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Swimming stimulates oocyte development in European eel

Arjan Palstra [a,*], Danilo Curiel [a], Madelon Fekkes [a], Merijn de Bakker [a], Csaba Székely [b], Vincent van Ginneken [a], Guido van den Thillart [a]

[a] *Integrative Zoology, Institute of Biology Leiden, van der Klaauw Laboratories, PO Box 9516, Kaiserstraat 63, 2300 RA Leiden, The Netherlands*
[b] *Veterinary Medical Research Institute, Hungarian Academy of Sciences, 1143, Hungária Krt. 21, Hungary*

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Abstract

In this study, we subjected eels from Lake Balaton (Hungary) to a swimming period of 1 week and 2 or 6 weeks. Most eels were silver and were 13–21 years old. Time dependent changes in morphometrical parameters and developmental characteristics of the oocytes were determined. Already after 1 week of swimming, the gonadal mass increased and oocytes became larger, filled with large numbers of lipid droplets. After 2 and 6 weeks of swimming we found in addition a significant enlargement of the eyes, which is a sign of sexual maturation. In contrast to the resting eels, that had oocytes in the primary growth phase (stage 1–2); the swimming eels had oocytes in stage 3; the cortical alveolus or lipid droplet stage. The results indicate that lipid mobilisation induced by swimming is a requirement for the natural incorporation of lipid droplets in the oocytes, a crucial step in oocyte maturation. As the Balaton eels responded stronger to swimming than young farmed eels, it is suggested that older eels are more sensitive for maturation triggers.

Keywords: *Anguilla anguilla* Linnaeus; Reproductive migration; Maturation; Lipid mobilisation; Silvering; Vitellogenesis

1. Introduction

European eels spend their feeding stage as immature yellow eels in the fresh and brackish European waters. It appears that at the end of each growth season many eels cease feeding and metamorphose (silvering) to prepare for oceanic migration. It is suggested that fat content is a key factor in the onset of migration (Larsson et al., 1990; Svedäng and Wickström, 1997) since reserves have to suffice to fuel migration (van Ginneken and van den Thillart, 2000; van den Thillart et al., 2004; van Ginneken et al., 2005) and are required for successful development of the oocytes. Drastic changes occur during silvering, most apparent though is the enlargement of the eyes discriminating the yellow and silver phase (Pankhurst, 1982). Durif et al. (2005) demonstrated recently that silvering and migration are closely related processes. As also the pectoral fins become longer (Durif et al., 2005) and shape changes (Tesch, 2003), Durif et al. (2005) proposed an index on basis of length, weight, eye diameter and pectoral fin length, which provides an estimate of the proportion of silver eels that are true migrants. This was needed since their abundance was overestimated as demonstrated by Svedäng and Wickström (1997) and Feunteun et al. (2000).

The ovaries of silver eels contain oocytes in the first developmental stages (Adachi et al., 2003) after

* Corresponding author. Tel.: +31 71 5274955; fax: +31 71 5274900.
E-mail address: A.P.Palstra@biology.leidenuniv.nl (A. Palstra).

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transformation of the oogonia. Further progression requires incorporation of lipids droplets (stage 3: cortical alveolus stage or lipid droplet stage) and vitellogenin. Although separated in other fish species, these two processes occur simultaneously in artificially matured Japanese eel (Adachi et al., 2003) and European eel (Palstra et al., 2005), which suggests an unnatural situation. Untreated silver eels are in a prepubertal stage, still far from sexual maturity (Larsen and Dufour, 1993; Dufour, 1994; Dufour et al., 2003) and remain as such when kept resting in aquaria. Further sexual development of silver eel appears to be blocked by dopaminergic inhibition of hypothalamus and pituitary resulting in insufficient FSH and LH levels (Dufour et al., 2003). This blockage is likely required in order to allow the long spawning migration. Obviously there must be natural conditions that lead to release of this blockage. As European eels have to swim about 5500 km to reach their spawning site, we hypothesize that swimming is the crucial trigger for releasing the dopaminergic inhibition.

Exercise has never been thoroughly investigated as a stimulating factor for maturation in fish. Eels have to cover an enormous distance to their spawning grounds, it is therefore likely that swimming is at least one of the factors that triggers natural maturation. Recently, van Ginneken et al. (2007) observed increased oocyte diameters in 3 year old hatchery eels after swimming 5500 km. Significantly higher levels of pituitary LH were found in the exercised group as compared to the controls. Those results indicate that long term swimming indeed has a stimulatory effect on maturation. Also in a recent study on swim performance based on short swim trials, we found indications that swimming triggered sexual maturation in Lake Balaton silver eels (Palstra et al., in press); the GSI in the swim group was higher than in rest group. Apparently Balaton eels are more sensitive to swimming than the farmed eels used by van Ginneken et al. (2007), as they responded already within a week. Therefore we used Lake Balaton silver eels in this study to investigate the effect of short swim trials (1–6 weeks) on oocyte development.

2. Materials and methods

2.1. Experimental animals

At the end of August 2003 and end of September 2004, a total of 120 eels were caught by electrofishing in Lake Balaton, Hungary, in the region of Keszthely and Tihany. They were transported to the laboratory in oxygen-filled plastic bags and marked individually by subcutaneous injection of PIT-TAGS (TROVAN, Aalten, The Netherlands) just behind the head. Eels were then packed into large oxygen-inflated nylon bags in Perspex and cardboard boxes after which they were sent to Leiden (The Netherlands) in early September 2003 and early October 2004 respectively.

2.2. Swim-flumes and conditions

Swim experiments were performed in 22 Blazka-type calibrated swim-flumes described in detail by van den Thillart et al. (2004). Swim-flumes were oriented towards the Sargasso Sea (WNW) in a climatized room of about 100 m^2. The total water content of about 7000 l was recirculated continuously over a bio-filter. The illumination in the climatized room was switched to 670 nm light (bandwidth 20 nm). Based on eye pigment changes during silvering, it can be assumed that this far-red light is invisible for eels (Pankhurst and Lythgoe, 1983). Indeed, the animals did not respond to movements of the experimenter during red light illumination. Experiments were performed in air-saturated (>75%) fresh water at 18±0.5 °C. The swim-flumes were 2 m long with a volume of 127 l, with an animal compartment of 110 cm suited for 1 individual eel.

