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Silvering of European eel (*Anguilla anguilla* L.): seasonal changes of morphological and metabolic parameters

VINCENT VAN GINNEKEN [1,*], CAROLINE DURIF [2], S. PAUL BALM [3], RON BOOT [1], MARTIN W.A. VERSTEGEN [4], ERIK ANTONISSEN [1], GUIDO VAN DEN THILLART [1]

[1] *Integrative Zoology, Institute Biology Leiden (IBL), van der Klaauw Laboratorium, P.O. Box 9516, 2300 RA Leiden, The Netherlands*
[2] *Cemagref, Unite Ressources Aquatiques Continentales, 50 avenue de Verdun, 33612, Cestas, France*
[3] *Animal Physiology, Department of Biology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*
[4] *Department of Animal Nutrition, Wageningen Agricultural University, Marijkeweg 40, P.O. Box 338, 6700 AH Wageningen, The Netherlands*

Abstract—The transformation of yellow eel into silver eel is called 'silvering', and takes place prior to migration. We found the sedentary yellow phase in spring, the migratory silver phase in autumn, while August was a cross-over month. We used principal component analysis (PCA) to characterise the morphological and physiological changes that accompany silvering in the European eel (*Anguilla anguilla* L.). Silvering is positively related to external parameters such as eye size, internal maturation parameters like GSI, vitellogenine (VIT), and blood-substrates such as phospholipids, Free Fatty Acids (FFA), and cholesterol. The Hepatosomatic Index was not significantly different between yellow and silver groups. In contrast, a significant difference was observed for parameters of body constitution (fat, protein, dry matter) between yellow and silver stages. Furthermore, the process of silvering is accompanied with increased levels of cortisol in autumn, which plays a role in mobilisation of metabolic energy from body stores towards migratory activity and gonadal growth. Based on Principal Component Analysis (PCA) with physiological, morphological and endocrinological parameters, it is concluded that during the process of silvering, several developmental stages can be recognised, with a timeframe of the premigratory sedentary yellow phase from April until July, August is a cross-over month, and the migratory silver phase is found from September until November.

Keywords: blood substrates; body constitution; cortisol; European eel (*Anguilla anguilla*, L.); metamorphosis; silvering.

*Corresponding author; e-mail: V.J.T.van.Ginneken@biology.leidenuniv.nl

INTRODUCTION

During its life cycle, the European eel (*Anguilla anguilla* L.) experiences two periods of metamorphosis. The first is transformation from the planktonic marine stage (*Leptocephalus* larvae) into glass eel. This occurs during its oceanic migration from the presumed spawning grounds in the Sargasso Sea to the coasts of Europe before entering fresh water. The second (partial) metamorphosis occurs after the juvenile growth and differentiation phase (>4 years for males, >7 years for females) in the inland waters. Eels transform then from yellow eel into silver eel, a process called 'silvering'. During the latter transformation there is some proliferation of the gonads and an increase in eye size (Pankhurst, 1982; Pankhurst and Lythgoe, 1983). Furthermore, the body colour becomes silvery due to differentiation of pigment cells (Pankhurst and Lythgoe, 1982); the alimentary tract shows regression, and the animal becomes fatter. These changes are part of the 'silvering' process, which precedes the spawning migration to the Sargasso, 6000 km away from Europe.

The mechanisms involved in the onset of silvering of eels are largely unknown, as are the different stages which characterise this metamorphosis. Only two extensive studies have been performed of the morphological and physiological characteristics at the different stages of eel silvering (Durif, 2003; Durif et al., 2005). Based on principal component analysis (PCA) some of the morphological and physiological characteristics associated with silvering were characterised using the following parameters: body length, eye index, fin index, condition factor, gonad weight, liver weight, gut weight, gonadotropine and growth hormone (Durif et al., 2005). Seasonal, monthly changes over the year in parameters from fat metabolism, morphological and physiological parameters have never been described before for female eels from the Grevelingen lake, a brackish water population. The Grevelingen lake is the largest brackish/saltwater lake of Western Europe with a total area of 14 000 hectares. The lake is situated on the boundary between Zuid-Holland and Zeeland, The Netherlands, and has a large standing stock of eels.

This study can be seen as a further refinement of the studies of Durif (2003) and Durif et al. (2005) due to a monthly sampling protocol and taking into account more physiological and metabolic parameters.

We hypothesise that the silver eels caught in autumn, which are on their seaward migration, have totally different body characteristics than the sedentary phase eels caught earlier in spring and summer. Through PCA we will determine which morphological and physiological characteristics are most altered during silvering. Thus the aim of this study was, by monthly sampling of female European eels at a fixed location (Grevelingen lake, The Netherlands), to describe the transient changes which are characteristic for the process of silvering, and to determine when these changes first appear. This will help us to understand the dynamics of the transformation process, which is an adaptation to a migration phase in an oceanic environment.

