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Precocious Silvering of Farmed Eels in Relation to Their Physiological Aspects

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ABSTRACT

In hatcheries, a precocious onset of silvering occurs, which is a pre-adaptation to the oceanic migration phase and is accompanied with an increase of fat content, a regression of the alimentary tract while the eyes enlarge. In the present study, the differences between yellow and silver European eel (*Anguilla anguilla* L.) from a hatchery were analysed by studying their physiological parameters like blood and body compositions, as well as the concentrations of the thyroid hormones (T3 and T4). The condition factor (C.F.), the eye index (E.I.), cholesterol, free fatty acids (FFA), and thyroid hormones T3 & T4, as well as the amount of fat, protein and dry matter were all significantly higher in silver eel than in yellow eel. These morphological and physiological parameters are therefore good for defining the status of silvering in order to obtain a 'silver-index'. The Gonadosomatic index (GSI) does not change in weight between yellow males (0.144 ± 0.082; n=13) and silver males (0.156 ± 0.049; n=29) (when $P \leq 0.585$). Also no histological differences were observed between the gonads of yellow and silver males. All above observations of the present study therefore proved that at least for male eels, maturation and silvering are separate processes, and that there is no harmonization of the gonadal maturation with silvering.

KEY WORDS: eels, Anguilla, silvering, maturation, fat metabolism, thyroid hormones, metamorphosis.

INTRODUCTION

During the life cycle, the European eel (*Anguilla anguilla* L.) experiences two periods of metamorphosis. The first transformation is from the planktonic marine stage (*Leptocephalus* larvae) into glass eel that occurs during its oceanic migration from the supposed 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. Then eels transform from yellow eel into silver eel. During the latter transformation, there is some proliferation of the gonads and an increase in eye size [1]. Furthermore, the body colour becomes silvery [2]; the alimentary tract shows regression, and the animal becomes fatter. These changes are part of the silvering process, which precedes to the spawning migration to the Sargasso, 6000 km away from Europe. Silver eels are in a prepuberal stage, even at the moment when they leave the coast. Maturation is likely postponed till the end of the journey, since otherwise two processes will compete simultaneously for energy reserves: muscle activity and gonad development.

The mechanisms involved in the onset of silvering are largely unknown. Until now the research at the mechanism of silvering of eels was mainly based on morphological characteristics like Eye Index and Digestive Tract Index [1]. The process of silvering of eels is important to understand for the following reason. The eel is becoming more important for aquaculture, but in eel farms, a precocious onset of silvering occurs, which impairs the productivity (pers.comm. Ir.J.van

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Rijsingen, Royaal BV, Helmond, The Netherlands). In nature, male eels are silvering at an age of 3-6 years and female's silver after a minimum growth period of 7-12 years. In farms, however, silvering can occur already after one year. This is disadvantageous for the fish farmer for two reasons. First, silver eel have a lower rate of food conversion compared to yellow eel, probably due to a regression of the alimentary tract. Secondly, the quality of the silver eel end product is low due to higher body fat content, compared to yellow eel.It is generally assumed that silvering is an adaptation of the eel for its 6000-km migration journey, since the swimming effort is a prerequisite for spawning, so it is hypothesised that morphological and physiological changes are more related to this swim-endurance than to maturation. It is more advantageous for the animal that maturation is postponed or slowed down till the end of the journey. The increase of metabolic rate due to swimming activity and the need for fuel mobilisation might thus be more relevant during silvering than the increase of the GSI. Thus, changes in fat mobilisation and thyroxin level might be expected. Therefore, the prepuberal stage of silvering with its corresponding morphological, physiological and endocrinological changes needs to be investigated. Therefore, the basic aim of the present study was to determine the status of silvering of eels based on a combination of morphological and physiological parameters. In this study morphological and physiological characteristics for 'silvering' in combination with gonad development (GSI) were described in order to investigate whether the process of 'silvering' is accompanied with a development of the gonad. The rationale of this study was therefore to elucidate the 'silvering' process by comparing yellow and silver eel for morphological, physiological parameters, blood and body constitutions, GSI, state of gonad development and the concentrations of the thyroid hormones T3 and T4.

