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Does a 5500-km swim trial stimulate early sexual maturation in the European eel (*Anguilla anguilla* L.)?

V. van Ginneken [a,*], S. Dufour [b], M. Sbaihi [b], P. Balm [c], K. Noorlander [a], M. de Bakker [a], J. Doornbos [a], A. Palstra [a], E. Antonissen [a], I. Mayer [d], G. van den Thillart [a]

[a] *Integrative Zoology, Institute Biology Leiden (IBL), van der Klaauw Laboratories, P.O. Box 9511, 2300 RA Leiden, The Netherlands*
[b] *UMR CNRS/MNHN/UPMC Biology of Marine Organisms and Ecosystems, Museum National d'Histoire Naturelle (MNHN), 7 Rue Cuvier, 75231 Paris Cedex 05, France*
[c] *Animal Physiology, Department of Biology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*
[d] *Department of Biology, University of Bergen, N-5020 Bergen, Norway*

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Abstract

The catadromous European eel (*Anguilla anguilla* L.) undertakes a 6000-km spawning migration from its freshwater habitats to the Sargasso Sea. In large Blazka swim tunnels of 127 l, the physiological effect of such a prolonged swimming performance on sexual maturation in adult female eels was investigated. Two groups of eels were placed in swim tunnels for 173 days, one group was able to swim at 0.5 body lengths/second (Swim group) covering a distance of c. 5500-km over the experimental period, and one group kept in static (End Control group). A control group was sampled at the start of the experiment in order to determine the initial stage of reproductive development (Initial Control group). At the end of the swim trial, the maturation parameters 11-ketotestosterone, pituitary levels of LH and plasma levels of estradiol were higher (although not significantly) in the Swim compared to the End Control group. In addition, no significant differences were observed in most measured morphometric and reproductive parameters, including eye-index, gonadosomatic index, hepatosomatic index, and plasma levels of vitellogenin, cortisol and melanophore-stimulating hormone (MSH). Also, pituitary levels of both MSH, and adrenocorticotropic hormone (ACTH) were unaffected. In contrast, the oocyte diameter was found to be significantly higher in the Swim compared to the End Control group. Based on these observations we conclude that a period of prolonged swimming might be a physiological stimulus necessary for the onset of maturation in the European eel.

Keywords: European eel; Gonad maturation; Spawning migration; Swim trial; Gonadotropins; Estradiol

1. Introduction

The catadromous European eel (*Anguilla anguilla* L. migrates 6000-km from its freshwater habitats to the Sargasso Sea where it spawns, following which the adults die (Tesch, 2003; van Ginneken and Maes, 2005). One of the mysteries of the life-cycle of the eel is the endocrinological mechanisms controlling gonadal maturation during its prolonged spawning migration. When silver eels start their oceanic migration in the autumn, they show only a limited degree of gonadal development, with a gonadosomatic index (gonad weight/body weight × 100; GSI) between 1 and 2. Silver eels maintained in captivity fail to show any further advance in gonadal development. In the migratory silver stage of the European eel it has been demonstrated that a pre-pubertal blockage occurs in the hypothalamus-pituitary-gonad (HPG) axis, resulting in the inhibition of sexual maturation (Dufour, 1994). At the hypothalamic level, this pre-pubertal blockage is a result of both a deficiency of gonadotropin-releasing hormone (GnRH) coupled with the inhibition of GHT-release by the neuropeptide dopamine (DA) (Dufour, 1994). The consequence of this dual blockage is an inhibition of pituitary GTH production, leading to the inhibition of pubertal development in captive silver eels.

* Corresponding author.
E-mail address: V.J.T.van.Ginneken@biology.leidenuniv.nl (V. van Ginneken).

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The endocrine mechanism(s) by which this dual blockage is naturally abolished in migrating silver eels is not yet clear. Recently the inhibitory control of the LH ovulatory peak in eel by dopamine (DA) could be abolished by triple treatment with testosterone (T), GnRH agonist (GnRHa) and DA-receptor antagonist (pimozide) (Vidal et al., 2004). The question however remains what is the environmental stimulus to abolish the prepubertal blockage and trigger the gonad maturation of European eel.

