

# Morphological discrimination of the silvering stages of the European eel

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*Abstract.*- Global concern has been raised on the future of eel populations. Outgoing fractions of eels need to be monitored. A lack of knowledge on this particular phase and particularly on the characteristics of migrating silver eels lead to a series of studies on the silvering process. A classification based on development of gonads, regression of digestive tract, levels of growth hormone and gonadotropin hormone was proposed by Durif (2003). The present study describes a non-invasive method to assign eels into each of the six silvering stages corresponding to a growth phase for (I to FII), a pre-migrant stage for females (FIII) and migrating stages for both sexes (FIV, FV and MII). Using discriminant analysis on four morphological parameters (body length, body weight, mean eye diameters, and length of pectoral fin), the derived classification functions are able to classify eels into each stage with an accuracy of 82%. This method, associated to proper sampling, could be utilized for the quantification of potential genitors a given year.

## Introduction

The scientific community agrees to say that the eel stock is seriously threatened and that urgent measures should be taken to monitor the remaining population. Indeed the International Council for the exploration of the Sea (ICES) considers that the stock is outside safe biological limits and that fisheries in recent years have not been sustainable (ICES 1998). In a recent communication, the European Commission has stated that escapement targets need to be established and that the highest initial priority is placed on assuring the survival and escapement of silver eels. Current conditions have to be measured and this can be realized through either the quantification of silver eels as they migrate to the sea or through the identification of potential migrants a given year.

1 Before eels actually start their downstream migration, they undergo internal and external  
2 changes, which will prepare them to the 6000 km journey to the spawning grounds in the  
3 Sargasso Sea. This metamorphosis is called silvering and corresponds to the beginning of the  
4 reproductive phase. Silver eels are potential genitors leaving the continental waters a given  
5 year. Physiologically, the differences between the so-called yellow and silver stages are  
6 important. Silver eels have stopped feeding and their alimentary tract regresses (Pankhurst  
7 and Sorensen 1984; Fontaine 1994; Marchelidon et al. 1999). Osmoregulatory mechanisms,  
8 which will allow adaptation to seawater, are already active before the eel leaves freshwater  
9 (Fontaine 1975 ; Dutil et al. 1987; Lecomte-Finiger 1990). Gonads have started to develop  
10 following production of gonadotropin hormone (Marchelidon et al. 1999 ; Durif et al. 2000).  
11 Above a certain size, eels are generally classified into two stages: the yellow resident stage  
12 and the silver (and implicitly migrant) stage. At present, methods for stage determination are  
13 varied and generally rely on the experimenter's impressions on skin color: an eel that displays  
14 a white silver belly well separated from a black dorsal region by the lateral line is considered  
15 at the "silver stage" and implicitly migrant. Increasing eye size and darkening of pectoral fins  
16 are sometimes also used to determine the stage of the eel. Yet on the field, eels showing  
17 intermediate features are very common. In such cases, stage determination remains empirical  
18 and will depend on the observer's experience. The most "standard" criterion was established  
19 by Pankhurst (1982), who observed that eye diameter of the eel is correlated to oocyte  
20 diameter. The author develops an eye index, which corresponds to the approximate eye  
21 surface area divided by total body length, and a threshold of 6.5 is set for the identification of  
22 "sexually maturing eels". However, part of the results was based on artificially sexually  
23 matured females and no direct link to downstream migration was made. A previous study  
24 (Durif and Elie submitted) has shown that the silvering process is a gradual phenomenon in  
25 female eels and it can be decomposed into five successive stages corresponding to a growth  
26 phase (I and FII), to a pre-migrating stage (III), and to two migrating stages (IV and V).  
27 Variability is much less important in male eels and results suggested that silvering and sex  
28 differentiation in males is simultaneous; thus two stages were identified for males (I and MII).  
29 The aim of this study is to develop a non-invasive method to determine the stage of an eel  
30 based on this classification. Discriminant Analysis was used on the same set of data, first, to  
31 determine which morphological parameters best distinguished between all six stages and  
32 second, to develop an index based on these parameters to classify eels into their respective  
33 stage.  
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## Methods

