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Coupling of heart rate with metabolic depression in fish: a radiotelemetric and calorimetric study

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Abstract

This study of the goldfish (*Carassius auratus* L.) combines two techniques: heat measurements via direct calorimetry and radio telemetry, using small implantable telemetry transmitters (3 g). These record overall metabolic rate, and electrocardiogram (ECG) and heart rate frequency (fHR), respectively. The metabolic rate decreased at hypoxia levels of 40, 20, 10, and 3% air-saturation (AS) almost linearly to 94, 84, 69, and 55% of the standard metabolic rate (SMR), respectively. This implies that metabolic depression is flexible, depending on the supply of oxygen. From the deconvoluted heat-flow signal it can be concluded that the metabolic depression per hypoxia level takes place within 20 min. At 3% AS anaerobic metabolism was strongly activated. The fHR 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 fHR suggesting a relationship between level of metabolic depression and the HR. These results support the hypothesis that blood flow reduction is the proximate cause for the observed metabolic depression.

Keywords: *Carassius auratus*; Anoxia; Metabolic depression; Heart rate; Radio-telemetry; Calorimetry

1. Introduction

Down-regulation of energy metabolism below the minimal energetic costs for homeostasis can be observed in many animals, such as diving turtles, insects in diapause, lungfish in aestivation during periods of drought, and in certain fish species during anaerobiosis [1–3]. It is described (depending on the environmental stress-factor and the organism) using terms such as torpor, hibernation, aestivation, and metabolic depression. In our laboratory, using direct calorimetry, metabolic depression has been demonstrated in several fish species. European eel (*Anguilla anguilla* L.) reduced its metabolic rate by 70% during anoxia [4], tilapia (*Oreochromis mossambicus* P.) showed a 50% metabolic depression during extreme hypoxia [5–7] but not under conditions of water acidification [6–8], while goldfish (*Carassius auratus* L.) showed a 70% metabolic depression under anoxia [9–12]. In contrast, common carp (*Cyprinus carpio* L.) does not show a metabolic depression under low oxygen conditions [13]. In addition, in *Sipunculus nudus* the process of metabolic depression was demonstrated by direct calorimetry under conditions of anaerobioses [14].

The mechanism has not yet been identified in any species. Obviously, the mechanism of metabolic depression is species dependent. Measuring heat production of fish via direct calorimetry has the disadvantage that, because of the slow response time of the calorimetric system, little information can be acquired about the kinetics of the biological process. With deconvolution techniques, using the time constant of the calorimeter, it is possible to correct for the lag-time and increase the time resolution to about 10 min. In an earlier study, we concluded that reduction of the metabolic rate was directly correlated with the decline of the oxygen concentration [4,11]. In addition, we could demonstrate with a Noldus–video analysis system that metabolic depression was not caused by reduction of the external activity of the

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animal [15]. Thus, we hypothesized that the cause of the process of metabolic depression must have its origin in internal physiological changes.

Coulson [16], proposed a 'blood-flow' theory, which states that cellular metabolism is primarily regulated by blood circulation and the factors it carries (substrates and oxygen). Until now the theory of Coulson [16] has been neither rejected nor supported.

The aim of this study was to test the hypothesis that regulation of the blood flow (indicated by the heart rate frequency (fHR)) of the organism, is directly correlated with the level of metabolic depression. Thus, we may demonstrate that reduction of the blood flow can induce metabolic depression. In this study, we used radio-telemetry, to measure in vivo cardiovascular parameters (ECG, heart rate) in combination with direct calorimetry.

2. Material and methods

2.1. Animals

Goldfish (*C. auratus* L.) were obtained from a commercial fish dealer (Boon, Hardinxveld, 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 daily with Trouvit pelleted food (Trouw, Putten, The Netherlands). The weight of the animals was 112.7 ± 11.3 g ($n = 5$).

2.2. Calorimeter

The calorimetric system is described before [9,10]. 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 (Fig. 1, right: calorimeter). 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 (1–120 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 [10].

Before the goldfish was placed in the vessel the sensitivity coefficient, which relates signal level to power input, was determined. The sensitivity coefficient of the performed experiments was 75.2 ± 2.17 μV/mW. The flow through the system was 100 ml/min. The baseline stability was ±0.01 mW per 24 h. At the beginning and the end of an experiment the heat flux signal was checked by calibration.