Although no adult eel has ever been caught in the Sargasso Sea to determine the GSI at spawning, based on observations from hormone-treated eels it has been concluded that fully mature adults attain a GSI value of between 40–70 (references vide van Ginneken et al., 2005d). In view of the pronounced pre-pubertal blockage evident in captive silver eels it has been proposed that the unique environmental factors experienced by the eel during its 6000-km spawning migration may, in some way, interact upon the endogenous neuroendocrine mechanisms controlling sexual maturation. These environmental factors include temperature (Boëtius and Boëtius, 1967), light, salinity (Nilsson et al., 1981) and water pressure (Fontaine, 1993). The latter factor is based on one observation of a migrating eel with swollen belly at the Bahamas at 2000-m depth (Robins et al., 1979). The influence of temperature, light, and salinity on maturation in silver eels has been studied, but showed no clear effect on the HPG-axis (Boëtius and Boëtius, 1967; Nilsson et al., 1981). As it has been assumed that maturing eels migrate at considerable ocean depths, water pressure has been investigated in the laboratory (Sebert and Barthelemy, 1985; Simon et al., 1988) and under simulated conditions in the field (Dufour and Fontaine, 1985). In laboratory studies using pressure chambers, exposing eels to high hydrostatic pressures of either 2.5 Mpa (Nilsson et al., 1981) or 101 atm (Sebert and Barthelemy, 1985; Simon et al., 1988) had no effect on gonadal maturation, although changes in metabolism were observed. This was even the case following long-term exposure to high pressure for a period of one month (Simon et al., 1988) or 4 months (Nilsson et al., 1981). In contrast, a pronounced stimulation on the HPG-axis was recorded in a field study, where female silver eels were kept in cages at a depth of 450 m in the Mediterranean Sea (Dufour and Fontaine, 1985). In this study, eels kept for 3 months at this depth showed a slight increase in gonad development (GSI=2.3) compared to the controls (GSI=1.6). However, most strikingly, the pituitary GTH content in the eels kept at depth was 27-times higher than in the controls (Dufour and Fontaine, 1985).

Remarkably, exercise has never been investigated as a potential stimulating factor. Major physiological and endocrinological changes are known to occur as a result of prolonged exercise in catadromic and anadromic fish species (Smith, 1985). As European eels are assumed to swim 5000–6000-km over a period of 6 months to their spawning grounds (Tesch, 2003), we hypothesized that the physiological demands of this prolonged migration may influence the mechanisms controlling sexual maturation.

The main aim of the work presented in this manuscript was to study the effect of exercise on maturation of the European eel.

In order to examine the natural trigger for maturation we aimed at comparing the changes in various pituitary hormones under the effect of a swimming challenge. We choose to measure ACTH (as representative of the control of stress and metabolism), and LH as representative of a gonadatropin hormone. Also we measured αMSH in plasma and the pituitary. The rationale behind this is that ACTH and αMSH are the products of one pituitary hormone precursor, proopiomelanocortin (POMC) (Alrubian et al., 2003).

To that end, using experimental swim tunnels, we investigated the effect of prolonged swimming exercise on adult female silver eels. Changes in morphometric and physiological parameters associated with sexual maturation were investigated in both eels allowed to swim for 6 months and in resting eels. In particular, the effects of prolonged swimming on parameters of the hypothalamus–pituitary–gonad (HPG) axis and ACTH-cortisol axis were investigated.

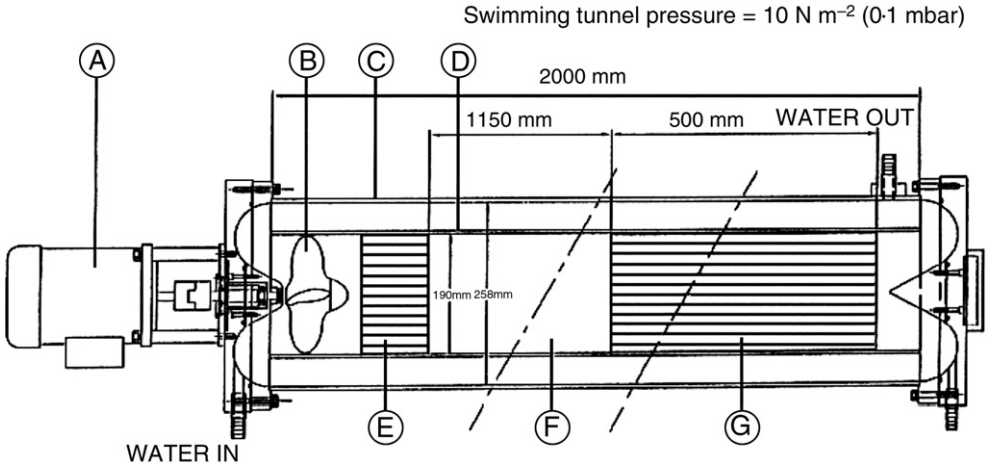

Fig. 1. Schematic drawing of a 2.0-m swim tunnel. The tunnel consists of two concentric Perspex tubes of 2 m and two PVC endcaps. A: electromotor, B: propeller, C: Perspex outer swim tunnel tube, D: Perspex inner swim tunnel tube, E: PVC end-streamer, F: animal compartment, G: PVC front streamer. The propeller pushes water into the outer ring and 'sucks it' out from the inner tube. The cross-section area of the inner tube and of the outer ring have the same surface area. This results in equal flow rates at both sides. The turbulent water is pushed through streamers that have internal diameters of *c.* 10 mm.

