GREECE

Methodology and Data

Quality Assurance Framework

for anadromous and catadromous species

National Data Collection Programme

2020

Contents

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1. Introduction

1.1. General Information

European eel (*Anguilla anguilla* Linnaeus 1758) has an unusual and complex life cycle (Tesch 2003). Depending on the life stage (leptocephalus, glass, yellow and silver eel), this species occurs in different habitats along Europe (Tesch 2003). In the 1990's a decline in the European eel's population had been observed (Castonguay & Durif 2016, Aschonitis *et al.* 2017a, Drouineau *et al.* 2018). Factors contributing to the reduction of existing eel population include overfishing, habitat loss, pollution, parasitism, increased migration barriers etc. (Castonguay & Durif 2016, Aschonitis *et al.* 2017a, Drouineau *et al.* 2018).

Since 2008, European eel has been characterized as Critically Endangered by IUCN (International Union for Conservation of Nature) and its trading and shipping are falling under the CITES regulations, as it is included in the species list on the Appendix 2 (Jacoby & Gollock 2014). Also, European eel has been included in the European Union's fisheries data collection regulations (Council Regulation 1543/2000 and Commission Regulations 1639/2001, 1581/2004).

The Data Collection Framework of the EU (DCF EU MAP) requires the collection of data on biological parameters and landings (commercial and recreational) of European eel, in its expansion range in the European continent. For this purpose, different methodological approaches have been developed concerning the monitoring of the commercial and recreational eel fishery in inland waters, through years among the EU countries.

1.2. Regulations about eel fishery in Greece

Greece is one of the first EU countries that have instituted measures for the eel fishery in early 1970. Specifically, according to the Royal Decree 142/1971 both fishing and the commercial exploitation of eels smaller than 30 cm, is entirely prohibited. In 2007, by the Article 7 (5) of Regulation No 1100/2007, the eel fishery was further limited. Recreational eel fishery is strictly prohibited throughout the year all over the country, while commercial eel fishery is allowed only in lagoons during the reproductive migration of the species from November 1st of each year until January 31st of the following year (HEMP 2009). Eel fisheries is allowed only for commercial purposes in rivers after issuing a special fishing permit by the General Fishery Department and the fishermen are obliged to declare the quantities of captured eels and the catch area to the Regional Authorities. Data are communicated to the Ministry of Rural Development and Food and stored in a database.

The Greek Management Plan was formulated and approved in 2009 (HEMP 2009). Since 2017

European eel was included in the DCF and the Greek Working Plan for eel initiated as a pilot study. Within the framework of the Hellenic Eel Management Plan, four Eel Management Units have been defined (HEMP 2009). In each of these areas, EU MAP requires the collection of data on landings and biological parameters of eels. It should be noted that Greek EMUs were initially coded with a number, 1 to 4, which was changed into a new coding system used in all Data Calls from General Fisheries Commission for the Mediterranean (GFCM) and Joint ICES/EIFAC/GFCM Working Group on Eels (WGEEL), i.e. EMU-1 is GR_NorW, EMU-2 is GR_WePe, EMU-3 is GR_EaMT and EMU-4 is GR_CeAe.

The majority of eels are caught in the lagoons, in North and Western Greece. The regional authorities are responsible for the management of these lagoons, while some belong to the Ministries of Development and Economics and others to local municipalities. In any case, the economic exploitation of the lagoons is performed for a certain period of time by fishing cooperatives, which lease the lagoons (in most cases for 10 years). The local fishing cooperatives have the exclusive right to exploit the fishes of the lagoons (Koutrakis *et al.* 2007). According to the EU Directive 1100/2007/, the lessees of the lagoons are obliged to release the 30% of the annual eel landings for reproductive migration and to promptly inform the fisheries service for the upcoming fishing eels, so that there is applicability of the proposed procedures (HEMP 2009).