2.3. Protocol experiment 1

Protocol 1 was performed in 2003 as part of a study on the influence of the swim-bladder parasite *Anguillicola crassus* on the swim performance (Palstra et al., in press). As part of the swim performance protocol, fish were exposed to up to 12 h swimming per day over a 7 day period that cumulatively examined the effect of a week of heightened and sustained but non-continuous swimming activity. The results are included here as they provided the basis for longer term experiments described in the following protocol.

For the control group 10 eels were upon arrival randomly chosen, then anaesthetized, measured, sacrificed and sampled. Before handling eels were anaesthetized with 1–1.5 ml diluted clove oil per l water (oil of cloves:ethanol=1:10). Forty eels in two groups of 20 were subjected to a swim fitness test as described by Palstra et al. (2006a). In short, eels were anaesthetized, measured and introduced into swim-flumes two days before the experiment started.

The swim fitness protocol consisted of 7 daily experimental trials: 2 speed tests and 5 endurance tests. On day 1, eels were subjected to a first speed test. Eels started to swim at a speed (U) of 0.5 m s^{-1} for 2 h. After these 2 h at 0.5 m s^{-1}, U was raised with 0.1 m s^{-1} to 0.6 m s^{-1} for 2 h. Subsequently, this was repeated with steps of 0.1 m s^{-1} for a U up to 1.0 m s^{-1}. On day 2

the eels were subjected to endurance tests starting at 0.5 m s^{-1} for 12 h. On the following days the eels were swum at respectively 0.6, 0.7, 0.8, and 0.9 m s^{-1}. On day 7, the protocol was finished with a second speed test. When fish fatigued during trials, the flow was lowered to 0.1 m s^{-1}.

A group of 10 eels were kept resting during the same period in a 1500 l tank connected to a 2400 l recirculation system under dark conditions. PVC pipes were added as shelter. At the end of the swim test both swimming and resting eels were anaesthetized, measured, sacrificed and sampled.

2.4. Protocol experiment 2

After arrival, 10 randomly chosen eels were anaesthetized, measured, sacrificed and sampled as control group. Twenty-one randomly chosen eels were measured and introduced in the swim-flumes. They were allowed to swim continuously at a speed of 0.5 BL s^{-1}. Six eels showed problems with swimming during the experiment and were stopped. We investigated the swim-bladder of these eels and found that three out of six eels were infected, and that five out of six eels had heavily damaged swim-bladders confirming the negative interference of the parasite with swimming (Palstra et al., in press). Only one out of twenty-one eels showed problems with swimming that could not be connected with swim bladder dysfunction. Data of these eels were not included for analysis. Resting eels were kept as described above. After 2 weeks, 6 randomly chosen swimming eels were stopped, anaesthetized, measured, sacrificed and sampled as well as a group of 10 resting eels. After 6 weeks, the remaining 9 eels were stopped, anaesthetized, measured, sacrificed and sampled as well as a group of 6 remaining resting eels.

2.5. Measurements and sampling

Morphometric parameters included body-length (BL), body-weight (BW), eye diameter horizontal (EDh), eye diameter vertical (EDv), pectoral fin length (PFL) and pectoral fin width (PFW; see for abbreviations also Glossary). The following indices were calculated according to the formulae below: condition factor (K), eye index (EI), pectoral fin length index (PFLI) and pectoral fin width index (PFWI).

1. Condition factor (K) = $100 * BW\ BL^{-3}$
   BW: body weight (g), BL: body length (cm)
2. Eye index (EI) = $100 * (((EDh + EDv) * 0.25)^2 \pi * (10 * BL)^{-1})$
   EDh: eye diameter horizontal (mm), EDv: eye diameter vertical (mm)
3. Pectoral fin length index (PFLI) = $100 * PFL\ BL^{-1}$
   PFL: pectoral fin length (cm)
4. Pectoral fin width index (PFWI) = $100 * PFW\ BL^{-1}$
   PFW: pectoral fin width (cm)

Table 1
Effect of swimming (protocol 1) on morphometric and gonad parameters (mean ± SD)

Parameters	Control	1 week		
		Pre-swim	Post-swim	Rest
n (eels)	10	40	40	9
External				
BL (cm)	67±4	69±6	69±6	66±5
BW (g)	475±77	525±142	**510±142**$^{\&}$	437±123
K	0.16±0.01	0.16±0.02	**0.15±0.02**$^{\&}$	0.15±0.02
PFLI	4.68±0.39	4.96±0.43	4.88±0.42	4.68±0.49
EI	7.38±3.30	9.14±2.99	8.64±3.12	7.31±2.49
SI	3±1	4±1	4±1	3±1
Internal				
GSI	0.59±0.34		0.82±0.43	0.59±0.32
DTSI	2.42±0.65		**1.66±0.67***	2.01±0.52
HSI	0.92±0.09		0.84±0.13	0.86±0.19
Oocytes				
OS	2.7±0.3		**2.8±0.4***	**2.9±0.2***
OD (μm)	103±36		**155±47***	128±48

Control eels ($n = 10$) were sampled at the start. Swimming eels ($n = 40$) were subjected to a swim fitness test (*see text for explanation*), corresponding to about a week of swimming, or kept resting ($n = 9$). Swimming eels were measured before (pre-swim) and after swimming (post-swim). Significant differences ($P < 0.05$) are indicated in bold. Symbols mark differences vs. the controls (*) or pre-swim values ($^{\&}$) (for abbreviations see Glossary).

The silver index (SI) was calculated according to the procedure described by Durif et al. (2005).

Blood samples were taken from the caudal vein with heparin flushed (10.000 IU ml^{-1}) 1 ml syringes, which were immediately placed on ice. The blood was centrifuged for 5 min at 14,000 rpm and bloodplasm was stored at −80 °C.

Liver, digestive tract and gonads were dissected and weighed. The following indices were calculated according to the formulae below: the gonadosomatic index (GSI), the digestive tract somatic index (DTSI) and the hepatosomatic index (HSI; see for abbreviations also Glossary).

