Chapter 3

Swim efficiency and reproductive migration of silver eels

are severely impaired by the swim-bladder parasite *Anguillicola crassus*

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CHAPTER 3

ABSTRACT

Infection with the swim-bladder parasite *Anguillicola crassus* is suggested as one of the causes of the collapse of eel populations worldwide. This nematode has been introduced 20 to 30 years ago from Asia and parasitised in a short time various eel species in different geographical regions of the world. The effects are energy drain due to its sanguivorous activities and mechanical damage of the swim-bladder wall by its migratory activity. These effects are hypothesized to impair the spawning migration of the European eel. In this study, we have investigated the effects of infection on swim performance. We hypothesized that parasitic sanguivorous activities reduce swim endurance while the mechanical damage impairs buoyancy control. Eighty eels suffering various degrees of infection have been introduced in the swim-tunnels and subjected to a swim fitness test. For the first time, oxygen consumption was measured of large infected silver eels swimming at different swim speeds, allowing to determine swim efficiencies. We found that especially silver eels are target of infection. Infected eels have lower cruise speeds and higher cost of transport. Eels that are not infected but contain a swim-bladder damaged by previous infection, show similar effects. Almost half of these eels stopped swimming at low speeds < 0.7 m/s. Effects thus seem to be associated with swim-bladder disfunction and the resulting loss of neutral buoyancy. This leads to the conclusion that infected eels with damaged swim-bladders will likely fail to reach the spawning grounds. Simulated migration trials confirmed fast migration failure (<1,000-km). This study shows that *A. crassus*-infection severely impairs the reproductive potential of eel. Recent studies indicated similar roles for PCB pollution and virus infection. We can therefore conclude that the downfall of quality of future genitors may well be a major acting force behind the eel's world-wide collapse.

INTRODUCTION

Eel populations worldwide are dangerously close to collapse (Anonymous, 2003). Rapid decline started in the 80s and ever since no signs of recovery have been observed. Several causes have been suggested such as over fishing, habitat destruction, pollution and introduction of new diseases. Between 20 and 30 years ago two new diseases were introduced from Asia *e.g*. a virus EVEX (van Ginneken et al., 2004, 2005c) and a nematode infection with *Anguillicola crassus*, originally a parasite of the Japanese eel *A. japonica.* It took about one decade to spread the *Anguillicola crassus* infection over large parts of Europe (Neumann, 1985; Székely et al., 1991; Moravec, 1992; Evans & Matthews, 1999) and more recently it also reached the United States (Johnson et al., 1995). In a short time, various eel species in different geographical regions of the world were parasitised (Moravec & Taraschewski, 1988), likely due to worldwide eel shipments.

Since its introduction in Europe, many authors described its life cycle (Haenen et al., 1989; De Charleroy et al., 1990; Thomas, 1993). Adults of *A. crassus* reside in the swim-bladder. Eels are physostomes, which is considered a primitive condition and means that the swim-bladder has an open connection with the environment through the gut. The swim-bladder can be filled with O_2 from the gas gland or, with eel, also by gulping air (Bone et al., 1999). The main function of the swim-bladder is to obtain neutral buoyancy. In the lumen, the parasites feed on eel blood. Here the females produce eggs, which are

passively transported via the pneumatic duct to the oesophagus and finally through the digestive tract into the environment. The hatched larvae are eaten by copepodes that serve as intermediate hosts. The copepods are eaten by a number of fish species (Thomas & Ollevier, 1992; Székely, 1994; Pazooki & Székely, 1994) and other animals like aquatic insects, crustacea, snails and amphibians that serve as new paratenic hosts (Kennedy & Fitch, 1990; Thomas & Ollevier, 1992; Moravec & Konecny, 1994; Pazooki & Székely, 1994; Székely, 1995; Moravec, 1996; Moravec & Skorikova, 1998). All may serve as eel's prey. Larvae of *A. crassus* migrate directly through the wall of the digestive tract of the eel to the swim-bladder wall and finally end up in the lumen where they mature.

The life cycle of *A. crassus* in Japanese eel lasts for about one year (Egusa, 1979) while in European eel it takes only two months (De Charleroy et al., 1990). The infection causes lesions in European eel in contrast to infection of Japanese eel (Egusa, 1979). In addition, the number of parasites per infected eel is much higher in European eel (Egusa, 1979). Furthermore, parasites display higher survival rates and a higher reproductive success (Knopf & Mahnke, 2004). Clearly the European eel is more sensitive and less effective in its defense against *A. crassus*. Growth of infected eels was found to be reduced (Boon et al., 1990a), but mass mortality is thus far only observed in combination with additional unfavourable conditions, such as unusually long lasting high water temperature in Lake Balaton (Molnár et al., 1991; Molnár et al., 1993), and serious bacterial infections in Dutch eel farms (Van Banning and Haenen, 1990).

