

# ACUTE STRESS SYNDROME OF THE YELLOW EUROPEAN EEL (*ANGUILLA ANGUILLA* LINNAEUS) WHEN EXPOSED TO A GRADED SWIMMING-LOAD

by

VINCENT J.T. VAN GINNEKEN<sup>1,\*</sup>, PAUL BALM<sup>2</sup>, VINOD SOMMANDAS<sup>1</sup>, MARJOLIJN ONDERWATER<sup>1</sup> and GUIDO VAN DEN THILLART<sup>1</sup>

<sup>1</sup>*Integrative Zoology, Institute of Evolutionary and Ecological Sciences (EEW), Department of Biology, van der Klauuw Laboratories, P.O.Box 9511, 2300 RA Leiden, The Netherlands;* <sup>2</sup>*Animal Physiology, Department of Biology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands)*

## ABSTRACT

In a Blazka swim tunnel (length 170.0 cm; outer diameter swim-tunnel tube 28.8 cm and an inner diameter of 19.0 cm) short-term swim experiments with groups of 120 g eel (≈40 cm) at different swimming velocities varying from 0.25 to 3 body lengths (BL) per second were performed. In these experiments, substrates (FFA, glucose), the stress hormone cortisol, parameters from the ionic balance (sodium, potassium and chloride) and lactic acid were measured in the blood plasma at 0 (control group), 0.25, 0.5, 0.75, 1, 1.5, 2.0, 2.5 and 3.0 BL/sec. It is concluded that a swimming speed up to 2 BL/sec is not stressful for yellow eel because the ionic balance is maintained, there is no evidence for activation of the pituitary-interrenal axis and the anaerobic metabolism is not activated. However swimming performances above 2 BL/sec showed a dichotomous pattern: some animals showed no changes while others showed the 'acute stress syndrome' resulting in elevated cortisol levels, glucose mobilisation, ionic imbalance and lactate accumulation. Based on these observations it can be concluded that eel-like (anguilliform) swimming is more suitable for long term sustained swimming than for burst activity.

KEY WORDS: acute stress-syndrome, eels, *Anguilla anguilla*, cortisol, optimal swimming speed, swim-tunnel.

## INTRODUCTION

In the 1960s and 1970s swimming experiments were performed with salmon in a stream gutter (BRETT, 1965, 1967, 1973; BRETT & GLASS, 1973) with, respectively, goldfish (SMIT, 1965, SMIT *et al.*, 1971), trout (WEBB, 1971) and mackerel (HUNTER *et al.*, 1971) in swim-tunnels. Only recently the research with swim-tunnels or similar devices was continued in work on plaice (PRIEDE & HOLLIDAY, 1980), trout (JOHNSTON

---

\* Corresponding author: e-mail: ginneken@rulsfb.leidenuniv.nl

& MOON, 1980; Wood *et al.*, 1983; RISTORI & LAURENT, 1985), salmon (VIRTANEN & FORSMAN, 1987), Arctic charr (CHRISTIANSEN & JOBLING, 1990) and carp (VAN DIJK *et al.*, 1993). The latter study for example (performed at our laboratories) demonstrated that the combination of water acidification and labour works synergistically (VAN DIJK *et al.*, 1993). Stress induction by exercise may be an interesting research topic exclusively suitable for migrating anadromic fish species such as salmon, catadromic fish species such as eel and ocean rangers such as tuna (SMITH, 1985). The resulting physiological and endocrinological changes observed in fish after exposure to exercise can only be studied systematically in laboratory studies.

Limited studies on the swimming performance of eels or other anguilliform swimming teleosts are available (WEBB, 1975; MCCLEAVE, 1980; MÜLLER *et al.*, 1995). In particular, data at low swimming speeds (<3BL/sec) for eels are lacking, despite the indications that cruising speeds at this level are preferred by this fish species (see discussion). Earlier, MCCLEAVE (1980) tried to obtain swimming data of elvers at speeds of 3BL/sec or below. He did not succeed because of the characteristic 'searching for shelter behaviour' of eels. Recently two kinematic studies with eels emerged, studying the pattern of undulatory locomotion in water and on land (GILLIS, 1998), and the changes in muscle performance between yellow and silver eels (ELLERBY *et al.*, 2001). Little is known about swimming performance and maximum swimming speed of yellow and silver eels. Because it recently became clear from the work of ELLERBY *et al.* (2001) that there are changes in muscle performance prior to migration, we hope in future studies to extend this work with similar studies on silver eel.

