

Is the silvering process similar to the effects of pressure acclimatization on yellow eels?

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Abstract

To reproduce eels need to migrate over 6000 km and at pressure but before this migration they metamorphose into silver eels (silvering). The question raised in this study is to determine whether and how the silvering process contributes to pressure resistance. As migration represents a long swimming activity mainly performed with slow muscles, the red muscle energetics of pressure exposed silver eels was studied. By comparing these results to what has already been shown in yellow eels, we point out that the effects of the silvering process on pressure resistance are similar to the effects of pressure acclimatization in yellow eels. As previously shown, success of pressure acclimatization depends on membrane fluidity, but we raise the hypothesis that the high-pressure resistance of silver eels is due to more fluid membranes.

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1. Introduction

The European eel, *Anguilla anguilla*, is a particular species in that to reproduce, it must migrate over about 6000 km from the European coasts to its supposed breeding area: the Sargasso Sea. Migration is a very important step in the eel life cycle because it represents many changes: sedentary to migratory behaviour, atmospheric to high pressure, freshwater to seawater, temperature and light variations and all this is done

without feeding (Tesch, 2003). Before departure, eels metamorphose from the yellow non-migratory and sexually immature stage to the silver migratory one, where sexual maturation begins. This metamorphosis, called silvering, is not reduced to a colour change in livery. Numerous studies have reported other morphological transformations such as eye and pectoral fin enlargement (Pankhurst, 1982b; Durif et al., 2000); as well as physiological ones such as the increase in haematocrit and in haemoglobin concentrations (Johansson et al., 1974), higher volume of slow (red) muscle, the latter becoming more fatty with a higher mitochondria content in silver form (Lewander et al., 1974; Pankhurst, 1982a). Furthermore, silver eels show an

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increase in aerobic enzyme activities (Boström and Johansson, 1972) together with higher slow muscle output and power (Ellerby et al., 2001). Several hormonal changes (Marchelidon et al., 1999) have also been reported indicating the beginning of sexual maturation. All these transformations constitute the silvering process and seem to prepare the eel to its new environment and activity pattern.

Among the environmental changes linked to the migratory activity, high pressure is a particular challenge. It is known that among fish species, the eel is characterized by a particular pressure resistance (Sébert, 2003). In fact, after a critical period of 2–3 days following compression, the yellow eel is able to acclimatize to high pressure by modifying its aerobic and anaerobic muscle metabolism in order to cope with pressure effects (Simon et al., 1989, 1992; Sébert, 1997; Theron et al., 2000). The question raised in this study is to determine whether and how the silvering process, described above, contributes to pressure exposure response. As migration represents a long swimming activity mainly performed with slow muscles, the red muscle energetics of pressure exposed silver was studied.

2. Methods

2.1. Animals

2.1.1. Storage

Twelve silver freshwater European eels (*A. anguilla* L.) (body mass, 567 ± 184 g; mean \pm S.E.M.) from the Loire River were used. At least 10 days before the experiments, they were stored without feeding in 40 L polyethylene tanks with continuously renewed and aerated tap water. The experiments were performed during the winter, in order to maintain water temperature (14.2 ± 0.1 °C) and to have a photoperiod as close as possible to natural conditions, the tanks were placed in a room open to the outside.

2.1.2. Morphometry

Some days after their arrival in the laboratory, the eels were slightly anaesthetised (*Eugenia caryophyllata*, clove oil, 1 mL for 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), volume (ml), horizontal and vertical eye

diameters (ODh, ODv, mm) and pectoral fin length (PFL, mm). Different indexes were calculated from these data: condition factor ($CF = (BM/BL^3) \times 10^5$); ocular index ($OI = [((ODh + ODv)/4)^2 \times \pi]/BL \times 100$); and pectoral fin index ($PFI = (PFL/BL) \times 100$). The CF has been proposed by Fulton (in Ricker, 1975), OI by Pankhurst (1982b) and PFI by Durif et al. (2000). Density was calculated from volume and BM, and surface from BM and BL according to Sébert et al. (2004).