### *Description of data*

The set of data consists of 1188 eels collected in six different locations in France with different types of fishing gear (electrofishing, eel pots, fyke nets, weir, stow net) in order to obtain resident eels as well as individuals at the migrating silver stage and all other possible intermediate stages. Sampling was carried out between 1994 and 2002. Several morphological and physiological parameters were measured on these individuals in order to determine their degree of silvering. Evaluation was based on levels of gonatropin hormone (GTH-II) and growth hormone (GH) as well as development of gonads, regression of digestive tract and liver weight. All eels in the sample were classified into five stages for undifferentiated and female eels and one silver migrating stage for males. Details can be found in Durif (2003) and Durif and Elie (submitted).

### *Morphological Measurements*

The following external measurements were made on eels: total body length (L), wet body weight (W), length of the pectoral fin (FL) and mean eye diameter (MD) based on vertical and horizontal eye diameters. For these, the distance measured corresponds to the visible part of the eye and not just the iris (Figure 1). The pectoral fin is measured from the insertion to the tip of the fin and corresponds to the greatest possible length.

### *Data analysis*

Discriminant analysis is used to determine which variables discriminate between two or more naturally occurring groups and to classify cases into the different groups with a better than chance accuracy. A backward stepwise analysis was carried out on the data using a cross-validation procedure: a model is developed in a 'learning sample' and the predictive accuracy of the model is evaluated in a test sample. If the model performs as well in the test sample as in the learning sample, it is said to cross-validate well. Classification functions are derived from the model and are used to determine to which group each case most likely belongs. There are as many classification functions as there are groups. Classification scores for each case are computed for each group according to the formula:

$$S_i = c_i + w_{i1} * x_1 + w_{i2} * x_2 + \dots + w_{im} * x_m$$

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2 Where  $i$  denotes the respective group;  $n$  denote the  $n$  variables;  $c$  is a constant;  $w_{in}$  is the  
3 weight for the  $n$ 'th variable in the computation of the classification score for the  $i$ 'th group;  $x_n$   
4 is the observed value for the respective case for the  $n$ 'th variable.  $S_i$  is the resultant  
5 classification score. A case is assigned to the group for which it has the highest classification  
6 score. The classification matrix shows the number of cases that are correctly classified (on the  
7 diagonal of the matrix) and those that are misclassified. Classification functions can be used  
8 to directly compute classification scores for some new observations. In this study, groups  
9 correspond to the silver stages determined in Durif and Elie (Durif and Elie submitted) and  
10 the variables used in the discriminant analysis are morphological measurements (L, W, MD  
11 and FL).

## 12 13 **Results**

### 14 15 *Description of silvering stages (Durif 2003)*

16 Morphological and physiological characteristics of eels according to their stage are  
17 presented in Table 1. Eye index (EI) was calculated according to Pankhurst (1982). Fin length  
18 was measured on 1156 individuals out of the 1188 eels in the total sample.

19 Gonads of stage I eels are hardly developed: ovaries appear as translucent strips and testes  
20 are not visible. GSI is less than 0.5%. DTI is highly variable and its variations are seasonal.  
21 GTH-II production is close to zero and GH levels are variable, ranging from 0.02 to 0.77  
22  $\mu\text{g}\cdot\text{g}^{-1}$  suggesting different growth rates among individuals.

23 At stage FII gonads are significantly more developed ( $p<0.01$ ); GSI is equal to 0.54%. DTI  
24 is still variable and presents seasonal fluctuations linked to nutrition. HSI and K follow the  
25 same pattern, but on the whole, mean K is higher than at stage I while HSI is lower. Eels are  
26 still in the growth phase and GTH-II levels are close to zero.

27 Stage FIII corresponds to the beginning of the metamorphosis, in other words the pre-  
28 migrant stage (potential migrants for the given year). GSI is intermediate between the resident  
29 and migrant phases (mean of 0.8%). This stage is characterized by a high increase in GH. A  
30 slight increase in GTH-II level testifies of the induction of the gonadotropic axis. Eye index  
31 and fin index are significantly higher ( $p<0.01$ ).

