Vol-3, Issue-7 PP. 552-559 ISSN: 2394-5788

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

SUITABILITY OF EMPIRICAL METHODS FOR THE MANAGEMENT OF THE CRITICALLY ENDANGERED EUROPEAN EEL: SKIN COLOUR CHANGES AS DESCRIPTOR OF MIGRATING SILVER EEL STAGE

Fabrizio Capoccioni
Council for Research in Agriculture and Agricultural Economy Analysis (CREA), Livestock and aquaculture (ZA), Research Centre for the production of meat and breeding (PCM) Via Salaria, 31, 00016 - Monterotondo (Rome) Italy.
fabrizio.capoccioni@crea.gov.it

Corrado Costa
(Corresponding author)
Council for Research in Agriculture and Agricultural Economy Analysis (CREA), Research Unit for Agricultural Engineering (ING), Via della Grazing, 16, 00015, Monterotondo Scalo (Rome), Italy.
corrado.costa@crea.gov.it

Eleonora Ciccotti
Department of Biology,
University of Rome "Tor Vergata" Via Scientific Research,
Romee, 00133,
Italy.
ciccotti@uniroma2.it

ABSTRACT

The European eel is a critically endangered species since stocks are in decline in most of their geographical distribution and their status is considered below safe biological limits. A number of management and conservation measures are being implemented in European countries, in particular applied to silver eels starting their unique spawning migration. A supervised multivariate classification approach (PLSDA) was applied to investigate the relationship between developmental stages of European eel on the basis of skin colour change, and several external and internal parameters. A first model was build using data of European eels collected in the Tiber River in Italy, to explain the co-variation between external morphological measures, and three developmental stages selected a priori, and a second one adding three parameters (otoliths radius, liver and gut weight). Satisfactory percentages of correct classification were obtained for both models. A validation test, based only on external measurements, was performed with the addition of new samples from two Italian coastal lagoons. Results confirm that changes in skin colour reflect the parameters considered, but the efficiency of the PLSDA model is highly site-dependent. The most evident application of this work is the confirmation of the validity of the empirical staging "by eye" between yellow and silver eel that is widely used by different actors of European eel conservation actions.

General Terms

Partial least Square Discriminant analysis, skin colour pattern

Keywords: European eel, *Anguilla anguilla*, silvering, conservation, PLSDA, morphometrics, skin colour

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

1. INTRODUCTION

The European eel (*Anguilla anguilla* L., 1758) is a catadromous fish whose larvae, the leptocephali, cross the Atlantic Ocean from the spawning area, in the Sargasso Sea, to reach the coasts of Europe and North Africa. After metamorphosis to glass eels, fish complete the migration into continental habitats. There they grow into yellow eels, and after a growing period of several years they make the final transition into silver eels that migrate back to the Sargasso Sea, where they mate and spawn only once prior to die (Tesch, 2003). This last transformation from yellow to silver eels is a gradual and crucial process, preparing the future spawners ready to embark on their seaward reproductive migration. Thus silvering defines the transition from the growing phase to the sexually maturing: by the silvering process the last developmental stage in European eel is attained, even if still sexually immature (Durif et al., 2006).

Distinguishing properly silver eels from yellow eels has been a complicated task for long time in eel biology (Durif et al., 2009). This is because there is no exact boundary between yellow and silver eels, and silvering progresses slowly without distinct structural changes due to the effect of different physiological and environmental conditions (Tesch, 2003).

Numerous studies have investigated the morpho-physiological changes that occur during the transformation from a resident yellow eel to actively migrating silver eel (reviewed by Durif et al., 2009). Silvering can be characterized by several morphological parameters like change in integument structure and colour (van den Thillart et al., 2009), increase in eye diameter (Pankhurst, 1982), increase of liver weight, and finally a regression of the alimentary tract partly related to natural starvation and cessation of body growth (Sorensen and Pankhurst, 1989). Some of these characteristics can be used to assess the developmental and migratory status of each eel, resident or migrating, by taking into account one or more of these features (Cottrill et al., 2002). Several classifying methods with a different number of stages for the silvering process have been developed: the silvering process has been divided into two (e.g. Pankhurst, 1982), three (Durif et al., 2000; Acou et al., 2005), or five stages (Durif et al., 2005). Morphological characteristics including skin colour are often used as criterion for staging silvering eels in many studies (Okamura et al., 2007).