The release of the eels takes place under the supervision of a representative of the Department of Fisheries of the Administrative Region, to which the respective lagoon belongs. The main biological parameters (length and weight) of these eels are recorded. The release of the 30% of eel production is validated with the issuance of the «Eel Release Certificate», which is a prerequisite for the legal marketing and movement of eels within the EU Member States (MD 643/39462/01-04-13).

2. Sampling plan

For the data collection for each life stage of the species, a different methodological approach (sampling plan) is applied, following the already used methodologies for eel samplings by other EU countries.

2.1. Sampling plan for glass eels

Since 2018, there was an effort to catch glass eels performed by implementing two types of traps made specifically for this purpose (Fig.1A & 1B). The first one, are pipes specially designed to create a favorable habitat for glass eels inside them, that offers shelter and protection from predators (Fig.1A). While, the water is renewed inside the traps They are placed near reed beds and in rocky substrates with flowing waters.

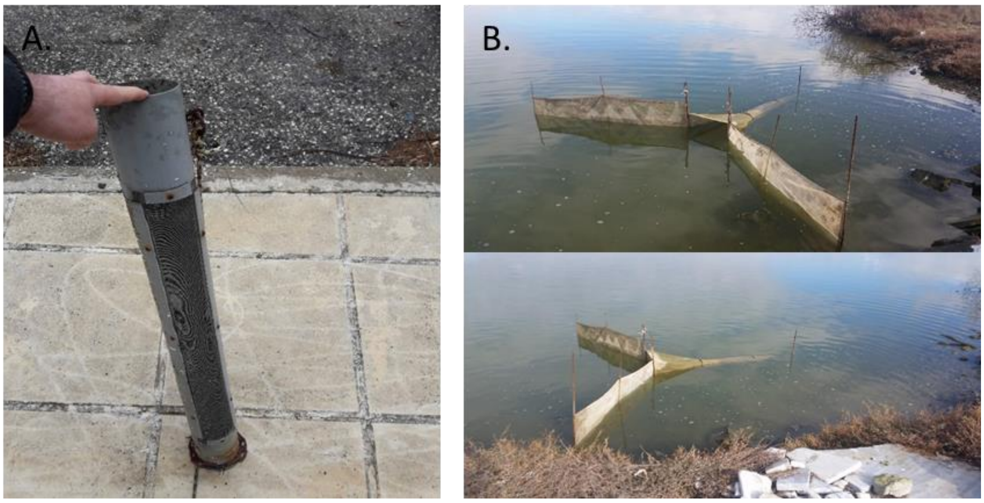

Figure 1. Types of traps used to catch glass eel samples:
A. pipes and
B. fyke nets for glass eels.

The second type of trap used for capture glass eels are fyke nets (Fig. 1B). These special made fyke nets have 1.5 mesh size, a cylindrical shape with three spaces and two vertical sections of netting (wings), on either side of the mouth, which guide the glass eels into the net. They are placed at the entrance of the lagoons or at the river mouths.

Both these gears were placed at different stations in the streams of the river Nestos and in the Lagoon of Porto Lagos during the period of glass eel's migration to coastal and freshwater habitats, and they were checked once per week. The number of these traps depends on the specific characteristic of each estuarine system. However, none of the above methodologies were successful and it was decided to discontinue any further effort to capture glass eels.

2.2. Sampling plan for yellow eels

The sampling plan for the yellow eel standing population, which occur in lagoons or lakes, includes the use of fyke nets (Fig.2). These fyke nets differ from those used for capture glass eels, as they are two cylindrical shape structures with three compartments joined by a leader. The number of fyke nets used depends on the total area covered by each ecosystem.

Figure 2. Types of traps used to catch yellow eel samples.

The first implementation of this methodology in Greece took place in Lake Vistonida (total surface of 45 km). 20 fyke nets, with 22 mm mesh size and total length almost 172 m, used consecutively and placed in random stations. Their position changed every week in order to cover the whole area of this lake. The captured yellow eels were stored in refrigerators and transported to the laboratory for further analysis (measurements of biological variables, abundance, etc.). The same methodology will be applied in the other EMUs (one each year), in order to have enough data to estimate the standing population in Greece.

