

## The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage

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The identification of five stages for female and two stages for male eels *Anguilla anguilla* using multivariate analysis was carried out on a large sample of individuals collected at six different locations in France. Stages corresponded to a growth phase (stages I and II), a pre-migrant phase (III) and two migrating phases (IV and V). It is likely that an important period of growth triggered silvering through the production of growth hormone (GH) in stage III eels. In migrating eels gonad development, gonadotropin hormone (GTH-II) production and increase of eye surface were similar at all sites. Differences among locations were found in gut regression and pectoral fin length. As variability for these increased with the size of the watershed and values were highest for the most downstream locations, fin length and gut regression may indicate the time since an eel started its migration. © 2005 The Fisheries Society of the British Isles

Key words: *Anguilla*; biometry; gonadotropin; growth hormone; silvering.

### INTRODUCTION

Since the 1980s, the landings of eel *Anguilla anguilla* (L.) have indicated a general decline (Moriarty & Dekker, 1997; Dekker, 2003). Because of their peculiar life cycle, eels are subject to both continental and oceanic factors and natural and anthropogenic perturbations. Once larvae have arrived from their spawning grounds, thought to be the Sargasso Sea (Schmidt, 1922), to the European and North African continental shelf, they metamorphose to become glass eels and large numbers of individuals swim upstream to colonize inland waters. The growth phase commonly called the yellow stage lasts several years and its time span varies in male and female eels. At the end of this phase, eels undergo a second metamorphosis called 'silvering'. This metamorphosis corresponds to physiological and morphological changes that prepare the fish for the oceanic migration back to the Sargasso Sea and reproduction there.

Physiologically, the differences between the yellow and silver stages are important. Modifications are linked to the transition between fresh and salt

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water, to the preparation of the *c.* 6000 km migration in terms of energy resources and most of all to the beginning of the reproductive phase. The silver livery of eels at the time of their downstream migration is the most apparent external change. Although, the use of skin colour to identify ready-to migrate eels has been highly criticized (Pankhurst & Lythgoe, 1982), it is generally this 'standard' that is most used for stage determination. Once an eel displays a white silver belly well separated from a black dorsal region by the lateral line then it is considered to be at the 'silver stage' and implicitly a migrant. Increasing eye size and darkening of the pectoral fins are also commonly used to determine the stage of the eel. Yet no physiological or morphological validation has been undertaken. Available information comes from laboratory studies of hormone-injected eels (Pankhurst, 1982; Pankhurst & Sorensen, 1984); thus no link to the ecology of the fish in its natural environment has ever been made. As a result of this, the migrating stage has not been characterized and questions remain on the sequence of events that lead to this stage, or on the factors that trigger silvering. Eels at the time of their migration are thought to cease feeding, although Facey & Helfman (1985) described silver American eels *Anguilla rostrata* (Lesueur) containing food items in their stomach with no signs of gut degeneration. More recently Westin (2003) also observed silver eels with full stomachs.

The aim of this study was to describe the evolution of some of the physiological and morphological transformations which lead to the silver migrating stage; in particular to examine the different stages from the yellow resident to the silver migrating phases in male and female *A. anguilla*. In order to obtain as much variability as possible in the sample, the characterization of silvering stages was carried out on eels from various locations (large watersheds, small coastal rivers, estuary and marsh), caught at several time periods in the year and with different types of fishing gear.

## MATERIALS AND METHODS

### FISH SAMPLING

This study is based on data from six different locations in France (Fig. 1). At all locations and when it was feasible two types of samples were collected: an 'all-stages' sample (using electrofishing gear, eel pots and fyke nets) and a 'migrant' sample of eels only caught during their downstream run in autumn and using typical silver eel fishing gear (weir and stow net). All samples were collected between 1994 and 2002.

### MORPHOLOGICAL AND PHYSIOLOGICAL MEASUREMENTS

Eels were anaesthetized in a 1:10 solution of clove oil dissolved in ethanol (70%). Several concentrations were tested (unpubl. data). Responses (induction and recovery from anaesthesia) were varied, and they depended more on water temperature than on size or stage of eels. Between 1–2 ml of this solution was added to the water bath (10 l). At 18° C, 1.5 ml was sufficient to immobilize the eels, 2 ml for lower temperatures (*c.* 14° C), and 1 ml for water *c.*  $\geq 20^\circ$  C.

The following measurements were made on all eels: body mass ( $M$ ), total length ( $L_T$ ), pectoral fin length ( $L_{PF}$ ) and horizontal ( $D_h$ ) and vertical ( $D_v$ ) eye diameters. Three morphometric indices were calculated: eye index  $I_E$ ,  $I_E = [0.25 (D_v + D_h) \pi L_F^{-1}]$

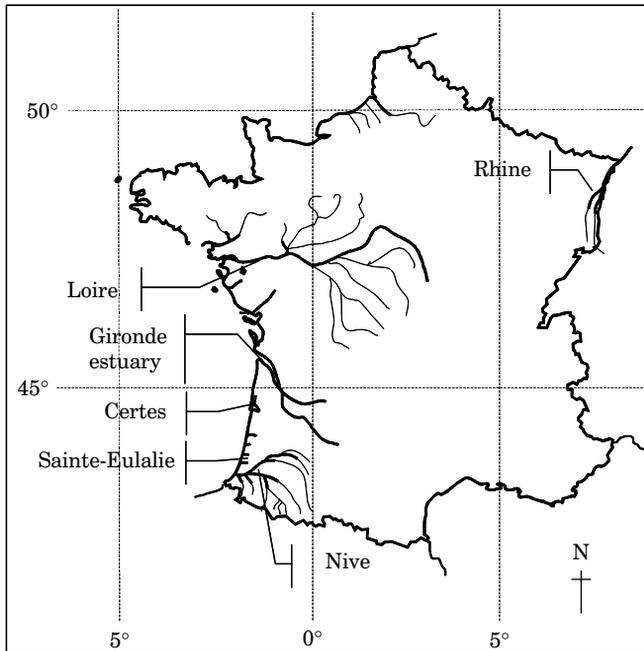


FIG. 1. Sampling sites.

(Pankhurst, 1982), fin index  $I_F$ ,  $I_F = 100 L_{PF} L_T^{-1}$ , and Fulton's condition factor  $K$ ,  $K = 10^5 M L_F^{-3}$ .