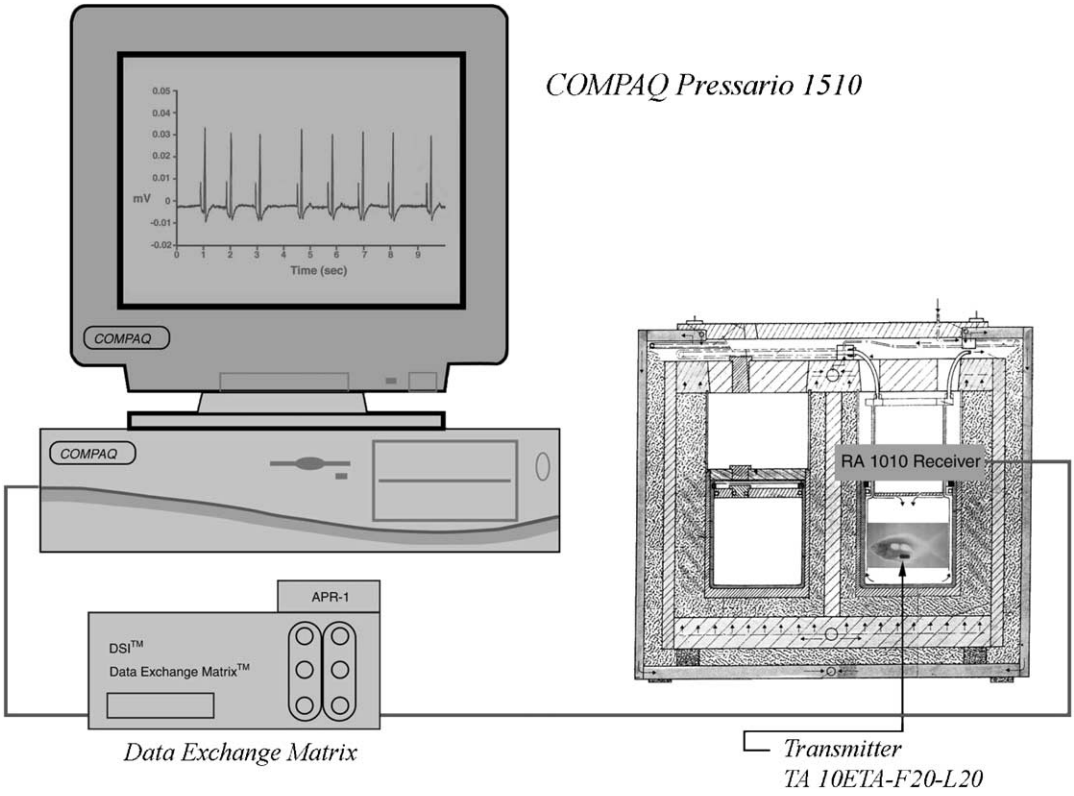

Fig. 1. Schematic drawing of the radio-telemetry system for recording ECG and heart rate of a freely swimming fish with implanted radio-transmitter, in the 1-l vessel of the calorimeter. Right: twin-detection, 1 l differential flow-through calorimeter (Sétaram GF 108). One vessel contains the fish with implanted radio-telemetry transmitter. The other vessel contains only water.

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 31.64 V which is applied to a resistor of 1000 Ω mounted in the measurement vessel. This resulted in a power output signal of 99.7 mW. The calorimetric apparatus was placed in a climatized 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 with specially developed software for data recording and graphical presentation [10].

2.3. Deconvolution techniques: desmearing of calorimetric data

The time constant, τ, of the developed 1 l differential flow-through calorimeter (Sétaram GF 108) was estimated to be 33 min [11]. Data were desmeared using the time constant of 33 min based on the method of Hand and Gnaiger [17], with one time constant.

$$\text{Å}_\text{d}(t) = \text{Å}(t) + \frac{t}{\Delta t}\{\text{Å}(t) - \text{Å}(t-1)\}$$

With $\mathring{A}_\text{d}(t)$ is deconvoluted signal, $\mathring{A}(t)$ is original data point to be corrected, $\text{Å}(t-1)$ is data point registered one minute prior to $\mathring{A}(t)$ and, τ, the time-constant corresponding to 33 min, Δt is the sampling rate equal to 1 min. In addition to the method of Hand and Gnaiger [17] data were filtered in the spreadsheet. For every new smoothed point the mean value of 10 data points prior to this point were used according to:

$$\text{Å}_\text{a}(t) = \frac{1}{10}\{\text{Å}(t) + \text{Å}(t-1) + \cdots + (\text{Å}(t-9)\}$$

with $\mathring{A}_\text{a}(t)$ as the mean value of 10 data points.

2.4. Oxygen registration system and establishing hypoxic conditions

The oxygen meter was a digital oxygen analyzer, Radiometer Copenhagen type PHM 72c with a $p(O_2)$ module type PHA 932. A platinum-silver $p(O_2)$ electrode (Radiometer Copenhagen E5046) was mounted in a thermostatted cell (Radiometer Copenhagen D616) and connected to the meter. All parts from calorimeter to oxygen electrode were in stainless steel to prevent oxygen diffusion. From the outflowing water of the calorimeter, a flow of 2 ml/min was drawn over the electrode using a Gilson persistaltic pump. This was sufficient to neglect the small internal blank oxygen consumption of the electrode. Rates of oxygen consumption were calculated following:

$$\acute{v}O_2 = v(c_\text{r} - c_\text{m})\ \text{mmol}\ O_2/\text{h})$$

where v was the water-flow through the vessels (for both vessels, $v = 100$ ml/min) and c_r and c_m were, respectively, the oxygen concentration measured in the out-flowing water of the reference and measurement vessels. The oxygen concentration of the in-flowing 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 ($n = 6$) [10]. After passage through the calorimeter (without fish) the oxygen concentration corresponded to a value of 8.84 ± 0.062 mg/l ($n = 6$), so the blank oxygen consumption (due to microorganism in the apparatus) was 0.1 mg/l [10]. Oxygen consumption data of the animals were corrected for the blank oxygen consumption of the calorimeter. Hypoxic conditions were established by equilibrating the water with a gas mixture of nitrogen and air produced by a gas-mixing pump (Wösthoff, West Germany, 2 M, 301/a-F). Each animal was exposed to 40% hypoxia for 1 h followed by hypoxia levels (20, 10, and 3% air-saturation (AS)) for a period of 5 h each. All animals survived the experiments and totally recovered from the oxygen stress.