2.1.3. Sampling

The fish were sacrificed by decapitation and the blood was collected in glass capillaries to determine the haematocrit. Three sections of eel, cut backwards from the swimbladder, were frozen in liquid nitrogen, then stored at -80 °C for further measurements of cytochrome oxidase (COX) activities, nucleotides and protein contents. Thereafter, fresh red and white muscles were sampled as follows: red muscle was dissected along the lateral line and 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. These samples were then prepared to perform direct oxygen consumption measurements. The gonads and livers were weighed to calculate gonado-somatic index (GSI), and hepato-somatic index (HSI) respectively. The white muscles and gills were sampled then weighed before and after desiccation in order to determine water contents. Finally swimbladder parasites were counted if present.

2.1.4. Sample preparation

Freshly removed muscle samples were immediately placed in an ice-cold extraction medium (Sébert and Theron, 2001). Before oxygen consumption measurements, the muscle fibres were permeabilised using a saponin solution (150 mg/L). The technique used was adapted to fish from Veksler et al. (1987) and Letellier et al. (1992) by Sébert and Theron (2001).

2.2. Protocol

2.2.1. Acclimatization

The eels were placed in an experimental tank (14.9 L), placed in a hyperbaric chamber and connected to a high-pressure water circulation system (Sébert et al., 1990). The water circulation system allowed a

continuous renewal of water, so that temperature and oxygen concentration could be controlled.

After 5 days at atmospheric pressure, the hyperbaric chamber was compressed at a rate of 2 bar/min to 101 ATA and this pressure was maintained for 21 days. At the time of compression, the pressure at which a strong motor activity began was recorded as the pressure threshold (P_{tr}). During the experiments water oxygen partial pressure (Pw_{O_2}) and temperature (T_w) in the experimental tank were regularly monitored; water flow was approximately $30 L \cdot h^{-1}$. The water flow was stopped for 30 min, twice a day, in order to calculate oxygen consumption from the decrease of Pw_{O_2} (Sébert and Barthélémy, 1985). After 21 days at this pressure, the chamber was decompressed at a rate of 2 bar/min. On reaching at atmospheric pressure the eels were immediately killed and the samplings were performed. 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 (1 ATA).

2.2.2. Short-term exposure

A vessel was specially designed, using an electrode with pressure compensation (YSI 5739) to measure the oxygen consumption of the red muscle sample of the control group under pressure and its reference at atmospheric pressure. Compression and decompression were performed at 10 bar/min.

2.3. Measurements

All the measurements were performed at 15 °C.

2.3.1. Muscle fibre oxygen consumption

The oxygen consumption of freshly permeabilised muscle fibres was performed according to the technique previously described by Theron et al. (2000) and Sébert and Theron (2001). At atmospheric pressure respiration was measured in a glass vessel by following its oxygen content decrease, using a Strathkelvin Instrument[®] O_2 microelectrode (accuracy: $\pm 0.2\%$ saturation). For measurements at 1 and 101 ATA, the electrodes were calibrated before the experiment, then the rates of oxygen consumption were measured using pyruvate plus malate at saturating concentrations (final concentrations were respectively 12 and 6 mM) to determine

state 4 and 5 min after ADP was injected at saturating concentrations (final concentration 5 mM) to determine state 3.

2.3.2. Cytochrome oxidase (COX) activity

The method was adapted from Simon et al. (1992). Tissue extracts were prepared from frozen sections of eels. Red muscle and white muscle samples were dissected, still frozen on a bed of liquid nitrogen. The tissues were homogenized 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 $11,000 \times g$ for 20 min at 4 °C. Filtered supernatants were directly used for COX activity determination. COX activity was determined by spectrophotometry at 550 nm in sodium phosphate buffer (0.33 M pH 7 at 15 °C) with $50 \mu mol L^{-1}$ reduced cytochrome *c* at saturating concentrations (Theron et al., 2000).

2.3.3. Energetic nucleotide contents

Following the technique used by Sébert et al. (1987), energetic nucleotides (ATP, ADP, AMP and IMP) were extracted with the acid solution of trichloroacetic acid. The extracts were immediately analysed using a HPLC method: the separation of different nucleotides was performed on a column Hypersil ODS 25 cm. The mobile phase was KH_2PO_4 (0.1 M), dissolved in deionised H_2O and filtered through $0.22 \mu m$ before pH adjustment to 6.35 with NH_4OH . The flow rate was 0.8 ml/min. Detection was performed by UV spectrophotometry (254 nm) as previously described by Cann-Moisan et al. (1988). From the results, energy charge was calculated as $EC = [ATP + (1/2)ADP]/[ATP + ADP + AMP]$.