2. Materials and methods

2.1. Rationale of the experiment, selection of the animals and experimental conditions

It was initially our intention to simulate the 5500-km migration of European eel (*Anguilla anguilla* L.) to the Sargasso Sea in 15 Blazka swim tunnels (Fig. 1) with approximately 20 years old silver eels caught during their spawning migration at a low temperature of 5–7 °C in sea water mimicking the deep-sea situation. However, for three consecutive years we were unable to perform these experiments because the animals caught in the Grevelingen (The Netherlands) during their seaward migration showed anaemia, blood in the abdominal fluid and hemorrhage all over the body during a simulated migration in sea water in the swim tunnels in the laboratory. Consequently, the animals stopped swimming after 1000–1500 km (van Ginneken et al., 2005a). Finally we detected EVEX (Eel Virus European X) a rhabdovirus in our animals (van Ginneken et al., 2005a). In a survey conducted in eel species from various geographic regions we found that viruses mainly EVEX, HVA (*Herpesvirus anguillae*) and EVE (Eel Virus European) were commonly present among eel populations (van Ginneken et al., 2005b). Therefore we choose to perform our endurance swimming experiments with yellow eels from an eel farm in fresh water at 18 °C. Eel farmers immunize their eels against eel viruses by means of bath exposure at the glass eel (elver) stage to adult, virus positive eels (van Ginneken et al., 2005a). With these animals we were able to make a simulated migration of 5500-km and calculate the energy costs of migration over this distance (van Ginneken et al., 2005c). In addition, we observed among these yellow eels, unexpectedly, significant signs of induced maturation onset which we found interesting and worth to mention in this manuscript.

2.2. Animals and husbandry conditions

Three-year old hatchery-reared eels were used, having a mean length and weight of 71.4±4.2 cm and 792±104 g respectively. These eels were known to be all females as under hatchery conditions and all males of similar age are considerably smaller (Tesch, 2003). They were kept in fresh water for a period of one month before being used in the experiment. The recirculation system and swim tunnels were placed in a climatized room with a constant temperature of 18±0.3 °C. The water temperature was kept at 18±0.1 °C, and the animals were kept under conditions of constant dark conditions. The NH_3 and NO value of the water was measured daily. At values >0.1 ppm NH_3 the water was refreshed from a 3000-l tank elsewhere (van den Thillart et al., 2004).

2.3. Blazka swim tunnels

The Blazka swim tunnels (Fig. 1) were calibrated with a Laser Doppler technique at the Delft Hydraulics Laboratory, Technical University Delft. The dimensions of each swim tunnel was: length of 2.0 m, diameter of the outer and inner swim tunnel tubes were 0.288 and 0.190 m respectively, with a volume of 127.1±0.9 l (n=5). The power of the engine is 400 W while the propeller consists of three 7.5 in. blades with a pitch of 7 in.. A PVC flow conditioner with a length of 0.05 m is located at the top end of the swim tunnel while at the propeller-end a flow conditioner of 0.12 m length is placed. At the bottom-end of the tunnel a screen is placed of plaited silver wire of 1-mm thickness to conduct the electrical current used for the stimulation of the fish to swim. The electrical current was sinusoid, with a peak of 6.5 V, a frequency of 1 s and a duration of c. 20 ms. This was applied *via* a central generator in a resuming way, sequential, to all 22 swim tunnels. The stimulation was only used initially when the eels had to learn to swim in the tunnels; in most cases this was not required. After several days the grids were not used any more (van den Thillart et al., 2004).

2.4. Experimental design

The female eels were randomly divided between three experimental groups: a Swim group (N=9), and an End Control group (N=6), and an Initial Control group (N=11). The Initial Control group was sampled at the start of the experiment to describe the initial maturational status of the animals. The remaining 15 eels were placed in individual 127 l Blazka swim tunnels. For the Swim group, depending on the size of each individual, the swimming speed of the water in the swim tunnels was set at 0.5 body length per second. This water speed equated to the eels swimming ±5500 km over the experimental period of 173 days, closely mimicking the exercise the eel performs during its natural migration (Ellerby et al., 2001). The End Control group experienced the same water quality conditions but without water current. All fish were not fed during the experimental period.

2.5. Sampling procedure

Animals 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 removed from the swim tunnel and blood was collected with a heparinized syringe (flushed with 3000 units heparin per mL blood). The fish were humanly killed by decapitation, following which the alimentary tract, heart, and liver were dissected and weighed. The gonads were then excised, and after weighing them to determine the GSI. A mid-portion of the gonad was fixed in Bouin's fixative. Finally, the pituitary was quickly dissected and directly homogenized in 0.3% NaCl with an ultrasonic thorax and stored at −80 °C pending analysis. In the pituitary LH, ACTH and MSH were determined with RIAs (see further). Blood was directly centrifuged at 10,000 rpm for 5 min, and the plasma divided in Eppendorf tubes (25 µl, 50 µl, 100 µl, 100 µl and 100 µl) for measurement of cortisol, vitellogenin (VTG), 11-ketotestosterone (11-kT), 17-β-estradiol (E_2) and melanophore-stimulating hormone (MSH) respectively, and stored at −80 °C pending analysis.

