

Eels at the Edge

Science, Status,
and Conservation
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John M. Casselman
and
David K. Cairns
editors

American Fisheries Society Symposium 58

Morphological Discrimination of the Silvering Stages of the European Eel

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Abstract.—A lack of knowledge on the transition from the resident to the migratory phase has led to a series of studies on the silvering process. Silvering marks the end of the sedentary growth phase and the beginning of the migratory phase. A six-stage classification was developed to describe the physiological and morphological events that occur during this metamorphosis and the subsequent migration. Stages corresponded to a growth phase (I to FII), a premigrant stage for females (FIII), and migrating stages for both sexes (FIV, FV, and MII). Here, the objective was to develop a “silver index” using only external measurements to assess the degree of metamorphosis of eels, based on the same data set that was used in the former study. It consisted of a large number of both resident and migratory eels that were sampled at different times of the year with different types of fishing gear and at several locations representing various types of habitats. Discriminant Analysis was applied on external measurements only: (body length, body weight, pectoral fin length, and eye diameters). Total percentage of correct reclassification into the six silvering stages was 82%. The silver index (classification functions) was able to identify 91% of the migratory eels. This method, associated with proper sampling, could be utilized for the quantification of potential spawners given that they all reach their spawning grounds in the Sargasso Sea.

Introduction

The scientific community agrees that the European eel *Anguilla anguilla* 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 eels are outside safe biological limits and that fisheries in recent years have not been sustainable (ICES 1999). In a recent com-

munication, 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. Whatever actions are taken, current conditions have to be measured, and this can be realized only through a better knowledge of the biological mechanisms that control the population dynamics (i.e., metamorphosis from the resident to the migratory stage). As catadromous fishes, European eels spend most of their life in freshwater until they head back to the spawning grounds in the Sargasso Sea. Only the freshwater phase

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of the eel is accessible to human observation, since no mature adults have ever been found in the open ocean. Therefore, the only way to evaluate the reproductive potential in a given year is to assess the proportion of potentially emigrating eels for that year. Although the eel's life cycle is well known and major traits of migratory individuals have been described (Bertin 1951; Tesch 2003), tools to identify which eels are physiologically ready to start their spawning migration are lacking.

Before individuals begin to swim downstream, they undergo a series of internal and external changes. These modifications prepare them for the 6,000-km journey back to their spawning grounds in the Sargasso Sea. This metamorphosis referred to as silvering, marks the end of the relatively sedentary growth phase (yellow stage) and the beginning of the migratory phase (silver stage).

Skin color is generally used to differentiate the two stages, as "migratory" eels most often display a white-silver belly and a black dorsal back. However, this counter-shading is not always present, and most migratory eels (especially large ones) often exhibit intermediate features, such as a bronze color on the belly and on the sides. Since the color criterion was found to be subjective and unreliable, Pankhurst (1982) proposed another method for distinguishing yellow and silver stages of female eels. The author developed an index based on eye surface area, which increases at the migratory stage, and set a threshold of 6.5 for sexually maturing European eels.

Physiologically, the differences between the so-called yellow and silver stages are important, since many complex mechanisms are involved in silvering. Migratory individuals stop feeding, and their alimentary tract regresses (Pankhurst and Sorensen 1984; Fontaine 1994; Marchelidon et al. 1999). Osmoregulatory mechanisms, which allow life in seawater, are already active before the eel leaves freshwater (Fontaine 1975; Dutil et al. 1987). Fat stores increase from 8% to 28%

(Bergersen and Klemetsen 1988; Larsson et al. 1990). But one of the most important changes at the silver stage is the initiation of the gonadotropic axis and the very beginning of gonad development (see review, Dufour et al. 2003).

As silvering involves many elaborate changes, a simple classification into two groups, namely yellow and silver stages, was not satisfactory. Therefore, we propose here and in Durif et al. (2005) a more detailed classification based on several internal and external parameters: gonad development, regression of digestive tract, gonadotropin and growth hormone levels, eye diameters, pectoral fin length, and condition factor. Five stages were defined for female eels: a growth phase (stages I and FII), during which eels feed and become sexually differentiated; and a premigrating stage (FIII), characterized by high levels of GH (growth hormone) and by the beginning of gonad development in females. Truly migratory individuals were divided into two groups: stage FIV, marked by the beginning of gonadotropin production and cessation of feeding; and stage FV, characterized by a significantly regressed digestive tract, higher gonadotropin levels, and elongated pectoral fins. Variability was found to be much less important in male eels, and results suggested that silvering and sex differentiation in males were simultaneous. Thus two stages were identified for males: the resident sexually undifferentiated stage (I) and the migratory stage (MII).