Eels were sacrificed and dissected. Sex was identified macroscopically and microscopically on histological preparations when necessary. Individuals were classified into three categories: females (differentiated ovaries), males (differentiated testes) and undifferentiated. Gonads were removed and weighed ( $M_G$ ) as well as the liver ( $M_L$ ). The gut was cut in the anal region and above the liver and its food contents were emptied before weighing ( $M_{GU}$ ). The gonado-somatic index  $I_G$ ,  $I_G = 100 M_G M^{-1}$ , hepato-somatic index  $I_L$ ,  $I_L = 100 M_L M^{-1}$ , and gut index  $I_{GU}$ ,  $I_{GU} = 100 M_{GU} M^{-1}$  were calculated.

The pituitary gland was removed from eels collected from the Loire and the Rhine (60% of total sample). Gonadotropin hormone (GTH-II or LH-like) and growth hormone (GH) were assayed at the Museum National d'Histoire Naturelle Paris, by specific radio immunoassays (RIA) on the pituitary extracts as described by Dufour *et al.* (1983) and Marchelidon *et al.* (1996). Levels are expressed in  $\text{ng g}^{-1}$  of total body mass for GTH-II and in  $\mu\text{g g}^{-1}$  of total body mass for GH.

## DATA ANALYSIS

Principal component analysis (PCA) was carried out on 'internal' variables:  $I_G$ ,  $I_L$  and  $I_{GU}$  to determine the silvering state of eels. Cluster analysis (Ward's method) was executed on the factorial scores of the PCA to differentiate yellow resident from silver migrating eels according to the different locations and validated by gonadotropin levels. Both analyses were further carried out on anatomical and morphometric variables to identify intermediate stages of metamorphosis and to describe the relationships between internal and external variables. The  $I_G$  of males varied over a much lower range than for females. In order to observe the possible individual differences between males, these were analysed separately from females. The sexually undifferentiated individuals were added to each set of data (male and female) to constitute a reference for the earliest stage of the silvering process in both analyses.

Statistical comparisons were performed on the physiological and morphological variables in order to validate the groups determined by PCA and cluster analysis. Morphometric data ( $L_T$  and  $I_F$ ) were compared by ANOVA followed by a multiple comparison test (Bonferroni) to detect which groups were different. Hormone levels and  $K$  were compared by Kruskal–Wallis tests as suggested by Bolger & Connolly (1989). To compare development of gonads, liver and gut regressions, ANCOVA was used on  $M_G$ ,  $M_L$  and  $M_{GU}$  values using  $M$  as a covariate to remove any size effect. Mean eye diameters were also compared by ANCOVA with  $L_T$  as a covariate followed by pairwise comparisons (Bonferroni). Links between physiological and morphological variables were evaluated by Pearson's correlation coefficient and their significance was determined by a Bonferroni test. Multivariate analyses and statistical tests were performed with ADE-4 (Thioulouse *et al.*, 1997) and SYSTAT 10.

## RESULTS

### SAMPLED FISH

The total sample was composed of 1021 female, 119 male and 48 undifferentiated eels. Both types of samples (all-stages and migrant) were collected at three sites (Table I), corresponding to three very different types of watershed (Fig. 1): the Loire (which represents a watershed of *c.* 110 000 km<sup>2</sup>), the Nive (*c.* 1000 km<sup>2</sup>) and the coastal marsh of Certes (*c.* 100 km<sup>2</sup>, brackish water). Samples from the Rhine (*c.* 224 000 km<sup>2</sup>) were collected at *c.* 900 km from the sea and comprised eels of all stages. Eels from the Sainte-Eulalie River (small coastal river in a watershed of *c.* 250 km<sup>2</sup>) were obtained from a commercial silver eel fisherman. A few individuals (15 eels) caught by trawling on the Gironde estuary were added to the data.

### CHARACTERIZATION OF MIGRATING FEMALES

The PCA, carried out on the three variables  $I_G$ ,  $I_L$  and  $I_{GU}$  of 1022 female and 48 undifferentiated eels, indicated that the first two components of the analysis represent 64 and 24% of the total inertia. The first component is highly correlated to  $I_G$  and  $I_{GU}$ , whereas the second component is correlated to  $I_L$ .

TABLE I. Characteristics of eel samples according to each location

Site	Sample type	<i>n</i>	Time of sampling	Fishing gear
Loire	Migrants	74	Autumn 2000 and 2002	Stow net
	All stages	334	1994–2002	Eel pots, fyke nets, electrofishing
Nive	Migrants	31	Autumn 2000 and 2002	Trap
	All stages	52	September 2000	Electrofishing
Certes	Migrants	27	December 1999 and October 2001	Trap
	All stages	70	Summer 2002	Fyke net
Rhine	All stages	524	1996–2002	Electrofishing, fyke nets
Sainte-Eulalie	Migrants	62	November 2001 and 2002	Eel weir
Gironde estuary	All stages	15	April and May 2001	Trawl net

Eels are distributed along the first axis in a 'horseshoe' pattern; this 'Guttman' effect (Guttman, 1950) reflects a gradient between eels with a low  $I_G$  and a high  $I_{GU}$  to metamorphosing eels that have developing gonads (high  $I_G$ ) and a regressing digestive tract (low  $I_{GU}$ ). Thus the first axis represents the silvering process. Migrating eels are clustered in a tight group on the left side of this axis. They had the most developed gonads and the most regressed gut at all sampled locations (Loire, Nive and Sainte-Eulalie). Some eels from the Rhine had the same characteristics as migrating eels even though they were from the all-stages sample and thus have not been captured during migration. These individuals were probably 'ready' to migrate. Undifferentiated eels, rather than being confined to one side of the gradient, are distributed along the second axis and display highly variable values for  $I_L$ . This is not the case for migrating eels for which  $I_L$  was lower and more uniform.

Cluster analysis applied on the individual factorial scores, clearly separates two groups: one comprises all the eels from the migrant sample (except for one eel from the Nive and one from Sainte-Eulalie) and consequently corresponds to the silver migrating stage; the second group, more scattered, includes all undifferentiated and yellow resident eels. The significant difference in the GTH-II level between the two groups confirms that they correspond to maturing eels (silver migrants) and to yellow resident eels (Table II).