2.6. Analytical methods

2.6.1. Hormone analysis

Cortisol was measured by radioimmunoassay at Nijmegen University according to the protocol of Balm et al. (1994). VTG

Table 1
Morphometric parameters of female European eel (*Anguilla anguilla* L.) exposed to endurance swimming

PARAMETER	Swim group	End Control	Initial Control	Kruskal–Wallis	*P*-value	*P*-value	*P*-value
	(*N*=9)	(*N*=6)	(*N*=11)		Swim ⇔ End Control	Swim ⇔ Initial Control	End Control ⇔ Initial Control
Length (cm)	74.7±3.4	70.6±3.74	71.2±3.9	$P \leq 0.042$ AN	$P \leq 0.031$*	$P \leq 0.023$*	$P \leq 0.797$
Initial mass (g)	914.7±58.37	778.2±66.08	795.0±71.92	$P \leq 0.001$ AN	$P \leq 0.001$**	$P \leq 0.001$**	$P \leq 0.676$
Condition-factor	0.221±0.03	0.221±0.02	0.214±0.02	$P \leq 0.896$ AN			
End mass (g)	734.33±44.86	698.33±60.39	–	$P \leq 0.115$ AN			
Weight-difference (in %)	−20.58±4.37	−10.26±1.37	–	$P \leq 0.000$ AN	$P \leq 0.000$**		
Eye index	7.43±1.86	7.32±1.44	6.06±1.15	$P \leq 0.102$ AN			
Gonad mass (g)	7.51±1.9	8.52±1.58	7.28±2.60	$P \leq 0.363$ AN			
G.S.I.	1.05±0.23	1.22±0.19	1.06±0.31	$P \leq 0.292$ AN			
Liver mass (g)	5.42±0.88	5.12±1.20	4.91±1.36	$P \leq 0.294$ AN			
H.S.I.	0.72±0.09	0.73±0.12	0.75±0.18	$P \leq 0.722$ AN			
Heart (g)	1.0±0.26	1.13±0.14	1.07±0.19	$P \leq 0.379$ AN			
Heart-somatic-index	0.13±0.04	0.16±0.02	0.17±0.03	$P \leq 0.227$ AN			

Swim group: sampled after swimming during 173 days over 5500-km; Initial Control group sampled at the start of the experiment; End Control group sampled after a resting period of 173 days. Mean±SD is given. AN: ANOVA, MW: Mann–Whitney *U* test with Bonferroni correction. *: denotes significant difference $P \leq 0.05$; **: denotes significant difference $P \leq 0.001$.

was measured by immunoenzymatic assay (ELISA) (Burzawa-Gerard and Dumas-Vidal, 1991) at MHNH in Paris. The LH content in the pituitary homogenate was also determined at MHNH in Paris by radioimmunoassay following the protocol of Dufour et al. (1983). The pituitary MSH and pituitary ACTH content were determined by RIAs as described elsewhere (Balm and Pottinger, 1995).

Plasma levels of 11-kT and E_2 were measured specific by radioimmunoassay at Stockholm University, Sweden. Plasma ACTH and αMSH were analyzed using methodology described in Balm and Pottinger (1995).

2.6.2. Histological analysis

After fixation in Bouin's fixative, gonads were dehydrated in a graded ethanol series and embedded in HistoResin according to standard procedures (Romeis, 1968). They were sectioned at 5 μm and stained with haematoxylin and eosine. Per section the length and width of thirty oocytes were measured and then averaged.

2.7. Statistical analysis

The condition factor (CF) was calculated according to the equation $CF = 100 \times W \times L^{-3}$

For all three groups; Swim, End Control, and Initial Control group, the mean value of all measured parameters was compared pair-wise. A Kruskal–Wallis test was performed on the data to see whether an overall significant difference was present among the three groups. Normality of the data and homogeneity of variances were checked by Kolmogorov–Smirnov and F_{max} tests, respectively. When data were normally distributed, a one-way ANOVA was used with a Bonferroni-correction to specify between which groups the difference existed. Not normally distributed data were tested using a Mann–Whitney *U* test applying a manual Bonferroni-correction. Since a low significant difference ($P \leq 0.042$) existed in length between the experimental groups at the start of the experiment, analysis of covariance (ANCOVA) was applied for found differences with length as cofactor to exclude the potential size-effect. All statistical tests were performed in SPSS 12.0.1 for Windows.