The purpose of the present study was to develop a noninvasive method for classifying developmental life stages and silvering of eels based on morphological characteristics. Discriminant analysis was used, first, to determine which morphological parameters best discriminated among all six stages, and second, to develop a silvering index based on these parameters. This data set was also used to compare between this silvering index and Pankhurst's criterion.

Methods

Description of the Data Set

The data set consisted of 1,188 eels collected at six different locations in France. Different types of fishing gear were used (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. Four morphological measurements were performed (see below) on the eels. Gonads, liver, digestive tract (emptied), and the pituitary (for growth hormone and gonadotropin assays) were then sampled on sacrificed individuals. Based on these parameters, all the eels in the sample were classified into one of six stages: one for undifferentiated resident male and female (I), four for female eels (FII to FV), and one for silver migrating males (MII). The characteristics of each stage are described in Table 1, with the various characteristics summarized for each stage. The procedures used to determine the degree of silvering of eels, as well as the full description of each stage, can be found in Durif et al. (2005).

Morphological Measurements

Four external measurements were made on eels, related to the morphological changes that are most apparent during silvering. Total body length (BL) and wet body weight (W) were measured to reflect condition of the eels. Length of the pectoral fin (FL) was measured from the insertion to the tip of the fin and corresponded to the greatest possible length (Figure 1). Both vertical (Dv) and horizontal (Dh) eye diameters were measured on the left eye, along the visible part of the cornea (Figure captions)

Figure 1). Mean eye diameter (MD) was calculated as $MD = (Dh + Dv)/2$.

For comparison with Pankhurst's method for silver stage determination, eye index was calculated as $EI = (MD/2)^2 \cdot \pi/L$ (Pankhurst 1982).

Data Analysis

Discriminant analysis was conducted on morphological variables L, W, FL, and MD. Groups corresponded to the six silvering stages described in Durif et al. (2005): stages I, FII, FIII, FIV, FV, and MII. A backward stepwise analysis was carried out on the data using a cross-validation procedure: a model was developed from a model sample, and its predictive accuracy was evaluated with a test sample. Classification functions were derived from the model and were used to determine to which stage each individual most likely belonged. Classification scores for each case were computed for each stage according to the formula

$$S_i = c_i + w_{i1} \times x_1 + w_{i2} \times x_2 + \dots + w_{in} \times x_n$$

where i denotes the respective stage, n denotes the n variables, c is a constant, w_{in} is the weight for the n^{th} variable in the computation of the classification score for the i^{th} group, and x_n is the observed value for the respective case for the n^{th} variable. S_i is the resultant classification score. An eel was assigned to the stage for which it had the highest S_i . The efficiency of the analysis was evaluated through a classification matrix, which indicated the number of eels that were correctly classified and those that were misclassified.

Results

Silvering Index

The various characteristics were quite different among the stages and provided the basis for developing a silvering index (Table 1). The sample of 1,156 individuals for

Table 1. Mean and standard deviation of morpho-anatomical parameters of undifferentiated and female eels according to life stage and silvering (Durif et al. 2005). BL (body length), *K* (Fulton's condition factor), EI (eye index), FI (fin index) = pectoral fin length/body length X 100, GSI (gonadosomatic index)=gonad weight/body weight X 100; HSI (hepatosomatic index) = liver weight/body weight X 100; DTI (digestive tract index) = digestive tract weight/body weight X 100; GTH (pituitary gonadotropin hormone or LH-like); GH (pituitary growth hormone).

