A large silver eel swimming in one of the 22 swim-tunnels.

Chapter 2

Swim performance of European silver eels (*Anguilla anguilla*)

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ABSTRACT
The 5,500-km migration to the spawning grounds in the Sargasso is crucial for reproduction of European eel. Most certainly the effective genitors contributing to the future generation are therefore characterised by an excellent swim capacity and efficiency. Performance is likely to vary among silver eels within and between locations, mainly determined by trophic quality. In order to be able to compare the performance of farmed and wild eels from different locations and under different conditions, we developed a swim fitness test. Swim trials with 101 female eels (60-96 cm, 400 – 1500g) were performed in 22 Blazka-type swim-tunnels in a climatised room at 18ºC with running fresh or salt water. Speed and endurance swim trials started at 0.5 up to 1 meter per second (m/s) with increments of 0.1 m/s. Since both tests showed similar results, the single day speed test can be used to predict endurance. Eels reached maximum aerobic swim speeds of 0.81 up to 1.24 BL/s body-length per second (BL/s). At optimum swim speeds of 0.74-1.02 BL/s, the cost of transport (COT) values were 37-50 mg O_2/kg/km, which are very low in comparison to other fish species. Energy expenditure during exercise was 20% higher in SW than in FW. Wild silver eels showed lower performance than farmed silver eels. Overall, we can conclude that silver eels can be considered as cruising specialists. If silver eels cruise at their optimum swim speeds, they would travel in less than 3.5 months to the Sargasso instead of the generally assumed 6 months.

INTRODUCTION

The 5,500-km migration to the spawning grounds in the Sargasso (Schmidt, 1923) is crucial for the semelparous reproduction of European eel. Swim capacity and swim efficiency are therefore primary necessities and presumed to be subjected to strong selection pressures that enhance evolutionary Darwinian fitness (Videler, 1993). Most certainly the effective genitors contributing to the future generation must therefore be characterised by an excellent swim fitness. A strong correlation between Darwinian fitness and swim fitness for silver eels is therefore to be expected.

The onset of migration is preceeded by and correlated to metamorphosis of 'continental' yellow eels into silver eels ('silvering'), a physiological and morphological preparation for their oceanic journey (Tesch, 2003; Lokman et al., 2003; Durif et al., 2005). Because of the cessation of feeding, silver eels have to primarily rely on their fat stores for swimming and reproduction. Fat, protein and carbohydrate are metabolised in the same proportion, since body constitution does not change during migration (Van Ginneken et al., 2005b) and maturation (Palstra et al., 2006). Drastic changes occur during silvering. Most apparent is the enlargement of the eyes which is used to discriminate between the yellow and silver phase in an index developed by Pankhurst (1982). Recently, Durif (et al., 2005) developed a more detailed index in which silvering is correlated to migration. Silvering also involves aquadynamic adaptations. The head becomes more acute and streamlined (Lokman et al., 2003). The pectoral fins become longer (Tesch, 2003; Durif et al., 2005) and change shape (Tesch, 2003). These changes allow them to act as hydroplanes that provide lift. Neutral buoyancy is obtained by the high fat percentages up to 35% ((Svedäng and Wickström, 1997) and by the swim-bladder which also shows increased capacity

(Kleckner, 1980a; Eggington, 1987; Kleckner, 1980b) in order to migrate at depths between 200-600 m (reviewed by Tesch & Rohlf, 2003). Migration in the field is generally assumed to last for 6 months at cruising swim speeds of around 0.4 m/s or 0.5 BL/s for average female silver eels of 80 cm. A 30-year old history of tracking studies revealed speeds for migrating eels in the wild of 0.50-2.09 km/h (reviewed by Tesch, 2003; McCleave & Arnold, 1999; Jellyman & Tsukamoto, 2002) corresponding with 0.14-0.58 m/s or 0.18-0.73 BL/s for an eel of 80 cm. Female eels leave the continent in September-November and spawning is believed to occur primarily in March and April (McCleave, 2003). The much smaller male silver eels (40 cm) should leave earlier to arrive in time.

In the 60s and 70s intensive research has been performed on the fundamentals of salmonid, swimming. Jones and Randall (1978) reviewed the pioneer work of especially Brett (1964, 1965ab) and Glass (1973), providing the parameters for quantification of sustained exercise. When measuring sustained exercise, it is generally assumed that respiratory and circulatory adjustments are adequate to meet increased energy demands aerobically. It is assumed that the anaerobic energy contributions are negligible and that there are no changes in the mode of propulsion. An exponential relation exists between oxygen uptake and swim speed (*U*) like Brett (1964) found in sockeye salmon (*Oncorhynchus nerka*) and which depends on water temperature (Brett, 1964) and body size (Brett, 1965; Brett & Glass, 1973). The maximum oxygen uptake is achieved just before fatigue in an incremental velocity test. Since the drag on fish increases in proportion to U^2, so does the cost of transport (COT; also Fry, 1971). Since there is an exponential relationship between oxygen uptake and swim speed, an U-shaped relationship exists between COT and swim speed. The optimum swim speed is defined by the situation when COT is minimal. Only recently, such experiments have been continued, again mainly with salmonids (Lee et al., 2003ab).

Experimental data available on swim performance of *anguillids* is limited and concerns only small eels either juveniles < 15 cm (reviewed by Langdon & Collins, 2000) or intermediate sized, mostly yellow phase, eels (Schmidt-Nielsen, 1972; Webb, 1975; Van Ginneken, 2002). However, the silver phase is characterised by eel's impressive swim performance. Recently, we subjected large female eels to long term swim trials in several studies aimed to estimate the ability and the energy costs to migrate and to compare efficiency with other fish species (Van Ginneken & Van den Thillart, 2000; Van den Thillart et al., 2004; Van Ginneken et al., 2005b). Results were quite revealing as we found that eels swim 4 to 6 times more efficient than non eel-like fish and utilise only *c*. 60g fat per kg for migration. High efficiency might be expected for migrating silver eels in the field. However, biomechanical efficiency of anguilliform swimming is considered low (e.g. Lighthill, 1970; Videler, 1993) which is inconsistent with our findings (Van Ginneken et al., 2005b).