There are basically two kinds of adverse effects of *A. crassus* infection (Höglund et al., 1992): 1) energy drain due to sanguivorous activities of the parasite per se, and 2) mechanical damage of the swim-bladder wall. Concerning effect 1, Boon et al. (1990b) found that the sanguivorous activities of the parasites decrease the number of circulating erythrocytes and therefore the oxygen carrying capacity. Highly infected active eels are therefore presumed to have lower aerobic performance. Molnár (1993) proved that in decreasing oxygen content of the water severely infected eels die first,while uninfected specimens endure the hypoxic condition for a long time. Concerning effect 2, the migratory activity of the larvae in the swim bladder wall and the direct invasion of the pre-adults and adults in blood vessels result in extensive damage of the bladder wall (Molnár et al., 1993). Pathological changes include haemorrhages, formation of parasitic nodules, inflammatory cell proliferation, hypertrophy of connective tissue, necrotic areas and oedema. These changes eventually cause substantial thickening of the swim bladder wall (Molnár et al., 1993; Beregi et al., 1998) and shrinkage of the swim bladder.

Effects of severe *A. crassus* infection are hypothesized to impair the migration to the spawning grounds in the Sargasso Sea and therefore also impair reproduction. Since eels migrate about 5,500-km, probably at great depths, a decrease of oxygen carrying capacity and disfunctionality of the swim bladder will likely reduce the swimming capacity. Parasitism does not seem to impede pressure resistance (Vettier et al., 2003). However, eels rested during pressure exposure, so this experiment did not provide evidence for a functional swim-bladder. Two earlier studies investigated the influence of *A. crassus* on swimming of eel. Sprengel & Lüchtenberg (1991) found reduction of maximum swimming speed of eels between 17 and 45 cm. Heavy infected eels showed a reduction of maximally 18.6%, lowering average swim speeds from 0.725 to 0.590 m/s. However, in contrast, Münderle et al. (2004) could not verify those results for similar sized eels (40.3 ± 2.7 cm,

81 ± 16 g) at similar speeds of 0.62 m/s. Maximum swim speed, swimming performance and oxygen consumption were found similar between infected and uninfected eels. However, these studies were performed with small eels (maximum 45 cm) without specification of sex and life stage (silver males or yellow). Obviously large silver eels should be tested over long distance and periods, as not only swim speed but particularly a low cost of transport and a high endurance are crucial for long distance migration. Moreover, until now no discrimination was made between the direct and indirect effects of infection on swimming. When for instance a silver eel with a perfect condition and a functional swim-bladder is infected with many small larvae, then the initial migration phase of the eel would proceed without any problems. However, with increasing parasite load, more and more blood will be drained, resulting after some time in impaired swim performance. On the other hand, when an eel recovered from a severe infection, it would have a thickened swim-bladder wall. In that case the condition of the eel may be perfect, however, it would be unable to control buoyancy, and will thus still be incapable to reach the spawning site.

Recently, we developed an experimental test to quantify swim performance (*chapter 2*). This single-day 'swim fitness' test is an incremental speed test that can be used to predict endurance performance. The objective of this study is to investigate the relation between swim endurance and the adverse effects of *A. crassus* infection: 1) energy drain by parasites, and 2) buoyancy loss due to the mechanical damage of the swim-bladder wall.

MATERIALS AND METHODS

Choice of experimental eels

For this study, we used eels from Lake Balaton because of two reasons. Firstly, the population of Lake Balaton eels generally displays high infection levels, especially at the end of the summer, which caused massive mortality in the past (Molnár et al., 1991, 1993). Secondly, Lake Balaton eels were at least 12 years old at the time of experiments since the lake was last restocked with glass eels in spring 1991 and has no endemic eel population (Bíró, 1992).