2.3.4. Protein contents

Protein contents were measured following the indirect technique of Lowry et al. (1951), on extracts prepared for COX activity determinations.

2.4. Analysis of data

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

Table 1

(A) Morphometric data of group measured at the beginning of experiment and (B) morphometric and metabolic data measured after 21 days

	1 ATA	
(A) Morphometric data		
<i>N</i>	6	
Body mass (g)	371 ± 14	
Body length (cm)	607 ± 8	
BMI (kg/m ²)	1.00 ± 0.01	
Condition factor (g/m ³)	0.16 ± 0.00	
Density	0.81 ± 0.01	
BS (cm ²)	676 ± 16	
Ocular index	9.3 ± 0.2	
Eye surface/BS (10 ⁺⁴)	8.9 ± 0.2	
Pectoral fin index	5.3 ± 0.1	
	1 ATA	101 ATA
(B) Morphometric and metabolic data		
<i>N</i>	6	6
Haematocrit (%)	31.7 ± 1.5	32.9 ± 2.5
White muscle protein (mg/g)	28.5 ± 0.9	29.2 ± 1.0
Red muscle protein (mg/g)	21.5 ± 0.6	22.0 ± 0.7
HIS (%)	1.20 ± 0.06	1.02 ± 0.04 ^a
GSI (%)	1.46 ± 0.03	1.58 ± 0.08
Muscle water content (%)	62.8 ± 0.9	61.2 ± 1.1
Gill water content (%)	81.0 ± 0.3	81.7 ± 0.2
Number of worms	3.2 ± 1.4	0.7 ± 0.4

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

^a Significant difference at 5% with the corresponding control group.

3. Results

The morphometric data measured at the beginning of the experiment are shown in Table 1A. All of them are representative of those obtained in female eels at the silver stage (Pankhurst, 1982b; Durif et al., 2000). After sacrifice, other morphometric and metabolic parameters were measured (Table 1B): HSI is significantly lower ($P < 0.05$) after exposure to hydrostatic pressure and GSI tends to increase (not significant). The swimbladder is very slightly infested by parasites (number of worms $< 2.25 \pm 0.95$). The other parameters were not affected by pressure exposure.

Global sensitivity to compression is represented, both by pressure threshold (P_{tr}) which is high: $P_{tr} = 81 \pm 7$ bar, and by MO_2 at the end of compression ($MO_2 = 1.75 \pm 6$ mmol/h/kg) which increased by 60% when compared to control values before compression.

Table 2

Oxygen consumption of muscle fibres ($\mu\text{mol}/\text{min}/\text{g}$) in control group (1 ATA) and pressure acclimatized group (101 ATA)

	1 ATA	101 ATA	% variation
Red muscle fibres			
State 4	0.12 ± 0.01	0.12 ± 0.01	0
State 3	0.42 ± 0.04	0.37 ± 0.03	-12
RCR	3.68 ± 0.48	3.31 ± 0.25	-10
White muscle fibres			
State 4	0.08 ± 0.01	0.08 ± 0.00	0
State 3	0.12 ± 0.02	0.12 ± 0.01	0
RCR	1.52 ± 0.13	1.41 ± 0.03	-7

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

sion. The average time taken to return to control values was 1.5 days.

When fibres were directly pressure exposed state 4 of the respiratory chain increased (% variation = $+33.2 \pm 13.62\%$, $P < 0.05$). State 3 did not change ($-12.3 \pm 7.4\%$) and consequently RCR (=state 3/state 4, respiratory control ratio) decreased substantially ($-33.4 \pm 6.3\%$, $P < 0.01$). Table 2 shows that after acclimatization, in white and red muscles, maximum oxygen consumption (state 4 and state 3) and RCR did not change by remaining at 101 ATA. Table 3 shows that pressure acclimatization had no effect on EC (energy charge) and increased COX activity (44%, not significant).