MATERIALS AND METHODS

Animals

Every month from April-November 2002, eels were caught by local fisherman using fyke nets at the Grevelingen lake. The eight largest animals (females) were selected. Water temperature was measured and the eels were classified as 'yellow' or 'silver' by a fisherman according to external features. These features were an enlargement of the eyes and a silvery body colour for the 'silver' stage. The fish were rapidly anaesthetised with benzocaine (100 ppm). Blood was collected with a heparinised syringe and stored on dry ice for further analysis. The carcasses were taken to the laboratory to determine the body weight, eye index (E.I.), the digestive tract index (D.T.I.), hepato-somatic index (H.S.I.) and gonado-somatic index (G.S.I.).

Blood analysis

In freshly collected blood samples, treated with anticoagulant, the haemotocrit was measured directly in 9 μl whole blood sample using a haematocrit micro-centrifuge (Bayer, FRG). Haemoglobin content in 20 μl blood was detected after 3 min using the cyan-methaemoglobin method (Boehringer Mannheim, FRG). Blood was directly centrifuged (10 000 rpm for 5 min). The plasma was divided in eppendorf tubes (10, 40, 50, 50, 20, 20, 20, 33, 33, 33 μl for, respectively, total protein, FFA (Free Fatty Acids), glucose, lactate, cholesterol, triglycerids, phospholipids and sodium, potassium and chloride analysis) and stored at -80°C pending analysis. For the glucose measurements, 50 μl plasma was mixed with 200 μl 6% trichloric acid solution to precipitate plasma proteins and stored at -80°C. Glucose was determined by colorimetric assay (Sigma, St. Louis, USA). FFA was measured with a commercial test-kit WAKO (NEFA C method, Instruchemie, Hilversum, The Netherlands). Lactic acid was determined with an enzymatic test-combination of Boehringer Mannheim: 139084 for L-lactate. Total protein, cholesterol, triglycerids, and phospholipids were measured with Boehringer Mannheim test kits (MPR3 124281, MPR1 CHOD-PAP 1442341, GPO-PAP 701882 and MPR2 691844, respectively). Plasma sodium, potassium and chloride levels were measured by flame photometric and colorimetric procedures (Technicon). For cortisol- and vitellogenine measurements the plasma was divided in Eppendorf tubes (25 μl, 50 μl) and stored at -80°C pending analysis. Cortisol was measured by radioimmunoassay at Nijmegen University according to the protocol of Balm et al. (1994). VIT was measured by immunoenzymatic assay according to the protocol of Burzawa-Gerard et al. (1991).

Carcass analysis

After weighing, the total carcass was cut into pieces of about 3 cm and nearly submerged in water in a glass beaker. The samples were autoclaved at 2 atmospheres at 120° for 4 h. They were then homogenised with a laboratory disperser and

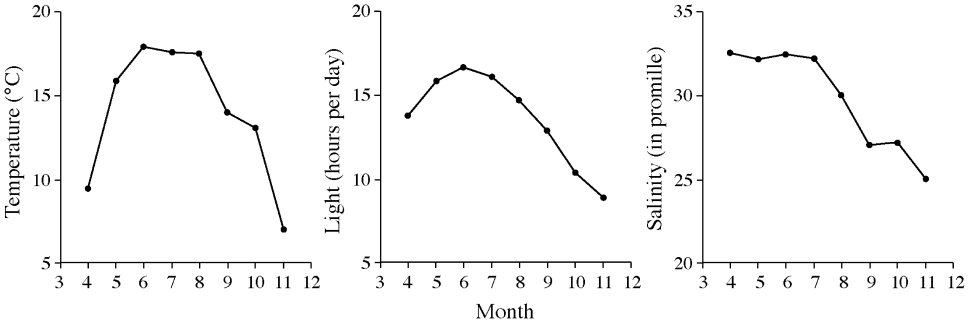

Figure 1. Monthly evolution of temperature (°C), salinity, and light (hours) in the Grevelingen in 2002 at the time of sampling. (April (month 4)-November (month 11)).

subsequently sampled in triplicate for dry matter, protein and fat analyses. The dry matter content was measured by freeze-drying the sub-samples to constant weight. Plate temperature started at −20°C and rose to 27°C after a vacuum of 40 Pa was reached. Condenser temperature was −90°C. The protein was measured according to ISO 5983 (Anon., 1979). For fat determination, sub-samples were freeze-dried, as described for dry matter, and subsequent extraction of the fat was performed as described in ISO/DIS 6492 (Anon., 1996).

Environmental factors for temporal relationships (correlations)

Water-temperature, salinity and day-length over the period April-November 2002 are depicted in figure 1.

Calculations and statistics

Fulton's condition-factor (K) was calculated according to the equation $K = 100 * W * L^{-3}$. The eye index was calculated according to the method of Pankhurst (1982) where $E.I = ([(A + B)^2/4 * \pi]/L) * 100$ where A is the horizontal eye diameter, B is the vertical diameter, and L is the total body length (mm).