3. Results

The eels swam 5533±354 km over a period of 173 days (for details see van Ginneken et al., 2005c). Mean (±SD) values of all measured morphometric parameters between the three experimental groups are shown in Table 1.

A significant difference was observed between the Swim and End Control groups in final body weight at the end of the period of 6 months. In the Swim group the mean body weight decreased by 20% in the Swim group compared to 10% in the End-Control group at the end of the 6-month swim trial. No significant differences were found in the other measured morphometric parameters between the three groups, including eye-index, gonad-weight, GSI, liver-weight, HSI, heart-weight and heart-somatic-index.

Mean values of the measured maturational parameters for the three experimental groups are shown in Table 2. For most measured parameters, including plasma VTG, 11 kT and ACTH levels, and pituitary levels of MSH and ACTH, no significant differences were recorded between the different groups. Only for plasma cortisol and plasma MSH, significant differences of respectively $P \leq 0.025$ and $P \leq 0.049$ were observed. For the maturation parameters 11 kT, pituitary-LH and 17β-estradiol (E_2) measured values were not significantly different between the Swim group and the End Control group (Fig. 2). The fact that the means are higher can be ascribed to the fact that some animals were 'responding' to the treatment, while others were not. For the different hormones, we found the following values of 'Swim'—animals with an increased level: Cortisol: 5 animals between 86–137 ng/mL, 4 animals between 32–65 ng/mL; Pituitary-LH: one animal 65 ng/pituitary, 4 animals between 7.1–10.3 ng/pituitary; 4 animals between 1.0–5.8 ng/pituitary;

Table 2
Endocrinological and maturation parameters of female European eel (*Anguilla anguilla* L.) exposed to an endurance swimming

Parameter	Swim group ($N=9$)	End Control ($N=6$)	Initial Control ($N=11$)	Kruskal–Wallis	*P*-value Swim ⇔ End Control	*P*-value Swim ⇔ Initial Control	*P*-value End Control ⇔ Initial Control
Vitellogenin (μg/mL)	148.0±56.9	Below detection limit <130.0	184.53±189.08	$P \leq 0.751$ MW			
11-ketotestosterone (ng/mL)	4.89±4.86	2.68±0.46	2.52±0.71	$P \leq 0.131$ AN			
Cortisol (ng/mL)	80.5±38.8	139.2±62.5	63.8±31.7	$P \leq 0.025$* AN	$P \leq 0.035$*	$P \leq 0.280$	$P \leq 0.003$**
Pituitary-ACTH (ng/pituitary)	48.96±22.43	54.3±12.63	48.9±10.06	$P \leq 0.485$ AN			
Plasma -ACTH (pg/mL)	146.8±98.49	130.67±57.28	164.33±96.37	$P \leq 0.841$ AN			
Pituitary-α-MSH (ng/pituitary)	877±833	1072±620	928±671	$P \leq 0.448$ AN			
Plasma-α-MSH (pg/mL)	521.6±239.1	631.8±256.3	359.2±129.3	$P \leq 0.049$* AN	$P \leq 0.399$	$P \leq 0.056$	$P \leq 0.008$**

Swim group: sampled after swimming during 173 days over 5,500-km; Initial Control group sampled at the start of the experiment; End Control-group sampled after a resting period of 173 days. Mean±SD is given AN: ANOVA, MW: Mann–Whitney *U* test with Bonferroni correction. *: denotes significantly difference $P \leq 0.05$; **: denotes significant difference $P \leq 0.001$.

estradiol: one animal 7.1 ng/mL, all other animals between 3.3–5.9 ng/mL; 11-kT: one animal 18.4 ng/mL, all other animals between 2.4–5.8 ng/mL. No correlations were found between the different hormones (elevated levels of certain hormones didn't indicate that all hormones were elevated for the same animal).

The oocyte diameter was significantly higher in the Swim group in comparison to the End Control group (Fig. 2). The oocyte diameter of 4 animals was between 0.17–0.19 mm, of the other 5 animals between 0.14–0.16 mm. It is interesting to notice that the response of the whole swim group in oocyte diameter was rather uniform with enlarged oocytes with increased number of secondary lipid vesicles. This explains why significant differences were recorded in oocyte-diameter between the End Control and Swim group (Fig. 2). The histological appearance of an ovary from a female from both an End Control and Swim group is shown in Fig. 3. The ovary from the Swim group clearly shows a higher proportion of secondary oocytes with numerous secondary lipid vesicles in the cortex.

ANCOVA showed that the differences of pituitary-LH ($P=0.003$), 17β-estradiol (E_2) ($P=0.02$) and oocyte diameter ($P=0.01$) in the Swim group were not related to differences in length between the groups. Both the average values as the amount of individuals scoring the highest values were higher in the Swim group.