Silvering stages	I	FII	FIII	FIV	FV	MII
	Undifferentiated males and females resident	Females resident	Females pre-migrant	Females Migrant		Males migrant
<i>N</i>	381	400	72	32	186	85
BL (mm)	399 655	526 662	658 682	746 6110	644 6122	393 623
<i>K</i>	0.172 60.026	0.186 60.030	0.197 60.025	0.218 60.022	0.182 60.026	0.177 60.022
EI	4.5 60.9	5.6 61.1	7.6 61.3	10.8 61.7	9.9 61.6	9.5 61.6
FI	3.7 60.5	3.9 60.6	4.3 60.6	4.3 60.4	5.0 60.7	4.7 60.6
GSI	0.21 60.14	0.54 60.19	0.82 60.24	1.47 60.15	1.71 60.31	0.16 60.11
HSI	1.72 60.59	1.41 60.44	1.26 60.37	1.40 60.17	1.24 60.30	1.41 60.26
DTI	4.75 61.90	4.64 61.60	3.76 61.30	1.84 60.61	1.18 60.55	1.59 60.48
GTH-II (ng.g ⁻¹)	0.03 60.06	0.02 60.06	0.06 60.15	0.24 60.25	0.49 60.36	1.08 60.95
GH (µg.g ⁻¹)	0.20 60.14	0.18 60.15	0.25 60.21	0.14 60.10	0.15 60.16	0.07 60.6

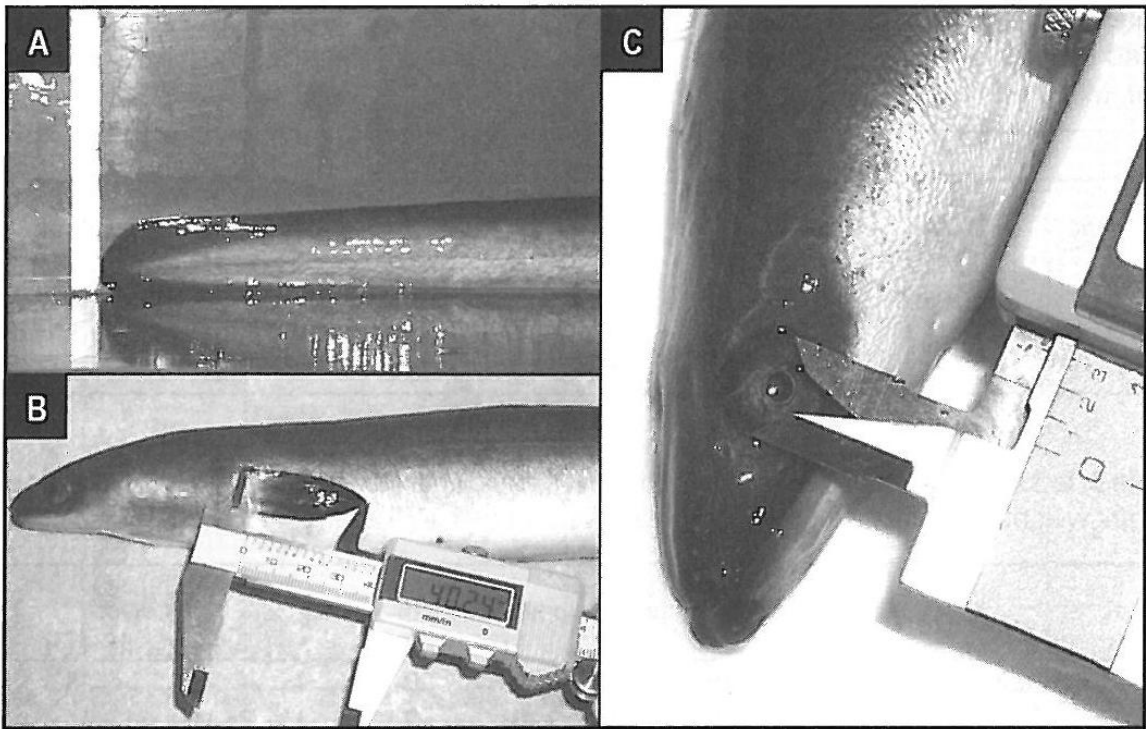


Figure 1. A) Measurement of body length; B) Measurement of pectoral fin length; C) Measurement of horizontal eye diameter.

which pectoral fin length was available was randomly split into a sample to develop the model and a test sample representing 66% (768 individuals) and 34% (388 individuals), respectively. A significant discriminant model was obtained when using all four variables: body length, body weight, mean eye diameter, and pectoral fin length. The first canonical variable accounted for more than 77% of the total dispersion of the groups. Standardized canonical discriminant function coefficients indicated the relative contribution of each variable to the overall discrimination. Mean eye diameter was the major contributor, followed by fin length and body weight (respectively, -1.034 , -0.770 , and 0.634); the contribution of body length was the lowest (0.183).