2.5. The radio-telemetry technique

With the radio-telemetry transmitters, cardiovascular parameters can be recorded like electrocardiogram (ECG), and heart rate frequency, but also activity of the animals and body temperature (BT) [18–21]. We were the first research group which demonstrated that we were able to implant a small telemetry transmitter (TA10ETA-F20-L20, Data Sciences International (DSI), St. Paul, MN, USA) in the peritoneal cavity of a small gold fish (100 g) (Fig. 2A and B).

A receiver system was built in the calorimetric vessel to pick up the signal from the transmitter implanted in the peritoneal cavity of the fish (Fig. 1). In this way we were able to measure the fHR under several conditions of hypoxia, in a freely moving fish with radio-transmitter, in the 1-l vessel of the calorimeter.

2.6. Surgery

Telemetry transmitters of the type TA 10ETA-F20-L20 (Data Sciences International, St. Paul, MN, USA) were used: length: 21 mm; height: 10 mm; width: 11 mm; weight in air: 3.3 g and implanted in five goldfishes of approximately 110 g. First, the fishes were anaesthetized with a 100 parts per million (ppm) MS-222 solution in water. A 1.5 cm incision above the pectoral fin was made and the transmitter body was fixed in the abdominal cavity with one stitch to the basipterygia of the pelvic girdle. The positive electrode was glued with dextroflavum in water solution to a longitudinal grinded hypodermic needle and inserted from the abdominal cavity through the septum transversum into the pericardial cavity. Injecting the hypodermic needle with tepid physiological salt solution dissolved the glue. Then the needle was withdrawn while leaving the electrode in place. The same procedure was used to place the negative electrode in the hypaxial musculature. After this procedure the fishes were returned individually to their aquariums. The experiments

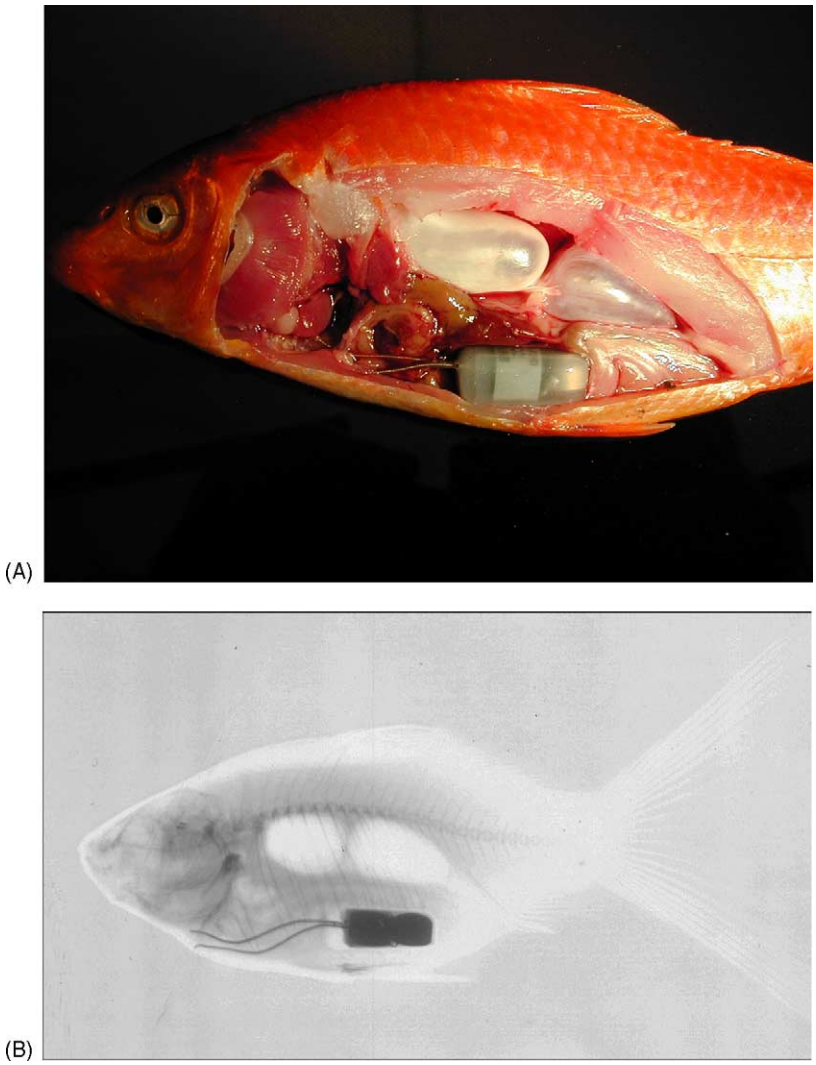

Fig. 2. (A) Photo of a goldfish (*Carassius auratus* L.) of 103 g with a radio-telemetry transmitter (3.36 g) implanted in the peritoneal cavity. (B) X-ray of a goldfish (*Carassius auratus* L.) of 85 g with the same radio-telemetry transmitter (3.36 g) implanted in the peritoneal cavity (TA10ETA-F20-L20, Data Sciences International, St. Paul, MN, USA).

were authorized by the ethical committee of Leiden University on animal experiments.