Swim performance is likely to vary among silver eels. Simulated migration trials would take too long to test differences between groups of different locations, and under different conditions. Our first objective is therefore to construct a swim fitness test providing a fast impression of swim capacity and swim efficiency. For this purpose, it is necessary to establish the relation between speed and endurance performance. This test can then be applied to investigate the swim efficiency of silver eels and the constraints. Constraints concern 1) size, 2) salinity, and 3) habitat. A larger eel is expected to perform

relatively worse but absolutely better than a smaller eel since performance is proportional to a fractional power of body length. Higher energy expenditure in SW is expected because of additional costs for osmoregulation (Kirschner, 1993, 1995). A better performance of wild eels is expected since they are less constrained than farmed eels in fat content and condition factor.

MATERIALS & METHODS

Experimental eels

Eels were obtained from three locations:

1) Farmed eels were obtained from a commercial eel farm (Royaal BV, Helmond, The Netherlands) in October 2001 and September 2004. The October 2001 batches were acclimated to SW during a 2-week period and used in SW experiments in November and December 2001. The September 2004 batch was used in FW experiments within a week after arrival.
2) Wild migratory eels were caught during their seaward migration in a SW-habitat in the brackish Lake Grevelingen (The Netherlands). They were caught in November 2001 at the North Sea sluice at 32 ppt (Bout, Bruinisse, The Netherlands). They were used in experiments in January 2002.
3) Wild migratory eels from a FW-habitat in River Loire (France) were caught in November 2003 by local fishermen at Saint-Florent le Vieil between Angers and Nantes. They were used in experiments in December 2003.

Swim-tunnel set-up and oxygen consumption

A set of 22 Blazka-type 127-L swimtunnels (Blazka, 1960; Smith & Newcomb, 1970) as described by Van den Thillart et al. (2004) were 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 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 was assumed that this far-red light is invisible for eels (Pankhurst & Lythgoe 1983). Indeed the eels did not respond to movement of the experimentor during red light illumination. The oxygen level in the tunnel was measured continuously by an oxygen electrode (Mettler Toledo). The oxygen level in the tunnels was controlled as described before (Van den Thillart et al., 2004). If oxygen levels came below 75% air saturation, rinsing occurred automatically raising it up to 85%. The oxygen consumption 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 oxygen consumption rate was calculated following the formula 1 (Table 1).

Pre swimming measurements

Before introduction into the swim tunnels, eels were anaesthetized (MS222 250 ppm, benzocain 80 ppm or oil of cloves; 1:10 dissolved in 100% ethanol with 1-1.5 ml/l water) and tagged with small passive transponders for individual identification (TROVAN, EID Aalten BV, Aalten, The Netherlands). Morphometric parameters included bodylength (BL),

bodyweight (BW), eye diameters horizontal and vertical (EDh, EDv) and pectoral fin length (PFL). 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.

Withdrawal of blood (500µl) was performed in the dorsal aorta in the tail with heparin flushed (10.000 IU/ml) 1 ml syringes which were immediately placed on ice. Hematocrit (Hct) values were measured in 9 µl whole blood samples in triplo using a micro-centrifuge (Bayer, F.R.G.). Haemoglobin (Hb) content in 10 µl was determined in duplo by a spectrophotometer (LS50B, Perkin Elmer) measuring the absorbance at a fixed λ of 550 nm using the MPR 3 kit (1 ml, Roche Diagnostics GmbH). The MCHC (Mean Cellular Haemoglobin Content) was calculated dividing Hb by Hct.

Table 1 Used formulas.

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. $PFI = 100 * PFL/BL$

Experimental protocols

Eels were introduced into the swim tunnels at least two days before the experiment started. Trials were performed at either fresh water (FW) or artificial salt water (SW). Salinity and water temperature were measured just before every trial. Salinity values of SW during experiments were 32±1 ppt. Water temperature values during experiments were 18±1ºC. Oxygen electrodes were callibrated with natriumsulfite (0%) and air (100%).

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 *U* of 0.5 m/s for 2 h. During these 2 h, we measured the decreasing oxygen content in the tunnel over the first 1.5 h, thereafter the tunnel was rinsed for 0.5 h. After these 2 h at 0.5 m/s, *U* was raised with 0.1 m/s to 0.6 m/s for 2 h, again measuring oxygen consumption over the first 1.5 h. Subsequently, this was repeated with steps of 0.1 m/s for a *U* up to 1.0 m/s. From day 2 to 6 eels were subjected to endurance tests. During endurance tests, the oxygen content in the tunnels was measured continuously. Rinsing occurred automatically so eels were swimming between 75 up to 85% air saturation. On day 2, eels swam at 0.5 m/s for 12 h. On day 3, eels swam 1 h at 0.5 m/s followed by 11 h at 0.6 m/s. On day 4, eels were subjected to1 h steps of 0.1 m/s up to swimming for 10 h at 0.7 m/s. Subsequenlty on day 5, 1 h steps of 0.1 m/s up to swimming for 9 h at 0.8 m/s. Finally, on day 6, 1 h steps of 0.1 m/s up to swimming for 8 h at 0.9 m/s. On day 7, the protocol was finished with a second speed test with the purpose to quantify conditioning effects during the experimental period.

When fish fatigued during trials, the flow was lowered to 0.1 m/s. This speed was considered as resting since eels had the choice either to swim or to rest while mixing of the

water in the tunnel was sufficient. Oxygen consumption in a resting state was measured for a period of 3-4 h.

Swim parameters

To characterise swim capacity and efficiency we derived five parameters as illustrated in Figure 1:

1) Oxygen consumption in rest ($\dot{M}O_{2\ rest}$) in mg O_2 per hour and per kg eel,
2) The critical swim speed (U_{crit}) calculated according to Brett (1964),
3) Maximum aerobic oxygen consumption ($\dot{M}O_{2\ max}$) in mg O_2 per hour per kg eel,
4) The optimum swim speed (U_{opt}) or the speed at which eels swim most efficient by and the amount of work per distance reaches a minimum (Tucker, 1970),
5) The cost of transport (COT) at optimum swim speeds in mg O_2 per km per kg eel.

