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Chapter 3

Male European eels are highly efficient long distance swimmers: Effects of endurance swimming on maturation

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Abstract

Eels do not mature naturally in captivity only by using hormonal treatments. Low gamete quality and survival of offspring may reflect the unnatural treatments. The use of natural triggers to induce maturation may improve gamete quality and therefore be of high interest for eel aquaculture. European eels (*A. anguilla*) migrate ~6000 km from the European and North-African coasts towards their spawning area in the Sargasso Sea. Eels are still premature at the onset of this migration. It was hypothesized that simulation of the migration by swimming exercise would be the natural trigger inducing maturation. A previous study showed that maturation in wild male silver eels was stimulated when subjected to swimming exercise for 3 months covering ~900 km. It was therefore hypothesized that male eels will become fully mature when covering a longer distance or swimming for a longer period of time.

In the present study two groups of farmed male silver eels were subjected to either endurance swimming or resting for a maximum of 6 months. It was found that male eels were able to swim continuously for a total distance of 6670 km within 6 months, corresponding to swimming at ca. 1.0 BL s^{-1}. This is the first study that shows that male eels are able to cover the distance to the Sargasso Sea within expected time. In contrast to our expectation, swimming exercise did not induce maturation in farmed male silver eels, suggesting that swimming is not sufficient as a trigger for sexual maturation.

Introduction

Reproduction of freshwater eels (*Anguilla* spp.) intrigued many scientists and is studied for almost 80 years. Over the last decade progress has been made especially for the Japanese eel (*A. japonica*), e.g. by obtaining feeding larvae (Tanaka et al., 2001), glass eels (Tanaka et al., 2003: Kagawa et al., 2005) and recently an F2 generation (Ijiri et al., 2011). However, the success rates are far from suitable creating a sustainable eel aquaculture. Presumably, major problems such as low egg quality and poor fertilization rates, are caused by the unnatural stimulation of maturation by weekly injections of gonadotropins and pituitary extracts (Fontaine, 1936; Fontaine et al., 1964; Ohta et al., 1997), causing e.g. high transient hormonal peak levels (Sato et al., 2000, 2003), and possibly asynchronous oocyte development (Palstra et al., 2005).

Hormonal treatment is necessary as eels do not mature naturally in captivity. At the onset of their reproductive migration they are still in a prepubertal state (Dufour et al., 2003); i.e. oocytes are still in a pre-vitellogenic stage (Versonnen et al., 2004). Maturation in female eels is suppressed by a deficit gonadotropin-releasing hormone (GnRH) secretion and inhibition by dopamine (dopaminergic inhibition). Dopamine inhibits synthesis and secretion of luteinizing hormone (LH) (Dufour et al., 1988; Vidal et al., 2004, Weltzien et al., 2006, 2009). The neurohormone GnRH stimulates the pituitary to release the gonadotropins follicle-stimulating hormone (FSH) and LH. However, injections with GnRH is not effective in silver eels, indicating that blockage of sexual maturation is more complex in eels as compared to other teleosts (Vidal et al., 2004; Dufour et al., 2005). Internal and external stimuli are of importance in triggering the brain to relieve this dopaminergic inhibition. Relieving this inhibition takes place during or after migration to the spawning area, leading to complete maturation.

Major barriers in several phases of the reproductive cycle such as induction of early maturation (e.g. previtellogenic growth, vitellogenesis) and release of the dopaminergic inhibition might be overcome by using natural triggers (e.g. photoperiod, temperature, pressure, swimming exercise. Maturation and reproduction of many fish species can be controlled by manipulation of natural conditions, especially by changing photoperiod and temperature (Taranger et al., 2010; Wang et al., 2010). Natural triggers to induce maturation in eels are still insufficiently studied (e.g. Palstra et al., 2007; Sébert et al., 2007; van Ginneken et

al., 2007, Perez et al., 2011; Sudo et al., 2011). In addition, information from the field is scarce, which is mainly due to the fact that the natural conditions encountered during their oceanic phase are still for the larger part unknown (e.g. Jellyman & Tsukamoto, 2002; Tesch, 2003; Aarestrup et al., 2009, Manabe et al., 2011).

