Milošević, D., Mrdak, D., Pešić, A., Ikica, Z., Kovačević, A. (2022). Migration wawes and stage of pigmentation of glass eels from river Bojana (Montenegro). Agriculture and Forestry, 68 (3): 103-110. doi:10.17707/AgricultForest.68.3.08

DOI: 10.17707/AgricultForest.68.3.08

Dragana MILOŠEVIĆ[1*], Danilo MRDAK[1], Ana PEŠIĆ[2], Zdravko IKICA[2], Anastasija KOVAČEVIĆ[1]

MIGRATION WAWES AND STAGE OF PIGMENTATION OF GLASS EELS FROM RIVER BOJANA (MONTENEGRO)

SUMMARY

The endangered European eel biological life cycle consists of five stages of metamorphosis: leptocephalus, glass eel, elver, yellow eel, and silver eel. Montenegro has limited data on biological and population parameters of glass eel e.g. accurate time and the number of migration waves, condition, rate of survival, recruitment success, etc. Since the early 1980s, the occurrence of glass eels in European waters has decreased significantly. Accordingly, after almost 25 years the first research was conducted on glass eel in the river Bojana (Montenegro). This study was done during the winter and spring of 2021 and 2022. Stage of pigmentation and condition were examined on 50 registered individuals of glass eel. Four pigmentation stages: VIA_0, VIA_1, VIA_2, and VII were registered. Its biometric characteristics indicate that one migration wave was registered with the domination of stage VIA_1. This research indicates the necessity of further research on the glass eel, as well as the threats they are facing in the river Bojana and another small river in the Adriatic catchment of Montenegro, with the aim of establishing the management plan for better protection conservation of this important but vulnerable species.

Keywords: European eel, Adriatic catchment, pigmentary stage, condition, migration

INTRODUCTION

The panmictic and catadromous European eel, *Anguilla anguilla* (Linnaeus, 1758) was listed as critically endangered (CR) on the IUCN Red List of Threatened Species in 2008, and remained in the same category by the most recent assessment published in 2020 (Freyhof & Kottelat, 2010; Pike *et al.*, 2020). The endangered European eel biological life cycle consists of five stages of metamorphosis: leptocephalus, glass eel, elver, yellow eel, and silver eel. After hatching in the Sargasso Sea, eel leptocephalus larvae drift with the gulf stream

[1] Dragana Milošević* (corresponding author: draganam25@gmail.com), Danilo Mrdak, Anastasija Kovačević, Department of Biology, Faculty of Natural Sciences and Mathematics, University of Montenegro, Podgorica, Montenegro.
[2]Ana Pešić, Zdravko Ikica, Institute for Marine Biology, University of Montenegro, Kotor, Montenegro.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online
Received: 27/07/2022 *Accepted: 24/08/2022*

(Tesch, 1977) until they reach the continental shelf where the larvae metamorphose into glass eels and migrate up the estuaries and colonize freshwater habitats. The glass eels sense the Earth's magnetic field and use it as a reference compass mechanism to orient (Cresci *et al*., 2017), as well as glass eels at sea swimming oriented towards the azimuth of the moon at the new moon when the moon rose above the horizon and was invisible but not during the other moon phases (Cresci *et al*., 2019). The duration of this migration is still controversial and could range from 7 to 9 months or at least 2 years (Lecompte-Finiger, 1994; Kettle & Hainse, 2006).

In the Adriatic Basin of Montenegro, the European eel occurs in all rivers draining into the Adriatic Sea. Despite their globally and commercial importance the data on the European eels in the Montenegrin coastal region are scarce and mainly concerned with the silver stage (Milošević & Mrdak, 2016; Rakočević *et al*., 2018; Kanjuh *et al*., 2018; Milošević *et al*., 2021; Marić *et al*., 2022). The first and only published data about the characteristics of glass eel migration in Montenegro based on research at the mouth of river Bojana that has been carried out from 1998-2002 was published by Hegediš *et al*., 2005. The Bojana River with the neighboring Porta Milena wetland complex and Lake Šasko represent the hydrologically largest and the most biologically complex aquatic unit in the entire coastal zone of Montenegro and important habitat for the overall stock of European eel in Montenegro.

This study had the following objectives: (1) to establish the number, time, and duration of migration waves of glass eel, and (2) to determine the morphometric characteristics and condition of presented pigmentary stages. The objectives are set in a way to check the parameters that were mentioned after almost 25 years of the latest survey.