The objective of this study was to examine the swimming performance of yellow eel at different swimming speeds in order to determine the range of the aerobic scope. We hypothesise that at intermediary swimming velocities in the range of the aerobic scope, metabolite and ionic levels will remain constant in the blood, but at a certain threshold there is a collapse of the animal resulting in changes of primary and secondary stress parameters in the blood. This threshold point when anaerobic metabolism becomes activated is not strictly defined among animals and will depend on factors such as respiration capacity, energy stores, and capacity for end product elimination of individuals. Therefore, an individual variation of the aerobic scope among animals may be expected. To test this hypothesis we investigated in this study the sustained swimming speed (which is defined as the swimming speed at which an animal is capable of swimming at that speed for hours (HUNTER, 1971)) of yellow eel from a hatchery.

Short-term swim experiments with groups of 120 g eel ( $\approx 40$  cm) were performed in a Blazka swim tunnel at different swimming velocities

varying from 0.25 to 3 body lengths (BL) sec. In order to avoid the problems of MCCLEAVE (1980) with eels refusing to swim, the animals were forced to swim by an electrical grid placed at the bottom end of the tunnel. In the performed experiments, substrates (FFA, glucose), the stress hormone cortisol, parameters from the ionic balance (sodium, potassium and chloride) and lactate which is an indicator of activation of the anaerobic metabolism, were measured in the blood plasma at several levels of exercise.

In this study with yellow eel, we investigated at which swimming velocities the animals remained energetically in homeostasis and at which swimming velocity the aerobic scope is ended, resulting in a collapse of the animals with elevated cortisol levels, glucose mobilisation, ionic imbalance and lactate accumulation. Based on these observations we can determine the sustained swimming speed of yellow eel.

## MATERIAL AND METHODS

### *Animals*

Eels were obtained from a commercial eel farm *Royaal BV*, Helmond, the Netherlands. At the moment of selection, fish were aged from 1-3 years. In the laboratory the animals were kept for 1 month with a 14:10 light-dark cycle in running local tap-water in aquaria at  $19 \pm 1^\circ\text{C}$  and were fed daily with *Provimi* pelleted food (*Provimi*, Rotterdam, The Netherlands).

The day before the experiment the animals were weighed, their length was measured and they were placed in a group of six to seven animals in the swim-tunnel, which was supplied with a fresh water supply of 20 l per min. The mean body length of the total group, corresponding to  $39.6 \pm 5.6$  cm, was determined and was defined as 1 body length (1 BL) while the bodyweights were  $118.2 \pm 15.38$  g.

### *Blazka swim-tunnel*

The Blazka swim-tunnel was calibrated with a Laser Doppler technique at the Delft Hydraulics Laboratory, Technical University Delft. The Blazka swim-tunnel is given in detail in figure 1.

### *Experimental protocol and sampling procedure*

We selected the following swimming speeds for the sustained swimming effort for a period of 6 h: 0 (control group), 0.25, 0.5, 0.75, 1, 1.5, 2.0, 2.5 and 3.0 BL/sec. Fish were placed the evening before the day of the swimming effort in the swim-tunnel at  $19^\circ\text{C}$ . After an introductory period

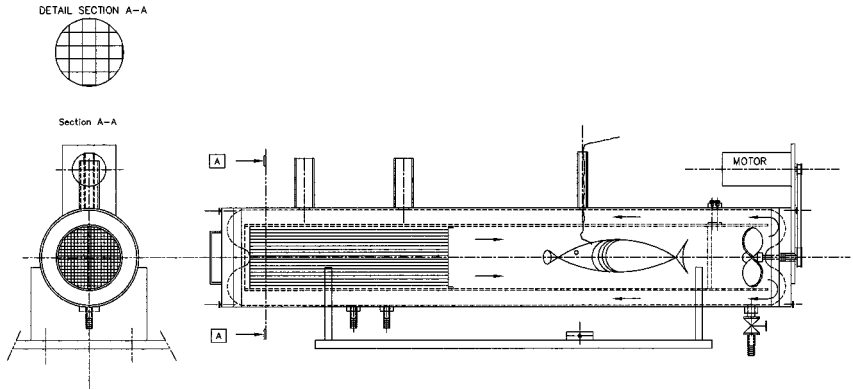


Fig. 1. Blazka swim tunnels used in the experiments with yellow eel. It has a length of 170.0 cm, an outer swim-tunnel tube diameter of 28.8 cm and an inner swim-tunnel tube diameter of 19.0 cm. The power of the engine is 400 W while the propeller consists of three blades of 7.5 in with a pitch of 7 in. At the top end of the swim-tunnel is a PVC flow conditioner with a length of 60 cm while at the propeller-end is placed a flow conditioner of 20 cm. The swimming compartment for the fish is 90.0 cm. At the bottom end of the tunnel a screen is placed of plaited silver wire of 1 mm thickness to conduct the electrical current to stimulate the fish to swim. The electrical current is a sinusoid with a peak of 10 V with a frequency of 1 sec. The Blazka swim-tunnel has earlier been depicted elsewhere (VAN DIJK *et al.*, 1993b).