#### RELATIONSHIP BETWEEN INTERNAL AND EXTERNAL VARIABLES IN FEMALE EELS

Two additional PCA were carried out on both of the groups previously defined: yellow resident and silver migrating eels. Morphological measurements and indices were included in the analyses. On the first factorial plot of the yellow eel analysis [Fig. 2(a)] undifferentiated eels are all located on the left side of axis 1; this is consistent with the fact that they represent the least mature individuals. For the silver eel analysis, [Fig. 2(b)], eels from the migrant sample are clustered together and display the highest scores on both axes. Relationships between

TABLE II. Mean  $\pm$  s.d. values of physiological and morphological variables (see Fig. 2) of yellow and silver female eels

	Yellow eels	Silver eels
<i>n</i>	839	230
$L_T$ (mm)	483 $\pm$ 102	658 $\pm$ 124*
<i>K</i>	0.180 $\pm$ 0.029	0.187 $\pm$ 0.028*
$I_E$	5.3 $\pm$ 1.4	9.9 $\pm$ 1.7*
$I_F$	3.9 $\pm$ 0.6	4.9 $\pm$ 0.7*
$I_G$	0.42 $\pm$ 0.27	1.68 $\pm$ 0.31*
$I_L$	1.58 $\pm$ 0.53	1.27 $\pm$ 0.31*
$I_{GU}$	4.61 $\pm$ 1.73	1.27 $\pm$ 0.60*
GTH-II (ng g <sup>-1</sup> )	0.03 $\pm$ 0.06	0.41 $\pm$ 0.35*
GH ( $\mu$ g g <sup>-1</sup> )	0.19 $\pm$ 0.15	0.14 $\pm$ 0.14*

\*, significant difference ( $P < 0.05$ ) between yellow and silver eels.

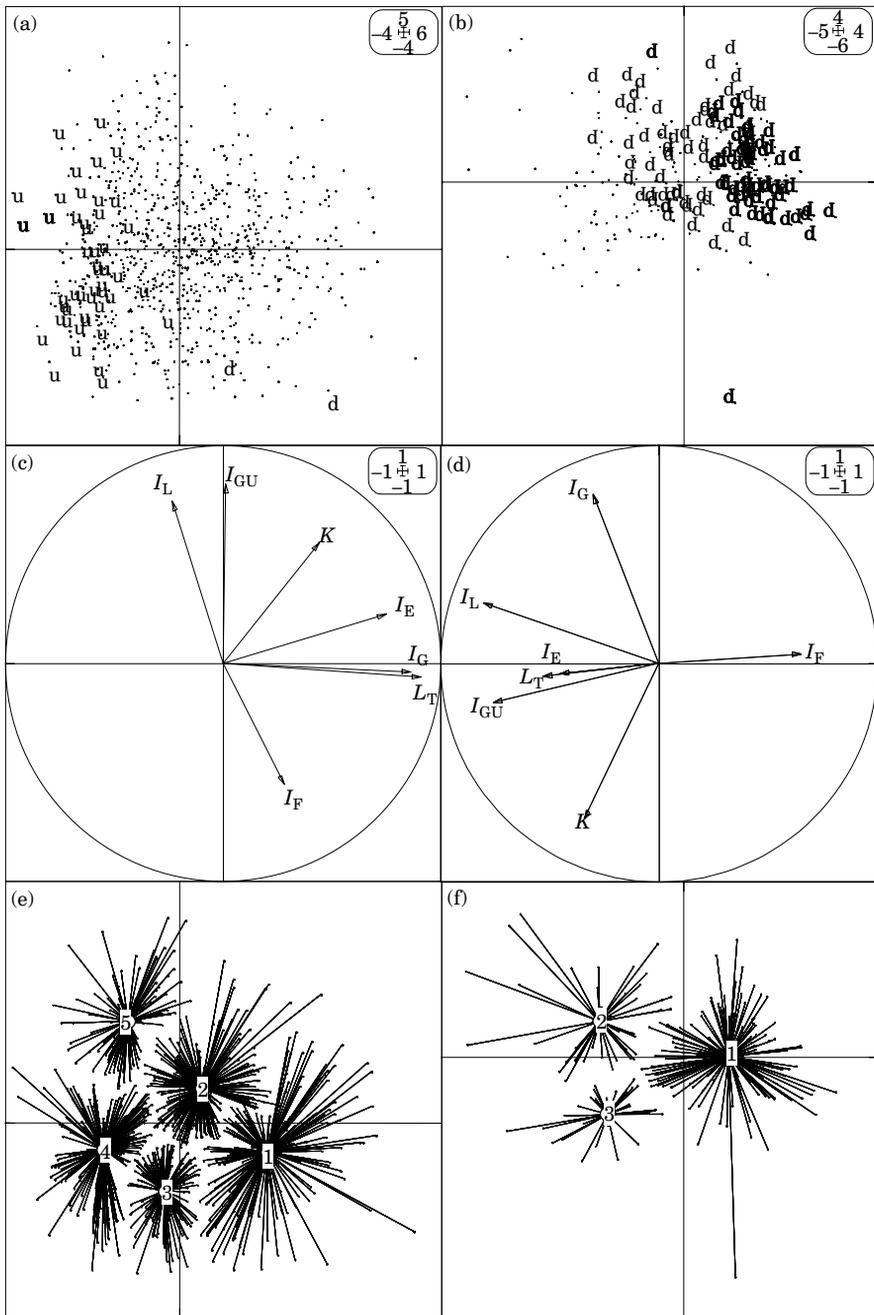


FIG. 2. Results of the two PCA (a), (c), and (e) yellow and (b), (d) and (f) silver eels carried out on morphometric and anatomical variables: total length ( $L_T$ ), condition factor ( $K$ ), eye index ( $I_E$ ), fin index ( $I_F$ ), gonado-somatic index ( $I_G$ ), hepato-somatic index ( $I_L$ ) and gut index ( $I_{GU}$ ). (a) and (b) factorial scores of individuals; only downstream migrants (d) and undifferentiated eels (u) are labelled. Unlabelled eels correspond to the all-stages sample (c) and (d) correlation circles of variables for yellow and silver eels. (e) and (f) corresponding groups determined by cluster analyses. Each cluster is labelled with a number (1–5 for both yellow and silver eels).

internal and external variables are different in the two analyses as indicated by both corresponding maps of variables or correlation circles [Fig. 2(c), (d)].

For yellow eels,  $I_G$  and  $I_E$  displayed size effects as they were both significantly correlated to  $L_T$  ( $r = 0.72$  and  $0.63$ , respectively,  $P < 0.05$ ). Thus, gonads developed and eye surface increased proportionally to  $L_T$ . The value of  $K$  was also significantly correlated to these variables but the link was weaker ( $r = 0.21$  to  $0.33$ ,  $P < 0.05$ ). The  $I_F$ ,  $I_L$  and  $I_{GU}$  did not show a size effect. These three variables as well as  $K$  represent the main source of variability on axis 2. Thus, eels in the top part of the factorial plot had a well developed gut and liver, both variables being significantly correlated to  $K$  ( $r = 0.37$  and  $0.15$ , respectively,  $P < 0.05$ ). In contrast, eels with negative scores on axis 2 had lower  $K$ ,  $I_L$  and  $I_{GU}$  values.

