Otolith of a 26-year old eel. Age is a determinant for successful eel reproduction since: a) older eels were more susceptible to swimming-induced oocyte development; b) older eels showed increased capacity to incorporate more fat from the muscle into the oocytes determining higher egg quality; and c) older eels were more sensitive to hormonal stimulation.

Chapter 7

The fate of fat in silver eels: lipid requirements for spawning migration

A.P. Palstra, E. Antonissen, M.E. Clavero, M. Nieveen, P. Niemantsverdriet, V.J.T. van Ginneken, G.J.E.E.M. van den Thillart

Integrative Zoology, Institute of Biology Leiden, van der Klaauw Laboratories, PO Box 9516, Kaiserstraat 63, 2300 RA Leiden, The Netherlands.

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CHAPTER 7

ABSTRACT

The energy budget of semelparous eels is a true example of biological efficiency. They metamorphose (silvering) preparing for their 5,500-km oceanic reproductive migration to the Sargasso. Since they cease feeding, their stored energy, mainly as lipids in muscle and under the skin, should suffice for both migration and incorporation in the oocytes. Few attempts however were made to estimate the energy costs. Recently, we found that optimum swim speeds of silver eels are around 0.8 BL/s, higher than the generally assumed cruise speeds of 0.5 BL/s. At those speeds, large silver eels may reach the spawning grounds in about 105 days. In this study we therefore subjected farmed eels and wild large silver eels to simulated migration at those speeds and calculated cost of transport (COT) from oxygen consumption ($\dot{M}O_2$) rates. We found that farmed eels swam at COTs of 34 ± 5 mg O_2/kg/km during 2,173 ± 305 km migration, while wild silver eels swam at higher COTs of 52 ± 12 mg O_2/kg/km during 1,232 ± 172 km migration. COTs were rather constant and similar to values obtained from short term 2h swim tests. Wild silver eels spend 78 ± 4 g fat /kg on a complete 5,500-km migration run. These relatively low values are proof of a high metabolic efficiency. We artificially matured eels from the same batch of wild silver eels by hormonal injections to determine the amount of fat that was incorporated in the oocytes. We found that eels incorporate 57 ± 22 g fat /kg in the oocytes during artificial maturation. The amount of fat transported to the gonads was found to be positively related to the age of the eel. In total, European eels may therefore spend about 135 g fat/kg on their spawning run. Fat stores of silver eels from high quality trophic habitats should suffice for successful reproduction.

INTRODUCTION

A true example of biological efficiency concerns the energy budget of eels for reproduction. All eel species exhibit an impressive reproductive migration of which the European eels swim the longest distance i.e. some 5,500-km to the assumed spawning grounds in the Sargasso Sea (Schmidt, 1923; Tesch 2003). They spend their feeding stage as immature yellow eels in the fresh and brackish European waters. At the end of each growth season, some eels cease feeding and metamorphose (silvering) in preparation of their oceanic journey. Probably their fat content plays a major role in the onset of silvering and their seaward migration (Larsson et al., 1990; Svedäng & Wickström, 1997). Their stored energy, mainly as lipids in muscle and under the skin, should suffice for two major purposes: migration and reproduction.

Eels are believed to exhibit a semelparous strategy meaning that individuals spawn only once in their lifetime. Semelparous spawners exceed a boundary to survive and they die shortly after reproducing. The boundary between semelparous and iteroparous lifestyles lies within a 60-70% energy depletion range (Wootton, 1990). Iteroparous trouts spend 40-50% energy on spawning with 3-4% of gonadal energy (Jonsson, 2005). Semelparous Pacific salmons (*Oncorhynchus* spp.) spend 75-82% with 10% of gonadal energy (reviewed by Lucas & Baras, 2001). A legitimate estimation of the cost of reproduction of eel is still lacking.

In his 'new solution to the Atlantic eel problem', Tucker (1959) suggested, that the European eel could never reach the *Anguilla* breeding area in the Sargasso. Since then, few attempts were made to estimate the energy costs of migration and maturation. Simulated migration trials that were recently carried out by our group provided new insight. They were performed in 22 Blazka-type 127-L swimtunnels in which the oxygen levels were measured continuously by oxygen electrodes (Van den Thillart et al., 2004). Van Ginneken & Van den Thillart (2000) extrapolated results of a 387 km swim trial and estimated that eels swim at 0.573 kJ/kg/km, a cost of transport (COT) 2.4-3.0 times lower than that calculated by Schmidt-Nielsen (1972). Van Ginneken et al. (2005b) calculated energy consumption of a full 5,500-km simulated migration by both oxygen consumption ($\dot{M}O_2$) and bomb-calorimetry. They came to the same conclusion and found that eels spent only 0.418-0.611 kJ/kg/km. Van den Thillart et al. (2004) extrapolated results of a 2,850-km swim trial and found slightly higher values of 0.833 kJ/kg/km. The latter two long distance experiments were performed with 3 year old farmed eels swimming in fresh water at a speed of 0.5 boy length per second (BL/s), the presumed cruise speed to the Sargasso (Tesch, 2003). This might, however, not be a correct representative for the natural situation. Palstra et al. (*chapter 2*) found optimum swim speeds for farmed eels in fresh water of 0.96 BL/s and for wild eels from Lake Grevelingen in salt water lower, of 0.77 BL/s. Although COT was rather constant at all swim speeds, it was the lowest at swim speeds around 0.8 BL/s. Therefore it was assumed that this speed may represent the actual cruise speed to the Sargasso. These speeds of 0.8 BL/s are much higher than the cruise speeds that were presumed until now (Tesch, 2003). The natural situation differs however in more perspectives. Wild silver eels have for instance a lower condition factor and lower fat percentages than farmed eels. Also is migration in reality performed in salt water and at lower temperatures. Palstra et al. (*chapter 2*) found that energy costs of swimming are 20% higher in SW in comparison to FW.