The catadromous eels migrate long distances to their spawning grounds; in the case of the European eel (*A. anguilla*) even over 6000 km, which is assumed to be covered within 6 months. The latter is based on the time difference between the onset of the migration in autumn and the occurrence of larvae in spring (Schmidt, 1923; Tesch, 2003). It was hypothesized that simulation of the migration by swimming exercise would release the dopaminergic inhibition and thereby induce maturation. Several studies showed that swimming exercise induced early maturation in female eels, such as incorporation of fat into the oocytes (van Ginneken et al., 2007; Palstra et al., 2007; reviewed by Palstra & van den Thillart, 2010). However, vitellogenesis and final maturation remained suppressed during longterm swimming exercise (Palstra et al., 2008; Palstra et al., 2010a). Similar effects of swimming exercise on maturation were recently found for rainbow trout (Palstra et al., 2010b).

In contrast to female silver eels, wild male silver eels showed a different response when subjected to long term swimming exercise. Palstra et al. (2008) found a significant increase of the GSI in male silver eels after three months of swimming, covering ca. 900 km (average velocity of 0.12 m s^{-1}). In addition, the expression of luteinizing hormone β subunit (LHβ) in the pituitary was two to three-fold higher as compared to resting males. Spermatogenesis was also stimulated; demonstrated by >80% spermatogonia of late type b, and one male even showing spermatocytes. These results suggest that swimming stimulates maturation in male silver eels, and that full maturation may occur when swimming for a longer period or distance (Palstra et al., 2008). In addition, it was stated that the dopaminergic inhibition as found in female eels, may not be effective in male eels based on the maturation response to injections with GnRH analogue (GnRHa).

It is currently unknown whether male eels are able to swim continuously covering a distance of ~6000 km within 6 months, which corresponds to a mean speed of 0.40 m s^{-1} (ca. 1 BL s^{-1}). Recently, it was found that farmed male eels are

efficient swimmers; i.e. relative low costs of transport and an optimal swimming speed of ca. 0.6 m s^{-1} (ca. 1.5 BL s^{-1}) (Burgerhout et al., unpublished data). Van Ginneken et al. (2007) showed that female eels were able to swim continuously for 6 months covering ca. 5500 km. The optimal swimming speed of males was found to be similar to that of females while the cost of transport was even lower (Tudorache, Burgerhout & van den Thillart, unpublished data). Therefore, it was expected that also male eels would be able to cover ca. 6000 km within 6 months.

During the present study a long term swimming trial was performed with farmed male silver eels for a maximum of 6 months to test two hypotheses: 1) farmed males will be stimulated to full maturation when subjected to continuous long term swimming, and 2) male eels are able to cover 6000km within 6 months. When spermiation is induced by a natural trigger such as swimming it may result in improved sperm quality, and therefore will be of much interest for eel aquaculture.

Methods

Animals and housing

Farmed male silver eels (*Anguilla anguilla*; n = 70; 119.9 ± 2.2 g; 38.2 ± 0.3 cm; average ± SE) were obtained from a commercial eel farm (Nijvis-Holding B.V., Nijmegen, The Netherlands), where they were kept in fresh water at 24°C. Prior to the trial, the eels were acclimated for two weeks and housed in a ca. 2500L recirculation system, supplied with natural seawater (32 ± 1 ppt) at 18 ± 0.5°C. The fish were kept under red light conditions (670nm, bandwidth 20nm), 12:12 L:D. This wavelength is likely invisible for silver eels as during silvering eye pigment changes to a blue spectral band (Pankhurst & Lythgoe, 1983). Light intensity above the experimental set-up was 0.06 lx. As silver eels do not feed, they were not fed. Black conservation sacks (120 x 80cm, Spro Strategy Conservation Sack) were added as shelter. The experiments complied with the Dutch law on animal experiments and were approved by the animal ethical committee of Leiden University (DEC# 09020).

Swimming exercise trial

At the start of the trial, morphometric data - including body length (BL), body weight (BW), eye diameter horizontal and vertical (Edh and Edv, respectively) and the pectoral fin length (PFL) - of all eels were obtained. Data were used to calculate the eye index following Pankhurst (1982) and the silver index following Durif et al. (2005). The males were not PIT-tagged to identify them individually, as the tag might have an influence on the swimming performance due to possible muscle damage.

Eels were randomly divided into seven groups (n=10 per group). One group was sampled (see next section) as an initial control (t=0), the other six groups were each introduced into six 127 L Blazka-type swimming tunnels (described by van den Thillart et al., 2004). The swimming tunnels were covered with plastic sheets which reduced the light intensity to 0.02 lx.

