ELSEVIER

Thermochimica Acta 373 (2001) 23–30

thermochimica acta

www.elsevier.com/locate/tca

Metabolic depression and investigation of glucose/ethanol conversion in the European eel (*Anguilla anguilla* Linnaeus 1758) during anaerobiosis

Vincent J.T. van Ginneken*, Marjolijn Onderwater, Olga Lamúa Olivar, Guido E.E.J.M. van den Thillart

Integrative Zoology, Department of Biology, Institute of Evolutionary and Ecological Sciences (EEW), Van der Klaauw Laboratories, University Leiden, P.O. Box 9516, 2300 RA Leiden, The Netherlands

Received 25 May 2000; received in revised form 20 December 2000; accepted 25 January 2001

Abstract

The European eel (*Anguilla anguilla* Linnaeus 1758) is an important commercial fish species for aquaculture and fisheries. In aquaculture, one of the main hazards for the farmer is low oxygen condition due to technical disturbances or calamities. Therefore, in this study, we investigated the tolerance and metabolic response of European eel to anoxic conditions. In a 1 l flow-through Sétaram microcalorimeter we measured a 70% reduction of the Standard Metabolic Rate (SMR) during a period of 1 h anoxia, a process called metabolic depression. This strategy has the advantage that the survival time during anaerobioses can be extended because of a reduction of energy consumption and reduction of end product accumulation. Correcting for the time constant of the calorimeter by deconvolution techniques (time lag correction), we could describe the dynamics of the process of metabolic depression in European eel. From the deconvoluted signal it can be concluded that the 70% metabolic depression of European eel under anoxia takes place within 30 min. In parallel asphyxia experiments with European eel, no increased levels of ethanol were observed in blood plasma or ambient water. Ethanol concentrations in blood plasma were even significantly lower in the asphyxia group, probably indicating a decreased microbial activity. The 18-fold increase of plasma lactic acid is indicative for activation of Embden–Meyerhof glycolysis during anaerobioses.

Keywords: *Anguilla anguilla*; European eel; Anoxia; Anaerobioses; Oxygen; Metabolic depression; Deconvolution; Lactate; Ethanol; Calorimeter; Aquaculture; Biological system

1. Introduction

The European eel (*Anguilla anguilla* Linnaeus 1758) is an important fish species for inland fisheries as well as for aquaculture. On a worldwide scale the eel culture was estimated to be between 100,000 to 110,000 tons in 1987 (fisheries and fish culture) which corresponds with 4–4.4 billion guilders per year [1]. In 1999 for aquaculture only, it was estimated worldwide 200,000 ton of eel was cultured while it is expected this production will expand with 50% (pers. commun. Ir. J.C.M. van Rijsingen). With regard to its commercial value, per total yield, the eel ranks 6–8th in importance when compared with other fish species. In price, per unit weight, however, the eel occupies the second position after the salmon [2].

* Corresponding author. Fax: +31-71-5274900.
E-mail address: ginneken@rulsfb.leidenuniv.nl (V.J.T. van Ginneken).

0040-6031/01/$ – see front matter
PII: S0040-6031(01)00463-4

The oxygen concentration of the water is one of the main factors, which determines survival of fish, fish production and fish growth [3]. For eel fisheries, hypoxic conditions of the ecosystem are disadvantageous. The seriousness of this matter can be demonstrated by the ecological study of Aalderink who demonstrated that the oxygen concentration in the Dutch surface water has decreased in the period 1978–1989 by 20% mainly due to pollution with oxygen consuming compounds [4]. Also in aquaculture, eel production is dependent on the oxygen concentration of the water and often in intensive eel culture pure oxygen is donated to the water [5]. However, in practice of intensive eel culture, in case of technical disturbances and calamities, hypoxic or anoxic conditions may occur for periods of several hours. This may lead in the worst cases to mass mortality and wipeouts and in case of less severe calamities in decreased food consumption and retarded growth of the elvers.