Female eels in this sample started to silver at 50 cm  $L_T$  (Fig. 3). In silver eels, the  $I_G$  was not correlated to  $L_T$  or  $I_E$  ( $r = 0.06$  and  $0.16$ , respectively,  $P > 0.05$ ). The  $I_F$  was negatively correlated to  $I_{GU}$  ( $r = -0.43$ ,  $P < 0.05$ ). Females from the migrant sample, grouped on the right-hand corner [Fig. 2(b)], had the lowest  $K$  values; their  $I_G$  was higher, their pectoral fins were longer, and their gut was the most regressed among silver eels.

#### GEOGRAPHICAL COMPARISON OF SILVER FEMALE EELS

Several observations can be made from the distributions of factorial scores from the silver eel PCA according to sampling location (Fig. 4). Distributions of scores for the first axis (linked to regression of gut, liver mass and  $I_F$ ) are

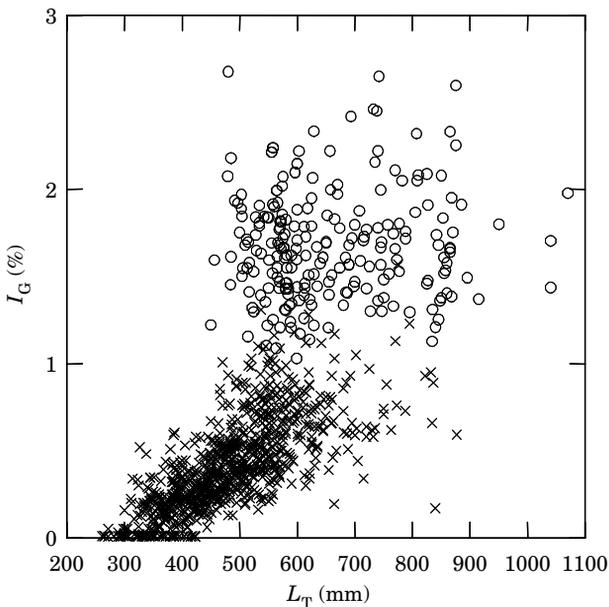


FIG. 3. Relationship between the gonado-somatic index and total length of yellow ( $\times$ ) and silver ( $\circ$ ) undifferentiated and female eels.

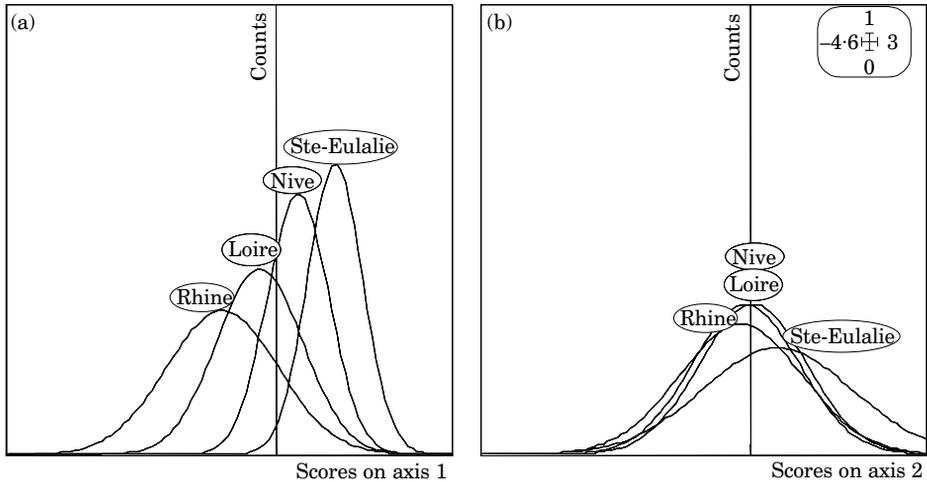


FIG. 4. Standardized distributions of factorial scores of the silver eels on (a) the first and (b) second axis of the PCA (silver eel analysis) according to their location. Rank of mean scores correspond to the size of each watershed: Sainte-Eulalie ( $250 \text{ km}^2$ ) < Nive ( $1000 \text{ km}^2$ ) < Loire ( $100\,000 \text{ km}^2$ ) < Rhine ( $224\,000 \text{ km}^2$ ).

significantly different at each site (Kolmogorov–Smirnov test,  $P < 0.05$ ). Eels from the smallest watershed (Sainte-Eulalie,  $250 \text{ km}^2$ ) present the highest scores, a narrow distribution (these eels display very similar characteristics in terms of  $I_{\text{GU}}$ ,  $I_{\text{L}}$  and  $I_{\text{F}}$ ), while the scores for the largest catchment (Rhine,  $224\,000 \text{ km}^2$ ) are the lowest and their distribution is wide. The scores for the Loire ( $100\,000 \text{ km}^2$ ) and the Nive ( $1000 \text{ km}^2$ ) are also ordered according to the size of the catchment [Fig. 4(a)]. This suggests that variability in gut regression, liver mass and fin length of migrating silver eels was higher in large watersheds. Moreover,  $I_{\text{GU}}$  and  $I_{\text{L}}$  were the highest for the eels from the Rhine where the sampling site was located furthest away from the sea (*c.* 900 km). The  $I_{\text{GU}}$  was lower in eels from sites, which were closer to the sea (Nive and Sainte-Eulalie, *c.* 15 km; Loire, 60 km). This suggests that eels from the Rhine being further upstream, and which were probably at the beginning of migration, were less advanced in the silvering process.

Scores on the second axis overlap and only the Nive is significantly different from the Rhine and Sainte-Eulalie (Kolmogorov–Smirnov test,  $P < 0.05$ ). This means that the  $I_{\text{G}}$  and  $K$  did not differ markedly according to site. Indeed, there was no significant difference ( $P > 0.05$ ) in  $I_{\text{G}}$  of silver eels between the four locations. Differences in  $K$  were slight but statistically significant ( $P < 0.05$ ): eels from Sainte-Eulalie had the lowest  $K$  value and this explains the slight shift of the distribution of scores for this site [Fig. 4(b)].