Catch, selection and x-ray of experimental eels

At the end of August of the subsequent years 2002 (n=40) and 2003 (n=40), eels were caught by electrofishing in Lake Balaton (Hungary) in the region of Keszthely and Tihany. Eels were transported to the laboratory in oxygen-filled plastic bags and then kept in concrete basins or plastic tanks with flow-through water until they were scanned by means of x-ray (Fig. 1) using the method described by Beregi et al (1998) and Székely et al. (2004, 2005). X-ray scans were used to measure the swim-bladder length (SBL) and to determine the actual swim-bladder status of the given eel specimen.. Eels were marked individually by injecting Passive Integral Transponder (PIT)-tags (TROVAN) subcutanously just behind the head. After a few days rest, the eels were packed into large oxygen-inflated nylon bags in boxes and sent to Leiden by air-mail early September (2002 and 2003).

Swim-tunnel set-up and oxygen consumption

A set of 22 Blazka-type 127-L swimtunnels as described by Van den Thillart et al. (2004) were used for the swim trials. The tunnels are 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 pigment changes during silvering, it was assumed that this far-red light is invisible for eels (Pankhurst & Lythgoe, 1983). The oxygen level in each tunnel was measured continuously by an oxygen electrode (Mettler Toledo). 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:

$\dot{M}O_2 = 127 \cdot \Delta[O_2]/\Delta t$ (mg O_2/kg/h),

where: $\Delta[O_2]/\Delta t$ is the decrease of the oxygen content per hour.

Experimental protocol

Experiments were performed in 2002 and 2003. Eels (n=80) were introduced into the swim tunnels in fresh water at a constant temperature of 18 ± 1ºC at least two days before the experiment started. Before introduction, eels were anaesthetized with oil of cloves (1:10 dissolved in 100% ethanol using a dosage of 1-1.5 ml / 1 water). Oxygen electrodes were calibrated with sodium sulfite and air. Oxygen consumption was measured for a period of 3-4 h in rest. Eels were subjected to a swim fitness test described in *chapter 2*. In short: Eels started to swim at a swim speed (U) of 0.5 m/s for 2 h. During these 2 h, we measured the decreasing oxygen content in the tunnel for the first 1.5 h after which 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. Subsequently, this was repeated with steps of 0.1 m/s for U up to 1.0 m/s. After each step the oxygen consumption was measured over the first 1.5 h, while during the last 0.5 h the water in the tunnel was refreshed. If oxygen levels came below 75% saturation, flushing occurred automatically raising AS level within 15 min to 85%. The swimming behaviour of the eels and their position in the swim tunnel was registered every 15 min. When the fish fatigued during the trials, the velocity was lowered immediately to 0.1 m/s. This velocity can be considered as resting state as eels had the choice either to swim or to rest.

Swim parameters

To characterise swim capacity and efficiency we derived five parameters (see *chapter 2*):

1) Oxygen consumption at rest ($\dot{M}O_{2\,rest}$) in mg O_2/h/kg,
2) The critical swim speed (U_{crit}) calculated according to Brett (1964),
3) Maximum $\dot{M}O_2$ at subcritical swim speeds ($\dot{M}O_{2\,max}$) in mg O_2/h/kg,
4) The speed at which the amount of work per distance reaches a minimum (Tucker, 1970): the optimum swim speed (U_{opt}),
5) The cost of transport (COT) at U_{opt} in mg O_2/h/kg.

The U_{opt} 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 U_{opt} 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.

Measurements and sampling

Morphometric parameters of the eels were measured before they were introduced into the swim tunnels including: bodylength (BL), bodyweight (BW), eye diameter horizontal (EDh) and vertical (EDv), pectoral fin length (PFL), BL (cm) and BW (g) and:

Fulton's condition factor $K = 100^* BW/BL^3$.

The eye index according to Pankhurst (1982) $EI = 100^* ((EDh+EDv)/4)^2\pi/10^*BL)$

The pectoral fin index according to Durif et al. (2005) $PFI = 100^* PF/BL$

The silver index according to Durif et al. (2005) based on BL, BW, ED and PF.

From the eels of 2003, 0.5 ml blood was taken before and after swimming. Haemotocrit (Hct) was determined immediately upon sampling. The remaining blood was centrifuged for 5 min at 14,000 rpm and bloodplasma was stored at -80˚C for later analysis of total blood protein (TP). Pre- and post swimming bloodplasma was defrosted on ice, 30* diluted and measured for TP content with a bicinchoninic acid protein assay reagent (assay #23225, Pierce Chemical Company, USA).