The aim of this study is to investigate the tolerance of European eel to anoxic conditions. Furthermore we want to investigate if eel has protective mechanisms to cope with low oxygen conditions. For some other fish species two important survival strategies can be observed to extend survival during anaerobiosis: (a) metabolic depression: a reduction of the metabolic rate below the standard metabolic rate (comparable with hibernation and/or aestivation) [6,7]. This mechanism is observed in fish species like, goldfish (*Carassius auratus*) [8–12] and the commercially important tilapia (*Oreochromis nilotica* Peters) [13,14]; (b) glucose–ethanol conversion: this is observed in three *Cyprinid* species: the goldfish (*C. auratus*), crucian carp (*Carassius carassius*) and the bitterling (*Rhodeus amarus*) [15]. The main advantage of this conversion is that ethanol can be excreted to the aquatic environment which prevents acidosis of the internal milieu [15]. We used a specially developed 1 l flow-through microcalorimeter, enabling the investigator to measure under constant environmental stress-free conditions [16]. For the first time we demonstrated the process of metabolic depression during anaerobiosis in the European eel. Further, correcting for the time constant of the calorimeter by deconvolution techniques (time lag correction), we could describe the dynamics of the process of metabolic depression in European eel.

In this study we aimed to elucidate the metabolic response of the commercially important European eel under anoxic conditions.

2. 2. Material and methods

2.1. Animals

Eels (*A. anguilla* L.) were obtained from a commercial fish farm (Royaal BV, Helmond, The Netherlands). The animals were acclimated to 20°C and kept under normal laboratory conditions (14 h light, 10 h darkness) and normoxic oxygen saturation values of 80%. The animals were fed with Provimi pelleted food (Rotterdam, The Netherlands). Two kinds of experiments were performed: (a) calorimetric experiment: in this case study an eel of 78 g and 38 cm length, was used; (b) respirometric experiments: here eels were exposed to asphyxia (i.e. no oxygen supply $\approx$ suffocation) overnight. The control normoxic group was 762 ± 34 g with a length of 72.4 ± 4.5 cm ($n = 10$) while the anoxic group was 738 ± 26 g with a length of 70.9 ± 3.9 cm ($n = 10$).

2.2. Calorimeter

The calorimetric system is described elsewhere [11,16]. In short, the heat production of the animals is measured in a differential flow through calorimeter (Sétaram GF 108, Lyon France), which measures continuously the rate of heat production of the fish in a vessel with a volume of 1 l. The concept of continuous perfusion of the measurement vessel is applied to ensure constant experimental conditions for the animals. In this way, long term monitoring of aquatic animals (1–80 g) under stress-free conditions is possible because waste products (NH_3, NO_2, NO_3, and CO_2) are flushed out while new oxygen is supplied with the incoming water [11,16]. The oxygen concentration of the inflowing air-saturated water in the calorimeter at this temperature of 20°C was earlier before determined with a Winkler titration and corresponded to 8.94 ± 0.063 mg l^{-1} ($n = 6$) [11]. After passage through the calorimeter (without fish) the oxygen concentration corresponded to a value of 8.84 ± 0.062 mg l^{-1} ($n = 6$), so the blank oxygen

consumption (due to microorganism in the apparatus) was 0.1 mg l^{-1} [11]. Before the eel was placed in the vessel the sensitivity coefficient, which relates signal level to power input, was determined. The sensitivity coefficient of the pilot experiment was 84.3 μV/mW. The flow through the system was 70 ml min^{-1}. The baseline stability was ±0.005 mW per 24 h. At the beginning and the end of an experiment the heat flux signal was checked by a calibration. Calibration was performed with a known electrical current and voltage (Sétaram EJ2 joule calibrator) by a current of 3.15 mA with a voltage of 3.164 V which is applied to a resistor of 1000 Ω mounted in the measurement vessel. This resulted in a power output signal of 9.97 mW. The calorimetric apparatus was placed in a thermostatically controlled room set at 19.3 ± 0.3°C. The operating temperature of the calorimeter was 20.0°C. The heat flux was recorded on an IBM compatible computer (Laser 386 SXE) with specially developed software for data recording and graphical presentation [11].