Besides the costs of migration, the development of the gonads up to gonadosomatic indices (GSIs) of 28 to 60 (*chapter 5*) requires a substantial part of the energy reserves. Boëtius & Boëtius (1980) measured lipid and protein contents in the gonads of two strip-ripe eels. Eel 1 had an initial body weight of 940 g, a gonad weight of 467 g (GSI=46.8) of which 43.4 g was fat and 36.9 g protein. Eel 2 had an initial body weight of 780 g, a gonad weight of 442 g (GSI=45.5) of which 23.4 g was fat and 29.2 g protein. By using conversion factors of 38.9 kJ/g for lipids and 17.2 kJ/g for protein, they calculated that 1.41 and 2.32 MJ was utilised for gonadal development. Until now this is the only calculation available.

In order to establish a legitimate estimation of the total cost of eel reproduction, we need 1) to determine the costs of transport at higher, near optimal, swim speeds, 2) to mimic natural migration conditions e.g. subjecting wild silver eels to swimming in SW at low temperature and 3) to determine the energy costs of gonad development of wild silver eels of the same batch

Therefore, two kinds of experiments were performed in this study: simulated migration and artificial maturation by hormonal stimulation. To investigate the effects of higher swim speeds during long-term swimming (1), we subjected 5 year-old farmed female eels to a swim trial in fresh tap-water (FW at 18°C) at speeds of 0.8 BL/s. We expect lower COTs at this speed in comparison with the generally applied 0.5 BL/s. To

mimic natural conditions (2), a similar swim trial was performed with wild female silver eels from Lake Grevelingen but in artificial sea water (SW) at low temperature (from 18°C down to 10°C). Swimming in SW results in a 20% higher COT (*chapter 2*). Like with speed, a temperature exists at which $\dot{M}O_2$ and swim performance are optimal (reviewed by Beamish, 1983) but which is, however, unknown for eel. To determine the energy costs of gonad development of these eels (3), fat contents in muscle and gonad tissue were determined in artificially matured eels. The total cost of eel reproduction was established as the sum of the cost of migration and the cost of gonad development.

MATERIAL AND METHODS

Formulas used for calculation of different parameters are given in table 1.

Table 1 Formulas used for calculation of parameters.

Formulas for sampling parameters:

1. $\dot{M}O_2$ = 127* $\Delta[O_2]/\Delta t$ ($mgO_2/kg/h$), where: $\Delta[O_2]/\Delta t$ is the decrease of the oxygen content per hour
2. $K = 100 * BW/BL^3$
3. $EI = 100 * ((EDh+EDv)/4)^2\pi/10*BL)$
4. PFLI= 100* PFL/BL
5. GSI= (Weight gonads / Body weight) *100%

Formulas for calculation of cost of transport (COT) from weight loss:

6. COT_{tot} (kJ/kg/km)= $E_{fat} + E_{protein} + E_{carbohydrate}$,
7. $dBW_{loss} = 0.50 * wBW_{loss}$
8. COT_{fat} (g fat/kg/km)= 0.68* dBW_{loss}
9. COT_{fat} (kJ/kg/km)= 39.5* COT_{fat} (g fat/kg/km)
10. $COT_{protein}$ (g protein/kg/km)= 0.28* dBW_{loss}
11. COT_{fat} (kJ/kg/km)= 23.6* COT_{fat} (g fat/kg/km)
12. $COT_{carbohydrate}$ (g carbohydrate/kg/km)= 0.01* dBW_{loss}
13. $COT_{carbohydrate}$ (kJ/kg/km)= 17.2* $COT_{carbohydrate}$ (g carbohydrate/kg/km)

Formulas for calculation of cost of transport (COT) from oxygen consumption:

14. COT_{tot} (kJ/kg/km)= $COT_{fat} + COT_{protein} + COT_{carbohydrate}$
15. COT_{fat} (kJ/kg/km)= 13.72*(0.798*COT (g O_2/kg/km))
16. COT_{fat} (g fat/kg/km)= (1/39.5) * COT_{fat} (kJ/kg/km)
17. $COT_{protein}$ (kJ/kg/km)= 13.36*(0.196*COT (g O_2/kg/km))
18. $COT_{protein}$ (g protein/kg/km)= (1/23.6) * $COT_{protein}$ (kJ/kg/km)
19. $COT_{carbohydrate}$ (kJ/kg/km)= 14.76*(0.005*COT (g O_2/kg/km))
20. $COT_{carbohydrate}$ (g carbohydrate/kg/km)= (1/17.2) * $COT_{carbohydrate}$ (kJ/kg/km)