There were no differences in hormone levels (GTH-II or GH) between eels from the Loire and the Rhine (only available data). The  $I_{\text{E}}$  was the same at all sites whereas the mean  $I_{\text{F}}$  of eels from the Nive and Sainte-Eulalie were the highest.

## DETERMINATION OF INTERMEDIATE STAGES FOR FEMALE EELS

Yellow female eels displayed a high variability in terms of gonad development and mass of the gut. Moreover, the GTH-II concentrations were also extremely variable among this group and fairly high for some individuals: between 0.00 and 0.56 ng g<sup>-1</sup>. This suggests that the silvering process is gradual and can be divided into several intermediate stages.

In order to determine the changes that occur from the yellow resident stage to the silver migrating stage in more detail, cluster analysis was carried out on the factorial scores of the previous two PCA. In the yellow eel analysis, the first axis reflected growth and development towards the silver migrating stage whereas the second axis separated eels according to their general state through the mass of liver, gut and *K*. In the silver eel analysis, axis 1 was linked to the regression of the gut and increase of the pectoral fin length. Based on the eight clusters, five stages were defined to describe the silvering process [Fig. 2(e), (f)].

### Stage I (clusters 5 and 4) [Fig. 2(e)]

This stage corresponds to the smallest eels in the sample. Their mean  $L_T$  was equal to 40 cm (Table III). The  $I_G$  or GTH-II levels were not significantly different between clusters 5 and 4, so both were grouped into the same stage. The gonads started to develop slightly and the ovaries appeared as translucent strips. The  $I_G$  was <0.5%. The  $I_{GU}$  was highly variable and its variations are linked to nutrition: during winter and spring, mean  $I_{GU}$  <4%, while in summer and autumn it ranged from 4 to 7% (Fig. 5). The  $I_L$  and *K* followed the same trend. The production of GTH-II was close to zero and GH levels were variable, ranging from 0.02 to 0.77 µg g<sup>-1</sup> suggesting different growth rates among individuals. The  $I_E$  and  $I_F$  were low (4.5 and 3.7, respectively).

TABLE III. Mean ± s.d. values of physiological and morphological variables (see Fig. 2) of undifferentiated and female eels according to the silvering stage

Stage	I	II	III	IV	V
<i>n</i>	381	400	72	32	186
$L_T$ (mm)	399 ± 55	526 ± 62*	658 ± 82*	746 ± 110*	644 ± 122*
<i>K</i>	0.172 ± 0.026	0.186 ± 0.030*	0.197 ± 0.025*	0.218 ± 0.022*	0.182 ± 0.026*
$I_E$	4.5 ± 0.9	5.6 ± 1.1*	7.6 ± 1.3*	10.8 ± 1.7*	9.9 ± 1.6
$I_F$	3.7 ± 0.5	3.9 ± 0.6*	4.3 ± 0.6*	4.3 ± 0.4	5.0 ± 0.7*
$I_G$	0.21 ± 0.14	0.54 ± 0.19*	0.82 ± 0.24*	1.47 ± 0.15*	1.71 ± 0.31*
$I_L$	1.72 ± 0.59	1.41 ± 0.44*	1.26 ± 0.37*	1.40 ± 0.17	1.24 ± 0.30
$I_{GU}$	4.75 ± 1.90	4.64 ± 1.60*	3.76 ± 1.30*	1.84 ± 0.61*	1.18 ± 0.55*
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
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

\*, significant difference ( $P < 0.05$ ) between yellow and silver eels.