Three groups were subjected to a water velocity of 0.57 m s^{-1} (swim group), which is the optimal swimming speed found for males in a study with individuals as well as with groups (Burgerhout et al., unpublished data). The other three groups were kept resting (rest group) at a water velocity of 0.05 m s^{-1}. The latter was necessary to keep the water within the tunnel well mixed, while low enough for the eels to remain rested.

When individuals within a swim group started to fall back, the velocity was decreased to 0.40 m s^{-1} for the rest of the experiment. This velocity is the estimated minimum speed to cover 6000 km within 6 months. Eels that could not sustain this speed were removed from the tunnel within ca. 15 minutes. After 1.5, 3, and 6 months, eels from the respective swim and rest groups were taken out of the tunnel, anesthetized, dissected and sampled (see next section).

Sampling procedure

The eels were sacrificed using an overdose of clove oil (1:10 dissolved in 96% ethanol, dose 5 mL L^{-1}), followed by decapitation. Blood was obtained from the tail (dorsal aorta or caudal vein), using a heparin (10.000 IU in 0.9% saline) rinsed needle and syringe. Blood was centrifuged for 5 minutes at 13.000 RPM at 4°C) to obtain the blood plasma, which was stored at -80°C until further analysis. A testis sample (left side) for histological analysis was fixed overnight in 4% paraformaldehyde (PFA), and afterwards stored in 70% ethanol at 4°C.

Blood analysis

The levels of the gonadotropins were measured in the blood plasma using the recently developed bioassays based on the eel-specific FSH and LH receptors (Minegishi et al., 2012). Briefly, the bioassays consist of human embryonic kidney cells (HEK293 cells), which stably express the LH receptor of the European eel or the FSH receptor of the Japanese eel and contain a stably integrated luciferase reporter gene driven by a cAMP responsive-element. After incubation at 37°C for five hours for cell stimulation, a luciferase assay was performed using Steadylite plus Reporter Gene Assay System (Perkin Elmer, Waltham, MA, USA). The luminescence signal was measured on a multilabel plate reader (Victor, PerkinElmer). A serial dilution of pure hCG (Sigma-Aldrich) in DPBS (Invitrogen, Paisly, UK) or salmon pituitary extract in eel blood plasma was used as a reference for LH and FSH, respectively.

Blood plasma levels of testosterone were measured using a Testosterone ELISA kit (HUMAN Diagnostics Worldwide GmBH) following manufacturer's instructions.

Histological analysis

The testis samples were first dehydrated in a series of ethanol (70%-80%-90%-100%), followed by incubation in 100% Histo-Clear (National Diagnostics) and a 1:2 mixture of Histo-Clear (National Diagnostics, Biozym TC B.V., The Netherlands) with paraffin (Paraclean, Klinipath B.V., The Netherlands), respectively. Afterwards, the tissue samples were then embedded in paraffin. Sections (7 µm thick) were obtained using a Leica microtome (Leica RM2165) and, after rehydration, nuclei and cytoplasm were stained using Mayer's haematoxylin and eosin (H-E) staining. The stages of the testis were determined following Peñaranda et al. (2010).

Statistics

All data were checked for normal distribution by Kolmogorov-Smirnoff tests. As the data were not normally distributed (Kolmogorov-Smirnoff; $p<0.05$) a Mann-Whitney U non-parametric test was used to analyze the results. Differences in biometry (EI, HSI, GSI, DTWI) and blood plasma levels of FSH, LH, T were tested between and within the groups (swimmers and resters) over the consecutive time

points (0, 1.5, 3 and 6 months). At $p<0.05$ the statistical difference was considered significant. In all cases values are expressed as average ± standard error (SE).

Results

Endurance swimming

During the first 26 days of the trial, three groups of males (n=10 per group) swam against a water flow of 0.57 m s^{-1}. Four swimmers (two of the 1.5 months group, one of the 3 months group and one of the 6 months group) that dropped out after 4 weeks were removed from the tunnels. The water velocity in all tunnels of the swim groups was thereafter decreased to 0.40 m s^{-1} for the rest of the trial. After 1.5 month 8 out of 10 males had swum 1970 km. In the period between 1.5 and 3 months, three swimmers of the 6 months group, and one rester of the 3 months group had dropped out, and were taken out of the tunnel. After 3 months of swimming 9 out of 10 eels had covered a total of 3525 km. Six remaining males of the swim group continued swimming for 6 months, covering 6670 km. Drop-outs in the swim groups were probably occurred due to fatigue. The one resting eel that dropped out showed several wounds along its body probably due to frequent contact with the rear part of the tunnel.

