# Chapter 5
# High Pressure Resistance and Adaptation of European Eels

**Philippe Sébert, Aurélie Vettier, Aline Amérand, and Christine Moisan**

**Abbreviations** BL: Body length; COX: Cytochrome c oxidase; d: Density; EC: Energy charge; FPT: Final preferred temperature; HP: Hydrostatic pressure; PRI: Pressure resistance index; Ptr: Pressure threshold; PUFA: Polyunsaturated fatty acids; ROS: Reactive oxygen species; SB: Swim bladder

## 5.1 Introduction

The fish is often used as a model for experiments under pressure because it has three main advantages (Barthélémy 1985): (a) It is a vertebrate which has an organisation not very different from a mammal; for some authors, due to the high number of species, the fish is even the typical vertebrate (Bone et al. 1995); (b) The ectothermic quality of fish enables study of pressure/temperature interactions; (c) Because they breathe water, fish can be used to study separately the effects of hydrostatic pressure and/or the effects of gas pressure, which is useful in understanding mammalian physiology (see Sébert 1997).

The adverse effects of high pressure on fishes have been known since the 19th century (Bert 1878; Regnard 1885) and are well reviewed in the literature (Gordon 1970; Sébert and Macdonald 1993; Sébert 2003). However, the great majority of these studies are concerned with the biological effects of high hydrostatic pressure (HP) *per se* considered, like temperature, as a thermodynamic factor (see Somero 1991; Sébert 2003). In other words, the effects of pressure on fishes have been studied more from a fundamental than from an ecophysiological point of view. In fact, in regard to pressure, we can consider three types of fishes: those which never encounter variations in pressure, and are unable to adapt to pressure effects (Sébert 2003); those which always live at great depth and have a poor resistance to low (atmospheric) pressure (Siebenaller and Somero 1989; Somero 1991); and finally those which, as the eel, *Anguilla*, live a part of their life under pressure and thus must adapt to its adverse effects. In terms of

P. Sébert, A. Vettier, A. Amérand, and C. Moisan
Laboratoire ORPHY, UFR Sciences, 6, Avenue Le Gorgeu, CS 93837, 29238 Brest Cedex 3 – France

G. van den Thillart et al. (eds.), *Spawning Migration of the European Eel,*

environmental adaptation, the eel is a fascinating animal, and an excellent model to examine almost any environmental effects in the field of fish biology (Owen 2001).

## 5.2 Eel and Hydrostatic Pressure

Eel biology and the eel life cycle are largely described in other chapters of this book and are not reviewed here. Most of the eel life cycle is in the yellow non-migratory stage, at atmospheric pressure in lakes, rivers or estuaries: this is mainly a period (the longest) of development and growth. However, the most important periods are under pressure at sea: the hatching and migration of larvae from the Sargasso Sea to the rivers, and many years later, the migration of silver eels from the rivers back to the Sargasso Sea. The time under pressure is relatively short, but requires many adaptations in order to cope with the high energy demand in a new environment (adaptation to temperature, salinity, pressure, swimming, etc.).

The pressures to which the eels are exposed and how they dive are not known. The possible range is from 200 to 700 m (2 to 7 MPa; Fricke and Kaese 1995; Aoyama et al. 1999; McCleave and Arnold 1999; Jellyman and Tsukamoto 2002; Tesch 2003; Van Ginneken and Maes 2005), although one eel has been photographed at 2,000 m (Robins et al. 1979). Moreover it seems that during its migration under pressure, eels perform daily vertical migrations (Tesch 1978). Most of the results reported in this chapter concerns experiments performed at 10.1 MPa hydrostatic pressure (10.1 MPa # 100 atm # 100 bars # 1,000 m $H_2O$) in fresh water.

## 5.3 Is the Yellow Non-migrating Eel Able To Cope with Pressure Effects?

Before discussing the ability of silver eels to migrate at great depths, i.e. to explore the ecophysiological aspects of the migration, it is necessary to report what is known about the effects of high pressure on fishes. In fact, migration towards the Sargasso Sea is a big challenge for the eel which must acclimate to changes in salinity and temperature, to starvation, absence of solar light and, above all, to continuous swimming at high pressure. In other words, migration represents a high energy demand which must be satisfied in order to ensure the survival of the species. In this chapter, we will focus on the energetic aspects knowing that pressure has evidently other specific, sometimes adverse effects (see for review; Macdonald 1984; Sébert and Macdonald 1993; Sébert 1997).

It is clear that the silvering process (the metamorphosis from yellow non-migrating eel to silver migrating one) is a preparation for migration i.e. to all the concomitant changes in environmental factors. In this sense, its importance in the pressure acclimatisation processes can be only deduced and elucidated by comparing pressure effects on silver and yellow eels. Numerous physiological data have been obtained in yellow eels before and after pressure exposure (Tables 5.1 to 5.4). When a fish is exposed to high hydrostatic pressure, at constant temperature and normoxia, it exhibits a strong motor activity concomitant

with an increase in oxygen consumption. The pressure at which these symptoms appear depends on species, size, rate of compression, water temperature, salinity (Ponat 1967; Flügel 1972; Vettier et al. 2005) and the metabolic rate before compression (see Sébert and Macdonald 1993; Table 5.5). In order to compare different conditions and /or species,

**Table 5.1** Physiological data (mean ± SEM) in heart, brain and plasma from yellow freshwater eels, *Anguilla anguilla*, under normal and high pressure

| | | 10.1 MPa | | |
|---|---|---|---|---|
| | 0.1 MPa | 1–3 days | 30 days | References |
| **Heart** | | | | |
| Isometric twitch tension, mN | 3.2 | 3.7 (SW) | – | Gennser et al. 1990 |
| Fc, bpm | 28 ± 1.2 | 50 ± 2.0* | – | Sébert and Barthélémy 1985b |
| Oxygen consumption, mmol $h^{-1}$ $kg^{-1}$ | 1.0 ± 0.03 | 6 (end of compression) | 0.67 ± 0.05* | Simon et al. 1989 |
| Respiratory frequency $min^{-1}$ | 35 | 50 | – | Belaud 1975 |
| Q10 | 2.3 ± 0.3 | – | 1.2 ± 0.3 | Sébert et al. 1995 |
| **Plasma** | | | | |
| Ht,% | 28.8 ± 0.9 | – | 27.1 ± 1.1 | Sébert et al. 1991 |
| Proteins, g $L^{-1}$ | 32.5 ± 1.5 | – | 31.4 ± 0.8 | Sébert et al. 1991 |
| Lactates, mM | 1.65 ± 0.42 | – | 0.71 ± 0.12 | Sébert et al. 1991 |
| $Na^{+}$, mEq $L^{-1}$ | 144 ± 6 | – | 151 ± 5 | Sébert et al. 1991 |
| $Cl^{-}$, mEq $L^{-1}$ | 59.5 ± 2.9 | – | 73.3 ± 3.1* | Sébert et al. 1991 |
| $K^{+}$, mEq $L^{-1}$ | 8.6 ± 0.6 | – | 7.1 ± 0.5 | Sébert et al. 1991 |
| $Ca^{++}$, mEq $L^{-1}$ | 7.6 ± 0.3 | – | 7.3 ± 0.4 | Sébert et al. 1991 |
| $Mg^{++}$, mEq $L^{-1}$ | 3.6 ± 0.1 | – | 4.6 ± 0.2 | Sébert et al. 1991 |
| Osmolarity, mosm $L^{-1}$ | 333 ± 17 | – | 365 ± 11 | Sébert et al. 1991 |
| Glucose, g $L^{-1}$ | 1.1 ± 0.2 | 1.5 ± 0.2 | – | Simon 1990 |
| Total FFA, nmol $L^{-1}$ | 501 ± 22 | 265 ± 61* | – | Simon 1990 |
| NE, nmol $L^{-1}$ | 5.7 ± 1.7 | 14.7 ± 1.7* | – | Sébert et al. 1987 |
| E, nmol $L^{-1}$ | 11.1 ± 1.6 | 20.2 ± 2.1* | – | Sébert et al. 1987 |
| **Brain** | | | | |
| NE, nmol $g^{-1}$ | 1.40 ± 0.072 | 1.42 ± 0.085 | – | Sébert et al. 1986 |
| E, nmol $g^{-1}$ | 0.24 ± 0.010 | 0.28 ± 0.009 | – | Sébert et al. 1986 |
| DA, nmol $g^{-1}$ | 0.83 ± 0.041 | 1.01 ± 0.032 | – | Sébert et al. 1986 |
| DOPAC, nmol $g^{-1}$ | 0.27 ± 0.016 | 0.30 ± 0.022 | – | Sébert et al. 1986 |
| 5-HT, nmol $g^{-1}$ | 0.89 ± 0.095 | 1.06 ± 0.136 | – | Sébert and Barthélémy 1985a |
| 5-HIAA, nmol $g^{-1}$ | 0.21 ± 0.011 | 0.29 ± 0.028* | – | Sébert and Barthélémy 1985a |
| Gly, nmol $g^{-1}$ | 1,394 ± 59 | 1,390 ± 76 | – | Sébert and Barthélémy 1985b |
| Gln, nmol $g^{-1}$ | 14,377±439 | 15,834 ± 674 | – | Sébert and Barthélémy 1985b |
| GABA, nmol $g^{-1}$ | 2,560 ± 99 | 2,530 ± 76 | – | Barthélémy et al. 1991 |
| MDA, nmol $g^{-1}$ | 6.1 ± 0.5 | 24.2 ± 6.5* | – | Sébert and Barthélémy 1985a |

Experimental pressure 10.1 MPa (1,000 m depth); rate of compression 1.0 MPa $min^{-1}$.
TW = 15–17°C. SW = seawater.
*Statistically different ($p < 0.05$ or better) from controls at 0.1MPa. Values are mean ± SEM

**Table 5.2** Physiological data (mean ± SEM) in gills and liver from yellow freshwater eels, *Anguilla anguilla*, under normal and high pressure