The swim-bladder was dissected and photographed on paper with a reference mm grid (Fig. 1). The bladder was cut open and the number of parasites was determined (Fig. 1). Parasites were preserved in 4% buffered formalin. These samples were used for wet weight determination of parasites (PW). For the determination of the direct effects of the infection i.e. the sanguivorous activities of the pre-adult and adult parasites, we calculated the weight of the parasites relative to the weight of the eel as parasite index (PI):

$PI = (PW / BW)$,

where PW is the parasite total weight (mg) and BW is the eel body weight (kg).

For determination of indirect effects of infection by mechanical damage of the swim-bladder wall, we calculated the length of the swim bladder relative to length of the eel as swim-bladder index (SBI):

$SBI = (SBL / BL)$,

where SBL is the swim-bladder length (cm) and BL is the eel body length (cm).

Statistics

Normality of data distribution was tested with Kolmogorov-Smirnov tests. For comparison of parameters before and after swimming one-tailed paired t-tests were performed. For comparison of parameters between swimmers and eels that fatigued at low speeds (drop-outs) and between eels of various silver stages, one-tailed unpaired t-tests were performed. For comparison of swim parameters between healthy, infected and damaged groups of eels, one-tailed univariate analyses of covariance (ANCOVA) was performed. Bodylength or -weight was used as cofactor. In case the cofactor did not have significant influence and to estimate between which groups the effect was significant, ANOVA with post-hoc Bonferoni correction was performed. Comparison of the number of eels that either stopped swimming before reaching a swim speed *U* of 0.7 m/s or continued swimming thereafter was tested with a Mann-Whitney U test. For correlation analyses, one-tailed Pearson tests were performed. All tests were performed in SPSS 10.0 for Windows. Results were calculated and plotted as means ± SD.

RESULTS

Status of eels before swimming

The experimental eels (n=80) measured 67 ± 6 cm (range 54-82 cm), weighed 466 ± 145 g (range 228-865 g), and had a condition factor K of 0.15 ± 0.02 (Table 1). The mean eye index (EI) was 8.38 ± 2.50; 71% of the eels had EI>6.5 and could thus be considered as silver. Among the experimental eels only 4% were in stage FII (residents), 56% in stage FIII (premigrants) and 40% in stage FV (active migrants), no eels were in stage FIV. The pectoral fin index (PFI) showed little variation between (yellow and silver) eels and was found 4.80 ± 0.44%. When considering characteristics of eels in the various migratory stages, stage FII residents (n=3) were the smallest at 57 ± 1 cm weighing 279 ± 12 g with a K of 0.15 ± 0.01. Stage FIII premigrants (n=39) were larger and measured 64 ± 5 cm weighing 390 ± 97 g with a K of 0.15 ± 0.01. Stage FV migrants (n=35) were the largest measuring 71 ± 5 cm weighing 573 ± 124 g with a K of 0.16 ± 0.02.

Table 1 Morphometric parameters (mean ± SD) of experimental eels (BL= body length, BW= body weight, K= condition factor, EI= eye index, PFI= pectoral fin index, SI= silver stage).

parameters	mean	SD	range
BL (cm)	67	6	54-82
BW (g)	466	145	228-865
K	0.15	0.02	0.11-0.20
EI	8.38	2.50	4.99-16.34
PFI	4.80	0.44	3.80-6.05
SI	3.9	1.1	2,3,5

Anguillicola crassus infection and swim-bladder damage

The swim-bladder length (SBL) as indicator of shrinkage by damage was non-invasively determined by X-ray before swimming of 78 eels (Fig. 1a), two eels were not scanned. The SBL was 7.22 ± 2.45 cm (range 2.1-12 cm; Table 2). Relatively to the length of the eel (swim-bladder index SBI) these values were 10.8 ± 3.5%. After swimming, swim-bladders were dissected. The numbers of parasites were found between 0 up to 28 (Fig. 1b; Table 2). Parasite weight (PW) was between 0 up to 1.93 g. Relatively to the weight of the eel (parasite index PI) these values were 29.9 ± 51.2 mg/kg. We observed that the SBL was correlated to its volume, transparency, and thickness of the wall (Fig. 1c). Eels had swim-bladders with SBIs in the range 5.0-15.6 (Fig. 2). Swim-bladders with SBI<10 contained 25% of the accumulative parasite weight while those with SBI>10 contained 75% of the accumulative parasite weight. Thus, larger swim-bladders exhibited higher parasite loads. Accordingly, when the swim-bladder was smaller, parasite load became smaller. Non-infected swim-bladders were found of all sizes. Large swim-bladders had thin, semi-transparent walls and showed only slight signs of damage (thickening) or pre-infection. The smallest swim-bladders had damaged and thickened walls that had severely

reduced the swim-bladder volume. This condition reflected the reaction of the swim-bladder to high pre-infection loads making it unsuitable even for re-infection.