Despite this, a number of aspects remain largely unclear, such as the effective duration of the silvering process, the progression by intermediate phases, the effective relationship between internal modifications and external and skin colour changes. Moreover, Rousseau et al. (2009) have stressed the importance of taking into account qualitative aspects of the silvering: they asserted that this process corresponds to a pubertal event because metamorphic changes are induced by the gonadotropic axis.

The European eel shares the fate of many diadromous species that because of the complexity of their life cycle, and of the fact that they use different habitats in marine and continental waters, are particularly vulnerable to consequences of anthropogenic effects pressures, both direct and indirect. That is particularly true for eel, that lives in both freshwater and marine waters, , and that is exploited by fisheries across the whole distribution area, targeted at all life stages.

For this species, major problems exist, in relation to a continent-wide decline in recruitment observed in the course of the last decades, and to a contraction in adult eel capture fisheries (Moriarty and Dekker, 1997; ICES, 2006). The severity of this decline was formally recognized in 1998 (ICES, 1998; Dekker, 2003; Bilotta et al., 2011), with eels now thought to be seriously threatened (Freyhof and Kottelat, 2012). Moreover, in September 2007 a Council Regulation (EC 1100/2007) established a comprehensive framework for the protection and sustainable use of the stock of European eel and in 2008 the species was listed as critically endangered in the IUCN Red List of Threatened Species. With this background, different parties should be involved in the conservation and management measures of the European eel all over the Europe such as fishermen and also civil society (i.e. within citizen science projects) in order to contribute to the collection of biological data that are basic information for the setting up of restoration programs for the European eel stock. Considering that, simple and reliable indices describing eel different developmental stages will represent useful tools not only for the scientific community interested in this topic.

Against this background, the aim of this study is to verify discrimination of eel developmental stages on the basis of some external morphometric parameters. These should be strictly related with the change of skin colour, which could be measured by a simple method, functional even in field surveys, to determine whether an eel is immature and sedentary, preparing its metamorphosis or about to migrate. In the bargain, the distinction in yellow and silver eels is widely used in current practices and it is hence quoted in most management documents. Indeed, a useful method that avoid animals sacrifice (e.g. histological analysis of gonads), is needed for a species of great scientific interest but also relevant for conservation of biodiversity.

2. MATERIALS AND METHODS

The work has foreseen two stages: a first phase was the development of the best approach by setting up two possible models and a second step was the validation of the definitive model.

2.1 Development of the model

Eels were collected in a single location, the low course of the Tiber river (41°47'57''N - 12°24'50''E) (Figure 1), at regular intervals along an entire commercial fishing season (March-December), by fyke nets. The stage of captured eels would vary according to the

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

time of capture (within migration season or not), but it is common that during the migration period, that in Mediterranean environments is in winter (Capoccioni et al., 2014) both yellow and silver eels are found together inside the fykenets (Lumare et al., 2010).