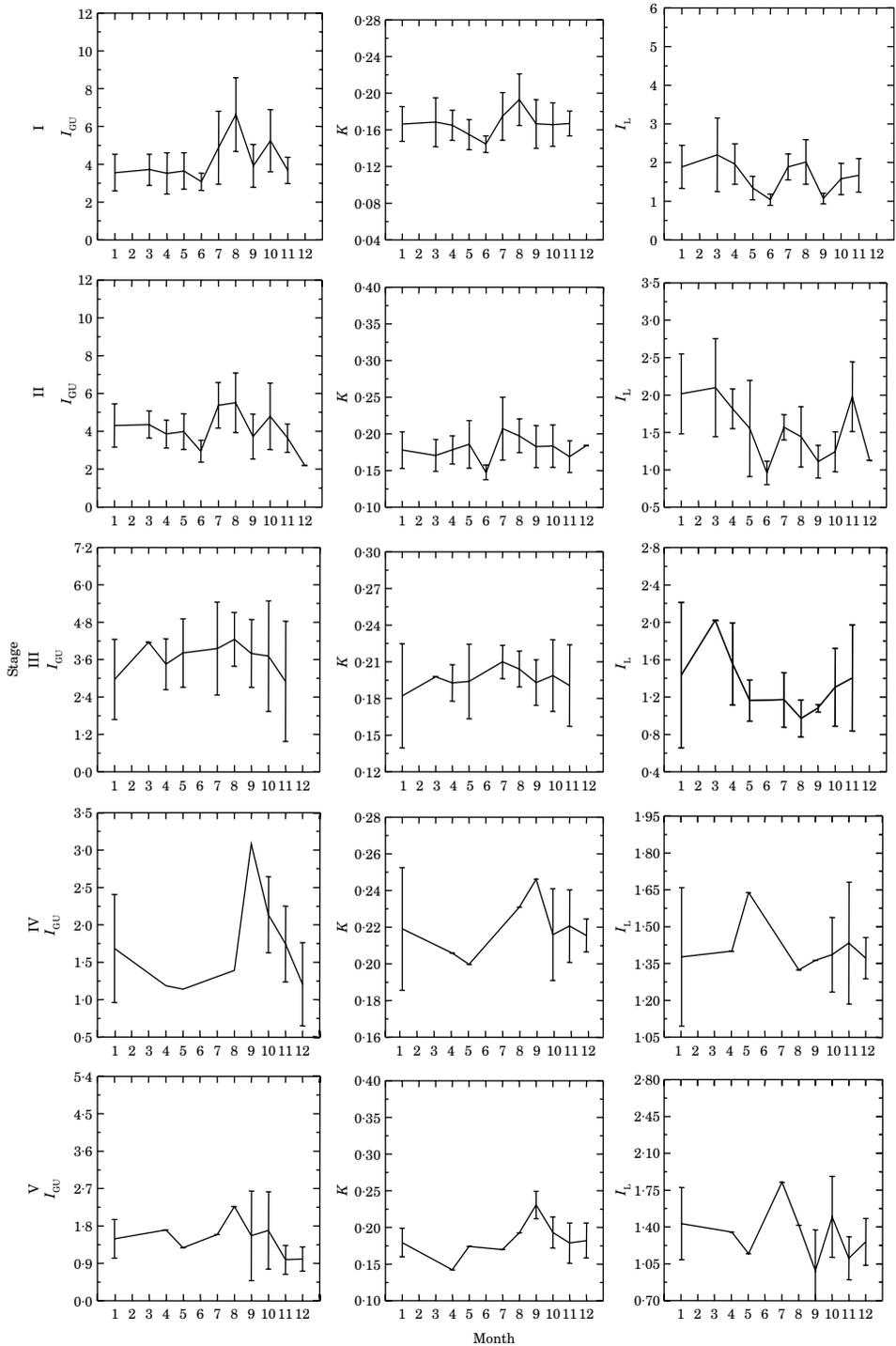


FIG. 5. Mean  $\pm$  s.d values monthly evolution of gut index, condition factor and hepato-somatic index according to stage (I to V) of female eels.

*Stage II (clusters 3 and 2) [Fig. 2(e)]*

Mean  $L_T$  of females was  $>50$  cm. The  $I_E$  and  $I_F$  increased accordingly. The gonads were significantly more developed ( $P < 0.05$ );  $I_G = 0.54\%$ . The  $I_{GU}$  was still variable and fluctuated seasonally with values ranging from 2 to 5% in winter and spring and from 3 to 6% in summer and autumn (Fig. 5). The  $I_L$  and  $K$  followed the same pattern, but on the whole, the mean  $K$  was higher than at stage I while  $I_L$  was lower (Fig. 6). Eels were still in the growth phase and the GTH-II levels were close to zero for the majority of individuals.

*Stage III (cluster 1) [Fig. 2(e)]*

This stage corresponds to the beginning of the metamorphosis, the pre-silver stage. Eels in the sample were between 50 and 85 cm  $L_T$  and 50 cm appeared to be the threshold for the start of silvering (Fig. 3). Eels at stage III displayed the first modifications linked to the metamorphosis. The  $I_G$  was intermediate between the yellow and silver phases (mean of 0.8%). Seasonal fluctuations of  $I_{GU}$  and  $K$  were less visible (Fig. 5). This stage was characterized by a peak of GH (with a maximum of  $0.94 \mu\text{g g}^{-1}$  for one eel). This suggests that growth was very high at the beginning of metamorphosis; eels must accumulate sufficient energy stores before the transatlantic migration. A slight increase in GTH-II level indicated the very beginning of sexual development. The  $I_E$  and  $I_F$  were significantly higher ( $P < 0.05$ ) than the previous stage.

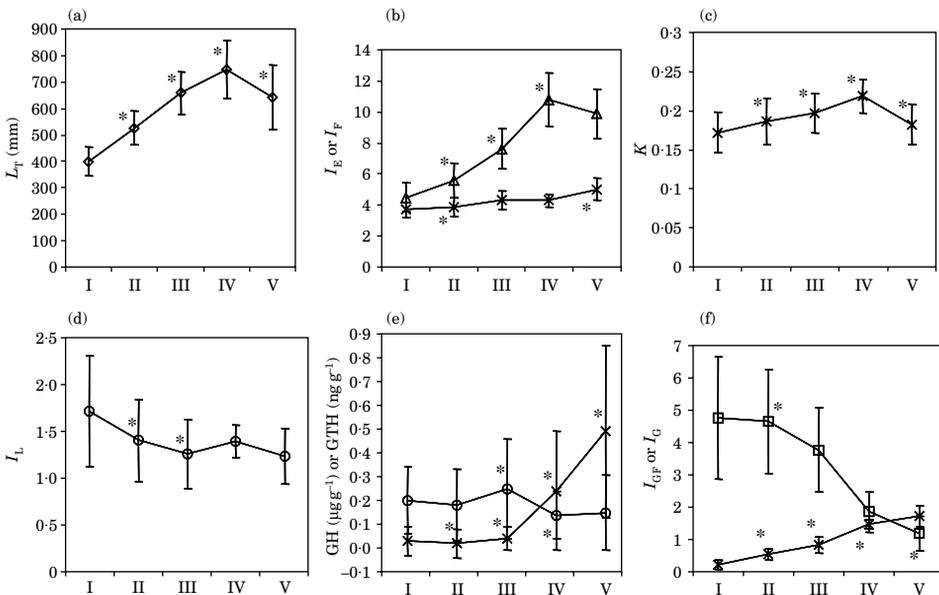


FIG. 6. Mean  $\pm$  s.d. (a) total length, (b) eye ( $\Delta$ ) or fin index ( $\times$ ), (c) condition factor, (d) hepato-somatic index, (e) pituitary growth hormone ( $\circ$ ) or pituitary type II gonadotropin ( $\times$ ) concentrations and (f) gonado-somatic ( $\times$ ) or gut ( $\square$ ) index at stages I to V for female eels. \*, Significant difference,  $P < 0.05$ .

*Stage IV (cluster 2) [Fig. 2(f)]*

At this stage eels began their first downstream movements and growth stopped: GH was significantly lower than at stage III. The  $K$  value was at its maximum. The GTH-II level was high ( $0.24 \text{ ng g}^{-1}$ ) compared to previous stages. The mean  $L_T$  of eels was 75 cm. Gonads were considerably more developed and  $I_G$  was independent of  $L_T$  and its mean was 1.5%. The  $I_{GU}$  was low (mean of 1.8%) and this value was lower than for individuals at stages I to III, even during the winter season. This indicated that eels had stopped feeding and this happened at the same time as the first downstream movements. Moreover, the  $I_{GU}$  decreased from September to December (Fig. 5) as the migrating season advanced and feeding probably stopped. The  $I_L$  and  $I_F$  did not vary significantly, ( $P > 0.05$ ). The  $I_E$  was high (10.8).

*Stage V (clusters 1 and 2) [Fig. 2(f)]*

This stage corresponds to the migrating stage. The  $I_G$  was equal to stage IV (mean = 1.7%). The  $I_{GU}$  was even lower than previously (mean = 1.2%). GTH-II was still high whereas GH did not increase. The  $I_E$  (9.9) did not increase but  $I_F$  was significantly higher with a maximum of 6.6.