2.3. Deconvolution techniques: desmearing of calorimetric data

The time constant τ of the developed 1 l differential flow-through calorimeter (Sétaram GF 108) at a flow rate of 50 ml min^{-1} was estimated to be 33 min [12]. Data were desmeared using the time constant τ of 33 min based on the method of Hand and Gnaiger [17], with a single time constant.

$$Q_d(t) = Q(t) + \frac{\tau}{\Delta t}\{Q(t) - Q(t-1)\}$$

with $Q_d(t)$ as the deconvoluted signal, $Q(t)$ the original data point to be corrected, $Q(t-1)$ the data point registered 1 min prior to $Q(t)$ and τ the time constant corresponding to 33 min, Δt is the sampling rate equal to 1 min.

2.4. Respirometer

The eels were individually starved for 2 days in a 35 l flow-through respirometer at normal oxygen levels (85% air saturation) [18]. The supply of water to the respirometer was regulated by an EIL oxygen controller (EIL LTD Richmond, Surrey, England, model 9402) connected to a valve. Oxygen tension was registered on a recorder. The temperature in the experimental set up was kept at 20 ± 0.5°C. Each individual of the control group was anaesthetized the 3rd day by adding 300 ppm MS222 (3-aminobenzoic-acid-ethyl-ester methanesulfonate salt, Sigma) to the respirometer. After 3 min a blood sample was collected with a heparinized syringe (flushed with 3000 units heparin/ml blood). From the respirometer also a water sample was taken. For the asphyxia group, on the 3rd day, anoxia was introduced by stopping the supply of freshly aerated water to the respirometer. After asphyxia of each individual eel in the respirometer, a plasma sample was taken the next morning in combination with a water sample of the water in the respirometer. Here again a heparinized syringe was used like in the control group for the plasma sample.

2.5. Lactate and ethanol measurements in plasma

Consequently, blood was directly centrifuged at 10,000 rpm for 5 min. The plasma was stored at −80°C for further analysis. The water sample, for measuring ethanol concentration, was stored in a plastic tube of 100 ml at −30°C. Ethanol in the water samples together with plasma ethanol and lactic acid concentrations were measured with Boehringer–Mannheim enzymatic test combinations: 176290 (ethanol) and 139084 (L-lactate).

2.6. Statistics

Statistics were performed using a one-way ANOVA. Normality of the data and homogeneity of variances were checked by Kolmogorov–Smirnov and F_{max} tests, respectively. $P \leq 0.05$ was considered as statistically significant.

3. Results

The result of the pilot calorimetric experiment with an eel of 78 g under anoxic conditions is presented in Fig. 1. The experiment starts with a calibration procedure for the heat signal. This results in a heat production of approximately 10 mW. Thereafter, the fish is introduced in the vessel. After 2 days at normoxia (100% oxygen) at $t = 72$ h the eel is exposed to 1.5 h of anoxia (0.5 h transition phase:

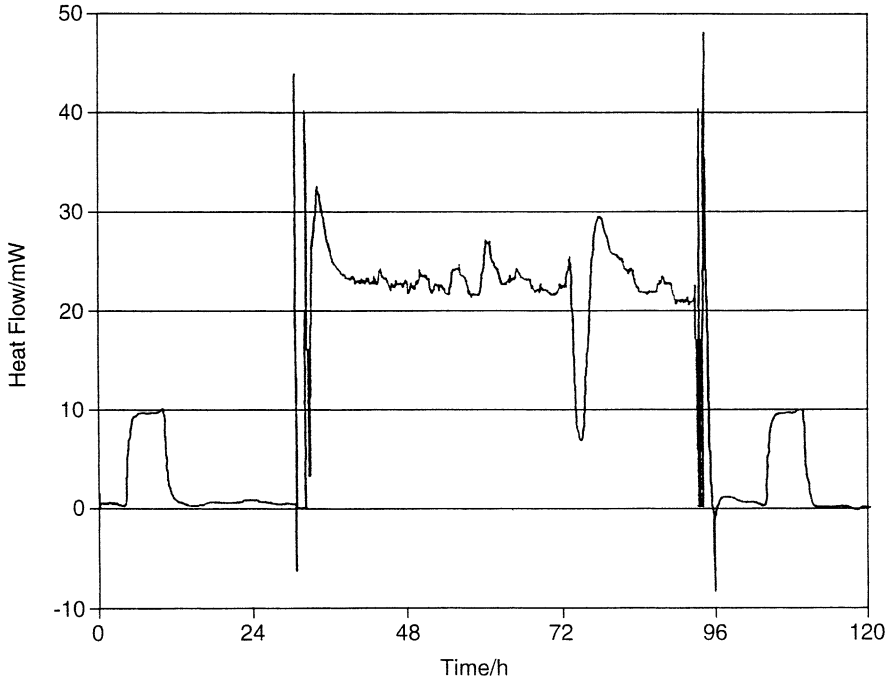


Fig. 1. Time course of a calorimetric experiment which is started and finished with an electrical calibration procedure resulting in a heat production of approximately 10 mW. After 1 day an European eel (*A. anguilla* L.) of 78 g is introduced in the calorimeter. At day 3 the eel is exposed to anoxia for 1.5 h (0.5 h transition phase: hypoxia → anoxia; 1 h 0% oxygen) resulting in a metabolic depression of 70%. The total calorimetric experiment last 4 days.

hypoxia → anoxia; 1 h 0% oxygen). During the anoxic period the heat production drops from 22 to 6.7 mW, a metabolic depression in eel of 70%. After regaining oxygen supply, the animal reacts with an increased heat production until a level of 29 mW, the so-called oxygen debt (132% compared to normoxia), which lasted 8 h. At day 4 the eel is taken out of the calorimeter and the experiment is finished again with a calibration.

In Fig. 2 is the process of metabolic depression enlarged depicted, correcting for the time constant of the calorimeter by deconvolution techniques (time lag correction). From the deconvoluted signal it can be concluded that the 70% metabolic depression of European eel under anoxia takes place within 30 min.

No ethanol was recorded in the normoxic or anoxic water samples in the respirometer. Ethanol concentration of the eels in the respirometer was 5.39-fold higher in the normoxic group (3.45 ± 0.50 mM ($n = 10$)) compared to the anoxic group (0.64 ± 0.02 mM ($n = 10$)) and was significantly higher ($P \leq 0.0001$) (Fig. 3a). Lactic acid in blood plasma was significantly higher in the anoxic group (31.69 ± 6.2 mM ($n = 10$)) compared to the normoxic group (1.74 ± 0.69 mM ($n = 10$)) ($P \leq 0.00001$)(Fig. 3b). The nearly 18-fold increase of lactic acid in the anoxic eel group is indicative for activation of Embden–Meyerhof glycolysis.

4. Discussion

A calorimetric system build by Sétaram (Lyon, France) has been further developed in our laboratory to study heat production and oxygen consumption for large aquatic animals during long-term experiments under stress-free conditions [16]. To conduct this kind of experiment, the calorimeter is equipped with a flow-through system. Constant experimental conditions can be maintained because fresh water is supplied and metabolic waste products are flushed out.