Experimental eels

Five year-old female eels (600-1,400 g; 60-90 cm; n=12) were obtained from a commercial hatchery (Royaal BV Helmond, The Netherlands; Table 2). Silver female eels (500-1,700 g; 60-90 cm; n=31) were caught in the fall of 2001, 2002 and 2003, during their seaward migration in the brackish Lake Grevelingen (Bout, Bruinisse, The Netherlands) at the North Sea sluice at 32 ppt. After arrival in the lab, all eels were anaesthetized with oil of cloves (1:10 dissolved in 100% ethanol using a dosage of 1.5 ml/l water). Six farmed eels and six eels from Lake Grevelingen were killed and sampled as untreated control groups. Other eels were tagged with small passive transponders (TROVAN, EID Aalten BV, Aalten, The Netherlands).

Experimental swim-tunnel set-up

A set of 22 Blazka-type 127-L swimtunnels as described by Van den Thillart et al. (2004) was used for the swim trials. The swim tunnels were placed in the direction of the Sargasso Sea (WNW) in a climatized room of about 100-m^2. The total water content of the system was about 7000-L and was recirculated continuously over a bio-filter, a sand-filter, and UV-lamps. The illumination in the climatized room was switched to 670-nm light (bandwidth 20-nm). Based on pigment changes during silvering, it is very unlikely that this far-red light is invisible for eels (Pankhurst & Lythgoe 1983). The oxygen level in all swim tunnels was measured continuously by oxygen electrodes (Mettler Toledo). The latter were calibrated with sodium-sulfite (0%) and air (100%). When the oxygen levels came below 75% air saturation (AS), the water refreshment was switched on by the controller, automatically raising it up to 85% AS. The $\dot{M}O_2$ rate was calculated from the oxygen decline after automatic closure of the water-inlet by a magnetic valve. From the decline of the O_2-concentration, the $\dot{M}O_2$ rate was calculated following formula (1). The body weight (BW) of each eel was calculated for each day of migration from a linear relation between BW before and after the trials.

Protocol experiment 1: swim trial with 5 year old farmed eels in fresh water

In May 2003, 6 eels were anaesthetized and morphometric parameters were measured. They were introduced in the swim tunnels in running fresh water (FW) of 18 °C. Swimming was started after two days of rest. During the first 4 days, the swim speed was increased from 0.5 to 0.8 BL/s with increments of 0.1 BL/s per day. The eels were kept at 0.8 BL/s for 27 days followed by 17 days at 0.7 BL/s. After this swim period, eels were removed from the tunnels, anaesthesized, measured, killed and sampled.

Protocol experiment 2: swim trial with Lake Grevelingen eels in salt water

In January 2003, 6 eels were anaesthetized and morphometric parameters were measured upon arrival from Lake Grevelingen. Thereafter, they were introduced in the swim tunnels in salt water (SW; 32 ppt) at 18 °C. After two days of rest, they started to swim at 0.5 BL/s and swim speed was increased like described above. Starting at day 4, water temperature was lowered with 0.5 ºC per day from 18 ºC down to 10 ºC. This temperature was kept stabile during further swimming. The experiment was stopped after 26 days, the eels were immediately removed from the tunnels, anaesthetized, measured, killed and sampled.

Protocol Experiment 3: Artificial maturation of Lake Grevelingen eels

Thirteen Lake Grevelingen eels were anaesthetized and morphometric parameters were measured before they were treated with carp pituitary extract (*chapter 5* and *6*). They were administered weekly injections of Carp Pituitary Extract (CPE: 20-mg/kg) until oocytes were displaying germinal vescicle breakdown and ovulation could be induced with 17,20β-dihydroxy-4-pregnen-3-one (DHP: 2-mg/kg), and they could be hand-stripped (*see for detailed description chapter 5*).

Morphometric measurements and sampling

Morphometric parameters included bodylength (BL), bodyweight (BW), eye diameters horizontal and vertical (EDh, EDv) and pectoral fin length (PFL). Eels (control, swim and hormone treated groups) were sampled for gonad tissue. With these measurements we determined:

- Fulton's condition factor K using formula (2)
- The eye index according to Pankhurst (1982) EI using formula (3)
- The pectoral fin length index according to Durif et al. (2005) PFLI using formula (4)
- The silver index (SI) according to Durif et al. (2005) based on BL, BW, ED and PFL.
- Gonadosomatic index (GSI) using formula (5)

Otolithes (sagitta) were removed for age determination according to the method by Daverat (2005a) as described in *chapter 2*. From artificially matured eels and their controls, a gonad tissue sample (at a standardised rostral location) and a muscle tissue sample (2x2x1cm, taken dorsal from the lateral line at the position of the genital pore) were stored at -20° for fat measurement.

Fat measurement

Portions of 1 g were homogenized in 2 ml ultra pure water. Isopropanol (2 ml) was added, mixed and subsequently fat was extracted using 3 portions of 4 ml hexane/di-ethyl-ether (97:3). After evaporation of the solvent, fat was determined gravimetrically.