## CHARACTERIZATION OF MIGRATING MALE EELS

The external and internal characteristics that were studied displayed much less variability in males than in females. The  $L_T$  of males was  $\leq 45$  cm. The testes were difficult to identify and remained almost invisible macroscopically at all the stages examined. Thus  $I_G$  values were very low ( $< 0.25\%$ ). A PCA was carried out on the following variables:  $L_T$ ,  $I_E$ ,  $I_F$ ,  $K$ ,  $I_G$ ,  $I_L$  and  $I_{GU}$  for 217 male and undifferentiated eels (the latter being the same individuals as in the female analysis). The structure on the first factorial plot (89% of the total inertia) was very similar to the female analysis. The first axis reflected the changes in  $I_G$  and  $I_{GU}$ , whereas axis 2 was correlated to  $K$  and  $I_L$ . Migrating eels were the most advanced individuals and undifferentiated eels were

TABLE IV. Mean  $\pm$  s.d. values of physiological and morphological variables (see Fig. 2) of undifferentiated and male yellow and silver eels

Stage	Undifferentiated eels	Yellow males only	Silver males
$n$	48	17	86
$L_T$ (mm)	$339 \pm 40$	$369 \pm 29^*$	$393 \pm 23^*$
$K$	$0.167 \pm 0.027$	$0.169 \pm 0.029$ NS	$0.177 \pm 0.022$ NS
$I_E$	$4.2 \pm 1.1$	$5.4 \pm 2.1^*$	$9.5 \pm 1.6^*$
$I_F$	$3.7 \pm 0.5$	$4.1 \pm 0.5^*$	$4.7 \pm 0.6^*$
$I_G$	$0.04 \pm 0.05$	$0.04 \pm 0.05$ NS	$0.16 \pm 0.11$ NS
$I_L$	$1.76 \pm 0.64$	$1.63 \pm 0.76$ NS	$1.41 \pm 0.26^*$
$I_{GU}$	$3.88 \pm 1.27$	$3.19 \pm 0.90$ NS	$1.59 \pm 0.48^*$
GTH-II ( $\text{ng g}^{-1}$ )	$0.04 \pm 0.04$ ( $n = 17$ )	$0.07 \pm 0.05$ ( $n = 7$ ) NS	$1.08 \pm 0.95$ ( $n = 21$ )*
GH ( $\mu\text{g g}^{-1}$ )	$0.20 \pm 0.14$ ( $n = 34$ )	$0.09 \pm 0.04$ ( $n = 8$ )*	$0.07 \pm 0.6$ ( $n = 49$ ) NS

\*, significant difference ( $P < 0.05$ ) between yellow and silver eels; NS, non-significant.

distributed along the second axis. Cluster analysis performed on the factorial scores of the PCA clearly isolates a migrating stage from the other individuals. Only 17 eels of the resident stage were identified as males in the sample. These yellow males differed slightly from undifferentiated eels in  $L_T$ ,  $I_E$ ,  $I_F$  and GH level ( $P < 0.05$ ). Differences between the yellow and silver males were more perceptible (Table IV). Testes were visible in silver males, although the increase in  $I_G$  was not significant and values remained very low (mean = 0.16%). The GTH-II level, however, was significantly higher for silver males ( $P < 0.05$ ) (mean = 1.08 ng g<sup>-1</sup>). The  $I_{GU}$  at the time of migration was about half its previous value (from 3.19 to 1.59%). The  $I_E$  and  $I_F$  increased significantly between the two stages. In males, silvering seemed to coincide with gonad differentiation and no intermediate stages could be found such as for females. Nevertheless, silver males that were not captured during their migration (all-stages sample) were grouped together on the factorial plot; these eels had a higher  $K$  than the migrants and may have corresponded to eels that were just about to start migrating, such as stage IV females.

## DISCUSSION

### VARIABILITY IN PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF EELS

One of the objectives of this study was to characterize silver migrating eels. Variability in the characteristics examined was linked to the existence of intermediate stages, time of collection and origin of eels. The modifications that the eels underwent were described by clustering the samples into several stages. Characterisation was first based on the physiological and morphological traits, mainly gonad mass, gonatropin level and mass of the gut. Variability in the data was also linked to the time of the year, as this was observed to fluctuate seasonally in particular in  $I_{GU}$  (regression of the gut),  $I_L$  (liver mass) and  $K$  (condition factor). Migrating eels from the different sampling locations only differed in  $L_T$ , gut regression and length of the pectoral fin. These variables appeared to change according to the size of the watershed and the distance from the sea.

### CHARACTERIZATION OF THE SILVER MIGRATING STAGE

The mean  $I_G$  of female migrating eels was 1.7% and was always >1.2%. There were no significant differences in  $I_G$  or GTH-II levels between the different locations. Maximum values ( $\leq 2\%$ ) given for stages IV and V are probably the upper limit for *A. anguilla* in its continental phase. These values are similar to those observed in other studies (Bertin, 1951; Vøllestad & Jonsson, 1986; Fontaine, 1994; Svedång & Wickstrom, 1997; Marchelidon *et al.*, 1999; Durif *et al.*, 2000). Gonads do not develop any further since the gonadotropic function is blocked until eels reach the ocean (Dufour & Fontaine, 1985). Ovaries in *A. anguilla* are much less developed than in other species of eels (*A. rostrata*, *Anguilla dieffenbachii* Gray, *Anguilla australis* Richardson

and *Anguilla japonica* Temminck & Schlegel) where they can reach 4% (Jessop, 1987; Lokman & Young, 1998; Yamada *et al.*, 2000).

There have been very few studies on silvering in male eels. In the present study it was possible to characterize a silver migrating stage for males, which was similar to migrating females (stages IV and V) except for  $I_G$ . Only 17 out of 1188 eels were identified as yellow males. This stage appeared to be transient. This is supported by the histological study of sex differentiation by Colombo & Grandi (1996), who observed that fully differentiated testes were only found in eels  $>35$  cm  $L_T$  and which were in the process of silvering. Thus silvering happens at a very early stage in males probably almost as soon as their gonads are differentiated.

Migrating eels cease to feed and their gut begins to regress. As mentioned in Bertin (1951), fasting yellow eels have the same regressive alterations of the gut as silver eels do. In the present study, some eels during the growth phase had low  $I_{GU}$  values due to seasonal fluctuations. These low values were, nevertheless, higher than for eels at stages IV and V for which  $I_{GU}$  was  $<2\%$ . Thus other mechanisms than fasting must accelerate gut regression. Cessation of feeding seems to occur at the same time or very close to the time of downstream movements; regression of the gut is then amplified as migration progresses. The  $I_F$  increases significantly when eels start their downstream migration and this variable is negatively correlated to  $I_{GU}$ . Pectoral fin length could be a useful indicator as to when eels have started their migration. Swimming may trigger this growth. Pectoral fins do not actually propel the eel in the water, but they contribute to its stabilization and this may explain the increase in length at the time of migration.