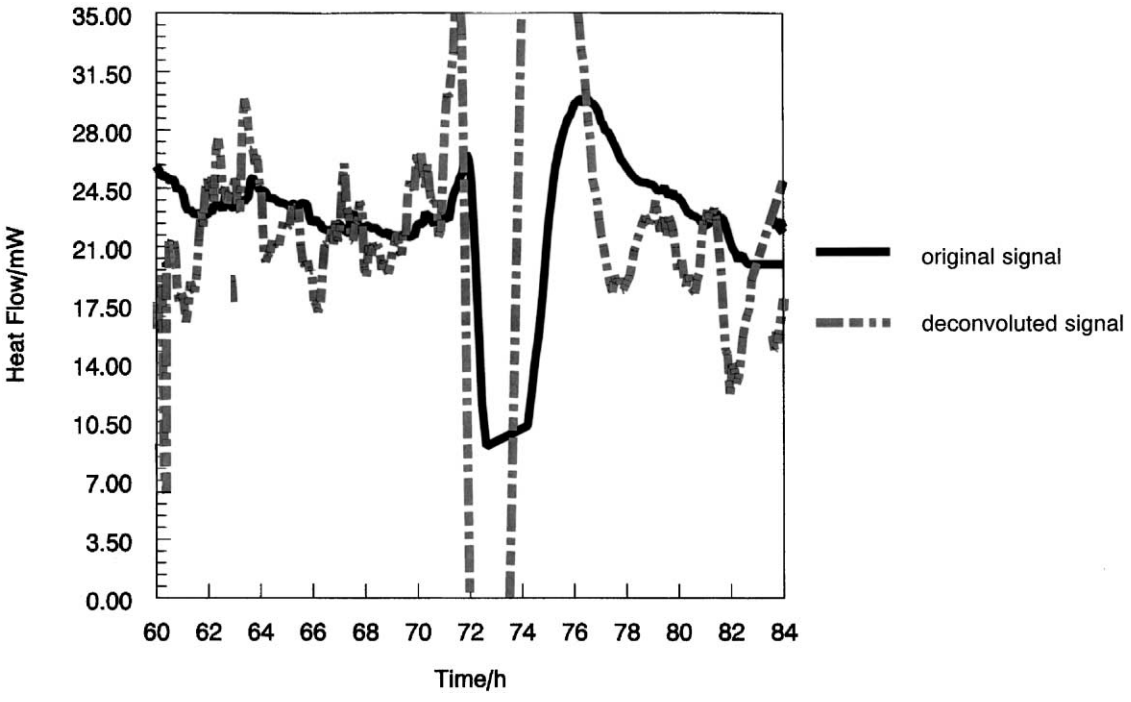
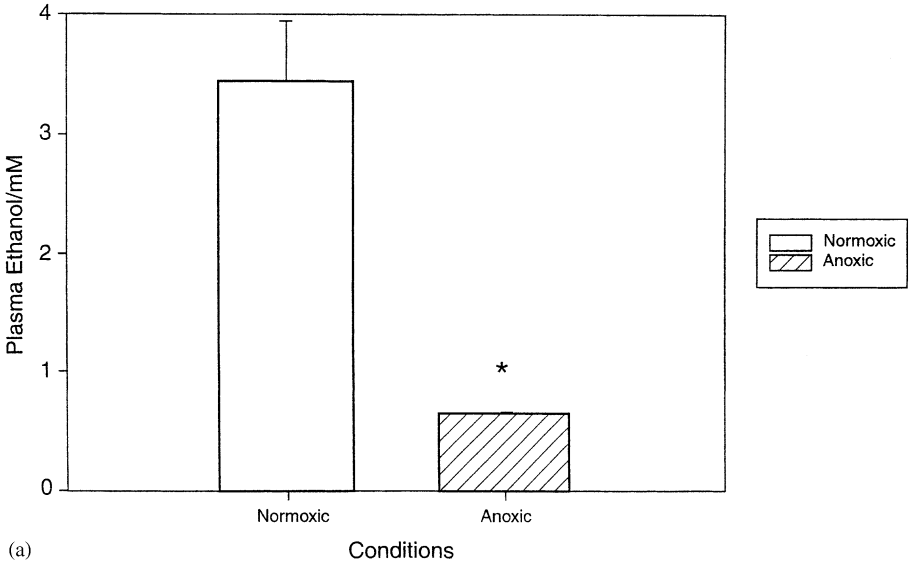