Classification functions, using the values in Table 2, were used to compute classification scores for new observations (test sample). The percentage of correct classification was equal to 83% for the model sample,

77% for the test sample, and 82% for the total jackknifed value (Table 3). The analysis usually assigned misclassified individuals to adjoining groups. Resident eels, stages I and FII, showed high percentages of correct classification: respectively, 84% and 86%. Errors were higher for the classification of pre-migrant eels (stage FIII), of which 30% were classified into FII, FIV, and FV stages. Misclassification was greatest for stage FIV eels, since only 55% of eels were correctly classified; however, none of those eels were assigned as resident. This group also comprised the smallest number of individuals. Twenty-six percent of the FV eels were misclassified, and 1.5% were assigned to the FII stage (2 individuals). Two were considered males (MII), but they could be manually reclassified since they were longer than is usual for European male eels (35–40 cm). The classification of migrant males was 98% accurate, and only one eel was misclassified into stage FV. Likewise, this individual was easily reclassified

Table 2. Classification functions for stage determination (I to FV and MII) of eels. Values correspond to the weights to be assigned to each variable. BL (body length in mm), W (body weight in g), MD (mean eye diameter in mm), FL (fin length in mm).

	I	FII	FIII	FIV	FV	MII
Constant	-61.276	-87.995	-109.014	-113.556	-128.204	-84.672
BL	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

Table 3. 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; $p < 0.0001$.

Initial group		Predicted group membership						% correct classification	
		I	FII	FIII	FIV	FV	MII		
I	Resident	206	30	0	0	0	4	86	95
FII		21	221	18	0	0	3	84	
FIII	Pre-migrant	0	6	32	2	6	0	70	70
FIV	Migrant	0	0	5	12	5	0	55	91
FV		0	2	13	19	100	2	74	
MII		0	0	0	0	1	60	98	
Total		227	259	67	35	111	69	82	92

into its correct stage by using body length, since males rarely exceed 45 cm.

Classification efficiency, when grouping stages into resident, premigrant, and migrant (male and female) groups, was 95%, 70%, and 91%, respectively (Table 3), with an overall mean of 92%

Comparison with Pankhurst's Eye Index

Using the classification functions, or silver index, based on body length, body weight, mean eye diameter, and fin length gave an accuracy of 91% for the estimation of migrants (stages FIV and FV). In comparison, when using Pankhurst's threshold, almost all migratory eels (FIV, FV, and MII) were correctly classified (Figure 2). Two individuals had an eye index under 6.5 and would have been considered yellow eels. These were the same two eels that were misclassified by the silver index. How-

ever, a large proportion of the resident eels (I and FII), as they were initially defined by their physio-anatomical characteristics, had an eye index above this limit although they did not display any sign of silvering: 3% of stage I and 25% of FII eels would have been considered silver (Figure 2). This percentage increased in FIII eels, since 81% had an eye index over 6.5. In total, applying Pankhurst's limit to our sample would have overestimated the number of migratory eels by 20%.

Application: Seasonal Variation in Silvering Stages

The seasonal variation of silvering stages in our sample was analyzed to illustrate a possible use for this classification method. Samples from the different years were pooled and monthly proportions were calculated (Figure 3). Stages I and FII represented more than 50%