of about 30 min at low speed (0.2 BL/sec) the fish were forced to swim at the selected swimming speed for a period of 6 h. Because it is known that eels do not swim voluntarily in swim tunnels (see MCCLEAVE, 1980), an electrical grid was placed at the bottom end of the tunnel to stimulate the fish to swim. After exposure to the exercise protocol, the fish were quickly anaesthetised with 300 PPM MS222 (3-aminobenzoic-acid-ethyl-ester methanesulfonate salt, Sigma, St Louis, USA). After 3 min the anaesthetised fish were taken out of the swim-tunnel and blood was collected with a heparinised syringe (flushed with 3000 units heparin per ml blood). Above 2 BL/sec we observed that some eels showed signs of fatigue and refused to swim. Those eels were directly removed from the tunnel *via* a special entry with valve at the bottom end of the Blazka respirometer. Thereafter, the animals were checked macroscopically for parasites on gills and in the swim bladder, or for signs of injury or external wounds. All animals looked healthy. Therefore, we can conclude that according to our initial expectation (see introduction) the threshold point when anaerobic metabolism becomes activated and the animals ultimately collapse will show an individual variation among animals for the factors 'swimming speed' and period of 'swim exercise'. Those animals which stopped swimming were called the 'exhausted group' (see table 1).

Since in the 'exhausted group' (except for lactate, see discussion) no trends and/or significant differences were observed between the different swimming velocities or swimming endurance periods (see table 2), data were pooled. Because at 3.5 BL/sec all animals collapsed and stopped swimming only data for the 'exhausted group' were available at this swimming speed (tables 1 & 2).

#### *Analytical method blood sample*

In the freshly collected blood samples treated with anticoagulant, haematocrit was measured directly in 9  $\mu$ l whole blood sample using a haematocrit micro-centrifuge (Bayer, FRG). Haemoglobin content in 20  $\mu$ l blood was detected after 3 min using the cyanmet-haemoglobin method (Boehringer Mannheim, FRG). Blood was directly centrifuged (10,000 rpm for 5 min). The plasma was divided in eppendorf tubes (50, 40, 50, 50, 33, 33  $\mu$ l, respectively, for cortisol, FFA, glucose, lactate and sodium, potassium and chloride analysis) and stored at  $-80^{\circ}\text{C}$  pending analysis. For the glucose measurements, 50  $\mu$ l plasma was mixed with 200  $\mu$ l 6% trichloric acid solution to precipitate plasma proteins and stored at  $-80^{\circ}\text{C}$ . Glucose was determined by colorimetric assay (Sigma, St Louis, USA). Cortisol was measured by radioimmunoassay (BALM *et al.*, 1994). FFA was measured with a commercial test-kit WAKO (NEFA C method, Instruchemie, Hilversum, The Netherlands). Lactic acid was determined with an enzymatic test-combination of Boehringer Mannheim: 139084 for L-lactate. Plasma sodium, potassium and chloride levels were measured by flame photometric and colorimetric procedures (Technicon) (BALM & POTTINGER, 1993).

#### *Calculations and statistics*

The mean value of every group (swimming velocity) was compared to the mean value of the control group (table 1). Mean  $\pm$  SD are given in the tables. Statistics were performed using a one-way ANOVA. Comparisons of mean squares of the ANOVA were tested using F-tests.  $P \leq 0.05$  was considered as statistically significant. Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov and  $F_{\max}$  tests, respectively.

## RESULTS

For the groups between 0 and 3.0 BL/sec, with the exception of plasma lactate at 1.5 and 2.0 BL/sec, no significant differences were observed

TABLE 1

Parameters measured in plasma of yellow eel (*Anguilla anguilla* L.) in a Control group (Co), exhausted group (Exh) and at different swimming velocities (0.25, 0.5, 1, 1.5, 2, 2.5, 3 Body Lengths (BL) per second, with 1 body length = 39.6 ± 5.6 cm). The mean value of six to eight animals is given with the standard deviation between brackets. \* denotes significant difference ( $P \leq 0.05$ ) from control group.