| | | 10.1 MPa | | |
|---|---|---|---|---|
| | 0.1 MPa | 1–3 days | 30 days | References |
| **Gill** | | | | |
| Water content, % | 75.0 ± 0.5 | – | 76.0 ± 0.5 | Sébert et al. 1991 |
| $Na^+$, µEq $g_{ww}^{-1}$ | 52.2 ± 3.8 | – | 59.5 ± 1.7 | Sébert et al. 1991 |
| Cl-, µEq $g_{ww}^{-1}$ | 24.0 ± 1.5 | – | 31.3 ± 1.5* | Sébert et al. 1991 |
| $K^+$, µEq $g_{ww}^{-1}$ | 64.3 ± 0.8 | – | 62.9 ± 2.0 | Sébert et al. 1991 |
| $Na^+$,$K^+$ATPase, µmol$_{Pi}$ $mg_{Prot}^{-1}$ $h^{-1}$ | 9.8 ± 0.6 | – | 4.5 ± 0.04* | Sébert et al. 1991 |
| $Mg^{++}$ATPase, µmol$_{Pi}$ $mg_{Prot}^{-1}$ $h^{-1}$ | 7.51 ± 0.8 | – | 3.0 ± 0.3* | Sébert et al. 1991 |
| Anisotropy | 0.220± 0.001 | – | 0.215±0.001* | Sébert P et al., 1989 unpublished data |
| Unsaturation index | 191 ± 8 | – | 197 ± 7 | Sébert P et al., 1989 unpublished data |
| Saturation ratio | 0.48 ± 0.03 | – | 0.37 ± 0.05* | Sébert P et al., 1989 unpublished data |
| Number $Cl^-$, cells $mm^{-2}$ | 798 ± 174 | – | 3,095 ± 403* | Dunel-Erb et al. 1996 |
| Fractional area, $\mu m^2$ $mm^{-2}$ | 6,804 ± 1,316 | – | 46,194±4,470* | Dunel-Erb et al. 1996 |
| Number of mucus cells, aff | 17.8 ± 1.1 | – | 4.4 ± 0.6 | Dunel-Erb et al. 1996 |
| **Liver** | | | | |
| Proteins, mg 100 $mg_{ww}^{-1}$ | 10.0 ± 0.5 | 9.5 ± 0.4 | 10.1 ± 0.3 | Simon 1990 |
| Lactate, µmol $g_{ww}^{-1}$ | 0.81 ± 0.32 | 0.84 ± 0.28 | 0.95 ± 0.43 | Simon 1990 |
| Glycogen, µg 100 $mg_{ww}^{-1}$ | 0.90 ± 0.17 | 0.67 ± 0.10 | 0.65 ± 0.11 | Simon 1990 |
| Total FA, µmol $g_{ww}^{-1}$ | 2.2 ± 0.6 | 5.0 ± 0.5* | 4.2 ± 0.5* | Simon 1990 |
| IDH, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 10.1 ± 0.99 | 11.3 ± 2.5 | 15.1 ± 0.75 | Simon 1990 |
| MDH, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 608 ± 34.4 | 671 ± 45 | 782 ± 50.9 | Simon 1990 |
| CS, µmolsubst $min^{-1}$ $kg^{-1}$ | 6.19 ± 0.735 | 9.06 ± 1.22 | 6.47 ± 0.819 | Simon 1990 |
| COX, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 1.38 ± 0.178 | 0.713 ± 0.71[a] | 1.58 ± 0.118* | Simon 1990 |
| GOT, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 92.2 ± 10.23 | 134 ± 24.2 | 142 ± 21.97 | Simon 1990 |
| GPT, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 33.7 ± 3.25 | 33.9 ± 3.8 | 37.1 ± 1.7 | Simon 1990 |
| PFK, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 0.12 ± 0.019 | 0.15 ± 0.01 | 0.16 ± 0.023 | Simon 1990 |
| PK, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 5.23 ± 0.559 | 4.53 ± 0.43 | 5.02 ± 0.372 | Simon 1990 |
| LDH, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 10.28±1.289 | 13.2 ±1.1 | 9.78 ±0.896 | Simon 1990 |
| GPD, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 1.43 ± 0.422 | 1.68 ± 0.31 | 2.22 ± 0.416 | Simon 1990 |
| CPK, µmol$_{subst}$ $min^{-1}$ $kg^{-1}$ | 10.7± 1.7 | 7.29± 0.87 | 7.75 ± 0.715 | Simon 1990 |
| ATP, nmol $g_{ww}^{-1}$ | 834 ± 119 | – | 749 ± 109 | Simon 1990 |
| ADP, nmol $g_{ww}^{-1}$ | 589 ± 36 | – | 730 ± 108 | Simon 1990 |
| AMP, nmol $g_{ww}^{-1}$ | 321 ± 34 | – | 449 ± 80 | Simon 1990 |
| IMP, nmol $g_{ww}^{-1}$ | 1,108±775 | – | 490 ±122* | Simon 1990 |
| EC | 0.64 ± 0.03 | – | 0.57 ± 0.04 | Simon 1990 |

(continued)

**Table 5.2** (continued)

| | 0.1 MPa | 10.1 MPa | | References |
|---|---|---|---|---|
| | | 1–3 days | 30 days | |
| **Membrane extracts** | | | | |
| PC, % total | 59.2 ±2.27 | – | 50.5 ± 1.2* | Sébert et al. 1994 |
| PE, % total | 11.6 ±1.4 | – | 19.8 ±1.5* | Sébert et al. 1994 |
| Cholesterol, nmol $mg_{Prot}^{-1}$ | 11.5 ± 2.3 | – | 20 ± 3.6* | Sébert et al. 1994 |

The experimental pressure was 10.1 MPa (1,000 m depth) and the rate of compression was 1.0 Mpa $min^{-1}$. TW = 15–17°C.
*Statistically different ($p < 0.05$ or better) from controls at 0.1MPa. PC: phosphatidylcholine; PE: phosphotidylethanolamine. Values are mean ±SEM.

**Table 5.3** Physiological data (mean ± SEM) in red (R) and white (W) muscles from yellow and silver freshwater eels, *Anguilla anguilla*, under normal and high pressure

| | | 0.1 MPa | 10.1 MPa | | References |
|---|---|---|---|---|---|
| | | | 1–3 days | 30 days | |
| **Red muscle fibres respiration** | | | | | |
| ADP/O | R | 2.52 ± 0.04 | – | 2.87 ± 0.05* | Théron et al. 2000 |
| MO2, µmol $min^{-1}$ $g^{-1}$ | W | 0.10 ± 0.01 | – | 0.11 ± 0.01 | Vettier and Sébert 2004 |
| | R | 0.29 ± 0.05 | – | 0.28 ± 0.04 | Vettier and Sébert 2004 |
| $OH^{\bullet}$ $ng.g_{ww}^{-1}$ $min^{-1}$ | R | 6.5 ± 0.9 | – | 5.5 ± 1.2 | Amérand et al. 2005 |
| **Membranes** | | | | | |
| Anisotropy, | R | 0.247± 0.002 | – | – | Vettier et al. 2006 |
| Unsaturation, index | R | 336 ± 3 | – | – | Vettier et al. 2006 |
| Saturation ratio | R | 045 ± 0.01 | – | – | Vettier et al. 2006 |
| Cholesterol, nmol $nmol^{-1}$ Phospholipids | R | 0.109 ± 0.005 | – | – | Vettier et al. 2006 |
| **Glycolysis** | | | | | |
| Glycolytic flux, $nmol_{G6P}$ $min^{-1}$ $mg_{Prot}^{-1}$ | W | 8.11 ± 0.9 | – | 14.8 ± 1.3* | Sébert et al. 1998 |
| Transition time, s | | 0.6 | | 0.4* | Sébert et al. 1998 |
| **Muscle composition** | | | | | |
| Mean fibre area, $\mu m^2$ | W | 1,747 ± 55 | – | 1,458 ± 50 | Simon et al. 1991 |
| | R | 291 ± 7 | – | 285 ± 71 | Simon et al. 1991 |
| Water content, mg 100 $mg^{-1}$ | W | 70 ± 1 | – | 71 ± 1 | Simon et al. 1991 |
| Protein content, mg 100 $mg^{-1}$ | W | 3.7 ± 0.3 | – | 2.5 ± 0.2 | Simon et al. 1991 |
| | R | 2.2 ± 0.2 | 2.2 ± 0.3 | 2.3 ± 0.2 | Simon et al. 1991 |
| Lactate, µmol $g_{ww}^{-1}$ | W | 49.4 ± 4.5 | 43.2 ± 1.6 | 42.2 ± 6.7 | Simon 1990 |
| | R | 3.1 ± 4.2 | 22.3 ± 6.1 | 23.4 ± 3.0 | Simon 1990 |
| Glycogen, µg $g_{ww}^{-1}$ | W | 0.4 ± 0.04 | 0.1 ± 1.6* | 0.3 ± 0.0 | Simon 1990 |
| | R | 2.1 ± 0.4 | 1.6 ± 0.2 | 1.8 ± 0.3 | Simon 1990 |
| Fatty acids, µmol $g_{ww}^{-1}$ | W | 1.7 ± 0.3 | 2.1 ± 0.4 | 1.9 ± 0.3 | Simon 1990 |
| | R | 2.7 ± 0.5 | 0.2 ± 0.1* | 2.8 ± 0.6 | Simon 1990 |

The experimental pressure is 10.1 MPa (1,000 m depth) and the rate of compression is 1.0 MPa $min^{-1}$. TW = 15–17°C.
*Statistically different ($p < 0.05$ or better) from controls at 0.1MPa. the data from Simon (1990) and Simon et al. (1991) concern yellow eels. Values are mean ± SEM

**Table 5.4** Enzymes activities (μmolsubst $min^{-1}$ $kg^{-1}$) and energetic nucleotides (nmol $gww^{-1}$) in red and white muscles from yellow freshwater eels, Anguilla anguilla, under pressure (mean ± SEM)

| | White muscle | | | Red muscle | | |
|---|---|---|---|---|---|---|
| | 0.1 MPa | ST | LT | 0.1 MPa | ST | LT |
| IDH | 0.74 ± 0.06 | 0.79 ± 0.08 | 1.25 ± 0.23 | 0.68 ± 0.30 | 2.33 ± 0.30* | 1.47 ± 0.31 |
| MDH | 43.6 ± 6 | 59 ± 58 | 51.3 ± 2.5 | 39.5 ± 7.4 | 180 ± 23* | 80 ± 15 |
| CS | 1.29 ± 0.16 | 1.22 ± 0.1 | 1.38 ± 0.11 | 1.16 ± 0.17 | 5.09 ± 1.53 | 2.28 ± 0.59 |
| COX | 0.025 ± 0.007 | 0.012 ± 0.0045 | 0.05 ± 0.01* | 0.060 ±0.019 | 0.065 ± 0.01 | 0.088 ± 0.027 |
| GOT | 5.11 ± 0.09 | 7.0 ± 0.8 | 7.38 ± 0.37* | 6.02 ± 0.66 | 20.36 ± 3.31* | 11.03 ± 1.90 |
| GPT | 0.34 ± 0.02 | 0.28 ± 0.02 | 0.48 ± 0.04* | 0.26 ± 0.06 | 0.36 ± 0.03 | 0.26 ± 0.03 |
| PFK | 3.78 ± 1.97 | 2.53 ± 1.22 | 6.85 ± 1.59 | 0.77 ± 0.441 | 0.34 ± 0.15 | 1.40 ± 0.29 |
| PK | 130 ± 6 | 111.4 ± 12.8 | 150 ± 18 | 95.2 ± 22.6 | 93.9 ± 12.6 | 97.3 ± 13.0 |
| LDH | 376 ± 43 | 529 ± 25 | 406 ± 33 | 226 ± 22 | 370 ± 50* | 262 ± 36 |
| GPD | 0.057 ± 0.007 | 0.092 ± 0.008 | 0.068 ± 0.01 | 0.081 ± 0.015 | 0.160 ± 0.008* | 0.132 ± 0.013* |
| CPK | 891 ± 38 | 756 ± 36 | 946 ± 68 | 559 ± 83 | 578 ± 115 | 504 ± 87 |
| ATP | 2,937 ± 267 | – | 2,457 ± 218 | 1,536 ± 212 | – | 2,130 ± 340 |
| ADP | 900 ± 46 | – | 870 ± 105 | 346 ± 59 | – | 522 ± 81 |
| AMP | 112 ± 28 | – | 143 ± 42 | 39 ± 9 | – | 45 ± 5 |
| IMP | 915 ± 321 | – | 759 ± 246 | 220 ± 78 | – | 277 ± 66 |
| EC | 0.85 ± 0.02 | – | 0.85 ± 0.03 | 0.89 ± 0.01 | – | 0.88 ± 0.01 |

The experimental pressure was 10.1 MPa (1,000 m depth) and the rate of compression was 1.0 MPa $min^{-1}$. TW = 15–17°C.
*Statistically different ($p < 0.05$ or better) from controls at 0.1MPa; ST: short-term pressure exposure; LT: long-term pressure exposure. From Simon (1990). Values are mean ± SEM

**Table 5.5** Oxygen consumption ($mmol.h^{-1}$ $kg^{-1}$) of eels at different stages

| | 0.1 MPa | End of compression | Ratio |
|---|---|---|---|
| Glass | 4.2 | 24 | 5.7 |
| Yellow | 1.2 ± 0.04 | 3.7 ± 0.1 | 3.1 |
| Silver | 1.6 ± 0.2 | 2.5 ± 0.5 | 1.6 |

The fish were compressed at a rate of 1.0 MPa $min^{-1}$ up to 10.1MPa. data from Vettier and Sébert (2004). Note the lower pressure sensitivity of silver eels. Values are mean ± SEM (N = 5 in each group of adults). Results for glass eels have been obtained from one group of 120 individuals (Sébert, P 1992, unpublished data)

it is useful to consider some parameters such as the pressure threshold (Ptr) which is the pressure level corresponding to the appearance of the above symptoms and /or the maximal oxygen consumption generally observed at the end of the compression period (Table 5.5). It has been recently suggested that at least a part of the strong motor activity observed during compression is due to disturbance of the hydrodynamical regulation of buoyancy resulting in a deflated swim bladder (see Section 5.4.2; Speers-Roesch et al. 2004).