The optimum swim speed was determined by plotting a polynomial trendline through COT values vs. swim speeds per individual eel. The point on this trendline with the lowest COT was considered the optimum swim speed (Fig. 1) and was calculated by equaling the derative of the function of the polynomial trendline to zero. By filling in this value again in the function of the polynomial trendline, the corresponding COT could be obtained.

Otolithometry

On the day after the last speedtest, the eels were removed from the swim tunnels, anaesthetized and sacrificed by decapitation. Otoliths (sagitta) of the wild eels were removed and collected for age estimation. For farmed eels, this procedure was not necessary since the duration of their residence at the farm was known. The age estimation was carried out in the laboratory of Cemagref, Bordeaux, France by otolithometry according to the method described by Daverat (2005a). After their 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 (Synolithe), then polished to the nucleus with a polishing wheel (Streuers Rotopol-35) using 2 different grits of sandpaper (1200 and 2400). Fine polishing was done by hand with Al_2O_3 (1µm grain) on a polishing cloth. Etching was done using 10% EDTA. A drop of this solution was applied on the mold for a duration of 15 minutes. The otoliths were then rinsed with distilled water and stored in dry conditions. Yearly increments were revealed by staining with a drop of 5% Toluidine blue on the otolith and letting it dry. Growth rings were then 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 1 of the eel's life.

Statistics

Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov tests. With a univariate general linear model (GLM), analysis of covariance (ANCOVA) was performed on log transformed data in search for group effects in swim parameters with BL and BW as cofactors. In case of occurrence of significant group effects, ANOVA with post-hoc Bonferroni test was performed to specify the effects between particular groups. Pearson tests were performed for correlation analysis between BL, BW, EI and swim parameters. For comparison of general parameters between groups (table 2) and of swim parameters between speed and endurance trials, t-tests were used

either paired for values of the same eels or unpaired for values of different eels. All statistical analysis were performed in SPSS 10.0 for Windows.

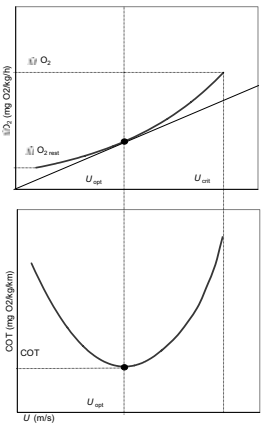

Figure 1 Measured and theoretically derived swim parameters. On the y-axis is given the oxygen consumption $\dot{M}O_2$ and determined were the $\dot{M}O_2$ in rest and the maximum $\dot{M}O_2$. On the x-axis is given the swim speed and determined were the optimum swim speed U_{opt} and the critical swim speed U_{crit}. At the U_{opt}, the cost of transport (COT) is lowest (*see text for detailed explanation*).

CHAPTER 2

RESULTS

Experimental eels

All farmed eels arrived as glass eels and spent 1.7 years at the farm (Table 2). They had eye indices ≥ 9.6 and were therefore all considered silver according to Pankhurst (1982). According to the silver index of Durif et al. (2005), only one was premigrant (stage 3) while the rest was migrant (stage 4-5). The wild eels from the salt water habitat Lake Grevelingen (n=19) were considerably older than the farmed eels with 11 ± 3 years old in a wide range between 7 and 20. They were also significantly larger ($P<0.01$) with 79 ± 5 cm, 949 ± 156 g ($P<0.05$) and had a significantly lower condition factor ($P<0.01$) of 0.19 ± 0.02 (Table 2). All eels were silver and pre-migratory or migratory (stage 3 or 4). Hct was significantly ($P<0.05$) lower than in farmed eels with 24.4 ± 7.3%. Hb was significantly ($P<0.01$) lower with 4.52 ± 1.62 mM and also the resulting MCHC was found significantly ($P<0.01$) lower with of 0.19 ± 0.03 mM, possibly by cell swelling. The wild eels from the fresh water habitat River Loire (n=20) were even older ($P<0.01$) with 16 ± 4 years old in a wide range between 10 and 28. All Loire eels were silver but not all were migratory since two silver eels were still pre-migrants at SI stage 3 (Durif et al., 2005). The pectoral fins of Loire eels were significantly ($P<0.01$) larger than the farmed eels. Hct was significantly ($P<0.01$) higher than in farmed eels with 39.6 ± 5.7%. However, Hb was lower with 5.52 ± 0.70 mM. The MCHC was found significantly ($P<0.01$) lower with of 0.14 ± 0.01 mM.

In general, the wild migratory silver eels were much older than the farmed silver eels. They were also 8 to 11 cm longer and were about 150 g heavier. Their condition factor was however lower. The wild migratory eels had significantly longer pectoral fins ($P<0.01$). Overall, the MCHC was much lower ($P<0.001$) in the wild migratory eels.

Swimming of experimental eels and measuring their oxygen consumption

All experimental eels were females so no sex differences could be observed. The eels appeared to be in good health. No eels died during experimental periods except for some accidents. Incidentally, a few eels did not swim at the beginning of an experiment, but those were easily stimulated by shining a flashlight on their eyes or by knocking on the swimtunnel. Also incidentally, some eels were "brushing the wall" (Brett, 1964), swimming close to the wall of the swim tunnel in search for a lower flow in the first 2 cm from the swim tunnel wall (Van den Thillart et al., 2004), however this only occurred with some smaller eels (<60 cm) when swimming at high speeds (0.9-1.0 m/s). Data of those eels were rejected for analysis. Eels mainly swam a few cm below the center in the front part of the tunnel near the grid. We did not observe any irregularities in swimming of eels at any size (up to 1 m).

Speed and endurance of farmed eels (SW)

The O_2 content in the swim-tunnels showed a gradual decrease during swimming of experimental eels. Figure 2 shows an example during swimming of a single experimental farmed eel during a speed test. With every increment, the slope becomes steeper indicating increase of $\dot{M}O_2$. The gradual decrease shows the ability to stabilise during 2 h swimming for each *U*. Endurance test results showed that eels were able to stabilise their $\dot{M}O_2$ rates during 12 h swim periods. Figure 3 shows a typical example of an endurance test for a

Table 2 Means, standard deviations (SD) and their range of parameters measured on experimental eels of all groups before the start of swim trials and their age either known or estimated by otolithometry.

