INDIRECT CALORIMETRY METHOD TO STUDY ENERGY METABOLISM OF AN AIR-BREATHING FISH, *HOPLOSTERNUM LITTORALE* (SILURIFORM, CALLICHTHYIDAE)

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ABSTRACT

The aim of the present work was to implement indirect calorimetry method to study energy metabolism of an air-breathing neotropical fish, *Hoplosternum littorale*. A closed respirometer was designed to measure the gas exchanges of this air-breathing fish in both aerial and aquatic media. The accuracy and the validity of the method were tested. The energy expenditure of fish fasted for 10 days and the involvement of energy-yielding body stores were calculated from the measurements of O_2 uptake, CO_2 output, and the nitrogen excretion of fasted Atipas. The average energy expenditure was 1.58 kJ/kg/hr and endogenous lipids were the main energy purveyor.

Atipa (*Hoplosternum littorale* Hancock, 1828) is an air-breathing siluriform fish of the Callichthyidae family living in the marshy areas of northern South America. To date, knowledge of this species concerned mainly its respiratory system (Carter and Beadle, 1931; Gee and Graham, 1978) and its behaviour (Winemiller, 1987; Boujard *et al.*, 1990). No data on its nutrition physiology are available. The present work is the first step to obtain basic knowledge in this area.

Energy requirements and the mode of substrates utilization to meet such needs take on a special dimension with tropical species, which are assumed to make better use of available energy than cold-water fish (Luquet and Moreau, 1989).

Indirect calorimetry allows qualitative and quantitative evaluation of the energy substrates used by fish (Gnaiger, 1983; Van Waversveld *et al.*, 1988). In this method, the calculation of heat production is based on respiratory gas exchanges which reflect cellular oxidation of energy-supplying substrates and on nitrogen excretion arising from catabolism of nitrogen compounds. One of the first trials in this line was made by Kutty (1972) to study metabolic rate associated to swimming activity in *Oreochromis mossambicus*. The attraction of this method in evaluating the standard metabolic rate of fish has recently been pointed

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out by Brafield (1985), and compared to direct calorimetry by Van Waversveld *et al.* (1989). Indirect calorimetry was also used in the context of a study on the nutrition of the trout and the siberian sturgeon (Medale and Kaushik, 1991). But to our knowledge such measurements have never been taken on an air-breathing fish although a respirometer for *Clarias lazera* had been described by Hogendoorn *et al.* (1981). The aim of this study was to implement a simple system to carry out indirect calorimetric measurements with this warm water bi-modal breathing fish.

MATERIALS AND METHODS

Respirometer

Atipa being a bi-modal breather, the respirometer had to deal with both aquatic and aerial media. The closed respirometer designed with this aim is shown in Fig. 1. The inner chamber (1) was a 0.21 m diameter glass barrel containing 10 l water and 1.5 l gas. Gas pressure

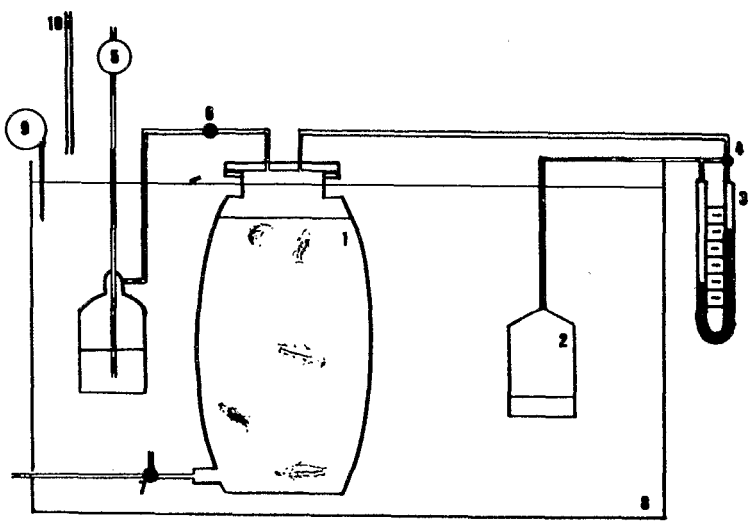

Fig. 1. Diagram of the closed respirometer system. 1—chamber, 2—reference bottle, 3—manometer, 4—shunt manometer valve, 5—air pump, 6—air inlet valve, 7—filling/sampling valve, 8—water bath, 9—temperature controller, 10—laboratory circuit water inlet.

variations were measured by means of a water U manometer (3) against a reference bottle (2) containing air and distilled water. According to Carter and Beadle (1931), oxygen uptake of this fish occurs in both media, while CO_2 and nitrogen wastes are mainly excreted in water. In order to confirm this assumption, the CO_2 variations in the air above the fishes were evaluated during preliminary trials with an IR gas analyser (Licor 6200). The values of CO_2 excretion in air were less than 1% of the total CO_2 excretion of the fish. Therefore,

analyses of air were limited to oxygen measurements. The oxygen depletion in air was estimated by manometric method. The chamber and the reference bottle were both immersed in a water bath (8) in order to control the temperature homogeneity of the system (9).