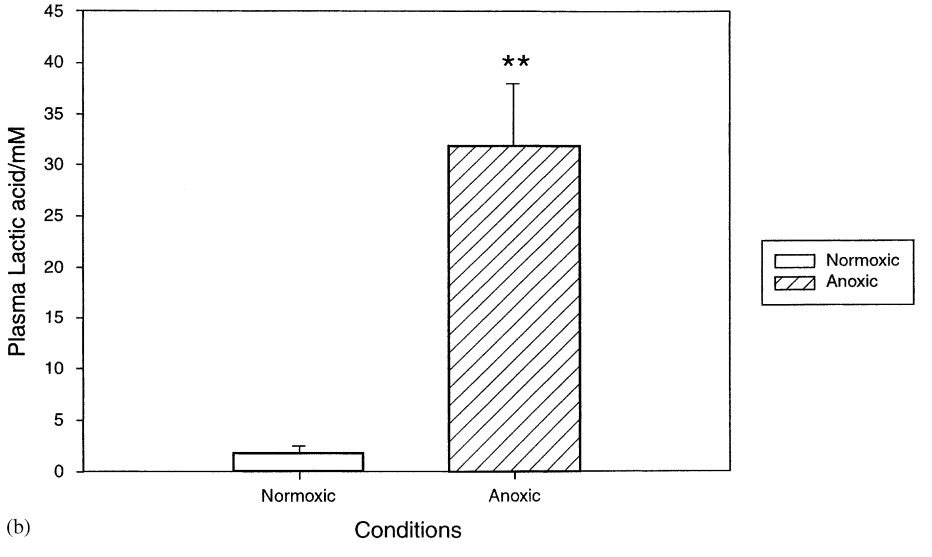
Fig. 2. Enlargement of the registration of the process of metabolic depression as depicted in Fig. 1. Solid line: recorded signal; dotted line: deconvoluted signal.

The system was improved by providing an automated data acquisition system, which enables us to register both heat production and oxygen consumption of large aquatic poikilotherms [11]. In this way, we studied the effects of adverse environmental conditions like hypoxia [9,19], anoxia [9–12] and acidification [14]. In nature, under extreme conditions, in several organisms, a down regulation of the energy metabolism can be observed like in diving turtles (*Chrisemys picta*), the African lungfish (*Protopterus aethiopicus*) during periods of drought, insects in diapause or bears and ground squirrels in wintertime [6]. This down regulation of the energy metabolism below the minimal energetic costs for homeostasis under standard conditions is indicated (depending on the environmental stress factor and the organism) with terms as torpor, hibernation, aestivation or in our case for fish, metabolic depression.

The reduction of heat production, called metabolic depression, can be measured only by the technique of direct calorimetry. As demonstrated in this study, European eel reduces the metabolic rate to 70% during anoxia. In other calorimetric studies with fish, similar values were observed. Tilapia showed a 50% metabolic depression during extreme hypoxia [13,14], while goldfish showed a 70% metabolic depression under anoxia [8–12]. The question remains what is the underlying mechanism of metabolic depression. In a calorimetric study we measured the metabolic rate in combination with activity measurements via a video motion and analyzing system. We concluded that metabolic depression was not caused by a reduction of the external activity, but is based on another mechanism, probably a reduction of the muscle tension and the blood flow [13]. Furthermore, there are strong indications that the inhibiting neurotransmitter γ-amino-butyric acid (GABA) is involved in the process of metabolic depression. In a comparative study three fish species tilapia (*Oreochromis mossambicus*), goldfish (*C. auratus*), and carp (*Cyprinus carpio*) were exposed to anoxic conditions. Under conditions without oxygen, goldfish showed a 70% metabolic depression in combination with a glucose/ethanol conversion; carp has no metabolic depression, and



(a)



(b)

Fig. 3. (a) Mean ± S.D. of the plasma ethanol concentration in a normoxic and anoxic European eel group. Each group consists of 10 individuals. The single astesisk (∗) denotes significant differences ($P \leq 0.0001$); (b) Mean ± S.D. of the plasma lactic acid concentration in a normoxic and anoxic European eel group. Each group consist of 10 individuals. The double asterisks (∗ ∗) denote significant differences ($P \leq 0.00001$).

tilapia has an intermediate metabolic depression. It was found GABA levels increased in goldfish brain, no changes were observed in carp brain while tilapia showed an intermediate position [20]. Probably, GABA has an inhibiting effect on the sympathetic nervous system, resulting in a reduction of the muscle tone and in general relaxation of the animal.