MATERIAL AND METHODS

Fish sampling

Eels were obtained from a commercial eel farm of Royaal BV, Helmond, the Netherlands during the year 2010 to 2012. The glass eel originated from France. From glass eel stage, until the time of sampling, the eels were kept in a recirculation system, with fish tanks, upflow filters for sedimentation, and trickling filters for nitrification. Husbandry conditions in the hatchery were as described earlier by Van-Ginneken *et al.*[3]. The sampling protocol for subdivision of the eels into yellow and silver was as described earlier by Van-Ginneken *et al.*[4]. In this way, 36 'silver' animals and 36 'yellow' animals were selected with a bodyweight in the range of 100-150 grams. In the laboratory, the animals were kept in aquaria for one month with a 14:10 light/dark cycle, in running local tap water at 20°C and were fed daily with Provimi pelletted food (Provimi, Rotterdam, The Netherlands).

Experimental protocol and sampling procedure

The animals were classified according to gender by the experimentation based on paraffin slices of the gonads stained with Gill's Hemotoxiline-eosine staining. The following classes were distinguished as follows;

1. Undifferentiated sex,
2. male,
3. female,
4. intersex

   Based on gonad characteristics such as, thickness of the layer of connective tissue, type, stage and number of germ-cells and formation of tubuli in the testis.

Only yellow and silver males, and yellow females were used in the analysis for blood and body composition. The fish were quickly anaesthetized with 300 PPM MS222 (3-aminobenzoic-acid-ethyl-ester methanesulfonate salt, Sigma, St. Louis, USA). After three minutes, the anaesthetized fish were taken out of the aquarium. Thereafter, the mean weight and body length of the eel was measured prior to sampling. Blood was collected with a heparinized syringe (flushed with 3000 units' heparin per ml blood). The horizontal and vertical eye diameters of left and right eye were measured to calculate the eye index (E.I) [5]. The animal was dissected, and the weight of the gonads, liver, and alimentary tract were determined.

Analytical methods blood sampling

After collection of the blood samples, blood was directly centrifuged (10,000 rpm) for 5 min. The plasma was divided in eppendorf tubes (20, 20, 10, 40, 60, and 60 µl for analysis of cholesterol, triglycerids, total protein, free fatty acids [FFA], andthyroid hormones [T3 and T4], respectively. Then stored at -80°C for further analysis. Cholesterol, triglycerides and total protein were measured with Boehringer Mannheim test kits (MPR1 CHOD-PAP 1442341, GPO-PAP 701882 and MPR3 124281), respectively. FFA was measured with a commercial test kit WAKO (NEFA C method, Instruchemie, Hilversum, The Netherlands). T3 and T4 were measured with a radioimmunoassay according to the method of Brown & Eales [6].

Carcass analysis

After weighing, the fish samples were cut into pieces of about 3.0 cm and nearly submerged in water in a glass beaker. To run the analyses for total fat, protein and dry weight, two animals were pooled. The samples were autoclaved at 2 atmospheres at 120°C for 4 hours. The samples were homogenised with a laboratory mixer and subsequently sampled in triplicate for dry matter, protein and fat analyses. The dry matter content was measured by freeze drying of the sub-samples to constant weight. Plate temperature started at -20°C and raised to 27°C after a vacuum of 40 Pa was reached. Condenser temperature was -90°C. The protein was measured according to ISO 5983. For the fat determination, the sub-samples were freeze dried, as described for dry matter, and subsequent extraction of the fat was performed as described in ISO/DIS 6492 [7].

Calculations and statistics

The condition-factor (C.F.) was calculated according to the equation 1 as follows;

$C.F.=100*WxL^{-3}$…… (1).

The eye index was calculated according to the method of Pankhurst [5] by equation 2 as follows;

$E.I.=\{[(A+B)^2/4*\pi]/L\}*100$ ……..(2)

where A is the horizontal eye diameter, B is the vertical diameter, and L is the total body length (mm).

The gonadosomatic index (G.S.I.) was calculated according to equation 3 as follows;

(Gonad weight/body weight)*100%…….(3).