Prior to each trial, the external water bath (8) was filled with water from the laboratory recirculated water system (10) and allowed to stand overnight. At the beginning of the experiment, the inner chamber was completely filled from the external water bath by means of the three-way valve at the bottom of the chamber (7). Fish were introduced through the large aperture at the top of the chamber. The chamber was closed with a Plexiglas airtight lid. An initial water sample was taken by aspiration through the bottom outlet, the air inlet valve (6) open and the manometer disconnected. The water level in the chamber was adjusted to 10 l and all valves closed before connecting the manometer. Two hours later, the air depression in the chamber was read on the manometer and the bath temperature was recorded. The manometer was disconnected and the chamber carefully emptied by air injection (5). Emptying time only took a few minutes, which avoided any fish disturbance. The final water sample was collected during emptying time. Furthermore, to avoid excessive fish disturbance, the water level of the chamber was not allowed to fall under 5 cm. Then the chamber was refilled for the next two-hour step of measurement.

Analyses

The precision of the manometer used for air pressure measurement was of 1 mm H_2O (0.073 Torr). The manometric readings were converted into oxygen quantity equivalents using capacitance tables given by Dejours (1981). The dissolved oxygen level was measured with a Clark type electrode (Orion 9708) standardized on air according to the manufacturer's recommendations. A CO_2 ion-selective probe (Orion 9502) was used for the determination of the CO_2 concentration in water. The two electrodes were each connected to an Orion SA720 pH/Ionometer which displayed results in mg/l after suitable conversions of the signal given by the electrode. The ammonia concentration in water was analysed by colorimetric method (Golterman *et al.*, 1978). Using the accuracy specified by the manufacturers for probes, manometer, and anlytical method, the expected reproducibility of all measurements was calculated for a temperature of 28°C (Table 1). Even though the measurements were

Table 1. The accuracy of measurement given by apparatus or analytical methods for concentration mg/l or µmol/l (a, b) and uptake (or excretion) of 100 g experimental fish biomass in µmol/kg/h during the two hours of measurement (c)

	mg/l (a)	µmol/l (b)	µmol/kg/h (c)
Air			
O_2	1.25	3.90	30
Water			
O_2	0.05	1.56	185
CO_2	0.27	6.14	610
$N\text{-}NH_4$	0.035	0.25	25

taken over two-hour periods with 25 g fish, the results are nevertheless expressed in µmol/kg/hr to allow a more convenient comparison with other sets of data. This must be kept in mind particularly when comparisons are made with fish of different weights.

Only NH_4 was considered as the end product of nitrogen catabolism. While it is well known that ammonia is the major excreted nitrogen waste of teleosts (Vellas, 1981), the amount of excreted urea could be significant (Kaushik, 1980). In order to verify the importance of urea as an excretory product of Atipa, the urea content of final water samples was estimated according to the method described by Aminot and Kerouel (1982). The urea-N concentration was below the sensitivity threshold of the analysis, which means <1 µmol N/l.

Experimental test

The tests were carried out with Atipas produced in our experimental fish farm (Kourou, French Guiana). Fish were fasted 15 days before the experiment. The experiment was performed with a batch of 5 fishes of 25.6 g mean weight (range: 22.0 to 28.4).

Trials were performed over 48 hours. The experimental room was submitted to the natural photoperiod. A red light of low intensity was used for night work. This red light was not switched off during the day.

Energy expenditure and body stores involves were calculated from oxygen uptake, CO_2, and ammonia excretions using formulae given by Van Waversveld *et al.* (1988).

RESULTS

As can be seen in Fig. 2, the oxygen uptakes from air and from water did not show any specific diurnal rhythm. In this experiment, the amount of oxygen derived from air represented 36.6% (range: 23.3 to 46.3%) of the total oxygen uptake. Calculated from the

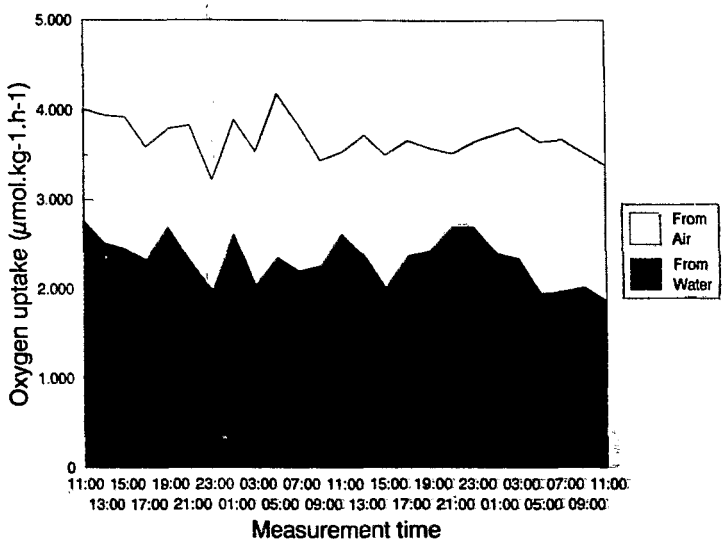

Fig. 2. Total oxygen uptake by fasted Atipa, *Hoplosternum littorale*, with the share between atmospheric and dissolved oxygen