32 Stage FIV eels begin their first downstream movements and their growth has stopped: GH  
33 is significantly lower than at stage FIII. Condition factor is at its maximum. GTH-II level is  
34 high ( $0.24 \text{ ng}\cdot\text{g}^{-1}$ ) compared to previous stages. Gonads are considerably more developed and

1 GSI is independent of size of eels and its mean is 1.5%. Eels have stopped feeding (DTI has a  
2 mean of 1.8%) and this happens concurrently to the first downstream movements. HSI does  
3 not vary significantly.

4 Stage FV corresponds to the migrating stage. GSI is equivalent to stage IV; its mean is  
5 equal to 1.7%. DTI is even lower than previously (mean of 1.2%). GTH-II is still high  
6 whereas GH does not evolve.

7 Stage MII represents migrant males. Values for all parameters (except for GSI and  
8 condition factor), are significantly higher than at stage I. Increase in GSI is not significant and  
9 values remain very low (mean of 0.16%). However, GTH-II level is significantly higher for  
10 migrant males ( $p < 0.01$ ): its mean is equal to  $1.08 \text{ ng.g}^{-1}$ . DTI at the time of migration is  
11 considerably lower than at stage I, (from 4.75 to 1.59%).

### 12 *Stage determination by morphological measurements*

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14 The sample (1156 individuals on which pectoral fin length was measured) is randomly  
15 split into a learning sample and a test sample representing respectively 66% (768 individuals)  
16 and 34% (388 individuals) of the dataset. A significant discriminant model was obtained  
17 when using all four variables: length (L), weight (W), mean eye diameter (MD), and pectoral  
18 fin length (FL). The first canonical variable accounts for more than 77% of the total  
19 dispersion of the groups. Standardized canonical discriminant function coefficients indicate  
20 the relative contribution of each variable to the overall discrimination. MD is the major  
21 contributor followed by FL and W (respectively -1.034, -0.770 and 0.634); the contribution  
22 of L is the lowest (0.183).

23 Eels can be assigned to a stage using the values in Table 3 (see Methods). The percentage  
24 of correct classification is equal to 83% for the learning sample, 77% for the test sample, and  
25 82% for the Jackknifed value. Errors are greatest for the classification of stage IV eels (55%  
26 of eels are correctly classified). This group also comprises the smallest number of individuals.  
27 On the contrary, the classification of migrant males is 98% accurate and only one eel is  
28 misclassified into stage V. Since males rarely exceed 45 cm for the European eel and  
29 migrating females are always bigger than this limit, this individual can be easily reclassified  
30 into its correct stage (MII). This information can also be used to reclassify females that are  
31 bigger than 45 cm and which have been misclassified into males.

32 Practically, to identify resident eels, one would take into account both I and FII stages,  
33 FIII eels can be considered as potential migrants and finally migrant eels correspond to stages  
34 FIV, FV and MII. Overall efficiency for the classification into resident, pre-migrant and

1 migrant (male and female) groups would then be 92% with values respectively corresponding  
2 to 95%, 70% and 91%.

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## Discussion

5 Up to now the identification of silver eels (and implicitly downstream migrants) was  
6 generally based on Pankhurst's eye index and a value of 6.5 was the threshold between yellow  
7 and silver eels. Eye index does reflect increasing GSI, however it is also strongly correlated to  
8 body length at the resident stage; thus a large sized eel may have a high eye index without  
9 necessarily being migrant. Moreover, the 6.5 limit was based on artificially matured  
10 individuals and no direct link to downstream migration was made. Pankhurst's criterion of 6.5  
11 overestimates the number of migrants (Figure 2). A detailed description of the silvering  
12 process was first realized on one subpopulation of eels from the lake of Grand-Lieu in France  
13 (Durif et al. 2000). This work was further generalized to other watersheds in France with  
14 different environmental characteristics (marsh, large rivers, small coastal river, estuary). Eels  
15 were classified into six groups (male and female), which represent a growth phase (stages I  
16 and FII), a pre-migrant stage (FIII), and migrating stages (FIV, FV, MII) for male and female  
17 eels (Durif 2003; Durif and Elie submitted). This classification gives a finer and more  
18 ecological description than does the restrictive yellow/silver classification. Given the large  
19 variability in length of "silver eels" a more appropriate description was needed.