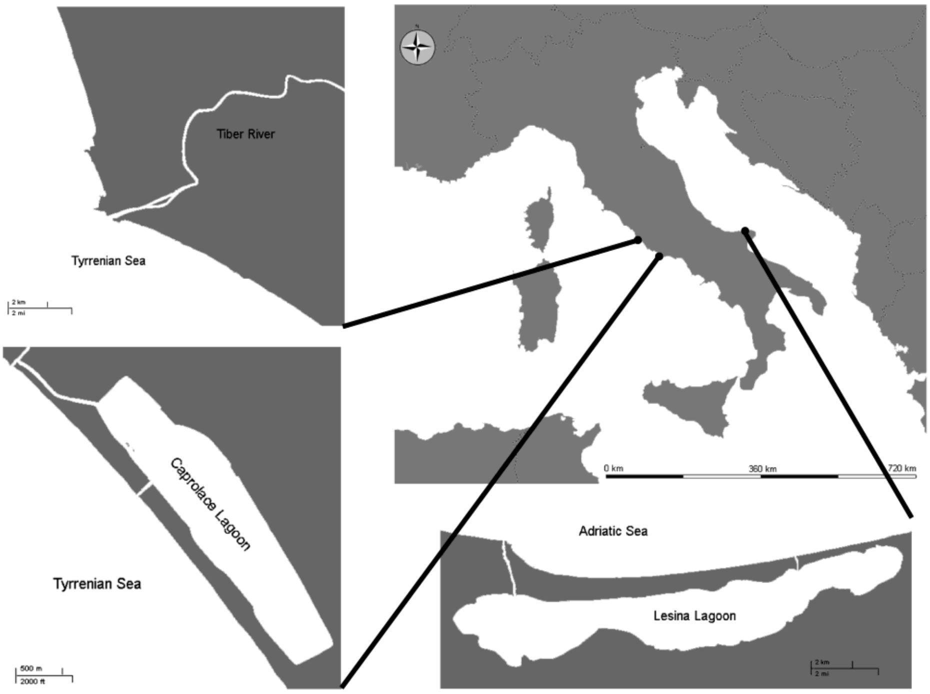

Fig. 1: The three different locations of selected sampling sites in Italy

A total of 454 individualswere examined by the same operator to assign developmental stage on the basis of the contrasting body colour (dark dorsal surface and a white ventral surface;Amilhat et al., 2013): yellow eel (Y), silver eel (S) and intermediate (Y/S), the latter assigned to eels that could not be allocated definitely to any of the two previous stages. Each animal was measured for length, weight, and three morphometric parameters (i.e. pectoral fin length, vertical and horizontal eye diameters), all informative of European eels silvering according to (Durif et al., 2005). A subsample of 229 individuals was then sacrificed respecting legislation and minimising suffering, in order to determine also liver and emptied gut weight and to measure otolith maximum radius length as a continuous value related to age (Svedäng, 1999).

The development of a definitive model has foreseen the setting up of two different procedures, in order to evaluate the accuracy of the stage attribution on the basis of body colour, using two sets of input variables. To achieve this purpose, two different datasets were used as inputs to the models, the first consisting of a complete set of variables referred to a subsample of eels (ALLVAR,) and the second (REDVAR) consisting of a reduced set of variables on a larger sample of eels, including the former and other eels as well. Thus, the first data set consisted of eight input parameters (total length, total weight, pectoral fin length, vertical and horizontal eye diameter, otolith radius, liver weight and gut weight), for a subsample of 118 eels (ALLVAR) and the second data set (REDVAR) consisted of five input variables, *i.e.*, the external morphometric measures, using a total of 223 eels.

Eel total lengths spanned within narrow ranges, the same for yellow and for silver, to avoid the risk of overestimating a parameter as total length that is clearly fundamental during the process of silvering (Durif et al., 2006).

Both models aimed to explain the co-variation between the parametersand the assigned stage. A supervised class-modelling approach, Partial Least Squares Discriminant Analysis (PLSDA; Sjöström et al., 1986; Costa et al., 2013; Taiti et al., 2015) is a PLS regression where the response variable is categorical, expressing the class membership of the statistical units. The objective of PLSDA is to find a model, developed from a training set of observations of known class membership, that separates classes of objects on the basis of their X-variables. PLSDA was used as a supervised modelling classmethod (Forina et al., 2008) using SIMPLS algorithm (de Jong, 1993) to classify the developmental stages (Y, S, and Y/S) from the input parameters (*i.e.*, 8 for ALLVAR, 5 for REDVAR) pre-processed with the "normalization" Matlab procedure. The dataset was divided into a calibration set composed by 75% of samples and an internal validation set represented by the remaining 25%. This partition was done optimally choosing the Euclidean distances based on the algorithm of Kennard and Stone (1969) that selects objects without the a priori knowledge of a regression model. The percentages of correct classification were calculated for calibration and validation phases, and then used for model selection. The PLSDA model selection was mainly based on the efficiencies and robustness parameters described above. The models were developed using a procedure written in the MATLAB 7.1 R14 environment.