Swimming males tended to aggregate in the front section of the tunnel close to the water inlet. As observed in a previous study, they often swam parallel to each other in a synchronized phase. The eels in the front frequently changed position with the eels in the rear of the tunnel as observed earlier (Burgerhout et al., unpublished data). Resting males showed three different kinds of behaviour: they moved around in the tunnel, kept oriented against the stream, or stayed clumped together at the rear of the tunnel. While resting in a clump at the rear, they still kept moving along each other.

Biometry

The results of the biometry data can be found in Table 1. All males used in this study were assigned silver eels following the silver index of Durif et al. (2005).

At all consecutive sample points, average body weight (BW, Fig. 1) and condition factor (CF) decreased in both swim and rest groups as compared to the pre-measurements. After 3 and 6 months of swimming exercise average BW was

significantly decreased in the swim group as compared to the pre-swim measurements and the initial control ($p<0.05$). After 1.5 and 6 months of resting the average body weight (BW) decreased significantly in the rest group as compared to the pre-rest measurements ($p<0.05$), and at 6 months also to the initial control ($p<0.05$).

In all groups the CF decreased significantly as compared to the pre-measurements and initial control ($p<0.05$). In addition, the average monthly BW decrease was calculated. At 1.5 months the decrease was 10.1 and 7.8 g per month for the swim and rest group, respectively. The rest group showed on average a reduction in monthly BW decrease at 6 months as compared to 1.5 months, while that of the swim group was similar (3.3 g and 9.9 g per month, respectively). At 6 months, swim and rest group showed a monthly BW decrease of 3.7 g and 4.6 g per month, respectively.

An increase of the eye index (EI) is associated with an increased production of sex steroids and gonadal development (Pankhurst, 1982; Sbaihi et al., 2001; Peñaranda et al., 2010), and therefore an external marker for maturation. In both swim and rest groups average EI increased at all consecutive sample points. Although on average the EI increase was higher in the rest group as compared to the swim group, EI was not significantly different between the swim and rest groups ($p>0.05$).The average EI of the swim group was significantly increased at 6 months as compared to the initial measurements ($p<0.05$); the average EI of the rest group was significantly increased as compared to the initial measurements and initial control at 1.5, 3 and 6 months ($p<0.05$).

Maturation in eels is accompanied by increases in liver weight (LW) and gonad weight (GW), and a decrease of the digestive tract weight (DTW) (e.g. Pankhurst & Sorensen, 1984; Rohr et al., 2001, Durif et al., 2005). Between the swim and rest groups no significant differences were found in liver weight (LW), gonad weight (GW) and digestive tract weight (DTW) at all consecutive sample points. The LW of the swim and rest groups was found significantly lower than the initial control at 6 months, and at 3 and 6 months, respectively ($p<0.05$). As compared to the DTW of the initial control, the DTW of the swim and rest groups were found significantly lower at 1.5, 3 and 6 months, and 3 and 6 months, respectively ($p<0.05$). The GW of the swim and the rest groups was significantly lower as compared to the initial control group at 3 and 6 months ($p<0.05$).

