Thermochimica Acta 483 (2009) 1–7

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Review

Metabolic depression in fish measured by direct calorimetry: A review

Vincent van Ginneken [a,*], Guido van den Thillart [b]

[a] Plant Research International, Agrosystems Research, Wageningen UR, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[b] 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

ARTICLE INFO

Article history:
Received 14 November 2007
Received in revised form
23 September 2008
Accepted 29 September 2008
Available online 5 November 2008

Keywords:
Direct calorimetry
Biological system
Heat
Fish
Metabolic depression
Activity
Heart rate
Radio-telemetry
Thyroid hormones
Review

ABSTRACT

In nature under adverse conditions like low oxygen conditions or starvation fish often lower their metabolism: 'metabolic depression'. This strategy of lowering the metabolic rate is a survival strategy and is used to save energy stores and diminish end-product accumulation. The overall metabolic rate of animals can be deduced by measuring metabolic processes such as oxygen consumption, but the ultimate method is measuring heat flow. In this review, we will summarise the available data about metabolic depression measuring heat flow, i.e. by direct calorimetry in fishes, which were carried out almost exclusively with a 1-l flow through calorimeter. Using deconvolution techniques the time constant of this calorimeter was measured that allowed to estimate the time course of metabolic depression, which was found to take place on a time scale of 20–30 min. We demonstrated that metabolic depression is species dependent. Goldfish, eel and tilapia show metabolic depression under low oxygen conditions while this is not the case for common carp. In addition it is shown that metabolic depression is flexible and increases with decreasing oxygen availability. Furthermore using a video analysing system we demonstrated that metabolic depression is not caused by a reduction of external activity. As heart rate falls dramatically during metabolic depression as shown by small wireless transmitters, we hypothesise that blood flow reduction might be the proximate cause for metabolic depression.

Contents

1. Introduction

In this mini-review we will give an overview of direct, in combination, with indirect calorimetric experiments we performed with large aquatic fish species (range 5–110 g) over the last 15 years in a flow through Setaram twin detection calorimetric system with an 1-l vessel. We will discuss the process of 'metabolic depression': a lowering of the metabolic rate under conditions of anaerobiosis (hypoxia/anoxia). We will demonstrate that it is flexible, species dependent, not caused by a reduction of the external activity and that it takes place on a time scale of 20–30 min. With small implantable telemetry transmitters we will demonstrate that metabolic depression is flexibly dependent on the heart rate (HR) suggesting that blood flow reduction is the proximate cause for the observed metabolic depression.

* Corresponding author. Tel.: +31 317481742; fax: +31 317423110.
E-mail address: Vincent.vanginneken@wur.nl (V. van Ginneken).

0040-6031/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2008.09.027

2. Direct calorimetry method

For a long time it was thought that measuring the metabolic rate via direct calorimetry was not possible for aquatic animals. First, the metabolic rate and thus the amount of produced heat, is low compared to mammals. For poikilotherms, the "standard metabolic rate" (SMR, for more information see Section 5) is only 17 kJ $kg^{0.75}$ day^{-1}, while for homeotherms a mean value of 308 kJ $kg^{0.75}$ day^{-1} has been reported [1,2]. Second, the water medium has a large specific heat capacity—some 800 times higher than that of air and is therefore rather insensitive to a temperature change [3,4]. Third, to determine the SMR, studies have to be performed over a period of several days under constant experimental conditions to eliminate handling stress [4]. This can only be accomplished by a flow-through system, which requires high technology skills in order to reach acceptable signal to noise levels.

Studies with small animals (<1 g) are feasible as they are not limited by slow time constants. Over the last 30 years direct calorimetry has been applied mainly on small aquatic organisms like Salmonid embryos [5] and Crustaceans from the genus *Gammarus* and *Idotea* recently [6–9]. For most fish species we have the technical difficulty for direct calorimetry that we need larger vessels as animal chamber. Therefore, thus far most studies are performed with indirect calorimetry based on oxygen consumption [10]. Oxygen consumption is however, only a derivative of total energy consumption, in many cases aerobic steady state and other preconditions are not reached. In case of an anaerobic component (e.g. during recovery, or under hypoxic and anoxic conditions) direct calorimetry is the only method for the determination of the metabolic rate.

Despite the above-mentioned technical difficulties we developed in 1990 a system capable of performing direct calorimetry with larger aquatic animals like fish, in collaboration with a calorimeter manufacturer, Sétaram, Caluire, France. The system has a 1-l vessel, a maximal flow through of 100 ml/min, a signal resolution of 50 μW, and a drift <0.1 mW per 24 h [11]. After introduction of 1–4 fish up to a total of 110 g, the baseline stability is reached within 8 h. For a resolution of <50 μW the ΔT between the heat sink and the animal chamber must be $<10^{-4}$ °C. This temperature

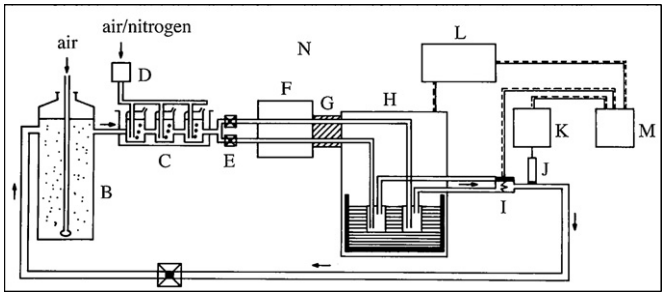

Fig. 1. Schematic drawing of the experimental set-up: (A) pump; (B) storage tank with aeration; (C) glass bottles with gas exchangers in thermostatted waterbath (Tamson TMV 45) for aeration with, air, nitrogen or a mixture; (D) gas mixture pump (Wösthoff, West Germany, 2M301.α-F) to create hypoxic conditions; (E) Veder pumps (Veder V006.10/2031); (F) heat exchanger for temperature stabilisation (Bioactivity Monitor LKB2277); (G) vacuum space; (H) microcalorimeter (Setaram GF108); final temperature stabilisation by means of three heat exchangers inside the calorimeter; (I) automatic alternating Bürckert three-way valve controlled by the computer; (J) oxygen electrode (Radiometer, Denmark, E5046-0) which is fed by a 2 ml water flow drawn with a Gilson peristaltic pump; (K) radiometer (Digital-acid base analyser PHM 72 with a pO_2 module PHA 932) connected with a special developed interface to the computer (L) miscellaneous equipment for the calorimetric set up like amplifier (Setaram A85), Voltmeter (Setaram VN), thermometer (Setaram TN2) temperature programmer and controller (Setaram), thermal safety unit (Setaram TS1); (M) personal computer (Laser 368 SXE) for data registration; (N) thermostatted room.

