research showed the presence of these individuals in both sampling years in February and March, which may indicate that monitoring of migration waves should begin. and earlier. We should also not ignore the fact that, although in a small percentage, the finding of stage VIA2 is significant since it is considered the limit of metamorphosis and the animal begins to feed again.

4.4. Condition factor

Considering that the glass eel changes its pigment status and body proportions during migration, one of the important parameters for assessing its quality is the condition factor as well as the connection of the condition factor with the pigment stage and biometric measures (TL and W). On the sample from 2021, it was not possible to do a more detailed analysis of the condition factor by pigment stages due to the small number of individuals. Analysis of the condition factor of individuals collected during 2022 showed that the average value of the condition factor was 0.82 with a range of minimum and maximum values of 0.39-1.55. Figure 13 shows the relationship between the conditioning factor and the pigment stages registered during the recorded migration wave in 2022. The general trend shows that individuals with a lower pigment stage have higher values. What is interesting is that since stage VII there has been a significant increase in this factor, which is in line with the fact that in stage VII glass eels are exogenously fed, which certainly leads to changes in body proportions and explains this trend in fitness factor.

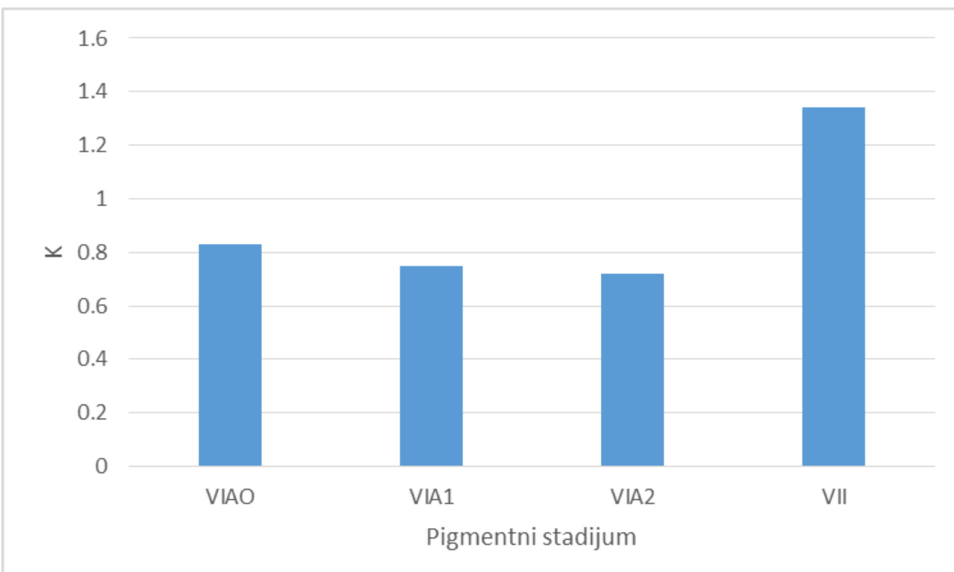


Figure 13. The ratio of the coefficient of condition and pigment stages during the migration wave in February 2022.

4.5. Recorded pressures on the glass eel on the Bojana River

In the area of the river Bojana, there is no tradition of hunting glass eels, which indicates that its mortality is completely dependent on natural predators that eat it at this stage. This research does not include the analysis of the diet of fish species that are its potential predators. Previous research on glass eel suggests that different stages of glass eel have been registered in the gastric contents of *Alosa fallax,* sea bass (*Dicentrarhus labrax*) as well as pond turtles (*Emys orbicularis*) (Hegediš, 2007).However, we should not neglect the anthropogenic factor, which implies the excessive construction of soybeans on the banks of the Bojana in this part of the estuary, which certainly affect the elements of biodiversity, and thus the younger eels.

5. Conclusions

1. During the quarterly (March-June) sampling of the glass eel in 2021 and the sampling in February 2022, 50 individuals were registered in the area of the river Bojana. In 2021, 10 were caught, and in 2022, 40 individuals. The small number of registered individuals during 2021 can be explained by the fact that the research period did not include the month of February, when the largest number of individuals in 2022 was caught. The explanation for the difference in the number of registered individuals during the first and second year of sampling can be explained by the change in the sampling position. The existence of the second and third migration waves was not confirmed by this research, which was to be expected somewhere, considering that this type of research was conducted for the first time after 20 years in significantly changed conditions than the previous ones. However, we can state that one migration wave was recorded during this research.

2. During this research, four pigment stages were recorded: VI Ao, VI A1 VI A2 and stage VII. In the literature data, the appearance of the final stage of pigmentation (VII) is stated for the third migration wave (April - May), while in our research these individuals were registered on March 12, 2021 and February 14 - 22, 2022. Also, we should not ignore the fact that, although in a small percentage, the finding of the VIA2 stage is significant since it is considered the limit of metamorphosis and the animal begins to feed again. The above indicates that in the next research, the monitoring of migration waves should start a calendar earlier. During the recorded migration wave, the VI A1 stage was dominant.

3. The analysis of the length-weight ratio on the units collected during 2022 showed a positive allometric growth. The obtained values of the condition factor indicate favorable conditions for the glass eel in the river Bojana. The analysis of the relationship between the condition factor and the pigment stages showed that individuals with a lower pigment stage have higher values of the condition factor. In stage VII, a significant increase in fitness is observed, which is in line with the fact that in stage VII, glass eels are exogenously fed, which certainly leads to changes in body proportions and explains this trend in the value of fitness factor. However, due to the small number of individuals in

stages VI A2 and VII in future research, this should be taken into account.

4. In the area of the river Bojana, there is no tradition of hunting glass eels, which means that its mortality is completely dependent on natural predators that eat it at that stage. However, the anthropogenic factor, which implies the excessive construction of soybeans on the banks of the Bojana in this part of the estuary, certainly affects the elements of biodiversity, and thus the younger eels.

5. Here are the results of the first research on glass eel in Montenegro after more than 20 years. The overall global situation influenced the fact that in the first year of the research, the work started in March, and in the second year of the research, the research lasted for a month and a half. Nevertheless, significant results were obtained which represent a good basis for further research and justified the original idea of a two-year project. During the research, four pigment stages were registered in good condition. Based on biometric characteristics, we can conclude that one migration wave was registered. Therefore, for a finer assessment of the state of the glass eel, it would be necessary to start the research much earlier (December / January) in order to determine with greater reliability the main periods of migration of the glass eel into the river system. It should be emphasized that due to global climate change, there were probably minor disturbances in the periods of mass appearance of young glass eels, which are completely dependent on sea currents, and their mass occurrences are related to periods of lunar darkness.

6. Having in mind all the above, and in order to get a clearer picture of the number and duration of migration waves, as well as the number and state of the present stages of the glass eel, it would be very important to continue this research.

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