		Farm, small (SW)	Farm, large (SW)	Farm, large (FW)	Lake Grevelingen (SW)	Loire (FW)
age	mean	1.7	1.7	1.7	11	16
	SD				3	4
	range				7-20	10-28
BL	mean	64	71	71	79	82
	SD	3	4	4	5	6
	range	60-70	64-80	63-75	69-88	70-96
BW	mean	536	799	824	949	1018
	SD	81	129	135	156	253
	range	409-648	659-1191	560-1062	635-1238	668-1473
K	mean	0.20	0.23	0.23	0.19	0.18
	SD	0.02	0.02	0.02	0.02	0.02
	range	0.17-0.23	0.20-0.29	0.19-0.28	0.16-0.24	0.15-0.20
EI	mean			11.0	11.7*	11.2
	SD			1.0	1.9	1.3
	range			9.6-13.3	8.2-14.7	8.7-13.5
PFI	mean			3.88	4.52	4.87
	SD			0.46	0.36	0.33
	range			3.27-4.89	4.08-5.04	4.15-5.25
SI	mean			4.0	3.9*	4.3
	SD			0.4	0.32	0.7
	range			3-5	3-4	3-5
Hct	mean	29.1	31.2	29.8	24.4	39.6
	SD	6.6	3.7	6.5	7.3	5.7
	range	17.5-45.2	23.3-40.3	9.5-38.6	9.5-36.5	27.5-46.6
Hb	mean			7.49	4.52	5.52
	SD			1.24	1.62	0.70
	range			4.97-9.54	2.03-7.76	4.05-6.44
MCHC	mean			0.26	0.19	0.14
	SD			0.07	0.03	0.01
	range			0.20-0.52	0.15-0.23	0.12-0.17

* data taken from similar sized eels (n=13) from the same batch for comparison

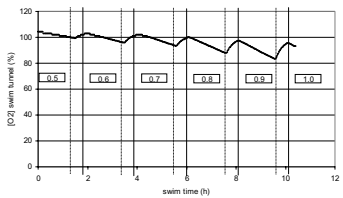

Figure 2 The $[O_2]$ in a swim tunnel as percentage of air saturation expressed against the swim time of an experimental farmed eel swimming (SW) in the tunnel during a speed test. During each U (solid lines), valves were closed for 1.5 h and opened for 0.5 h (dashed lines) to rinse the system. The downward slopes of $[O_2]$ at a specific U indicates $\dot{M}O_2$. Slopes were gradual without irregularities indicating steady consumption rates. Slopes became steeper during increasing Us showing increased $\dot{M}O_2$. This particular eel fatigued after swimming 17 minutes at 1.0 m/s.

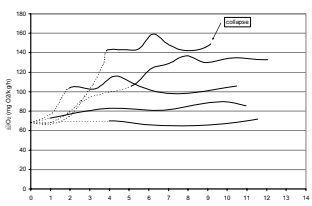

Figure 3 Typical experiment of an endurance test ($\dot{M}O_2$ profiles vs. swim time). A female silver eel (856 g, 73 cm) was exposed to step wise increasing water flow, the first day to 0.5 m/s and the fifth day to 0.9 m/s. The start speed was 0.5 m/s, the increments 0.1 m/s per hour. Each run lasted 12 hours, overnight the eels were rested. The starting point on the x-axis is the group average. Dashed lines indicate incremental U steps. $\dot{M}O_2$ rates were higher at every speed. Once reached the targeted U, $\dot{M}O_2$ rates were stable within a range of 20 mg O_2/kg/h.

farmed silver eel. $\dot{M}O_2$ rates per speed stayed well within a range of 20 mg O_2/kg/h. $\dot{M}O_2$ rates of the two grouped of farmed eels swimming in SW were pooled and expressed vs. U (Table 3). Paired observations are given of the same experimental eels for the two speed tests and the five endurance tests. Results of the speed tests indicated a conditioning effect, as during the second speed test eels fatigued later. Results for speed tests and endurance tests were similar. Only at a U of 0.8 m/s we found a significant difference in $\dot{M}O_2$ of the first speed test and the endurance tests. This difference was not significant between the second speed test and the endurance tests showing that this was due to conditioning during the experiment. In Table 3 we also expressed COT vs. U. COTs were rather constant between 45 and 53 mg O_2/kg/km at U/s of 0.5 up to 0.9 m/s. Only 10 out of the total 42 farmed eels were able to swim at 1.0 m/s for longer periods during the speed tests. COTs at these speeds were higher at 58 and 62 mg O_2/kg/km. Since results for speed and endurance tests were similar for these eels, we subjected other groups only to a single speed test.

Table 3 Pooled mean $\dot{M}O_2$ and COT data of farmed eels (SW) during the various U/s of speed test 1, 2 and the endurance tests. Comparison shows that $\dot{M}O_2$ data of the first speed test and endurance tests are similar. Only at 0.8 m/s, values were different probably due to conditioning. COTs were found between 45 and 53 mg O_2/kg/km and constant for speeds between 0.5 and 0.9 m/s. The asterix marks a significant (P<0.05) difference between the first speed test and the endurance test.

			swim speed (m/s)					
			0.5	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
$\dot{M}O_2$	**speed test 1**	**mean**	89	101	125	140	147	161
		SD	15	14	14	16	25	17
		n	35	36	30	17	8	3
	speed test 2	**mean**	87	100	126	150	167	173
		SD	15	17	17	21	21	17
		n	40	41	41	39	31	7
	endurance tests	**mean**	85	104	129	153*	161	
		SD	12	12	14	19	23	
		n	39	42	39	32	11	
COT	**speed test 1**	**mean**	49	47	50	49	45	58
		SD	8	7	6	6	8	6
		n	35	36	30	17	8	3
	speed test 2	**mean**	49	46	50	52	52	62
		SD	8	8	7	7	6	6
		n	40	41	41	39	31	7
	endurance tests	**mean**	47	48	50	53	50	
		SD	6	6	10	7	7	
		n	39	42	40	32	11	