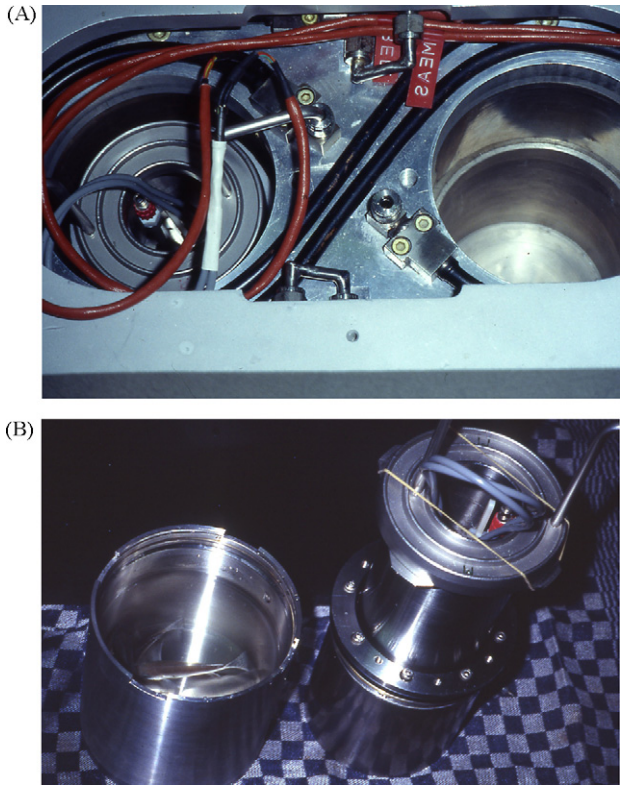

Fig. 2. (A) Calorimetric block with twin detection system identical for both, measurement (right, empty) and reference vessel (left, in place). The connections (stainless tubes) for water in- and out-flow are visible on the left side. (B) Left: 1 l vessel for fish up to 120 g. A low heat loss of <5% was reached by using 3 counter current heat exchangers, 2 within the calorimeter block (A, right) and 1 on top of the animal chamber within the measuring system (B, right).

stability and heat flow sensitivity can only be reached with a twin detection system in combination with a flow through system. A low heat loss of <5% was reached by using 3 counter current heat exchangers, 2 within the calorimeter systems and 1 on top of the animal chamber within the measuring system [11]. An overview of the total system is given in Fig. 1. The calorimetric block (Fig. 2A) contains identical measurement and reference vessels (Fig. 2B). The signals of both vessels are affected by environmental (=temperature) changes in an equal way such that subtraction of both signals results in a stable baseline (Fig. 3).

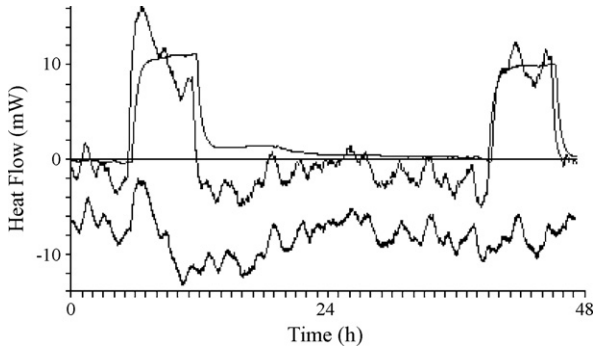

Fig. 3. Principle of the twin detection system. Typical signals of the twin detection system. The signals of both vessels are affected by environmental (=temperature) changes in an equal way such that subtraction of both signals gives a stable baseline indicated by the bold curve at the top showing also the two calibration events in the beginning and the end of the experiment.

3. Metabolic depression takes place on a time scale of 20–30 min

The 1 l flow-through calorimeter (Sétaram GF 108, Fig. 1) contains two stainless steel vessels (twin-detection system), a calorimetric block (Fig. 2A) functioning as a heat-sink, a flow through (dependent on the size of the fish) of 50–100 ml/min and the fish which produces metabolic heat (0.10–100 mW). For the following heat balance model we assumed that the water in the vessel is perfectly mixed and that the temperature of the stainless steel vessel corresponds to the temperature of the water T_w.

The change of the heat content of the system can then be described by

$$C_{tot}\left(\frac{\delta T}{\sigma t}\right) = Å_f - Å_{out} \quad (1)$$

where C_{tot} is the total capacity of the water (w), stainless steel (ss) and $Å_f$ the heat produced by the fish (f) while the heat capacity of the water and stainless steel corresponds to

$$C_{w+ss} = c_{p,w}M_w + c_{p,ss}M_{ss} \quad (2)$$

with c_p is the specific heat of water (w) and stainless steel (ss) and M_w is the mass of water and M_{ss} is the mass of the stainless steel.

The heat flow due to the flow of the water through the calorimeter can be described by

$$Å_{in} - Å_{out} = c_{p,w}\rho\phi(T_{in} - T_{out}) \quad (3)$$

where $\boldsymbol{\phi}$ denotes the volume flow and $\boldsymbol{\rho}$ is the density of water.