The hepatosomatic index (H.S.I) was calculated according to equation 4 as follows;

(liver weight)/(body weight)*100 percent.

The mean value of the yellow eel group was compared to the mean value of the silver eel group. All statistical analysis of data were performed via SAS (Statistical Analysing Software) using a one-way ANOVA for differences between yellow and silver groups when $P \leq 0.05$ was considered as statistically significant. Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov and F_{max} tests, respectively.

RESULTS

The results of the present study were presented in Tables 1-3, respectively.

Table 1: Differences between silver and yellow eel for body characteristics and blood parameters (Mean ± S.D. of 30-36 animals per group, except for fat, protein and dry matter: N=18). N= Number of samples; C.F=Condition Factor; E.I=Eye index; G.S.I= gonadosomatic index; the H.S.I=hepatosomatic index; ** = show highly significance when $P \geq 0.01$; * shows significance when $P \geq 0.05$.

Parameter	Mean ± S.D. Silver (N=29)	Mean ± S.D. Yellow (N=26)	ANOVA
Bodyweight (g)	126.2 (21.9)	118.7 (43.7)	P<0.3555
Length (cm)	40.4 (2.2)	40.5 (4.1)	P<0.9186
Condition factor (C.F.)	0.189 (0.019)	0.171 (0.023)	P<0.0003**
Alimentary tract (g)	9.75 (2.88)	12.80 (7.45)	P<0.0269*
Gonad weight (g)	0.24 (0.21)	0.37 (0.42)	P<0.0879
G.S.I.	0.19 (0.19)	0.29 (0.29)	P<0.0697
Liver	1.507 (0.479)	1.675 (0.587)	P<0.124
H.S.I.	1.21 (0.33)	1.45 (0.27)	P<0.0017**
Eye-index (E.I.)	10.27 (2.06)	6.27 (1.52)	P<0.0001**
Triglycerids in blood (mM)	18.87 (5.50)	16.81 (7.49)	P<0.1762
Cholesterol in blood (mM)	17.99 (2.51)	15.47 (3.62)	P<0.0006**
FFA in blood (mM)	0.203 (0.009)	0.153 (0.082)	P<0.0161*
Total Protein in blood (mM)	1209.0 (133.3)	1163.8 (215.0)	P<0.3231
T3 (nM)	5.11 (3.21)	3.35 (3.04)	P<0.0139*
T4 (nM)	2.68 (1.32)	2.02 (1.37)	P<0.0398*
Fat (g/kg)	307.02(15.38) (N=18)	290.98 (26.33) (N=18)	P<0.0338*
Protein (g/kg)	159.64 (4.83) (N=18)	155.33 (5.59) (N=18)	P<0.0201*
Dry matter(g/kg)	479.77 (11.74)(N=18)	460.93 (22.56) (N=18)	P<0.0037**

Table 2: Differences between silver and yellow male eel for body characteristics and blood parameters ((Mean ± S.D.).

Parameter	Mean ± S.D. Silver males (N=29)	Mean ± S.D. Yellow males (N=13)	ANOVA
Bodyweight (g)	128.00 (22.78)	100.41 (32.97)	P<0.002**
Length (cm)	40.6 (2.3)	38.4 (2.9)	P<0.011*
Condition factor	0.190 (0.020)	0.172 (0.027)	P<0.018*
Alimentary tract (g)	10.07 (2.98)	9.71 (3.09)	P<0.722
Gonad weight (g)	0.199 (0.071)	0.132 (0.059)	P<0.004**
G.S.I.	0.156 (0.049)	0.144 (0.082)	P<0.585
Liver weight (g)	1.543 (0.433)	1.487 (0.486)	P<0.707
H.S.I.	1.22 (0.28)	1.53 (0.33)	P<0.002**
Eye-index (E.I.)	9.74 (1.62)	7.20 (1.85)	P<0.0001**
Triglycerids in blood (mM)	19.10 (5.79)	18.60 (7.81)	P<0.506
Cholesterol in blood (mM)	18.01 (2.68)	14.91 (3.68)	P<0.007*
FFA in blood (mM)	0.209 (0.009)	0.127 (0.078)	P<0.007**
Total Protein in blood (mM)	1219.5 (133.9)	1168.2 (229.1)	P<0.407
T3 (nM)	5.2 (3.16)	3.74 (3.45)	P<0.191
T4 (nM)	2.71 (1.26)	2.23 (1.41)	P<0.297