Swim characteristics of farmed eels (SW)

$\dot{M}O_{2\ rest}$ of the smaller farmed eels was on average 38 ± 5 mg O_2/kg/h with a range between 27 to 48 (Table 4). The mean U_{crit} during the first speedtest was 1.24 ± 0.15 BL/s or 0.80 ± 0.08 m/s (Table 4). The mean U_{crit} during the second speedtest was 1.31 ± 0.13 BL/s or 0.84 ± 0.06 m/s. This difference was significant (P<0.05) and expressed a mean training effect of 5.6%. For endurance trials a U_{crit} value was found of 1.25 ± 0.12 BL/s. $\dot{M}O_{2\ max}$ values were often reached at speeds below U_{crit}. Swimming near or at U_{crit} involved apparantly a large anaerobic component creating an oxygen debt. $\dot{M}O_{2\ max}$ was on average 136 ± 23 mg O_2/kg/h for the first speed test, 165 ± 32 mg O_2/kg/h for the second speed test

Table 4 Means, standard deviations (SD) and their range of swim parameters measured on experimental eels of all groups a to e. For both groups of farmed eels swimming in SW, two speed tests and an endurance test were performed, for other groups just one speed test. The given COT is the lowest at the optimum swim speed. Bold are statistic differences (P<0.05; Table 5) between groups as indicated.

		a) Farm, small, SW (20)			b) Farm, large, SW (22)			c) Farm, large, FW (20)	d) Lake Grevelingen SW (19)	e) Loire FW (20)
		speed 1	speed 2	endurance	speed 1	speed 2	endurance	speed 1	speed 1	speed 1
U_{crit}	mean	**1.24 (ae)**	1.31	1.25	1.05	1.11	1.13	**1.15 (ce)**	0.91	**0.81 (ace)**
(BL/s)	SD	0.15	0.13	0.12	0.12	0.13	0.12	0.20	0.20	0.24
	range	1.02-1.54	1.01-1.50	1.04-1.48	0.83-1.32	0.82-1.32	0.89-1.34	0.69-1.43	0.48-1.21	0.48-1.29
U_{crit}	mean	0.80	0.84	0.80	0.74	0.78	0.79	0.81	0.71	0.66
(m/s)	SD	0.08	0.06	0.06	0.08	0.08	0.07	0.13	0.14	0.18
	range	0.71-0.93	0.71-0.92	0.73-0.90	0.62-0.91	0.61-0.93	0.66-0.90	0.51-0.94	0.42-0.96	0.40-0.91
U_{opt}	mean	1.02	0.98	0.92	0.96	0.85	0.82	0.94	0.77	0.74
(BL/s)	SD	0.16	0.13	0.09	0.23	0.07	0.06	0.11	0.15	0.09
	range	0.74-1.41	0.82-1.37	0.79-1.07	0.74-1.69	0.74-1.00	0.70-0.95	0.70-1.15	0.56-1.02	0.58-0.87
U_{opt}	mean	0.65	0.63	0.59	0.68	0.60	0.58	0.66	0.62	0.61
(m/s)	SD	0.10	0.06	0.04	0.18	0.05	0.03	0.06	0.12	0.05
	range	0.50-0.89	0.54-0.82	0.53-0.66	0.52-1.22	0.52-0.72	0.52-0.65	0.49-0.74	0.45-0.82	0.51-0.69
$\dot{M}O_{2\ rest}$	mean	38			**35 (be)**			38	**35 (de)**	**43 (bde)**
(mg/kg/h)	SD	5			4			5	8	13
	range	27-48			28-41			28-49	26-50	31-77
$\dot{M}O_{2\ max}$	mean	136	165	158	134	159	148	130	152	138
(mg/kg/h)	SD	23	32	26	16	16	18	26	39	38
	range	105-175	93-218	115-212	106-161	122-189	105-178	93-191	80-224	91-230
COT	mean	43	45	49	**46 (bc)**	45	45	**37 (bcd)**	**50 (cd)**	44
(mg/kg/km)	SD	5	6	6	8	5	4	5	8	11
	range	32-52	30-56	35-62	23-57	37-56	37-54	23-43	36-65	31-69

and 158 ± 26 mg O_2/kg/h for endurance tests (Table 4). Maxima were found up to even 218 mg O_2/kg/h. The mean U_{opt} during the first speedtest was 1.02 ± 0.16 BL/s or 0.65 ± 0.10 m/s (Table 4). The mean U_{opt} during the second speedtest was similar with 0.98 ± 0.13 BL/s or 0.63 ± 0.06 m/s. The mean U_{opt} for endurance trials was found slightly lower with 0.92 ± 0.09 BL/s or 0.59 ± 0.04 m/s, significant only vs. the second speedtest (P<0.05). COT values at U_{opt} were similar for both speed tests with 43 ± 5 and 45 ± 6 mg O_2/kg/km. Higher values (P<0.01) were found at the endurance trials with 49 ± 6 mg O_2/kg/km.

$\dot{M}O_{2\ rest}$ of the larger farmed eels was on average 35 ± 4 mg O_2/kg/h with a range between 28 to 41 (Table 4). The mean U_{crit} during the first speedtest was 1.05 ± 0.12 BL/s or 0.74 ± 0.08 m/s (Table 4). The mean U_{crit} during the second speedtest was 1.11 ± 0.13 BL/s or 0.78 ± 0.08 m/s. This difference was significant (P<0.05) and expressed a training effect of 5.7%, similar to the training effect found for smaller eels. For endurance trials a U_{crit} value was found of 1.13 ± 0.12 BL/s. $\dot{M}O_{2\ max}$ was on average 134 ± 16 mg O_2/kg/h for the first speed test, 159 ± 16 mg O_2/kg/h for the second speed test and 148 ± 18 mg O_2/kg/h for endurance tests (Table 4). Maxima were found up to 189 mg O_2/kg/h. The mean U_{opt} during the first speedtest was 0.96 ± 0.23 BL/s or 0.68 ± 0.18 m/s (Table 4). The mean U_{opt} during the second speedtest was lower (P<0.05) with 0.85 ± 0.07 BL/s or 0.60 ± 0.05 m/s. The mean U_{opt} for endurance trials was also lower (p<0.05) with 0.82 ± 0.06 BL/s or 0.58 ± 0.03 m/s. COT values at U_{opt} were similar for speedtests with 46 ± 8 and 45 ± 5 mg O_2/kg/km and for endurance trials with 45 ± 4 mg O_2/kg/km.