The heat loss from the vessel is given by

$$Å_{out} = \rho c_{p,w}\phi(T_w - T_{in}) + \kappa(T_w - T_{hs}) \quad (4)$$

where $\boldsymbol{\kappa}$ is the heat transfer coefficient dependent on the amount and type of thermopiles and *hs* and *w* are the abbreviations for heat sink and water respectively.

In conclusion the heat balance for the 1 l flow-through calorimeter can be written as

$$C_{tot}\left(\frac{\delta T}{\delta t}\right) = Å_f - \rho c_p\phi(T_w + \kappa T_{in}) - \kappa(T_w - T_{hs}) \quad (5)$$

The temperature of the vessel in the equilibrium situation ($T_{w,eq}$) is dependent on the temperature of the in-flowing water, the temperature of the heat sink and the metabolic rate of the fish

$$T_{w,eq} = \frac{c_p\rho\phi T_{in} + \kappa T_{hs} + Å_f}{\kappa + c_p\rho\phi} \quad (6)$$

The time constant $\boldsymbol{\tau}$ of the system is given by

$$\tau = \frac{C_{tot}}{c_p\rho\phi + \kappa} \quad (7)$$

The time constant $\boldsymbol{\tau}$ of the developed 1 l differential flow-through calorimeter (Sétaram GF 108) was estimated to be 33 min and agreed with a model for a first-order process [12]. Data were desmeared using the time constant $\boldsymbol{\tau}$ of 33 min based on Tian equation and the method of Hand and Gnaiger [13], with one time constant.

$$Å_d(t) = Å(t) + \tau/\Delta t\{Å(t) - Å(t-1)\} \quad (8)$$

with $Å_d(t)$ is deconvoluted signal, $Å(t)$ is original data point to be corrected, $Å(t-1)$ is data point registered one minute prior to $Å(t)$ and $\boldsymbol{\tau}$ is the time constant corresponding to 33 min, $\boldsymbol{\Delta t}$ is the sampling rate equal to 1 min. In addition to the method of Hand and Gnaiger [13] data were filtered in the spreadsheet. For every new smoothed point the mean value of ten data points prior to this point were used according to:

$$Å_a(t) = \frac{\{Å(t) + Å(t-1) + \cdots(Å(t-9)\}}{10} \quad (9)$$

with $Å_a(t)$ = the mean value of 10 data points.

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 goldfish (*Carassius auratus* L.) [12], eel (*Anguilla anguilla* L.) [14], and tilapia (*Oreochromis mossambicus* Peters) [4] (Fig. 5). From the deconvoluted signal it can be concluded that the 70% metabolic depression in goldfish and European eel under anoxia takes place on a time

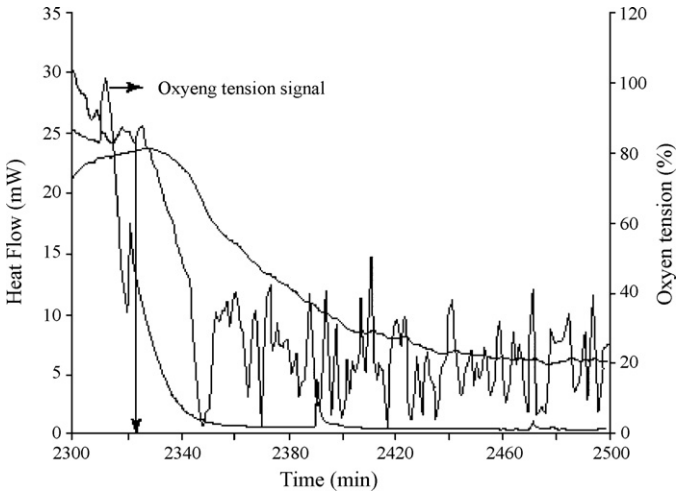

Fig. 4. Enlargement of the registration of the process of metabolic depression of goldfish (n = 4; 6.1; 10.2; 7.5 and 6.5 g respectively) exposed to anoxic conditions. Bold line recorded signal, dashed line deconvoluted (or desmeared) signal. At T = 2310 min anoxia was introduced by saturating the inlet water with pure nitrogen gas. The arrow indicates the start of metabolic depression. Metabolic depression takes place on a time scale of 23 min. (Modified from [12].)

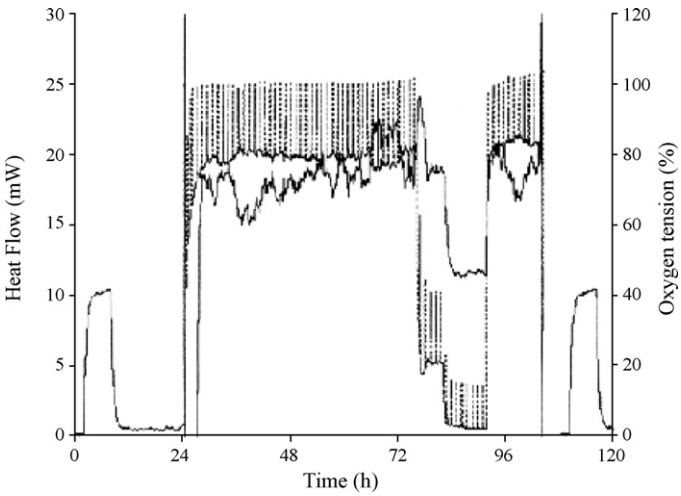

Fig. 5. Metabolic depression of a group of Tilapia (n = 6, total biomass 33.25 g) exposed to hypoxic conditions of 41% and 14.6% AS. The top signal, alternating between reference and measurement vessel of the calorimetric system, is the oxygen tension signal (right *Y*-axis). The difference in oxygen tension multiplied with the flow through the system gives the oxygen consumption. The lower line is the heat production signal of the fish (left *Y*-axis). The experiment starts the first 20 h with an electrical calibration until 10 mW and finishes at 72 h after removing the fish from the vessel with an electrical calibration. During hypoxia of 14.6% AS a metabolic depression with 45% takes place.

