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A portable unit for direct calorimetry of small aquatic animals ☆

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Abstract

A portable direct calorimeter for aquatic animals is described. The unit has been designed to be operated in the laboratory as well as in the field. All parts needed for construction are readily available and inexpensive (about US $1600). The unit delivered reproducible results from the first experiments with freshwater fish. The sensitivity of the calorimeter is 130 μV mW^{-1} or 1.4 μA mW^{-1}, the time-constant being about 20 min.

Keywords: Aquatic animal; Calorimetry; Direct calorimeter; Fish

1. Introduction

Direct calorimetry of fish and other aquatic animals is still a rather difficult task, which is unavoidable if the total energy metabolism has to be determined [1]. Indirect calorimetry, by oximetry, is a more easy method from the experimental point-of-view. But several species of amphibia and fish are able to switch their metabolism partially or totally to anaerobic conditions, depending on the availability of oxygen [2,3]. Oximetry naturally fails to detect the anaerobic part of the metabolic heat production. In direct calorimetry the high heat capacity of the water (unavoidable as this is the environment for aquatic animals) causes the system to have a high time constant, which extends the measuring periods. Unfortunately, many aquatic

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species have a very limited tolerance for low oxygen tension. These two principally contradicting facts have to be taken into consideration in the design of a direct calorimeter for fish.

The intention was to construct a portable unit which delivers reliable results while maintaining an affordable price. An additional goal to be accomplished in the design was to enable measurements in the field even in tropical regions.

One cannot overcome the heat capacity or avoid the use of water as the measuring medium. To solve this problem, three approaches remain: (1) reducing the volume of the water in the measuring chamber (however, the lower the volume the faster the critical oxygen tension for the animal is reached); (2) keeping the outer environment (reference) as stable as possible; (3) increasing the sensitivity and response of the heat sensors. Implication of point (1) has only very limited application. So one has to concentrate on points (2) and (3), which can be handled technically.

2. Experimental

2.1. Equipment

The complete unit (Figs. 1 and 2) consists of five main components. (1) A commercially available "Thermo-box" (internal volume 18 l). Such a box is commonly known as a car-accessory "camping cooler"; any brand that does not

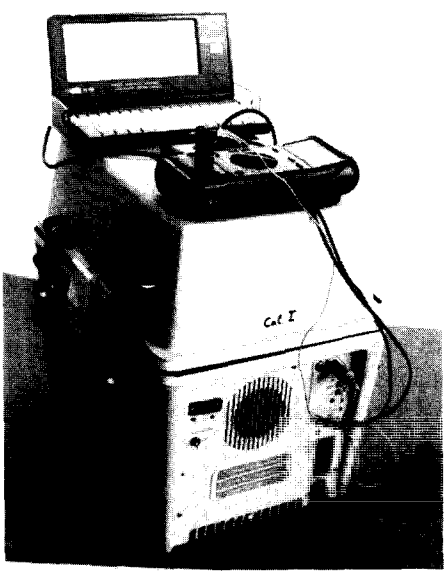

Fig. 1. Photograph of the closed direct calorimeter unit ready for operation.

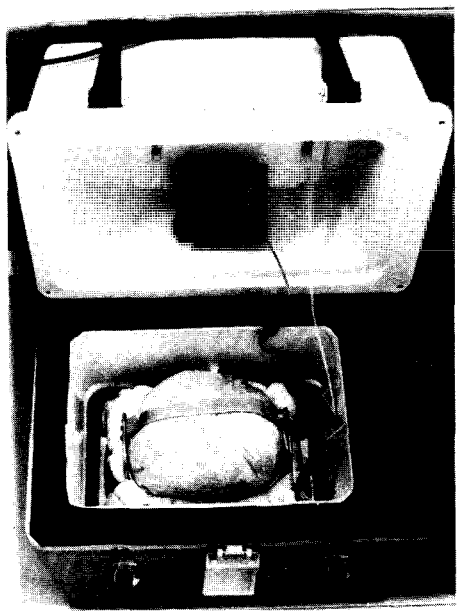

Fig. 2. Photograph of the outer cooling-box with open cover and the closed calorimeter unit inserted.

have a cooling unit integrated into the top cover can be used; (2) a temperature control unit (stability $\pm 0.2^{\circ}C$); (3) the 'real' calorimeter: a measuring chamber (internal volume ($105 \times 155 \times 75$) $mm^3 \approx 1$ l, using 550–650 ml H_2O, depending on fish size); (4) a digital data recording facility (Multimeter METEX M-4650-CR with an integrated RS-232 port (Conrad Elektronik, Germany) or similar connected to a small DOS-compatible, portable computer ("Pocket-PC") with appropriate