2.7. Experimental set up

A schematic drawing of the experimental set up with a goldfish with radio-telemetry transmitter implanted in the peritoneal cavity and placed in the calorimeter is given in Fig. 1. A receiver plate for measuring fHR was build in the aquatic phase of the vessel with the freely moving fish below it while heat production was registered with thermopiles in the wall of the calorimetric block. In this way five different experiments with five individual goldfish, all implanted with radio-telemetry transmitters, were performed. The experimental set up is of the twin detection system: one vessel contains only water, the other vessel the fish and water. Subtraction of the signal of both empty vessels gives a stable baseline.

2.8. Statistics

Statistics were performed in SPS6 using a one-way ANOVA. Normality of the data and homogeneity of variances were checked by Kolmogorov–Smirnov and F_{max} tests, respectively. $P < 0.05$ was considered as statistically significant. The Pearson correlation technique, programmed in SPS6, was used in order to calculate the correlation coefficient between level of metabolic depression and fHR, fHR and oxygen consumption, heat production and oxygen consumption, metabolic depression and oxygen consumption.

3. Results

In Fig. 2A a photo of a goldfish (103 g) with a small telemetry-transmitter (3 g) implanted in the peritoneal cavity is depicted while in Fig. 2B an X-ray of a goldfish (85 g)

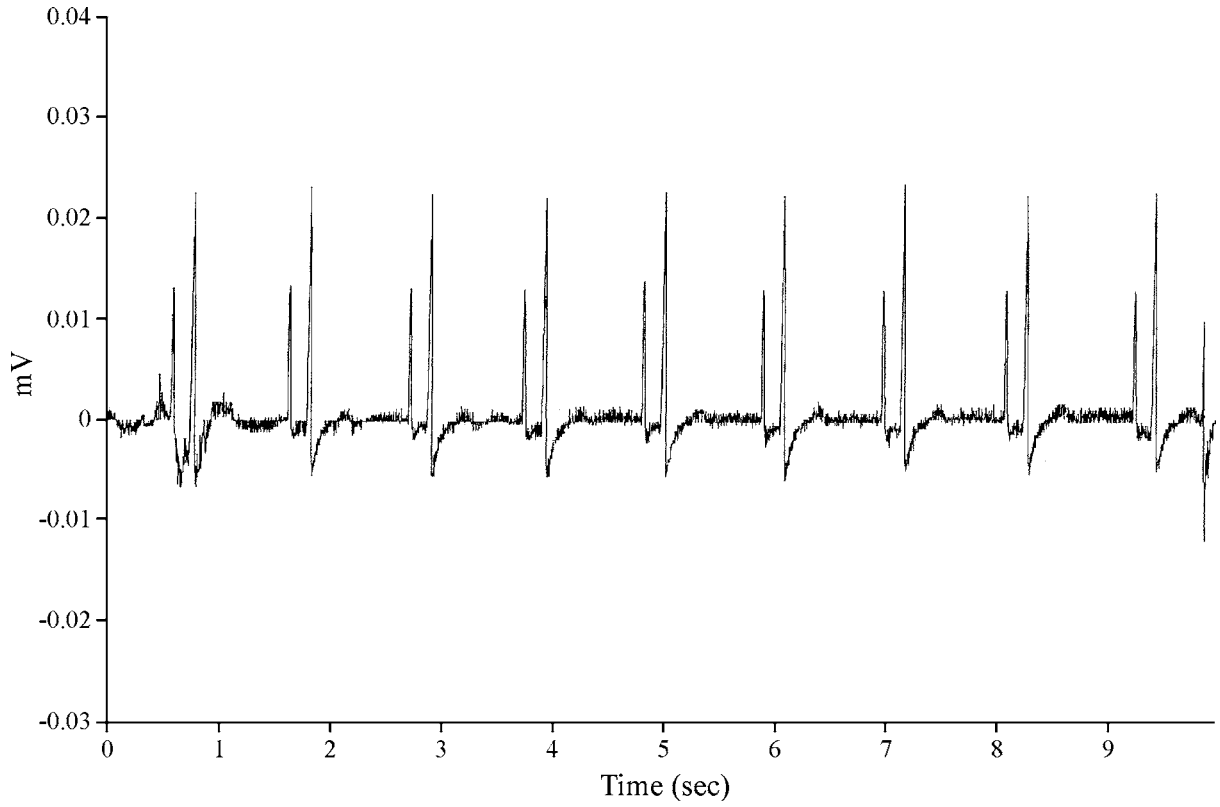

Fig. 3. Some ECG registrations of a freely swimming goldfish (103 g) in the 1 l calorimeter vessel. Note that for fish the cardiovascular system can be characterized by two heart chambers in series, an atrium and a ventricle, which is expressed in the two peaks of the ECG.

is given. Recovery after the operation took a minimum of 2 weeks.