Table 1
Calorimetric data of metabolic depression in different fish species during, facultative anaerobioses, estivation and dormancy. Data from van Waversveld et al. [16,17] and van Ginneken et al. [3,4,12,14,15,36] can be compared with the data of Gnaiger [5] by dividing the energy production by a factor 1277 to obtain mW g^{-1}.

Fish species	Bodyweight (g)	Condition	Energy	% Rate of SMR	Source
Salmonid embryos (*Salvelinus alpinus*) T = ??°C	–	Normoxia	1.5 mW g^{-1}	100.0	5
		Anoxia	0.25 mW g^{-1}	16.7	
Goldfish (*Carassius auratus*) T = 20 °C	10	Normoxia	709 J h^{-1} $kg^{-0.85}$	100.0	16[a]
		Anoxia	206 J h^{-1} $kg^{-0.85}$	29.1	
Goldfish (*Carassius auratus*) T = 20 °C	10	Normoxia	555 J h^{-1} $kg^{-0.85}$	100.0	17[a]
		Hypoxia 10%	335 J h^{-1} $kg^{-0.85}$	60.4	
		Hypoxia 5%	290 J h^{-1} $kg^{-0.85}$	52.3	
Goldfish (*Carassius auratus*) T = 20 °C	6.4–9.6	Normoxia	738.0 J h^{-1} $kg^{-0.85}$	100.0	3[a]
		Hypoxia 42.5%	763.6 J h^{-1} $kg^{-0.85}$	103.5	
		Hypoxia 27.1%	710.0 J h^{-1} $kg^{-0.85}$	96.2	
		Hypoxia 16.7%	538.3 J h^{-1} $kg^{-0.85}$	72.9	
		Anoxia	167.8 J h^{-1} $kg^{-0.85}$	22.7	
Tilapia (*Oreochromis mossmabicus*) T = 20 °C	25	Normoxia day 1 pH 7.6	349.6 J h^{-1} $kg^{-0.85}$	83.3	4[a]
		Normoxia day 2 pH 7.6	369.3 J h^{-1} $kg^{-0.85}$	88.0	
		Normoxia day 3 pH 7.6	419.6 J h^{-1} $kg^{-0.85}$	100.0	
		Normoxia day 4 pH 4.0	452.6 J h^{-1} $kg^{-0.85}$	107.9	
		Hypoxia 40% pH 4.0	462.0 J h^{-1} $kg^{-0.85}$	110.1	
		Hypoxia 25% pH 4.0	433.0 J h^{-1} $kg^{-0.85}$	103.2	
		Hypoxia 15% pH 4.0	396.1 J h^{-1} $kg^{-0.85}$	94.4	
		Hypoxia 5% pH 4.0	234.6 J h^{-1} $kg^{-0.85}$	55.9	
Tilapia (*Oreochromis mossambicus*) T = 20 °C	25	Normoxia	502.1 J h^{-1} $kg^{-0.85}$	100.0	15[a]
		Hypoxia 40%	519.8 J h^{-1} $kg^{-0.85}$	103.5	
		Hypoxia 25%	488.2 J h^{-1} $kg^{-0.85}$	97.2	
		Hypoxia 15%	407.0 J h^{-1} $kg^{-0.85}$	81.1	
		Hypoxia 5%	274.6 J h^{-1} $kg^{-0.85}$	54.7	
European eel (*Anguilla anguilla* L.) T = 20 °C	78	Normoxia	887.9 mW	100.0	14[a]
		Anoxia	270.4 mW	30.4	
Goldfish (*Carassius auratus*) T = 25 °C	113	Normoxia	1036.2 J h^{-1} $kg^{-0.85}$	100.0	17[a]
		Hypoxia 40%	957.8 J h^{-1} $kg^{-0.85}$	94.1	
		Hypoxia 20%	849.9 J h^{-1} $kg^{-0.85}$	84.2	
		Hypoxia 10%	690.4 J h^{-1} $kg^{-0.85}$	68.5	
		Hypoxia 3%	552.6 J h^{-1} $kg^{-0.85}$	55.0	

[a] Indicates authors made an attempt to estimate the SMR.

scale of 20–30 min. In this study with goldfish we demonstrated that a 70% metabolic depression takes place on a time scale of 23 min (Fig. 4).

The fact that the heat flow dropped almost simultaneous with the fall in PO_2 (Fig. 4), suggests a fast control via the CNS [12]. As under all conditions the animals switched almost immediately to a lower heat flow and remained thereafter at that level.

4. Species differences

In our laboratory, using direct calorimetry, metabolic depression has been demonstrated in several fish species. European eel reduced its metabolic rate by 70% during anoxia [14], tilapia showed a 45% metabolic depression during extreme hypoxia [15] (Fig. 5), but not under conditions of water acidification [4], while goldfish showed a 70% metabolic depression under anoxia [3,12,16,17]; (see Table 1). In contrast, common carp (*Cyprinus carpio* L.) does not show metabolic depression under low oxygen conditions (Fig. 6).

In experiments exposing goldfish to anoxia we demonstrated that after 2 h of anoxia the heat flow reached a stable level for more than 8 h. The results were reproducible, indicating that this response is typical for goldfish [3,12,16,17]. It has been demonstrated that goldfish can resist such a long period of anoxia by a lactate/ethanol conversion [18] in combination with the 70% metabolic depression. In contrast to this long-term anoxia resistance of goldfish, European eel could resist 1 h of anoxia by a 70% metabolic depression but showed no lactate/ethanol conversion. A few years ago a calorimetry study was carried out with tilapia. Since this species cannot be exposed to anoxia for longer than 2 h, hypoxia experiments were carried out. Heat flow measurements showed a constant rate at 100, 40, 25 and 15% airsaturation (AS) of around 500 J h^{-1} $kg^{-0.85}$, which is rather close the value found for resting goldfish. Further reduction of the PO_2 to 5%AS resulted in a decline to about 284 J h^{-1} $kg^{-0.85}$ (56% SMR), the anaerobic component of the heat flow was 52 J h^{-1} $kg^{-0.85}$ (10% SMR) [4].