4. Discussion

The eye index (eye size relative to length) in our experimental groups was between 6.1–7.43. According to Pankhurst (1982) the cut-off point that separated 'yellow' (non-migratory) with 'silver' (migratory) animals is an eye index of 6.5. So in our experiment the animals achieved just the silvering stage.

In contrast with to salmonids, eels can adapt quickly to saltwater whatever the stage (Rankin et al., 2006). Yellow eels can be found both in fresh and saltwater (Tsukamato et al., 1998), so changes in the osmoregulatory function are less determinant. Nevertheless, yellow eels seem to adapt more slowly to saltwater than migratory eels according to Boucher-Firly and Fontaine

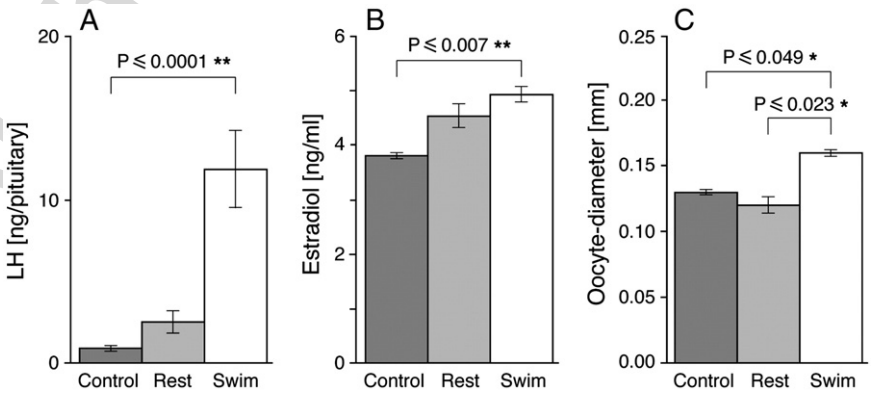

Fig. 2. Effect of a swim-trial of 5500-km on the following maturation parameters: A) pituitary LH, B) plasma E_2 levels and C) oocyte diameter. For the three groups: Initial Control ($n=11$), End-Control ($n=6$) and Swim group ($n=9$) the mean±SD values given. *: denotes significantly difference $P \leq 0.05$; **: denotes significantly difference $P \leq 0.01$; ***: denotes significantly difference $P \leq 0.001$.

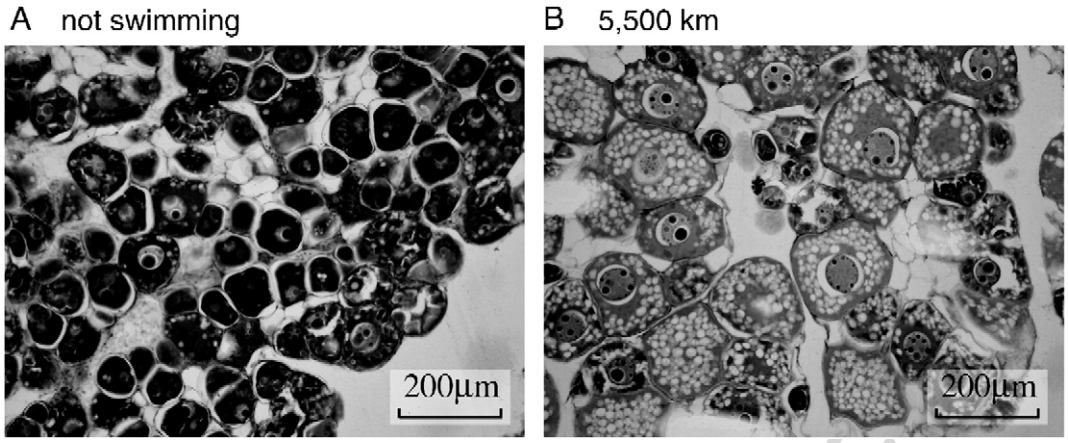

Fig. 3. Transverse section of an ovary from a female eel from the A) End Control and B) Swim group. 5 µm HistoResin sections, stained with haematoxylin and eosine.

(1933). At the silver stage, the eel stops feeding and it therefore displays a salt-deficit. As long as silver eels stay in freshwater, they will undergo a progressive demineralization (Fontaine, 1975; Dutil et al., 1987). According to Fontaine (1985) this state would urge silver eels to migrate and leave freshwater. We are aware that our experimental set up with animals just at the silvering stage in freshwater is an unnatural situation, probably counteracting the maturation process. We explained in Section 2.1 the rationale behind this possible flaw in the experiment. The fact that we observed under these suboptimal conditions (freshwater) an early maturation possibly implicates that under saltwater conditions the maturation effect may be stronger. Therefore in future studies a 5500-km migration experiment in saltwater has to be performed.