The group of large farmed eels was significantly larger (P<0.0001), heavier (P<0.0001) and had a higher condition factor (P<0.0001) than the group of smaller farmed eels (Table 2). Therefore the two groups could be used to study the size effects.
Group-wise comparison by ANCOVA showed that the $\dot{M}O_{2\ rest}$ and U_{crit} of the larger eels was lower but not significantly different (Table 5). On average the performance of larger eels was 9.6% lower for endurance tests and 15.3% for speed tests (resp. 1.4 and 2.2% per cm BL). Mean U_{opt} values were slightly lower for larger eels but also not significantly different. Absolute values were similar. COT values were slightly higher for larger eels but not significantly.

Individual comparison by correlation analysis (Table 6) showed that BL and BW significantly correlated with all absolute values for swim parameters. Positive correlations existed between size and $\dot{M}O_{2\ rest}$ (mg O_2/h; P<0.0001), $\dot{M}O_{2\ max}$ (mg O_2/h; P<0.0001), U_{opt} (m/s; P<0.05) and COT (mg O_2/km; P<0.0001). A negative correlation existed between size and U_{crit} (m/s; P≤0.05). Relatively, BL and BW significantly correlated negatively with $\dot{M}O_{2\ rest}$ (mg O_2/kg/h; P<0.01) and U_{crit} (BL/s; P<0.0001; Table 6). The negative correlation with the relative $\dot{M}O_{2\ rest}$ expresses the underestimation of values for smaller eels when extrapolating to 1 kg or, in other words, the metabolic weight of a smaller eel is larger than a larger eel. This relation was found in all groups (Fig. 3). The negative correlations between size and U_{crit} shows that larger eels not only reach relatively lower U_{crit} but also absolutely lower U_{crit}. Thus, small eels were able to swim significantly faster.

Table 5 Significant differences of swim parameters between groups by a) ANCOVA showing overall group effects and effects of BL and BW, followed by b) ANOVA showing the significant effects between particular groups. Values are P values and ns= not significant.

ANCOVA	group	BL	BW
$\dot{M}O_{2\ rest}$**	0.01	ns	<0.001
U_{crit}*	0.001	ns	ns
$\dot{M}O_{2\ max}$**	0.034	ns	<0.001
U_{opt}*	ns	ns	ns
COT**	<0.001	ns	<0.001

	ANOVA	Farm, large (SW)	Farm, large (FW)	Lake Grev. (SW)	Loire (FW)
Farm, small (SW)	$\dot{M}O_{2\ rest}$**		ns	ns	ns
	U_{crit}*		ns	ns	**0.005**
	$\dot{M}O_{2\ max}$**		ns	ns	ns
	U_{opt}*		ns	ns	ns
	COT**		ns	ns	ns
Farm, large (SW)	$\dot{M}O_{2\ rest}$**			ns	**0.02**
	U_{crit}*			ns	ns
	$\dot{M}O_{2\ max}$**			ns	ns
	U_{opt}*			ns	ns
	COT**		**0.008**	ns	ns
Farm, large (FW)	$\dot{M}O_{2\ rest}$**			ns	ns
	U_{crit}*			ns	**0.004**
	$\dot{M}O_{2\ max}$**			ns	ns
	U_{opt}*			ns	ns
	COT**			**<0.001**	ns
Lake Grev. (SW)	$\dot{M}O_{2\ rest}$**				**0.01**
	U_{crit}*				ns
	$\dot{M}O_{2\ max}$**				ns
	U_{opt}*				ns
	COT**				ns

* absolute values compared because no effects length and weight (U_{crit}: m/s, U_{opt}: m/s)
** relative value compared because effect weight (COT: mg/kg/km)

Swimming in SW vs FW

$\dot{M}O_{2\ rest}$ of farmed eels in FW was on average 38 ± 5 mg O_2/kg/h with a range between 28 to 49 (Table 4). A mean U_{crit} was found of 1.15 ± 0.20 BL/s or 0.81 ± 0.13 m/s (Table 4). $\dot{M}O_{2\ max}$ was on average 130 ± 26 mg O_2/kg/h (Table 4). A mean U_{opt} was found of 0.94 ±

Results were compared with results of the larger farmed eels swimming in SW. Both groups were of the same origin and very similar in age, morphological parameters and Hct and therefore considered as completely comparable (Table 2). The$\dot{M}O_{2\ rest}$ was higher but not significantly different for eels in fresh water (Table 4). The U_{crit} was found higher with 0.10 BL/s or 7 cm/s (9.5%) in fresh water but not significantly different. $\dot{M}O_{2\ max}$ was found similar. The U_{opt} was also found similar but the COT at these speeds was found significantly lower for swimming in FW (P<0.01; ANCOVA Table 5).

Table 6 Results of the swim fitness tests of Lake Grevelingen and River Loire groups were compared. $\dot{M}O_{2\ rest}$ was significantly higher but not for the Loire eels in Correlations between BL, BW and swim parameters, either as absolute (A) or relative values (R).