Statistics

Normality of the data distribution was tested with Kolmogorov-Smirnov tests. Paired t-tests with one-tailed probabilities were used for differences of parameters body weight (BW), condition factor (K), eye index (EI), pectoral fin index (PFI) comparing pre- and post swimming or pre- and post hormonal treatment. A Wilcoxon test with one-tailed probabilities was used to test for differences in pre- and post silver index (SI). Difference in BW between the farmed and Lake Grevelingen eels was tested with a unpaired t-test with two-tailed probabilities.

In order to test for a relation between water temperature and $\dot{M}O_2$, a Pearson correlation test with one-tailed probabilities was performed for mean datapoints during the trajectory of water temperature decrease from 18 to 10 °C during simulated migration of Lake Grevelingen eels. To test for energy differences of farmed eels swimming in FW vs. Lake Grevelingen eels swimming in SW, univariate analyses of variance (ANCOVA) with

co-factor BW was performed on oxygen consumption ($\dot{M}O_2$) and weight loss (BW_{loss}) data. A Pearson correlation test with one-tailed probabilities was used to test for correlation between $\dot{M}O_2$ and BW loss. For comparison between muscle and egg samples within groups, paired one-sided t-tests were performed. For comparison between control and treated groups, ANCOVA was performed. Pearson correlation with one-tailed probabilities between the start parameters age, body length (BL), BW vs. swim parameters ($\dot{M}O_2$, BW_{loss} and distance) and vs. maturation parameters (no inj, GSI, fat percentage muscle, fat percentage gonads, total fat gonads) was tested for predictive significance. Significant correlations were analysed with ANOVA to estimate the determinant. All tests were performed in SPSS 10.0 for Windows. Results were calculated and plotted as means ± SD.

RESULTS

1 Migration

Experiment 1: swim trial with 5 year old farmed eels in FW

Oxygen consumption and weight loss during migration of farmed eels

Mean $\dot{M}O_2$ rates peaked at day 5, a day after the last speed increment (Fig. 1). After this peak, during the period of swimming at 0.8 BL/s (day 6-31), values became fairly stable at a mean of 75 ± 11 mg O_2/kg/h corresponding to a COT of 34 ± 5 mg O_2/kg/km (Table 2). From day 31 to 45, eels swam at 0.7 BL/s. After 45 days of swimming, the eels were stopped at a mean distance of 2,173 ± 305-km (range 1,717-2,447-km). During this period they lost on average 64 ± 32 g corresponding to 30 ± 15 mg/kg/km (Table 2).

Morphometric changes during simulated migration of farmed eels

In table 3a an overview is given of external parameters before and after swimming. Eels were all 5 years old, 76 ± 4 cm long and weighted 951 ± 95 g at the start. They were silver as indicated both by the eye index (11.2 ± 1.9) and silver index (3.83 ± 0.41). After swimming, the eyes had increased significantly ($P<0.01$)! The eye index increased with 8% to 12.1 ± 1.8. However, the GSI did not differ from the control group.

Experiment 2: swim trial with Lake Grevelingen eels in SW

Oxygen consumption and weight loss during migration of Lake Grevelingen eels

The mean $\dot{M}O_2$ of the six swimmers is plotted per day in Fig. 2. During the first four days the speed is raised from 0.5 up to 0.8 BL/s which correlates with a rise in the $\dot{M}O_2$. After reaching a speed of 0.8 BL/s at day four, and during decrease of the water temperature from 18 to 10°C, the $\dot{M}O_2$ rates remained stable at an mean of 108 ± 5 mg/kg/h corresponding to a COT of 52 ± 12 mg O_2/kg/km (Table 2). No significant correlation was found between water temperature and $\dot{M}O_2$ rates. After 26 days of swimming, the eels were stopped at an mean distance of 1,232 ± 171 km (range 909-1361

km). During this period they lost on average 48 ± 19 g corresponding to 54 ± 30 mg /kg/km (Table 2).

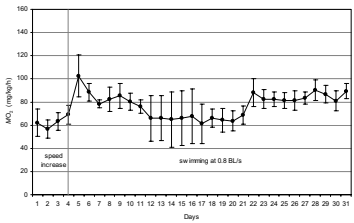

Figure 1 Daily mean of oxygen consumption rate ($\dot{M}O_2$) of farmed eels (n=6) in 18 °C fresh water (FW) swimming 27 days at 0.8 BL/s. The swim speed was increased the first four days from 0.5 bodylength per second (BL/s) to 0.8 BL/s with increments of 0.1 BL/s per day.

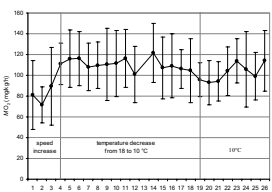

Figure 2 Mean $\dot{M}O_2$ of Lake Grevelingen eels (n=6) in salt water (SW) swimming 26 days (1.232 ± 171 km). Speed was increased the first four days from 0.5 BL/s to 0.8 BL/s with increments of 0.1 BL/s per day. Starting at day 4, the water temperature was lowered with 0.5 °C per day from 18 °C to 10 °C.