	Co (n = 6)	0.25 (n = 6)	0.5 (n = 6)	0.75 (n = 6)	1.0 (n = 7)	1.5 (n = 7)	2.0 (n = 6)	2.5 (n = 6)	3.0 (n = 6)	Exh (n = 8)
Haematocrit (%)	37.68 (4.80)	39.65 (4.26)	40.65 (4.17)	37.02 (5.62)	38.58 (4.28)	36.25 (5.30)	40.53 (5.52)	37.98 (2.17)	36.33 (2.30)	35.78 (3.86)
Haemoglobin (mM)	7.02 (1.30)	7.89 (1.10)	7.24 (0.67)	6.37 (0.94)	6.86 (0.56)	6.17 (0.57)	6.90 (0.42)	7.00 (0.55)	6.87 (0.26)	4.92 (0.92)
Plasma glucose (mM)	3.61 (1.10)	4.49 (1.44)	4.23 (1.16)	4.34 (1.24)	4.30 (1.68)	3.00 (1.52)	4.28 (1.46)	4.51 (0.93)	5.57 (1.57)	11.23* (2.30)
Plasma lactate (mM)	0.48 (0.08)	0.35 (0.07)	0.39 (0.07)	0.24 (0.05)	0.52 (0.11)	0.17* (0.03)	0.16* (0.04)	0.36 (0.06)	0.21 (0.06)	4.13* (1.73)
Plasma cortisol (ng/ml)	58 (23)	61 (14)	81 (20)	49 (34)	35 (9)	40 (16)	81 (26)	34 (24)	35 (26)	25 (19)
Plasma FFA (mM)	0.214 (0.107)	0.239 (0.016)	0.363 (0.068)	0.522 (0.189)	0.593 (0.159)	0.507 (0.159)	0.283 (0.045)	0.404 (0.085)	0.475 (0.127)	0.236 (0.107)
Plasma sodium (meq/l)	143.5 (15.0)	176.8 (17.2)	131.1 (10.8)	141.1 (18.4)	146.4 (33.8)	144.5 (12.0)	158.9 (19.6)	173.7 (24.8)	142.5 (7.2)	149.0 (13.2)
Plasma potassium (meq/l)	2.92 (0.60)	3.54 (0.60)	2.60 (0.40)	2.44 (0.40)	2.66 (0.60)	2.10 (0.20)	2.88 (0.40)	2.76 (0.60)	2.12 (0.40)	4.66* (0.71)
Plasma chloride (meq/l)	92.3 (17.6)	116.8 (15.2)	89.0 (9.4)	91.1 (12.0)	95.5 (21.4)	91.8 (11.6)	100.3 (15.0)	108.5 (15.4)	92.1 (8.0)	102.8 (20.6)

TABLE 2  
Overview of the composition of the exhausted group.

Animal	Time (min)	Swimming Speed (BL/sec)	Haematocrit %	Haemoglobin (mM)	Lactate (mM)	Glucose (mM)	FFA (mM)	Cortisol (ng/ml)	Na <sup>+</sup> (meq/l)	K <sup>+</sup> (meq/l)	Cl <sup>-</sup> (meq/l)
1	10	2.5	35	3.99	5.59	10.81	0.253	21.8	177.9	5.48	121.2
2	15	2.5	42.2	6.21	5.99	13.54	0.133	15.5	143.6	5.04	102.5
3	30	2.5	36.1	5.67	6.24	14.20	0.266	52.0	148.1	4.92	84.1
4	5	3	32.7	4.74	2.48	9.07	0.375	9.3	135.4	3.82	77.9
5	3	3	41.8	5.45	1.71	9.35	0.129	6.5	151.9	3.46	97.5
6	70	3.5	31.4	5.49	3.35	6.62	0.152	12.3	139.6	4.40	122.8
7	137	3.5	34.5	6.37	4.75	14.51	0.426	31.0	142.0	5.32	84.1
8	165	3.5	32.5	5.41	2.92	8.73	0.156	54.3	153.7	4.80	132.5
Mean	-	-	35.78	4.92	4.13	11.23	0.236	25.3	149.0	4.66	102.8
STD	-	-	3.86	1.92	1.73	2.30	0.107	18.8	13.2	0.71	20.6

with the control group for all parameters (table 1). In the 1.5 and 2.0 BL/sec groups, plasma-lactate was significantly lower compared to the control group and groups up to 1.0 BL/sec. The metabolism is probably still aerobic and in balance and the decline of plasma lactate can be explained because lactate acts like substrate that can be used by the exercised muscle *in situ*. MILLIGAN and WOOD (1986) demonstrated that more than 80% of lactic acid generated by the exercised muscle is retained in the muscle mass.

So up to a swimming speed of 2 BL/sec the eels are energetically and metabolically in equilibrium and can perform at this swimming speed without any collapse.

Above 2 BL/sec the anaerobic metabolism becomes activated resulting in a rise of plasma lactate. In those groups animals showed (in incidental cases) signs of fatigue and refused to swim from which the 'exhausted group' was created. It is clear that in those animals there is a collapse, the aerobic threshold has been passed, the anaerobic metabolism activated, and primary- (cortisol) and secondary- (glucose, lactate, potassium) stress parameters activated.