2.2 Validation and application of the best model

Following the setting up of the best model, three eel samples were submitted to the PLSDA model based only on external parameters. Three Italian local stocks from three sites were sampled: a new sampling was performed in the Tiber river, and two samples were obtained from two coastal lagoons along the Italian coast (Caprolace lagoon 41°20'54.56"N - 12°58'32"E and the Lesina lagoon 41°53'1"N - 15°26'58"E) (Figure 1). A total of 1214 eels were collected, with the same sampling methodology, *i.e.*, fyke netting at regular intervals during the season in order to obtain all migratory stages. Animals were examined in order to assess the developmental stage, again inferred by skin colour. Total length, weight and morphometric variables (pectoral fin length, vertical and horizontal eye diameter) were measured.

3. RESULTS

3.1 Building of the model

The PLSDA approach applied to the two data sets yields a cumulative good classification percentage of correct classification for both datasets (90.8 % and 88.3 % respectively for ALLVAR and REDVAR) compared with the random probability (33%).
In Table 1 the features of the two PLSDA are summarized: percentage of specificity and sensitivity for both are considerably high (respectively, 0,70 and 0,80 for the ALLVAR dataset and 0,74 and 0,77 for the REDVAR dataset).
The independent test (applied to the 25% of individuals of both dataset) yields 82.8% of correctly classified eels for the ALLVAR dataset, and 89.6% for the REDVAR dataset.

Table 1. Characteristics and principal results of the PLSDA model used to classify the developmental stages (Y, S, and Y/S).

	ALLVAR	REDVAR
N° individuals (X-block)	118	223
N° of X variables	5	8
n° units (Y-block)	3	3
n° LV	3	3
% Cumulated Variance X-block (%)	100	100
% Cumulated Variance Y-block (%)	69.61	67.34
Mean Specificity (%)	0.70	0.74
Mean Sensitivity (%)	0.80	0.77
Random Probability (%)	33	33
% Corr. Class. Model	83.14	78.58
% Corr. Class. Indep. Test	82.75	89.64
n° misclassified Y (test 25%)	0/22	0/40
n° misclassified Y/S (test 25%)	3/3	8/8
n° misclassified S (test 25%)	2/4	1/7

The misclassified eels of the test group, for the two datasets, are respectively 5 and 9. Examining the two datasets, one (ALLVAR) and two (REDVAR) silver eels were wrongly assigned to the Yellow eel stage. For both datasets all intermediate stages (Y/S) have been classified by the model as yellow eels, while all yellow eels were correctly classified.
The stage attributions yielded by the model for both datasets have been represented in a bi-dimensional space using the two latent variables (LV) that explain the higher percentage of total variance (Figure 2A and 2B).