4. Discussion

The calculated morphometric indexes (Table 1) undoubtedly prove that the studied eels are female at the silver stage. In fact ocular index is much greater than 6.5 according to Pankhurst (1982b), and even greater than 8 according to Marchelidon et al. (1999). The pectoral fin index is also within the female silver eel range, likewise for GSI and HSI according to Durif et al. (2000). In addition to these morphometric characteristics, we can see that protein contents in white muscle are lower in silver eels than in yellow eels previously studied in our lab (36.5 ± 0.3 mg/100 mg; Simon et al., 1991), and likewise for water contents (yellow eels, $69.7 \pm 1.27\%$; Simon et al., 1991). Moreover, haematocrit is higher in silvers (yellows: $Ht = 28.8 \pm 0.9\%$; Sébert et al., 1991). These three parameters tally with what was demonstrated to be silvering effects (Johansson et al., 1974;

Table 3
Energetic nucleotide contents and COX activity

Parameters	Muscle	1 ATA	101 ATA
ATP ($\mu\text{mol/g}$)	Red	1.44 ± 0.21	1.25 ± 0.13
SA ($\mu\text{mol/g}$)	Red	1.72 ± 0.22	1.48 ± 0.13
EC (-)	Red	0.91 ± 0.01	0.92 ± 0.01
EC' (-)	Red	0.87 ± 0.02	0.91 ± 0.01
ATP ($\mu\text{mol/g}$)	White	2.17 ± 0.17	1.61 ± 0.34
SA ($\mu\text{mol/g}$)	White	2.71 ± 0.15	1.99 ± 0.34
EC (-)	White	0.9 ± 0.01	0.9 ± 0.02
EC' (-)	White	0.89 ± 0.02	0.88 ± 0.02
COX activity (nmol/min/g)	Red	122.0 ± 18.2	175.5 ± 20.1
COX activity (nmol/min/g)	White	3.6 ± 0.4	2.9 ± 0.3

Results are expressed as mean \pm S.E.M. AS (adenylates sum) = ATP + ADP + AMP; EC (energy charge) = (ATP + (1/2)ADP)/AS; EC' = EC/(1 + (IMP/AS)); COX activities are expressed in nmol of substrate which disappear in 1 min/g of fresh tissues.

Lewander et al., 1974). It should be pointed out that, despite a decrease in worm numbers under pressure (not significant), parasitism has no influence in terms of pressure resistance (Vettier et al., 2003).

The metabolic characteristics of yellow and silver eels have already been described by many authors showing that silver eels exhibit better aerobic enzymatic activities (Boström and Johansson, 1972; Egginton, 1986). This is confirmed in this study: the silver COX activity of red muscle is higher than that of yellow eels studied in our lab (60 ± 19 nmol/min/g; Simon et al., 1992). The results of EC and EC' in white muscle are higher for silver eels than those at the yellow stage (EC = 0.85 ± 0.02 and EC' = 0.71 ± 0.06 ; Simon et al., 1992). Thus improvements linked to the silvering process, are manifest in the aerobic metabolism of red and white muscles and are in agreement with a better "quality" of muscle which could explain the better contractile performances (Ellerby et al., 2001). These different measurements point to a physiological and metabolic preparation prior to the migratory activity i.e. sustained swimming. However, even if silver eels are ready for the migration challenge (swimming activity, sexual maturation) there is no evidence to show what happens when they need to cope with hydrostatic pressure.

The pressure effects were evaluated on the whole animal and on energetic metabolism in white and red muscles. However, even if the white muscle is greater from the point of view of mass, it is the red muscle, which ensures energy production during sustained swimming and migration. Moreover, Simon et al. (1989) have shown that aerobic energy production predominates af-

ter several days at pressure: the discussion must therefore be focused on the results from red muscle. Long-term hydrostatic pressure exposure does not significantly modify cellular energetics as the results from Tables 2 and 3 testify. Mitochondrial respiration and/or oxidative phosphorylation did not change, likewise the COX^{max} activity, which has a tendency to increase by 44% (not significant). Therefore, the results obtained on silver eels are not different from what was previously observed in yellow eels with the same methods (Simon et al., 1989, 1992; Sébert, 1997; Theron et al., 2000). Subsequently, we should focus on the contribution of the silvering process to pressure resistance.