MATERIAL AND METHODS

Study area. Bojana River is a 41 km long river situated in the southern part of the Montenegrin coast. It is an outflow of Lake Skadar and flows into the Adriatic Sea, forming the border with Albania in some extent. By its water amount brought into the sea, it is the third river on the Mediterranean (after the river Nile and Po in Italy). The source of the Bojana River lies at the southern part of Skadar Lake, the largest lake in southeastern Europe. Due to the deposition of the sediment conveyed by the Bojana River into the Adriatic Sea for centuries the Ada Bojana island was formed and today presents a unique ecosystem in Europe (Gršić *et al*., 2018; Petković & Sekulić, 2019).

Sampling. Research on glass eel on river Bojana was conducted in 2021 (from February to June) and 2022 (from February to May) (Figure 1).

Glass eels were gathered with the help of a handmade fyke net trap with wings and two funnels (three chambers). Traps were made of aluminum profile and tulle according to Hegediš *et al*., 2005 (Figure 2a). They were set on the right side of the river, at the depth of 60 cm (locations I and II, Figure 1) and on the small stream that flows into Bojana (location III, Figure 1). The surface of the front entering part of the trap was 1.2 m^2. Traps were being emptied two times a week.

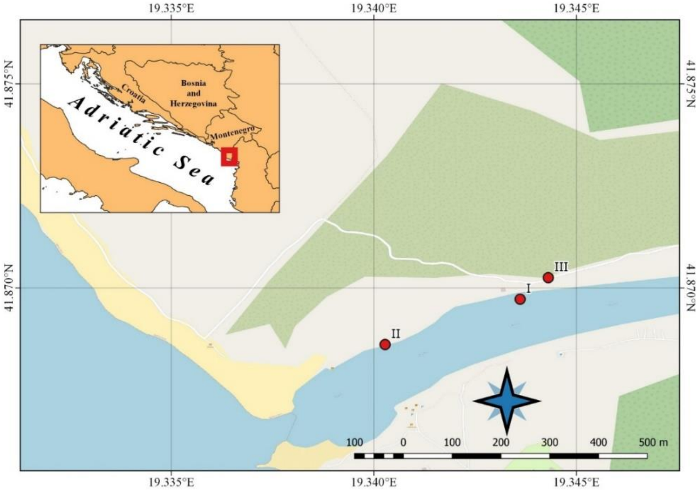

Figure 1. River Bojana and sampling locations of glass eel

The captured glass eel individuals were measured to the nearest 1 mm (total length, TL) and weighed to the nearest 0.1 g (weight, W), and then returned back to the water (Figure 2b). Stadium of pigmentation was determined according to Elie *et al*., 1982. Data of the Fulton's condition factor (CF) of the analyzed specimens was calculated from the W (g) and TL (cm) using the formula according to Fulton (1904).

Figure 2a (left). Fyke net at the sampling locations; **Figure 2b (right).** Glass eels from River Bojana (Montenegro). After measuring the glass eels were returned back to the water

Since the number of registered individuals in 2021 did not meet the statistically valid number of individuals for making relevant conclusions about their condition and quality, their basic biometric characteristics will be presented since these are the first findings and descriptions of these individuals after almost 25 years, and a more detailed analysis was done on a sample from 2022.

RESULTS

During the three-month survey in 2021, 10 glass eel individuals were recorded. The first individuals were registered on March 12, 2021. while the last ones were caught on April 5, 2021. During the months of April and May (until the beginning of June), not a single glass eel was registered in the catch. 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 average 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.

In the second year of the research, 40 glass eel individuals were recorded. The first individuals were registered in the first week of February, while the largest number of individuals was registered from the end of February to the middle of March.

Table 1. The summary of descriptive statistics of observed pigmentary stages of glass eels during 2022 (N-number of individuals, minimum, maximum, and average values of total length (TL in cm) and weight (W in gr), condition factor (CF)

Pigmentary stage	*N*	*TL (min-max; average)*	*W (min-max; average)*	*CF*
VI $_{A0}$	15	5.5-6.6; 6.02	0.1-0.2; 0.12	0.83
VI $_{A1}$	18	5.8-6.9; 6.22	0.1-0.2; 0.12	0.75
VI $_{A2}$	5	5.8-6.6; 6.2	0.1-0.2; 0.12	0.72
VII	2	8.1-9.1; 8.6	0.8-1.5; 1.15	1.34

During the research conducted in 2021, two pigment stages were recorded in registered individuals: VI A_1 and VII. In the second year of the research, four pigment stages were registered: VIA_0, VIA1, VIA_2, and VII. The descriptive statistics of observed pigmentary stages during 2022 sampling are given in Table 1. The analysis of the contributions of registered pigment stages during 2021 showed that the dominant stage was VI $A1_1$ with 70%, while stage VII was represented with 30%. In the second year of the research, the largest number of registered individuals belonged to stages VI A_1 and VI Ao (45% and 37.5%), while the other two stages (VI A_2 and VII) were significantly less represented (13% and 5% respectively). According to biometric characteristics of pigmentary stages, one migration wave was registered with the domination of stage VIA_1.