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

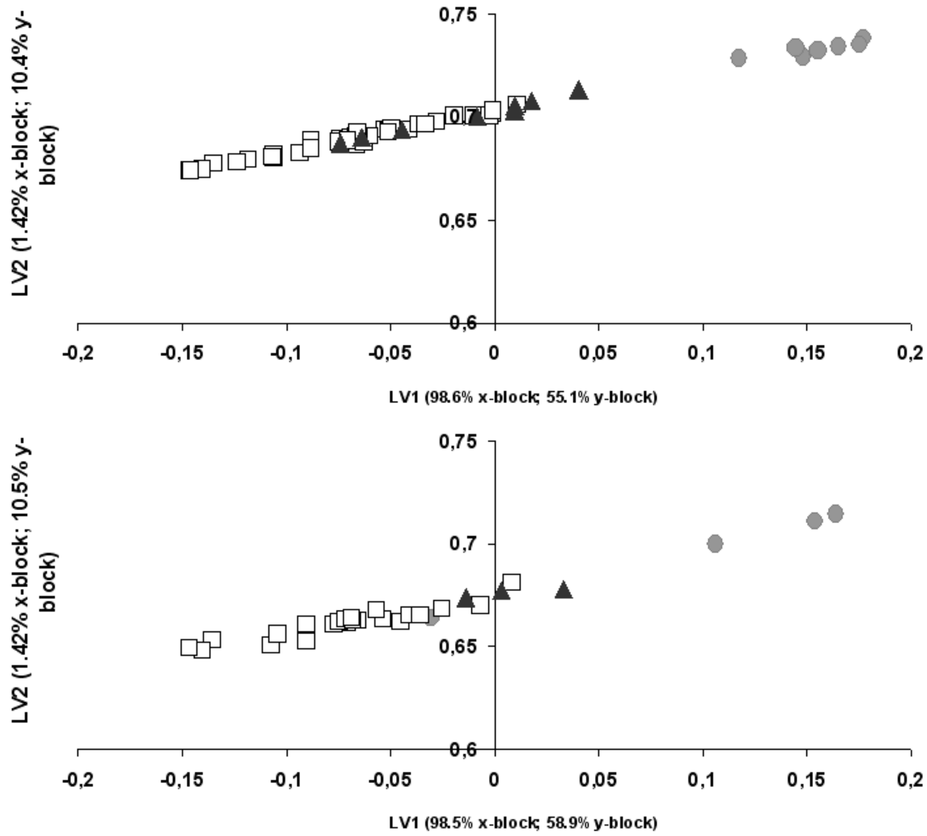

Fig. 2: Scatter plot of the first 2 LVs obtained by the ALLVAR dataset Yellow eels (white square), Intermediate eels (black triangle), Silver eels (grey circle) (A) Scatter plot of the first 2 LVs obtained by the REDVAR dataset Yellow eels (white square), Intermediate eels (black triangle), Silver eels (grey circle) (B)

The ordination relative to the ALLVAR, that yields a explained variance of the two latent variables amounting to 99,6%, shows two highly defined clusters for Yellow and Silver eels, while the Intermediate group (Y/S) appears distinct, but closer to the Y group than the S one.
The two latent variables of the ordination test relative to the REDVAR dataset explain a total variance of 100%. The ordination shows, again, Yellow and Silver eels grouped in two distinct clusters, while individuals of Intermediate stage partly overlap Y cluster.

3.2 Validation and application

In the validation phase, the PLSDA model has been run on the three samples (Tiber, Caprolace, Lesina), and yields a good correct classification percentage for all three local stocks. Table 2 shows the numbers of eels correctly assigned to the predetermined stage (grey cells), and the wrongly classified ones (white cells). For the Tiber sample, overall correct classification amounts to 81,5%: this value is similar to the one obtained by the model in the setting up phase (82.76%; Table 1). For the Caprolace and Lesina samples, this value is respectively 64,1% and 56,3%. Yellow and Silver eels have been correctly assigned by the model to the proper predetermined, with the exception of yellow eels of Lesina (57.5% of correct classification). For all samples only one eel (in Lesina) has been placed by the model to the Intermediate stage.

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

Table 2. Staged observed eels and confusion matrix of the PLSDA: predicted specimens with the disaggregate percentage of correct classification for each stage in the river Tiber, Caprolace Lagoon and Lesina Lagoon.

			n° predicted eels			
Sampling site	n° observed eels		S	Y/S	Y	%
Tiber river	S	52	**47**	0	5	**90.4**
	Y/S	69	33	**0**	36	**0.0**
	Y	294	3	0	**291**	**99.0**
Caprolace lagoon	S	55	**51**	0	4	**92.7**
	Y/S	63	57	**0**	6	**0.0**
	Y	286	78	0	**208**	**72.7**
Lesina lagoon	S	17	**17**	0	0	**100**
	Y/S	23	1	**1**	21	**4.3**
	Y	365	144	11	**210**	**57.5**

4. DISCUSSION

Results of the present study confirm, using an advanced statistical approach, the effective biological correlation between most of morphological variables involved during silvering and the skin colour.