The Hepato somatic Index (HSI) was calculated according to ([Liver weight]/[Body weight]) * 100%. The Gonado somatic Index (GSI) was calculated according to ([Ovary weight]/[Body weight]) * 100%.

Morphological and physiological descriptors were tested for normality (Kolmogorov-Smirnov, Lilliefors probability). Those that differed significantly from the normal distribution ($p < 5\%$) were log-transformed. To remove any size effect, and because mean length of eels slightly differed between samples, variables which were correlated to eel length were standardised according to: Var_std = Var − (M(L − L_mean)) (MacCrimmon and Claytor, 1986), where Var_std is the corrected variable, Var is the original variable, M is the slope of the regression of the descriptor on total body length, and L_mean is the mean length of the eels in the sample.

Table 1.
Description of the transformed variables.

Variable	Transformation	Abbreviation
Body weight	Fulton's condition factor	K
Gonad weight	Log-transformed, standardised	GW
Intestine weight	Log-transformed	IW
Liver weight	Log-transformed, standardised	SI
Vitellogenin	Log-transformed	VIT
Cortisol	Log-transformed	CORT
Haematocrit	None	HCR
Haemoglobin	None	HB
Sodium	None	SO
Potassium	None	PO
Chloride	Log-transformed	CL
Cholesterol	None	CHO
Glucose	None	GLUC
Phospholipids	None	PL
Free fatty acids	Log-transformed	FFA
Lactate	Log-transformed	LAC
Triglycerids	Log-transformed	TG
Total protein	Log-transformed	TP
Carcass fat	Log-transformed	CFAT
Carcass protein	None	PROTCARC
Dry matter	None	DRY

Means of variables were compared between the yellow eel group and the silver eel group using a two-sample t-test. The variables which did not follow a normal distribution regardless of the log-transformation were compared by Kruskal-Wallis tests. Pearson correlations were calculated between variables and their significance was determined with a Bonferroni test. $P \leqslant 0.05$ was considered as statistically significant for all tests. Statistics were performed via Systat SPSS Version 10.

Principal Component Analysis (PCA, correlation matrix) was carried out on 21 physiological parameters (transformed variables, see table 1), which were initially centred and standardised. These variables would presumably evolve with silvering to examine their seasonal evolution. The PCA was performed with ADE-4 (Thioulouse et al., 1997).

RESULTS

The animals caught in the period from April-July were all yellow, while the animals in the period September until November were all silver. The month of August comprised both yellow ($n = 4$) and silver ($n = 3$) animals while one animal was classified according to external parameters (skin colour, eye size) as 'half' silver. In addition, for all measured parameters, a one-way ANOVA was performed on

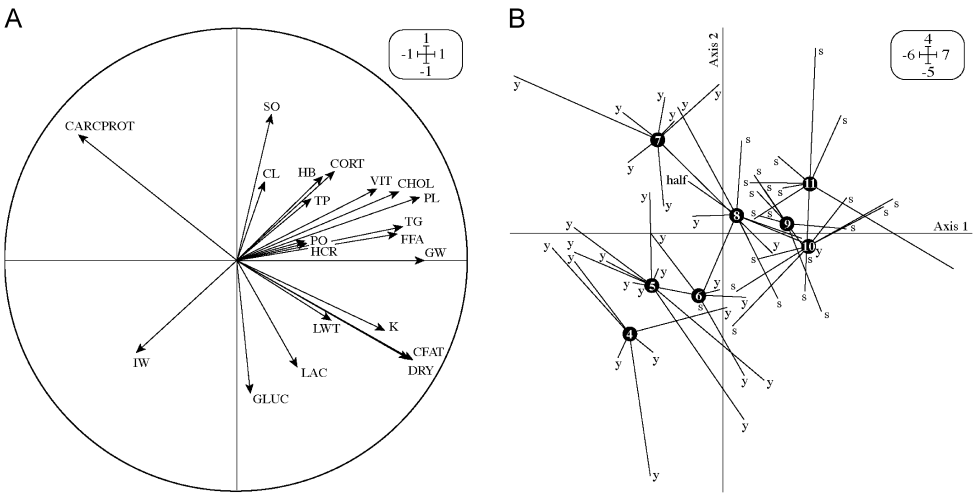

Figure 2. A: Principal Component Analysis (PCA) of body composition and blood substrates; correlation circle. A: shows the correlations between the principal components and the variables. Abbreviations: Carcprot = carcass-protein; SO = Sodium; CL = Chloride; HB = Haemoglobin; CORT = cortisol; TP = total protein; VIT = vitellogenine; CHOL = cholesterol; PL = phospholipids; PO = potassium; TG = triglycerids; FFA = free fatty acids; HCR = haematocrit; GW = gonad weight; K = Fullton's condition factor; LWT = liverweight; CFAT = carcass-fat; DRY = carcass dry matter; LAC = lactic acid; GLUC = glucose; IW = intestine weight. B: Factorial scores. Individuals (eels) are grouped by month (April (month 4)-November (month 11)). Y: yellow; S: silver. The stars link the eels that were sampled in the same month.

the average value of the yellow vs. the average value for silver animals. In this comparison, the 'half' silver animal caught in the month of August was eliminated.