		$\dot{M}O_{2\ max}$	$\dot{M}O_{2\ rest}$	COT	U_{crit}	U_{opt}
ABSOLUTE						
BL	corr.	**0.781**	**0.761**	**0.743**	-0.257	**0.293**
	P	**0.000**	**0.000**	**0.000**	0.052	**0.041**
	n	**36**	**40**	**36**	41	**36**
BW	corr.	**0.859**	**0.863**	**0.794**	**-0.294**	**0.359**
	P	**0.000**	**0.000**	**0.000**	**0.031**	**0.016**
	n	**36**	**40**	**36**	**41**	**36**
RELATIVE						
BL	corr.	-0.197	**-0.440**	**-0.064**	**-0.673**	-0.051
	P	0.124	**0.02**	**0.356**	**0.000**	0.383
	n	36	**40**	**36**	**41**	36
BW	corr.	-0.182	**-0.450**	**-0.083**	**-0.643**	0.042
	P	0.144	**0.002**	**0.315**	**0.000**	0.404
	n	36	**40**	**36**	**41**	36

Swimming of Lake Grevelingen (SW) and River Loire (FW) migratory eels

$\dot{M}O_{2\ rest}$ of Lake Grevelingen eels in SW was on average 35 ± 8 mg O_2/kg/h with a range between 26 to 50 (Table 4). A mean U_{crit} was found of 0.91 ± 0.20 BL/s or 0.71 ± 0.14 m/s (Table 4). The $\dot{M}O_{2\ max}$ was on average 152 ± 39 mg O_2/kg/h (Table 4). A mean U_{opt} was found of 0.77 ± 0.15 BL/s or 0.62 ± 0.12 m/s (Table 4). COT values were 50 ± 8 mg O_2/kg/km.

$\dot{M}O_{2\ rest}$ of River Loire eels in FW was on average 43 ± 13 mg O_2/kg/h with a wide range between 31 to 77 (Table 4). A mean U_{crit} was found of 0.81 ± 0.24 BL/s or 0.66 ± 0.18 m/s (Table 4). $\dot{M}O_{2\ max}$ was on average 138 ± 38 mg O_2/kg/h (Table 4). A mean U_{opt} was found of 0.74 ± 0.09 BL/s or 0.61 ± 0.05 m/s (Table 4). COT values were 44 ± 11 mg O_2/kg/km.

Results of the swim fitness tests of Lake Grevelingen and River Loire groups were compared. $\dot{M}O_{2\ rest}$ was significantly higher but not for the Loire eels in fresh water (P=0.01; Table 4; Table 5). The U_{crit} was found lower in Loire eels in fresh water but not significantly with 0.10 BL/s or 5 cm/s (11%), comparable with the 9.5% found in farmed eels in FW. $\dot{M}O_{2\ max}$ was found lower in Loire eels but not significantly. The U_{opt} was found similar and the COT was found lower in Loire eels but not significantly (Table 5).

Swimming of farmed eels vs wild migratory eels

Results of the swim fitness tests between the large farmed eels and the wild migratory eels were compared. For SW and FW identical differences were observed. The wild migratory eels had lower U_{crit}, lower U_{opt} and higher COT. ANCOVA showed however effects of bodyweight on $\dot{M}O_{2\ rest}$, $\dot{M}O_{2\ max}$ and COT. By eliminating effects of BW and effects of swimming in SW or FW, only U_{crit} was found significantly different between farmed and wild eels. Differences between farmed and wild eels were more pronounced in fresh water.

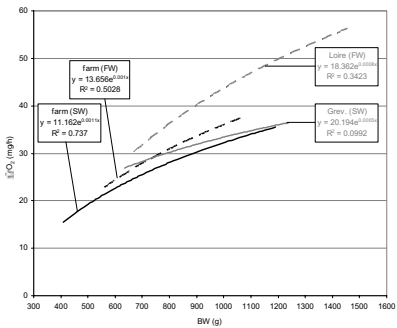

Figure 4 Trendlines of $\dot{M}O_{2\ rest}$ vs. BW for experimental eels from the farm in SW or FW and wild migratory eels in SW or FW.

DISCUSSION

Swim fitness of silver eels

This study has been the first study testing the swim capacity and efficiency of large female silver eels on a large scale. The enormous amount of 500,000 $\dot{M}O_2$-datapoints was collected for analysis. Earlier swim experiments were performed only on small eels (< 60 cm; Davidson, 1949; Schmidt-Nielsen, 1972; Webb, 1975; Tsukamoto et al., 1975; McCleave, 1980; Mitchell, 1989; Barbin & Krueger, 1994; Gilis, 1998; Langdon & Collins, 2000; Van Ginneken et al., 2002) and often in low numbers. At Leiden University we have a set-up of 22 Blazka swim tunnels. The swim performance of carp *Cyprinus carpio* and rainbow trout *Oncorhynchus mykiss* has been investigated in our tunnels (Van Dijk et al., 1993; Van Ginneken et al., 2005b) and provided similar results as reported in literature. Recent swim experiments with large eels mainly concerned long term simulated migration trials (Van Ginneken & Van den Thillart, 2000; Van den Thillart et al., 2004; Van Ginneken et al., 2005b).

In this study we found that silver eels showed similar swim performance during speed tests and endurance tests. We did not find a difference between swimming for 2 h and swimming for 12 h, except for minor conditioning effects. Farmed eels that were exercised at the second speed test fatigued later and showed about 5% higher U_{crit}. Also exercised rainbow trout *Oncorhynchus mykiss*, sockeye salmon *Oncorhynchus nerka* and coho salmon *Oncorhynchus kisutch* tend to fatigue later than unexercised individuals (reviewed by Beamish, 1978).Eels kept $\dot{M}O_2$ rates rather constant during swimming at all speeds, for 2 h as well as for 12 h periods. During 12 h swimming the rates stayed within a range of 20 mg O_2/kg/h (Fig. 3). The COTs at these *U*s between 0.5 and 0.9 m/s were also rather constant staying between 45 and 53 mg O_2/kg/km. This shows the ability to stabilise and maintain metabolic balance. Results of the speed test have therefore highly predictive value. This makes it possible to determine swim performance in a single speed test, thus according to a single day protocol. In this study, we have applied the speed test to measure the swim fitness of various groups of silver eels.