2.3. Sampling plan for silver eels

Regarding the silver eels, all data and samples come from the commercial fisheries in lagoons, from each EMU. As already mentioned, eel fishery is only allowed to be performed in lagoons by the local Fishermen Cooperatives, who are responsible for the lagoon exploitation. Fishing in the lagoons is based on the use of fixed barrier traps (Fig. 3), concrete constructions, which are installed at the interface between the lagoon and the sea and cover all the width of the connection channel. They are passive, V-shape traps, fixed gears which catch all migrating fish, eels in particular, during their seasonal and reproductive migration (for more details see Ardizzone *et al.* 1988, Koutrakis *et al.* 2007).

Figure 3. Permanent fish entrapment devices, which catch live fish as they move seawards (Vassova and Erateino Lagoons, Nestos Delta). The arrow shows the direction of fish movement (Source: E.T. Koutrakis).

In these traps, all migrating silver eels are caught during their reproductive spawning migration. Then, the captured silver eels are stored live in adequate infrastructure until their sale is complete. Initially, Greek NWP proposed the collection of 200 samples for every 20 t of silver eel landings from each EMUs. The samples from each area are stored in refrigerators and transported to the laboratory for further analysis (measurements of biological variables, abundance, etc.).

For the period 2020 – 2021 a new sampling scheme was adopted in the Greek NWP following the already used methodology by other countries. This methodology is spatially stratified based on the assessment of all EMUs', where eels are present, in a three-year period. Due to the critical condition of the stock and the already collected data, only 100 samples of the species will be collected as part of the biological sampling, from a different EMU every year, i.e. for 2020, 100 samples will be collected from EMU 3 and in the next year 2021 the number of samples will be collected from another EMU.

3. Methodology for Age determination

The age determination of eels was conducted according to the European Protocol of Age Assessment of ICES (2009), using otoliths of eels and not scales, which was modified accordingly by FRI in an effort to develop a methodology, which will result in the better resolution of the annual rings. The otoliths were processed according to the “modified Crack & Burn protocol by FRI”, following the steps below (Papadopoulou *et al*. 2019):

1. Drying otoliths in an oven.
2. Cracking of the otolith as close as to center of the nucleus.
3. Burn both pieces on a hotplate at 400°C.
4. Let them cool down to room temperature.
5. Cleaning and polishing of the broken face of the otolith using a 1000 and 2000 grit grinding paper.
6. Etching of the broken face with 1-2 drops of 1% HCl
7. Repeat steps 3-6 until the desired result, the emergence of the otoliths annual rings.
8. Observation of the otoliths under stereoscope immersed in glycerin.
9. Photo shooting

A similar methodology was developed for the age determination of *Capros aper* (Linnaeus, 1758) (boarfish) (Vagenas *et al*. 2021).

The age of the studied population (both yellow and silver eels) was determined from the photographs of the processed otoliths in Software to Work with Microscope Images. Otoliths were read by two independent observers. The uncertainty in the readings was addressed using the Otolith Uncertainty Index (OUI) according to Durif *et al.* (2020). Each age corresponded to one OUI that shows how much the observations differed between observers:

1. OUI level 1: differences <3 years
2. OUI level 2: differences between 3 and 5 years
3. OUI level 3: differences of more than 5 years.

In cases where the OUI level was higher than 1, the samples were re-evaluated or removed from the study.

4. Fecundity analysis

The determination of eel's sample fertility was carried out according to Barbin & McCleave (1997) and MacNamara & McCarthy (2012). The gonads were examined macroscopically for the sex determination (Tesch 2003) and were treated according to the protocols described by MacNamara & McCarthy (2012). The length of pectoral fin, the eye diameter and the weight of the gonads were measured (to the nearest 0.01 mm and 0.01gr). These measurements were taken to confirm the silvering stages (Durif *et al.* 2009) of each specimen of the sample, based on the GSI and Eye index.