Table 2 Individual values for parameters during 2 swim experiments: farmed eels in fresh water (FW) or Lake Grevelingen eels in salt water (SW). Data represent: their age in years; body length (BL) in cm; swim distance in km; BW in g before and after swimming; the body weight loss (BW_{loss}); oxygen consumption rate ($\dot{M}O_2$) and cost of transport (COT). Asterisks mark values of eels that contained much fluid in intestine and bladder after swimming resulting in higher BW.

					pre-swimming	post swimming					
		age	BL	swim distance	BW	BW	BW_{loss}			$\dot{M}O_2$	COT
group	no	(years)	(cm)	(km)	(g)	(g)	(g)	(g/kg)	(mg/kg/km)	(mg O_2/kg/h)	(mg O_2/kg/km)
Cultured eels	1	5	69	1717	883	877	6*	7	4	70 ± 11	35 ± 5
(FW, 18 °C)	2	5	75	2380	964	895	68	71	30	74 ± 11	34 ± 5
	3	5	78	2447	908	812	96	105	43	83 ± 9	37 ± 4
	4	5	81	1890	1127	1069	59	52	28	56 ± 25	24 ± 11
	5	5	76	2189	865	777	88	101	46	86 ± 13	39 ± 6
	6	5	78	2416	957	891	66	69	29	79 ± 11	35 ± 5
	av	**5**	**76**	**2173**	**951**	**887**	**64**	**68**	**30**	**75**	**34**
	stdev	**0**	**4**	**305**	**95**	**101**	**32**	**36**	**15**	**11**	**5**
Lake Grevelingen eels SW	1	7	71	1247	787	773	15*	19	15	82 ± 10	40 ± 5
(SW, 18 to 10 °C)	2	13	76	1343	852	787	65	77	57	130 ± 19	59 ± 9
	3	8	72	909	683	616	66	97	107	147 ± 15	71 ± 7
	4	9	68	1194	589	551	38	64	54	109 ± 13	55 ± 6
	5	6	78	1361	901	852	49	55	40	105 ± 11	47 ± 5
	6	7	76	1336	759	708	51	68	51	87 ± 9	40 ± 4
	av	**8**	**73**	**1232**	**762**	**714**	**48**	**63**	**54**	**108**	**52**
	stdev	**2**	**4**	**171**	**113**	**113**	**19**	**26**	**30**	**5**	**12**

Table 3 Values of morphometric parameters for eels before (pre) and after (post) the different swim experiments; a) simulated migration (2,173 ± 305 km) of farmed eels in FW, and b) simulated migration (1,232 ± 171 km) of Lake Grevelingen eels in SW. In bold are given significant differences (P<0.01) between pre and post measurements. GSI values before experiments (*italics*) are given from a control group that was sampled upon arrival in the lab.

	a) **Experiment 1 simulated migration farmed eels FW (n=6)**				b) **Experiment 2 simulated migration Lake Grevelingen eels SW (n=6)**			
	pre		post		pre		post	
parameter	mean	SD	mean	SD	mean	SD	mean	SD
age (years)			5	0			8	2
bodylength (cm)	76	4	76	4	73	4	73	4
bodyweight (g)	951	95	**887**	**101**	762	113	**714**	**113**
condition factor	0.22	0.03	**0.19**	**0.04**	0.19	0.02	**0.18**	**0.02**
ocular index	11.2	1.9	**12.1**	**1.8**	11.5	1.6	11.8	1.7
pectoral fin index	3.9	0.25	3.85	0.24	4.62	0.31	4.60	0.21
silver index	3.83	0.41	4	0.63	4.17	0.75	4.33	0.82
gonadosomatic index	*1.43*	*0.23*	1.19	0.09	*1.13*	*0.18*	1.24	0.26

Table 4 Values of morphometric parameters for eels before (pre) and after (post) inducing maturation by 17 ± 4 CPE injections of Lake Grevelingen eels. In bold are given significant differences (P<0.01) between pre and post measurements. GSI values before experiments (*italics*) are given from the control group that was sampled upon arrival in the lab.

	Experiment 3 artificial maturation Lake Grevelingen eels (n=13)			
	pre		post	
parameter	mean	SD	mean	SD
age (years)			11	4
bodylength (cm)	81	6	81	6
bodyweight (g)	1131	297	**1256***	**291**
condition factor	0.21	0.02	**0.23**	**0.03**
ocular index	11.7	1.9	**15.4**	**2.2**
pectoral fin index	4.81	0.25	5.16	1.04
silver index	3.90	0.32	4.10	0.32
gonadosomatic index	*1.13*	*0.18*	37.1	9.4

* weight taken at the moment of DHP injection

Morphometric changes during simulated migration of Lake Grevelingen eels

Eels were on mean 8 ± 2 years old (range 6-13; table 3b). They were 73 ± 4 cm long and weighed 762 ± 113 g at the start. They were silver as indicated both by the eye index (11.5 ± 1.6) and silver index (4.17 ± 0.75). After swimming, eye or silver indices had not increased significantly. Also the GSI did not differ from the control group.

2 Maturation

External changes after artificial maturation

Hormone-treated eels were on mean 11 ± 4 years old (range 6-20; Table 4). They were 81 ± 6 cm long and weighed 1,131 ± 297 g at the start. Upon hormone-treatment all females fully matured resulting in a significant increase of the GSIs to 37 ± 9% after 17 ± 4

weekly CPE injections. Other significant changes that indicated maturation were increases in body weight (11%, P<0.001; Table 2) and condition factor (10%, P<0.001). The eye index also increased significantly (32%, P<0.001; Table 2) from 11.7 ± 1.9 to 15.4 ± 2.2. Other signs of silvering like pectoral fin index and the silver index increased but not significantly.