Metabolic depression has the consequence that the survival time during anaerobiosis can be extended because of a reduction of the rate of energy

consumption and reduction of the rate of end product accumulation.

For European eel, the mean survival time (LT_{50} = the mean time when 50% of the animals died due to the experimental treatment) during anoxic conditions proved to be 5.7 h [21]. This seems to be rather long and does not correspond to eel culture in practice, where mass mortalities already occur within 30 min of anoxia (pers. commun. Ir. J.C.M. van Rijsingen). The study of van Waarde et al. [21] however, was performed with eel of approximately 80 g at night under stress-free conditions at a temperature of 15°C. In another study at 20°C, the following LT_{50} values were found for European eel: 0.60 mg O_2/l ([oxygen] = 6.5% of maximal oxygen saturation) during 1.4 h, 0.80 mg O_2/l during 4 h ([oxygen] = 8.7%), 1.00 mg O_2/l during 9 h ([oxygen] = 10.9%) and 1.18 mg O_2/l ([oxygen] = 12.8%) during 24 h [22].

For goldfish exposed to anoxia, Shoubridge and Hochachka discovered that this fish species has a modified glycolytic pathway, in which glycogen is converted to ethanol and CO_2 which are both excreted [15,23]. Indications that ethanol was not produced by microorganisms but was produced by the fish itself, came from the observation that a high activity of alcohol-dehydrogenase was found in red and white myotomal muscles [15,23]. The main advantage of ethanol formation is to prevent acidosis of the interior milieu and to prevent osmotic problems that arise from extensive glycogen degradation [15]. In their review, regarding the anaerobic metabolism of several fish species during anaerobiosis, van den Thillart and van Waarde stated that eel had no mechanism of glucose/ethanol conversion to limit acidosis induced by lactic acid production [24]. In contrast, in a high pressure study exposing eels to 101 ATA (9.967925×10^{-4} Pa) in a hyperbaric chamber of 130 l, a trend of increasing ethanol concentration was observed in red muscle (+78%) and plasma (+133%) during normoxia [25]. The combination of anoxia and high pressure even gave higher ethanol production rates in red muscle (+150%), liver (+91%) and plasma (+194%) compared to the control group [25]. For eels, there are several indications that they migrate at great depths during their return journey to the spawning grounds. Firstly, a migrating eel has been observed at a depth of 2000 m near the Bahamas [26]. Secondly, Dufour and Fontaine demonstrated in a field study where cages with silver eel were sunken in the Mediterranean Sea that eels could survive for a period of 3 months at a depth of 1650 m [27]. Sebert et al. [25] who observed ethanol production under high pressure in the laboratory, hypothesize that the ethanol production under high pressure could help to refluidify the membranes of the animals which could be helpful in the acclimatization of the eels to high pressure [25]. However, in this study no indications for glucose/ethanol conversion in European eel was observed. Ethanol concentrations in eel plasma were even 4.36-fold lower in the anoxic group compared to the control group, probably due to decreased microbial activity. The only way to clarify these controversial results (this study and [24] versus [25]) and elucidate the possibility of a glucose/ethanol conversion in the European eel is to investigate in future studies the existence of an alcohol-dehydrogenase.

Furthermore, we demonstrated under stress-free conditions, metabolic depression is an important survival strategy for European eel to survive temporarily adverse low oxygen conditions. In laboratory studies, survival time during anoxia is more extended (LT_{50}, 5.7 h [21]) compared to the situation at the eel farm, where mass mortality may occur within 30 min. This controversy can be explained because in intensive eel culture stocking densities are so high, and husbandry conditions so stressful, that the survival strategy of metabolic depression may not be fully expressed during anaerobiosis.

Acknowledgements

Vincent van Ginneken was supported by a grant of the Technology Foundation (STW). STW-project no. 427024. Ir. J. van Rijsingen (Royaal B.V., Helmond, The Netherlands) was member and sponsor of the utility committee of this STW-project. Drs. C. Vancoillie is kindly acknowledged for providing the eel.