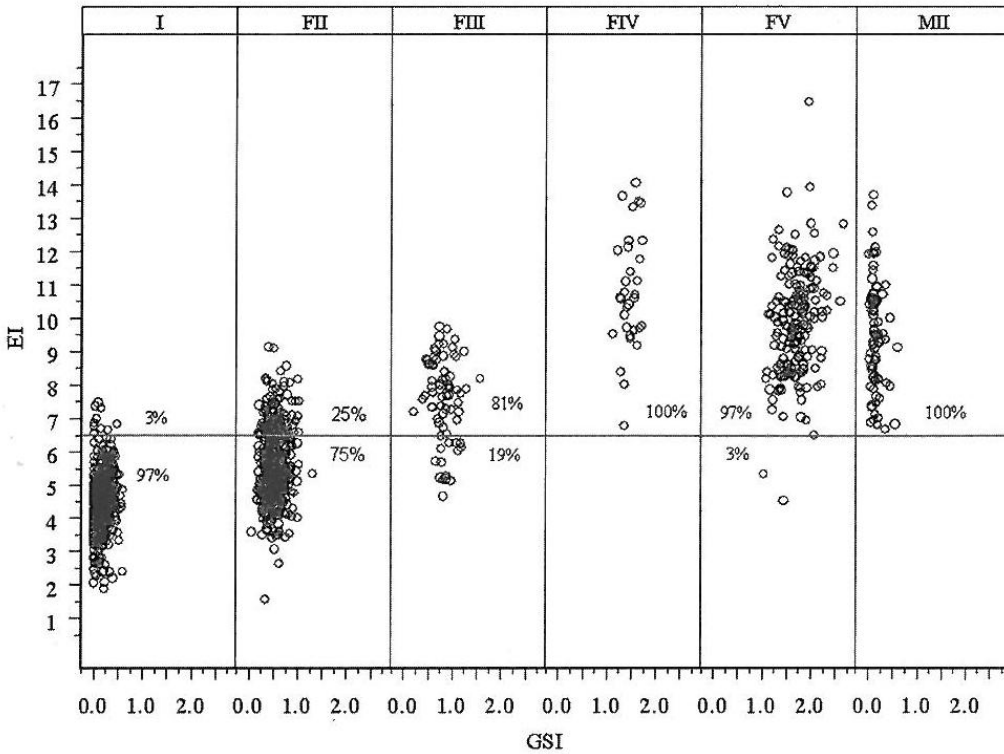


Figure 2. Relationship between gonadosomatic index (GSI) and eye index (EI) for each of the various stages associated with silvering. The horizontal (light) line is set at Pankhurst's 6.5 limit. Percentages indicate the proportions of eels that would have been defined as silver eels (above the limit) and yellow eels (below the limit).

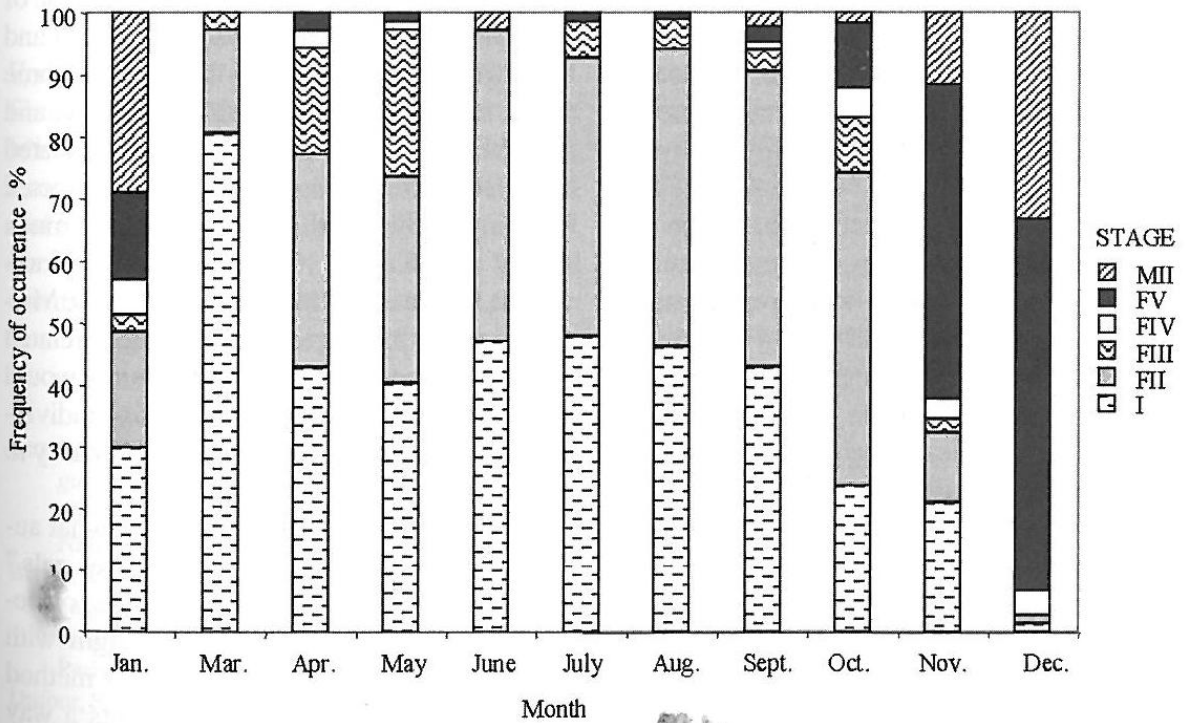


Figure 3. Proportions of the various stages associated with silvering (I, FII, FIII, FIV, FV, and MI) by month.