Fat incorporated in oocytes

Data on Lake Grevelingen eels as presented here have already been reported together with data of 7 eels from River Loire (France) in *chapter 6*. Control eels had a GSI of 1.13 ± 0.18% (Fig. 3a). When having reached a mean GSI of 37 ± 9% after treatment (Fig. 3a), the total fat in the gonads of the hormone-treated females was 57 ± 22 g per kg eel (Fig. 3b), 12 times higher than in control eels (4 ± 1 g per kg eel). Fat percentages in muscle tissue of control eels were 21 ± 8% (range 11 to 35%; Fig. 3c) and remained similar after treatment (20 ± 5%). Fat percentages in the gonad tissue of control eels were 33 ± 5% (Fig. 3d) and significantly lower (P≤0.01) after treatment. Significant differences (P≤0.01) existed between fat in muscle and gonad between control and treated eels

Correlations between status at the start and maturation performance

Correlation analysis showed a significantly (P<0.05) negative correlation between age and the number of injections needed to mature the females (Table 4). Furthermore age showed a negative correlation with muscle fat percentage (P<0.05) and positive with gonad fat percentage (P<0.05) and with total fat in the gonads (P<0.01). ANCOVA showed that age (P=0.02) was the significant determinant for the amount of incorporated fat. BL and BW showed a negative correlation (P<0.01) with GSI.

Table 5 Pearson correlations between parameters age, BL and BW at the start of hormonal injections and parameters at full maturation; the required number of CPE injections, GSI, fat percentage in muscle and gonads, and total fat in the gonads. Shown are the correlation, the P-value and the number of observations n. In bold, significant correlations.

		end parameters:				
start parameters:		**no inj**	**GSI**	**fat muscle (%)**	**fat gonad (%)**	**total fat gonad**
age	corr.	**-0.495**	0.069	**-0.519**	**0.514**	**0.743**
	P	**0.043**	0.411	**0.035**	**0.036**	**0.002**
	n	**13**	13	**13**	**13**	**13**
BL	corr.	-0.345	**-0.719**	-0.472	0.405	0.163
	P	0.12	**0.003**	0.052	0.085	0.298
	n	13	**13**	13	13	13
BW	corr.	-0.228	**-0.713**	-0.455	**0.63**	0.275
	P	0.226	**0.003**	0.059	**0.011**	0.182
	n	13	**13**	13	**13**	13

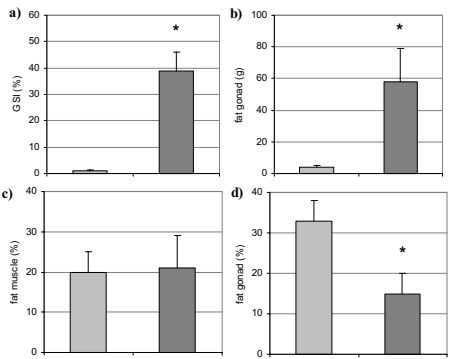

Figure 3 a) GSI (relative gonad weight), b) total fat in gonad (g/kg eel) in control (light grey) and hormone-treated (dark grey) eels, c) relative fat content in muscle and d) gonad (g fat/g tissue *100%). Significant differences (P≤0.01) are indicated by the asterisks.

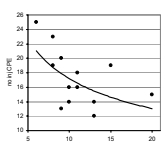

Figure 4 Significant relation (Pearson correlation P<0.05, ANCOVA P=0.02) between age and the number of injections CPE required to fully mature Lake Grevelingen silver eels.

DISCUSSION

Effect of swim speed

Farmed eels in FW swam for 28 days at 0.8 BL/s at oxygen consumption ($\dot{M}O_2$) values of 75 ± 11 mg/kg/h. The cost of transport (COT) was found 34 ± 5 mg/kg/km. These values were similar to the values observed during swim fitness tests (*chapter 2*). During this test, eels swam for just 2 h per speed. At a slightly higher speed of 0.85 ± 0.05 BL/s, the $\dot{M}O_2$ values were 82 ± 12 mg/kg/h and COT values were 39 ± 5 mg/kg/km. Van den Thillart et al. (2004) and Van Ginneken et al. (2005b) used similar sized farmed eels swimming in FW at 0.5 BL/s and found $\dot{M}O_2$ levels of resp. 37 ± 3 and 42 ± 6 mg/kg/h. COT values were resp. 28 ± 2 and 32 ± 3 mg/kg/km. This indicates that COT values remain similar when the swim speed is increased with 60% from 0.5 BL/s to 0.8 BL/s. This result agrees with the conclusion from swim fitness tests (*chapter 2*) that COT is very low and rather constant at all swim speeds. Eels are therefore excellent cruisers that, in absence of a strictly defined optimum swim speed, may easily alter the cruise speed of preference in a range of 0.5 to 1.0 BL/s.