The prediction models applied for the Tiber river population indicate that all external and internal physical changes allow a high percentage of correct classification (both more than 80%). The model seems to be more accurate for Yellow and Silver eels that for the Intermediate ones. The fact that all individuals, classified by skin colour on field as Intermediate stage (Y/S), have been placed by PLSDA in the Yellow stage is probably due to the fact that this is a short and transient phase and that main morphological changes are not conspicuous yet.

The misclassification performance of the PLSDA for Y/S stage, which is harder to evaluate visually, is in great measure due to an uncorrected a priori that influences the model.

This result is confirmed by the validation test, where samples from three different populations have been analysed using only external morphological parameters. No Y/S eels have been placed in the same a priori stage, but the model assigned them to the Yellow or to the Silver stage.

The percentage of correct classification obtained by the validation test is considerably high for the Tiber population and the model can be considered highly reliable even if only five parameters have been considered. This is confirmed by the low decrement of the Cumulated Variance Y-block (%) between the two models. In fact results of validation test for Tiber eel collected for confirmation are comparable with the model performed with initial data, thus confirming the validity of this approach applied within a single local stock.

On the contrary, the results of the validation test on the Caprolace and Lesina samples show a lower efficiency. This is probably due to a different colour pattern, in its turn related to the different ecological conditions of these two environments in comparison to these of the Tiber River, that influence the dynamics of the silvering process. Eels are more easily staged for Caprolace by the PLSDA model than the Lesina sample and this could be due to the fact that the variability in morphological and physiological traits decreases with the size of catchment (Acou et al., 2005). The relative influence of environmental factors is seemingly different according to the type of environment for many euryhaline species (Antonucci et al., 2012), although in the European eel the mechanisms through which they act on eel are not clearly understood (van den Thillart et al., 2009).

Finally, van den Thillart et al. (2009), describing the dynamics of the silvering process from both morphological and physiological parameters, found them uncoupled because silvering is strongly related to diversity of the growth habitat that influences somatic

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

growth (i.e. age). This study focused on a single and simple external descriptor, the skin colour colour, trying to understand the relationship existing between this parameter (expressed in three different stages) and the most important external and internal parameters involved in the silvering process.

Results confirm that changes in skin colour reflect these parameters, and evidence that the efficiency of the statistical model used is highly site dependent.

5. CONCLUSION

The most evident application of this work is the confirmation of the validity of the empirical staging "by eye" between yellow and silver eel that is widely used in current practices, in the majority of scientific literature and hence referred to in most management documents about the European eel, that avoid the necessity of animals sacrifice. Although the use of skin colour to identify ready-to migrate eels has been criticized in the past (Pankhurst and Lythgoe, 1982), it is generally this parameter that is most used for selling price determination among professional fishermen at fish markets (Bach et al., 1992).

Nonetheless, easy and reliable indices are important tools for non-scientist people, such as fishermen, involved in the management eel measures or always more frequently in citizens science project by non-professional scientists (Silvertown et al., 2013; http://www.zsl.org/conservation/regions/uk-europe/eel-conservation).

A subsequent technical step of this study could be a finer description of the pigmentation pattern evolution, by an appropriate colorimetric imaging technique and advanced statistical approaches. Moreover, advanced opto-electric technologies might allow a much rapid, reliable and economic operative measure method even on the field with more efficacy and attention to animal welfare.