Gonadosomatic index (GSI):

$$GSI = (\text{gonad weight} / \text{body weight}) \times 100$$

Eye index (Pankhurst 1982):

$$\text{Eye index} = [\{(\text{right horizontal eye diameter} + \text{right vertical eye diameter}) / 4\} \times (\text{left horizontal eye diameter} + \text{left vertical eye diameter}) / 4\} \times (\pi / \text{body length})] \times 100$$

To ensure eels were sufficiently mature, and to facilitate comparison with other studies (i.e. MacNamara & McCarthy 2012), only eels with an eye index >6.5 (Pankhurst, 1982) and GSI >1.2% (MacNamara *et al.* 2014) were considered for fecundity analysis.

The above methodology was used to estimate eel's sample fertility from Lake Vistonida by MacNamara *et al*. (2014).

For the time being and according to WP approved by EU, the estimation of fecundity is not required in the following years. But in case it is requested, the above methodology will be used for this purpose.

5. Morphometric relationships

Two types of morphometric relationships were examined, the length-age relationship (LAR) and the weight-length relationship (WLR) separately for males and females.

In the case of LAR, the following type of von Bertalanffy growth equation was used (Castaldelli *et al.* 2013):

$$L = L_\infty(1-e^{-kX})+L_0e^{-kX}$$

where L_∞ (cm) and k (year^{-1}) are regression coefficients. The parameter L_0, which describes the length of elvers at metamorphosis from the glass stage, either it is estimated by regression analysis or it is assumed constant at 7.5 cm according to De Leo & Gatto (1996). In the case of a large and well distributed dataset to various length and age classes, it is suggested to use L_0 as a third regression coefficient. In the case of a poor dataset, it is suggested to be used as a constant equal to 7.5 cm. The latter case is used to avoid deriving values for the regression coefficients, which deviate from their physical meaning due to the large flexibility of von Bertalanffy's equation.

In the WLR case, the following typical power function was used:

$$W = aL^b$$

where a and b are coefficients. Log-log plots of length and weight values were created for visual identification and removal of outliers before the fitting procedure (Froese 2006, Liu *et al.* 2013). In the case where Log-log plots indicated bimodal trends, the LinBiExp function of Buchwald (2007) was used as proposed by Lanzoni *et al.* (2018). LinBiExp function can detect changes in growth rates due to the combination of ontogenetic diet shift and other metabolic processes (e.g., beginning of sexual maturation) or other abrupt environmental changes. The LinBiExp provides smooth and fully parametrizable transitions between two linear segments maintaining a clear connection between them. The specific function is fitted on the log-transformed W, L variables and is given by the following function (Buchwald 2007):

$$W' = f(L') = c \cdot \log\{\exp[a_1(L' - L'_t)c^{-1}] + \exp[a_2(L' - L'_t)c^{-1}]\} + d \quad \text{for } c \neq 0$$

where W' and L' are the log-transformed weight [log(g)] and length [log(cm)], a_1 and a_2 are coefficients that regulate the slopes of the two linear segments, c is a parameter for adjusting the smoothness/abruptness of the transition and the form of angle between the two linear segments, d is a constant for shifting the curve along the vertical axis (log-W axis), and L_t' is a constant that defines the break point between the two linear segments at horizontal axis (log-L axis). The transition between the two linear segments does not require a sharp break-point, it can take place along a smooth, continuously differentiable, curved portion of adjustable width (with the deviation from linearity having an exponential character). Nevertheless, a model should be considered bilinear only if it shows linearity at both ends of its considered range (Buchwald 2007). Positive values of c coefficient indicate that the angle above the two linear segments is < 180° while negative c values indicate that the respective angle is > 180°. The larger the absolute value of c is, the smoother is the transition between the two linear segments. The c coefficient is generally sensitive and a large variation of its values only in a positive or a negative range just affects the steepness in the transition between the two linear segments. For this reason, a high statistical significance for the case of c coefficient is not required. The angle is regulated by the a_1/a_2 ratio and when it is equal or close to 1 indicates that bilinearity hardly exists. The only difference between the two exponent factors inside LinBiExp is the value of a_1 and a_2 coefficient and thus exchanging their values does not change the curve (e.g., for a_1 = 1 and a_2 = 4 or a_1 = 4 and a_2 = 1, the curve is the same). The coordinates of the breakpoint in the log(W)–log(L) plot are given by the following functions:

$$\text{for log-}L\text{ axis:} \quad x' = L_t'$$

$$\text{for log-}W\text{ axis:} \; y' = W_t' = c \cdot log(2) + d$$

The respective coordinates of the breakpoint in regular weight–length curves after removing the logarithmic transformation are provided by:

$$x = L_t = 10^{x'}$$

$$y = W_t = 10^{y'}$$

Regression analysis in both LAR and WLR cases was performed using bootstrap non-linear regression (Boot-NLR). Boot-NLR is based on the generation of a large number of new datasets by randomly sampling data with replacement (Efron and Tibshirani 1994) and it is considered among the most robust methods for assessing the variability of regression coefficients. Boot-NLR was performed for 1,000 iterations in R software using the "nls.lm" function of the {minpack.lm} package (Elzhov *et al.* 2016), which includes the Levenberg-Marquardt nonlinear least-squares algorithm. The range of 1000 solutions of each regression coefficient was defined by the 95% confidence interval of the highest posterior density (HPD) distribution. The 2.5% and 97.5% thresholds (HPD thresholds), which contain the central 95% interval of the HPD distribution, were estimated in R software using the "p.interval" function of the {LaplacesDemon} package (Bernardo 2005), which returns unimodal or multimodal HPD intervals, depending on the form of the probability distributions.

6. Eel Population Dynamics Model

The eel population dynamics were described using the model of Aschonitis et al. (2017b), which is an improved version of the old model of Gatto & Rossi (1979), which also includes a method for correcting fyke net selectivity provided by Bevacqua *et al.* (2009).

6.1. Correction of fyke net effects in yellow eel samplings

The small age class specimens can escape from fyke nets introducing error in the frequency distribution of individuals per age class of yellow eel population. The frequency of yellow eels in each age class i is defined as the ratio n_i/N (number n of yellow specimens in age class i versus the total number of yellow specimens N). The error was corrected using the method of Bevacqua *et al.* (2009) based on the calculation of fyke net selectivity φ, which is estimated as a function of eel body length and net mesh size using the following equation:

$$\varphi(L,m) = \{1 + exp[-\eta(m) \cdot [A(L) - A_{50}(m)]\}^{-1}$$

where L is body length (mm), m is the mesh size (mm), $\eta(m)$ is a shape parameter expressed as a function of the fyke net mesh size (mm^{-2}), $A(L)$ is the section size of the fish trunk expressed as a function of body length (mm^2), and $A_{50}(m)$ is the trunk section at 50% selectivity expressed as a function of the fyke net mesh size (mm^2). The functions of $\eta(m)$ and $A_{50}(m)$ are estimated by the following equations (Bevacqua *et al.* 2009):

$$\eta(m) = exp(-0.06m - 1.65) \quad \text{and} \quad A_{50}(m) = exp(0.09m + 3.26)$$

where m is mesh size (mm). The trunk section was estimated using the assumption that eel shape is a cylinder of body mass M, density ρ (equal to water density 0.001 g mm^{-3}), and body length L (Gatto & Rossi 1979). Eel mass and body size are significantly related, and M can be substituted as a function of L. The above define the function of trunk $A(L)$ as follows (Gatto & Rossi 1979, Bevacqua *et al.* 2009):