Table 2 Parasite characteristics (mean ± SD and range; n=80). Swim-bladder damage was indicated by its length (SBL) and was determined by X-ray. Infection load was given by the number of parasites and parasite weight (PW) and was determined by dissection.

			n	mean	SD	min	max
damage	**SBL**	**(cm)**	78	7.22	2.45	2.1	12.0
	SBI	**(%)**	78	10.8	3.5	3.0	19.0
infection	**parasites**	**n**	78	3.4	5.3	0	28.0
	PW	**(g)**	71	0.15	0.29	0	1.93
	PI	**(mg/kg)**	71	29.9	51.2	0	295.0

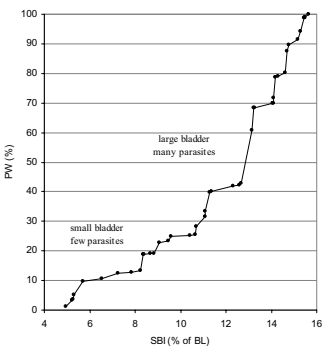

Figure 2 Accumulative parasite weight PW (%) plot against the swim-bladder index SBI (% of BL) of infected eels. The smaller swim-bladders have lower parasite loads, while larger swim-bladders exhibit higher loads.

We pooled data in three groups based on the presence of parasites (infected/not infected) and, in the not infected eels, the SBI (larger or smaller than the mean): 1) a relatively healthy group represented by 13 eels with large swim-bladders (SBI ≥ 10) and

without parasites, 2) an infected group represented by 43 eels with all sized swim-bladders with parasites and 3) a damaged group represented by 14 eels with small swim-bladders (SBI < 10) and without parasites. All groups contained eels of similar BL and BW.

Relation between silver stage, swim-bladder infection and damage

Parasite index (PI) and Swim-bladder index (SBI) were compared between the eels of the represented silver stages (Fig. 3). A clear relation was found between the silver stage and the level of infection. The infection load significantly increased comparing the resident stage FII with the premigrant stage FIII ($P<0.001$) and the stage FII with the active migrants stage FV ($P<0.05$). This did not account for damage. Thus, silver migratory eels experienced highest infection levels (Fig. 3).

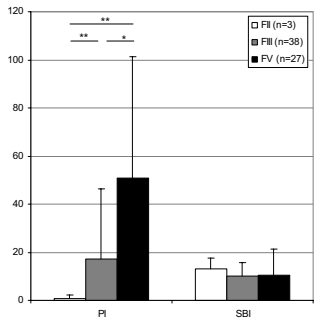

Figure 3 Relative parasite weight (PI in mg/kg) and swim-bladder length (SBI in % of BL) in experimental eels representing silver stage FII, FIII and FV. Stage FIV was not represented. Active migrant silver eels harboured significantly more parasites and had a higher PI (* $P<0.05$ and ** $P<0.001$) than residents and pre-migrants. The SBI was not different between stages.

Swimming of experimental eels

Of 74 eels a complete set of swim data was collected. In general, two groups of swimming eels could be distinguished. A group of eels (from here on referred to as "drop-

outs", n=27) stopped swimming before reaching a swim speed U of 0.7 m/s. They swam unsteady and were not able to maintain balance in the swim-tunnel. The number of data points did not suffice to derive the polynomial and thus to determine the optimum swim speed (U_{opt}) and cost of transport (COT) for these eels. Another group consisted of steady swimmers (from here on referred to as "swimmers", n=47) that continued swimming at swim speeds ≥0.7 m/s. The drop-outs had a critical swim speed (U_{crit}) of 0.54 ± 0.07 m/s vs. 0.73 ± 0.09 m/s for the swimmers. Their $\dot{M}O_2$ rates were significantly higher. In rest, the difference between drop-outs and swimmers was not significant (resp. 41.7 ± 9.7 vs. 38.4 ± 8.0 mg/kg/h). But already at the start of swimming at 0.5 m/s the difference was significant (P<0.05) with resp. 129 ± 34 vs. 101 ± 30 mg/kg/h. The drop-outs showed indications of lower haematocrit Hct (P=0.07) and lower SBI (P=0.06).