During its long 6-month spawning migration, the European eel undergoes pronounced morphological as well as physiological changes. In this study, using experimental swim tunnels, the effect of constant swimming over a 6-month period on both morphometric and physiological parameters was studied in female yellow eels. In particular, the effect of prolonged swimming performance on the hormones of the HPG-axis and the ACTH-cortisol axis was investigated by radioimmunoassay. We choose to use radioimmunoassays in the present pilot study in order to compare various pituitary hormones in the same individual pituitary extracts.

To date, it is still unclear whether sexual maturation is accompanied by increased corticosteroid levels in teleost fishes (reviewed by Idler and Truscott, 1972; Pickering, 1989). Impaired reproductive performance is a common phenomenon in fish (Donaldson, 1990; Barton and Iwama, 1991) and other vertebrates following exposure to stress (Moberg, 1985). On the other hand, a stimulating effect of cortisol on pituitary LH production in the eel was recently demonstrated both *in vitro* using pituitary cell cultures as well as *in vivo* by Huang et al. (1999). Several studies have reported on the negative effect of elevated cortisol levels on the HPG-axis during sexual maturation. For example, in the rainbow trout (*Oncorhynchus mykiss*) increased cortisol levels have been shown to suppress pituitary GTH release and also to inhibit ovarian steroidogenesis both *in vitro* (Carragher and Sumpter, 1990) as *in vivo* (Pankhurst and van der Kraak, 2000). Also, cortisol administration resulted in decreased plasma estradiol-binding capacity in immature female rainbow trout, resulting in the decreased effectiveness of estradiol (Pottinger and Pickering, 1990). Conversely, a few studies have reported a stimulatory effect of cortisol on the HPG-axis. For example, Huang et al. (1999) demonstrated in pituitary cell cultures as well as *in vivo*, a stimulating effect of cortisol on pituitary LH-production in the European eel.

A model for the interaction between ACTH/cortisol and the HPG axis can be *via* the Steroidogenic acute regulatory protein (StAR). This is a key molecule for steroid production by translocating cholesterol from the outer to inner mitochondrial membrane (Li et al., 2003). It is known for mammals that the conversion of cholesterol to pregnenolone, catalyzed by cytochrome P450 side chain cleavage enzyme (P450scc), is the rate-limiting step in steroidogenesis and that StAR is required for this process (Stocco, 2001). In general, StAR is principally expressed in steroidogenic tissues (Baeur et al., 2000; Kusakabe et al., 2002). For Japanese eel it was demonstrated that the distribution of StAR was indeed in steroidogenic tissues like head kidney and gonads, and to minor extent in brain (Li et al., 2003). For teleosts there are two indications that acute stress can have impact on steroid production *via* StAR. For rainbow trout it was demonstrated that acute stress increased StAR transcripts in the head kidney (Kusakabe et al., 2002). Furthermore, for *Anguilla japonica*, it was demonstrated that ACTH injection elevated both plasma cortisol and StAR mRNA levels in the head kidney 1.5 and 4.5 h after injection (Li et al., 2003). So, these literature data demonstrate that an interaction *via* both axis (ACTH/cortisol axis and HPG-axis) is possible *via* StAR. Therefore in this study the effect of prolonged swimming on parameters of the hypothalamus–pituitary–gonad (HPG) axis and ACTH-cortisol axis were investigated.

In the present study, plasma cortisol levels measured in the eels of all three experimental groups were in general agreement to those levels reported by van Ginneken et al. (in press) on the seasonal changes (April–November) in hormone levels in wild eels. In this study, female silver eels had mean plasma cortisol levels of c. 81 ng/mL, while in the present study the Swim and End Control groups had mean levels of 81 and 139 ng/mL respectively. This suggests that the stimulation of maturational

parameters in the swimming eels were not due to elevated cortisol levels, or an activation of the ACTH-cortisol axis *per se*.

The MSH regions in the Pars Intermedia of the pituitary are the fastest growing regions in the stages from elvers to 12–14 cm yellow eels. (Grandi et al., 2003). Therefore it can be concluded that the plasma level of αMSH increases with prolonged aging of the yellow eels in both Swim and End Control group after a period of 173 days as observed in this study.