20 This study supplements the description of silvering stages by providing a non- invasive  
21 method to determine whether an eel is sedentary, preparing its metamorphosis or about to  
22 migrate. Results show that it is possible to assign a stage (I to FV and MII) using only  
23 external measurements with a reasonable efficiency (82%). Practically and with an  
24 appropriate sampling method, one can evaluate the proportion of eels (stages IV, V and MII),  
25 which if triggered by the appropriate environmental factors, will start their downstream  
26 migration. Under the hypothesis that all will reach the Sargasso Sea, they will constitute the  
27 minimal estimation of genitors a given year. Applying Pankhurst's 6.5 limit on our sample  
28 would have overestimated migrants by 34.6% (wrongly comprising stages I, FII and FIII,  
29 Figure 3). Using the classification functions based on length, weight, eye diameters and  
30 pectoral fin length, gives an accuracy of 91% for the minimal estimation of migrants. A  
31 maximal estimation of potential genitors would include stage FIII eels (this estimation would  
32 not account for pre-migrant males as they have not been identified).



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Table 1. Mean and standard deviation of morpho-anatomical parameters of undifferentiated and female eels according to silvering stage. L (body length), K (Fulton’s condition factor), EI (eye index), FI (fin index) = pectoral fin length/body length X 100, GSI (gonado-somatic index)=gonad weight/body weight X 100; HSI (hepato-somatic index) = liver weight/body weight X 100; DTI (digestive tract index) = digestive tract weight/body weight X 100. \*: Significant difference with the previous stage p<0.01.

Silver stages	I	FII	FIII	FIV	FV	MII
	Resident males and females	Resident females	Pre-migrant females	Migrant females		Migrant males
Number	381	400	72	32	186	86
L (mm)	399±55	526±62*	658±82*	746±110*	644±122*	393±23 *
K	0.172±0.026	0.186±0.030*	0.197±0.025*	0.218±0.022*	0.182±0.026*	0.177±0.022 ns
EI	4.5±0.9	5.6±1.1*	7.6±1.3*	10.8±1.7*	9.9±1.6*	9.5±1.6 *
FI	3.7±0.5	3.9±0.6*	4.3±0.6*	4.3±0.4	5.0±0.7*	4.7±0.6 *
GSI	0.21±0.14	0.54±0.19*	0.82±0.24*	1.47±0.15*	1.71±0.31*	0.16±0.11 ns
HSI	1.72±0.59	1.41±0.44*	1.26±0.37*	1.40±0.17	1.24±0.30	1.41±0.26 *
DTI	4.75±1.90	4.64±1.60*	3.76±1.30*	1.84±0.61*	1.18±0.55*	1.59±0.48 *
GTH-II (ng.g <sup>-1</sup> )	0.03±0.06	0.02±0.06*	0.06±0.15	0.24±0.25*	0.49±0.36	1.08±0.95 *
GH (µg.g <sup>-1</sup> )	0.20±0.14	0.18±0.15	0.25±0.21*	0.14±0.10	0.15±0.16	0.07±0.6 ns

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Table 2: Jackknifed classification matrix for the test sample after step-wise discriminant analysis using length, weight, pectoral fin length and mean eye diameter. Wilks’ lambda = 0.057; p<0.00005

Initial group	Predicted group membership						% correct
	I	FII	FIII	FIV	FV	MII	
I	206	30	0	0	0	4	86
FII	21	221	18	0	0	3	84
FIII	0	6	32	2	6	0	70
FIV	0	0	5	12	5	0	55
FV	0	2	13	19	100	2	74
MII	0	0	0	0	1	60	98
Total	227	259	67	35	111	69	82

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2 Table 3: Classification functions for stage determination (I to FV and MII) of eels. Values  
3 correspond to the weights to be assigned to each variable.