of the sample every month except from November to January, the period of downstream migration. These two stages totaled 97% of the eels caught during March, with a high proportion of stage I eels (81%). It is very likely that these small undifferentiated individuals were eels that had just been recruited in the months prior to sampling; in France, glass eels migrate upstream from December to April. Stage FIII eels were most abundant in spring (17% in April, 24% in May), when growth conditions are optimal. They were also found from July to October, but in smaller proportions, between 4% and 9%. Migratory eels (FIV, FV, and MII) were present from October to January and comprised up to 97% of the individuals caught in December. Percentages decreased to 49% in January, when most of the eels would have emigrated.

Discussion

The silvering process of eels from Lake Grand-Lieu in France was first described by Durif et al. 2000. This work was then extended to different types of habitats in France (brackish-water marsh, large rivers, small coastal river, and estuary) with different environmental characteristics, to look for variability in physio-anatomical characteristics of migratory eels. A broad classification was defined based on this varied sample of eels from different locations. It consisted of six stages, which represented a growth phase (stages I and FII), a premigrant stage (FIII), and migrating stages (FIV, FV, MII) for male and female eels (Durif et al. 2005). This classification gives a more precise and ecologically appropriate description than do the limited terms yellow and silver phases. It reflects the continuous nature of the silvering process and the fact that although silvering may have started, some individuals may not be ready for active migration nor have started to mature. Since the duration of this process is currently unknown, this description of several silvering stages represents a first step towards

understanding the dynamics of silvering and migration.

Reliable criteria were needed to determine whether an eel was physiologically ready to start migration and sexual maturation. Up to now, identification of female silver eels and assumed emigration has generally been based on Pankhurst's eye index. Very importantly, if we had applied only Pankhurst's 6.5 limit on our sample, we would have overestimated emigrants by 20%. Eye index does reflect increasing GSI; however, it is also strongly correlated to body length in the resident stage. Thus, a large yellow eel (such as some stage FII eels) may have a high eye index without necessarily being silver. This partly explains why Pankhurst's criterion of 6.5 overestimated the number of potential female emigrants.

This study complements the description of silvering stages by providing a noninvasive method of determining whether an eel is sedentary, maturing, or physiologically ready to migrate. We found it reasonably accurate (82%) to use only external measurements to assign a developmental stage (I to FV and MII). The use of additional parameters, such as body weight and fin length, was necessary to differentiate some stages more specifically (mainly FIII, FIV, and FV). Misclassification occurred. Errors related to the sex of individuals were easily corrected by using body length, since males are much shorter than females. Other misclassified individuals were assigned to adjoining groups. Misclassification for stages that are closely related would be expected, since the attributes would naturally overlap for certain borderline individuals, but these stages were selected primarily to provide a practical description.

It is not always clear in literature what authors mean when they mention "silver eels," since they can refer to color, eye size, or behavior (actively emigrating eels caught with specific fishing gear). The silver index method for determining stages also represents a way of standardizing observations between different observers and watersheds and periods. To

obtain these data, eel populations need to be closely monitored and data must be collected carefully and consistently. Measurements used here are relatively simple and can easily be done on a large scale. Surveys could be carried out over different seasons within the same year: the evolution of the proportion of the different stages would help in understanding the dynamics of the silvering process (time and duration of the metamorphosis); these may vary according to location. Practically, and with an appropriate sampling method, the proportion of eels (stages FIV, FV, and MII) that will start their downstream migration can be evaluated if they are triggered by the appropriate environmental factors. Under the hypothesis that all eels will reach the Sargasso Sea, they will constitute the minimal estimation of progenitors for a given year. Analysis of year-to-year variations in the proportion of these migratory stages could also help to explain quantitative variation in the run of emigrant eels.

Acknowledgments

This study benefited from a grant from the European Union (project Q5RS-2001-01836). Financial support was also given by the French Ministry of Environment and Research, the Conseil Supérieur de la Pêche, the GRISAM, and the Cemagref. We also would like to thank the anonymous reviewers for their helpful comments on improving this manuscript.

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