	Initial control	Swim 1.5 months		Rest 1.5 months		Swim 3 months		Rest 3 months		Swim 6 months		Rest 6 months	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	n=10	n=10	n=8	n=10	n=10	n=10	n=9	n=10	n=9	n=10	n=6	n=10	n=10
BW	115.4 ± 5.3	115.5 ± 5.7	103.9 ± 6.1	131.5 ± 4.0*	**116.4 ± 5.6**	126.0 ± 4.4*	**96.3 ± 2.8***	109.2 ± 6.4	99.3 ± 6.9	120.0 ± 6.1	**98.1 ± 3.8***	121.4 ± 7.4	**93.8 ± 8.2**^{*b}
BL	37.3 ± 0.6	37.8 ± 0.7	38.2 ± 0.7	39.4 ± 0.4	39.3 ± 0.4	39.1 ± 0.5	38.3 ± 0.5	37.5 ± 0.8	37.6 ± 0.9	38.0 ± 0.8	38.9 ± 0.7	38.2 ± 0.8	37.8 ± 0.9
CF	0.22 ± 0.01	0.21 ± 0.00	**0.18 ± 0.00***	0.22 ± 0.00	**0.19 ± 0.01***	0.21 ± 0.01	**0.17 ± 0.01***	0.21 ± 0.01	**0.19 ± 0.01***	0.22 ± 0.01	**0.17 ± 0.01***	0.22 ± 0.00	**0.17 ± 0.01**^{*b}
EI	9.6 ± 0.5	10.4 ± 0.6	11.1 ± 0.7	9.7 ± 0.2	**11.2 ± 0.3***	9.4 ± 0.4	10.6 ± 0.7	9.7 ± 0.4	**11.9 ± 0.3***	9.1 ± 0.4	**11.1 ± 0.8**	9.3 ± 0.4	**11.6 ± 0.3***
PFLI	4.9 ± 0.1	4.8 ± 0.2	4.9 ± 0.1	4.7 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	4.8 ± 0.2	4.8 ± 0.1	5.1 ± 0.1^{c}
LW	1.29 ± 0.05		1.29 ± 0.12		1.49 ± 0.10		1.37 ± 0.24		0.96 ± 0.04^{*a}		1.02 ± 0.07*		0.90 ± 0.11^{*b}
GW	0.10 ± 0.00		0.09 ± 0.01		0.08 ± 0.02		0.06 ± 0.01^{*a}		0.07 ± 0.01*		0.06 ± 0.01*		0.07 ± 0.01*
DTW	2.21 ± 0.11		1.59 ± 0.15*		1.79 ± 0.17		1.51 ± 0.16*		1.59 ± 0.26*		1.32 ± 0.16*		1.58 ± 0.16*
HSI	1.13 ± 0.04		1.25 ± 0.10		1.33 ± 0.15		1.47 ± 0.32		1.00 ± 0.06		1.05 ± 0.08		0.96 ± 0.07^{*b}
GSI	0.09 ± 0.00		0.09 ± 0.01		0.06 ± 0.01*		0.06 ± 0.01^{*a}		0.06 ± 0.01*		0.06 ± 0.01*		0.07 ± 0.01*
DTSI	1.93 ± 0.08		1.55 ± 0.13*		1.54 ± 0.11*		1.60 ± 0.22		1.57 ± 0.19		1.33 ± 0.12**		1.70 ± 0.10

Table 1 (opposite page): Biometry of males subjected to endurance swimming (Swim) or resting (Rest) for 1.5, 3 and 6 months. Prior the experiment an initial control group was sampled (t=0), and external morphometry data of all eels were obtained. Significant differences ($p<0.05$) are indicated in bold (Pre vs Post conditioning), or by * (swim or rest vs initial control) or by ** (swim vs rest and initial control). Changes in biometric data over time of male eels subjected to endurance swimming (Swim) or resting (Rest) for 1.5, 3 and 6 months. Significant differences ($p<0.05$) are indicated by letters a (1.5 vs 3), b (1.5 vs 6) or c (3 vs 6). Abbreviations: BW: body weight; BL: body length; CF: condition factor; EI: eye index (Pankhurst, 1982); PFLI: pectoral fin index (Durif et al., 2005); LW: liver weight; GW: gonad weight; DTW: digestive tract weight; HSI: hepatosomatic index; GSI: gonadosomatic index; DTSI: digestive tract somatic index.

When body weight was taken into account to calculate the somatic indices of liver (HSI), gonads (GSI) and digestive tract (DTSI), only the DTSI was found significantly lower in the swim group as compared to the rest group at 6 months. No significant differences in HSI and GSI were found between the swim and rest groups at all consecutive sample points. The HSI of the rest group was significantly lower than the initial control at 6 months ($p<0.05$). The DTSI of the swim group was significantly lower than the initial control at 1.5 and 6 months, and significantly lower than the rest group at 6 months ($p>0.05$). The rest group showed as compared to the initial control a significantly lower DTSI at 1.5 months ($p<0.05$).

Plasma hormone levels

Testosterone is an important marker for sexual maturation in fish (Nagahama, 1994) and was measured in the blood plasma using an ELISA. No significant differences were observed between the swim and rest groups at all consecutive sample points ($p>0.05$, Fig. 2a). A significantly lower in T plasma level was observed after 6 months in resting group as compared to the initial control group, and after 1.5 months of swimming and resting ($p<0.05$, Fig 2a).