Table 3: Differences between yellow male and female eel for body characteristics and blood parameters (Mean ± S.D.). N=Number of samples

Parameter	Mean ± S.D. Yellow males (N=13)	Mean ± S.D. Yellow females (N=13)	ANOVA
Bodyweight (g)	100.41 (32.97)	137.85 (50.30)	P<0.03*
Length (cm)	38.39 (2.86)	43.11 (4.34)	P<0.03*
Condition factor	0.172 (0.027)	0.165 (0.014)	P<0.409
Alimentary tract (g)	10.07 (2.98)	9.71 (3.09)	P<0.034
Gonad weight (g)	0.132 (0.059)	0.606 (0.423)	P<0.0001**
G.S.I.	0.144 (0.082)	0.461 (0.349)	P<0.003**
Liver weight (g)	1.487 (0.486)	1.876 (0.675)	P<0.097
H.S.I.	1.534 (0.334)	1.407 (0.206)	P<0.251
Eye-index (E.I.)	7.20 (1.85)	5.41 (0.745)	P<0.003**
Triglycerids in blood (mM)	18.60 (7.81)	15.40 (7.24)	P<0.290
Cholesterol in blood (mM)	14.91 (3.68)	15.70 (3.30)	P<0.583
FFA in blood (mM)	0.127 (0.078)	0.187 (0.076)	P<0.068
Total Protein in blood (mM)	1168.2 (229.1)	1146.5 (216.6)	P<0.823
T3 (nM)	3.74 (3.45)	3.47 (3.27)	P<0.852
T4 (nM)	2.23 (1.41)	2.12 (1.23)	P<0.761

In Table 1, different physiological and endocrinological parameters obtained from yellow and silver are presented. From Table 1, it becomes clear that the body weight and length are not significantly different between the yellow and silver eel groups used in the present study. The length and the body weight in the silver group were respectively 126.2 g and 40.4 cm, while in yellow group were 118.7 g and 40.5 cm, respectively. The condition factor (C.F.) was significantly higher in silver eel (silver eel 0.189, yellow eel 0.171), while the weight of the alimentary tract was significantly lower in silver eel (silver eel: 9.75 g, yellow eel: 12.80 g). Furthermore, it was observed that the eye-index (E.I.), an indicator of maturation status, was significantly higher in the silver eel group compared to the yellow eel group (silver eel: 10.27, yellow eel: 6.27). The substrate cholesterol was significantly higher in the silver eel group compared to the yellow eel group (17.99 mM in silver eel, 15.47 mM in yellow eel). The FFA also show a rise from 0.153 mM in the yellow eel group to 0.203 mM in the silver eel group. The triglycerids (silver eel: 18.87 mM, yellow eel: 16.81 mM) and [total protein] in plasma (silver eel: 1209 mM, yellow eel: 1164 mM) were not significantly different between groups. For thyroxin, it was concluded that T3 and T4 were significantly higher in the silver eel group compared to the yellow eel group (silver eel: T3=5.11 nM, T4=2.68 nM; yellow eel: T3=3.35 nM, T4=2.02 nM). Based on these observations, it was concluded that the concentrations of the active hormone T3 were significantly higher than the prohormone T4.From the data of the carcass analysis, it is obvious that the fat, protein and dry matter contents of silver eels are significantly higher, compared to yellow eels (fat silver: 30.7 %, fat yellow 29.1 %; protein silver: 16.0 %, protein yellow 15.5 %, dry matter silver 48.0 %, dry matter yellow 46.1 %).