5. Gonadosomatic index (GSI) = $(GW\ BW^{-1}) * 100\%$
   GW: gonad weight (g), BW: body weight (g)
6. Digestive tract somatic index (DTSI) = $(DTW\ BW^{-1}) * 100\%$
   DTW: weight digestive tract (g)

7. Hepatosomatic index (HSI) = (LW BW^{-1}) * 100%
   LW: liver weight (g)

Gonads of all eels solely contained oocytes so all eels were females. From the gonad at a standardised posterior location a piece of about 1 g was placed in Bouin solution at room temperature. From all swimming eels, the otoliths (sagitta) were dissected for age determination.

2.6. Otolithometry

Age estimation was carried out in the laboratory of Cemagref, Bordeaux, France by otolithometry according to the method described by Daverat et al. (2005). After extraction, otoliths were cleaned of all organic matter in distilled water, dried with ethanol, and then stored in eppendorf tubes. The otoliths were later embedded in synthetic resin (Synolith, Euroresins Benelux BV, The Netherlands), then polished to the nucleus with a polishing wheel (Streuers Rotopol-35, West Lake, Ohio, USA) using 2 different grits of sandpaper (1200 and 2400). Fine polishing was done by hand with Al_2O_3 (1 μm grain) on a polishing cloth. Etching was done using 5% EDTA. A drop of this solution was applied on the mold for 3 min. The otoliths were then rinsed with distilled water and stored in dry conditions. Year rings were visualised by staining with a drop of 5% Toluidine blue and counted under a microscope. The age of each eel was determined by the number of increments starting from the nucleus which was considered as year one of the eel's life.

2.7. Histology

To remove the Bouin fixative, the gonads were washed in 0.1 M phosphate buffer and 70% ethanol until the solution became transparent. After dehydrating through an accumulating alcohol series the samples were embedded in air-free Technovit 7100 (Kulzer Histo-Technik) in Peel-A-way molds (Polysciences Inc.) covered with a layer of air-free paraffin oil during polymerization to exclude oxygen (de Jonge et al., 2005). Sections of 10 μm thick were cut using a sledge microtome (Jung Polycut E). Three sections were put on a slide and five slides per sample were made and stained with Mayers Haematoxylin–Eosin. The oocytes were studied visually under the microscope (Nikon Eclipse E400) and overview pictures were taken (Nikon Coolpix 4500). Per section, ten (protocol 1) or twenty (protocol 2) oocytes were randomly selected that were cut through the nucleus. For each oocyte the developmental stage (OS; see for abbreviations also Table 1) was determined. The diameter of the oocytes (OD) was determined using UTHSCSA Image Tool 2.0. The number and diameter of lipid droplets occurring in stage 3 oocytes were measured with the same method. In fact, what we call lipid droplets are the empty lipid vesicles from which the lipids have been extracted by washing in ethanol.

2.7.1. Statistics

Normality of the data and homogeneity of variances were checked by Kolmogorov–Smirnov tests. Paired observations before and after swimming of morphometric parameters were tested with student *t*-tests with one-tailed probabilities. For SI, a Wilcoxon test with one-tailed probabilities was used.

With an univariate general linear model (GLM), analysis of covariance (ANCOVA) with one-tailed probabilities was performed on log transformed unpaired observations in search for group effects in swim parameters with either BL (for PFL, PFW, ED) or BW (for GW, DTW, LW, OD, number lipid droplets, diameter lipid droplets; for abbreviations see Glossary) as cofactors. ANCOVA was similarly performed for comparison between the 2 weeks and the 6 weeks swim groups. In case of occurrence of significant group effects, ANOVA with a post-hoc Bonferroni test was performed to specify the effects between particular groups. ANCOVA was especially required for the scale difference between swim and rest groups in experiment 2 (Quinn and Keough, 2002).

Kruskal–Wallis tests with one-tailed probabilities were performed for comparison of SI and oocyte stage. Spearman correlation tests with one-tailed probabilities were performed between start parameters (BL*, BW*) vs. silvering parameters (EI*, SI*, HSI, DTSI) vs. maturation parameters (GSI, OS, OD) for control groups, but also for pre-swim groups for parameters marked with asterisks.

Pearson correlation tests with one-tailed probabilities were performed for comparing between OD and lipid droplet number and size. OD between stages was tested with student *t*-tests with one-tailed probabilities. All statistical tests were performed in SPSS 10.0 for Windows. P-values < 0.05 were considered to indicate statistically significant differences. Results were calculated and plotted as mean ± standard deviation.

3. Results

3.1. Protocol 1

In Table 1 the external measurements are given of the control and swim group. The pre-swim and post-swim

data are from the same eels. Paired observations showed that after 1 week of swimming, experimental eels showed a significant weight loss of 15 g (Table 1). Also the K was significantly lower. There was a significant decline of the DTSI (compared to the controls). No increase was observed of silvering indicators.

Based on an eye index threshold of 6.5 (Pankhurst, 1982), 70% of all the eels was scored as silver eels. No change in EI and silver index occurred as a result of 1 week of swimming. Still, histology data revealed significant increases of oocyte stage and oocyte diameter. The GSI of eels that had swum was on average 39% higher than control and rest groups but individual variation remained high and this difference was not significant (Table 1). Percentages of eels with GSIs > 1 were 11% in the rest group and 33% in the swim group.

Eels of the control group in protocol 1 showed oocytes in stage 1, 2 and 3 with the latter oocytes just having entered this stage containing only a few lipid droplets. The average diameter of these oocytes was 103 ± 36 μm. After 1 week, eels from the swim group showed a significant change in the average oocyte stage (Table 1). 85% of the eels in the swim group had ovaries with exclusively stage 3 oocytes with a larger diameter of 155 ± 47 μm and containing much more lipid droplets (20–100).