The processes of spermatogenesis can be induced by human chorionic gonadotropin (hCG, Miura et al., 1991a), a hormone analogue to luteinizing hormone (LH). Stimulation of spermatogenesis is suggested one of the major functions of follicle stimulating hormone (FSH) (reviewed by Planas & Swanson,

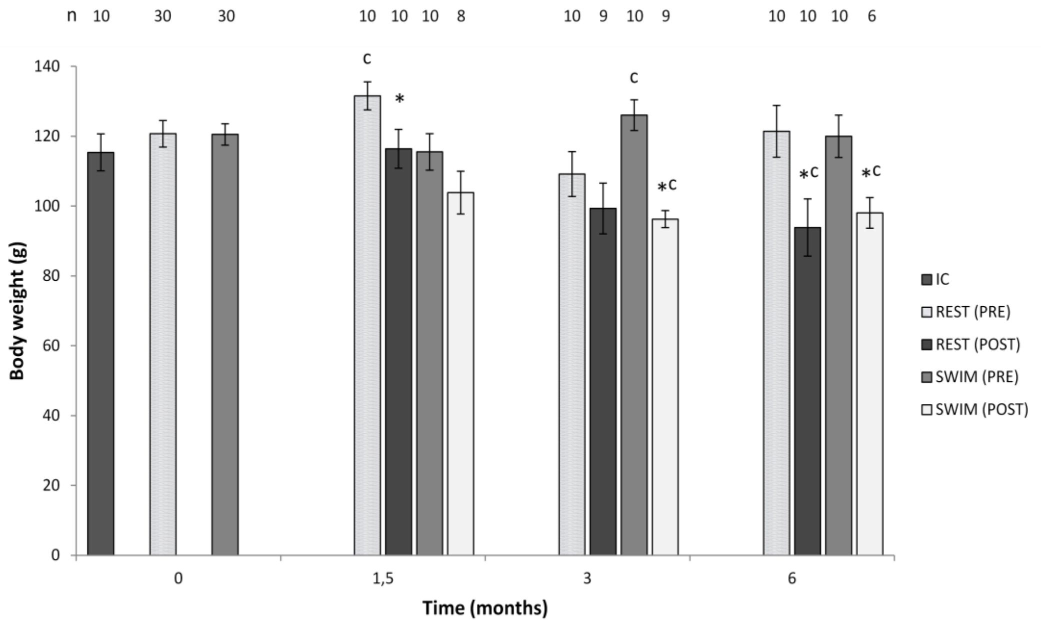

Figure 1. Body weight overview. Initial body weight (g; average ± SE) measurements (t=0 months, PRE) and final measurements (POST) at consecutive sample points (t=1.5, 3 6 months) after swimming and resting treatment (Swim and Rest, respectively). Statistical differences ($p<0.05$) between PRE and POST are indicated asterisk (*). Significant differences ($p<0.05$) as compared to initial control (IC) are indicated by letter c. Number of males measured is indicated by values (n) at the top of the figure above each bar.

2008). Both hormones were therefore measured in blood plasma. No significant differences were found in the blood plasma levels of FSH for all groups at all consecutive sample points based on fluorescence counts per second (CPS) ($p> 0.05$, Fig 2b). After 1.5 months of swimming and resting, the blood plasma levels of LH were significantly higher than the LH levels of the initial control based on CPS ($p<0.05$, Fig. 2c). However, after 3 and 6 months of swimming the LH levels were significantly lower than the values of the swim and rest group at 1.5 months, and significantly lower than the swim group at 3 months, respectively ($p<0.05$). The LH levels of the rest group at 6 months was significantly lower as compared to the levels of the swim and rest group at 1.5 months ($p<0.05$). No significant

differences in LH levels were found between the initial control and the swim and rest group at 3 and 6 months ($p>0.05$).

Histological analysis

Testis showed spermatogonia type a and b as the most advanced developmental stages in all groups, corresponding to testis development stages 1-2 as described by Penaranda et al. (2010). No further development such as formation of spermatocytes was observed. No differences in testis development were observed between swimmers and resters at all consecutive sample points (data not shown).

Discussion

The application of natural triggers, e.g. photothermal stimulation, pressure or exercise, may ultimately lead to breakthroughs in eel reproduction, but they are still hardly studied. Maturation is suppressed due to a deficit release of GnRH and inhibition by dopamine acting on the synthesis and release of LH (Dufour et al., 1988; Vidal et al., 2004; Weltzien et al., 2006, 2009). Simulation of migration by swimming exercise was hypothesized to be a natural trigger by releasing eel from this deficiency and inhibition (van Ginneken et al., 2007; Palstra et al., 2007). Palstra et al. (2008) showed that swimming exercise stimulated maturation in wild male silver eels. Based on the latter study, it was hypothesized that, when wild male eels would be subjected to endurance swimming covering a larger distance or longer time period, full maturation may be expected. In the present study, farmed male silver European eels were subjected to long-term swimming exercise for up to 6 months to test the effects of long-term swimming on maturation.