The average value of the condition factor during 2022 was 0.82 with a range of minimum and maximum values of 0.39-1.55. The relationship between the condition factor and pigmentary stages registered during the observed migration wave in 2022 is given in Figure 3. The general trend shows that individuals with a lower pigment stage have higher values of CF while the increase of CF was observed in stage VII, again.

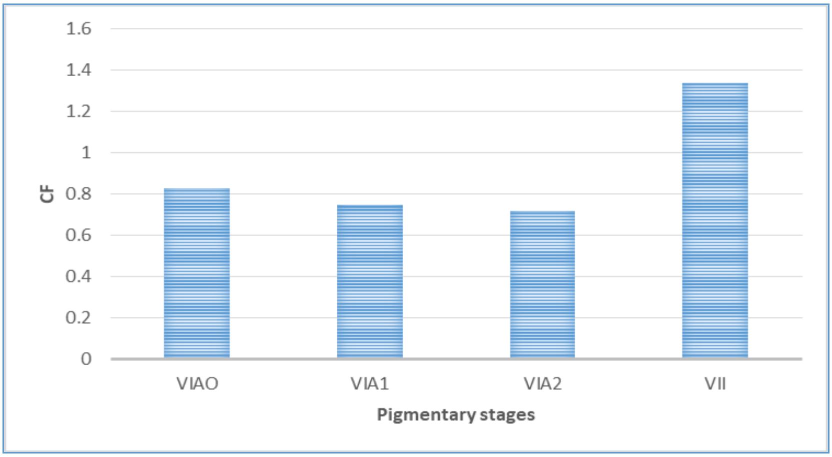

Figure 3. Condition factor of pigmentary stage of glass eel during migration wave in 2022.

DISCUSSION

The catadromous European eel *A. anguilla* reproduces in the Sargasso Sea from March to July (Schmidt, 1922; McCleave *et al*., 1998). In Europe, glass eels enter estuaries mostly from October to April/May (Elie, 1979). Since Montenegro has limited data on biological and population parameters of glass eels for almost 25 years the first research was conducted on glass eels in the river Bojana (Montenegro). It was established that the river Bojana can represent a significant source of high-quality glass eel, which can produce between 320 and 427 kg annually (Hegediš *et al*., 2005).

This study was done during the winter and spring of 2021 and 2022 i.e. from February to May. During the three-month survey in 2021, the first individuals were registered on March 12, 2021. while the last ones were caught on April 5, 2021. In the second year of the research, the first individuals were registered in the first week of February, while the largest number of individuals was registered from the end of February to the middle of March. In studies conducted 25 years ago in the same river, three migration waves were shown (Hegediš *et al*., 2005). The first wave occurred at the end of February and the beginning of March. The second wave occurred at the end of March and the beginning of April, while the third migration wave occurred at the end of April (Hegediš *et al*., 2005). Although the research presented in this paper covered the same research period, our research did not record the other two migration waves. It should not be overlooked that in the cited literature data, the sampling was carried out over a period of five years (cumulatively observed). In addition, the fact that the conditions on the Bojana River have changed significantly in the previous 24 years since the last survey was conducted. Two disastrous floods in 2009–2011 have significantly altered the bifurcation of the Bojana River, by forming a barrier of tree trunks and sediment bars in the right river reach. The civil engineering solution for restoring the flow capacity, based on dredging sand bars, barrier removal, and bank consolidation, is opposed by environmentalists on the ground that these works are detrimental to the unique ecosystem (Petković & Sekulić, 2019). On the other hand as a result of global warming, the Sub Tropical Gyre in the Sargasso Sea inhibits thermocline overturn in the spring (Knights, 2003). Also, the sea warming in the eel spawning area since the early 1980s has modified marine production and affected the survival rate of European eels in early life (Bonhommeau *et al*., 2008) which consequently decrease larval growth and survival. Results of the study Borges *et al*., 2019 suggest a reduction in glass eel survival under a 4°C temperature increase. Decreased survival likely reflects lower body condition, which is considered a major cause for eels not to venture to full riverward migration (Edeline *et al*., 2006; Edeline *et al*., 2009). Thus, less amount of lipids would be available for glass eel energy stores during metamorphosis and this could lead to an increase in the estuarine settlement process (Bureau Du Colombier *et al*., 2007).