3.2. Protocol 2

At the start of the 2 week protocol the eels were 62 ± 4 cm, 347 ± 76 g (Table 2) and 83% of them were silver (EI > 6.5). At the start of the 6 week protocol the eels were 63 ± 5 cm long, weighted 429 ± 137 g (Table 2) and 33% was silver. This difference occurred despite the random procedure for assigning eels to the different trials. Experimental eels were assigned to SI stages 2, 3 and 5, while not any fish was assigned to stage 4. The experimental eels that had swum aged between 13 and 21 years (mean 16 years; Table 2). Paired observations showed that after 2 weeks of swimming, experimental eels showed a significant weight loss of 14 g (Table 2). Also the K was significantly lower. PFLI and PFWI did not show changes. Paired observations showed that after 6 weeks of swimming, experimental eels showed a significant weight loss of 63 g (Table 2). Again the K was significantly lower. PFLI and PFWI did not show changes in the swim group. The PFWI was however smaller in the rest group.

The EI increased significantly after 2 weeks of swimming from 8.32 ± 2.20 to 10.31 ± 2.84, also a significant increase in comparison with the rest group (Table 2). After 2 weeks, all eels showed increases of the

Table 2
Effect of swimming (protocol 2) for 2 weeks and 6 weeks on morphometric and gonad parameters (mean ± SD)

Parameters	Control	2 weeks			6 weeks		
		Pre-swim	Post-swim	Rest	Pre-swim	Post-swim	Rest
n (eels)	10	6	6	10	9	9	6
Age (years)			16 ± 1			16 ± 3	
External							
BL (cm)	59 ± 5	62 ± 4	62 ± 4	55 ± 2	63 ± 5	63 ± 5	53 ± 2
BW (g)	267 ± 68	347 ± 76	**333 ± 63**$^{\&}$	212 ± 26	429 ± 137	**366 ± 114**$^{\&}$	174 ± 23
K	0.13 ± 0.01	0.14 ± 0.02	**0.14 ± 0.01**$^{\&}$	0.13 ± 0.02	0.16 ± 0.03	**0.14 ± 0.02**$^{\&}$	0.12 ± 0.02
PFLI	4.55 ± 0.19	4.89 ± 0.25	4.89 ± 0.21	4.39 ± 0.25	4.78 ± 0.55	4.78 ± 0.57	4.33 ± 0.25
PFWI	2.32 ± 0.26	2.64 ± 0.43	2.45 ± 0.46	2.30 ± 0.17	2.52 ± 0.18	2.52 ± 0.16	**2.03 ± 0.21***
EI	6.20 ± 1.81	8.32 ± 2.20	**10.31 ± 2.84**$^{\&\#}$	5.55 ± 0.43	6.89 ± 3.16	**9.09 ± 2.91**$^{\&\#}$	5.53 ± 0.60
SI	3 ± 1	3 ± 2	4 ± 1	2 ± 0	2 ± 2	**3 ± 1**$^{\&}$	2 ± 0
Internal							
GSI	0.26 ± 0.31		0.74 ± 0.48	0.13 ± 0.12		**0.80 ± 0.35***$^{\#}$	0.38 ± 0.18
DTSI	2.58 ± 0.81		1.95 ± 0.77	2.40 ± 0.52		2.14 ± 0.54	**2.18 ± 0.20***
HSI	1.13 ± 0.20		1.02 ± 0.15	**0.87 ± 0.09***		0.77 ± 0.13	**0.72 ± 0.12***
Oocytes							
OS	1.7 ± 0.5		2.4 ± 0.7	1.5 ± 0.2		**2.4 ± 0.6***$^{\#}$	**1.7 ± 0.1***
OD (μm)	83 ± 39		**136 ± 46**$^{\#}$	61 ± 14		109 ± 41	94 ± 17

Control eels ($n = 10$) were sampled at the start. Eels swam two ($n = 6$) or 6 weeks ($n = 9$) at 0.5 BL s^{-1} or were kept resting for 2 ($n = 10$) or 6 weeks ($n = 6$). Swimming eels were measured before (pre-swim) and after swimming (post-swim). Significant differences ($P < 0.05$) are indicated in bold. Symbols mark differences vs. the controls (*), pre-swim values ($^{\&}$) or the rest group ($^{\#}$). The 6 weeks rest group had an average OS value of 1.73 and was found significantly different by a Kruskal–Wallis test from the control group. The control group consisted of 9 fish that together had an average value of 1.58 with one fish that solely had stage 3 oocytes. (for abbreviations see Glossary).

EI between 11 to 41% (Fig. 1), corresponding to a rise in SI. The EI increased further after 6 weeks of swimming between 10 and 66% (Fig. 1) resulting in 100% silver eels. This change in eye diameter caused a significant change in the SI.

The GSI was found on average 3–5 times higher in the 2 week swim group than in the controls though not significantly (Table 2). The number of eels with a GSI>1 was 50% in the swim group, which was significantly different from the control and rest group. There were no eels at all in the control and rest group with GSI>1. The GSI in the 6 week swim group was significantly higher than in the control and rest group. Also in comparison with 2 weeks of swimming, the GSI was significantly higher both in the swim group and rest group.

DTSI and HSI values of the 2 and 6 week swim groups were lower than the controls but not significantly. HSI was found significantly lower in the rest group both vs. control and 2 week swim group. DTSI and HSI were lower in the 6 week swim group but only significantly in the rest group. Also in comparison with 2 weeks of swimming, the HSI was significantly lower (Table 2).

3.3. Oocyte developmental status

Eels of the control group contained oocytes representing stages 1, 2 and 3 according to Wallace and Selman (1981), Tyler and Sumpter (1996) and Adachi et al. (2003).

Stage 1 oocytes represented small oocytes (30–90 μm), still in nest structure or individually organised. An acellular zona radiata (zona pellucida, chorion vitelline envelop) could not be identified yet. Oocytes were oval shaped and the cytoplasm was darkly coloured (Fig. 2A). The nucleus was centred and round, contained 1 to 3 large nucleoli and about 20 smaller ones dispersed throughout the nucleus. The stage 1 oocytes that were found should be considered late stage 1 oocytes since size was already considerable in comparison with Tyler and Sumpter (1996) stating that oogonia in teleosts generally measure less than 10 μm in diameter.

Stage 2 oocytes represented larger oocytes (40–150 μm) with less darkly coloured cytoplasm. In this phase a zona radiata could be identified. The number of nucleoli was higher. Islands of lightly stained cytoplasm could be distinguished in the oocyte. Lipid and connective tissue were surrounding the oocytes (Fig. 2A).