In Fig. 3, a typical ECG registration of a goldfish under normoxic conditions is given. A graphical presentation of a typical experiment performed in the calorimeter is given in Fig. 4. At 24 h the fish is placed in the calorimeter. After approximately 6 h, the calorimeter is stabilized and the heat production signal of the fish is stable and remains at a constant level. When hypoxia is introduced the fish responds for every new hypoxia level with an overshoot of heat production, probably because the animal becomes disturbed due to the hypoxic water conditions. This overshoot of heat production is followed by a lower steady state such that at each hypoxia level the metabolic rate is lower. In Fig. 5, the heat-flow signal during metabolic depression is enlarged, together with a second line which shows the actual heat flow after correcting for the time constant of the calorimeter by deconvolution (time lag correction). From the deconvoluted signal it can be concluded that the metabolic depression per hypoxia level takes place within 20 min. In detail, metabolic depression at 20% hypoxia took place within 22 min (spike 1, from 81.08 to 33.91 mW); metabolic depression at 10% hypoxia in 16 min (spike 2, from 58.96 to 29.90 mW), while for 3% hypoxia this process took place with 25 min (spike 3, from 34.67 to 23.82 mW). Recovery was rapid and took place within 25 min (spike 4, from 22.94 to 60.76 mW). After hypoxia when normoxia is reinstalled the fish regains a stable heat production again, comparable with the initial normoxic heat production prior to the establishment of hypoxia. We can see from the increase of the oxycaloric equivalent (Table 1) at all hypoxia levels that the anaerobic metabolism starts to become activated: the decline of oxygen consumption is stronger than the heat production drops. At 3% AS, the value of the oxycaloric equivalent of 678 kJ/mol is indicative that the anaerobic metabolism is strongly activated, despite the earlier mentioned metabolic depression.

In Table 1, the heat production data, the percentage of metabolic depression during hypoxia in comparison to the normoxic situation, the oxygen consumption data, the fHR, and the oxycaloric equivalent are given. The metabolic rate of goldfish (*C. auratus* L.) decreased at hypoxia levels of 40, 20, 10, and 3% to 94, 84, 61, and 55%, respectively. The fHR at normoxia (100% oxygen) was 34 beats per minute (bpm) and decreased at hypoxia levels of 40, 20, 10, and 3% AS until 26, 22, 14, and 9 bpm, respectively. The oxygen consumption during normoxia (which corresponds to the standard metabolic rate (SMR)) was 0.38 mmol O_2/h per 100 g fish and decreased at hypoxia levels of 40, 20, 10, and 3% AS until 0.35, 0.28, 0.22, and 0.14 mmol O_2/h per 100 g fish, respectively. The correlation coefficient between (a) level of metabolic depression and fHR, (b) fHR and oxygen consumption, (c) heat production and oxygen consumption, and (d) metabolic depression and oxygen consumption was 0.974, 0.963, 0.996, and 0.971, respectively.