Because there was a large individual variation in the moment of exhaustion, the 'exhausted group' consisted of a pooled group of eight animals as given in table 2. Comparison of the 'exhausted group' with the control group revealed no significant differences for hematocrit, hemoglobin, FFA, cortisol,  $\text{Na}^+$  and  $\text{Cl}^-$ . BALM *et al.* (1994) however observed a rapid sampling-associated elevation of cortisol (BALM *et al.*, 1994). This was also observed in this study, giving the following cortisol values ranking according to fish number: 1)  $6.61 \pm 2.78$  (n = 9); 2)  $47.8 \pm 6.9$  (n = 9); 3)  $50.4 \pm 12.1$  (n = 9); 4)  $70.5 \pm 11.8$  (n = 9); 5)  $73.8 \pm 22.1$  (n = 9); 6)  $60.41 \pm 22.6$  (n = 9) (ng/ml). Therefore, it is more accurate to compare the first animal of the groups for plasma cortisol until 2 BL/sec with the 'exhausted group'. This gives more information about stimulation of the pituitary interrenal axis. First the cortisol data of 'first sampled animals' were checked with the Kolmogorov-Smirnov test for normality. They were distributed normally ( $P \leq 0.016$ ). Using this approach a strongly significant difference is observed between the exhausted group (cortisol:  $25.34 \pm 18.80$  ng/ml) and the pooled group of 'first sampled animals' (cortisol:  $6.61 \pm 2.78$  ng/ml) ( $P \leq 0.003$ ).

GILHAM and BAKER (1985) gave for unstressed and stressed European eel a plasma cortisol concentration of 21.4 vs 127.6 nmol/l respectively, which corresponds to 7.32 vs 43.6 ng/ml. Comparing our cortisol data with those values it is clear that the cortisol plasma value of the pooled group of 'first sampled animals' in our study corresponds with the value of GILHAM and BAKER (1985) for unstressed eels. However the cortisol



value found in our exhausted group is half of the value found by those authors in stressed eel (GILHAM & BAKER, 1985). Despite this lower value in our exhausted stressed group, the difference between control (pooled group of first sampled animals) vs exhausted eel group in our study is significantly different. In addition the rise of cortisol in the exhausted group corresponds with 383%. Based on these plasma cortisol values we conclude that the eels in the exhausted group were stressed by the experimental protocol (exhaustive exercise). Furthermore significant differences were observed between the control group and exhausted group for lactate (increase 860%), glucose (increase 311%) and  $K^+$  (increase 160%) (table 2). Those parameters are primarily used as secondary indicators of stress (WENDELAAR BONGA, 1997).

These results are indicative that up to 2 BL/sec swimming eels are energetically and metabolically in homeostasis. In general, the anaerobic threshold is above 2 BL/sec. Within the selected time interval of 6 h this leads in incidental cases to a collapse of animals with primary and secondary stress effects. It may be clear that there is an individual variation in the moment of this collapse. We found for individual animals a collapse at 2.5 BL/sec after 10, 15 and 30 min, respectively, at 3 BL/sec after 3 and 5 min, and at 3.5 BL/sec after 70, 137 and 165 min, respectively.

## DISCUSSION

The most important conclusion from this study is that at intermediary swimming velocities, within the aerobic scope, the swimming eels remain in homeostasis. Similar results were found with cannulated carp (VAN DIJK *et al.*, 1993a). However, beyond the aerobic scope, at a certain threshold there is a collapse of the animal, resulting in changes of metabolite levels and ion in the blood. This threshold point when anaerobic metabolism becomes activated is not strictly defined among animals and will depend on the aerobic/anaerobic capacity of the animal. Therefore an individual variation of the aerobic scope among animals may be expected in swimming speed and time of exposure before a collapse occurs. The 'exhausted group' is created from this pool of collapsed animals. In this respect, it can be questioned if in this way no selection is made for the weakest, injured or parasitised animals. This is partly true. Because we checked the animals macroscopically, injuries or parasites can be excluded, but in this way a selection can be made for the animals with the narrowest aerobic scope and the most limited anaerobic capacity. However, collapse of these animals all occurred above 2 BL/sec,

which does not undermine our main conclusion that the optimal swimming speed for yellow eel from a hatchery is up to 2 BL/sec. From the performed short-term swim experiments with eels of  $\approx 120$  g, it is concluded that a swimming speed up to 2 BL/sec is not stressful for yellow eel because the ionic balance is maintained, there is no evidence for activation of the pituitary-interrenal axis and the anaerobic metabolism is not activated. Above 2 BL/sec the animals showed signs of exhaustion which can be associated with the 'acute stress syndrome' resulting in lactate accumulation, elevated cortisol levels, glucose mobilisation and ionic imbalance. From studies with other fish species it was concluded that the 50% fatigue-time during a sustained swimming effort was 4 sec (BRETT, 1967) or 3 to 4 BL/sec (BLAXTER, 1969). The energy cost and efficiency for eel-like swimmers may be different compared to other fish species and probably this type of propulsion is more suitable for long distance swimming (see further). The tremendous rise of lactate in plasma in the 'exhausted group' demonstrates that the metabolism became anaerobic and that swimming was strenuous. In the 'exhausted group' it is characteristic that animals which at an earlier moment failed to swim and showed the 'acute stress syndrome' had higher levels of plasma-lactate. Probably the plasma-[lactate] is negatively correlated with the period of swimming. In animals which showed a longer endurance period of activity, plasma-[lactate] was lower. This observation probably can be explained because lactate acts like substrate. More than 80% of the lactic acid generated by the exercised muscle is retained in the muscle mass (MILLIGAN & WOOD, 1986).