At the end of the compression period, performed at 0.2 MPa $min^{-1}$ or 1MPa $min^{-1}$ depending on the experiment, the oxygen consumption progressively decreases despite the fact that a strong motor activity is maintained (Fig. 5.1). This pattern appears to be common to several fish species (see, Fontaine 1929; Naroska 1968). Measurements performed after 3 h at 10.1 MPa hydrostatic pressure show a decrease in muscle ATP content and energy charge together with a decrease in cytochrome oxidase activity. As oxygen transport is not altered with pressure (the circulatory convection is generally increased: Belaud et al. 1976; Gennser et al. 1990; Pennec and LeBras 1988; together with catecholamine release: Sébert et al. 1986), it has been proposed that hydrostatic pressure induces a state resembling histotoxic hypoxia, by decreasing membrane fluidity (Sébert et al. 1987; Sébert 1993). The first 48 h under pressure appear critical. In fact, if a fish has an efficient anaerobic metabolism to compensate for the deleterious pressure effects on aerobic processes, it can survive: this is the case for eel and goldfish, *Carassius auratus*, which can produce sufficient energy from the anaerobic pathway but not for trout, *Oncorhynchus mykiss*. After this critical period, the animal is generally able to acclimate to the effects of high hydrostatic pressure (Johnstone et al. 1989; Simon et al. 1989). The term "generally" is chosen because our experience shows that since about 10–15 years ago, the resistance of yellow eels to compression seems to decrease concomitantly with the observed decline in population. Now, when yellow eels are exposed to 10.1 MPa hydrostatic pressure for a long period (about 1 month), there exist numerous acclimatization processes which concern energy production. A complete description of these acclimatization processes has been given earlier (Sébert 2003), and will be summarised below. From the maximal value reached at the end of the compression period, oxygen consumption decreases progressively (over 6–8 days) to a level which represents about 65% of the oxygen consumption before compression (Fig. 5.1). Note that such a decrease (−35%) observed in pressure acclimatised fishes corresponds to the effects of a 3–4°C decrease in temperature, considering $Q_{10} = 2$: this is in the range of what is generally observed for the relationship temperature/pressure for several physiological processes i.e. about −2°C to −5°C for 10.0 MPa (Brauer et al. 1985). This acclimatization process includes restoration of muscle nucleotides (ATP, ADP, AMP) and energy charge and, although the aerobic pathway appears restored to the level observed at atmospheric pressure (0.1 MPa), also of the anaerobic capacity (Sébert et al. 1998). The restoration of the aerobic pathway during pressure acclimatization is accompanied by an improvement of oxidative phosphorylation efficiency together with readjustment of relative activities of the respiratory chain complexes (Théron et al. 2000). This seems to lead to a decrease in electron leaks and consequently to a decrease in reactive oxygen species, ROS, production (Amerand et al. 2005, 2006). If the eel is recompressed during the week after decompression, the acclimatization process takes place within some hours, at least as judged from the steady state in oxygen consumption (Simon et al. 1989). This indicates that the mechanisms which have been involved in the acclimatization process have produced effects which are still present. Measurements performed after 1 month under pressure show that the acclimatization and thus the improvement in oxidative phosphorylation is due, at least in great part, to the restora-

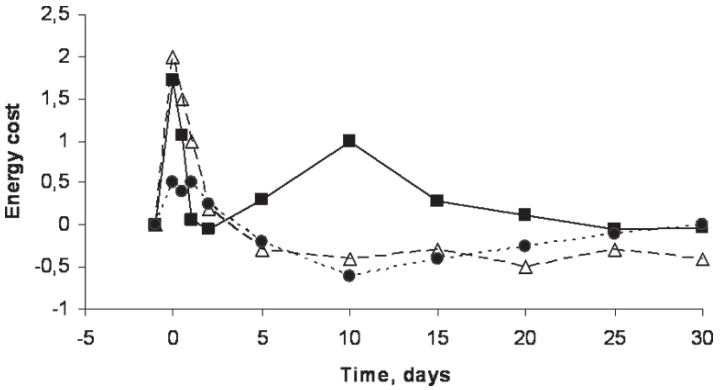


**Fig. 5.1** Energy cost of pressure exposure (mmol $h^{-1}$ $kg^{-1}$). Eels are compressed at 0.2 MPa $min^{-1}$ to 10.1 MPa (yellow eels: triangles and silver eels: squares) or to 6.1 MPa (circles-silver eels) at 1.0 MPA.$min^{-1}$ (for 12 h) then decompressed (for 12 h)

tion of membrane fluidity by increasing unsaturated fatty acids proportions, at the expense of saturated ones, in the phospholipid bilayer (Section 5.4.3.7). To explain all these modifications, which take place if the yellow eel is exposed to high pressure for a long time, we have proposed that at atmospheric pressure yellow eels have a suprafunctioning of the mitochondrial apparatus (despite the resulting energy cost, but they are still feeding). When pressure is applied, its effects are to restore a normal mitochondrial functioning allowing the eel to cope with pressure without a deleterious decrease in energy production (Sébert and Théron 2001).

It is interesting to point out that at least two morphological changes are observed after 1 month under pressure. The first is at the white muscle level, where there is an increase in small diameter fibres at the expense of large fibres, leading to an overall 16% decrease in mean fibre area (Table 5.3) together with a 32% decrease in protein content (Simon et al. 1991). The second is that, in studies of the gill epithelium of freshwater yellow eels acclimatised to HP, Dunel-Erb et al. (1996) found a significant decrease in the number of mucus cells and a large increase in density and in fractional area of chloride cells on the apical surface (Table 5.2). This is thought to compensate, at least partly, for the impairment of ATPase by high pressure (Sébert et al. 1991). It is surprising that the yellow eel under pressure increases its ability to excrete salts although it stays in fresh water.

Concluding this section, yellow freshwater non-migrating eels are able to acclimate to the hydrostatic pressure effects. The acclimatization process mainly concerns the aerobic pathway (improvement of oxidative phosphorylation efficiency) by way of homeoviscous adaptation. The question could be raised about the ability of yellow eels to migrate. In this stage, the eel is able to cope with pressure effects,

changes in salinity and /or temperature and is known to have the capacity to swim for a long distance (Van Ginneken et al. 2005a). But the yellow eel is unable to reproduce. Thus we must consider that what is observed in yellow eels reflects potential ability to migrate but they do not migrate because they are not sexually ready! The metamorphosis, i.e. the silvering process, appears strictly necessary.

## 5.4 Silver Eels Under Pressure

### *5.4.1 Effects of Metamorphosis (Silvering Process) on Energy Metabolism*

The morphological changes which appear during the different eel life stages are reviewed by Tesch (2003) and Durif et al. (2005). It is known that during the silvering process, red muscle volume increases (Pankhurst 1982) as is observed in pelagic fishes which can swim at depth (Altringham and Ellerby 1999). The volume increase is probably due more to an increase in fat and mitochondria than to an increase in number of muscle fibres (Lewander et al. 1974; Pankhurst 1982). Moreover, activities of enzymes involved in the aerobic pathway are higher in silver than yellow eels

**Table 5.6** Effects of long term exposure of male silver eels to high pressure

| | Muscle | 0.1 MPa | 10.1 MPa 3 days | 10.1 MPa 25 days | 10.1 MPa 50 days |
|---|---|---|---|---|---|
| Muscle fibre respiration, μmol $min^{-1}$ $g^{-1}$ | R | 0.48 ± 0.0 | 0.59 ± 0.05 | 0.57 ± 0.04 | 0.53 ± 0.03 |
| | W | 0.14 ± 0.01 | 0.21 ± 0.02 | 0.20 ± 0.02 | 0.14 ± 0.01 |
| Water muscle content, % | W | 61.9 ± 1.6 | 63.7 ± 1.3 | 61.2 ± 1.1 | 60.2 ± 1.8 |
| Protein content, mg $g^{-1}$ | R | 20.7 ± 1.0 | 18.7 ± 0.7 | 20.3 ± 0.6 | 26.7 ± 1.4 |
| | W | 23.0 ± 0.6 | 23.4 ± 1.3 | 24.1 ± 0.8 | – |
| ATP, μmol $g^{-1}$ | R | 1.7 ± 0.1 | 1.14 ± 0.24 | 1.19 ± 0.18 | 1.6 ± 0.7 |
| | W | 2.3 ± 0.3 | 2.4 ± 0.2 | 2.5 ± 0.3 | 2.4 ± 0.4 |
| AS, μmol $g^{-1}$ | R | 1.95 ± 0.1 | 1.38 ± 0.25 | 1.58 ± 0.24 | 2.1 ± 0.12 |
| | W | 2.8 ± 0.3 | 2.8 ± 0.2 | 3.1 ± 0.3 | 3.2 ± 0.52 |
| EC | R | 0.93 ± 0.01 | 0.90 ± 0.01 | 0.87 ± 0.01 | 0.89 ± 0.01 |
| | W | 0.92 ± 0.01 | 0.92 ± 0.01 | 0.98 ± 0.01 | 0.97 ± 0.01 |
| Gill water content, % | – | 81 ± 0.3 | 81 ± 0.4 | 82 ± 0.2 | 80 ± 0.3 |
| COX activity, μmol $min^{-1}$ $g^{-1}$ | R | 79.0 ± 2.0 | 71.0 ± 5.0 | 82.2 ± 2.0 | 120.6 ± 19.3 |
| | W | 9.3 ± 1.6 | 7.6 ± 1.3 | 6.1 ± 0.4 | 8.4 ± 2.2 |
| Myoglobin, mg $g^{-1}$ | R | 1.7 ± 0.13 | – | 1.7 ± 0.24 | – |

Values post decompression: Vettier and Sébert (2004) and unpublished data. The experimental pressure was 10.1 MPa (1,000 m depth) and the rate of compression was 1 MPa $min^{-1}$. R is for red muscle and W for white muscle. Values are mean ± SEM

(Boström and Johansson 1972; Egginton 1986) together with a change in the main energy stores from glycogen in the yellow stage to fat in migrating fish (Lewander et al. 1974; Barni et al. 1985; Zara et al. 2000). It is probable that the hormonal changes observed during metamorphosis (Marchelidon et al. 1999; Chapter 3) help in the use of fat as the main substrate for aerobic energy production. Concomitantly, an increase in myoglobin content is observed (Egginton 1986). Clearly, silver eels have higher aerobic capacities than yellow eels and this is in agreement with the observation that the mass-specific power output of silver phase slow muscle is greater than that of yellow phase slow muscle (Ellerby et al. 2001). Note that twitch tension of swimming muscle of seawater adapted eels increases with pressure and is maximal at 20 MPa (Wardle et al. 1987), together with absence of pressure sensitivity for cardiac contraction (Gennser et al. 1990). In terms of muscle mechanics, it cannot be excluded that fast white muscle is recruited to help in obtaining optimal swimming performance (Ellerby et al. 2001); the fact that aerobic capacities increase in white muscle during silvering (Boström and Johanson 1972) and/or pressure exposure argues in this sense (Tables 5.4 and 5.6).

Thus, metamorphosis from yellow to silver stages prepares the eel to cope with many of the special conditions linked to migration at depth: energy metabolism (see above), morphological changes (Tesch 2003; Durif et al. 2005), ability to swim (Ellerby et al. 2001; Durif et al. 2005; Thillart in this book), salinity (Fontaine 1975; Lecomte-Finiger and Yahyaoui 1990; Bertin 1951; Thomson and Sargent 1977), obscurity (Carlisle and Denton 1959; Pankhurst and Lythgoe 1983; Tesch 2003), floatability (Kleckner 1980; Pelster 1997), absence of feeding activity (Zara et al. 2000; Durif et al. 2005), and evidently reproduction by way of sexual maturation. But what about pressure?