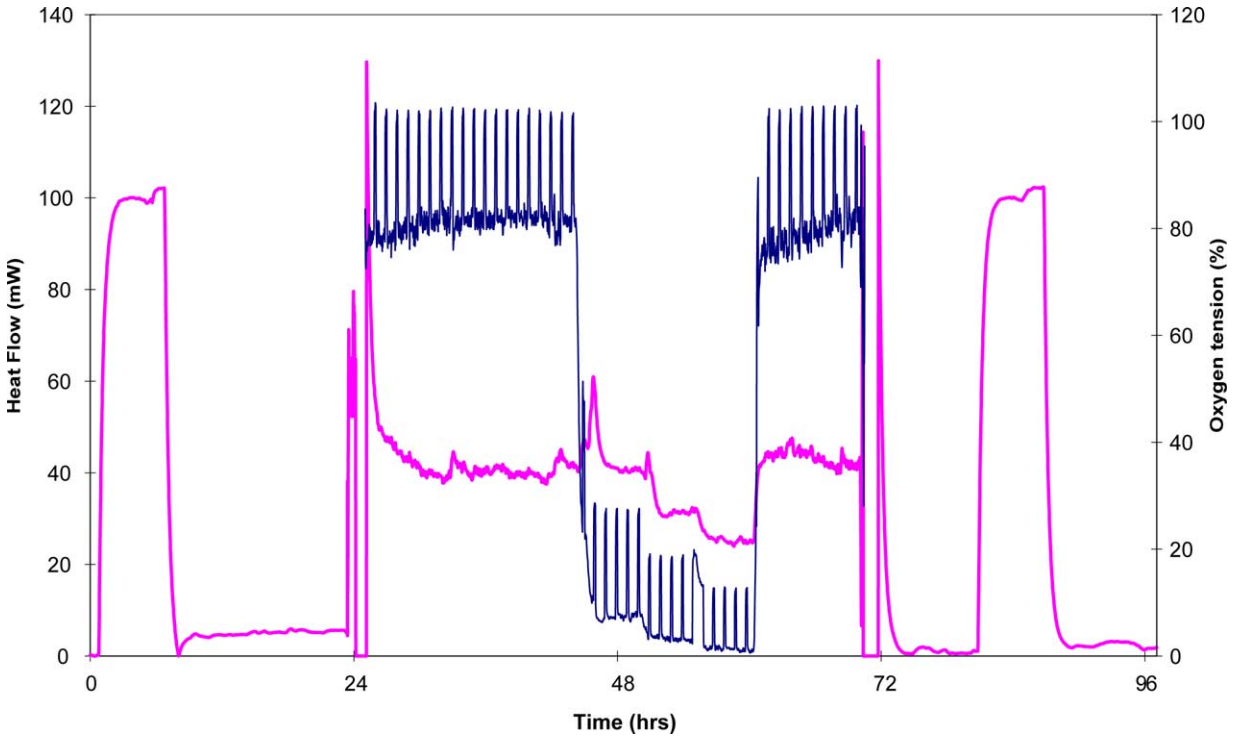

Fig. 4. Time course of a calorimetric experiment, which is started and finished with an electrical calibration procedure, resulting in a heat production of approximately 100 mW. The initial and final position of the base line of the heat production signal indicates almost no base line drift. After calibration a goldfish (*Carassius auratus* L.) of 103 g with the radio-telemetry transmitter in the peritoneal cavity to measure ECG and HR is introduced in the calorimeter. The top line corresponds to the oxygen tension signal, alternating measuring the measurement and reference vessel. The oxygen tension of the water flowing in the calorimeter is given by the top of the spikes in the $p(O_2)$ signal switching every 50 min for 10 min to reference position. The difference between measurement and reference vessel is caused by the oxygen consumption of the fish and, when multiplied with the flow through the system (100 ml/min) corresponds to the oxygen consumption At Day 2 the goldfish is exposed to 40% hypoxia for 1 h, and 20, 10, 3% AS hypoxia for a period of 5 h per level each. The animal survived the experiments and totally recovered from the oxygen stress.

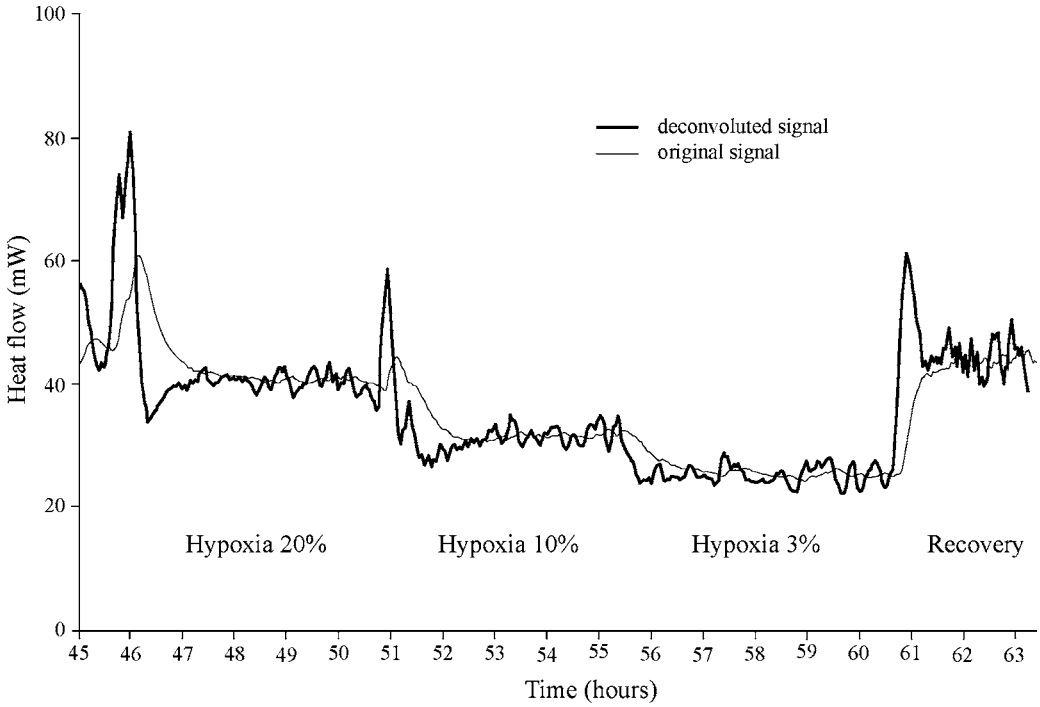

Fig. 5. Enlargement of the registration of the process of metabolic depression as depicted in Fig. 4. Thin line recorded signal; bold line: deconvoluted signal.