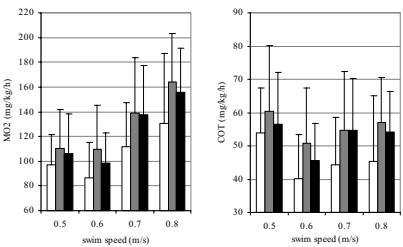

Figure 4 Oxygen consumption levels ($\dot{M}O_2$) and cost of transport (COT) of healthy eels (white bars), infected eels (grey bars) and damaged eels (black bars) at swim speeds between 0.5 and 0.8 m/s. $\dot{M}O_2$ was higher (ANCOVA; P<0.01) for infected and damaged eels at all swim speeds. COT tended to be higher for infected and damaged eels at all swim speeds.

Influence of infection and damage on swimming

To analyse the influence of infection and damage on swimming, we compared values of swim parameters between the healthy, the infected and the damaged group by ANCOVA. Figure 4 shows oxygen consumption ($\dot{M}O_2$) and cost of transport (COT) levels at the various swim speeds. $\dot{M}O_2$ levels (P=0.01) and COT (ns) were found higher in the infected (13%) and damaged groups (9%) at all speeds. No difference was found in oxygen consumption in rest ($\dot{M}O_{2rest}$) but a significant effect of BW on $\dot{M}O_{2rest}$ (P<0.001) existed (Fig. 5a). Maximum oxygen consumption ($\dot{M}O_{2max}$) was higher in infected and damaged eels but not significantly (Fig. 5b). The effect of BW on $\dot{M}O_{2max}$ was significant (P<0.001).

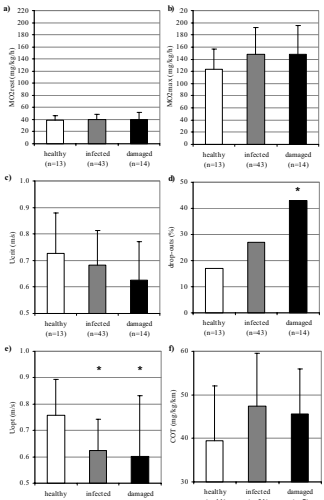

Figure 5 Swim parameters of healthy eels (white bars), infected eels (grey bars) and damaged eels (black bars). Healthy eels had large swim-bladders (SBI ≥ 10) without parasites. Infected eels had all-sized swim-bladders with parasites. Damaged eels had small swim-bladders (SBI < 10) without parasites. Significant differences (P<0.05) are indicated by asteriks. No significant differences were found for a) oxygen consumption in rest ($\dot{M}O_{2\,rest}$) b) and maximal oxygen consumption ($\dot{M}O_{2\,max}$), c) critical swim speeds (U_{crit}) tended to decrease with increasing damage and d) 43% of these eels dropped out before reaching U_{opt} (Mann-Whitney; P=0.03), e) eels with small swim-bladders had lower optimum swim speeds U_{opt} (ANCOVA; P=0.01) and f) cost of transport (COT) tended to increase with increasing damage.

Critical swim speed (U_{crit}) was lower in the infected group and even more in the damaged group (Fig. 5c) but not significantly different. There was a significant effect of BL on U_{crit} (P=0.01). In the healthy group of eels, the percentage of drop-outs was 17% (Fig. 5d). In the infected group of eels, this percentage was higher with 27%. Of the damaged eels, 43% dropped out which was significantly different (P<0.05) from the healthy eels (Fig. 5d). The optimum swim speed (U_{opt}) was 18 and 21% lower (P=0.01) in resp. infected eels and eels with damaged swim-bladders (Fig. 5e). Healthy eels had COT values of 40 mg/kg/h that increased 18 and 21% in respectively damaged eels and infected eels (Fig. 5f).

Blood parameters before and after swimming

The average haematocrit (Hct) percentage before swimming (eels 2003 only; n=40) was 31.4 ± 7.6 % (Table 3). Total protein (TP) content was 52.8 ± 5.6 mg/ml bloodplasma. No relation was found with silver stage (premigrants vs. migrants). Stage FIII premigrants had a Hct 30.2 ± 9.3% (n=18 eels from 2003). TP content was 53.6 ± 6.0 mg/ml bloodplasma. Stage FV migrants had Hct values of 32.4 ± 5.9% (n=22 eels from 2003). TP content was 52.1 ± 5.2 mg/ml bloodplasma. Blood parameters of stage FV migrants were similar as those of stage FIII eels, indicating that there was no difference in physical condition.