During the research conducted in 2021, two pigment stages were recorded in registered individuals: VI A_1 (70%) and VII (30%). In the second year of the research, four pigment stages were registered: VIA_0, VIA_1, VIA_2, and VII. In the second year of the research, the largest number of registered individuals belonged to stages VI A_1 and VI Ao (45% and 37.5%), while the other two stages (VI A_2 and VII) were significantly less represented (13% and 5% respectively). According to biometric characteristics of pigmentary stages, one migration wave was registered with the domination of stage $VIA_{1.}$ The obtained results of this research somewhat coincide with the research of Hegediš *et al*., 2005. Namely, during the first migration wave (February - March), stage VI A_1 was dominant, which is in agreement with our results. Apart from this stage, stage VIA_0 was represented in a significant percentage. It is interesting that stage VII was not registered in the research of Hegediš *et al*., 2005, while during our research it was registered on two occasions, on 12.03. 2021 and 14-22.02.2022. Although the appearance of the final stage of pigmentation (VII) is reported in the literature for the third migration wave (April - May), our research showed the presence of these individuals in both years of sampling, in February and March, which indicates that the monitoring of migration waves should begin and before at the latest in December. Different authors give preference to different environmental parameters during the determination of the temporal and spatial profile of the migration. The most frequently associated factor is the tidal regime combined with the changes in water temperature, tidal regime, lunar cycle, and other environmental factors (Hegediš *et al*., 2005). Also, migration can be influenced by internal as well as environmental factors such as estuarine pollution, and temperature and salinity fluctuations. Relationships between CF and pigmentary stages show that individuals with a lower pigment stage have higher values. Further from stage VII, a significant increase in this factor is observed, which is in agreement with the fact that glass eels are fed exogenously at stage VII, which certainly leads to changes in body proportions and explains this trend in the value of the condition factor.

CONCLUSION

Taking in mind the surface of the inland Skadar Lake-Drim-Ohrid Lake system, delta of River Bojana is one of the most important area for the eel population in this part of the north-western Mediterranean. The presented result provide important data which, once again, confirms that delta of the Bojana river is an important nursery area for the young eels where this fish not only enter into the Skadar Lake- Drim-Ohrid Lake system but also implies the fact that this is the place where young eels undergo metamorphosis to the stage on which it starts with active feeding.

ACKNOWLEDGMENTS

The data for this paper were collected in the frame of Green Home project "Estimation of condition of the glass eel in the River Bojana" financed by the Critical ecosystem Partnership Fund and Bird Life International.

REFERENCES

Bonhommeau, S., Chassot, E. & Rivot, E. (2008). Fluctuations in European eel (*Anguilla anguilla*) recruitment resulting from environmental changes in the Sargasso Sea. Fisheries Oceanography, 17 (1), p. 32-44.

Borges, F.O., Santos, C.P., Sampaio, E., Figueiredo, C., Paula, J.R., Antunes, C., Rosa, R. & Grilo, T.F. (2019). Ocean warming and acidification may challenge the riverward migration of glass eels. Biol. Lett. 15: 20180627. http://dx.doi.org/10.1098/rsbl.2018.0627

Bureau Du Colombier, S., Bolliet, V., Lambert P. & Bardonnet, A. (2007). Energy and migratory behavior in glass eels (*Anguilla anguilla*). Physiology and Behaviour 92 (4). 684-90. DOI: 10.1016/j.physbeh.2007.05.013

Cresci, A, Paris CB, Durif CMF, Shema S, Bjelland RM, Skiftesvik AB, Browman HI. (2017). Glass eels (*Anguilla anguilla*) have a magnetic compass linked to the tidal cycle. Sci. Adv. 3, 1–9. (doi:10.1126/sciadv.1602007)

Cresci, A., Durif, C.M., Paris, C.B., Thompson, C.R.S., Shema, S., Skiftesvik, A.B. & Browman, H.I. (2019). The relationship between the moon cycle and the orientation of glass eels (*Anguilla anguilla*) at sea. R. Soc. open sci. 6: 190812. http://dx.doi.org/10.1098/rsos.190812

Edeline, E., Lambert, P., Rigaud, C. & Elie, P. (2006). Effects of body condition and water temperature on *Anguilla anguilla* glass eel migratory behavior. J. Exp. Mar. Biol. Ecol. 331, 217– 225. (doi:10.1016/ j.jembe.2005.10.011)

Edeline, E., Dufour, S. & Elie, P. (2009). Proximate and ultimate control of eel continental dispersal. In Spawning migration of the European eel, pp. 433 – 461. Dordrecht, The Netherlands: Springer.