Effect of salinity and water temperature

Farmed eels and Lake Grevelingen eels were of similar length (Table 3ab), which makes comparison of swim performance legitimate. However, at the same length farmed eels were significantly heavier (P=0.01), having significantly higher K and having, in contrast to the Lake Grevelingen eels, large amounts of intestinal fat. Both groups were also comparable with respect to their silver index. All experimental eels were silver eels (EI>6.5) and, except for one farmed and one Grevelingen eel, all were in a migratory phase (stage 4 or 5). Lake Grevelingen eels swimming in SW exhibited significantly (P<0.01) higher $\dot{M}O_2$ values (44%) and COTs (53%) than the farmed eels swimming in FW. Also in the former experiments such differences were found in $\dot{M}O_2$ values and COTs between farmed eels in FW with Lake Grevelingen eels in SW. Van den Thillart et al. (2004) and Van Ginneken et al. (2005b) used farmed eels in FW and found $\dot{M}O_2$ values of resp. 37 ± 3 and 42 ± 6 mg/kg/h, and COT values of 28 ± 2 and 32 ± 3 mg/kg/km. Van Ginneken & Van den Thillart (2000) used Lake Grevelingen eels in SW and found $\dot{M}O_2$ values of 66 ± 14 mg/kg/h, and COT values of 42 ± 10 mg/kg/km. Thus, in these studies $\dot{M}O_2$ values were at least 57% higher and the COT values were 31% higher of Lake Grevelingen eels in SW vs. farmed eels in FW. Comparing $\dot{M}O_2$ values of similar farmed eels in either FW or SW during swim fitness tests (*chapter 2*) showed a difference in COT of only 20%. Paired observations of the Lake Grevelingen eels showed no significant change in $\dot{M}O_2$ during the 8°C decrease, suggesting that energy costs of eel swimming is indepent from water temperature. So, the additional 33% increase in COT cannot be ascribed to differences in either salinity or water temperature and thus reflects a lower condition of wild Lake Grevelingen eels in comparison with farmed eels.

Table 6 Comparison of estimated energy costs for migration (COT) and maturation (COM) of eel in literature and this study with a) reference, experiment, conditions, and migration costs in kJ/kg eel/km (COT_{kg}) and required fat in g fat/kg eel/km, and b) maturation costs in required fat.

Energy cost of transport (COT)

experiment	conditions	COT_{kg} kJ/kg/km	COT_{fat} mg fat/kg/km	ref
<1 day	male (♂) yellow and silver (2.50 g) FW, 0.35-0.65 m/s, 15 °C	1.37-1.74	27.8-35.3	1
387 km	5 female silver eels (± 1 m) SW, 0.5 BL/s, 14 °C	0.57	11.5	2
2,850 km	5 female farmed eels (919 g) FW, 0.5 BL/s, 19 °C	0.833	16.7	3
5,533 km	9 female farmed eels (3 years old, 915 g) FW, 0.5 BL/s, 19 °C	0.418/0.611*	8.4/12.4*	4
2,173 km	6 female farmed silver eels (5 years old, 951 g) FW, 0.8 BL/s, 18 °C	0.469/0.522*	9.5/10.5*	5
1,232 km	6 female Lake Grevelingen silver eels (6-13 years old, 762 g) SW, 0.8 BL/s, 10-18 °C	0.702/0.860*	14.2/17.5*	5

* measured by two methods: resp. oxygen consumption and carcass analysis (van Ginneken et al., 2005) or weight loss (this study)

ref 1 Schmidt-Nielsen (1972)
2 Van Ginneken & Van den Thillart (2000)
3 Van den Thillart et al. (2004)
4 Van Ginneken et al. (2005b)
5 this study

Energy cost of maturation (COM)

conditions	COM_{fat} g fat/kg	ref
2 female silver eels (960 and 780 g) SW, 22-25 °C	29/46	1
13 female Lake Grevelingen silver eels (6-20 years old, 1131 g) SW, 18 °C	57	2

ref 1 Boetius & Boetius (1980)
2 this study

Cost of migration

In this study we found that farmed silver eels swim at COTs of 34 ± 5 mg O_2/kg/km during 2,173 ± 305 km swimming at a swim speed of 0.8 BL/s in FW. The found COTs were very similar to those found for such eels during short term swimming (2 h) in SW (*chapter 2*). With the swim fitness test we found COTs of 38 ± 5 mg O_2/kg/km at optimum swim speeds. The same accounted for Lake Grevelingen silver eels swimming at COTs of 52 ± 12 mg O_2/kg/km during 1,232 ± 172 km migration and at COTs of 50 ± 8 mg O_2/kg/km during the swim fitness test. These results illustrate eel's capability of sustained swim performance. This capacity together with the very high efficiency (4-6 times more efficient than salmon; van Ginneken et al., 2005b and *chapter 2*) make eels ultimate cruising specialists.