### *5.4.2 Pressure Effects and Pressure Resistance*

Unfortunately, adult migrating silver eels have rarely been caught (Van Ginneken and Maes 2005; with a camera: Robins et al. 1979). Consequently, it is impossible to describe the physiological adjustments which occur when the eel is really adapted to a deep environment and not to only one or several factors. However, the experimental data obtained and the comparison of silver and yellow eels helps in the understanding of the processes involved.

As for yellow eels, effects of pressure on silver eels have been studied only using a hyperbaric chamber (Johnstone et al. 1989; Nilsson et al. 1981) except for Fontaine et al. (1985) who used caging at different depths. They observed significant ovarian development and a strong increase in GTH level which was not due to decreased light (Dufour and Fontaine 1985). Moreover, recent data from our team (Vettier 2005) show a significant decrease in hepato-somatic index and a trend to increase in gonado-somatic index after 1 month under pressure, variations which are typical of the silvering process (Durif et al. 2005). These results suggest that hydrostatic pressure is involved in the triggering of sexual development and plays a positive role

in eel reproduction (Sébert et al. 2007), even if a certain level of energy release (by swimming) seems a prerequisite to induce sexual maturation (Nilsson et al. 1981). We cannot exclude the hypothesis that high pressure can modify gene expression (Bartlett et al. 1989, 1993, 1995; Simon et al. 1994) and thus protein synthesis involved in all life processes. How do silver eels cope with pressure effects (which require energy) at the same time as they must swim for 6,000 km to reach the Sargasso Sea?

As we have seen previously, specific pressure effects can depend on several factors, including rate of compression, temperature, pressure used, species, and developmental stage. Thus, different protocols can be used to expose silver eels to high pressure: Fig. 5.1 shows that the diving pattern is not very important, at least at the level of animal metabolism evaluated from oxygen consumption. It must be pointed out that nobody knows exactly how silver eels reach great depths: is it abruptly starting at the continental plateau or progressively? This latter pattern is the more evoked in relation to some works showing vertical migrations, at a weekly time scale, but for released fish (Tesch 1978; Fricke and Kaese 1995; McCleave and Arnold 1999). Indeed, information about the depth of spawning has been extrapolated from data on the release of hormone-treated females tagged with transmitters, and on larval catches. Again, the number of telemetry studies and the number of radio transmitter-tagged animals is low. Releasing female adults in the Sargasso Sea demonstrated a preference for the upper zone of the ocean at depths of 250–270 m and at temperature around 19°C (Fricke and Kaese 1995). However, in the study of Tesch (1989) the maximum swimming depth of hormone treated silver female eels in the Sargasso Sea was nearly 700 m. Hormone treated female Japanese silver eels, *Anguilla japonica*, tagged with ultrasonic transmitters were released at their supposed spawning grounds in the western Pacific Ocean. These eels preferred relatively shallow water, swimming at a depth ranging from 81–172 m and at relatively high temperatures of 18–28°C (Aoyama et al. 1999). Interestingly, the catch of *Anguilla* larvae of less than 5 mm length confirmed these observations. The smallest (probably just hatched) larvae were found at depths between 50 and 300 m with temperatures of 24°C to 18°C respectively (Castonguay and McCleave 1987). Those temperatures are close to the final preferred temperature (FPT) of sexually mature *Anguilla rostrata* (17.5°C), so spawning probably takes plays in the upper 200 m of the ocean at temperatures close to FPT (Haro 1991). Although the preceding authors suggest depths between 20 and 700 m for spawning, in reality it is perhaps higher during migration. Indeed, the increase in swim bladder function in silver when compared to yellow eels extends the maximal depth at which silver eels can maintain swim bladder volume (Kleckner 1980). Sébert (2007) has recently calculated that the migration depth is potentially around 2,000 m. This calculation is based on the measured pressure threshold values (Ptr, the pressure at which the animal exhibits tremors and/or convulsions indicating muscle dysfunction and consequently inability for swimming) in migrating silver eels compared to those observed in deep living fishes. Ptr is estimated to be about 240 atm at surface temperature which means that at depth temperature, Ptr is probably near to 400 atm. As Ptr generally corresponds to twice the depth of living, the latter can be estimated to be about 200 atm (2,000 m depth). However, the effects of hydrostatic pressure

on energy metabolism of silver eels appear of little importance (Vettier 2005 and Table 5.6). Submitting red permeabilized muscle fibres to 10.1 MPa HP induces a small decrease (from 0% to 25% depending on the eel origin) in oxygen consumption (Vettier and Sébert 2004). However, when the intact eel is exposed to 10.1 MPa for 3, 30 or 50 days, the observed changes are small and the eels are able to acclimate. An interesting point is that pressure exposure induces an improvement of aerobic capacities in red but also in white muscle. In the white muscle, this improvement is realised by way of a cytochrome c oxidase (COX) activity increase and a trend to increase ATP content (Vettier and Sébert 2004). COX seems to play an important role in pressure acclimatization (Theron et al. 2000; Sébert and Theron 2001), which is not surprising considering that it is the last enzyme complex of the respiratory chain and is responsible for oxygen consumption.

Interestingly, the COX activity appears to increase with the maturation stage and /or the pressure exposure (Fig. 5.2). The improvement in aerobic capacities, whatever the muscle type, is evidently in relationship with the high energy demand due to swimming activity during the migration. Clearly, silver eels have higher aerobic capacities than yellow eels at atmospheric pressure (Egginton 1986). Pressure exposure can improve these capacities, helping the silver eels to resist its effects. However, several parameters are able to modulate the pressure resistance and /or acclimatization. In order to estimate the role of different parameters in the acclimatization process, it was necessary to have a viable index. In this aim, knowing that pressure resistance and thus success of the acclimatisation, depends mainly on events during the first hours under pressure, a Pressure Resistance Index (PRI) has been proposed (Vettier 2005). The higher the PRI, the higher the fish fitness. The PRI is calculated from normalised values of Pressure threshold (the pressure at which the fish exhibits tremors) and maximal oxygen consumption observed at the end of compression period. These two parameters, which allow a good estimation of pressure sensitivity (Sébert and Macdonald 1993), are combined with the energy charge values, EC, observed after decompression because EC reflects the possibility for the fish to restore a "normal" energetic state i.e. to acclimate (Simon et al. 1992). To illustrate this, experiments performed in our laboratory have shown that trout, yellow eel and silver eel have PRI values of about –22, 1 and 16 respectively.

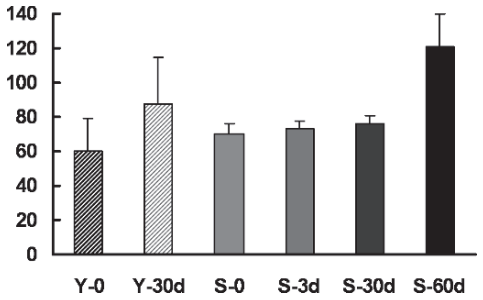


**Fig. 5.2** COX activity (mU.g$^{-1}$) in eel red muscle. Y: yellow stage; S: silver stage; the number besides the stage is the duration (days) of exposure to 10.1 MPa hydrostatic pressure

## *5.4.3 Factors Modulating Pressure Resistance*

### 5.4.3.1 Freshwater or Not

Yellow and silver eels can live in different aquatic environments, for example freshwater or marsh with different salinities. Knowing that these eels will migrate in seawater (silver eels are very well pre-adapted to sea water; Thomson and Sargent 1977; Lecomte-Finiger and Yahyaoui 1990), is there an advantage for them to live in brackish water? We have compared silver males from these two origins. If the two populations are similar in terms of size, some physiological characteristics at 0.1 MPa are very different: density and muscle water content are higher in saltwater but COX activity, energy charge, protein contents of red muscle and haematocrit are lower (Vettier and Sébert 2004). Clearly, red muscle from marsh eels has less good muscle quality (more water, less proteins) which induces apparent lower aerobic capacities at 0.1 MPa. However, it appears that after some days under pressure (10.1 MPa), many of these parameters are improved, in contrast to what is observed in fishes living in freshwater. Although the two groups were tested in freshwater (Vettier and Sébert 2004; Vettier 2005), it appears that growing in salt water (even if the salt concentration is low, as in the marsh) has disadvantages at 0.1 MPa but allows of a faster acclimatisation to pressure effects: about 3 days are needed in contrast to several weeks for freshwater eels. However, we cannot confirm that this result is exclusively due to differences in salinity because the two populations are different in origin and we do not know exactly what the physico-chemical properties of the two aquatic environments and the food quality and availability are.

### 5.4.3.2 Parasitism

Most eel populations are known to be highly infected by *Anguillicola crassus*, the development of which appears to depend on the temperature (Knopf et al. 1998). This nematode damages the swim bladder (Molńar et al. 1994; Würtz and Taraschewski 2000; Chapter 9). Damaging the gas gland epithelium could impair the production of salts, e.g. lactate, that play a central role in gas secretion (Kobayashi et al. 1989) which is necessary to compensate for the high density of muscle tissue and skeletal elements (Pelster 2004). If such damages, which seem to depend on the fish size (Lefebvre et al. 2002), appear not too deleterious for non-migrating eels, they can become severe in migrating silver eels where gas secretion rates are about five times greater in order to maintain swim bladder volume and thus extend the maximal depth at which they can migrate (Kleckner 1980). Thus it has been shown that these damages could impede the supposed vertical displacements that eels perform during their migration to the Sargasso Sea, although the effects on swimming capacities have also been discussed (Sprengel and Luchtenberg 1991; Kirk et al. 2000; Nimeth et al. 2000). Our experiments have shown that parasitism, whatever its level, does not modify pressure resistance

(Vettier et al. 2003), bearing in mind that the latter is evaluated from short term pressure exposure. However, parasitism does not seem to have drastic effects (Kelly et al. 2000; Roche et al. 2003) on the ability of the eels to acclimatise to sea water and hydrostatic pressure (caging experiments, Fontaine et al. 1990), although some of our results suggest that infected eels do not have the respiratory and metabolic capacities to migrate and to cope with all the changes the migratory activity entails prior to reproduction (Vettier and Sébert 2004).

#### 5.4.3.3 Nutritional State

Silver eels stop feeding even before migration (Zara et al. 2000; Durif et al. 2005); this goes together with a progressive reduction of the digestive tract (Tesch 2003). It is known that pressure acclimatisation is achieved mainly by a restoration of membrane fluidity by increasing the recruitment of polyunsaturated fatty acids, PUFA (see Sébert 2003 and Section 5.2). Synthesis of PUFA is more difficult in fishes living in seawater (Watanabe 1982; Bell et al. 1986; Green and Selivonchick 1987) which is the condition during migration. Thus, eels must probably have enough PUFA stored before starting towards the ocean. In this sense, it is probable that availability and quality of food in the aquatic environment prior to migration plays an important role (Vettier and Sébert 2004), dietary history being very important for metabolic machinery (Moon 1983). Now, the question is about the usefulness of fasting during migration. Several studies have shown that food deprivation for 6 months exerts little metabolic effect (Love 1970; Moon 1983; Cornish and Moon 1985) but can compromise muscle contractile proteins (Moon and Latham 1984). As it seems that eels have sufficient fat stores to migrate (Van Ginneken and Van Den Thillart 2000), it can be supposed that feeding is not necessary and that proteins from the digestive tract could be used to build up gonads. Another interesting aspect is that muscle fibres from long-term fasting eels are more resistant to high pressure (in terms of aerobic capacities) than fibres from eels having fasted for a short time (see Fig. 5.3). Perhaps this can be explained by the fact that, at least in ectotherms, dietary restriction allows locomotor superiority

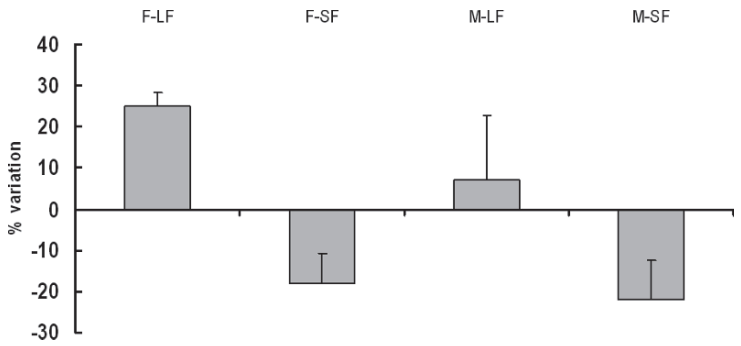


**Fig. 5.3** Muscle fibre respiration changes at 10.1 MPa (in % of variation by comparison with values at 0.1 MPa). F: females; M: males; LF: long fasting; SF: short fasting

(Le Galliard et al. 2004), and reduces the cost of protein assimilation (Owen 2001), although some results seem to show that digestion can increase maximal oxygen capacity (Hicks and Bennet 2004). Perhaps it can be concluded that fasting helps migration by enhancing pressure resistance. But fasting could have also "mechanical" effects. Indeed, as they use only fat stores, the density of the fish is expected to progressively increase (Vettier 2005) as it spends longer in seawater (McCleave 1977). Indeed, although the relative composition of the body does not change during swimming (Van Ginneken et al. 2005b; Chapter 8), the volume of fat decreases together with the body mass. Due to lower lipid density, the eel loses more volume than mass and consequently animal density is expected to increase, which could help it to swim deeper and deeper and/or to counteract the higher density of coldwater encountered at depth without extra effort to maintain neutral floatability.