Another characteristic of the 'exhausted group' is a hyperglycaemia. This can probably be attributed to glycogenolytic effects of catecholamines on the liver (NAKANO & TOMLANSON, 1967; MAZEAUD *et al.*, 1977). Those hormones only play a role during burst or violent exercise (RISTORI & LAURENT, 1985). It is generally accepted that at a sustained cruising speed, fish use lipid metabolism to drive red muscle (GORDON, 1968; BLAXTER, 1969; BILINSKI, 1974). However this was not reflected in the levels of the FFA which showed no significant difference with the control group at any swimming velocity, nor was there any change in the 'exhausted group'. Another characteristic of the 'acute stress syndrome' due to exercise is an elevation of the  $K^+$  plasma concentration. This result was confirmed in a study with rainbow trout (*Oncorhynchus mykiss*). In a moderate exercised group (1.5 BL/sec) potassium was significantly increased from controls after 2 h of exercise until the end of the experiment after 24 h. In this study, it was hypothesised that the elevated plasma potassium concentrations after moderate exercise may be the result of an efflux of potassium from the muscle tissue caused by

a blockage of the  $\text{Na}^+/\text{K}^+$  ion pumps due to a limited production of ATP (NIELSEN *et al.*, 1994). This hypothesis is confirmed by two observations: a) the level of the increased potassium concentration is dependent on the intensity level of exercise in rainbow trout (THOMAS *et al.*, 1987); b) muscular contraction can induce an efflux of potassium ions from the myoplasma into the extracellular fluid (SJOGAARD, 1990).

A question remains regarding the cause for the swimming failure in the exhausted group. Several mechanisms are plausible. First, the rise of the [lactate] by 860% may interfere with muscle contractility. A second key toxic event as proposed by WOOD *et al.* (1983) is an intracellular acidosis of the intracellular compartment. With *in vivo*  $^{31}\text{P}$ -NMR the intracellular pH of muscle tissue can easily be determined based on the chemical shift between creatinephosphate (PCr) and Inorganic Phosphate (VAN DEN THILLART *et al.*, 1989; VAN GINNEKEN *et al.*, 1995, 1996, 1999). In combined studies using a Blazka respirometer and a  $^{31}\text{P}$ -NMR spectrometer, carp and trout were exposed to severe exercise in a swim-tunnel. The dynamics of the recovery process of energy rich compounds and intracellular pH were followed with *in vivo*  $^{31}\text{P}$ -NMR, while the response of energy rich compounds were determined in different body compartments (blood, white-, red muscle and liver) *via* conventional biochemical methods. In this study, we observed a dichotomous response of trout and carp to exhaustive exercise: 50% of the animals recovered while 50% died. Comparing the data of the survival groups with the mortality groups for intracellular pH and depletion of the energy stores revealed that there was no significant difference for both fish species between survivors and non-survivors for intracellular pH which dropped to a value around 6.6-6.7. However, the difference between the survivors and non-survivors in depletion of energy stores was tremendous, resulting in a near depletion of the PCr and ATP pool in the non-survivors. Based on these observations, we concluded that the cause for fish mortality after extreme exercise is a depletion of energy stores and not an internal acidosis of the muscle compartment (VAN GINNEKEN *et al.*, 2001).

In literature, limited data on swimming performance of eels or other anguilliform swimming teleost are available (WEBB, 1975; MCCLEAVE, 1980, MÜLLER *et al.*, 1995). From the scarce field data the optimal swimming speed of eel can be calculated. ELLERBY *et al.* (2001) assumed a 60 cm eel with a speed of 0.48 BL/sec (=0.29 m/sec), at a constant swimming speed, would perform the distance from the European coast to the Sargassosea (approximately 5000 km) in 28.5 weeks. This swimming speed is extremely low for fish because burst activity, *i.e.* for salmon may correspond to 10-11 BL/sec (VIDELER, 1993). Results of this study are also indicative that eel-like (anguilliform) swimming is more suitable for long-term sustained swimming than for burst activity.

In conclusion, this relatively low cruising speed of up to 2 BL/sec for eel could be characteristic for this catadromic long distance traveler.

### *Perspectives*

Because ELLERBY *et al.* (2001) demonstrated a difference in muscle performance and properties between yellow or silver (=migratory stage) eel stages, this study with a Blazka swim tunnel and yellow eel has to be extended in future studies with similar experiments with silver eel.