	I	FII	FIII	FIV	FV	MII
Constant	-61.276	-87.995	-109.014	-113.556	-128.204	-84.672
L	0.242	0.286	0.280	0.218	0.242	0.176
W	-0.108	-0.125	-0.127	-0.103	-0.136	-0.116
MD	5.546	6.627	9.108	12.187	12.504	12.218
FL	0.614	0.838	1.182	1.230	1.821	1.295

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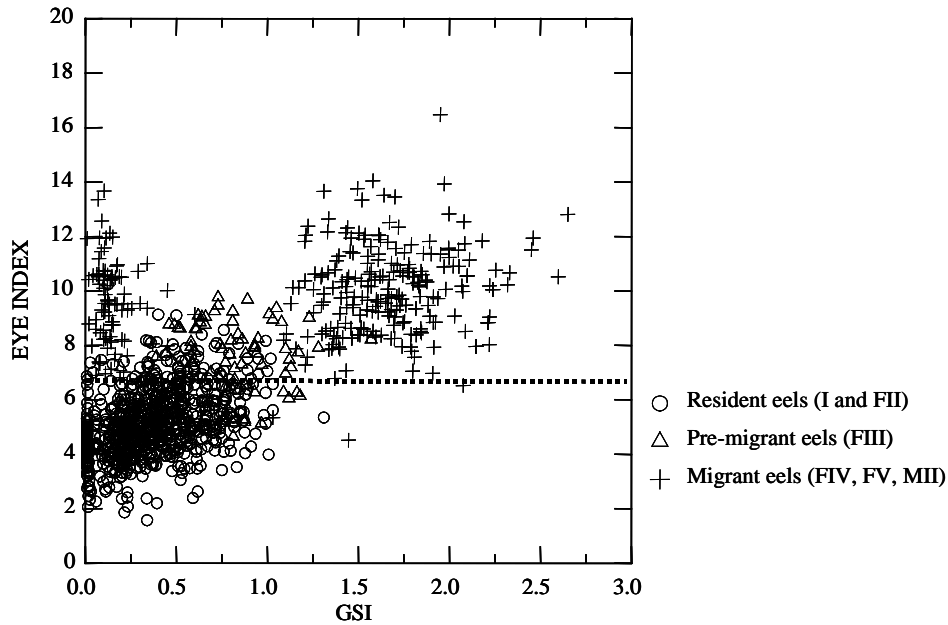
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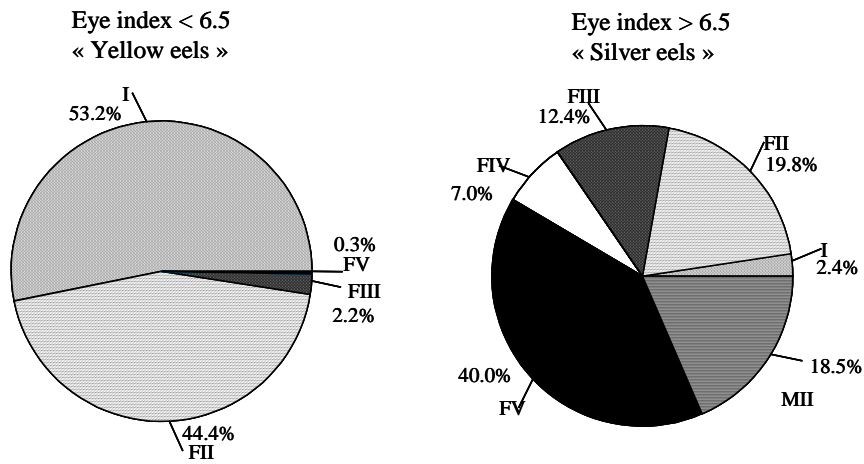
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7 Figure 1: A. Measurement of total body length; B. Measurement of pectoral fin length; C.  
8 Measurement of horizontal eye diameter.

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 2 Figure 2: Relationship between GSI and Pankhurst's eye index. The dotted line separates the  
 3 silver and yellow eels according to the 6.5 limit.  
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 7 Figure 3: Comparison of stage determination using Pankhurst's eye index (silver and yellow)  
 8 and silvering stage of eels based on physio-anatomical parameters. When using a threshold of  
 9 6.5, one overestimates the percentage of migrant eels as eels at stages I, II and III can be  
 10 defined as silver