$$A = M/\rho L \rightarrow A(L) = M(L)/\rho L$$

$$M(L) = aL^b \quad \text{and} \quad A(L) = \alpha \cdot \rho^{-1} L^{b-1}$$

where a and b are regression coefficients for M in g and L in mm. In this study, the morphometric relationship $M(L)$ is equivalent to the WLR power function $W=a \cdot L^b$, whose coefficients are obtained through regression analysis. Using the above formulas, correction was performed to the number of individuals n_i of yellow eel age classes, which were affected by fyke net selectivity. The morphometric relationships WLR and LAR were applied using only the specimens of L<400 mm in order to increase their accuracy for the smaller specimens. This threshold was chosen using indications from selectivity graphs of different mesh size obtained by Bevacqua *et al.* (2009). After the correction of n_i values of the age classes affected by selectivity, their values were used to recalculate the frequency n_i/N of yellow eels per age class, symbolized as G_i.

Correction was not required for the data of silver eels since they were not affected by the fyke net selectivity. The abundance S_i (ind. ha^{-1}) for each age class i of silver eels was estimated by multiplying the frequency for each age class n_i/N with the density factor f (ind. ha^{-1}) ($S_i=f \cdot n_i/N$). The G_i and S_i were used for the calculation of a) the eel survival curve and the survival rates per age class of the entire population to the time of migration, b) the stock of yellow eels, c) the recruitment, and d) the rate of metamorphosis from yellow to silver eels.

6.2. Modelling approach to estimate the survival curve, stock of yellow eels, recruitment and metamorphosis

The survival curve describes the degree of survival of both yellow and silver eels per age class after the occurrence of natural mortality. The survival for each age class i is symbolized F_i and is given by values relative to G_i. The F_i is equal to G_i for the first age classes in which metamorphosis to silver eels was not observed. A preliminary analysis was performed to select the optimum form of the survival curve using the known F_i values of the first four age classes versus the parametric age class AC. The aim of the analysis was to find the optimum transformations of F and AC for providing a linear relationship of the two transformed parameters with an intercept which can be used for the derivation of the recruitment when AC=0. The optimum transformations for F and AC were $F'=F^{-1/2}$ and $AC'=[\ln(AC+1)]^2$, respectively, and the general form of the transformed and non-transformed survival curve is the following:

$$F' = c \cdot AC' + d \quad \text{and} \quad F = \{c[ln(AC+1)]^2 + d\}^{-2} \quad \text{for} \quad c,d > 0$$

The value of 1 in the natural logarithm of AC transformation was used to solve the equation for AC=0. For AC=0 the value of F is equal to d^{-2}, which is used in the following steps for the estimation of glass eel recruitment. The survival rate RF of an age class i is described by the ratio of sequential F values as follows:

$$RF_i = F_i / F_{i-1}$$

The parameters of the survival curve were estimated based on the concept that the G_i of an age class i of yellow eels is equal to the value of survival F_i minus the proportional frequency of silver eels of the same age class. The proportional frequency of silver eels is equal to S_i of each age class i multiplied by a correction factor k. The factor k is also used: (a) to convert the frequencies G_i to abundance Y_i (ind. ha^{-1}) of yellow eels per age class according to $Y_i=G_i/k$, (b) to convert the F_i values to total population abundance per age class according to F_i/k (ind. ha^{-1}) and (c) to convert the parameter d^{-2} to recruitment abundance according to d^{-2}/k (ind. ha^{-1}). The concept of the conversion factor k was first proposed by Gatto & Rossi (1979). The connection between G_i, F_i and S_i using k is performed by the following expressions:

$$G_i = F_i - k \cdot S_i \quad \text{for } k > 0$$

$$G_i = \{c[ln(AC_i + 1)]^2 + d\}^{-2} - k \cdot S_i \quad \text{for } c, d, k > 0$$

The value of k is also used to estimate the relative rate (i.e. values 0-1) of metamorphosis to silver eels TR_i for each age class according to the following:

$$TR_i = \frac{k \cdot S_i}{G_i + k \cdot S_i}$$

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