The largest animals (silver) above 1 kg were found in the period September-November (table 2). The GSI and eye indexes are the highest in the silver eels (table 2).

Principal Component Analysis (PCA) was carried out on a total of 21 physiological variables. The first two axes account for 44% of total inertia. Relationships between factorial axes and variables are indicated on the correlation circle for body constitution and blood substrates (fig. 2, left panel). The first axis accounts for most of the variation (31%); it is significantly correlated to GW and VIT which are the parameters that reflect the silvering process. Other main contributors to this axis are the following parameters: carcass proteins for negative scores, and carcass fat, dry mass, FFA, triglycerids, phospholipids and cholesterol for positive scores. Main contributors to the second axis (13% of total inertia) are glucose, carcass-fat, and dry mass for negative scores, and the blood hormone cortisol, carcass proteins, and sodium for positive scores.

All of these parameters have a seasonal evolution, which coincides with the silvering of eels (fig. 2, right panel). From May to June, eels show no sign of metamorphosis: they still have a low GSI and a high DTI. The levels of glucose and lactate are relatively high. The eels sampled in July are slightly isolated on

Table 2.
Mean ± Standard Deviation of morphological parameters that have been studied over their annual cycle of female European eel (*Anguilla anguilla* L.). From April until July the animals are yellow (non-migratory phase) while from September until November the animals are silver (migratory phase). In August, half of the animals are yellow and half are silver. The mean value of the yellow eel group was compared to the mean value of the silver eel group. Statistics were performed via Systat SPSS using a one-way ANOVA for differences between yellow and silver groups. $P \leqslant 0.05$ was considered as statistically significant.

Morphology	April N = 8	May N = 8	June N = 8	July N = 8	August N = 8	September N = 8	October N = 8	November N = 8	Yellow N = 36	Silver N = 28	P-value
Body weight (g)	861 ± 194	969 ± 187	908 ± 187	751 ± 175	771 ± 184	1248 ± 302	1092 ± 132	1178 ± 293	855 ± 195	1132 ± 262	$P \leqslant 0.0001^{**}$
Length (cm)	74.9 ± 5.95	77.1 ± 4.76	75.8 ± 5.20	73.4 ± 3.47	70.1 ± 4.90	83.4 ± 5.78	78.4 ± 4.37	79.5 ± 5.81	74.7 ± 5.18	79.2 ± 6.36	$P \leqslant 0.003^{**}$
Condition factor	0.20 ± 0.02	0.21 ± 0.02	0.21 ± 0.01	0.19 ± 0.02	0.22 ± 0.03	0.21 ± 0.02	0.23 ± 0.01	0.23 ± 0.03	0.20 ± 0.02	0.23 ± 0.02	$P \leqslant 0.0001^{*}$
Eye index	7.34 ± 0.98	7.28 ± 2.38	7.26 ± 0.72	6.69 ± 0.85	7.99 ± 0.93	10.42 ± 0.62	9.95 ± 0.92	10.91 ± 0.86	7.14 ± 1.31	10.17 ± 1.04	$P \leqslant 0.0001^{**}$
Gonad weight (g)	5.75 ± 2.74	6.61 ± 3.31	7.01 ± 2.60	6.19 ± 3.45	7.08 ± 3.83	18.09 ± 5.28	17.02 ± 4.75	16.58 ± 6.25	6.17 ± 2.87	16.16 ± 5.68	$P \leqslant 0.0001^{**}$
G.S.I.	0.65 ± 0.15	0.65 ± 0.20	0.76 ± 0.24	0.79 ± 0.28	0.87 ± 0.28	1.44 ± 0.17	1.54 ± 0.30	1.38 ± 0.26	0.71 ± 0.22	1.40 ± 0.28	$P \leqslant 0.0001^{**}$
H.S.I.	1.49 ± 0.45	1.28 ± 0.24	1.54 ± 0.50	1.23 ± 0.30	1.17 ± 0.20	1.32 ± 0.09	1.36 ± 0.28	1.37 ± 0.12	1.35 ± 0.39	1.34 ± 0.16	$P \leqslant 0.924$
Digestive tract (g)	23.9 ± 3.1	33.9 ± 12.4	32.5 ± 16.1	23.4 ± 7.8	13.9 ± 3.8	17.8 ± 4.7	13.4 ± 2.3	13.2 ± 4.5	27.1 ± 11.9	14.8 ± 4.4	$P \leqslant 0.0001^{**}$

