Are yellow eels from Lake Balaton able to cope with high pressure encountered during migration to the Sargasso sea? The case of energy metabolism

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Abstract—Eels from Lake Balaton are unique because they do not undergo the silvering process and do not migrate. The question is whether these eels, despite such particularities, retain their ability to cope with migration constraints, usually high pressure. To ascertain this, eels were exposed for 3 days to 10.1 MPa of hydrostatic pressure (HP) and the effects of this on aerobic metabolism were evaluated by measuring oxygen consumption (MO_2), Cytochrome Oxydase activity (COX) and energetic nucleotide contents in red and white muscles. The results show that Balaton eels survive HP. However, 3 days under pressure induces an alteration in aerobic metabolism. Moreover, when only muscle fibres are exposed to HP, there is a significant decrease in maximal aerobic capacities (−20%). The results are discussed in terms of the ability of these eels to migrate, bearing in mind that this activity represents a high percentage of maximal aerobic capacity when compared with other populations.

Keywords: aerobic metabolism; *Anguilla anguilla* L.; hydrostatic pressure; parasitism; yellow eels.

INTRODUCTION

The European silver eel is known to encounter hydrostatic pressure (HP) during its migration to the supposed spawning area, the Sargasso Sea (Tesch, 1977). Such a physical performance requires an efficient aerobic metabolism because the slow aerobic muscles are the ones most used. Silver eels, which have begun to metamorphose in view of imminent migration, exhibit an improvement in aerobic metabolism, mainly in red muscles (Bostrom and Johansson, 1972; Egginton, 1986).

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Recent studies have shown that yellow and sexually immature freshwater eels are also pre-acclimated to HP, and therefore to migration. In fact, they present an optimal metabolic design (Sébert et al., 1998) with an over-functioning of oxydative phosphorylation at atmospheric pressure, allowing normal mitochondrial functioning under pressure (Theron et al., 2000; Sébert and Theron, 2001). This pre-acclimation relates to eels living in rivers or open ponds, enabling them to migrate and to complete their life cycle. The case of Lake Balaton eels, about whose energy metabolism little is known, is particular, since these fishes never become silver and do not migrate (Bíró, 1992). In fact they were introduced into the lake (Bíró, 1992), and this new and unknown environment, together with nematode infestation, probably impedes metamorphosis and migratory activity (Székely et al., 1991; Molnár et al., 1991, 1993; Bekesi et al., 1997; Nimeth et al., 2000; Sures et al., 2001).

This study has two objectives: 1) to present a general view of the energy metabolism of this specific eel population; and 2) to test its pressure resistance in order to evaluate its migratory capacities or, more specifically, to see if impossibility of migration decreases or cancels the eel's acclimation capacity. With this objective, aerobic metabolism was evaluated before, during and after 3 days HP exposure in order to assess energy metabolism status. Such a timescale was chosen because, in our experience, it has been shown that after compression there is a critical 2-day period, but once this threshold has been passed, long-term survival is possible.

MATERIAL AND METHODS

Animals

Sixty yellow freshwater European eels (*Anguilla anguilla* L.) were caught by electro-fishing in November 2002 in the Tihany region of Lake Balaton, Hungary. Sixteen were selected by radiography in Hungary to determine the severity of swimbladder changes caused by anguillicolosis (Beregi et al., 1998). Pre-selected specimens were sent to France by air in polyethylene bags (six specimens with mild, and six specimens with severe swimbladder changes). Only 12 specimens were used for HP experiments (mass 155 ± 5 g; mean $\pm$ S.E.M.). They were stored without feeding for at least 10 days prior to the experiments, in 40 litre polyethylene tanks with continuously renewed and aerated tap water. The experiments were performed during the winter and, in order to maintain water temperature ($15.3 \pm 0.2°C$) and photoperiod as closely as possible to natural conditions, the tanks were placed in a room open to the outside.