6. ACKNOWLEDGMENTS

This work was supported by a grant to EC by the Italian Ministry of University and Scientific Research – PRIN Project 2006: "An Integrated Approach to the Conservation and Management of the European Eel in the Mediterranean Region". The Authors wish to thank their professor, Stefano Cataudella, for sharing research topics and partaking facilities at the Laboratory of Experimental Ecology and Aquaculture of the Biology Department. The Authors are also grateful to the fishers of the Tiber for hospitality on the river and help for catching eel.

7. REFERENCES

[1] Acou, A., Boury, P., Laffaille, P., Crivelli, A.J. and Feunteun, E. 2005. Towards a standardized characterization of the potentially migrating silver European eel (*Anguilla anguilla*, L.). Archiv für Hydrobiologie 164, 237-255.

[2] Amilhat, E., Fazio, G., Simon, G., Manetti, M., Paris, S., Delahaut, L., Farrugio, H., Lecomte-Finiger, R., Sasal, P. and Faliex, E. 2013. Silver European eels health in Mediterranean habitats. Ecology of Freshwater Fish 23(1), 49-64.

[3] Antonucci, F., Boglione, C., Cerasari, V., Caccia, E. and Costa, C. 2012. External shape analyses in Atherina boyeri (Risso, 1810) from different environments. Italian Journal of Zoology 79, 60-68.

[4] Bach, P., Legendre, P., Amanieu, M. and Lasserre, G. 1992. Strategy of eel (*Anguilla anguilla*, L) exploitation in the Thau lagoon. Estuarine Coastal and Shelf Science 35, 55–73.

[5] Bilotta, G.S., Sibley, P., Hateley, J. and Don, A. 2011. The decline of the European eel *Anguilla anguilla*: quantifying and managing escapement to support conservation. Journal of Fish Biology 78, 23–38.

[6] Capoccioni, F., Costa, C., Canali, E., Aguzzi, J., Antonucci, F., Ragonese, S. and Bianchini, M. L. 2014. The potential reproductive contribution of Mediterranean migrating eels to the *Anguilla anguilla* stock. Scientific Reports doi:10.1038/srep07188

[7] Costa, C., Antonucci, F., Boglione, C., Menesatti, P., Vandeputte, M. and Chatain, B. 2013. Automated sorting for size, sex and skeletal anomalies of cultured seabass using external shape analysis. Aquaculture Engineer 52, 58-64.

[8] Cottrill, R.D., McKinley, R.S. and Van Der Kraak, G. 2002. An examination of utilizing external measures to identify sexually maturing female American eels, Anguilla rostrata, in the St Lawrence River. Environmental Biology of Fishes 65, 271-287.

[9] De Jong, S. 1993. SIMPLS: an alternative approach to partial least squares regression. Chemometrics and intelligent laboratory 18(3), 251-263.

[10] Dekker, W. 2003. Status of the European eel stock and fisheries. In: Eel Biology: 237-254 (K. Aida, K. Tsukamoto and K. Yamauchi, Eds.). Springer Verlag, Tokyo.

[11] Durif, C.M.F., Dufour, S. and Elie, P. 2005. The silvering process of the European eel: a new classification from the yellow resident stage to the silver migrating stage. Journal of Fish Biology 66, 1025-1043.