As the sexes of all animals was determined histologically [4], thus, comparisons were made between silver and yellow eel males (see Table 2) and yellow males and yellow females (see Table 3). Table 2 gives the variancesfor body characteristics between yellow and silver males. The body-length, body-weight and condition-factor of the silver male group were significantly higher in comparison to the yellow male group. No significant difference was observed in the weight of the alimentary tract between yellow and silver males. Silver males had a significant higher gonad weight but not a significant higher G.S.I., in comparison to yellow males. No significant difference in liver weight was observed between silver or yellow males.But in contrast the hepatosomatic index (H.S.I) was significantly higher in yellow males. The eye-index (E.I), plasma -cholesterol and FFA were significantly higher in the silver eel group when compared to the yellow eel group. The triglycerids-, total protein and T3 and T4 in plasma were not significantly different between yellow and silver male groups. Thus, from the Table 3, it had been proved that for yellow animals, sexing can be based on morphological parameters such as, bodyweight, length, gonad weight, GSI and eye-index (E.I), which were all significantly higher for females.

DISCUSSION

In the present investigation, eel was found to be a very fatty fish as compare to the other fish species. For example, silver eel contain high fat content, that is 30.7%, whereas, yellow eel also contain highest fat percentage, that is 29.1% in their bodies, as presented in the Table 1. Our present results were in consistency to theHirt-Chabbert and Young [8], whoalso reported high fat content in eel ranged from 20 to 22%. Accordingly, such highest percentage of fats will define the flavour, texture and aroma of any fish products. While in contrast, the common carp contain only small percentage of fats that is 2% in their body [8]. While the concentration of plasma-free fatty acids [FFA] were measure as 0.15 for yellow eel and 0.20 mM for silver eel, which were found to be very low as compared to carp, that is 0.59 mM as previously reported by Van Ginneken *et al.* [10]. Such low FFA content in plasma of yellow and silver eels was might be related to the location of the fat stores or the spawning season [11], becausefatty acidvariations in muscle tissues of the yellow European eel (*Anguilla anguilla*) had been observed during short-term migratory adaptation between fresh and marine waters under food deprivationbecause eel fish appear to use lipids in muscle to provide metabolic and osmoregulatory energy.In case of carp fish, these FFA are located around the intestines, whereas in eel, most fat is located in and between the muscles. FFA's are formed by lipolysis and transported from the adipose tissue to the muscle via blood. In eels, this distance is very short and hardly requires blood as a transport medium. Thus, in eels, FFA probably play a minor role as a transport form of lipids [12]. In silver eel, the FFA is significantly higher than in yellow eel as shown in Table 1 that was in accordance with Larsson & Fänge [13] and Lewander *et al.* [14]. This is likely related to a higher level of lipolysis and a large demand for FFA as an energy source during muscular work. In general, active fish have higher plasma levels of FFA, while sluggish and lethargic fish have lower FFA [12].

As lipid reserves are quite necessary to fullfilthe energy requirements for migration and reproduction in eel[15], so, changing in the fats concentration in eel may also have some relation with sex of individual eels, or various environmental variables such as changing temperature, decreasing eutrophication, food availability and trophic status as previously reported by Boëtius and Boëtius [16]and Jonsson and Jonsson [17].More recently, the energy required for eel migration was calculated as 7.8% and 6% fat as reported by Palstra et al. [18] and Van Ginneken et al. [19-20].

eels achieved 20–22%
total fat, starting from wild fish with 7% fat content

The increased content of blood lipids like triglycerids, FFA, 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 life phase of migration must be characterised by an increased use of fat as an energy source for swimming. Eels have an impressive amount of storage fat, which must be mobilised during migration. Recent results showed that 30-40% of the fuel storage is used for the migration, which obviously leaves enough for gonad development [3]. High fat levels are required for the spawning

migration, and so high fat levels might play a role in the silvering process. The other possibility is that in the silver phase, the animal converts all available fuels into fat. For example, when the intestines degenerate, the amino acids can be used for fat deposition. In silver eel, higher levels of cholesterol were observed, as shown in Table 1. This was in consistent with studies in maturing salmons, where increased levels of cholesterol in plasma and gonads occurs during the spawning season as reported by Idler & Tsuyuki [21] and Idler & Bitners [22]. According to Lewander *et al.* [14], a redistribution of cholesterol occurs from other tissues to the gonads in silver eel, which might be related to produce steroid hormones for later maturation. Increasing level of plasma cholesterol might be related to the onset of spawning, because gonads are immature. The level of plasma cholesterol might be related to the activity of fish like age, growth, sex, diet, nutritional state, salinity, temperature that can effect this cholesterol level in blood plasma of fish.Lewander et al.[14] had described the presence of high levels of cholesterol in the blood plasma of silver eels ready for migration.Diwan and Krishnan[23] observed the cholesterol aspioneer for the synthesis of steroids, which in turn can influence on maturation phenomena.