the factorial plane as they display relatively high values for carcass proteins, the blood parameters cortisol, and sodium compared to previous months. This may reflect the very beginning of the metamorphosis. This sample is also particular, as sodium, chloride and haemoglobin have increased, and values for carcass proteins are at their maximum. In contrast, glucose values are at their minimum. The August sample is situated at the very centre of the factorial plane, meaning it is composed of eels having intermediate characteristics between the yellow and silver stages. Some of these individuals present signs of metamorphosis, such as a degeneration of the digestive tract as attested by the decrease in IW, indicating they stopped feeding. However, GW and VIT concentrations are still very low. It is only during the following period (September-November) that eels show distinctive silver eel features. The low summer GSI of females increases from September until peak migration time in fall. The alimentary tract shrinks during the period of silvering to approximately one third of the values found in spring and summer (table 2). Increased plasma levels of VIT in autumn suggest that excess precursor (VIT) was available for producing yolk, the main chemical component of oocyte and ovarian growth: the vitellogenesis process. The correlation coefficient between vitellogenin and GW is statistically significant: 0.57 ($p < 5\%$, table 2). This implies that vitellogenin concentration in the blood is a good estimator for gonad growth (GSI). Several other parameters are significantly correlated to GW: cholesterol, triglycerids, phospholipids, dry mass, carcass proteins, condition factor, FFA and carcass fat.

By September and November, plasma cortisol levels are at their highest values during the season just prior to the migration period. It is likely that mobilisation of energy reserves is greatest during this period. Both cholesterol and phospholipids are low until August, and high from September-November, peaking in October. Triglycerids show the same tendency but remain low in September. Although FFA displays some fluctuations, they tend to increase in fall. Haemoglobin continues to increase from August to November. Carcass fat is low during the first part of the year and high during the second; Carcass protein shows the reverse pattern. Carcass dry matter is low during the first part of the year, and peaks in October.

In table 2, the morphological parameters have been tested for significant differences between yellow (May-mid-August) and silver (half August-November) animals, while this has been performed in table 3 for blood substrates and body carcass constitution. Significant differences ($P \leqslant 0.05$) were observed for potassium, cholesterol, triglycerids, lactic acid, vitellogenin, dry matter, carcass fat and carcass protein, while highly significant differences ($P \leqslant 0.0001$) were observed for body weight, eye index, G.S.I., Digestive Tract, cortisol, phospholipids, Hb and Hct.

Many significant correlations were found between descriptors (table 4). GW was positively correlated with E.I. (=eyediameter) ($R = 0.69$), VIT ($R = 0.57$), CHOL ($R = 0.52$), phospholipids (PL, $R = 0.58$), triglycerids (TG, $R = 0.57$). Fat content (CFAT) was significantly related to condition factor (K, $R = 0.59$) and free fatty acids (FFA, $R = 0.56$). Moreover, the eels with the highest fat stores displayed the

Table 3.

Mean ± Standard Deviation of ions, blood-substrates parameters in combination with the steroid cortisol and constitution of the carcass have been studied over their annual cycle of female European eel (*Anguilla anguilla* L.). From April until July the animals are yellow (non-migratory phase) while from September until November the animals are silver (migratory phase). In August, half of the animals are yellow and half are silver. The mean value of the yellow eel group was compared to the mean value of the silver eel group. Statistics were performed via Systat SPSS using a one-way ANOVA for differences between yellow and silver groups. $P \leqslant 0.05$ was considered as statistically significant.