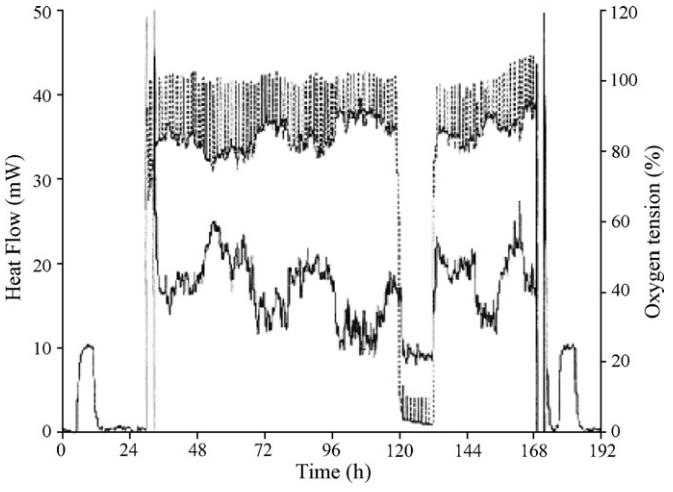

Fig. 6. Common carp of 42 g (n = 1) shows periods of reduced activity but no metabolic depression at severe hypoxia of 10% AS.

5. Metabolic depression is flexible

Metabolic depression has been studied mainly in animals under extreme conditions. Under those conditions it is as if metabolism is switched back in one step to a much lower level. Our experience with fishes, however, indicate that the depression of the metabolic rate is related to the severity of the condition, for example metabolic rate appears to be flexible also below the SMR. In our laboratory calorimetric studies were performed with goldfish [3,12,16,17], tilapia [4,15] and European eel [14] during hypoxia/anoxia. In Table 1 studies are summarised in which the metabolic rate of fish was measured by direct calorimetry. Heat flow data are presented from experiments where animals were kept under normoxia and hypoxia/anoxia. From the data can be concluded that metabolic depression varies from 0 to 83%. These studies indicate there is evidence that animals apply a form a flexible metabolic depression. Under hypoxic conditions, the animal has the possibility of regenerating ATP by aerobic and anaerobic processes. In fact, by reducing the pO_2, we can manipulate the aerobic capacity, and consequently we can measure the extent by which the anaerobic processes are activated and how much both processes contribute to the energy production.

Direct calorimetry with goldfish exposed to normoxia, anoxia, and 5 and 10% air saturation showed heat flows of respectively 700, 181, 290, and 335 J h^{-1} $kg^{0.85}$, corresponding to respectively 30, 53, and 59% of the SMR value [16,17]. In these calorimetric studies with goldfish, it was found that the heat flow stayed constant starting from 1 h after the change in condition. The oxygen supply to the fish was restricted both by flow and by pO_2; because the heat flow measurements would otherwise be disturbed. The flow was kept constant at 50 ml/min, which results in an O_2 difference between in and outflow of 10–15% AS. In this case, the pO_2 of the outflowing water was zero when the inflow was kept at either 5 or 10% air saturation (1 and 2 kPa). Since the ethanol production rate did not differ much between the different hypoxic conditions, it is concluded that the animal used restricted aerobic energy as a supplement to anaerobic energy. In another study, with goldfish the oxygen level of the water outflow was 2–3% AS, indicating that the animals could not use all the available oxygen because of physical reasons (flow 50 ml/min). Under these conditions of severe hypoxia, the heat flow was reduced to 73% of the SMR value, while in the same study, anoxia caused a decrease to 23% of the SMR [3]. So, goldfish apparently have a flexible metabolic depression depending on oxygen availability. This same type of flexible metabolic depression has been shown by Dalla Via co-workers [22] in sole (*Solea solea* L.) using indirect calorimetry. Their data were based on a combination of oxygen consumption and changes in metabolite levels in different tissues.

From these calorimetric and respirometric studies, it can be concluded that during anaerobioses, the anaerobic component remains depressed, but the heat flow increases when the oxygen availability increases. These results indicate that the controls of anaerobic and aerobic processes are rather independent of each other. So, with two fish species we found the same strategy: depression of metabolic rate in relation to the available oxygen, and only partial compensation by anaerobic pathways.

6. Metabolic depression is not caused by a reduction of external activity

A reference point to determine whether an organism shows metabolic depression is the SMR. This is defined for fish as the metabolic rate of postprandial, stress-free fish with a locomotor activity of zero and acclimated at a particular temperature [19,20].

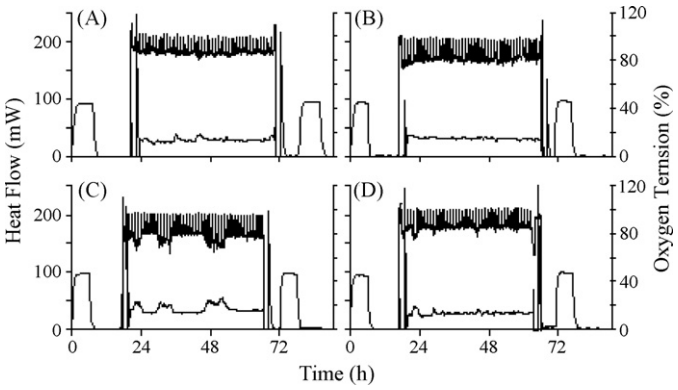

Fig. 7. Registration of 4 typical experiments during a period of 3.5 days to determine the standard metabolic rate (SMR). Each experiment is performed with one eel. (A) Control animal (102.9 g), (B) goitrogenic (=thyroid gland destroyed) animal (105.9 g), (C) T4-treated animal (96.3 g), (D) T3-treated animal (93.4 g). The top signal, alternating between reference and measurement vessel of the calorimetric system, is the oxygen tension signal (right *Y*-axis). The difference in oxygen tension multiplied with the flow through the system gives the oxygen consumption. The lower line is the heat production signal of the fish (left *Y*-axis). The experiment starts the first 20 h with an electrical calibration until 100 mW and finishes at 72 h after removing the fish from the vessel with an electrical calibration. (Source: [27].)