In silver eel, higher levels of blood lipids and a higher fat constitution of the body are observed compared to yellow eel. However, Svedäng and Wickström [24] proposed that migration of eels can still take place at low fat-concentrations in the muscles. In that case, the stimulus for migration might not directly be related to the lipid concentration, but by an endocrinological signal like T3 or T4. According to Svedäng and Wickström [24], silver eels contain a very low fat concentration, but still have the urge for migration, might arrest their migration temporarily for resumption of feeding. Only by applying this strategy they might be able to swim the 6000-km. In contrast, in another study, the fat content of the eels was considered as a factor directly inducing migratory behaviour [25]. Table 1 shows that T3 and T4 are significantly higher in silver eels when compared with yellow eels. In other studies with eel, also increased thyroid and pituitary activity were observed during silvering, which was in accordance with Callamand and Fontaine [26], Bertin [27] and Knowles & Vollrath [28]. In salmons during parr-smolt transformation thyroxin is involved [29]. Also, in salmons and other fish species, similar endocrinological changes in thyroxin were observed before or during the spawning season. Treatment of juvenile sockeye salmon with thyroxin resulted in an increased migration activity [30]. In the perch, *Anabas testudineus*, it was observed that T4 reaches its maximal concentration at the beginning of the spawning season [31]. However, It remains unclear whether thyroxin plays a role in the precocious onset of silvering. In a study of Timmermans *et al.* [32], with juvenile inbred carp, the T3 concentration in plasma was artificially increased by administration of low amounts of T3 to the water. This resulted in a precocious onset of spermatogenesis in the juvenile carp. A direct effect of T3 on spermatogenesis cannot be excluded, but it is also possible that gonadotropine release from the pituitary is stimulated, or that there is an interaction with steroids [32]. Still, these results suggest that thyroxin may play a role in the silvering process.

In the present study, no differences in G.S.I. of the testis of silver or yellow eels were observed. Therefore, the most important conclusion from this study is that for males silvering and gonad-development (maturation) are processes that occur separately. Silvering, a pre-adaptation to the oceanic migration phase is accompanied with an increase of fat content, a regression of the alimentary tract and enlargement of eyes. The male gonad does not change in weight or in developmental stage [4]. Hence, all above observations suggested that maturation and silvering are separate processes and there is no synchronisation of the gonadal maturation with silvering.

Perspectives: In future studies we hope to elucidate the following related matters:

1) Develop a technique for adipocyte isolation from eel muscle in order to investigate the endocrinological regulation of lipolysis (FFA-mobilisation) in adipocytes of yellow and silver eel.
2) To assess, using thyroidectomized- *vs.* control- fish; to what extent the thyroid of ectotherms is involved in the control of the overall metabolic rate. Do thyroid hormones in ectotherms have a calorigenic effect and are involved in the control of metabolic rate [33] or do they only play a role in metamorphosis like for amphibians in larvae to adult transformation [34], for salmon during parr-smolt transformation [29] or for eel during silvering [35]? Minimal changes in metabolic rate in cold-blooded animals can be measured with the very sensitive and state of the art technique of direct calorimetry [36].