References

[1] L.T.N. Heinsbroek, Aquacult. Fish. Manage. 22 (1991) 57–72.
[2] F.W. Tesch, Helgoländer Meeresunsters 35 (1982) 263–277.
[3] C.E. Boyd, Water quality in warm water fish ponds. Agricultural Experiment Station, Auburn University, Auburn, AL, 1979, p. 359.

[4] R.H. Aalderink, Analysis of the oxygen content in the Dutch surface water 1978 until 1989. Agricultural University Wageningen, Department of Nature Conservation, Section Waterquality, 1991.
[5] A. Usui, Fishing News Books, Blackwell Scientific Publications Ltd, The University Press, Cambridge, ISBN 0-85238-182-4, 1991, 148 pp.
[6] P.W. Hochachka, G.N. Somero, Biochemical Adaptation. Princeton University Press, Princeton, NJ, ISBN 0-691-08343, 1984, 537 pp.
[7] K.B. Storey, Can. J. Zool. 66 (1987) 124–132.
[8] J. van Waversveld, A.D.F. Addink, G. van den Thillart, H. Smit, J. Therm. Anal. 33 (1988) 1019–1026.
[9] J. van Waversveld, A.D.F. Addink, G. van den Thillart, J. Comp. Physiol. 159 (1989) 263–268.
[10] J. van Waversveld, A.D.F. Addink, G. van den Thillart, J. Exp. Biol. 143 (1989) 325–335.
[11] V.J.T. van Ginneken, A. Gluvers, R.W. van der Linden, A.D.F. Addink, G.E.E.J.M. van den Thillart, Thermochim. Acta 247 (1994) 209–224.
[12] V.J.T. van Ginneken, J. Vanderschoot, A.D.F. Addink, G.E.E.J.M. van den Thillart, Thermochim. Acta 249 (1995) 143–159.
[13] V.J.T. van Ginneken, A.D.F. Addink, G.E.E.J.M. van den Thillart, Thermochim. Acta 276 (1996) 7–15.
[14] V.J.T. van Ginneken, A.D.F. Addink, G.E.E.J.M. van den Thillart, L. Noldus, M. Buma, Thermochim. Acta 291 (1997) 1–13.
[15] A. van Waarde, G. van den Thillart, M. Verhagen, in: P.W. Hochachka, P.L. Lutz, T. Sick, M. Rosenthal, G. van den Thillart (Eds.), Surviving Hypoxia: Mechanisms of Control and Adaptation, CRC Press, Boca Raton, 1993 (Chapter 11), pp. 157–170, ISBN 0-8493-4226-0.
[16] A.D.F. Addink, G. van den Thillart, H. Smit, J. van Waversveld, Thermochim. Acta 193 (1991) 41–48.
[17] S.C. Hand, E. Gnaiger, Science 239 (1988) 1425–1427.
[18] G. van den Thillart, R. Verbeek, Physiol. Zool. 64 (1991) 525–540.
[19] I. Hardewig, A.D.F. Addink, M.K. Grieshaber, H.O. Pörtner, G. van den Thillart, J. Exp. Biol. 157 (1991) 143–160.
[20] V. van Ginneken, M. Nieveen, G. van den Thillart, A. Addink, Comp. Biochem. Physiol. 114A (1996) 189–196.
[21] A. van Waarde, G. van den Thillart, F. Kesbeke, J. Comp. Physiol. 149 (1983) 469–475.
[22] M. Wozniewski, Roczniki nauk rolniczych seria H.T. 101 (Z4) (1988) 177–182.
[23] E.A. Shoubridge, P.W. Hochachka, Science 209 (1980) 308–309.
[24] G. van den Thillart, A. van Waarde, Mol. Physiol. 8 (1985) 393–409.
[25] P. Sebert, B. Simon, L. Barthelemy, Comp. Biochem. Physiol. 101A (1992) 233–235.
[26] C.R. Robins, D.M. Cohen, C.H. Robins, Bull. Mar. Sci. 29 (1979) 401–405.
[27] S. Dufour, Y.A. Fontaine, Bull. Soc. Zoll. Fr. 110 (1985) 291–299.