Thus, the SMR corresponds to the minimum rate of energy expenditure to keep the organism alive [20]. In the literature several attempts have been made to estimate the SMR. Beamish used a warm bulb flow-meter to measure the activity of the fish, which allowed him to extrapolate to activity zero [21]. van den Thillart et al. [22] used a statistical method based on a frequency distribution to estimate the SMR. In our studies using the Sétaram 1 l flow through calorimeter [3,4,12,15–17] the space in the vessel was too limited to allow the animals sufficient large pattern of activity. We applied video motion analysis system to determine the spontaneous activity of the experimental animals. With this system we could indeed confirm the low activity level of the animals in the calorimeter (Fig. 7, [17]). In this study we demonstrated for hypoxia-exposed tilapia that despite a metabolic depression of 50% under severe hypoxia (5% AS) there was no complete reduction of the external activity (locomotion). So metabolic depression has its origin in the animal itself, and does not appear to have a constant value. The SMR may change in dependence of the nutritional state (increasing with feeding [23]), but also it depends on the season, and even the daily cycle. This may become clear from Fig. 6 were a common carp of 42 g is in the calorimetric vessel. The space is limited so external activity is nearly zero and we will measure SMR. With the introduction of hypoxic conditions (5% AS) we see that there is nearly no further decline below the SMR so there is no metabolic depression in this fish species.

The reason for these changes in SMR may be due to differences in hormone level and in the state of muscle relaxation during the resting period. The muscle tension can be recorded, but any interference with the animal may affect its behaviour and therefore the observed resting rate. Still, it seems reasonable that the SMR may be influenced markedly by the level of muscle tension. This certainly makes sense for fish, since these animals have a very high somatic index for muscle (>45%). Calorimetric data of excised tissues of cold-blooded animals are lacking, but from the data of warm-blooded animals it can be concluded that muscle tissue is one of the major heat generating tissues. This can be illustrated with the heat production data from Woledge [24]: striated muscle at rest: 2.6–17 W/kg and during activity: 200 W/kg, smooth muscle at rest: 0.6–1.1 W/kg and during activity: 5 W/kg, heart-muscle: 1.8–2.6 W/kg, brown adipose fat tissue: 3.6–11 W/kg and in stimulated tissue 82 W/kg, brain tissue 4.2–7.7 W/kg, and kidney tissue

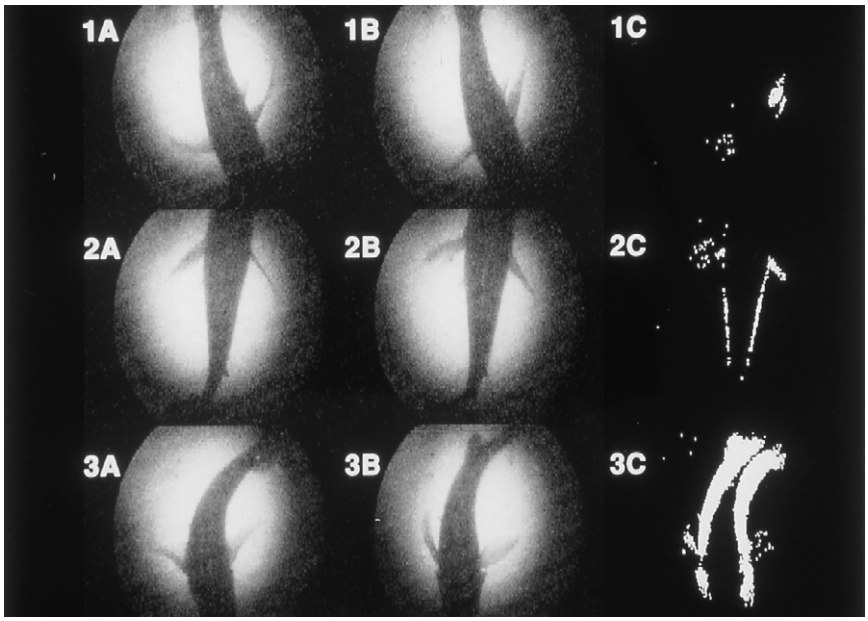

Fig. 8. Photo series for which the inter-image interval between image A and B is 120 ms. Image C is the difference image where the white area reflects the changed pixels due to the difference between A and B. From this white area (composed of individual pixels) mobility can be quantified. Photo-series 1 depicts only mobility due to movement of the pectoral fins of the fish. Photo-series 2 and 3 depict mobility of the fish with a displacement of the body's centre of gravity. (Source: [15].)

30 W/kg. Furthermore, in mammalian thermogenesis, shivering or physical thermogenesis is one of the main mechanisms for upward regulation of metabolism via muscle activity [25,26]. In this respect, the question of whether or not ectothermal animals can achieve *down* regulation of metabolism through muscle regulation (e.g. by depression of muscle tone) is yet to be resolved. The activity level of an animal is predominantly determined by the sympathetic nervous system, which is responsible for increase in muscle tone, blood pressure, changes in blood distribution, and increased alertness of the animal; conversely parasympathetic stimulation will depress all these functions. Therefore, it can be expected that processes that interfere with the autonomic nervous system can influence metabolic depression.