	April N = 8	May N = 8	June N = 8	July N = 8	August N = 8	September N = 8	October N = 8	November N = 8	Yellow N = 36	Silver N = 28	P-value
Ions & Substrates											
Sodium (μM)	345 ± 25	365 ± 36	394 ± 30	422 ± 40	395 ± 48	411 ± 33	388 ± 14	412 ± 44	380 ± 44	403 ± 31	$P \leqslant 0.028^{*}$
Potassium (μM)	3.4 ± 1.1	4.7 ± 1.3	5.6 ± 1.6	4.4 ± 1.5	5.0 ± 2.0	5.2 ± 0.9	6.0 ± 1.5	5.3 ± 1.4	4.4 ± 1.5	5.5 ± 1.5	$P \leqslant 0.005^{*}$
Chloride (μM)	328 ± 40	327 ± 40	322 ± 55	335 ± 26	320 ± 33	329 ± 17	315 ± 26	332 ± 38	326 ± 39	326 ± 29	$P \leqslant 0.992$
Total protein (g/l)	49.7 ± 10.9	49.9 ± 6.6	66.7 ± 18.8	57.1 ± 9.3	52.3 ± 7.4	56.8 ± 6.7	53.8 ± 7.1	49.4 ± 5.5	56.1 ± 13.2	52.4 ± 6.8	$P \leqslant 0.295$
Cholesterol (mmol/l)	12.5 ± 4.6	12.1 ± 2.5	14.8 ± 2.0	13.0 ± 3.5	14.2 ± 3.3	17.8 ± 4.2	18.3 ± 7.3	16.5 ± 4.6	13.3 ± 3.2	17.1 ± 5.2	$P \leqslant 0.001^{**}$
Phospholipids (mmol/l)	13.1 ± 5.5	13.6 ± 2.6	17.2 ± 2.9	14.1 ± 3.9	15.1 ± 3.6	19.5 ± 2.8	20.4 ± 6.5	19.3 ± 4.9	14.6 ± 3.9	19.2 ± 4.8	$P \leqslant 0.0001^{**}$
Triglycerids (mmol/l)	5.6 ± 3.2	5.9 ± 2.6	13.1 ± 6.1	7.7 ± 3.5	9.7 ± 4.8	7.5 ± 1.6	12.2 ± 5.8	11.7 ± 4.3	8.5 ± 5.0	10.4 ± 4.7	$P \leqslant 0.044^{*}$
FFA (mmol/l)	0.17 ± 0.12	0.21 ± 0.14	0.37 ± 0.20	0.24 ± 0.14	0.30 ± 0.11	0.19 ± 0.05	0.24 ± 0.13	0.34 ± 0.12	0.26 ± 0.16	0.26 ± 0.12	$P \leqslant 0.272$
Hb (mmol/l)	7.13 ± 1.40	6.93 ± 1.18	4.84 ± 0.73	7.09 ± 0.87	8.24 ± 2.63	9.11 ± 1.00	7.92 ± 0.56	8.96 ± 0.98	6.75 ± 1.64	8.52 ± 1.51	$P \leqslant 0.0001^{**}$
Hct (%)	29.0 ± 7.51	34.7 ± 5.29	33.8 ± 5.30	31.5 ± 4.17	33.5 ± 8.41	49.0 ± 12.1	40.3 ± 3.13	35.8 ± 4.43	32.5 ± 5.65	40.3 ± 9.98	$P \leqslant 0.0001^{**}$
Glucose (mmol/l)	1.82 ± 0.43	1.26 ± 0.28	1.52 ± 0.34	0.71 ± 0.18	0.92 ± 0.19	1.43 ± 0.47	1.53 ± 0.21	1.00 ± 0.16	1.28 ± 0.51	1.27 ± 0.38	$P \leqslant 0.921$
Vitellogenin (μg/ml)	104.27 ± 85.31	84.03 ± 8.49	133.77 ± 62.45	159.48 ± 140.12	133.84 ± 34.10	1023.05 ± 1680.97	766.81 ± 1279.3	2438.99 ± 2626.92	122.01 ± 84.53	1189.05 ± 1883.15	$P \leqslant 0.001^{**}$
Lactate (mmol/l)	0.70 ± 0.54	0.20 ± 0.12	0.29 ± 0.21	0.10 ± 0.06	0.10 ± 0.04	0.65 ± 0.28	0.76 ± 0.56	0.17 ± 0.08	0.29 ± 0.34	0.45 ± 0.40	$P \leqslant 0.030^{*}$
Cortisol (ng/ml)	26.1 ± 10.3	31.5 ± 25.3	27.1 ± 15.5	70.2 ± 38.5	40.7 ± 24.7	108.1 ± 27.7	70.1 ± 55.0	85.8 ± 25.8	38.2 ± 29.1	80.7 ± 42.0	$P \leqslant 0.0001^{**}$
Carcass											
Dry matter (g/kg)	427 ± 41	423 ± 42	428 ± 45	410 ± 38	451 ± 25	450 ± 25	454 ± 17	448 ± 31	424 ± 40	452 ± 23	$P \leqslant 0.002^{**}$
Fat (g/kg)	264 ± 52	236 ± 55	244 ± 55	215 ± 48	269 ± 34	269 ± 33	276 ± 21	270 ± 41	235 ± 51	273 ± 31	$P \leqslant 0.001^{**}$
Protein (g/kg)	172 ± 11	169 ± 13	168 ± 10	177 ± 38	177 ± 8	165 ± 8	163 ± 5	164 ± 10	171 ± 11	164 ± 8	$P \leqslant 0.006^{**}$

Table 4.
Correlation matrix of the parameters sampled on the eels.