## THE DIFFERENT STAGES OF METAMORPHOSIS

Ecologists often try to classify individuals of similar characteristics into groups to visualize a continuous phenomenon. Up to now silvering was split into two separate stages: yellow and silver, which did not take into account a possible preparatory phase. Feunteun *et al.* (2000) classified eels into three stages: yellow, silver and yellow/silver, however, these stages were only based on external and visual variables (skin colour, visibility of the lateral line and eye surface) and no physiological or morphological validation was made. In the present study, the initial objective was to obtain a standard classification, which accounts for the existing and observed variability. In diadromous salmonids, smoltification corresponds to the pre-migratory metamorphosis, which allows the young fishes to acquire the necessary adaptations to live in salt water. In this sense, it can be compared to silvering of eels, even though none of the changes are related to sexual maturation. Smoltification is a progressive phenomenon and the intermediate stages have been described (Hoar, 1988; Boeuf, 1994). Fontaine (1994) was the first to suggest more than two stages to describe the silvering of eels. In the present study a growth phase (stages I and II), a pre-migrating stage (stage III) and a migrating phase (stages IV and V) have been defined. Using this classification to describe a population, rather than classifying eels into the restrictive yellow and silver stages, will give a more realistic and complete image of its dynamics.

## REGRESSION OF SILVERING

Is silvering reversible? It has been shown in diadromous salmonids that smoltification can be reversed when fishes are kept in fresh water (Lundqvist & Fidberg, 1982; Ombredane *et al.*, 1996; McCormick *et al.*, 1999). The fishes may again smoltify the following year (Shrimpton *et al.*, 2000). The physiological 'window' during which Atlantic salmon *Salmo salar* L. smolts are able to migrate is very narrow (McCormick *et al.*, 1998). The loss of physiological smolt characteristics is more rapid when fishes are held at higher temperatures. Individuals keep their silvery colour but lose their capacity to osmoregulate in salt water. No such information is available for eels. The only published experiments have been performed on artificially maturing silver eels. It was observed that even after ovulation and spermiation, eels are capable of feeding again and their gut regenerates (Fontaine *et al.*, 1982; Dollerup & Graver, 1985). Reversibility of silvering has been mentioned by Svedäng & Wickstrom (1997). In their study, the authors measured fat contents of silver eels from different parts of Sweden. Some individuals displayed very low values, often lower than the threshold of 20%, which would be needed to swim 6000 km according to Boëtius & Boëtius (1980). The authors conclude that silvering is much more flexible than assumed, and eels can stop metamorphosing and start feeding again if chances of successfully migrating are compromised. Even if estimations of necessary energy stores have been lowered to 13% by Van den Thillart *et al.* (2004), it is still highly probable that eels optimize their chances of a successful reproduction by delaying migration and reverting back to a yellow resident stage.

In the present study, a small number of stage IV eels were captured in April. These individuals were either developing towards stage V or were more likely eels that did not migrate and were regressing to a resident stage. A behavioural study of downstream migration (Durif *et al.*, 2003) has shown that downstream migration itself is flexible and that if environmental conditions were not favourable eels stopped their migration such as during daylight but sometimes for longer periods of time (up to 1 month). The presence of dams could also stop or delay the run. Therefore, an eel that has missed favourable 'environmental windows' would probably stop silvering and revert back to the growth phase. Moreover several mark-recapture experiments have shown that presumed silver eels could be recaptured 1–4 years after they had been tagged (Vøllestad *et al.*, 1994; Westin, 1998; Feunteun *et al.*, 2000). As early as 1975, Hain (1975) suggested that *A. rostrata* makes several trial runs before finally leaving for the Sargasso Sea; he indicated that migratory characteristics weaken after each false start until the next migratory season. This physiological and behavioural flexibility of eels could explain the high variability in age and length of female silver eels as this has been well described in the literature (Frost, 1945; Hansen & Eversole, 1984; Helfman *et al.*, 1984; Vøllestad, 1992; Svedäng *et al.*, 1996; Holmgren *et al.*, 1997; Oliveira, 1999). This variability is much less important in male eels: their  $L_T$  does not exceed 45 cm and their gonads hardly develop even at the migrating stage. Prerequisites for migration and reproduction for males are probably more resilient than for females as less energy is demanded for gonad development. Regression of silvering is probably less common in male eels.

## TIMING AND TRIGGER OF SILVERING

The eels at stage IV, V and silver males first appeared in the samples during September. Transition from stage III to IV probably occurs at the end of the summer season (Durif, 2003). This is supported by a study on the seasonal changes of the pituitary gland of the eel, which produces GTH-II and GH (Evans, 1940). The author showed that the anterior and posterior lobes increase significantly 2 months prior to downstream migration, in September and October.

Internal or external factors responsible for the initiation of the silvering process are not known. Larsson *et al.* (1990) hypothesized that a certain level of lipid stores in the fish are required for metamorphosis and they set a threshold of 28%. Whatever the limit, lipid stores are probably not a prerequisite for silvering but they are for the final migration to the Sargasso Sea. An important increase in GH was observed in stage III individuals (or pre-migrants). Huang *et al.* (1998) suggest that insulin-like growth factor IGF-I (produced by the liver under GH stimulatory control) is the link between body growth and induction of puberty. The IGF-I acts on the pituitary gland by stimulating GTH-II production and exerts a negative feedback on GH (Rousseau *et al.*, 1998). Consequently a peak in GH could stimulate secretion of IGF-I. This would occur during an important growth period such as summer. This situation would trigger the initial GTH-II synthesis and decrease in GH. By stimulating the synthesis of sexual steroids (namely androgens), this hormone would induce morphological modifications such as colour changes and increase of eye surface (Huang *et al.*, 2001; Rohr *et al.*, 2001). In the present study, an increase in GH in pre-migrant individuals (stage III) was observed. Silvering may be triggered by an important growth period. Indeed, favourable growth conditions cause eels to silver rapidly (Vøllestad, 1988, 1992; De Leo & Gatto, 1995) such as is the case in aquaculture, under experimental conditions (Tesch, 1991; Beullens *et al.*, 1997) or in brackish water and at low latitudes (Lee, 1979; Fernandez-Delgado *et al.*, 1989). In this sense, environmental factors may play a role in the triggering of silvering.

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