In our study, 11-kT was slightly, but not significantly, elevated in the Swim group. Compared to the End Control group, the Swim group showed no significant changes in eye index, gonad-weight, G.S.I., liver-weight, H.S.I. heart-weight and heart-somatic index. In our study at least, this suggests that 11-kT is not directly involved in the physiological changes associated with sexual maturation in female eels during their prolonged spawning migration. However, a possible role in the control of maturation in male eels cannot be excluded. Physiologically, 11-kT is the most important androgen in male teleost fishes, and has been shown to be the major androgen involved in spermatogenesis in the Japanese eel, *A. japonica* (Miura et al., 1991a,b, 2003). Despite the fact that 11-kT is generally a male-specific androgen, elevated levels of this androgen has recently been reported in wild New Zealand female eels, *A. dieffenbachia* and *A. australis* (Lokman et al., 1998). In teleost fishes, as in other vertebrates, some androgens can be converted to estrogens by the enzyme complex called aromatase, located mostly in the brain (Timmers et al., 1987). However, 11-kT is a teleost specific non-aromatizable androgen and for this reason was assumed not to be involved in female reproductive development, including oogenesis. While not involved in the control of oogenesis, Lokman et al. (1998) suggested that 11-kT may play a role in preparing maturing animals for their spawning migration. Indeed, in some studies (Rohr et al., 2001; Lokman et al., 2003) it has recently been demonstrated that 11-kT administration induced silvering-related changes in immature *A. australis* eels, including change in head shape and pectoral fin appearance, structural changes of the skin, and an increase in liver weight and eye index, larger ovaries and more advanced oocytes as compared to controls.

For European eel high FSH-β mRNA expression was detected in females at the pre-vitellogenic stage while very low or no LH-β mRNA expression was detected at this stage (Degani et al., 2003). For Japanese eels similar results were found. In young and pre-vitellogenic fish high FSH-β mRNA expression was observed while in late vitellogenic and maturing fish LH-β mRNA was expressed by (Suetake et al., 2002). Also in ovulating animals LH-β mRNA was expressed (Yoshiura et al., 1999). The mRNA expression profiles of females of European eel, where FSH is expressed prior to LH, suggest that FSH regulates gametogenesis and vitellogenesis while LH, on the other hand, is involved in oocyte maturation and ovulation (references: vide Degani et al., 2003). This pattern of expression where FSH is expressed during vitellogenesis and LH during maturation resembles the pattern of salmonids (Gomez et al., 1999; Degani et al., 2003).

The presence of vitellogenin granules marks the beginning of 'exo-vitellogenesis' which is known to be the result of FSH secretion (Degani et al., 2003). However for European eel the respective roles of FSH and LH in the early steps of vitellogenesis in the eel may not be as clearly distinct, especially as silvering is marked by a significant increase in LH (Marchelidon et al., 1999; Sbaihi et al., 2001; Durif et al., 2005). Also, recent work of Schmitz et al. (2005) demonstrated that both LHβ and FSHβ are submitted to an opposite regulation (increase in LH and decrease in FSH) during experimental maturation (gonadotrope treatment) or under steroid treatments. For the Japanese eel, injected with salmon pituitary homogenate, it was observed that LH-β gene expression correlated with serum estradiol-17β and testosterone levels during oocyte maturation (Nagae et al., 1996; Suetake et al., 2002).

The data from the Paris research group show that during experimental maturation, and under the feedback of steroids, there is an opposite regulation of the expression of LH and FSH (increase in LH and decrease in FSH) (Schmitz et al., 2005). So, it is clear that LH will accumulate in the pituitary throughout vitellogenesis and be ready for triggering the final steps (oocyte maturation and ovulation). But it is still not clear whether FSH alone controls the entire vitellogenic process.

It has now been demonstrated that a pre-pubertal blockage occurs in the HPG-axis in silver eels maintained in captivity, resulting in the inhibition of sexual maturation (Dufour, 1994; Dufour et al., 2003). It has been suggested that the unique environmental factors experienced by the eel during its prolonged spawning migration may influence reproductive development. Although not conclusive, evidence suggests that hydrostatic pressure can influence gonadal development, including stimulating GTH release in the eel (Dufour and Fontaine, 1985). In the present study, we investigated whether the physiological demands associated with long-term swimming over a distance of 5500-km can also have an influence on sexual maturation. Our results show that female yellow eels allowed to swim constantly for almost six months did indeed show early signs of sexual maturation. Compared to the End Control group, eels in the Swim group showed (not-significantly), higher levels of pituitary LH and plasma E_2. Further, although there was no difference in GSI between the groups, mean oocyte diameter was significantly higher in eels from the Swim group. Most noticeably, a large proportion of oocytes from eels in the Swim group contained numerous lipid vesicles throughout the cortex. The still low GSI value is a clear indication that exogenous vitellogenesis, the principal event responsible for the enormous growth of the oocytes (Nagahama, 1994), had not started. This is supported by the low plasma VTG levels measured in all eels. In conclusion, the results indicate that prolonged swimming had the effect of stimulating the HPG-axis resulting in the start of oogenesis, although at a very early, pre-vitellogenic stage.

In conclusion, using experimental swim tunnels, we have demonstrated that exposing female yellow eels to endurance swimming over a distance of 5,500 km has a stimulating effect on the HPG-axis resulting in the start of oogenesis. These results suggest that a prolonged period of swimming may be a

necessary physiological cue stimulating the initiation of sexual maturation in the European eel.

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