Stage 3 oocytes were characterised by the presence of lipid droplets. In eels from control groups, stage 3 oocytes contained very few lipid droplets, generally dispersed in the periphery of the ooplasm near the zona radiata (Fig. 2B). Mostly the nucleus was roundly shaped and nucleoli were found in the periphery of the nucleus. Eels from swim groups contained stage 3 oocytes with a large number of lipid droplets (Fig. 2C).

Yolk globuli, such as is characteristic for vitellogenesis, were not found. For comparison, Fig. 2D shows an oocyte during final maturation in an artificially matured silver eel (Palstra et al., 2005). Numerous yolk globuli could be observed. The number of lipid droplets is decreased and the size increased indicating extensive fusion of the lipid droplets. The oocyte showed considerable increase in size indicating a hydration response.

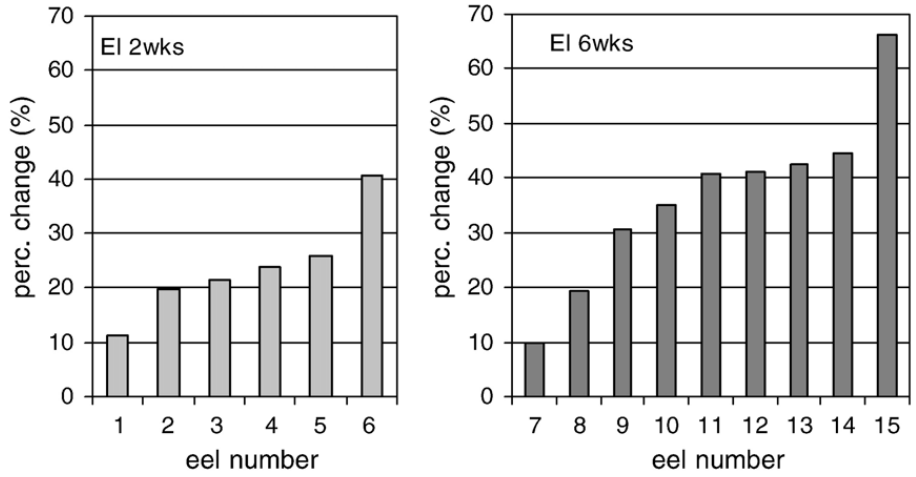

Fig. 1. Swimming induced changes of eye diameter. Eels were sampled after swimming continuously at 0.5 BL s^{-1} for 2 and 6 weeks (also Table 2). The EI had increased significantly after 2 and 6 weeks of swimming. This bar graph shows that the EI of each individual had increased. After 2 weeks of swimming, the EI had increased by 23.9±9.6% (range 11–41%). After six swimming, the EI had increased by 36.6±16.0% (range 10–66%). Individual increase was more pronounced after 6 weeks of swimming.

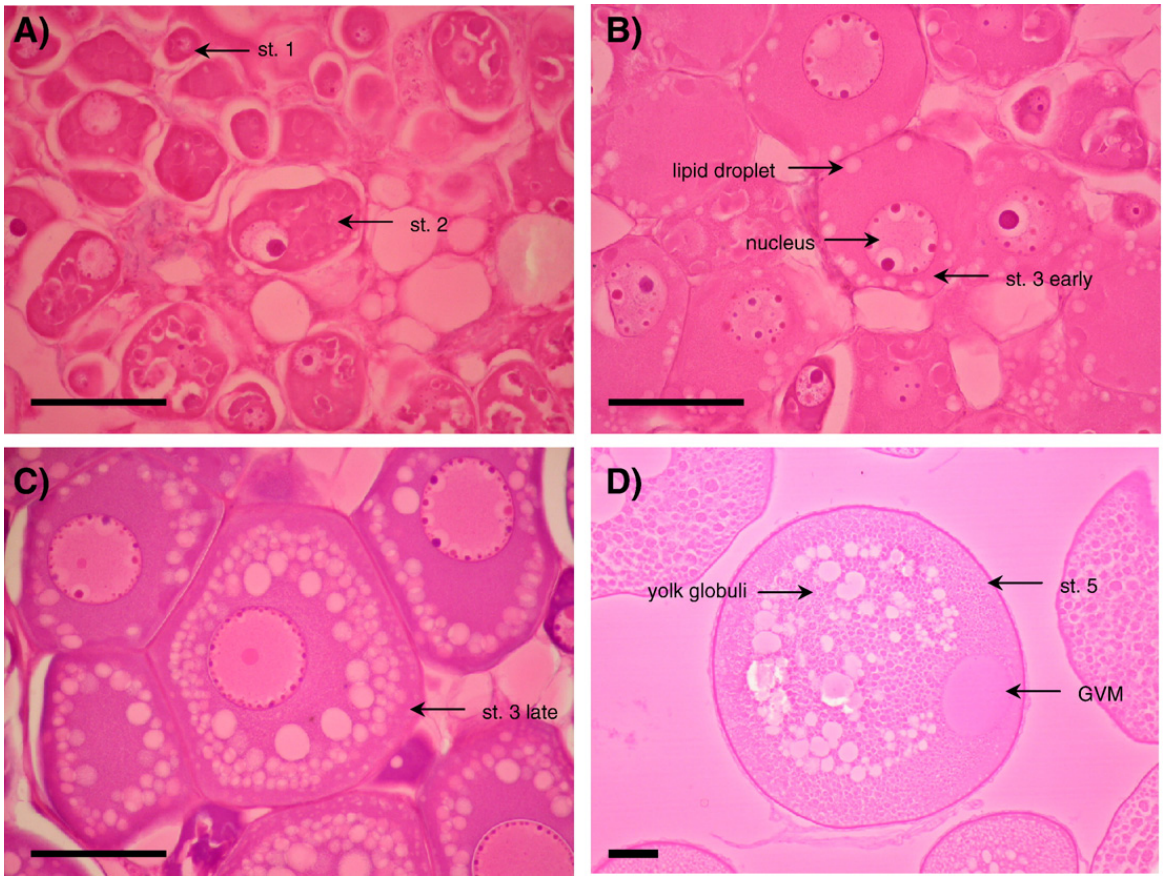

Fig. 2. Gonad histology. Technovit 7100 sections of 10 μm after staining with Mayers Haematoxilin–Eosin. A) Oocytes in stage 1 and 2 (small cells with dense cytoplasm and nucleus). B) Oocytes early stage 3 (larger cells with light cytoplasm, few lipid droplets swollen nucleus). C) Oocytes late stage 3 (larger cells with a large number of lipid droplets, swollen nucleus with many nucleoli). D) Oocytes in late stage 5 displaying: large cells with packed lipid droplets and yolk globuli, and very transparent nucleus in stage of Germinal Vesicle Migration (GVM). This picture is from an artificially matured eel, and included for comparison (Palstra et al., 2005). Scale bars are 100 μm. Note that the mature stage 5 oocyte (D) is much larger than the oocytes in stage 1, 2 and 3 (A, B, C). Stages were assigned according to Wallace and Selman (1981), Tyler and Sumpter (1996) and Adachi et al. (2003).