Morphometry

The eels were slightly anaesthetised (*Eugenia caryophyllata,* clove oil, 1 ml to 10 l of water; ethanol, 10 ml for 10 l of water) in order to perform morphometric

measurements: body length (BL, mm), body mass (BM, g), horizontal and vertical eye diameters (ODh, ODv, mm) and pectoral fin length (PFL, mm). Different indexes were calculated from these data:

$$\text{Condition Factor} \left(CF = \left(\frac{BM}{BL^3} \right) * 10^5 \right);$$

$$\text{Ocular Index} \left(OI = \left[\frac{(\frac{ODh + ODv}{4})^2 * \pi}{BL} \right] * 100 \right);$$

and pectoral fin index

$$\left(PFI = \frac{PFL}{BL} * 100 \right).$$

The CF (Durif et al., 2000) is commonly used in fisheries to follow fish growth; OI (Pankhurst, 1982) and PFI (Durif et al., 2000) are silvering indexes.

Protocol

All the experiments were performed at $15°C$. The eels were placed in an experimental tank (14.9 l), connected to a high-pressure water circulation system, and the tank was placed in a hyperbaric chamber (Sébert et al., 1990). The water circulation system allowed continuous renewal of the water, so that temperature and oxygen concentrations could be controlled.

After 1 day at atmospheric pressure, the hyperbaric chamber was compressed at a rate of 1 MPa min^{-1} to 10.1 MPa and this pressure was maintained. During the experiments, water oxygen partial pressure (Pw_{O_2}) and temperature (Tw) in the experimental tank were regularly monitored; water flow was approximately 30 l h^{-1}. The water flow was stopped twice a day for 30 min in order to calculate oxygen consumption from the decrease in Pw_{O_2} (confinement method, Sébert and Barthélémy, 1985). After 3 days at this pressure, the chamber was decompressed at the same rate of 1 MPa min^{-1}. On arrival at atmospheric pressure, the eels were immediately killed by decapitation and their blood was collected in glass capillaries to determine the haematocrit. Three sections of the eel were frozen in liquid nitrogen then stored at $-80°C$. Red muscles (the colour of which was noted) and white muscles were sampled to perform direct oxygen consumption measurements and, finally, white muscle and gills were sampled and weighed before and after desiccation in order to determine water content. The same procedures were used for the control group which was kept in the same experimental tank under the same environmental conditions (water temperature and oxygen content, light, noise, time) but at atmospheric pressure (0.1 MPa). However, the red muscle sample of the control group was also used for direct measurement of oxygen consumption under pressure.

MEASUREMENTS

Fibre oxygen consumption

After decapitation, the red muscle was dissected along the lateral line and the white muscle was dissected on the left side, at about 30% of total length, close to the vertebral column. Dissection was completed within 4 min. The muscle samples were immediately placed in an ice-cold extraction medium (Sébert and Theron, 2001). The technique used to perform the permeabilisation of muscle fibres was adapted to fish from Veksler et al. (1987) and Letellier et al. (1992) by Sébert and Theron (2001). Muscle fibres were permeabilised using a saponin solution to alter the cell membrane but not the mitochondrial membrane, so as to perform mitochondrial respiration measurements.

The methods used have been previously described by Theron et al. (2000) and Sébert and Theron (2001). At atmospheric pressure, respiration was measured in a glass vessel following its oxygen content decrease, using a Strathkelvin Instrument® O_2 microelectrode (accuracy: ±0.2% saturation). A vessel was specially designed, using an electrode with pressure compensation (YSI 5739), to measure oxygen consumption under pressure and its control at atmospheric pressure. For both conditions, the electrodes were calibrated before the experiment then the rates of oxygen consumption were measured on freshly permeabilised fibres using pyruvate plus malate and ADP at saturating concentrations (final concentrations were respectively 12 and 6 mM and 5 mM).