#### 5.4.3.4 Water Temperature

Water temperature is probably not a problem for the migrating eel because, at least in the yellow stage, it is known to tolerate a large temperature range. However, we do not know at what depth and thus temperature the eel migrates: some authors suggest that with the optimal temperature for sexual development being a little over 20°C, the spawning depths could be about 200 m (Nilsson et al. 1981). If low temperature is known to increase aerobic capacity of fish swimming muscle (Guderley 1990), it has negative effects on membrane fluidity, at least for short term exposure (Hazel 1995) and interacts with pressure effects at the mitochondrial level (Sébert et al. 2004). Moreover, it is well established that acute temperature changes have detrimental effects on both central nervous system processes and contractile performance in muscle of fishes (see Block 1991). Thus we can assume that eels migrate at a depth where temperature is not too low, in the 13–22°C range, indicating their great thermal tolerance during oceanic migration (Tesch 2003). However, it is not clear if the eels swim towards a rising temperature gradient or avoid colder water (Ekman 1932; Westin and Nyman 1979). The vertical migrations (up to 300 m, deeper during the day than at night) can induce large temperature variations: do they have a metabolic regulation role (Block 1991) or is it to avoid predators? We have no answer.

The conjunction of low temperature and high pressure is not good in terms of thermodynamics, but it has been shown that, in eels acclimated to high pressure, which is the case for migrating silver eels, temperature has little effect on metabolism (Sébert et al. 1995).

#### 5.4.3.5 Buoyancy

Due to a density (d = 1.05–1.20) higher than seawater (d = 1.026) fishes must use different mechanisms to maintain neutral buoyancy and thus to stay in the water column (Pelster 1997). If this is not the case, they sink. The best known apparatus

is a gas-filled cavity, the swim bladder (SB). Active and passive mechanisms also exist. The main active process consists in producing hydrodynamic lift, due to water flow over wing-shaped fins and due to hovering, swimming at higher speed and/or at the optimal tilt angle by combining pectoral fin lift, body lift and lift from the tilted thrust vector. These active processes are independent of depth but represent an energy cost (Strand et al. 2005). The passive mechanisms consist mainly, in fishes living at depth, accumulating lipids (even in the SB) and/or water in different tissues; decreasing content of heavy ions, in order to decrease the fish density (Pelster 1997).

The swim bladder raises interesting problems in the migrating silver eels. If physoclistous fishes (closed SB) are placed under increased hydrostatic pressure, they secrete gases into their SB and if the pressure is reduced, they reabsorb gases. Physostomatous fishes have a SB with a pneumatic duct and sometimes also a gas gland and a rete: this is the case for the eel. Fishes in seawater, such as the migratory eel, need a SB occupying about 5% of the body volume (Bone et al. 1995). The very efficient counter-current system of the *rete mirabile* allows accumulation of gas in the SB with pressure gradients which may be up to several hundred atmospheres (Kobayashi et al. 1989; Pelster and Scheid 1992; Pelster 1997 for reviews) but the energy cost to pressurise gases is very low (Strand et al. 2005). The diffusive gas loss is reduced by an increase in the crystalline guanine content of the SB wall during the yellow-silver eel metamorphosis (Kleckner 1980; Bone et al. 1995). It is known that nitrogen, $CO_2$ and even argon can accumulate depending on the depth but the main gas is oxygen (Schölander and Van Dam 1953; Pelster and Scheid 1992). This has two consequences. The first is illustrated by the following calculation. Considering a migrating silver eel with a body volume of 1 L (body mass around 1.1 kg); it has a SB volume of about 50 mL. If they dive from the surface to 1,000 m depth (10.1 MPa), maintenance of the SB volume requires a gas secretion of 5,000 mL which represents about 220 mmol $O_2$! The secretion rate values are in the range of 1 to 3 mL $h^{-1}$ (Tytler and Blaxter 1973; Kleckner 1980; Goolish 1992): it increases during the yellow-silver stages transition in the eel but decreases with the ambient pressure (Kleckner 1980). So, even using a high rate value of 2 mL $h^{-1}$ (0.1 mmol $O_2$), it takes 2,500 h (more than 3 months) to restore the SB volume, knowing that gas density increases with pressure and thus is less efficient in terms of buoyancy. This suggests that when starting their migration, silver eels must dive slowly and progressively in order to limit excessive oxygen consumption. A second consequence is that at high pressure oxygen becomes toxic for tissues by way of reactive oxygen species, ROS. D'Aoust (1969) has shown that the high oxygen pressures which can be measured inside the SB can induce rigidity of the fins, alterations of breathing and gill function and then tetanic contraction or muscle paralysis. These symptoms are evidently not compatible with swimming activity. However in the eel SB epithelium, a high activity of glutathione reductase, which degrades ROS, has been measured whilst the enzyme was not detectable in muscle tissue: this protects the tissues from the $O_2$ damage (Pelster and Scheid 1992).

Now, at what depth does the eel migrate and does it undergo vertical migrations as suggested by different authors (Tesch 1978; McCleave and Arnold 1999; see also

the review by Van Ginneken and Maes 2005)? Kleckner (1980) has calculated from experiments on silver eels that they can maintain their SB volume up to 150 m depth, which is not very deep when compared to the observations of Robins et al. (1979) or Bailey et al. (2005). As a starting hypothesis, an average depth of 1,000 m can be considered and Tesch (1978) has observed vertical daily migrations up to 400 m during eel tracking experiments. Considering the eel model used above (1 kg body mass with a SB volume of 50 mL) that means that every day, if the volume of SB is maintained for neutral buoyancy, it must alternatively secrete and reabsorb about 30 mL of gas. Gas reabsorption is more rapid than secretion (Tytler and Blaxter 1973; Kleckner 1980) which probably needs more than 12 h (see above) to be completed and thus cannot be realized in the frame of daily vertical migrations. All the above observations suggest that SB is probably insufficient to ensure neutral buoyancy and *a fortiori* vertical migrations as suggested by Speers-Roesch et al. (2004) for *Goregonus hoyi*. This is particularly relevant if we consider that migrating eels have their SB more and more infested with *Anguillicola crassus*. Clearly, it seems reasonable to think that, as in other deep-living fishes (Bone et al. 1995; Pelster 1997), migrating eels progressively abandon gas as a source of static lift because there are difficulties in regulating buoyancy with gases over a wide range of pressure (and thus vertical migrations). The extensive review of buoyancy at depth by Pelster (1997) shows that tissue composition in terms of lipids, water, proteins and ions can help in the process but all the mechanisms involved require energy which becomes unavailable for swimming and reproduction. Some authors have suggested that the lipid content of eggs can help with buoyancy (see Pelster 1997) but this strategy, or that concerning the increase in lipid tissue content, are without relevance in the eel because it does not eat during migration: lipids must come only from fat stores which progressively decrease, probably inducing an increase in density. We raise the hypothesis that maintaining buoyancy using the swim bladder is not really feasible at low energy cost in the migrating eel: consequently, it probably progressively sinks from Europe to the Sargasso Sea. However, this phenomenon is probably somewhat limited, firstly by the fact that temperature decreases with depth making water denser, secondly by using the hydrodynamic lift induced by the swimming activity and helped by the enlarged pectoral fins. Results from possible changes of membrane fluidity and COX activity (Vettier 2005) during the migration suggest that this particular fish progressively improves its pressure resistance and thus can dive deeper and deeper. This agrees with the hypothesis and can explain why adult eels have never been found in the Sargasso Sea.

#### 5.4.3.6 Sex

Studying silver eels from different origins has shown that some differences exist due to the sex of the animal. This is true in terms of aerobic capacities or pressure resistance (Vettier and Sébert 2004) although the sex expression is not always synchronized with metamorphosis (Beullens et al. 1997). In fact, maximal oxygen consumption of muscle fibres has been measured after 1 month under pressure. Then, considering that red muscle represents 30% of total body mass and white muscle

6% of total muscle mass (Goolish 1991) with an increase in red muscle proportion (about +60%) with the silvering process (Eggington 1986) we have estimated the maximal aerobic capacity of males and females under pressure. It is clear that such estimations are only indicative (but realistic, see Kimberly et al. 1997) because composition is probably somewhat different in the two sexes as indicated by the difference in densities. The swimming cost has been estimated to about 2.2 mmol $O_2$ $h^{-1}$ $kg^{-1}$ by Van Ginneken and Van den Thillart (2000) which represents, under pressure, more than 40% of the maximal abilities for females but less than 30% for males. Thus, males have a lower (but good) pressure resistance (Pressure Resistance Index: about 12 and 20 for males and females respectively) with a better aerobic capacity (about 7.5 mmol $O_2$ $h^{-1}$ $kg^{-1}$ and 4.5 mmol $O_2$ $h^{-1}$ $kg^{-1}$ for males and females respectively) with the same characteristics for contraction of isolated fibres (Rossignol et al. 2006). The swimming cost has been estimated on a basis of a 0.5 Body Length $s^{-1}$ speed (BL $s^{-1}$). That means that, if the travel represents 6,000 km, females need about 230 days (about 7 months) and males about 350 days (about 12 months) to reach the spawning area. This is evidently impossible to ensure reproduction! However, it is known that males generally start their migration sooner in the season, i.e. about 50 days before the females: thus they can migrate for 280 days (230 + 50) to meet the females at the same time in the Sargasso Sea. That means they must swim at a speed of about 21.5 km $day^{-1}$ or 0.25 m $s^{-1}$ which represents, in our example, about 0.65 BL $s^{-1}$. Considering that the cost of swimming increases with resistance i.e. with the square of speed, which means that the relative cost of swimming under pressure for the males would be about 40% to 50% of the total aerobic capacities, as in females, which is acceptable as is the estimated speed of 0.6–0.7 BL $s^{-1}$ (Vettier et al. 2006). This simple calculation suggests that males meet the females by starting sooner and swimming faster, which is possible due to their higher aerobic capacities under pressure. However, several questions stay without clear answers. It is known that a linear relationship exists between swimming speed *vs* tail beat frequency (McCleave 1977) which depends on water temperature (Ellerby et al. 2001). Is it the same in males and females? Moreover, we have no clear data to confirm that the males have sufficient fat stores to ensure such an energy requirement, their high density being probably a sign of lower fat content. Indubitably, physiology of migrating males must be studied more because the majority of the available data are from females.

#### 5.4.3.7 Silvering

Effects of silvering on eel physiology have been comprehensively described and many data are available in this book. However, as we have previously shown that yellow eels are able to acclimatize to high hydrostatic pressure, our question is "how the silvering process can help and /or participate in the acclimatization processes?" Considering that the ocular index can give an estimation of the silvering stage, it appears that pressure resistance, evaluated with the PRI, is higher overall

when the silvering process is advanced. However, whatever the silver stage, all the silver eels are able to acclimatize to high pressure (Nilsson et al. 1981; Fontaine et al. 1985; Johnstone et al. 1989; Vettier and Sébert 2004). Moreover, results reported above show that long term exposure to high pressure (10.1 MPa) does not generally change the energetic metabolism features of the silver eel significantly, leading to the hypothesis (Vettier et al. 2005) that the silvering process induces, in the yellow eel, several effects similar to those observed after a long term pressure exposure: increases in COX activity, in gill chloride cell number, decreases in pressure sensitivity, muscle protein contents and reactive oxygen species.