Endurance swimming and efficiency

It is still uncertain whether eels swim continuously during their ca. 6000 km spawning migration, or whether they use the oceanic currents, as suggested by Fricke & Kaese (1995). Female eels were shown to be able swimming 5500 km in 6 months (van Ginneken et al., 2007). The present study showed for the first time that farmed male silver eels are able to swim continuously for 6 months covering a total distance of 6670 km (corresponding to 0.42 cm s^{-1}, ca. 1.0 BL s^{-1}). Although males were subjected to their optimal swimming speed of 0.57 m s^{-1} (Burgerhout

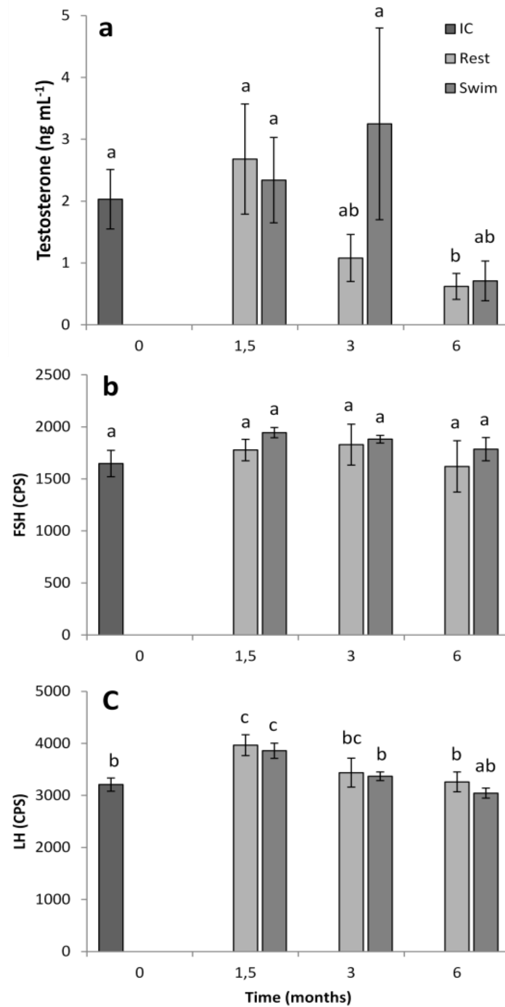

Figure 2. Changes in blood plasma hormone levels. Plasma samples were obtained from initial control (IC) and after swimming or resting (Swim and Rest, respectively). Testosterone (a) was measured by ELISA. FSH (b) and LH (c) were measured using the bioassay as described in Minegishi et al. (2012) and expressed as fluorescence counts per second (CPS). There were no significant differences ($p>0.05$) in plasma hormone levels between the swim and rest groups at all consecutive sample points. Significant differences ($p<0.05$) over time between groups (Swim and Rest) and as compared to the initial control (IC) are indicated by letters.

et al., unpublished data), during the trial several males dropped out possibly due to fatigue. As the applied swimming speed was based on short-term swimming trials (Burgerhout et al., unpublished data), the drop-outs after 4 weeks indicate that the optimal swimming speed (U_{opt}) is probably lower for long-term swimming. However, U_{opt} might be different between individuals, and during their natural spawning migration it may be expected that not all males will survive, due to e.g. insufficient energy stores (Svedäng & Wickström, 1997; Clevestam et al., 2011).

At 1.5 and 6 months there were no significant differences in the average monthly BW decrease between the swim and rest groups, indicating that the swimming costs at the applied speeds tested were close to the routine metabolic rate. The drop-outs in the swim groups were not included in this comparison. It is quite possible that they had a higher metabolic rate, thus using up their energy stores faster than the others. Recently we found that the oxygen consumption per individual of group-wise swimming males at a velocity of 0.40 m s^{-1} was not significantly higher than the standard metabolic rate (Burgerhout et al., unpublished data). The present results indicate that the energy costs during resting (e.g. spontaneous activity, social interaction) are similar as those for swimming at 0.40 m s^{-1}.