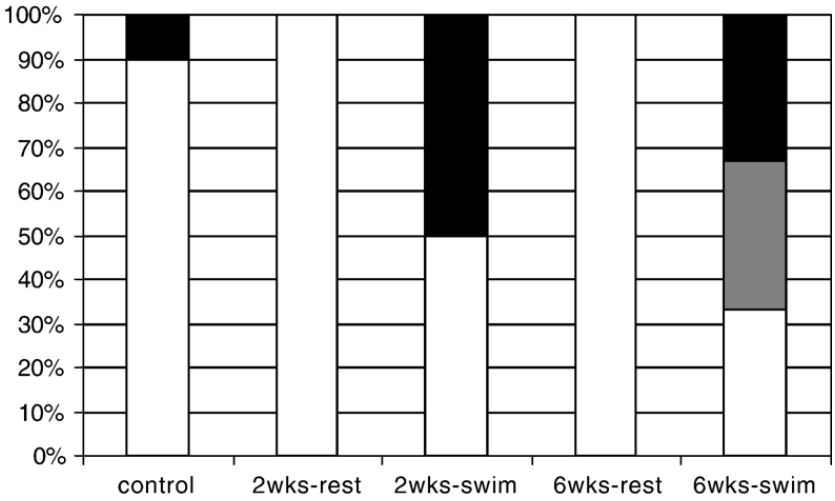

Fig. 3. Gonad developmental in relation to swimming. The % of silver eels with oocytes in stage 1+2 (white bars), stage 2+3 (grey bars), and stage 3 (black bars) is presented in control eels (initial situation), resting eels, and in eels after continuous swimming at 0.5 BL s^{-1} for 2 and 6 weeks. In each eel the developmental stage of the oocytes was found rather homogeneous. The difference in stage for 2 weeks of swimming was just not significant vs. the controls but significant vs. resting eels ($P<0.01$). The difference for 6 week of swimming was significant vs. the controls ($P<0.01$) vs. resting eels ($P=0.05$).

The nucleus (germinal vesicle) was migrating to the periphery (Germinal Vesicle Migration — GVM).

Eels of the control group in experiment 2 contained oocytes in stage 1 and 2, except for one eel that showed only stage 3 oocytes. The average diameter of the oocytes was 83±39 μm. After 2 weeks of swimming, eels showed oocytes in a more advanced stage of development. Change in average stage was just not significant, but oocyte diameter had significantly increased ($P<0.01$; Table 2). Fifty percent of the eels had exclusively stage 3 oocytes in the ovaries with large numbers of lipid droplets in contrast to the eels in control and rest groups which only had stage 1 and 2 oocytes (Fig. 3).

After 6 weeks, eels from the swim group showed further oocyte development. The oocyte stage in the swim group was significantly different vs. control and rest group (Table 2). In the swim group, the oocytes were on average larger (not significantly). In the rest group, eels had only oocytes representing stage 1 and 2. In the swim group, only three eels showed stage 1–2 oocytes. The other six eels showed stage 2–3 or 3 oocytes with large numbers of lipid droplets (Fig. 3). The change in the rest group was also significantly