Indeed, the increase in COX activity agrees with the improvement of aerobic metabolism by pressure or silvering (Boström and Johanson 1972; Lewander et al. 1974; Egginton 1986). Improvement of aerobic metabolism corresponds to a better efficiency of the respiratory chain and coupled oxidative phosphorylation (Theron et al. 2000; Sébert et Theron 2001) by way of a lower electron leak, explaining the observed decrease in reactive oxygen species as hydroxy/radical $OH^{\bullet}$ (Amerand et al. 2005, 2006). At the same time, it appears that pressure sensitivity, estimated from the pressure threshold (Ptr) at which eels exhibit a strong motor activity during the compression, is the same in pressure-naïve silver eels at 0.1 MPa as in yellow eels after 1 month under pressure. Such an increase in Ptr is known as an index of pressure adaptation (Sébert and Macdonald 1993). Thus silver eels appear prepared for pressure effects and when they encounter pressure for the first time they have the same behaviour as pressure-acclimated yellow eels. It is a well known fact that most of the pressure acclimatisation process is performed by the way of restoring a normal membrane fluidity (homeoviscous theory) (White and Somero 1982; Cossins and Macdonald 1984; Macdonald and Cossins 1985), the cell membrane appearing as a metabolic pacemaker (Else and Hulbert 2003) and the major target for the HP effects. Evidently, other features of membrane organisation can also influence membrane function (Lee 1991; Hazel 1995) but membrane fluidity, determined by anisotropy measurements, is a good index of the membrane state. Consequently, if the main effects of hydrostatic pressure concern cell membranes and that we consider silver eels as pre-adapted to high pressure we must observe a higher membrane fluidity in silver eels (lower anisotropy) than in yellow eels after 1 month under pressure. This hypothesis has been verified and confirmed by measuring, at atmospheric pressure, red muscle membrane fluidity and composition in silver and yellow eels. The higher fluidity observed in silver eels is ensured by increasing the recruitment of polyunsaturated fatty acids in the membrane phospholipids (Vettier et al. 2006), which is the common way to ensure homeoviscosity with the help of cholesterol as a modulator (Dave et al. 1975).We can thus consider that one of the aims of the silvering process is to perform the main adaptations before migration because they have a non-negligible energy cost. Thus, on entering the migratory process the silver eel can devote all its available energy to swimming activity and gonad development, which would be probably problematic if the fishes must ensure them together with adaptation to their new environment.

## 5.5 Potential Pressure Effects on Reproduction, Eggs and Larval Development

As seen in the preceding sections, high hydrostatic pressure (10.1 MPa) has potential effects on fish energy metabolism and consequently on migration for reproduction. As seen previously, we estimate the maximum migration depth at about 2,000 m. Such a depth and the corresponding pressure of 20.1 MPa probably has no greater effects than the pressure tested (10.1 MPa) on yellow and silver eels' muscle energy metabolism as such. However, nothing allows us to reject the possibility that, when the fish has to swim (using its muscles) and to resist high pressure (that is to say when the fish is in the natural conditions of migration), things could be different. This needs further experiments which are scheduled in our lab.

Most of the studies actually performed on eels have the aim of explaining the decline of the population and to find a solution to stop this phenomenon. One of the often explored possibilities is to control reproduction by artificially obtaining eggs, then larvae able to survive and to develop into glass eels. Although high pressure is known to induce significant increases in pituitary LH content and gonadosomatic index (Fontaine et al. 1985; Vettier et al. 2005) experiments conducted until now to obtain larvae have not been very successful. We are convinced that one of the main reasons is that the pressure factor has been neglected.

Spawning seems to be a surface-related event. Eggs were found to rise in the water column (Balon 1975) up to the surface with maximum speeds of 2.24 m per hour. The main hatching times for European eels are between 47 and 60 h after fertilization (see Van Ginneken et al. 2005). During these times eggs will rise 105–134 m. Assuming that hatching should take place in the food-rich upper water layers, these shallow depths represent spawning depths. However, for the Japanese eel it was observed that high pressure delays embryonic development and hatching times (Hiroi et al. 2003). However, egg ascent in the water column must be accompanied by a volume increase. This latter has to be compensated by some material; it can be supposed that water entry will be facilitated. Indeed, Beney et al. (1997) have pressurized vesicles of egg yolk phosphatidylcholine in pure water and their volumes were measured by optical microscopy. It appears that the volume of vesicles decreases at high pressure (250 MPa) by 16% which is more than can be accounted for by bulk compression of water (10%). This arises from the lateral compression of the bilayer reducing the area, and hence the volume, of the vesicle. Water moves out to accommodate the change. We can reasonably suppose that reversal of this physical effect occurs when pressure decreases, which can modify buoyancy of eggs and then embryos. The buoyancy of eggs has often been used as an indicator in the assessment of egg quality, especially in studies of marine teleosts that spawn pelagic eggs, such as red sea bream *Pagrus major* (Watanabe et al. 1984a, b), sea bass *Dicentrarchus labrax* (Carrillo et al. 1989), yellowtail *Seriola quiqueradiata* (Mushiake et al. 1994) and the Japanese eel (Seoka et al. 2003). In these species, the ratio of buoyant eggs to total eggs spawned, measured several hours after fertilization, correlates positively with egg hatchability, which is based on the fact that

most of the unfertilized eggs and the fertilized eggs that have ceased developing do not remain buoyant for very long.

Till the 1960s, pressure shocks were used to induce triploidy (Dasgupta 1962) in amphibians or in fishes (Rottmann et al. 1991; Gillet et al. 2001) or even tetraploidy in fish embryos (Yamazaki and Goodier 1993). Marsland (1938, 1950, 1970) had shown that high pressures cause inhibition of embryonic development in shallow-water marine invertebrates. It was shown on sea urchin embryos that pressure inhibits the formation of the furrow, normally formed when cells enter into cellular division (Begg et al. 1983). The effects of pressure are different in deep-sea organisms, which silver eels can be considered to be after several months under pressure. Studies done on high hydrostatic pressure effects on embryonic development have also been performed on echinoderms living at depth. It was demonstrated that these organisms nonetheless did not present normal development at too high temperatures but they also need pressure. Experiments have demonstrated that the best pressure is the pressure at which they normally live (Young and Tyler 1993; Tyler and Young 1998). In *Echinus affinis*, the embryos are truly barophilic and could not develop under pressures lower than 1,000 m depth. Moreover, in *Echinus acutus*, which lives at about 1,000 m depth, development at atmospheric pressure is possible but the number of abnormalities decreases with the depth. In the same manner, the asteroid *Plutonaster bifrons* has the highest percentage of normal development near the peak of the species distribution (2,000 m), developmental rate being retarded at pressures higher and lower than that at this depth (Young et al. 1996). Similar conclusions concerning the requirement of high pressure for embryos to develop have been drawn from the vent worms *Alvinella pompejana* and *Riftia pachyptila* (Pradillon et al. 2001). Although they are not well known, pressure effects on egg buoyancy, on larval development and viability are certainly of importance and need further experiments.

## 5.6 Concluding Remarks

Hydrostatic pressure has potential effects on living organisms from the cell to the organismic levels. The eel is thus concerned mainly during its migration for reproduction but eggs and larvae are also potential targets for mechanical effects of pressure. Most of the pressure effects on the eel have been studied on the non-migratory yellow stage. When hydrostatic pressure is applied for a short term period, aerobic metabolism is altered by the way of a decrease in membrane fluidity which can induce inhibition of oxidative phosphorylation. However, the yellow eel is able to acclimatize to the pressure effects by modifying membrane phospholipid composition (which has an energy cost) in order to maintain homeo-viscosity i.e. optimal fluidity. Indeed, it appears clearly that pressure acclimatization induces a state where yellow eels resemble silver eels at atmospheric pressure. Probably, such an acclimatized yellow eel has the metabolic ability to migrate but the metamorphosis to the silver eel stage (silvering process) is required for sexual

maturation and reproduction, the aim of the migration. The results obtained from silver eels show that they have low sensitivity to pressure effects (they are pressure resistant). Several observations suggest that silver eels are really ready to cope with the new environment they will encounter during the migration. The set-up of adaptative mechanisms before migration allows them to save energy during it and consequently to optimize the energy budget which is restrained to fat stores. For example, the silvering process allows the eels to modify their membrane composition in order to counterbalance the potential damaging pressure effects without energy loss. However, pressure resistance of silver eels appears modulated by several factors such as sex, origin, nutritional state, temperature. The combination of factors such as, for example, pressure exposure and swimming activity remains to be studied.

**Acknowledgements** This work has been supported by the European contract EELREP N°Q5RS-2001-01836.

## References

Altringham JD, Ellerby DJ (1999) Fish swimming: patterns in muscle function. J. Exp. Biol. 202(23):3397–3403

Amerand A, Vettier A, Sébert P, Cann-Moisan C (2005) In vitro effect of hydrostatic pressure exposure on hydroxyl radical production in fish red muscle. Redox Rep. 10:25–28

Amerand A, Vettier A, Sébert P, Moisan C (2006) Does hydrostatic pressure has an effect on reactive oxygen species in the eels. Undersea Hyperb. Med. 33:157–160

Aoyama J, Hissmann K, Yoshinaga T, Sasai S, Uto T, Ueda H (1999) Swimming depth of migrating silver eels *Anguilla japonica* released at seamounts of the West Mariana Ridge their estimated spawning sites. Mar. Ecol. Prog. Ser. 186:265–269

Bailey DM, Genard B, Collins MA, Rees JF, Unsworth SK, Battle EJV, Bagley PM, Jamieson AJ, Priede IG (2005) High swimming and metabolic activity in the deep-sea eel *Synaphobranchus kaupii* revealed by integrated *in situ* and *in vitro* measurements. Physiol. Biochem. Zool. 78:335–346

Balon EK (1975) Reproductive guilds of fishes: a proposal and definition. J. Fish. Res. Board Can. 32:821–864

Barni S, Bernocchi G, Gerzeli G (1985) Morphohistochemical changes in hepatocytes during the life cycle of the European eel. Tissue Cell 17:97–109

Barthélémy L (1985) Le poisson modèle scientifique en hyperbarie. Bull. Inst. Oceanogr. 4:9–31

Barthélémy L, Cann-Moisan C, Simon B, Caroff J, Sébert P (1991) Concentrations encéphaliques d'acide gamma-aminobutyrique (Gaba) et de glutamine chez un poisson (*Anguilla anguilla* L.) soumis à une pression hydrostatique de101 ATA. Med. Sub. Hyp. 1:35–47

Bartlett D, Wright M, Yayanos AA, Silverman M (1989) Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium. Nature 342:572–574

Bartlett DH, Chi E, Wright ME (1993) Sequence of the Omph gene from the deep-sea bacterium *photobacterium* Ss9. Gene 131:125–128

Bartlett DH, Kato C, Horikoshi K (1995) High pressure influences on gene and protein expression. Res. Microbiol. 146:697–706

Belaud A, Barthélémy L, Lesaint J, Peyraud C (1976) Trying to explain an effect of *per se* hydrostatic pressure on heart rate in fish. Aviat. Space Environ. Med. 47:252–257

Begg DA, Salmon ED, Hyatt HA (1983) The changes in structural organization of actin in the sea urchin egg cortex in response to hydrostatic pressure. J. Cell. Biol. 97:1795–1805

Belaud A (1975) Contribution à l'étude de quelques réactions physiologiques de l'anguille (*Anguilla anguilla* L.) soumise à diverses conditions hyperbares. Ph.D. thesis, UBO, Brest, pp. 271