	K	VIT	AW	CORT	GW	HSI	HCR	PO	CHOL	GLUC	PL	DRY	PROTCARC	CL	FFA	TP	LAC	CFAT	TG	HB	SO
K	1.00																				
VIT	0.28	1.00																			
AW	−0.10	**−0.53**	1.00																		
CORT	0.11	0.43	−0.31	1.00																	
GW	**0.71**	**0.57**	−0.44	0.41	1.00																
HSI	0.17	0.01	0.32	0.04	0.10	1.00															
HCR	−0.03	0.29	−0.13	0.25	0.14	−0.12	1.00														
PO	0.13	0.13	−0.28	0.11	0.34	0.08	0.00	1.00													
CHOL	0.28	0.46	−0.36	0.31	**0.52**	−0.10	0.26	0.19	1.00												
GLUC	0.01	−0.10	0.10	−0.14	−0.07	0.23	0.18	−0.10	0.09	1.00											
PL	0.34	**0.51**	−0.35	0.34	**0.58**	−0.06	0.33	0.22	**0.94**	0.05	1.00										
DRY	**0.58**	0.24	−0.25	0.14	**0.56**	−0.20	0.13	0.24	0.31	0.16	0.40	1.00									
PROTCARC	**−0.58**	−0.25	0.08	−0.18	**−0.51**	0.01	−0.13	−0.15	−0.19	−0.24	−0.29	**−0.85**	1.00								
CL	0.02	0.16	−0.02	0.07	0.05	0.08	0.06	0.03	0.07	−0.17	0.14	0.01	0.03	1.00							
FFA	0.48	0.31	−0.26	0.24	**0.52**	−0.07	−0.07	0.14	**0.51**	−0.20	**0.59**	**0.53**	**−0.51**	−0.02	1.00						
TP	0.08	0.11	0.07	0.17	0.18	−0.03	0.15	0.03	0.40	−0.07	0.43	0.06	−0.06	0.09	0.48	1.00					
LAC	0.15	0.16	0.00	0.22	0.20	0.00	0.35	−0.11	0.18	**0.62**	0.20	0.28	−0.32	0.01	−0.19	−0.05	1.00				
CFAT	**0.59**	0.27	−0.24	0.17	**0.59**	−0.17	0.15	0.25	0.30	0.17	0.40	**0.99**	**−0.88**	0.01	**0.56**	0.05	0.27	1.00			
TG	0.38	0.44	−0.28	0.12	**0.57**	−0.07	0.20	0.17	**0.59**	−0.10	**0.69**	0.47	−0.38	0.07	**0.64**	0.44	−0.04	0.48	1.00		
HB	0.10	0.33	−0.43	0.31	0.27	0.02	0.47	0.03	0.27	−0.04	0.35	0.13	−0.07	0.26	0.13	0.09	0.04	0.13	0.17	1.00	
SO	−0.09	0.24	−0.29	0.46	0.07	0.08	−0.02	0.04	0.21	−0.26	0.21	−0.05	0.11	0.45	0.10	0.02	−0.23	−0.04	0.05	0.21	1.00

greatest ovarian development, as all the descriptors indicative of the animal energy stores were also correlated with GW (table 4). PL, FFA, CHO, TG, CFAT were all significantly correlated, with eye index as well (table 4). Lactic acid (LAC) and glucose (GLU) were only significantly correlated with each other ($R = 0.62$).

DISCUSSION

The aim of the PCA analysis was to describe the transient changes that are characteristic of the process of silvering. Results of the PCA analysis indicate that silvering is positively related to internal maturation parameters such as VIT and blood-substrates such as phospholipids, triglycerids, FFA and cholesterol. Silvering is also positively related to constituents of the carcass, such as protein content and parameters for fuel mobilisation such as cortisol. In contrast, the PCA analysis indicated that silvering is negatively correlated with intestine weight.

Principal Component Analysis (PCA) was carried out on a total of 21 physiological parameters. The first two axes amounted to 44% of total inertia (first axis 31% and second 13%). So one third of the variation can be explained by one axis, and both axes explain nearly 50% (44%). This is not extremely high for a PCA but it can be remarked that this study concentrates on metabolic parameters and cortisol while, in another study (van Ginneken et al., 2007), important maturation parameters such as hormones (oestradiol, testosterone, thyroid hormones and growth hormone), together with oocytediameter, and gonad weight are presented. Those parameters accounted for 57% of the total inertia (respectively 42 and 15% for axes 1 and 2).

Pankhurst (1982) indicated that the eye index (eye size relative to length) might be used to define an increased stage of silvering. The cut off point that separated immature and mature eels was an eye index of 6.5. In our study, the eye index of yellow eels was higher, varying between 6.5 and 7.3. An intermediate value of 8 was found in August, exactly when the migration season started; half of the animals at this time were yellow and half were silver. The differences between the absolute values of Pankhurst (1982) and our values are likely due to a different hormone treatment of Pankhurst's and our wildlife animals. The HSI is more or less constant during the whole year and does not correlate with the levels of VIT and the weight of the alimentary tract ($r = 0.01$ and $r = 0.32$, respectively). Thus the HSI is not determined by the vitellogenine process or by the supply of food. The HSI is also not significantly different between yellow or silver eel stage ($P \leqslant 0.924$). This result corresponds with the findings of Han et al. (2003) who observed that the mean HSI remained constant during silvering for Japanese eel. However, this observation is in contrast to other studies where increased HSI values in silver animals have been reported for European eel (*A. anguilla*) (Olivereau and Olivereau, 1979), and shortfin (*A. australis*) and longfin (*A. dieffenbachii*) New Zealand eel (Lokman, 1998).