## ACKNOWLEDGMENTS

We thank Ir. J. van Rijsingen and Dr Carolien Vancoillie, Royaal BV, Helmond, the Netherlands, for providing the yellow eel. Vincent van Ginneken is supported by a grant from the Foundation for Technical Research (STW), which is subsidised by the Netherlands Organization for Scientific Research (NWO), STW-project no. LBI66.4199.

## REFERENCES

- BALM, P.H.M. & T.G. POTTINGER, 1993. Acclimation of Rainbow Trout (*Oncorhynchus mykiss*) to low environmental pH does not involve an activation of the pituitary-interrenal axis, but evokes adjustments in branchial structure. *Can. J. Fish. Aquat. Sci.* **50**: 2532-2541.
- BALM, P.H.M., P. PEPELS, S. HELFRICH, M.L.L. HOVENS & S.E. WENDELAAR BONGA, 1994. Adrenocorticotrophic hormone (ACTH) in relation to interrenal function during stress in tilapia (*Oreochromis mossambicus*). *General Comparative Endocrinology* **96**: 447-460.
- BILINSKI, E., 1974. Biochemical aspects of fish swimming. In: D. Mallins and J. Sargent (Eds): *Biochemical perspectives in Marine Biology*, Vol. 1, 239-288. Academic Press, New York.
- BLAXTER, J.H.S., 1969. Swimming speeds of fish. *FAO (Food and Agricultural Organization, United Nations) Fish. Rep.* **62**: 69-100.
- BRETT, J.R., 1965. The swimming energetics of salmon. *Scientific American* **213**: 80-85.
- BRETT, J.R., 1967. Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. *J. Fish. Res. Bd. Can.* **24**: 1731-1741.
- BRETT, J.R., 1973. Energy expenditure of Sockeye Salmon *Oncorhynchus nerka*, during sustained performance. *J. Fish. Res. Board Can.* **30**: 1799-1809.
- BRETT, J.R. & N.R. GLASS, 1973. Metabolic rate and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *J. Fish. Res. Board Can.* **3**: 379-387.
- CHRISTIANSEN, J.S. & M. JOBLING, 1990. The behavior and relationship between food intake and growth of juvenile Arctic charr, *Salvelinus alpinus* L., subjected to sustained exercise. *Can. J. Zool.* **68**: 2185-2191.

- ELLERBY, D.J., I.L.Y. SPIERTS & J.D. ALTRINGHAM, 2001. Slow muscle power output of yellow- and silver-phase European eels (*Anguilla anguilla* L.): changes in muscle performance prior to migration. *J. Exp. Biol.* **204**: 1369-1379.
- GILHAM, I.D. & B.I. BAKER, 1985. A background facilitates the response to stress in teleosts. *J. Endocr.* **105**: 99-105.
- GILLIS, G.B., 1998. Environmental effects of undulatory locomotion in the American eel *Anguilla rostrata*: kinematics in water and on land. *J. Exp. Biol.* **201**: 949-961.
- GORDON, M.S., 1968. Oxygen consumption of red and white muscle from tuna fishes. *Science* **159**: 87-90.
- HUNTER, J.R., 1971. Sustained swimming speed of Jack Mackerel, *Trachurus symmetricus*. *Fishery Bulletin* **69**: 267-271.
- JOHNSTON, I.A. & T.W. MOON, 1980. Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). *J. Exp. Biol.* **87**: 177-194.
- MAZEAUD, M.M., F. MAZEAUD & E.M. DONALDSON, 1977. Primary and secondary effects of stress in fish: some new data with a general view. *Trans. Am. Fish. Soc.* **106**: 201-212.
- MCCLEAVE, J.D., 1980. Swimming performance of European eel (*Anguilla anguilla* L.) elvers. *J. Fish. Biol.* **16**: 445-452.
- MILLIGAN C.L. & C.M. WOOD, 1986. Intracellular and extracellular acid-base status and H<sup>+</sup> exchange with the environment after exhaustive exercise in the rainbow trout. *J. Exp. Biol.* **123**: 93-121.
- MÜLLER, U.K., J.H. SMIT, E.J. STAMHUIS & J.J. VIDELER, 1995. Flow field around a swimming juvenile eel. BIONA report 10, ed. W. Nachtigall. Fischer Verlag.
- NAKANO, T. & N. TOMLINSON, 1967. Catecholamine and carbohydrate metabolism in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. *J. Fish. Res. Bd. Can.* **24**: 1701-1715.
- NIELSEN, M. & L. BOESGAARD, 1994. Plasma levels of lactate, potassium, glucose, cortisol, growth hormone and triiodo-L-thyronine in rainbow trout (*Oncorhynchus mykiss*) during exercise at various levels for 24 h. *Can. J. Zool.* **72**: 1643-1647.
- PRIEDE, I.G. & F.G.T. HOLLIDAY, 1980. The use of a new tilting tunnel respirometer to investigate some aspects of metabolism and swimming activity of the plaice (*Pleuronectes platessa* L.). *J. Exp. Biol.* **85**: 295-309.
- RISTORI, M.T. & P. LAURENT, 1985. Plasma catecholamines and glucose during moderate exercise in the trout: comparison with bursts of violent activity. *Exp. Biol.* **44**: 247-253.
- SMIT, H., 1965. Some experiments on the oxygen consumption of goldfish (*Carassius auratus* L.) in relation to swimming speed. *Can. J. Zool.* **43**: 623-633.
- SMIT, H., J.M. AMELINK-KOUTSTAAL, J. VIJVERBERG & J.C. VON VAUPEL-KLEIN, 1971. Oxygen consumption and efficiency of swimming goldfish. *Comp. Biochem. Physiol.* **39A**: 1-28.
- SMITH, R.J.F., 1985. The Control of Fish Migration. Editors: B. Heinrich, W.S. Hoar, K. Johansen, H. Langer, G. Neuweiler, D.J. Randall, 243 pp. Springer-Verlag, Berlin Heidelberg.
- SJOGAARD, G., 1990. Exercise-induced muscle fatigue: the significance of potassium. *Acta Physiol. Scand.* **140**: 5-51.
- THOMAS, S., J. POUPIN, G. LYKKEBOE & K. JOHANSEN, 1987. Effect of graded exercise on blood gas tensions and acid-base characteristics of rainbow trout. *Respir. Physiol.* **68**: 85-97.
- VAN DIJK, P.L.M., G.E.E.J.M. VAN DEN THILLART, P. BALM & S. WENDELAAR BONGA, 1993a. The influence of gradual water acidification on the acid/base status and plasma hormone levels in the carp. *J. Fish Biol.* **42**: 661-671.