Bell MV, Henderson RJ, Sargent JR (1986) The role of polyunsaturated fatty acids in fish. Comp. Biochem. Physiol. 83B:711–719

Beney L, Perrier-Cornet JM, Wayert M, Gervais P (1997) Modification of phospholipids vesicles induced by high pressure: influence of bilayer compressibility. Biophys. J. 72:1258–1263

Bert P (1878) La pression barométrique. Masson, Paris: Reprinted in 1979 by the Editions du Centre National de la Recherche Scientifique, Paris

Bertin L (1951) les anguilles variation croissance euryhalinité toxicité hermaphrodisme juvénile et sexualité migrations métamorphoses. Payot, Paris

Beullens K, Eding EH, Ollevier F, Komen J, Richter CJJ (1997) Sex differentiation changes in length weight and eye size before and after metamorphosis of European eel (*Anguilla anguilla* L.) maintained in captivity. Aquaculture 153:151–162

Block BA (1991) Endothermy in fish: thermogenesis ecology and evolution. In: Hochachka PW and Mommsen TP (eds) Phylogenetic and Biochemical Perspectives. Elsevier, Amsterdam, pp. 269–311

Bone Q, Marshall NB, Blaxter JHS (1995) Biology of Fishes. Blackie Academy and Professional, London

Bostrom SL, Johansson RG (1972) Enzyme activity patterns in white and red muscle of the eel (*Anguilla anguilla*) at different developmental stages. Comp. Biochem. Physiol. 42B:533–542

Brauer RTW, Jordan MR, Miller CG, Johnson ED, Dutcher JA, Sheeman ME (1985) Interaction of temperature and pressure in intact animals. In: Péqueux AJR, Gilles R (eds) High Pressure Effects on Selected Biological Systems. Springer, Berlin, pp. 3–28

Carrillo M, Bromage N, Zanuy S, Serrano R, Prat F (1989) The effect of modifications in photoperiod on spawning time ovarian development and egg quality in the sea bass (*Dicentrarchus labrax* L.) Aquaculture 81:351–365

Carlisle DB, Denton EJ (1959) On the metamorphosis of the visual pigments of *Anguilla anguilla* (L.). J. Mar. Biol. Ass. UK 38:97–102

Castonguay M, McCleave JD (1987) Vertical distributions diel and ontogenic vertical migrations and net avoidance of leptocephali of *Anguilla* and other common species in the Sargasso Sea. J. Plankton. Res. 9:195–214

Cornish I, Moon TW (1985) Glucose and lactate kinetics in American eel *Anguilla rostrata*. Am. J. Physiol. 249:R67–R72

Cossins AR, Macdonald AG (1984) Homeoviscous theory under pressure: the molecular order of membrane from deep-sea fish. Biochim. Biophys. Acta 776:144–150

D'Aoust BG (1969) Hyperbaric oxygen: toxicity to fish at pressures present in their swimbladders. Science 163:576–578

Dasgupta S (1962) Induction of triploidy by hydrostatic pressure in the leopard frog *Rana pipiens*. J. Exp. Zool. 151:105–121

Dave G, Johansson-Sjobeck ML, 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. 52A:423–430

Dufour S, Fontaine YA (1985) La migration de reproduction de l'anguille européenne (*Anguilla anguilla* L.): rôle probable de la pression hydrostatique dans la stimulation de la fonction gonadotrope. Bull. Soc. Zool. Fr. 110:291–299

Dunel-Erb S, Sébert P, Chevalier C, Simon B, Barthélémy L (1996) Morphological changes induced by acclimatation to high pressure in the gill epithelium of the freshwater yellow eel. J. Fish. Biol. 48:118–122

Durif C, Dufour S, Elie P (2005) The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage. J. Fish Biol. 66:1025–1043

Egginton S (1986) Metamorphosis of the American eel *Anguilla rostrata* Leseur: changes in metabolism of skeletal muscle. J. Exp. Biol. 237:173–184
Ekman S (1932) Prinzipielles uber die wanderungen und die tiergeographische stellung des europaischen aales *Anguilla anguilla* (L.) Zoogeographica 1:85–106
Ellerby DJ, Spierts IL, Altringham JD (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
Else PL, Hulbert AJ (2003) Membranes as metabolic pacemakers. Clin. Exp. Pharmacol. Physiol. 30:559–564
Flügel H (1972) Adaptation and acclimatization to high pressure environments. In: Brauer RW (ed) Barobiology and the Experimental Biology of the Deep Sea. University of North Cardina Chapel Hill, NC, pp. 69–88
Fontaine M (1929) De l'augmentation de la consommation d'O des animaux marins sous l'influence des fortes pressions ses variations en fonction de l'intensité de la compression. C. R. Acad. Sci. 188:460–461
Fontaine M (1975) Physiological mechanisms in the migration of marine and amphihaline fish. Adv. Mar. Biol. 13:241–355
Fontaine YA, Dufour S, Alinat J, Fontaine M (1985) L'immersion prolongée en profondeur stimule la fonction hypophysaire gonadotrope de l'anguille européenne (*Anguilla anguilla* L.) femelle. C. R. Acad. Sci. Paris 300:83–87
Fontaine YA, Le Belle N, Lopez EBQ, Alinat J, Vidal B, Barthélémy L, Sébert P, Alinat J, Petter AJ (1990) Infestation de populations françaises d'anguilles (*Anguilla anguilla* L.) par des nématodes (*Anguillicola crassus*): essais thérapeuthiques évaluation de risques potentiels liés à l'écophysiologie de l'hôte. Ann. Parasitol. Hum. Comp. 65:64–68
Fricke H, Kaese R (1995) Tracking of artificially matured eels (*Anguilla anguilla*) in the Sargasso Sea and the problem of the eel's spawning site. Naturwissenschaften 85:290–291
Gennser M, Karpe F, Ornhagen HC (1990) Effects of hyperbaric pressure and temperature on atria from ectotherm animals (*Rana pipiens* and *Anguilla anguilla*). Comp. Biochem. Physiol. 95A:219–228
Gillet C, Vauchez C, Haffray P (2001) Triploidy induced by pressure shock in Arctic charr (*Salvelinus alpinus*): growth survival and maturation until the third year. Aquat. Living Resour. 14:327–334
Goolish EM (1991) Aerobic and anaerobic scaling in fish. Biol. Rev. 66:33–56
Goolish EM (1992) Swimbladder function and buoyancy regulation in the killifish *Fundulus heteroclitus*. J. Exp. Biol. 166:61–81
Gordon MS (1970) Hydrostatic pressure. In: Hoar WS, Randall DJ (eds) Fish Physiology. Academic Press, New York/London, pp. 445–464
Greene DHS, Selivonchick DP (1987) Lipid metabolism in fish. Prog. Lipid Res. 26:53–85
Guderley H (1990) Functional significance of metabolic responses to thermal acclimation in fish muscle. Am. J. Physiol. 259:R245–R252
Haro AJ (1991) Thermal *preferenda* and behaviour of Atlantic eels (genus *Anguilla*) in relation to their spawning migration. Environ. Biol. Fish 31:171–184
Hazel JR (1995) Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu. Rev. Physiol. 57:19–42
Hicks JW, Bennett AF (2004) Eat and run: prioritization of oxygen delivery during elevated metabolic states. Respir. Physiol. Neurobiol. 144:215–224
Hiroi J, Yasumasu S, Kawazu K, Kaneko T (2003) Hatching enzymes in the Japanese eel. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel Biology. Springer, Tokyo, pp. 445–456
Jellyman D, Tsukamoto K (2002) First use of archival transmitters to track migrating freshwater eels *Anguilla dieffenbachii* at sea. Mar. Ecol. Prog. Ser. 233:207–215
Johnstone DF, Macdonald AG, Mojsiewicz WR, Wardle CS (1989) Preliminary experiments in the adaptation of the European eel (*Anguilla anguilla*) to hydrostatic pressure. J. Physiol. 417:87
Kelly CE, Kennedy CR, Brown JA (2000) Physiological status of wild European eels (*Anguilla anguilla*) infected with the parasitic nematode *Anguillicola crassus*. Parasitology 120(2):195–202

Kimberly A, Hammond KA, Diamond J (1997) Maximal sustained energy budgets in human and animals. Nature 386:457–462

Kirk RS, Lewis JW, Kennedy CR (2000) Survival and transmission of *Anguillicola crassus* Kuwahara Niimi & Itagaki, 1974 (Nematoda) in seawater eels. Parasitology 120(3):289–295

Kleckner RC (1980) Swim bladder volume maintenance related to initial oceanic migratory depth in silver-phase *Anguilla rostrata*. Science 208:1481–1482

Knopf K, Wurtz J, Sures B, Taraschewski H (1998) Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. Dis. Aquat. Organ. 33:143–149

Kobayashi H, Pelster B, Scheid P (1989) Water and lactate movement in the swimbladder of the eel *Anguilla anguilla*. Respir. Physiol. 78:45–57

Lecomte-Finiger R, Yahyaoui A (1990) La microstrucutre de l'otholite au service de la connaissance du développment larvaire de l'anguille européenne. C. R. Acad. Sci. Paris 308:1–7

Lee AG (1991) Lipids and their effects on membrane proteins: evidence against a role for fluidity. Prog. Lipid Res. 30:323–348

Lefebvre F, Contournet P, Crivelli AJ (2002) The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. Parasitology 124(4):457–463

Le Galliard JF, Clobert J, Ferrière R (2004) Physical performance and Darwinian fitness in lizards. Nature 432:502–505

Lewander K, Dave G, Johansson ML, 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. 47B:571–581

Love RM (1970) The Chemical Biology of Fishes. Academic Press, New York

Macdonald AG (1984) The effects of pressure on the molecular structure and physiological functions of cell membrane. Phil. Trans. R. Soc. Lond. 304B:47–68

Macdonald AG, Cossins AR (1985) The theory of homeoviscous adaptation of membranes applied to deep-sea animals. Symp. Soc. Exp. Biol. 39:301–322

Marchelidon J, Le Belle N, Hardy A, Vidal B, Sbaihi 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. Pêche Pisc. 355:349–368

Marsland DA (1970) Pressure-temperature studies on the mechanisms of cell division. In: Zimmerman AM (ed) High Pressure Effects on Cellular Processes. Academic Press, New York, pp. 260–312

Marsland DJ (1938) The effects of high hydrostatic pressure upon cell division in Arbacia eggs. J. Cell. Comp. Physiol. 12:57–70

Marsland DJ (1950) The mechanisms of cell division: temperature-pressure experiments on the cleaving eggs of *Arbacia punctulata*. J. Cell. Comp. Physiol. 3:205–227

McCleave JD (1977) Migratory mechanisms in larval and adult American and European eels (*Anguilla rostrata* and *A anguilla*). Nat. Geog. Soc. 18:517–528

McCleave JD, Arnold GP (1999) Movements of yellow- and silver-phase European eels (*Anguilla anguilla* L) tracked in the western north sea. Ices J. Mar. Sci. 56:510–536

Molnar K, Szekely C, Perenyi M (1994) Dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection in eels of Lake Balaton Hungary. Folia Parasitol. (Praha) 41:193–202

Moon TW (1983) Metabolic reserves and enzyme activities with food deprivation in immature American eels *Anguilla rostrata* (Lesueur). Can. J. Zool. 61:802–811

Moon TW, Latham R (1984) Myofibrillar ATPase activities in red and white myotomal muscles of the American eel *Anguilla rostrata* (Lesueur). Comp. Biochem. Physiol. 79A:151–153

Mushiake K, Kawano K, Sakamoto W, Hasegawa I (1994) Effects of extended daylength on ovarian maturation and hCG induced spawning in yellowtail fed moist pellets. Fish Sci. 60:647–651

Naroska V (1968) Vergleichende untersuchugen über den einfluss des hydrostatischen druckes auf überlebensfähigkeit und stoffwechsel intensität mariner evertebraten und teleosteer Kieler. Meeresforschung 24:95–123

Nilsson L, Nyman L, Westin L, Ornhagen H (1981) Simulation of the reproductive migration of European eels (*Anguilla anguilla* L.) through manipulation of some environmental factors under hydrostatic compression. Spec. Sci. Technol. 4:475–484