The average Hct percentage of 32.8 ± 5.7% after swimming was slightly higher than before but not significantly different (Table 3). The same applied to TP content with 53.5 ± 4.7 mg/ml bloodplasm. No correlations were found between the parasite index (PI) vs. Hct and TP. Although weak, decreasing trendlines of PI vs. Hct and TP could be plot, values for infected eels fell well within the large range of indivual variation of parasite-free eels. Correlations found between PI and the difference of Hct and TP were not found *i.e.* high parasite loads did not correlate with the level of change in Hct and TP due to swimming.

Table 3 Blood characteristics before (pre) and after (post) swimming. Paired observations on eels from the 2003 experiment (n=40) of Hct and TP are shown. (SD= standard deviation, min= minimium and max= maximum).

			n	mean	SD	min	max
pre-swimming	**Hct**	**(%)**	40	31.4	7.6	10.2	48.4
	TP	**(mg/ml)**	40	52.8	5.6	38.3	64.6
post-swimming	**Hct**	**(%)**	40	32.8	5.7	20.6	44.4
	TP	**(mg/ml)**	40	53.5	4.7	46.9	64.3

DISCUSSION

Infection and damage

This study attempted for the first time to estimate the effects of the *A. crassus* infection on swimming with respect to energy drain and swim-bladder damage. We used the relative parasite weight as a parameter for infection load and the swim-bladder length as parameter for the degree of damage. We observed that the length of the swim-bladder was correlated to its transparency and thickness of the wall (Palstra et al, unpublished data). The level of infection may impair the eel's condition and its endurance by energy drainage. The

level of damage may impair buoyancy control without affecting the eel's condition. High numbers of parasites (n>20) were found in large swim-bladders. Shorter swim-bladders contained less parasites. Density of parasites is constrained by space (Van Banning & Haenen, 1990; Ashworth & Kennedy, 1999; Lefebvre et al. 2002ab). Recently, Lefebvre & Crivelli (2004) showed that the infection rate is lower among eels with severely damaged swim-bladders. The shortest swim bladders did not harbor any parasites. Damage of these swim-bladders was so high that they were considered as totally disfunctional. It seems plausible that this eventually represents the endstage for all heavily infected eels. The space in the swim-bladder has become very limited by the thickened walls in the shortened swim-bladder reducing the change for survival and making reinfection very unlikely.

We did not find significant correlations between infection and haematocrit (Hct) and total protein (TP) content. Results in literature are contradictory. Boon et al. (1989) did not find a significant correlation with Hct but in a later publication he did find negative correlations with Hct and proteins (Boon et al., 1990b). Höglund et al. (1992) did not find a correlation with Hct but these authors did find a significant positive correlation between infection and total serum protein. Kelly et al. (2000) did not find significant correlations between infection and Hct, plasma glucose and many other physiological parameters. Parasites do not seem to cause anaemia (also Höglund et al., 1992) like with the EVEX virus (van Ginneken et al., 2004). Würtz et al. (1996) concluded that it does not seem that parasites show any sanguivorous activities but feed on surrounding tissue as can be concluded from their proteolytic enzymes (Polzer & Taraschewski, 1993). Thus, evidence about sanguivorous activities of the swim-bladder parasite and energy drainage in this way is still controversial.

Effects on swimming: reduced cruising ability and efficiency

In this study, for the first time oxygen consumption ($\dot{M}O_2$) was measured during swimming of large infected eels at various speeds. We found that infection and, even more, damage had serious effects on cruising ability and efficiency. Both infection and damage caused higher $\dot{M}O_2$ levels (resp. 13 and 9% ; P=0.02) at all swim speeds. Eels with damaged swim-bladders had a 21% decreased optimum swim speed (U_{opt}). These eels tended to raise cost of transport (COT) up to 18% (not significant). Almost half of these eels (43% ; P<0.05) dropped out below a swim speed of 0.7 m/s. Effects of infection and damage were similar but more pronounced in the latter. We hypothesize that additional energy is required to maintain neutral buoyancy. In the case that the swim-bladder's volume is reduced (by parasites and shortening of the swim-bladder), neutral buoyancy may become lost. Lift may be provided dynamicly by the pectorals to compensate for the loss of neutral buoyancy by a reduced swim-bladder volume (Bone et al., 1999). This mechanism is also illustrated by the fact that scombroid species, which do not have a swim bladder, must swim continuously with pectoral fins extended which produces a lift to overcome negative buoyancy. The 2 species with the fastest speeds necessary to counter negative buoyancy; skipjack (kawakawa) *Euthynnus affinis*, and Pacific bonito *Sarda chiliensis*, do not possess a swim-bladder (Beamish, 1978). To achieve lift by the pectoral fins, eels need to change to a more tilted position in the water column. Increase of swim-bladder damage would cause eels to swim harder to compensate. We found that maximum aerobic swim speeds tended to be negatively affected by infection and damage levels, but not