Table 1
Mean value and standard deviation of heat production, metabolic depression, heart rate frequency (fHR), and oxygen consumption of five experiments with five different individual goldfish (*Carassius auratus* L.) under normoxic and hypoxic conditions at a temperature of 20 °C

Condition	Heat production rate (mW/g)	Heat production rate (J/h per 100 g fish) ($n = 5$)	Heat production rate of normoxic situation (Θ) (%) ($n = 5$)	Heart rate frequency (beats/minute)($n = 5$) between brackets (%)	Oxygen consumption (mmol O_2/h per 100 g fish) ($n = 5$) between brackets (%)	Oxycaloric Equivalent (kJ/mol) ($n = 5$)
Normoxia $\geq$ 24 h	0.451 ± 0.062	162.4 ± 22.2	100	34.3 ± 9.5 (100%)	0.38 ± 0.04 (100%)	432.8 ± 63.5
Hypoxia-40% $\geq$1 h	0.417 ± 0.042	150.1 ± 15.2	94.1 ± 17.5	25.6 ± 5.8 (74.6%)	0.35 ± 0.10 (92.1%)	444.5 ± 99.6
Hypoxia-20% $\geq$ 5 h	0.370 ± 0.054	133.2 ± 19.3	84.2 ± 21.1	22.4 ± 3.6* (65.3%)	0.28 ± 0.07* (73.7%)	479.9 ± 63.2
Hypoxia-10% $\geq$ 5 h	0.300 ± 0.047	108.2 ± 16.8**	68.5 ± 18.2**	13.5 ± 7.9** (39.4%)	0.22 ± 0.05** (57.9%)	491.6 ± 61.7
Hypoxia-3% $\geq$ 5 h	0.240 ± 0.066	86.6 ± 23.6***	55.0 ± 18.4***	9.2 ± 5.5*** (26.8%)	0.14 ± 0.06*** (36.8%)	677.8 ± 204.3 *

The five different fishes, all implanted with telemetry transmitters (TA10ETA-F20-L20, Data Sciences International, St. Paul, MN, USA) implanted in the peritoneal cavity were placed individually in a Setaram GF 108 flow-through calorimeter and exposed after 48 h to a graded hypoxia load (40% air-saturation (1 h), 20, 10, and 3% AS (for 5 h per hypoxia level each). (Θ) reciprocal is metabolic depression. Normoxia vs. hypoxia: (*) denotes $P \leq 0.05$, (**) denotes $P \leq 0.01$, and (***) denotes $P \leq 0.001$.

4. Discussion

For all adult fish living in an environment with variable oxygen availability, there has to be a close coupling between metabolic demand, respiratory gas exchange, and cardiac activity. If circulatory system delivers less oxygen due to environmental anaerobioses, the linkage with metabolic requirements can be maintained by a lowering of the metabolic rate: metabolic depression and an activation of anaerobic metabolism. Both processes were observed in this study. At normoxia we observed an oxycaloric value of 432.8 kJ/mol. This is close to an oxycaloric value with fat as substrate (439.04 kJ/mol) or protein (to ammonia-conversion, 427.52 kJ/mol) (review: [22]). It is possible that a mixed substrate is used. However, earlier we demonstrated that it is very difficult to obtain data accurately enough, such that conclusions can be made about the oxycaloric equivalent and substrate usage. If we assume an experimental error of 5% in both heat production and oxygen consumption measurements, we obtain an experimental error of $\pm\sqrt{5^2+5^2} = \pm 7\%$ corresponding to ±32 kJ/mol, which is within the range of differences between substrates [10]. At 3% AS (Table 1) we see that, despite a metabolic depression of 45%, the anaerobic metabolism is strongly activated, resulting in an oxycaloric equivalent of 678 kJ/mol. From our direct and indirect calorimetric measurements we can calculate the lactic acid production. At 3% AS, 0.14 mmol O_2/h per 100 g fish is consumed (Table 1, column 4)) which, based on the oxycaloric equivalent, measured under normoxia, of 432.8 kJ/mol (Table 1, column 5), corresponds to a heat production of 60.6 J/h per 100 g fish. The anaerobic and aerobic heat production, measured by direct calorimetry, corresponds to 86.6 J/h per 100 g fish (Table 1, column 1). Thus, the anaerobic heat production corresponds to 26.0 J/h per 100 g fish. The heat production in the conversion from glucose to lactic acid is −184 kJ/mol [23]. Thus, in a fish of 100 g the decline of glucose corresponds to 1.4 mM glucose/h which corresponds to a lactic acid production of 2.8 mM lactic acid/h. This is rather low in comparison to other values reported in literature [24], probably because anaerobic energy demand is strongly reduced by the 45% metabolic depression at 3% AS.

With our 1 l flow-through microcalorimeter, we have quantified in previous studies the process of metabolic depression in several aquatic animals (*C. auratus*, *O. mossambicus*, *A. anguilla*, and *S. nudus*) under adverse conditions such as anoxia/hypoxia and water acidification [4,6,10–12,14,15]. Using two approaches, we demonstrated that the degree of metabolic depression is fish species-specific. By direct calorimetry, measuring the overall metabolic rate, we observed a 70% metabolic depression in goldfish [10–12], 50% metabolic depression in tilapia [6,15], and no metabolic depression in carp [13]. In addition, we have demonstrated metabolic depression in fish species with the ^{31}P nuclear magnetic resonance (NMR) technique. This was based on the speed of depletion of energy rich compounds in muscle tissue and the rate of intracellular acidification of muscle tissue [5,7,25,26].