In fact, 3 weeks under pressure do not reveal any difference between yellow and silver eels whereas there is an obvious improvement in global quality shown above, in silver eel muscle metabolism. Therefore, we need to relocate cellular energetics within an organismic context and to consider that it acts upon the whole animal. In this way, pressure resistance could be evaluated by P_{tr} (pressure at which strong motor activity begins during compression), MO_2^{max} of animal at the end of compression (Sébert, 1993) (Fig. 1). This figure shows that the silver eel results are in agreement with what is observed in yellow eels, which were acclimatized for 30 days at 101 ATA hydrostatic pressure and then recompressed (Simon et al., 1989). Indeed when acclimatized yellow eels are recompressed, a lower increase in oxygen consumption during compression and a higher pressure threshold are observed in comparison with pressure-naïve animals. Moreover, there is also a quicker return to control values of about 1.5 days for silver eels versus about 5 days for yellow eels when com-

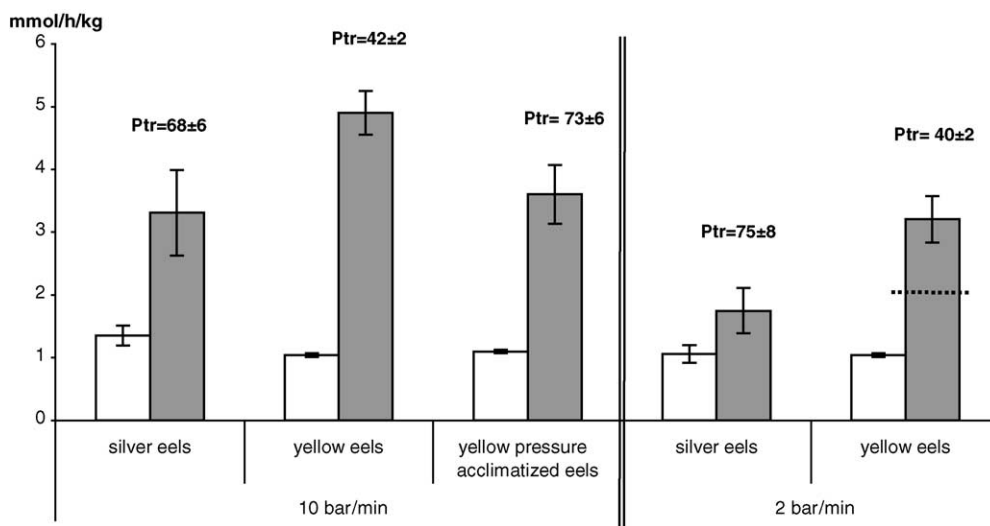


Fig. 1. Animal oxygen consumption (mmol/h/kg) measured at 1 ATA before compression (white columns), or at 101 ATA at the end of compression (grey columns). Results of silver eels compressed at 10 bar/min obtained from Vettier and Sébert (2004), the three groups of yellow eels from Simon et al. (1989) and silver eels at 2 bar/min from this study. We can note that the higher the compression rate, the greater the MO_2 increase. We can evaluate the cost of the quicker compression rate: it is about 1.5 mmol/h/kg. If we use the specific cost observed in yellow eels we can extrapolate to obtain acclimatized yellow eels compressed at 2 bar/min. Thus the extrapolated increase in MO_2 is represented by the dotted line, and it is similar to what is observed in silver eels.

pressed at 2 bar/min (Simon et al., 1989). The following hypothesis was put forward and verified: in response to the decrease in membrane fluidity induced by compression, pressure acclimatization allows the fluidity to return to normal by means of a change in membrane composition, thus demonstrating the existence of the homeoviscous theory. Consequently when the fish was again pressure exposed, it was “ready” and able to cope with the membrane pressure effects (Sébert, 1993). By analogy, we propose that at atmospheric pressure, silver eels are in the same state as yellow eels after pressure acclimatization; in other words the silvering process could well have the same effects as pressure acclimatization on yellow eels. Several experimental facts are in agreement with this hypothesis. Silver eel muscles have higher lipid contents than those of yellow eels (Dave et al., 1975, 1976; Tesch, 2003; Pankhurst, 1982a) and silver red muscle tends to have more unsaturated and longer fatty acids compared to yellow red muscle (Dave et al., 1974). It could thus be inferred that silver eel membranes are more fluid. In agreement with such a hypothesis, it has been shown that sexual hormones and especially 17β -estradiol, which rises during the silvering process (Han et al., 2003), increase membrane flu-

idity (Whiting et al., 2000). Moreover, COX activity is positively correlated to membrane fluidity (Vik and Capaldi, 1980): it is higher in silver than in yellow eels at 1 ATA (our results). In terms of pressure resistance, the silvering process appears to mimic pressure acclimatization, thus allowing the silver eels to cope with pressure effects with a minimal energy cost. Consequently, energy and fat stores can be kept for swimming and gonad development (van Ginneken and van den Thillart, 2000).