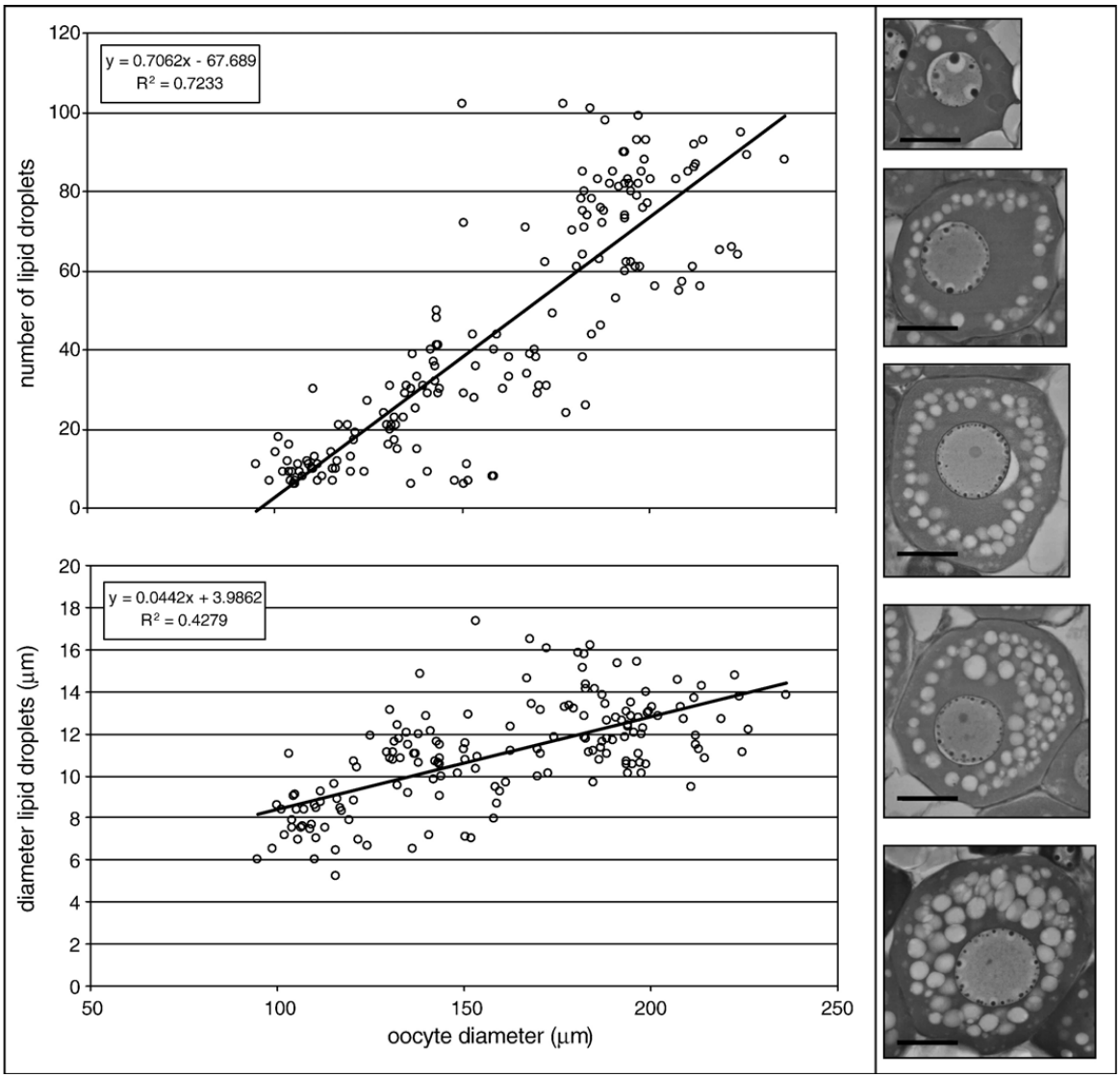

Fig. 4. Relationship between oocyte diameter and lipid deposition in stage 3 oocytes. Depicted are the relations between the oocyte diameter and the number of lipid droplets (upper panel) and the diameter of the lipid droplets (lower panel). All samples originate from eels from experiment 2. Only eels that had swum had stage 3 oocytes. These eels had stage 3 oocytes with an average diameter of 154±34 μm containing 38.4±26.8 lipid droplets with a diameter of 10.4±2.5 μm. Significant positive correlations were found for these averages per eel between oocyte diameter and the number of lipid droplets ($P<0.001$) and between oocyte diameter and the diameter of the lipid droplets ($P<0.001$). Representative pictures showing the relation between number and size of lipid droplets and oocyte diameter are given on the right side. Scale bars are 50 μm.

different from the control group. Average stage and diameter were significantly higher after 6 weeks of resting than after 2 weeks of resting (Table 2).

3.4. Variation of number and size of lipid droplets in stage 3 oocytes

In total 770 oocytes of 39 experimental eels in protocol 2 were histologically analysed. 32 eels contained stage 1 and 2 oocytes (without any lipid droplets) with diameters between 30 and 147 μm ($n=615$). Stage 1 oocytes were on average 56±14 μm ($n=220$). Stage 2 oocytes were significantly larger ($P<0.001$) and on average 87±23 μm ($n=395$).

Ten of the 39 experimental eels also had stage 3 oocytes: nine swimmers and one control eel. Stage 3 oocytes were on average 159±36 μm ($n=165$) and again significantly larger ($P<0.001$). A large variation in the number and size of lipid droplets in stage 3 oocytes was observed (Fig. 4). They contained on average 45±30 lipid droplets in a range between 6 and 102 which measured on average 11±2 μm in a range between 5 and 17 μm. Significant positive correlations were found for these averages per eel between oocyte diameter and the number of lipid droplets ($P<0.001$) and between oocyte diameter and the diameter of the lipid droplets ($P<0.001$). Also when oocyte data were pooled for all eels (Fig. 4), we observed a positive correlation between oocyte diameter and the number of lipid droplets ($P<0.01$) and between oocyte diameter and the diameter of the lipid droplets ($P<0.01$).

3.5. Correlations between size, silvering and oocyte developmental indicators

Data of experimental eels at the start (control and pre-swim measurements) were used for correlation analyses. Correlations between size (BL, BW), silvering (externally EI, SI and internally DTSI, HSI) and oocyte developmental indicators (GSI, OS, OD) were analysed (Table 3). Significant positive correlations were found between size and silvering indicators and between size and oocyte developmental indicators suggesting that the silvering and maturation status of larger eels was more advanced. Between silvering and oocyte developmental indicators, significant positive correlations were found only between EI, SI and OS (Table 3). Positive correlation with GSI was just not significant. No significant correlation was found

Table 3
Correlations between parameters of control eels ($n=19$–20) and eels before swimming ($n=75$)

		BW	EI	SI	DTSI	HSI	GSI	OS	OD
BL	Corr.	**0.944**	**0.740**	**0.680**	−0.218	**0.478**	**0.703**	**0.900**	**0.612**
	P	**0.000**	**0.000**	**0.000**	0.178	**0.017**	**0.000**	**0.000**	**0.003**
	n	**75**	**75**	**75**	20	**20**	**20**	**19**	**19**
BW	Corr.		**0.729**	**0.658**	−0.147	**−0.522**	**0.734**	**0.884**	**0.582**
	P		**0.000**	**0.000**	0.268	**0.009**	**0.000**	**0.000**	**0.004**
	n		**75**	**75**	20	**20**	**20**	**19**	**19**
EI	Corr.			**0.884**	−0.211	−0.062	0.311	**0.510**	0.212
	P			**0.000**	0.186	0.398	0.091	**0.013**	0.191
	n			**75**	20	20	20	**19**	19
SI	Corr.				−0.090	−0.020	0.351	**0.603**	0.269
	P				0.353	0.467	0.065	**0.003**	0.133
	n				20	20	20	**19**	19
DTSI	Corr.					−0.013	**−0.432**	−0.323	−0.203
	P					0.479	**0.028**	0.088	0.203
	n					20	**20**	19	19
HSI	Corr.						−0.342	**−0.390**	−0.229
	P						0.070	**0.049**	0.173
	n						20	**19**	19
GSI	Corr.							**0.721**	**0.730**
	P							**0.000**	**0.000**
	n							**19**	**19**
OS	Corr.								**0.744**
	P								**0.000**
	n								**19**

Significant correlations ($P<0.05$) are given in bold. Significant correlations were found between size (BL, BW) with silvering (EI, SI) and oocyte developmental indicators (GSI, OS, OD). DTSI and HSI were correlated with oocyte developmental indicators rather than silvering parameters (for abbreviations see Glossary).

between external indicators of the level of silvering EI and SI vs. DTSI and HSI, which are often considered to change in relation to the degree of silvering.