The increased content of blood lipids (phospholipids, cholesterol) at silvering, in combination with a higher fat content of the body, might be an adjustment of the silver eel to its new stage of life. This increased cholesterol level is consistent with studies in maturing salmonids where increased levels of cholesterol in plasma and gonads during the spawning season were reported (Idler and Tsuyuki, 1958; Idler and Bitners, 1960). According to Lewander et al. (1974), a redistribution of cholesterol occurs from other tissues to the gonads in silver eel. A low correlation coefficient ($r = -0.24$) between the weight of the alimentary tract and the carcass fat content does not support the view that the fat composition of the animals is directly dependent on the feeding season and that the fat stores are probably more important for the animals as a form of storage than nutritional deposition in the liver.

Mesenteric fat has been shown to be important for the hormonal regulation of maturation in Atlantic salmon *Salmo salar* L. (Rowe et al., 1991) and it has also been suggested that fat content regulates the onset of maturation in eel (Larsson et al., 1990). The assumption that fat is a controlling factor in the initial maturation process is supported by a study of Andersson et al. (1991). In the comparison of yellow Baltic eels vs. Kattegat eels, individuals originating from the Baltic metamorphose at a greater age. This observation was explained due to the slower accumulation of fat in Baltic yellow eels (Andersson et al., 1991). In our study, fat content of the carcass was very significantly higher in silver animals in comparison with yellow animals ($P < 0.001$).

Many diverse roles have been suggested for cortisol during such metabolic stresses as starvation, osmoregulation, mobilisation of energy stores for migration, gonad maturation, spawning (Wingfield and Grimm, 1977) and during stress itself (Wendelaar Bonga, 1997). Cortisol is released from the interrenal tissue when an animal is under exposure to stressors (Wendelaar Bonga, 1997) but changes in cortisol levels can also be attributed to a daily rhythm (Spieler, 1979) and to sexual maturity and season (Pickering and Christie, 1981). Although the stress of capturing the eels with fyke nets, holding them in storage tanks on the boat, and sampling the eels causes plasma cortisol to rise, we can clearly see a seasonal pattern in cortisol plasma levels. Those changes do indicate a changing activity of the interrenal system throughout the year. If we compare the pattern of secondary stress parameters of eel such as increased glucose, potassium or lactic acid (van Ginneken et al., 2002) with the pattern of the cortisol response, we find low correlation coefficient of respectively ($r = -0.14$, 0.11 and 0.22). These low values suggest that this cortisol is not solely involved in a stress-response. Currently, it is not understood whether sexual maturation is accompanied by increased corticosteroid levels (review Idler and Truscott, 1972; Pickering, 1989). Several studies have demonstrated a correlation between sex hormones, body constitution and cortisol (Mackinnon, 1972; Wingfield and Grimm, 1977). In general, vertebrates exposed to stress commonly show a reduction in reproductive performance (Moberg, 1985). Furthermore, exposure of fish to stress results in impaired reproductive capacity (Donaldson, 1990; Barton and Iwama,

1991). However, in other studies, seasonal elevations in plasma corticosteroids have been found, suggesting a positive stimulating role of cortisol in the pre-spawning period. This is not only in salmonid species such as Pacific salmon (Pickering and Pottinger, 1987) which only spawn once, but also in landlocked non-migratory salmonids which spawn several times, like the rainbow trout (Robertson et al., 1961).

In the study reported here, we found elevated cortisol levels in silver eels prior to migration and a rather high correlation with the salinity level ($r = -0.79$). A role of cortisol in the maturation process of eels is at this moment under investigation in our laboratories. However, from our observations in this study it has become clear that a role for cortisol may be in a mobilisation of energy stores, especially in European eel which have to cover a distance of 6000 km to its spawning areas in the Sargasso. Van Ginneken and van den Thillart (2000) demonstrated in large Blazka swimtunnels of 127 litres that, for this tremendous swim effort, 40% of the eels' energy reserves are needed while still 60% of their energy stores can be used for gonad development.

The observation that blood-substrates, such as phospholipids and cholesterol, are significantly increased in European silver eels corroborates the view that the major role of cortisol lies in the mobilisation of energy stores prior to migration.

Based on PCA analysis with physiological, morphological and endocrinological parameters we see (fig. 2B) that during the process of silvering, several developmental stages can be recognised. Up until now, silvering was split into two separate stages; yellow and silver. This classification 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). This is, in our opinion, the first study where physiological parameters (blood substrates, constitution carcass) together with endocrinological parameters (cortisol) have been incorporated in the analysis giving a more subtle seasonal description of the silvering process (start and duration) as well as of the physiological mechanisms involved (triggers and endocrine control).

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