High swim efficiency of silver eels

We found that silver eels swam highly efficient. At optimum swim speeds of 0.58-0.68 m/s or 0.74-1.02 BL/s, COT values were found of 37-50 mg O_2/kg/km which are very low. In comparison, Videler (1993) reviewed results for 12 undulatory swimming fish species and reported optimum swim speeds of 0.18-0.51 m/s or 0.8-2.8 BL/s with corresponding COT values of 113-475 mg O_2/kg/km. From biomechanics it is known that for physical reasons locomotory specialisations are mutually exclusive, e.g. cruisers like silver eels necessarily have poor accelerating capabilities and vice versa (Gemballa, 2005). Indeed, we found that silver eels did not swim fast relatively to other species. Critical swim speeds were found between 0.81 up to 1.24 BL/s or 0.66 up to 0.81 m/s for the various groups of eels. Burst speeds are supposed not to be much higher in comparison with the 1.9 BL/s (1.14 m/s) found for a 60 cm eel swimming for 2-5 s in a swim tunnel (Blaxter & Dickson, 1959). In comparison with eel elvers, McCleave (1980) found burst speeds of 7.5 BL/s or 0.53 m/s. These values are comparable to another migrating species with an elongated body, the sea lamprey *Petromyzon marinus*. This species has critical swim speeds of 1.01 up to 1.34 BL/s or 0.82 up to 1.19 m/s (Almeida et al., 2005). Values of eels are low in comparison to cyprinid and salmonid species having prolonged swim speeds above 3 BL/s and mean burst speeds of 10 BL/s (reviewed by Videler, 1993). We can therefore conclude that silver eels are highly efficient cruisers with poor performance at high swim speeds. This indicates a high degree of locomotory specialisation in favour of cruising as a probable result of 60 million years of selection pressure on Darwinian fitness.

Constraints of salinity and habitat on swim fitness

Energy expenditure during exercise was higher in SW than in FW. The COT of farmed eels was found 20% higher when swimming in SW. Also wild eels showed a higher COT in SW although this effect could be due to the difference in origin. These results agree with the hypothesis that osmoregulation would require higher energy expenditure. Measurements in literature are scarce and do not provide a clear picture. Energy expended for swimming by rainbow trout *Oncorhynchus mykiss* and tilapia *Tilapia nilotica* was found to be independent of salinity. However, changes in metabolic rate did occur, suggesting that

overall performance would still be reduced (reviewed in Beamish, 1978). Morgan & Iwama (1998) did not find significantly different oxygen consumption rates in juvenile coho salmon *Oncorhynchus kisutch* after increase of salinity. Possibly the osmotic difference together with the permeability of the gills determines how much extra energy must be spend by the animal which may be species specific. Increase in oxygen consumption may also be explained by the environment that is natural for the specific life-history stage like suggested for salmonids by Morgan & Iwama (1991). Recently, Rankin et al. (2006) found that marine osmoregulation in eels indeed improves during silvering, as shown by a positive correlation between eye index and plasma osmolality at 36h after transfer to seawater, and is maintained until ovulation. However, experimental eels that were used for comparison between swimming in fresh and salt water in our study were all silver so this would not account (partly) for a rise of oxygen consumption.

The wild eels used in this study were much older than the farmed eels. The silver eels from Lake Grevelingen were on average 11 ± 3 years when migrating and younger and less silver than the eels from River Loire of 16 ± 4 years. Surprisingly, these wild eels were not in more advanced silver stages in comparison with the farmed eels both according to the indices of Pankhurst (1982) and Durif (2005). They had however much longer pectoral fins than the farmed eels. Although we expected that the wild eels would be better cruisers than the farmed eels, we found trends for even lower U_{opt} (8-9% for m/s) and higher COT (9-20%). The U_{crit} was found significantly lower (4-19% for m/s). These findings accounted for silver eels of both locations. Evidently, the wild eels had a lower condition that might reflect a difference in trophic quality. Firstly, fat percentages in wild eels were lower as indicated by K and was observed during sampling. A higher fat percentage results in an easier maintance of neutral buoyancy (Seibel, 2005). Secondly, silver eels from the Loire River, which scored the lowest U_{crit} and U_{opt} values, suffered from severe infection with the swim-bladder parasite *Anguillicola crassus*. This suggests that *A. crassus* impairs of swim capacity and efficiency of silver eels.

Implications for reproductive migration to the Sargasso

Optimum swim speeds for wild silver eels in SW were 0.77 BL/s or 0.62 m/s. When we assume that silver eels cruise at optimum swim speeds, these speeds are much higher than the generally assumed cruise speed of 0.5 BL/s. These optimum swim speeds agree with sustained cruising speeds between 0.6 – 0.9 BL/s found for 11 silver eels (69 to 96 cm) tracked in the North Sea by Tesch (1974; reviewed by Beamish, 1978). As for other tracking studies on migrating silver eels, optimum swim speeds only correspond to the fastest migration speeds found in the wild (Tesch, 1978, 1989, 2003; Tesch et al., 1991; McCleave & Arnold, 1999; Jellyman & Tsukamoto, 2002). If female silver eels would cruise at optimum swim speeds, they would travel 3.5 months to the Sargasso instead of the generally assumed 6 months (Tesch, 1977). Consequently, female migration may start later and/or spawning may start earlier (reviewed by McCleave, 2003).

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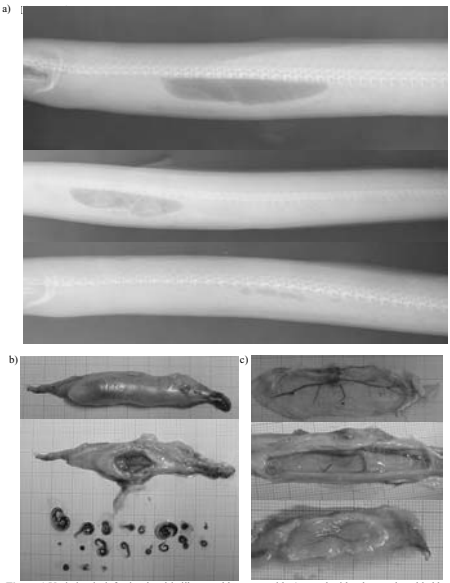

Figure 1 Variation in infection level is illustrated by x-ray with a) an eel with a large, clean bladder (top), an eel with a medium sized bladder with visible parasites (middle) and an eel with a small bladder with minimal volume (bottom), b) Dissection of the swim-bladder, whole (top) and cut open (bottom) showing abundance of 19 parasites of various sizes and c) three levels of swim-bladder wall transparency, -thickness and length showing a large, thin-walled transparent swim-bladder (top), a medium-sized swim-bladder with a thicker wall (middle) and a small thick-walled non-transparent swim-bladder (bottom).