7. Coulson's blood-flow theory

We performed recently a study with direct calorimetry of free-moving eels with manipulated thyroid status [27]. In birds and mammals, the thyroid gland secretes the iodothyronine hormones of which T4 (Tetrajodothyronin) is less active than T3 (Trijodothyronin). The action of T3 and T4 is calorigenic, and is involved in the control of metabolic rate. Across all vertebrates, thyroid hormones also play a major role in differentiation, development and growth. Although the fish thyroidal system has been researched extensively [28], its role in thermogenesis is unclear. In a recent study we measured overall heat production to an accuracy of 0.1 mW in comparison to the SMR by direct calorimetry in free moving European eel with different thyroid status. Hyperthyroidism was induced by the injection of T3 and T4 and hypothyroidism was induced by treatment with phenylthiourea. The results showed for the first time at the organismal level, using direct calorimetry, that neither overall heat production nor overall oxygen consumption in eels is affected by hyperthyroidism [27]. Therefore, we concluded that the thermogenic metabolism-stimulating effect of thyroid hormones (TH) is not effective in a cold-blooded fish species like the European eel (Fig. 8). This supports the concept that TH does not stimulate thermogenesis in poikilothermic species. Apart from muscle activity, which is responsible for the bulk of the metabolic rate, the blood flow is important too. Without sufficient blood flow the muscle tissues cannot generate sufficient power and consequently heat. In the control of the metabolic rate at the organismal level the blood flow is crucial for the transport of oxygen, fuels, and waste-products, but also of metabolic hormones.

Four decades ago Coulson et al. [29] proposed their 'blood-flow' theory, an interesting hypothesis regarding the relationship between blood flow and metabolic rate. Basically, they state that metabolic rates between animals differ by more than a factor of 1000 when expressed as oxygen consumption per gram and that this effect can be directly reflected to blood-flow. At this point we should consider the regular relationship between metabolic rate (rate of oxygen consumption) and the body size of an animal for endotherms which is described in the classical Brody: "mouse-to-elephant curve". If the data sets of metabolic rate and body size are plotted on logarithmic paper a straight regression line is obtained with a slope of approximately 0.75 [30]. The large difference in metabolic rate between the elephant and the mouse (more than a factor of 1000) cannot be ascribed to a different metabolic set up [31]. Remarkably, though, the metabolic machinery of the cells in the different sizes does not scale at all in relation to their metabolic weight. Of course there are size dependent differences like the observed correlation between the metabolic rate of tissues and the overall metabolic rate [32] but those are infinitely small when compared to differences in overall metabolic rate. Probably the major difference, which defines the metabolic rate between large and small animals, is the distance that blood has to travel between organs. Coulson et al. [29], proposed their 'blood-flow' theory, which states that cellular metabolism is primarily regulated by the blood circulation and the factors it carries (substrates, hormones and oxygen). Particularly of interest in this respect are thyroid hormone [33], beta-endorphines [34] prostaglandines [35], and growth hormone. It is obvious that in all animals under metabolic depression the blood flow is severely restricted. Until now the theory of Coulson et al. [29] was neither rejected nor supported. Recently, we tested the hypothesis that blood flow is a dominant factor in the control of metabolic depression. This can be achieved

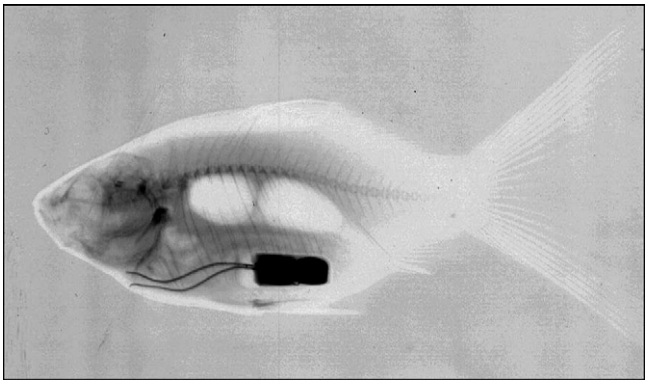

Fig. 9. X-ray of a goldfish (*Carassius auratus* L.) of 85 g with a radio-telemetry transmitter (3.36 g) implanted in the peritoneal cavity (TA10ETA-F20-L20, Data Sciences International, St. Paul, MN, USA).(Source: [36].)

by measuring with telemetry *in vivo* cardiovascular characteristics (heart-frequency and blood pressure) in combination with the overall metabolic rate. In this study with goldfish two techniques were combined: heat measurements via direct calorimetry, to measure overall metabolic rate, and radio telemetry by using small implantable telemetry transmitters (3 g) for measuring electrocardiogram (ECG) and heart rate (HR) in goldfish (Fig. 9, [36]). The metabolic rate decreased at hypoxia levels of 40, 20, 10 and 3% air-saturation almost linearly to 94, 84, 69 and 55% of the SMR, respectively. At 3% air-saturation (AS) anaerobic metabolism was strongly activated. The heart rate of 34 beats per minute (bpm) at normoxia fell at hypoxia levels of 40, 20, 10 and 3% AS to 26, 22, 14 and 9 bpm respectively. A correlation coefficient of 0.97 was calculated between the level of metabolic depression and decrease of HR suggesting a relationship between level of metabolic depression and the HR [36]. These results support the hypothesis that blood flow reduction is the proximate cause for the observed metabolic depression.

8. Calorimetry of aquatic animals: future perspectives and conclusion

Oxygen consumption is only a derivative of total energy consumption. Apart from the possible anaerobic processes, there are marked differences between the oxycaloric values of different substrates used for oxidation: i.e. carbohydrates, fat, and proteins have oxycalorific values of 21.1, 19.6, and 19.1 kJ/l O_2 [37]. However, for these values it is assumed that the animals are under steady state conditions, and that no incomplete oxidations occur nor conversion of one substrate to another. So, the measurement of metabolic rates by oxygen consumption is not always reliable. Under hypoxic and anoxic conditions direct calorimetry is of course *the only* method for the determination of the metabolic rate. Heat production measurements of fish is however complicated since fish are very sensitive to handling and pre-acclimation of several days is required before measurements can be started. To solve this problem, we developed a flow-through compartment, which requires high technology skills in order to reach acceptable signal to noise levels. The applications for direct calorimetry on aquatic organisms or fish are still tremendous. Apart from exposure to environmental stressors like hypoxia and water acidification as reviewed in this manuscript many studies can still be performed. Like e.g. the investigation of industrial pollutants like polychloro biphenyls (PCBs), or heavy metals like copper on metabolic rate of aquatic animals. Also the effect of adding pheromones to the aquatic environment, or injection of hormones in fish (like we did with thyroid hormones [27]), gives enormous unexplored research areas. Therefore we can conclude that direct calorimetry in biological aquatic systems has a great potential in future studies.