- VAN DIJK, P.L.M., G.E.E.J.M. VAN DEN THILLART & S. WENDELAAR BONGA, 1993b. Is there a synergistic effect between steady-state exercise and water acidification in carp? *J. Fish Biol.* **42**: 673-681.
- VAN GINNEKEN, V., G. VAN DEN THILLART, A. ADDINK & C. ERKELENS, 1995. Fish muscle energy metabolism measured during hypoxia and recovery: an *in vivo* <sup>31</sup>P-NMR study. *Am. J. Physiol.* **268**: R1178-R1187.
- VAN GINNEKEN, V., G. VAN DEN THILLART, A. ADDINK & C. ERKELENS, 1996. Synergistic effect of acidification and hypoxia: an *in vivo* <sup>31</sup>P-NMR study in fishes. *Am. J. Physiol.* **271**: R1746-R1752.
- VAN GINNEKEN, V., G. VAN DEN THILLART, H. MULLER, S. VAN DEURSEN, M. ONDERWATER, J. VISEE, V. HOPMANS, G. VAN VLIET & K. NICOLAY, 1999. Phosphorylation state of red and white muscle in tilapia during graded hypoxia: an *in vivo* <sup>31</sup>P-NMR study. *Am. J. Physiol.* **277**: R1501-R1512.
- VAN GINNEKEN, V., T. SUNDERMEIER, R. BOOT, K. COLDENHOFF, J. HOLLANDER, F. LEFEBER & G. VAN DEN THILLART, 2001. The question 'why do fish die after severe exercise?' revisited: an *in vivo* <sup>31</sup>P-NMR Study. *Am. J. Physiol.*, submitted.
- VAN GINNEKEN, V. & G. VAN DEN THILLART, 2000. Eel fat stores are enough to reach the Sargasso. *Nature* **403**: 156-157.
- VIDELER, J.J., 1993. Fish Swimming. Chapman & Hall, London, Fish and Fisheries Series 10, 260 pp.
- VIRTANEN, E. & L. FORSMAN, 1987. Physiological responses to continuous swimming in wild salmon (*Salmo salar* L.) parr and smolt. *Fish Physiol. and Biochem.* **4**: 157-163.
- WEBB, P.W., 1971. The swimming energetics of trout 11. Oxygen consumption and swimming efficiency. *J. Exp. Biol.* **55**: 521-540.
- WEBB, P.W., 1975. Hydrodynamics and energetics of fish propulsion. *Bull. Fish. Res. Board. Can.* **190**: 77-105.
- WENDELAAR BONGA, S.E., 1997. The stress response in fish. *Physiological Reviews* **77**: 591-625.
- WOOD, C.M., J.D. TURNER & M.S. GRAHAM, 1983. Why do fish die after severe exercise? *J. Fish. Biol.* **22**: 189-201.