Requirements for migration were calculated from body weight loss (BW_{loss}) and oxygen consumption ($\dot{M}O_2$) according to formulas in table 1 that are based on energy conversion and oxycaloric values of Brafield & Llewellyn (1982) and bomb calorimetry values of Van Ginneken et al. (2005b). For calculation of energy cost of transport from fat (COT_{fat}) from the BW_{loss}, we used formulas (6), (7) and (8). For calculation for total energy COT (COT_{tot}), we used formulas (9) – (13). For calculation of energy COT from fat (COT_{fat}) from the oxygen consumption we used formulas (14) and (15). For calculation for total energy COT (COT_{tot}), we used formulas (16) - (20). For extrapolation, we used a standardised distance to the Sargasso of 5,500-km. Farmed eels swim in fresh water (FW) at a total energy cost of transport of 0.469kJ/kg/km (by $\dot{M}O_2$) and 0.522 kJ/kg/km (by BW_{loss}; Table 6). The COT of fat is resp. 9.5 and 10.5 mg fat/kg/km. Lake Grevelingen eels swim in salt water (SW) at a higher total cost of transport of 0.702 kJ/kg/km (by $\dot{M}O_2$) and 0.860 kJ/kg/km (by BW_{loss}; Table 6). The COT of fat is resp. 14.2 and 17.5 mg fat/kg/km. In literature estimations have been made on costs for eel migration. Table 6 shows an overview of all these experiments. Data were modified on basis of 79.8% fat use (van Ginneken et al., 2005b) as fuel and not as sole energy provider, considered as such in most studies until now. When results are compared, we find that our results concerning simulated migration agree with results of former recent swim experiments (van Ginneken & van den Thillart, 2000; van den Thillart et al., 2004; van Ginneken et al., 2005b), however bomb calorimetry values were found lower (van Ginneken et al., 2005b). Results of these experiments did not agree with the experiments of Schmidt-Nielsen who used however small eels at very high speeds.

Cost of maturation

In this study we found that Lake Grevelingen eels incorporate 57 ± 22 g fat/kg (range 33-103 g fat/kg) in the oocytes. For a mean 1-kg silver eels with a fat reserve of 200 g this means that on mean 33% (range 17 to 52%) of the total fat reserve is transported into the gonads. We also calculated these percentages from fat droplet volumes in single oocytes (based on *chapter 5*) with similar results: 25 to 43% of the total fat reserve is incorporated in the oocytes. The values of Boětius & Boětius (1980), based on 2 matured females, were low in comparison with this study. Our study widens the range considerably. This is of major importance since the amount of fat transported into the gonads was found to be positively related to the age of the eel. This suggests an increased capacity of older eels to incorporate fat from the muscle into the eggs. This appears to determine the sensitivity to

mature and the start of vittelogenesis since age was negatively correlated to the number of hormonal injections to induce final maturation. As egg quality depends heavily on incorporation of reserves (Adachi et al., 2003), this increased capacity and sensitivity of older eels suggests a greater reproduction potency. As far as we know, age has never been considered as such for fish in literature.

Cost of reproduction

The total cost of migration can be calculated by multiplying the energy cost of transport by fat of Lake Grevelingen eels times the distance to the Sargasso of 5,500-km. We can conclude that a 1-kg silver eel requires 60 – 107 g fat for migration. We measured a cost of maturation of 33 – 103 g fat. Sucessful reproduction would require 93 – 210 g fat in total. Since a mean 1-kg silver eel has about 200 g fat (fat percentage of 20%), at least 47% up to 100% of the fat stores are used. Silver eels used in this study had percentages of 11 to 35%. In ecological surveys, similar values were found for the majority of the silver eels (Svedäng & Wickström, 1997) implying that fat requirements are not limiting for reproduction. However, since egg quality depends heavily on incorporation of reserves (Adachi et al., 2003), it might well be that silver eels with lower fat percentages (<15%) will not leave and that a next trial will be performed the year after (Larsson et al., 1990; Svedang & Wickstrom, 1997). According to the results of Schmidt-Nielsen (1972) and Boëtius & Boëtius (1980), only eels with the highest fat percentages would be able to migrate for 5,500-km and mature. The lowest estimate of fat costs would be 185 g fat/kg (Table 6 and 7: 139 g fat/kg for migration and 46 g fat/kg for oocyte incorporation). Spending on average 67% of the fat reserves combined with extensive degeneration of muscles, bone as calcium stores (Yamada et al., 2002) and the digestive tract makes survival after spawning improbable.

In this study we found indications that the body constitution remained constant during maturation. The fat percentage in the muscles remained 20% after complete maturation while major fat incorporation into the gonads occurred. Van Ginneken et al. (2005b) reported that the ratio of body constituents lipid, carbohydrate and protein remained constant during 5,500-km migration. These authors concluded that fat, protein and carbohydrate were metabolised in the same proportion. Considering the fats, this is important while they are required as fuel for continuous swimming, as food reserve for developing embryos, but also to keep neutral buoyancy at great depth. The fat percentage in the muscle remained 20% after treatment, a percentage typical for deep sea fish (Bone et al., 1999).

We can conclude that fat fuel stores of the majority of wild migratory silver eels are sufficient for reproduction; for its 5,500-km migration to the Sargasso sea and maturation reaching GSIs up to 60. However, reproductive success may be higher for the older eels. With this, fat percentage is an important discriminator that deserves more attention. Fat percentage reflects on the trophic habitat quality, generation time and amount of silver eels really migrating to the Sargasso and thus reproducing. An estimate for such numbers is crucial but still lacking.

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