Acknowledgements

Experiments were sponsored by the Foundation for Technical Research (STW) and EELREP. Th review was accomplished during an appointment of Vincent van Ginneken at Plant Research International, Agrosystems Research, Wageningen UR.

References

[1] W.R. Robinson, R.H. Peters, J. Zimmerman, Can. J. Zool. 61 (1983) 281.
[2] K. Blaxter, Energy Metabolism in Animals and Man, Cambridge University Press, 1990, p. 336.
[3] 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.
[4] V.J.T. van Ginneken, Influence of hypoxia and acidification on the energy metabolism of fish: an *in vivo* ^{31}P NMR and calorimetric study, PhD Thesis, Leiden University, 1996, p. 336.
[5] E. Gnaiger, M. Ortner, G.E. Rieger, W. Salvenmoser, Am. Zool. 27 (1987) 31A.
[6] M. Normant, E. Schmolz, I. Lamprecht, Thermochim. Acta 415 (2004) 135.
[7] M. Normant, T. Lapucki, E. Schmolz, I. Lamprecht, Thermochim. Acta 422 (1–2) (2004) 49.
[8] M. Normant, I. Lamprecht, J. Exp. Marine Biol. Ecol. 334 (2006) 158.
[9] T. Lapucki, M. Normant, M. Feike, G. Graf, A. Szaniawska, Thermochim. Acta 435 (1) (2005) 6.
[10] G. Winberg, Fish. Res. Bd. Can. Transl. Ser. 194 (1956) 41.
[11] A.D.F. Addink, G.E.E.J.M. van den Thillart, H. Smit, J. van Waversveld, Thermochim. Acta 193 (1991) 41.
[12] V.J.T. van Ginneken, J. Vanderschoot, A.D.F. Addink, G.E.E.J.M. van den Thillart, Thermochim. Acta 249 (1994) 143.
[13] S.C. Hand, E. Gnaiger, Science 239 (1988) 1425.
[14] V.J.T. van Ginneken, M. Onderwater, O. Lamua Olivar, G.E.E.J.M. van den Thillart, Thermochim. Acta 373 (2001) 23.
[15] V.J.T. van Ginneken, A.D.F. Addink, G.E.E.J.M. van den Thillart, F. Körner, L. Noldus, M. Buma, Thermochim. Acta 291 (1997) 1.
[16] J. van Waversveld, A.D.F. Addink, G.E.E.J.M. van den Thillart, J. Comp. Physiol. B 159 (1989) 263.
[17] J. van Waversveld, A.D.F. Addink, G.E.E.J.M. van den Thillart, J. Exp. Biol. 142 (1989) 325.
[18] A. van Waarde, G.E.E.J.M. van den Thillart, M. Verhagen, Ethanol formation and pH-regulation in fish, in: P.W. Hochachka, P.L. Lutz, T. Sick, M. Rosenthal, G.E.E.J.M. van den Thillart (Eds.), Surviving Hypoxia, Mechanisms of Control and Adaptation, CRC Press, Inc., Boca Raton, FL, 1993.
[19] F.E.J. Fry, The effect of environmental factors on physiology of fish, in: W.S. Hoar, D.J. Randall (Eds.), Fish Physiology V1, Academic Press, New York, 1971, p. 1.
[20] J.R. Brett, T.D.D. Groves, Physiological energetics, in: W.S. Hoar, D.J. Randall, J.R. Brett (Eds.), Fish Physiology V111, Academic Press, New York, 1979, p. 280.
[21] F.W.H. Beamish, Can. J. Zool. 42 (1964) 355.
[22] G.E.E.J.M. van den Thillart, J. Dalla Via, G. Vitali, P. Cortesi, Mar. Ec. Prog. Ser. 104 (1994) 109.
[23] M. Jobling, P.S. Davis, J. Fish Biol. 16 (1980) 629.
[24] R.C. Woledge, The heat production of intact organs and tissues, in: A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, New York, 1980, p. 145.
[25] P.W. Hochachka, Fed. Proc. 33 (1974) 2162.
[26] S. Prusiner, M. Poe, Nature 220 (1968) 235.
[27] V.J.T. van Ginneken, B. Ballieux, G.E.E.J.M. van den Thillart, Naturwissenschaften 94 (2007) 128.
[28] J.F. Leatherland, Am. Zool. 28 (1988) 319.
[29] R.A. Coulson, T. Hernandez, J.D. Herbert, Comp. Biochem. Physiol. 56A (1977) 251.
[30] S. Brody, R.C. Procter, U.S. Ashworth, Univ. Missouri Agric. Exp. State Res. Bull. 220 (1934) 1.
[31] K. Schmidt-Nielsen, Scaling: Why Is Animal Size so Important? Cambridge University Press, 1984, p. 241, ISBN 0-521-266572.
[32] A.W. Martin, F.A. Fuhrman, Physiol. Zool. 28 (1955) 18.
[33] E.M. Plisetskaya, N.Y.S. Woo, J.C. Murat, Comp. Biochem. Physiol. 74A (1983) 179.
[34] D.L. Margulus, Neurosci. Biobehav. Rev. 3 (1979) 155.
[35] M. Sancibrian, J.S. Serrano, F.J. Minano, Gen. Pharmacol. 22 (1991) 259.
[36] V.J.T. van Ginneken, P. Snelderwaard, R. van der Linden, N. van der Reijden, G.E.E.J.M. van den Thillart, K. Kramer, Thermochim. Acta 414 (2004) 1.
[37] J.M. Elliot, W. Davison, Oecologia 19 (1975) 195.