Nimeth K, Zwerger P, Wurtz J, Salvenmoser W, Pelster B (2000) Infection of the glass-Eel swimbladder with the nematode *Anguillicola crassus*. Parasitology 121(1):75–83

Owen SF (2001) Meeting energy budgets by modulation of behaviour and physiology in the eel (*Anguilla anguilla* L.). Comp. Biochem. Physiol. 128A:631–644

Pankhurst NW (1982) Changes in body musculature with sexual maturation in the European eel *Anguilla anguilla* (L.). J. Fish Biol. 21:417–428

Pankhurst NW, Lythgoe JN (1983) Changes in vision and olfaction during sexual maturation in the European eel *Anguilla anguilla* (L.) and American eel *Anguila rostrata* (Lesueur). J. Fish Biol. 23:229–240

Pelster B (1997) Buoyancy at depth. In: Randall DJ, Farell AP (eds) Deep Sea Fishes Fish Physiology. Academic press, San Diego, CA, pp. 195–238

Pelster B (2004) pH regulation and swimbladder function in fish. Respir. Physiol. Neurobiol. 144:179–190

Pelster B, Scheid P (1992) Countercurrent concentration and gas secretion in the fish swim bladder. Physiol. Zool. 65:1–16

Pennec JP, Le Bras YM (1988) Dial and seasonal rhythms of the heart rate in the common eel (*Anguilla anguilla* L.): role of cardiac innervation. Exp. Biol. 47:155–160

Ponat A (1967) Untersuchungen zur zellulären druckresistenz verschiedener evertebrates der nord-und ostsee Kieler. Meeresforschung 23:21–47

Pradillon F, Shillito B, Young CM, Gaill F (2001) Developmental arrest in vent worm embryos. Nature 413:698–699

Regnard P (1885) Phénomènes objectifs que l'on peut observer sur les animaux soumis aux hautes pressions. C. R. Sci. Soc. Biol. 37:510–515

Robins CR, Cohen DM, Robins C (1979) The Eels *Anguilla* and *Histiobranchus* photographed on the floor of the deep Atlantic in the Bahamas. Bull. Mar. Sci. 29:401–405

Roche H, Buet A, Ramade F (2003) Caractéristiques écophysiologiques d'une population d'anguilles de camargue exposée à une pollution clandestine par des polluants organiques persistants. Rev. Ecol. 58:103–126

Rossignol O, Sébert P, Simon B (2006) Effects of pressure acclimatization on silver eel (*Anguilla anguilla* L.) slow muscle contraction. Comp. Biochem. Physiol. 143A:234–238

Rottmann RW, Shireman JV, Chapman FA (1991) Induction and Verification of Triploidy in Fish. Southern Regional Aquaculture Center, pp. 421

Scholander PF, Van Dam L (1953) Composition of the swimbladder gas in deep sea fishes. Biol. Bull. Mar. Biol. Lab. 104:75–86

Sébert ME, Amérand A, Vettier A, Weltzien FA, Pasqualini C, Sébert P, Dufour S (2007) Effects of high hydrostatic pressure on the pituitary-gonad axis in the European eel *Anguilla anguilla* (L.). Gen. Comp. Endocrinol. 153:289–298

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 DJ, Farell AP (eds) Deep Sea Fishes Fish Physiology. Academic Press, San Diego, CA, pp. 279–324

Sébert P (2003) Fish adaptations to pressure. In: Val AL, Kapoor BG (eds) Fish Adaptations. Science Publisher, Enfield, pp. 73–95

Sébert P (2007) Fish muscle function and pressure. In: Sébert P, Onyango D, Kapoor BG (eds) Fish Life in Special Environments. Science Publisher, Enfield (in preparation)

Sébert P, Barthélémy L (1985a) Effects of high hydrostatic pressure per se 101 atm on eel metabolism. Respir. Physiol. 62:349–357

Sébert P, Barthélémy L (1985b) Hydrostatic pressure and adrenergic drugs (agonists and antagonists): effects and interactions in fish. Comp. Biochem. Physiol. 82C:207–212

Sébert P, Macdonald AG (1993) Fish. In: Macdonald AG (ed) Effects of High Pressure on Biological Systems. Springer, Berlin, pp. 147–196
Sébert P, Theron M (2001) Why can the eel unlike the trout migrate under pressure? Mitochondrion 1:79–85
Sébert P, Barthélémy L, Caroff J (1986) Catecholamine content (as measured by the HPLC method) in brain and blood plasma of the eel: effects of 101 ATA hydrostatic pressure. Comp. Biochem. Physiol. 84C:155–157
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. 86A:491–495
Sébert P, Péqueux A, Simon B, Barthélémy L (1991) Effects of long term exposure to 101 ATA HP on blood gill and muscle composition and of some enzyme activities of the fw eel (*Anguilla anguilla* L.). Comp. Biochem. Physiol. 98B:573–577
Sébert P, Meskar A, Simon B, Barthélémy L (1994) Pressure acclimation of the eel and liver membrane composition. Experientia 50:121–123
Sébert P, Simon B, Barthélémy L (1995) Effects of a temperature increase on oxygen consumption of yellow fresh water eel exposed to high hydrostatic pressure. Exp. Physiol. 80:1039–1046
Sébert P, Peragon J, Barroso JB, Simon B, Melendez-Hevia E (1998) High hydrostatic pressure (101 ATA) changes the metabolic design of yellow freshwater eel muscle. Comp. Biochem. Physiol. 121B:195–200
Sébert P, Theron M, Vettier A (2004) Pressure and temperature interactions on cellular respiration: a review. Cell. Mol. Biol. 50:491–500
Seoka M, Yamada S, Iwata Y, Yanagisawa T, Nakagawa T, Kumai H (2003) Differences in the biochemical content of buoyant and non-buoyant eggs of the Japanese eel *Anguilla japonica*. Aquaculture 216:355–362
Siebenaller JF, Somero GN (1989) Biochemical adaptation to the deep sea. CRC Crit. Rev. Aquat. Sci. 1:1–25
Simon B (1990) Métabolisme énergétique de l'anguille (*Anguilla anguilla* L.): effets d'expositions de courte durée (3 h) et de longue durée (1 mois) à 101 ATA de pression hydrostatique. Ph.D. thesis, UBO, Brest pp. 105
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. 102B:205–208
Simon B, Barthélémy L, Sébert P (1994) Comparison of some isoenzymes on the yellow and silver phases of the European eel (*Anguilla anguilla* L.). Aquaculture 25:937–942
Somero GN (1991) Hydrostatic pressure and adaptation to the deep sea. In: Prosser CL (ed) Environmental and Metabolic Animal Physiology. Wiley-Liss, New York, pp. 167–204
Speers-Roesch B, Lingwood D, Stevens ED (2004) Effects of temperature and hydrostatic pressure on routine oxygen uptake of the bloater (*Coregonus hoyi*). J. Great Lakes Res. 30:70–81
Sprengel G, Luchtenberg H (1991) Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla anguilla*. Dis. Aquat. Organ. 11:31–35
Strand E, Jorgensen C, Huse G (2005) Modelling buoyancy regulation in fishes with swimbladders: bioenergetics and behaviour. Ecol. Model. 185:309–327
Tesch FW (1978) Telemetric observations on the spawning migration of the eel (*Anguilla anguilla*) west of the European continental shelf. Environ. Biol. Fish 3:203–209
Tesch FW (1989) Changes in swimming depth and direction of silver eels (*Anguilla anguilla* L.) from the continental shelf to the deep sea. Aquat. Living Resour. 2:9–20
Tesch FW (2003) The Eel. Fifth edition. Thorpe JE, 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(19):3019–3023

Thomson AJ, Sargent JR (1977) Changes in the levels of chloride cells and ($Na^+ + K^+$)-dependent ATPase in the gills of yellow and silver eels adapting to seawater. J. Exp. Zool. 200:33–40

Tyler PA, Young CM (1998) Temperature and pressure tolerances in dispersal stages of the genus Echinus (*Echinodermata: Echinoidea*): prerequisites for deep-sea invasion and speciation. Deep-Sea Res. II 45:253–277

Tytler P, Blaxter JHS (1973) Adaptation by cod and saithe to pressure changes. Neth. J. Sea Res. 7:31–45

Van Ginneken V, Van Den Thillart G (2000) Eel fat stores are enough to reach the Sargasso. Nature 403:156–157

Van Ginneken V, Maes G (2005) The European eel (*Anguilla anguilla* Linnaeus) its lifecycle evolution and reproduction: a literature review. Rev. Fish Biol. Fish. 15:367–398

Van Ginneken V, Antonissen E, Müller UK, Booms R, Eding E, Verreth J, Van Den Thillart G (2005a) Eel migration to the Sargasso: remarkably high swimming efficiency and low energy costs. J. Exp. Biol. 208:1329–1335

Van Ginneken V, Vianen G, Muusze B, Palstra A, Verschoor L, Lugten O, Onderwater M, Van Schie S, Niemantsverdriet P, Van Heeswijk R, Eding E, Van Den Thillart G (2005b) Gonad development and spawning behavior of artificially-matured European eel (*Anguilla anguilla* L.). Anim. Biol. 55:203–218

Vettier A (2005) La migration de reproduction de l'anguille européenne (*Anguilla anguilla* L): effets de la pression hydrostatique et de la métamorphose. Ph.D. thesis, UBO, Brest, pp. 90

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. Anim. Biol. 53:329–338

Vettier A, Amérand A, Cann-Moisan C, Sébert P (2005) Is the silvering process similar to the effects of pressure acclimatization on yellow eels? Respir. Physiol. Neurobiol. 145:243–250

Vettier A, Labbé C, Amérand A, Da Costa G, Le Rumeur E, Moisan C, Sébert P (2006) Hydrostatic pressure effects on eel mitochondrial functioning and membrane fluidity. Undersea Hyperb. Med. 33:149–156

Wardle CS, Tetteh-Lartey N, Macdonald AG, Harper AA, Pennec JP (1987) The effect of pressure on the lateral swimming muscle of the European eel *Anguilla anguilla* and the deep sea eel *Histiobranchus bathybius*; results of Challenger cruise 6b/85. Comp. Biochem. Physiol. 88A:595–598

Watanabe T (1982) Lipid nutrition in fish. Comp. Biochem. Physiol. 73B:3–15

Watanabe T, Itoh A, Kitajima C, Fujita S (1984a) Effect of dietary protein levels on reproduction of red sea bream. Bull. Jpn. Soc. Sci. Fish. 50:1015–1022

Watanabe T, Itoh A, Murakami A, Tsukashima Y, Kitajima C, Fujita S (1984b) Effect of nutritional quality of diets given to broodstock on the verge of spawning on reproduction of red sea bream. Bull. Jpn. Soc. Sci. Fish. 50:1023–1028

Westin L, Nyman L (1979) Activity orientation and migration of Baltic eel (*Anguilla anguilla* (L.)) Rapp. Cons. Explor. Mer. 174:631–644

White FN, Somero GN (1982) Acid base regulation and phospholipid adaptation to temperature time courses and physiological significance of modifying the milieu for protein function. Physiol. Rev. 62:40–90

Würtz J, Taraschewski H (2000) Histopathological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. Dis. Aquat. Organ. 39:121–134

Yamazaki F, Goodier J (1993) Cytogenetic effects of hydrostatic pressure treatment to suppress the first cleavage of salmon embryos. Aquaculture 110:51–59

Young CM, Tyler PA (1993) Embryos of the deep-sea echinoid *Echinus affinis* require high pressure for development. Limnol. Oceanogr. 38:178–181

Young CM, Tyler PA, Gage JD (1996) Vertical distribution correlates with pressure tolerances of early embryos in the deep-sea asteroid *Plutonaster bifrons*. J. Mar. Biol. Ass. UK 76:749–757

Zara V, Palmieri L, Giudetti A, Ferramosca A, Capobianco L, Gnoni GV (2000) The mitochondrial tricarboxylate carrier: unexpected increased activity in starved silver eels. Biochem. Biophys. Res. Commun. 276:893–898