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REFERENCES

1. Pankhurst, N.W. & J.N. Lythgoe, 1983. Changes in vision and olfaction during sexual maturation in the European eel *Anguilla anguilla* (L.). J.Fish.Biol.,23: 229-240.
2. Pankhurst, N.W. & J.N. Lythgoe, 1982. Structure and colour of the integument of the Europeaneel *Anguilla anguilla* (L.). J.Fish.Biol.,21: 279-296.
3. Van Ginneken, V.J.T. & G.E.E.J.M. Van den Thillart, 2000. Eel fat stores are enough to reachthe Sargasso. Nature,403: 156-157.
4. Van Ginneken, V.J.T., H. Komen, K. Noorlander, V. Sommandas, S. Van Schie & G. van den Thillart, 2003. Precocious silvering of farmed eels 1: A study at gonad-development. Netherlands Journal of Zoology (submitted for publication).
5. Pankhurst, N.W., 1982. The relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* (L.). J.Fish.Biol.,21: 417-428.
6. Brown, S. & J.G. Eales, 1977. Measurement of L-thyroxin and 3,5,3'-triiodo-L-thyronine levels in fish plasma by radioimmunoassay. Can.J.Zool. 55: 293-299.
7. ISO/DIS 6492 (International Organisation for Standardisation), 1996. Animal feeding stuffs-Determination of nitrogen content and calculation of fat content, category B, 1996 (ISO/DIS 6492), Geneva, Switzerland.
8. Hirt-Chabbert, J. A. and Young, O. A. (2012), Modification in Body Fat Content and Fatty Acid Profile of Wild Yellow Shortfin Eel, *Anguilla australis*, through Short-term Fattening. Journal of the World Aquaculture Society, 43: 477–489.
9. Dirksen, S., T.J. Boudewij, L.K. Slager, R.G. Mes, M.J.M. van Schaick & P. de Voogt, 1995.Reduced breeding success of cormorants (*Phalacrocorax carbo sinensis*) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands. Environmental Pollution,88: 119-132.
10. Van Ginneken, V.J.T., P. van Caubergh, M. Nieveen, P. Balm, G. van den Thillart & A. Addink, 1998. Influence of hypoxia exposure on the energy metabolism of common carp (*Cyprinus carpio* L.). Netherlands Journal of Zoology,48: 65-82.
11. Ghazali, N., D. Boussoufa, J. C. Navarro & M. El-Cafsi, 2013. Lipid and fatty acid variations in muscle tissues of the 'yellow' stage of the European eel (*Anguilla anguilla*) during short-term adaptation to freshwater and seawater under food deprivation. Marine and Freshwater Behaviour and Physiology, 45(6): 385-395.
12. Larsson, A., & R. Fänge, 1977. Cholesterol and free fatty acids (FFA) in the blood of marinefish. Comp.Biochem.Physiol.,57B: 191-196.
13. Larsson, A. & R. Fänge, 1969. Chemical differences in the blood of yellow and silver phasesof the European eel (*Anguilla anguilla* L*).* Archives Internationales de Physiologie et de Biochimie, 77: 701-709.
14. Lewander, K., G. Dave, M.L. Johansson, A. Larsson & U. Lidman, 1974. Metabolic andhaematological studies on the yellow and silver phases of the European eel, *Anguilla anguilla* L. I: Carbohydrate, lipid, protein and inorganic ion metabolism. Comp.Biochem.Physiol.,47B: 571-581.
15. Larsson P., Hamrin S., Okla L., 1990. Fat content as a factor inducing migratory behavior in the eel (*Anguilla anguilla* L.) to the Sargasso Sea. Naturwissenschaften, 77: 488–490.
16. Böetius, I., Böetius, J., 1980. Experimental maturation of female silver eels, Anguilla anguilla. Estimates of fecundity and energy reserves for migration and spawning. Dana 1, 1-28.
17. Jonsson, B., Jonsson, N., 2005. Lipid energy reserves influence life-history decision of Atlantic salmon (Salmo salar) and brown trout (S. trutta) in fresh water. Ecology of Freshwater Fish 14, 296–301.
18. Palstra, A.P., Antonissen, E., Clavero, M.E., Nieveen, M., Niemantsverdriet, P., van Ginneken, V.J.T., van den Thillart, G.E.E.J.M., 2006. The fate of fat in silver eels: lipid requirements for spawning migration. In: Palstra, A.P. Energetic requirements and environmental constraints of reproductive migration and maturation of European silver eel (Anguilla anguilla L.). PhD dissertation. Leiden, The Netherlands: University of Leiden. 184 pp.
19. van Ginneken, V.J.T., C. Durif S. P. Balm, R. Boot, M. W.A. Verstegen, E. Antoissen, G. V.D. Thillart, (2007). Silvering of European eel (*Anguilla anguilla* L.): seasonal changes of morphological and metabolic