4. Discussion

4.1. Swimming triggers silvering

Continuous swimming at 0.5 BL s^{-1} resulted in an increase of the eye index (EI), which was significant after after 2 and 6 weeks swimming. The observed changes were even stronger after 6 weeks of swimming. In contrast no change could be observed in the EI of the rest groups, indicating that the effect was not caused by time and/or starvation. As increase of EI has been described as an external parameter for progression of sexual maturation (Pankhurst, 1982; Tesch, 2003; Durif et al., 2005), these observations suggest that swimming induces early maturation.

The mean eye index of all the eels that just arrived from Lake Balaton in September was 8.2±3.0. As an EI of 6.5 is used as the threshold for silvering (Pankhurst, 1982), 59% of the group can be classified as silver eels. The degree of silvering and oocyte development was positively correlated with body size (Table 3). This observation of silver eels in Lake Balaton contradicts Bíró (1992), who stated that in Lake Balaton eels never metamorphose into silver eels. However, we found even in the control group some silver eels in migrant stage 5. These eels had a GSI>1 and gonads with some stage 3 oocytes, although with few lipid droplets. Surprisingly, the migrant SI stage 4 (Durif et al., 2005) was not represented at all, not before nor after the swim experiments. This stage is characterised with elongated pectoral fins, however no changes of the pectoral fins were observed due to swimming. In field studies it was found that downstream silver eels have longer pectoral fins, from which it was concluded that swimming likely causes the fins to grow (Tesch, 2003; Durif et al., 2005). As in our swim trials no change in fin length occurred, we must conclude that the increase in fin size during downstream migration must be due to other factors than swimming.

4.2. Swimming triggers oocyte development

After 6 weeks of swimming, changes were much more pronounced than after 2 weeks of swimming, both GSI and oocyte diameter was significantly higher. More than 50% of the eels that had swum for 2 and 6 weeks had oocytes predominantly in stage 3, while in contrast resting eels had no stage 3 oocytes at all (Fig. 3). Oocytes in stage 3 showed a high variation in numbers and diameter of lipid droplets, in other words in total lipid content. The increase of lipid content is typical for stage 3. As resting eels showed a slight oocyte development but not any lipid deposition in the oocytes, we conclude that the deposition of lipids in oocytes occurs under conditions of increased lipid mobilisation.

Recently, van Ginneken et al. (2007) simulated a complete migration of 5500-km using 3 year old silver eels from the farm. Those eels were 71±4 cm long and weighed 792±104 g, bigger than the ones used in this study. Significant higher levels of pituitary LH and plasma estradiol were found in the swim group as compared to the controls, also the oocyte diameter was increased. Those results indicate that long term swimming had an effect on maturation of younger farmed eels. However, no changes in EI and GSI were found. Thus, despite the increased hormone levels, the gonads did not develop.

The more explicit changes in this study, already after 2 weeks of swimming, might be explained by the difference in age. The hatchery eels were young (3 years) while the Lake Balaton eels in our study were much older (13 to 21 years). This finding supports the hypothesis that older eels are more responsive and sensitive for maturation. Positive correlations between age vs. condition factor, liver weight and vitellogenin level (Durif et al., 2006), and the increased capacity to incorporate fat from the muscle into the eggs (Palstra et al., 2006b) suggest that older eels are more suited for reproduction. Indeed, we found recently that older eels require a shorter hormonal treatment to mature (Palstra et al., 2006c), indicating a higher sensitivity for maturation.

In this study, we observed swimming induced oocyte development up to stage 3, the lipid droplet stage. Stage 3 was found to be highly variable with respect to the arrangement, number and size of the lipid droplets (Fig. 4). Oocytes of eels that had swum contained more than 100 larger droplets. However, oocytes did not develop further than stage 3. Most developed oocytes had lipid droplets that covered >50% of the cytoplasm and formed a complete ring around the circumference of the oocyte (Couillard et al., 1997), which is typical for pre-vitellogenic oocytes (Colombo et al., 1984). In *Anguilla rostrata* this condition is shown to be the start of vitellogenesis (Cottril et al., 2001). However, we did not observe any yolk globuli in the oocytes of eels that had swum, quite in contrast to artificially matured oocytes which are packed with yolk globuli (Fig. 2D). Also the oocytes did not reach sizes that are characteristic for vitellogenesis. Vitellogenesis is the major cause for oocyte growth in teleosts in general (Tyler, 1991),

including eel (Nagahama, 1994). Cottril et al. (2001) found oocytes of 200 μm for *A. rostrata* and considered them already vitellogenic. Such values are, however, not typical for vitellogenesis. Adachi et al. (2003) showed for *Anguilla japonica* that vitellogenesis begins when oocytes are about 250 μm in diameter. In this study we found maximum oocyte diameters of 236 μm, which are quite close to the onset of vitellogenesis.

4.3. Perspectives

The eels in this experiment swam for max 6 weeks, still all individuals showed progression in sexual maturation. Earlier experiments with 3 year old farmed eels, that swam for 5500-km, showed hormonal changes but no increase in gonadal mass. Therefore it is possible that maturation sensitivity depends on age, and that older eels may develop further during continued swimming.

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Glossary

BL: bodylength
BW: bodyweight
DTSI: digestive tract somatic index
DTW: digestive tract weight
ED: eye diameter
EDh: eye diameter horizontal
EDv: eye diameter vertical
EI: eye index
GSI: gonadosomatic index
GW: gonad weight
HSI: hepatosomatic index
K: condition factor
LW: liver weight
OD: oocyte diameter
OS: oocyte stage
PFL: pectoral fin length
PFLI: pectoral fin length index
PFW: pectoral fin width
PFWI: pectoral fin width index
SI: silver index