Table 2. Gas exchanges and nitrogen excretion of fasted Atipa at 28°C. Values averaged over 48 hours, in µmol/kg/h

	Average	SD
O_2 uptake:		
Air	1351	48.8
Water	2336	51.2
Total	3687	
CO_2 output	2836	216
N-NH_4 excretion	351	7.67

data recorded over the 48 hours, the average total oxygen uptake was 3687 µmol/l/hr (Table 2). As was the case for oxygen, no rhythm could be noticed for CO_2 output and NH_4 excretion. From the data on respiratory exchanges and ammonia excretion over the trials, a respiratory quotient of 0.77 and an ammonia quotient of 0.095 were calculated.

According to these results the mean energy expenditure was 1.58 kJ/kg/hr. The quantities of oxidized proteins and lipids were similar (Table 3). Reduced to energy equivalent values, 32% of these oxidized compounds were endogenous proteins and 68% were body lipids.

Table 3. Energy metabolism of fasted Atipa at 28°C

Respiratory quotient	0.77
Ammonia quotient	0.095
Energy expenditure	1.58 kJ/kg/h
Quantity of oxidized body reserves	
Proteins	30.7 mg/kg/h
Lipids	30.1 mg/kg/h
Carbohydrates	–10.9 mg/kg/h

DISCUSSION

The designed respirometer enabled measurement of the gas exchanges and the nitrogen excretion in both aquatic and aerial media. The aim of the study being the evaluation of the *total* oxygen uptake, the possible O_2 equilibration between the air and the water compartments (which could influence the share of the partition between water and air oxygen uptake) was not to be taken into account. The use of the present manometer led to a measurement accuracy of aerial O_2 uptake similar to that of dissolved oxygen consumption measured by the oxygen probe. But it required the use of a closed system. Consequently, a particular attention should be paid to avoid adverse values of O_2, CO_2, or nitrogen wastes. As recommended by Van Waversveld *et al.* (1989), the measurements had to be performed with a group of fish and not with a single fish. In this way, the two-hour step was chosen as not too long but still allowing an acceptable precision for all measured parameters. During this experiment the oxygen level in the water did not fall under 3.15 mg/l (62 Torr or 41% of the saturation at 28°C) and NH_4 concentration was never higher than 0.2 mg/l at the end of any two-hour trial. *Hoplosternum littorale* being an air breather, no attention has been given to

the dissolved oxygen level under which *H. littorale* would become an oxygen conformer. Some available data are related to dissolved oxygen level under which facultative air-breathing species such as *Hypostomus plecostomus* or *Ancistrus chagresi* begin to take oxygen from the air. For both species this threshold is under 60 Torr (Graham and Baird, 1982). In fact, no increase in fish activity or abnormal behaviour (manifesting stress due to a dissolved oxygen deficiency) was registered at the end of each trial.

As the fish were fasted and no rhythm was noticed, each set of 25 values for gas exchanges and nitrogen excretion was averaged (Table 2). In fact, for indirect calorimetric procedure, one needs to know the quantity of the exchange as a whole. These averages can be used in the description of the general nutritional status of fish. The reliability of CO_2 output measurement must be borne in mind as its value strongly influences the results, i.e. the calculation of the distribution of non-protein energy sources.

In the present experiment, the negative oxidation value for carbohydrates could be interpreted as neoglucogenesis. Thus, further experiments on enzyme activity involved and direct estimation using labelled compounds (amino acids or fatty acids) might be useful to check the possible neosynthesis of carbohydrates and such nutrients' interconversion.

The measurements of gas exchanges and nitrogen excretion were carried out over a 48-hour period for two reasons: (1) Van Waversveld *et al.* (1989) recommended taking measurements during darkness for a better approximation of standard metabolism. Moreover, Boujard *et al.* (1990) reported that locomotor activity and air breathing of starved Atipa were increased in the light phase. (2) If the fishes were disturbed by handling, a long period of measurement allows for possible effects of handling stress.

In fact, in the present experiment, we did not notice any difference in parameters either between the dark and light phases or between the beginning and the end of the 48-hour measurement period. Furthermore, the standard metabolism of Atipa at a temperature of 28°C was similar to those of *Tilapia rendalli* (Caulton, 1978) at 23°C, of *Oreochromis mossambica* (Sukumaran, 1986), and of *Rhinomugil corsula* (Peer-Mohammed and Kutty, 1986) at 30°C. The standard metabolic rate of these fishes was respectively 1.88, 1.37, and 1.89 kJ/kg/hr.

When compared to data on endogenous protein and energy losses from body composition analyses data, the results obtained by the indirect calorimetry method were similar for fish starved from 10 to 50 days (Luquet *et al.*). The indirect calorimetry method turned out to be effective with a bi-modal breathing fish.

Trials are under way to study energy metabolism associated with the feeding of Atipa.

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