Cytochrome Oxydase (COX) activity

The method was adapted from Simon et al. (1992). Tissue extracts were prepared from frozen sections of eels. Superficial red muscle samples and white muscle samples were dissected, still frozen on a bed of liquid nitrogen. The tissues were homogenised in 100 mg/1 ml of extraction buffer (Tris, 0.1 M; EDTA, 2 mM; DTE, 2 mM; pH 7.4) at 4°C, using a Polytron. The obtained extracts were then centrifuged at 11 000 *g* for 20 min at 4°C. Supernatants were directly used for COX activity determination. COX activity was determined by spectophotometry at 550 nm in sodium phosphate buffer (0.33 M pH7 at 15°C) with 50 μmol L^{-1} reduced Cytochrome c at saturating concentrations (Theron et al., 2000).

Energetic nucleotide contents

Following the technique used by Sébert et al. (1987), energetic nucleotides (ATP, ADP and AMP) were extracted with an acid solution of trichloroacetic acid. The extracts were immediately analysed using an HPLC method associated with spectophotometry UV detection (254 nm) as previously described by Cann-Moisan et al. (1988).

Analysis of data

The results are expressed as mean ± S.E.M. The statistical signification of the results was evaluated at the 5% level with Student's *t*-test or ANOVA (to evaluate interactions), after having ensured the normality of the distributions and the homogeneity of their variances (Lilliefors test).

RESULTS

The animals were processed in only two groups: 0.1 and 10.1 MPa because ANOVA showed no interaction between pressure effects and parasitism levels. During pressure exposure, MO_2 of animals was maximum just at the end of compression (3.57 ± 0.08 mmol.h^{-1}.kg^{-1}) and decreased exponentially with time (time constant, τ, was 0.25 day) to return to control values (1.31 ± 0.02 mmol.h^{-1}.kg^{-1}). All animals survived 3 days pressure exposure and appeared to be in good condition. Table 1 shows that morphometric data are not significantly different between the two groups (0.1 and 10.1 MPa), despite a slight tendency to increase in OI and decrease in PFI and in haematocrit.

As regards the white muscle, 3 days under pressure does not modify either fibre oxygen consumption (MO_2) or COX activity, and thus the MO_2/COX ratio remains constant (table 2). As regards the red muscle, COX activity increases (+47%, not significant) but fibre MO_2 is not affected: consequently, the MO_2/COX ratio decreases (-37%, $p < 0.05$; table 2).

When the red fibres are exposed to high pressure, their respiration significantly decreases ($p < 0.05$) from 0.24 ± 0.02 μmol.min^{-1}.g^{-1} ($N = 6$) at 0.1 MPa to 0.17 ± 0.02 μmol.min^{-1}.g^{-1} ($N = 6$) when measured at 10.1 MPa. This corresponds to a $19.3 \pm 4.8\%$ decrease (paired *t*-test, $p < 0.01$).

Whatever the muscle (red or white), figure 1 shows that, despite a slight tendency for ATP and thus Adenylate Sum (AS = ATP + ADP + AMP) to increase after

Table 1.

	0.1 MPa	10.1 MPa
N	6	6
Body Length, mm	484.8 ± 7.4	484.3 ± 8.5
Body Weight, g	153.6 ± 7.8	156.8 ± 8.2
Condition Factor	0.14 ± 0.01	0.14 ± 0.00
OI	3.97 ± 0.27	4.69 ± 0.48
PFI	4.44 ± 0.10	3.9 ± 0.16
Hematocrit, %	32.7 ± 3.0	29.1 ± 1.6
Water content, %		
Gill	66.3 ± 2.5	67.9 ± 2.9
Muscle	80.8 ± 0.9	80.8 ± 0.3

Morphometric data. Results are expressed as mean ± S.E.M.

Table 2.

	0.1 MPa		10.1 MPa	
N	6		6	
Muscle	Red	White	Red	White
COX Activity	18.7 ± 4.1	1.3 ± 0.2	27.5 ± 6.1	1.4 ± 0.2
MO_2	0.29 ± 0.05	0.10 ± 0.01	0.28 ± 0.04	0.11 ± 0.01
MO_2/COX ratio ($\times 10^{-3}$)	18.5 ± 2.1	85.3 ± 11.2	$11.4 \pm 2.5^{*}$	78.9 ± 10.2

Metabolic values measured at 0.1 MPa on control fish (0.1 MPa) and fish exposed to 10.1 MPa of hydrostatic pressure for 3 days.