Elie, P. (1979). Contribution à l'étude des montées de civelles *d'Anguilla Anguilla* L., dans l'estuaire de la Loire: Pêche, écophysiologie et élevage. Thèse de 3ème Cycle Université Rennes I. p. 383.

Elie, P., Lecomte-Finiger, R., Cantarelle, I. & Charlon, N. (1982). Dèfinition des limites diffèrents stades pigmentaries durant la phase civelle d'*Anguilla anguilla* L. Vie Milieu, 32 (3): 149-157.

Freyhof, J.& Kottelat, M. (2010). *Anguilla anguilla*. The IUCN Red List of Threatened Species 2010. (Accessed September 3, 2016). http://www.iucnredlist.org/details/60344/1.

Fulton, T.W. (1904). The rate of growth of fishes. 22ndAnnual Report of the Fishery Board of Scotland 1904. 3, pp. 141–241.

Gršić, N., Popović-Đorđević, J. B., Kostić, A. Ž., Dojčinović, B. & Pantelić, N. Đ. (2018). Alkali and Alkaline Earth Metals in Water – Case if Study of the Bojana River, Montenegro. Agric For, 64 (4), 145.

Hegediš, A., Kalauzi, A., Mićković, B., Nikčević, M. & Anđus, K. R. (2005). Modeling of the migration of the European glass eel. Ann. N. Y. Acad. Sci. 1048: 85-91. doi: 10.1196/annals.1342.008

Kanjuh, T., Mrdak, D., Piria, M., Tomljanović, T., Joksimović, A., Talevski, T. & Milošević, D. (2018). Relationships of otolith dimension with body length of European eel *Anguilla anguilla* (Linnaeus, 1758) from Adriatic catchment of Montenegro. Acta Adriat. 59 (1), 91–96.

Kettle, A.J. & Haines, K. (2006). How does the European eel (*Anguilla anguilla*) retain its population structure during its larval migration across the North Atlantic Ocean? Can J Fish Aquat Sci 2006;63:90–106.

Knights, B. (2003). A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. Sci. Total Environ. 310, 237 – 244. (doi:10.1016/S0048-9697(02) 00644-7)

Lecompte-Finiger, R. (1994). Contribution de l'otolithométrie à l'étude de la dynamique de la migration larvaire de l'anguille européenne Anguilla anguilla. Bull Fr Peche Piscic;335:17–31.

Petković, S. & Sekulić, G. (2019). Erosion and Sedimentation Processes in the Bojana River Delta at the Adriatic Sea. J Coast Conserv, 23, 39

Marić, D., Burzanović, K. & Marić, S. (2022). Variability of length-weight relationship and condition factor of the European eel (*Anguilla anguilla* L.) – case study from the Lake Skadar (Montenegro). Agriculture and Forestry 68 (2): 175-191. doi:10.17707/AgricultForest.68.2.13

McCleave, JD, Brickley, PJ, O'Brien, KM, Kistner, DA, Wong, MW, Gallagher, M, & Watson, SM: (1998). Do leptocephali of the European eel swim to reach continental waters? Status of the question. J Mar Biol Assoc UK 1998; 78, 285–306.

Milošević, D. & Mrdak, D. (2016). Length-weight relationship of nine fish species from Skadar Lake (Adriatic catchment area of Montenegro). J. Appl. Ichthyol. 32, 1331–1333.

Milošević, D., Bigović, M, Mrdak, D., Milaševic, I & Piria Marina (2021). Otolith morphology and microchemistry fingerprints of European eel, *Anguilla anguilla* (Linnaeus, 1758) stocks from Adriatic Basin in Croatia and Montenegro. Science of Total environment 786: 147478 https://doi.org/10.1016/j.scitotenv.2021.147478

Pike, C., Crook, V. & Gollock, M. (2020). *Anguilla anguilla*. The IUCN Red List of Threatened Species 2020: e.T60344A152845178 https://doi.org/10.2305/IUCN.UK.2020-2.RLTS. T60344A152845178.en (Downloaded on 18 February 2021).

Rakočević, J., Šuković, D. & Marić, D. (2018). Distribution and relationships of eleven trace elements in muscle of six fish species from Skadar Lake (Montenegro). Turk. J. Fish. Aquat. Sci. 18, 647–657. https://doi.org/10.4194/1303-2712-v18_5_01.

Schmidt, J. (1922). The breeding places of the eel. Phil Trans R Soc; 211: 179–208.

Tesch, F.W. (1977). The eel, 5th edn. Oxford, UK: Blackwell Publishing.