significantly. Also Münderle et al. (2004) concluded that U_{crit} for smaller eels was unaffected. Maximum aerobic speeds up to 1.64 BL/s were found for Lake Balaton eels and were comparable to those found for different other groups of eels (*chapter 2*). We can conclude that infected and especially damaged swim-bladders are reduced in volume that may cause loss of neutral buoyancy. Swim efficiency in eels with damaged swim-bladders is significantly impaired as indicated by higher $\dot{M}O_2$ and COT levels and lower $U_{opt.}$. These eels stop swimming at a swim speed below 0.7 m/s.

Migration failure

Infected eels and eels with damaged swim-bladders experiencing impairment of swim efficiency are likely to fail migration. We did observe that all eels from Lake Balaton that stopped swimming during simulated migration trials had heavily damaged swim-bladders (Palstra et al., unpublished results); 80% of these eels stopped within 42 days and before reaching 1,000-km. This represented 30% of the total number of eels. Furthermore, in experiments where we hormonally stimulated infected silver eels from the Loire River (France; Palstra et al., unpublished results), we found that infection levels were still high after 6 months of captivity in salt water, while there was no chance of reinfection. This means that parasites survive longer or may have parasite larvae that develop such that swim-bladder damage progresses even under salt water conditions (also Kennedy & Fitch, 1990; Kirk et al., 2002ab). Székely et al. (2005) confirmed that during prolonged laboratory maintenance of *A. crassus* infected eels no improvement can be observed in the condition of the swimbladders.

Migratory silver eels are targets for infection

The old age of >12 years of the experimental eels was confirmed in another study by otolith analysis (Palstra et al., unpublished results). All eels in that study were between 13 and 21 years of age (n=20). Tátrai et al. (2003) examined 114 Lake Balaton eels (395-690 mm long and 112-760g weight) and confirmed the old age. Among the experimental eels were residents (FII) and premigrants (FIII), but also 40% active migrants (FV). It seems curious that migrant stage FIV was not represented. This might well be due to the absence of major differences in pectoral fin length, an important discriminator between stage 4 and 5 according to the PCA cluster analysis plots of Durif et al. (2005). The high percentage of active migratory Lake Balaton silver eels is in contrast with Bíró (1992), who stated that Lake Balaton eels never become silver and do not migrate. Accordingly also with Székely et al. (1991), Molnár et al. (1991, 1993), Békési et al. (1997), Nimeth et al., (2000), Sures et al. (2001) and Vettier et al. (2003) who stated that metamorphosis and migratory activity were impeded.

We found that the silver eels clearly displayed highest infection levels. We believe that a shift to higher quantity and quality (by e.g. piscivory) of food preceding silvering may be the proximate cause of higher infection chances and rates. The highest infection levels in Lake Balaton are found at the end of the summer at the time of silvering. Since especially migratory silver eels are targeted by infection, the impact of the adverse effects of infection is greater.

Reproductive failure by swim-bladder parasite

When results are extrapolated to the field, we can conclude that the damage of the swim-bladder wall caused by parasite infection with *A. crassus* very likely leads to a fast migration failure. In most European habitats 40 up to 90% of the eel population is infected (Sprengel & Lüchtenberg, 1991; Wurtz et al., 1998; Lefebvre et al., 2002ab; Audenaert et al, 2003; Dekker, 2004; Lefebvre & Crivelli, 2004). In this study we found that especially the migratory silver eels are heavily infected thus rising the impact of the effects. We found that effects concern a significant impairment of swim efficiency causing failure of long-term migration. Thus, for the first time strong evidence of *A. crassus* having major implications on recruitment is provided. Since the decline of European eel populations had already started when the swim bladder parasite was introduced, it might not have been the single cause of the decline. Devastating effects were also found for the virus EVEX on long term swimming (van Ginneken et al., 2005c) and contamination with PCBs on long term swimming (van Ginneken et al., to be submitted) and even more so on embryonic survival and development (Palstra et al., 2006). All these agents determine the spawner quality as a product of their habitat. Since recruitment is the product of quantity and quality of spawners, downfall of quality may well be a major acting force behind eel's worldwide collapse.

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