COX activity, mU.g^{-1}, one unit (U) correspond to 1 μmol of substrate which disappears in 1 min; MO_2, μmol.min^{-1}.g^{-1} wet weight. Results are expressed as mean $\pm$ S.E.M.

[*] Significant difference at 5% with the corresponding 0.1 MPa group.
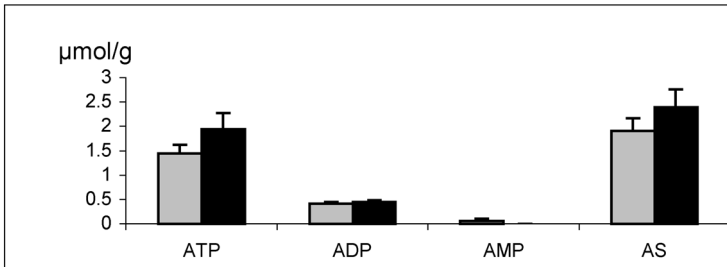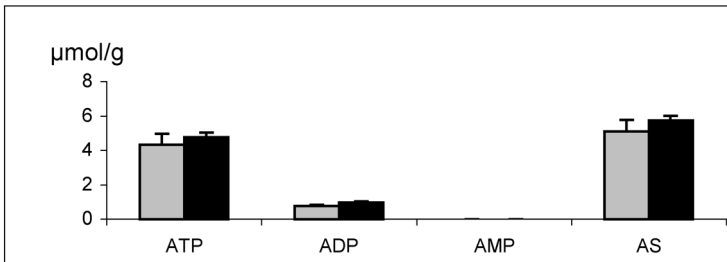

Figure 1. A and B are energetic nucleotide contents expressed as mean $\pm$ S.E.M., respectively, in red and white muscle. Control group (0.1 MPa) is in grey, and pressure group (10.1 MPa) in black. AS is the adenylates sum.

3 days under pressure, there is no significant change in adenylate content, and thus energy charge is not modified (EC in red muscle is 0.87 ± 0.01 and 0.90 ± 0.01 for control and pressure group, respectively, and in white muscle 0.92 ± 0.01 and 0.91 ± 0.01 for control and pressure group, respectively).

DISCUSSION

The yellow stage of the eels used is revealed by the values of ocular and pectoral fin indexes reported in table I (OI < 6.5: Pankhurst, 1982; PFI: Durif et al., 2000). The MO_2 values obtained at pressure in whole animal are similar to those reported by Sébert (1997). In the same manner, MO_2 of muscle fibres at 0.1 MPa and 10.1 MPa are in agreement with those obtained by Sébert and Theron (2001), and COX activities or nucleotide contents are the same as those obtained by Cann-Moisan et al. (1988) or Simon et al. (1989). Previous evidence, obtained for the same species, validates the results from this study and provides a general assessment of the aerobic metabolism of this particular eel population from Lake Balaton. One of the main objectives of this work was to investigate the pressure effects on this specific population. After 3 days under pressure they were in a good energy state, as shown by the Energy Charge values obtained (EC $\sim$ 0.90), higher COX activity values (+46.7%, NS), and MO_2 unmodified by pressure exposure. The Balaton eels are clearly resistant to HP. However, the fact that the eels survive 3 days pressure exposure does not mean that pressure has no effect at a cellular level and does not potentially impede their capacity for migratory activity. The pressure threshold (the pressure at which the eels begin to have a strong motor activity, P = 0.42 $\pm$ 0.02 MPa) is significantly lower ($p < 0.01$) in these eels than in others (0.68 $\pm$ 0.06 MPa, unpubl. data). This leads us to assume that their resistance to HP is lower. In fact, several other results corroborate this and we should like to focus our discussion on the red muscle because, unlike white muscle, its metabolism is aerobic and it ensures swimming activity during migration. Firstly, a decrease in the MO_2/COX ratio is observed in the red muscle fibres after 3 days under pressure. This is linked to an alteration in oxydative phosphorylation (OP) efficiency in contrast with the results from Theron et al. (2000) who observed an improvement in OP in yellow eels from the river Loire after 21 days under pressure. It is clear that 21 days is sufficient for pressure acclimation, which is not the case for 3 days (this study). The duration of pressure exposure is probably involved because when fibres are directly exposed to pressure their oxygen consumption decreases significantly (-20%, $p < 0.01$), whereas after 3 days, MO_2 is not modified (table 2). This means 3 days are sufficient to trigger the acclimation process, and/or pressure tolerance at the mitochondrial level requires the involvement of all the muscles and/or the whole organism. However, despite this, eels from Lake Balaton still have a lower pressure tolerance because pressure decreases their fibre oxygen consumption (measured at 10.1 MPa), which is not the case for eels from other locations (Sébert and Theron, 2001), and also alters oxydative phosphorylation efficiency (see above). A second fact, based on calculation, indicates a lower resistance to high pressure by these eels. Goolish (1991) estimated that in the yellow eel, red muscle represents 3% of the total body mass and 6% of muscle mass: thus, for a fish weighing 1 kg, red and white muscle mass can be evaluated at about 30 and 500 g, respectively. Considering the results for MO_2 measured at substrate saturation (i.e. maximal respiratory rate), the maximal muscle respiration rate under