The rest group at 1.5 months and the swim group at 3 months had initially a significantly higher BW as compared to the swim and rest group at 1.5 and 3 months, respectively. All other measured parameters did not differ between groups. Interestingly, in both groups the level of BW decrease was relatively higher as compared to the other groups. This phenomenon may be explained by a higher standard metabolic rate (SMR) for males with a relatively higher BW. However, it is currently unknown whether eels with a relative higher BW have a higher SMR. In conclusion, from almost similar costs of swimming and resting it is shown that farmed male silver eels are extremely efficient swimmers.

Induction of maturation

No significant differences in the maturation parameters – EI, GSI, plasma levels of T, FSH and LH – were found between the swimming and resting eels, clearly in contrast to our expectations. The increase in eye index in both swimmers and

resters at all consecutive sample points, suggests a progressed maturation, which might be due to the transition from freshwater to seawater prior to the trial.

The presented results indicate that endurance swimming is not sufficient to induce sexual maturation in farmed male silver eels. However, there were differences between the present study and the study of Palstra et al. (2008), which may explain the differences in final results.

First, the major difference between our study and the study performed by Palstra et al. (2008) is the use of farmed instead of wild eels. Those wild migratory males were obtained from Greece and caught in February (Palstra et al., unpublished data). Possibly, the stimulatory response shown by the wild males to swimming exercise could be due to the fact that maturation had farther progressed, which is reflected by a relatively high GSI found for several males of the initial control (GSI of 0.3; Palstra et al., unpublished data). In addition, after 3 months the resting males in the study of Palstra et al also showed a progression in maturation of the testis, although less apparent than in the swimming males (Palstra et al., 2008). Based on those data we suggest that before the trial those wild males have been stimulated in their maturation by other environmental factors (e.g. changes in photothermal period) which caused this progressed state of maturation. In many fish species, changes in photothermal period affects maturation (see reviews Taranger et al., 2010; Wang et al., 2010). It was also recently found for female eels that temperature decrease induces pre-vitellogenic growth. However, further sexual maturation was not observed indicating that other environmental factors, such as salinity or photoperiod, are involved (Sudo et al., 2011).

Second, the males in the study of Palstra et al. (2008) swam at an average velocity of 0.12 m s^{-1} (i.e. 912 km in 90 days). During the present study males were subjected to a much higher velocity – initially 0.57 m s^{-1} and 0.40 m s^{-1} after 26 days for the rest of the trial. The difference in swimming velocity may have contributed to the contrasting results concerning maturation found in the present study and the study of Palstra et al. (2008). This suggests that swimming at a low velocity (ca. 0.12 m s^{-1}), which was slightly above the resting velocity in our study (0.05 m s^{-1}), appears to stimulate maturation.

In addition, it was found that wild males responded to a GnRH-agonist (GnRHa) with increased expression of LHβ in the pituitary and with an increase of

the GSI (Palstra et al., 2008). Therefore, it was stated that the dopaminergic blockage as described for female eels (Dufour et al., 1988; Vidal et al., 2004; Weltzien et al., 2006, 2009) is not effective in male eels (Palstra et al., 2008). However, GnRHa did not affect the maturation (i.e. increase of GSI) in farmed male Japanese eels (Kagawa et al., 2009), suggesting that in farmed male eels maturation is blocked by dopamine. Therefore it appears that the major difference is the use of wild versus farmed male eels; i.e. the initial maturation status of the wild males used in the study of Palstra et al. (2008) being more advanced and therefore responsive to GnRHa treatments and swimming.

Although the dopaminergic system in male eels is still insufficiently studied, based on our present results, and the results of Palstra et al. (2008) and Kagawa et al. (2009), we hypothesize that inhibition of dopamine may be effective in males and released by environmental triggers than swimming exercise. Future studies should elucidate whether maturation in males is also suppressed by dopamine as found in females.

With the present study we have shown that one of the proposed natural triggers, i.e. swimming exercise, was found not sufficient to induce maturation in farmed male eels. We recommend that future research studies should focus more on other possible triggers (e.g. photoperiod, temperature). It may be expected that, as compared to the current artificial reproduction procedures by hormonal treatments, natural induction of maturation increases gamete quality and efficiency rates, which is a major priority for a sustainable aquaculture.

Conclusions

This is the first study that showed that farmed male silver eels are able to swim continuously for 6 months covering a total distance of 6670 km. The extreme swimming efficiency of male eels is indicated by similar decreases in body weight as found for resting eels. Furthermore our results suggest that swimming exercise does not trigger sexual maturation in farmed male silver eels in contrast to earlier observed effects on wild male silver eels.

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