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

[12] Durif, C.M.F., Dufour. S. and Elie, P. 2006. Impact of silvering stage, age, body size and condition on reproductive potential of the European eel. Marine Ecology Progress Series 327, 171-181.
[13] Durif, C.M.F., Elie, P., Dufour, S., Marcheridon, J. and Vidal, B. 2000. Analyse des paramétres morphologiques et physiologiques lors de la préparation à la migration de dévalaison chez l'anguille europénne (*Anguilla anguilla*) du lac de Grand-Lieu (Loire-Atlantique). Cybium 24, 63–74.
[14] Durif, C.M.F., van Ginneken, V., Dufour, S., Muller, T. and Elie, P. 2009. Seasonal evolution and individual differences in silvering eels from different locations. In: Spawning migration of the European eel: reproduction index, a useful tool for conservation management: 13-35 (G.E.E.J.M. Van den Thillart, J. C. Rankin and S. Dufour, Eds.) Springer Netherlands, Dordrecht.
[15] Forina, M., Oliveri, P., Lanteri, S. and Casale, M. 2008. Class-modeling techniques, classic and new, for old and new problems. Chemometrics and intelligent laboratory 93(2), 132-148.
[16] Freyhof, J. and Kottelat, M. 2012. *Anguilla anguilla*. In: IUCN 2011 IUCN Red List of Threatened Species Version 2012, wwwiucnredlist.org
[17] ICES (International Council for Exploration of the Sea), 1998. Report of the ICES Advisory Committee on Fisheries Management, ICES Cooperative Research Report, 229, 393–405.
[18] ICES (International Council for Exploration of the Sea), 2006. Report of the ICES/EIFAC Working Groupon Eels ICESCM2006/ACFM: 16.
[19] Kennard, R.W. and Stone, L.A. 1969. Computer aided design of experiments. Technometrics, 11, 137-148.
[20] Lumare, D., Lumare, L., Scirocco, T., Florio, M. and Lumare, F. 2010. Composizione strutturale e dinamica del pescato nel lago di Acquatina. Thalassia Salentina 31, 63–82.
[21] Moriarty, C. and Dekker, W. 1997. Management of the European eel. Fish Bulletin 15, 110.
[22] Okamura, A.K., Yamada, Y.K., Yokouchi, N., Horie, N., Mikawa, T., Utoh, N., Tanaka, S. and Tsukamoto, K. 2007. A silvering index for the Japanese eel Anguilla japonica. Environmental Biology of Fishes 80(1), 77-89.
[23] Pankhurst, N.W. 1982. Relation of visual changes to the onset of sexual maturation in the European eel (*Anguilla anguilla* L.). Journal of Fish Biology 21(2), 127-140.
[24] Pankhurst, N.W. and Lythgoe, J. N. 1982. Structure and colour of the integument of the European eel *Anguilla anguilla* (L.). Journal of Fish Biology 21(3), 279-296.
[25] Rousseau, K., Aroua, S., Schmitz, M., Elie, P. and Dufour, S. 2009. Silvering: Metamorphosis or Puberty In: Spawning migration of the European eel: reproduction index, a useful tool for conservation management, 39-55 (G.E.E.J.M. Van den Thillart, J.C. Rankin and S. Dufour, Eds.) Springer Netherlands, Dordrecht.
[26] Silvertown, J., Buesching, C.D., Jacobson, S.K. and Rebelo, T. 2013. Citizen science and nature conservation. In: Key topics in conservation biology, (2)124-142 (D. W. Macdonald and K.J. Willis, Eds.). John Wiley and Sons, Oxford.
[27] Sjöström, M., Wold, S. and Söderström, B. 1986. PLS Discrimination plots. In: Pattern recognition in practice II: 236-241 (E.S. Gelsema and L.N. Kanals, Eds.). Elsevier, Amsterdam.
[28] Sorensen, P.W. and Pankhurst, N.W. 1988. Histological changes in the gonad, skin, intestine and olfactory epithelium for artificially matured male American eels, Anguilla rostrate. Journal of Fish Biology 32(2), 297-307.
[29] Svedäng, H. 1999. Vital population statistics of the exploited eel stock on the Swedish west coast. Fisheries Research 40(3), 251-265.
[30] Taiti, C., Costa, C., Menesatti, P., Comparini, D., Bazihizina, N., Azzarello, E., Masi, E. and Mancuso, S. 2015. Class-modeling approach to PTR-TOFMS data: a peppers case study. Journal of the Science of Food and Agriculture 95, 1757-1776.
[31] Tesch, F. W., 2003. The Eel. Blackwell Science.
[32] Van den Thillart, G.E.E.J.M., Rankin, J. C. and Dufour, S. 2009. Spawning migration of the European eel: reproduction index, a useful tool for conservation management. Springer Netherlands, Dordrecht.