pressure can be estimated at 16.8 μmol.g^{-1}.h^{-1} and 6.6 μmol.g^{-1}.h^{-1} for red and white muscles, respectively. Therefore, the maximal oxygen consumption for an individual weighing 1 kg can be estimated at 3.8 mmol.h^{-1}. As it is generally accepted that swimming requires at least 2.2 mmol.h^{-1} for the same weight (Böetius and Böetius, 1980; van Ginneken and van den Thillart, 2000), swimming under pressure should correspond to at least 60% of maximal ability. The same calculation applied to the Loire eels gives a result of only 35%. Thus, given that eels must cope with changes in salinity and ensure reproduction (ovarian development is estimated to be about 20% of total energy; Böetius and Böetius, 1980), the reserve levels for other activities appear to be substantially reduced in the Lake Balaton eels.

At the time of the experiment it was noticed that the red muscle of Balaton eels was subjectively much redder than in other yellow eels, where the 'red' muscle tends to be more orange than red. This difference probably reflects a higher myoglobin content in Balaton eels. It is known that such an increase in myoglobin is a trait commonly observed in diving mammals with a negative relationship between blood flow and myoglobin content (Cherepanova et al., 1993). It can therefore not be excluded that, for an unknown reason, the Balaton eel red muscle suffers from hypoxia and/or hypo-perfusion, and this could also impede migratory activity. Moreover, this population is known to be infested by *Anguillicola crassus,* and in our study there were mildly and severely infested fish. This nematode damages the swimbladder (Molnár et al., 1993; Würtz and Taraschewski, 2000) and it has been shown that such damage can impede the vertical displacement that eels perform during their migration to the Sargasso sea although the effects on swimming capacities have been discussed (Sprengel and Luchtenberg, 1991; Nimeth et al., 2000). The parasite is known to induce stress in eels and to increase cortisol plasma levels (Sures et al., 2001) which increases energy metabolism and decreases fat store utilisation, when fat is the only substrate used during migration (van Ginneken and van den Thillart, 2000). This could explain why MO_2 of muscle fibres after 3 days HP exposure was higher in the group with severe swimbladder changes (+48%, $p < 0.05$), without any changes in the MO_2/COX ratio and without any interaction between the effects of high pressure and parasitism level. In other words, parasitism, at whatever level, does not modify pressure resistance.

In conclusion, the results of this work show that yellow eels from Lake Balaton globally resist high pressure exposure, and nematode infection does not modify their resistance. However, several of the results point to a lower pressure tolerance of these particular eels which suggests that they do not have the respiratory and metabolic capacity to migrate and to cope with all the changes migratory activity entails prior to reproduction.

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