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References

- Boström, S.L., Johansson, R.G., 1972. Enzyme activity patterns in white and red muscle of the eel (*Anguilla anguilla*) at different developmental stages. *Comp. Biochem. Physiol. B* 42, 533–542.

- Cann-Moisan, C., Sébert, P., Caroff, J., Barthélémy, L., 1988. Effects of hydrostatic pressure (hp = 101 ATA) on nucleotides and pyridine dinucleotides tissue contents in trout. *Exp. Biol.* 47, 239–242.
- Dave, G., Johansson-Sjöbeck, M.L., Larsson, A., Lewander, K., Lidman, U., 1974. Metabolic and hematological studies on the yellow and silver phases of the European eel, *Anguilla anguilla* L. II. Fatty acid composition. *Comp. Biochem. Physiol. B* 47, 583–591.
- Dave, G., Johansson-Sjöbeck, M.L., Larsson, A., Lewander, K., Lidman, U., 1975. Metabolic and hematological effects of starvation in the European eel, *Anguilla anguilla* L. I. Carbohydrate, lipid, protein and inorganic ion metabolism. *Comp. Biochem. Physiol. A* 52, 423–430.
- Dave, G., Johansson-Sjöbeck, M.L., Larsson, A., Lewander, K., Lidman, U., 1976. Metabolic and hematological effects of starvation in the European eel, *Anguilla anguilla* L. III. Fatty acid composition. *Comp. Biochem. Physiol. B* 53, 509–515.
- Durif, C., Elie, P., Dufour, S., Marchelidon, J., Vidal, B., 2000. Analysis of morphological and physiological parameters during the silvering process of the European eel (*Anguilla anguilla*) in the lake of grand-lieu (France). *Cybiurn* 24 (3), 63–74 (Suppl. S).
- Egginton, S., 1986. Metamorphosis of the American eel, *Anguilla rostrata* leueur. I. Changes in metabolism of skeletal muscle. *J. Exp. Zool.* 237, 173–184.
- Ellerby, D.J., Spierts, I.L., Altringham, J.D., 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.
- Han, Y.S., Liao, I.C., Tzeng, W.N., Huang, Y.S., Yu, J.Y.L., 2003. Serum estradiol-17 beta and testosterone levels during silvering in wild Japanese eel *Anguilla japonica*. *Comp. Biochem. Physiol. B* 136, 913–920.
- Johansson-Sjöbeck, M.L., Dave, G., Larsson, A., Lewander, K., Lidman, U., 1974. Metabolic and hematological studies on the yellow and silver phases of the European eel, *Anguilla anguilla* L. III. Hematology. *Comp. Biochem. Physiol. B* 47, 593–599.
- Letellier, T., Malgat, M., Coquet, M., Moretto, B., Parrot-Roulaud, F., Mazat, J.P., 1992. Mitochondrial myopathy studies on permeabilized muscle fibers. *Pediatr. Res.* 32, 17–22.
- Lewander, K., Dave, G., Johansson-Sjöbeck, M.L., Larsson, A., Lidman, U., 1974. Metabolic and hematological studies on the yellow and silver phases of the European eel, *Anguilla anguilla* L. I. Carbohydrate, lipid, protein and inorganic ion metabolism. *Comp. Biochem. Physiol. B* 47, 571–581.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Marchelidon, J., Le Belle, N., Hardy, A., Vidal, B., Sbahi, M., Burzawa Gerard, E., Schmitz, M., Dufour, S., 1999. Study of variations of anatomical and endocrine parameters in sedentary and downstream migrating female European eels (*Anguilla anguilla*): application to the characterization of the silver stage. *Bull. Fr. P. P.* 355, 349–368.
- Pankhurst, N.W., 1982a. Changes in body musculature with sexual maturation in the European eel *Anguilla anguilla* (L.). *J. Fish Biol.* 21, 417–428.
- Pankhurst, N.W., 1982b. Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* (L.). *J. Fish Biol.* 21, 127–140.
- Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish population. *Bull. Fish Res. Bd. Can.* 21, 507–512.
- Sébert, P., 1993. Energy metabolism of fish under hydrostatic pressure: a review. *Trends Comp. Biochem. Physiol.* 1, 289–317.
- Sébert, P., 1997. Pressure effects on shallow water fishes. In: Randall, D.J., Farrell, A.P. (Eds.), *Deep Sea Fishes*, Fish Physiology. Academic Press, San Diego, pp. 279–323.
- Sébert, P., 2003. Fish adaptations to pressure. In: Val, A.L., Kapoor, B.G. (Eds.), *Fish Adaptations*. Science Publisher, Enfield, pp. 73–95.
- Sébert, P., Barthélémy, L., 1985. Effects of high hydrostatic pressure per se, 101 atm on eel metabolism. *Respir. Physiol.* 62, 349–357.
- Sébert, P., Barthélémy, L., Caroff, J., Hourmant, A., 1987. Effects of hydrostatic pressure per se (101 ATA) on energetic processes in fish. *Comp. Biochem. Physiol. A* 86, 491–495.
- Sébert, P., Barthélémy, L., Simon, B., 1990. Laboratory system enabling long-term exposure to hydrostatic pressure of fishes or other animals breathing water. *Mar. Biol.* 104, 165–168.
- Sébert, P., Péqueux, A., Simon, B., Barthélémy, L., 1991. Effects of long term exposure to 101 ATA H.P. On blood, gill and muscle composition and of some enzyme activities of the fw eel (*Anguilla anguilla* L.). *Comp. Biochem. Physiol. B* 98, 573–577.
- Sébert, P., Theron, M., 2001. Why can the eel, unlike the trout, migrate under pressure. *Mitochondrion* 1, 79–85.
- Sébert, P., Vettier, A., Belhomme, M., 2004. A simple relationship to calculate eel surface area. *Ann. Biol.* 54, 131–136.
- Simon, B., Sébert, P., Barthélémy, L., 1989. Effects of long-term exposure to hydrostatic pressure per se (101 ATA) on eel metabolism. *Can. J. Physiol. Pharmacol.* 67, 1247–1251.
- Simon, B., Sébert, P., Barthélémy, L., 1991. Eel, *Anguilla anguilla* (L.), muscle modifications induced by long-term exposure to 101 ATA hydrostatic pressure. *J. Fish Biol.* 38, 89–94.
- Simon, B., Sébert, P., Cann-Moisan, C., Barthélémy, L., 1992. Muscle energetics in yellow fresh water eel (*Anguilla anguilla* L.) exposed to high hydrostatic pressure (101 ATA) for 30 days. *Comp. Biochem. Physiol. B* 102, 205–208.
- Tesch, F.S., 2003. *The Eel*. Blackwell Publishing, Oxford.
- Theron, M., Guerrero, F., Sébert, P., 2000. Improvement in the efficiency of oxidative phosphorylation in the freshwater eel acclimated to 10.1 mPa hydrostatic pressure. *J. Exp. Biol.* 203 (Pt 19), 3019–3023.
- van Ginneken, V.J., van den Thillart, G.E., 2000. Eel fat stores are enough to reach the Sargasso. *Nature* 403, 156–157.
- Veksler, V.I., Kuznetsov, A.V., Sharov, V.G., Kapelko, V.I., Saks, V.A., 1987. Mitochondrial respiratory parameters in cardiac tissue: a novel method of assessment by using saponin-skinned fibers. *Biochim. Biophys. Acta* 892, 191–196.
- Vettier, A., Sébert, P., 2004. Pressure resistance of aerobic metabolism in eels from different water environments. *Mitochondrion* 3, 347–354.
- Vettier, A., Szekely, C., Sébert, P., 2003. Are yellow eels from lake Balaton able to cope with high pressure encountered during migration to the Sargasso Sea? The case of energy metabolism. *Ann. Biol.* 53, 329–338.

Vik, S.B., Capaldi, R.A., 1980. Conditions for optimal electron transfer activity of cytochrome *c* oxidase isolated from beef heart mitochondria. *Biochem. Biophys. Res. Commun.* 94, 348–354.

Whiting, K.P., Restall, C.J., Brain, P.F., 2000. Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. *Life Sci.* 67, 743–757.