parameters. Animal Biology, 57(1): 63-77.
20. van Ginneken, V.J.T., Dufour, S., Sbaihi, M., Balm, P., Noorlander, K., de Bakker, M., Doornbos, J., Palstra, A., Antonissen, E., Mayer, I., van den Thillart, G. 2007. Does a 5,500-km swim trial stimulate early sexual maturation in the European eel (Anguilla anguilla L.)? Comparative Biochemistry and Physiology, in press, doi: 10.1016/j.cbpa.2007.03.021
21. Idler, D.R. & H. Tsuyuki, 1958. Biochemical studies on sockeye salmon during spawning migration. 1: Physical measurements, plasma cholesterol and electrolyte levels. Can. J. Biochem. Physiol., 36: 783-791.
22. Idler, D.R. & I. Bitners, 1960. Biochemical studies on sockeye salmon during spawning migration. IX: Fat, protein and water in the major internal organs and cholesterol in the liver and gonads of the standard fish. J.Fish. Res.Bd.Can., 17: 113-122.
23. Diwan, A.D. and L. Krishnan, 1986.Levels of Cholesterol in blood serum and gonads in relation to maturation in Etroplus suratensis (Block). Indian journal of fisheries, 33(2): 241-245.
24. Svedäng, H. & H. Wickström, 1997. Low fat contents in female silver eels: indications of insufficient energetic stores for migration and gonadal development. Journal of Fish Biology,50: 475-486.
25. Larsson, P., S. Hamrin & L. Okla, 1990. Fat content as a factor inducing migratory behaviour in the eel (*Anguilla anguilla* L.) to the Sargasso Sea. Naturwissenschaften, 77: 488-490.
26. Callamand, O. & M. Fontaine, 1942. L'Activité thyrodienne de l'Anguille au cours de son development. Arch. Zool. Exp. Gen., 82: 129-135.
27. Bertin, L., 1956. Eels, a Biological study. Cleaner-Hume, London, 192 pp.
28. Knowles, F. & L. Vollrath, 1966. Changes in the pituitary of the migrating European eel during its journey from rivers to the sea. Zeitschrift für Zellforschung, 75: 317-327.
29. Dickhoff, W.W., L.C. Folmar & A. Gorbman, 1978. Changes in plasma thyroxin during smoltification of coho salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology, 36: 229-232.
30. Iwata, M., 1995. Downstream migratory behaviour of salmons and its relationship with cortisol and thyroid hormones: A review. Aquaculture, 135: 131-139.
31. Chakraborti, P. & S. Bhattacharya, 1984. Plasma thyroxin levels in freshwater perch: influence of season, gonadotropins, and gonadal hormones. Gen. Comp. Endocrinol., 53: 179-186.
32. Timmermans, L.P.M., D.A. Chmilevsky, H. Komen & H. Schipper, 1997. Precocious onset of spermatogenesis in juvenile carp (*Cyprinus carpio* L., Teleostei) following treatment with low doses of L-thyroxin. European Journal of Morphology,35: 344-353.
33. Etkin, W., 1978. The thyroid-a gland in search of a function. Perspective in Biology and Medicine, 22:19-30.
34. Dent, J.N., 1988. Hormonal interaction in amphibian metamorphosis. Amer.Zool., 28:297-308.
35. Etienne, N., 1959. Influence de la maturation sexuelle provoquée sur l'activité thyroïdienne de l'Anguille européenne mâle, *Anguilla anguilla* L. Société de Biologie, pp. 41-44.
36. Van Ginneken, V.J.T., A. Gluvers, R.W. van der Linden, A.D.F. Addink & G.E.E.J.M. Vanden Thillart, 1994. Direct calorimetry of aquatic animals: automated and computerized data-acquisition system for simultaneously direct and indirect calorimetry in aquatic animals. *Thermochim. Acta*, 247:209-224.