The following features characterize the mechanism of metabolic depression. First, the process is tissue dependent. This was proved in a ^{31}P NMR study with three surface coils at three locations of muscle tissue (dorsal-, ventral-white muscle and red muscle) [25]. Second, metabolic depression is flexible. In earlier anoxia studies it appeared that the metabolic rate was reduced to its lowest level in one step for goldfish [12] and eel [4]. However, calorimetry studies under conditions of hypoxia show that metabolic depression is flexible and dependent on the oxygen availability. Through this mechanism of hypometabolism, metabolic demand is directly linked to the oxygen-delivery capacity to the tissues. Coulson [16] suggested in his 'blood-flow' theory that the dominant and interrelating factor might be the blood-flow, and that it is only through this general mechanism, that extracellular changes in metabolites, oxygen level, hormones, and substrates may have an influence on each of the thousands of different intracellular metabolic reactions. Other support for the theory of Coulson [16] comes from the correlation coefficient of 0.97 seen between the level of metabolic depression and decrease of fHR. This result suggest that there is a causal relationship between level of metabolic depression and the fHR. The fHR together with the stroke volume determines the cardiac output, the latter determines the blood flow. For a complete picture the cardiac output also has to be determined which we hope to perform in future studies with special telemetry transmitters (see Section 4.1). At this moment this research area of telemetric techniques for implanting small transmitters in vertebrates of 100–500 g is developing rapidly [18]. Physiological information about heart rate from individual freely swimming fish can be obtained by this radio-telemetry technique. This technique provides an alternative means of obtaining physiological parameters from conscious, free-moving animals, without introducing stress artifacts [18–21]. The resting fHR of ectothermic vertebrates (fish and tetrapods) with a size comparable to the fish used in this study (100 g) is in the range of 30–170 bpm [27]. In our experiment, we observed a mean frequency of 34 bpm at 20.0 °C, which is at the bottom end of the range. In the review of Farrel and Jones [28] a table with intrinsic fHR is included. For teleosts (with the exception of tunas) they are in the range of 22–57 bpm. Cameron et al. [29] mentions for goldfish an intrinsic fHR of 57 at a temperature of 20–25 °C, which is rather high in comparison with our data: 34 bpm at 20.0 °C at normoxia. We did not determine the intrinsic fHR in goldfish in vivo by pharmacological blockade with atropine and a β-adrenergic antogonist. However, the limited size of the 1 l calorimetric vessel implies that we may assume, that we measured the resting fHR without any locomotor activity of the animal. In general, the maximum fHR of lower vertebrates is, in most cases, no greater than 100–120 beats/min. For active fish this is 0.5–2 times the resting fHR [30].

More information about the process of metabolic depression can be derived from a temperature induced state of hypometabolism: hibernation. This situation can be characterized by a high Q_{10} value. In eel, for example, a Q_{10} value of 4.10 was observed over the temperature range 5–10 °C. The observed Q_{10} value is not caused by decreased enzymatic activities, but more probably by acidosis, which results in metabolic deactivation [31]. This results indicates that metabolic rate decreases more than can be expected from the normal Q_{10} values, thus indicating metabolic depression. Recently, a 70% metabolic depression under conditions of anoxia was demonstrated in European eel, by direct calorimetry [4]. The correlation coefficient of 0.97 observed in this study between the level of metabolic depression and decrease of fHR suggests a direct relationship between metabolic depression and the fHR. In line with this observation, for the intrinsic pacemaker rate, regulating the heart frequency in the eel, a similar Q_{10} of around 4.0–4.1 can be expected. This expectation is not confirmed by literature data. For most fish species the Q_{10} for intrinsic heart rate is 2.0 or more, e.g. for acclimated eel, a Q_{10} of 2.1 at 15–25 °C is reported [32]. Also from an in vitro study with, isolated goldfish hearts a Q_{10} in the range of 2.0–2.3 was reported [33]. Therefore, we can conclude that the situation is more complicated and that the state of hypometabolism (hibernation) not only relies on temperature induced adaptations in enzyme-kinetics but that regulation via the CNS may be involved probably via the fHR. It has been suggested that eels can down-regulate the resting cardiac output during hypoxia exposure by involvement of higher cerebral centers [28]. This statement is based on the observation that the eel myocardium, in vitro has a high hypoxia tolerance but the cardiac output is not maintained in vivo at water $p(O_2)$ below 40 Torr (5333 kPa) [34]. So, we hypothesize that in certain fish species adapted to hypoxic conditions (like the goldfish used in this study) via a CNS regulation of the fHR, in principle may regulate their metabolic rate and may reach a state of hypometabolism, metabolic depression.

In conclusion, we observed in this study with 'state-of-art' techniques like radio-telemetry and direct calorimetry that there is a close coupling of heart rate with metabolic depression in goldfish. A correlation coefficient of 0.97 was calculated between the level of metabolic depression and decrease of fHR. These results support the hypothesis that blood flow reduction is the proximate cause for the observed metabolic depression.

4.1. Perspectives

In future studies we hope to perform a similar calorimetric/telemetric experiment with transmitter for measuring blood pressure (BP), (TA11PA-C20, Data Sciences International, St. Paul, MN, USA). Those transmitters have the advantage that HR as well as stroke volume (derived from blood pressure) can be recorded which is a prerequisite for determining cardiac output. In pilot studies performed at our laboratories, we demonstrated that the BP transmitters can be implanted in fish of 100–120 g resulting in a good registration of HR and BP (unpublished results).

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