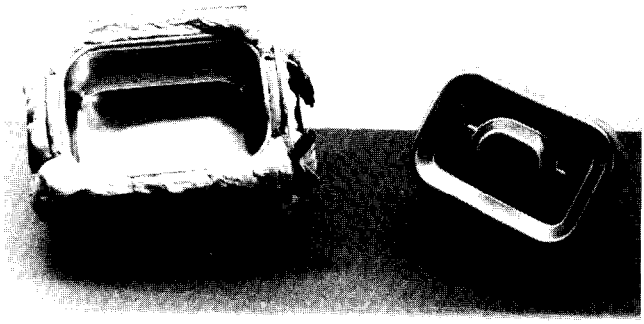

Fig. 3. The open calorimeter unit with the temperature-insulated top-cover (as described in the text).

data acquisition software) or a Yt-line recorder. The calorimeter unit (3) (Fig. 3) is constructed from a stainless steel container, with 6 Peltier elements, size (40 × 40) mm^2, attached to the sidewalls and the bottom of the container. The opposite side of the Peltier elements is attached to large passive heat sinks, as commonly used for cooling purposes in power transistors. All other openings of the container surface to the outside are heat insulated with polyurethane foam. The consequence of this construction is that any heat transport between the inner measuring chamber and the outer environment has to go through the Peltier elements.

3. Results

Due to the size of the measuring chamber the size of the animals that can be measured is restricted to a maximum of 13 cm in length. Due to the sensitivity of the calorimeter a fish should not be smaller than 5 cm or lighter than 5 g. One experiment takes 2–5 h.

The Peltier elements were used as-received without further testing. They can either be connected in series or in parallel. We chose a parallel connection to average the output signal of all Peltier elements. Alternatively, both thermo-voltage and thermo-current were monitored. The detection of thermo-current seems preferable in environments with high electromagnetic noise. In thermo-current mode, fewer interferences were received due to the low input impedance of the signal recording unit compared to the high input impedance needed for the necessary sensitivity in thermo-voltage mode. In both detection modes the sensitivity remains the same. The reaction time of the system is slightly faster in the current mode. There are not yet sufficient data to compare the linearity of the signal between the two modes. For semi-conductor photo-detectors it is established that the photo-current gives the better linearity.

The calorimetric unit is still under development and testing under laboratory conditions. For that reason all data presented here should be treated as preliminary. Improvements are still in progress. The following data are based on internal medium "water"/external medium "air". The unit should be switched on 1 day before use: minimal stabilization period before starting experiments, 6 h; re-equilibration period after sample insertion 30 min; time constant, 20–25 min; sensitivity, 130 $\mu V\ mW^{-1}$ or 1.4 $\mu A\ mW^{-1}$. (Average heat production in fish ≈ 1 mW g^{-1}; in shrimp (post-larval) ≈ 2 mW g^{-1}.)

The first measurements with fish conducted in parallel using indirect calorimetry/oximetry (equipment described in Ref. [4]) and the direct calorimeter, produced a recorded heat output from direct calorimetry that was lower than that from indirect calorimetry. A reasonable explanation for the missing/unrecorded energy can be provided. The calorimeter chamber is filled with water to about 2/3 of the total internal volume. The remaining 1/3 is air. During acclimatization of the chamber, water will evaporate and the air in the chamber will become water-saturated. To insert a fish, the chamber has to be opened and the air will be exchanged almost completely with the outside air. Usually the air inside a lab is extremely dry.

Preliminary calculations assuming a complete exchange of the internal water-saturated air with dry external air and a subsequent re-saturation of the air by evaporation of water inside the chamber, resulted in energy values which would account very well for the energy difference detected in the comparison between direct and indirect calorimetry with the two pieces of equipment. An additional factor contributing to different results for the two methods of calorimetry has not yet been tested. That factor is light. Oximetry is conducted under ambient light; direct calorimetry, however, proceeds in complete darkness. Most organisms reduce their general metabolism to a resting state during total darkness. Illumination of the direct calorimeter chamber through a fiber optic, without imparting or reducing considerable heat energy, is possible but has not yet been implemented. Conducting oximetry in a dark-room could provide an alternative determination of the "dark-/resting metabolism".

4. Discussion

A similar "cool-box calorimeter" has been described by Wesolowski et al. [5]. The main difference to the unit described here is that the whole cool-box as used by Wesolowski et al. was in fact the calorimeter. Instead of using the integrated Peltier element to cool the inner compartment by connecting it to a power source, they just connected a voltmeter to the element and recorded the reverse effect (thermovoltage or Seebeck-effect). A prerequisite of this design is a relatively stable external temperature as reference, which means that the time-constant of temperature changes should be smaller inside the box (measuring chamber) than outside (reference) and the heat capacity of the heat-conducting media inside and outside should be as similar as possible. These pre-conditions are not fulfilled if water has to be used as the medium inside the calorimeter chamber and air outside. For that reason, in our design the cool-box is used in its original function. Equipped with control circuitry, the temperature of the inner compartment of the cool-box is closely regulated to compensate for the low heat capacity of the air/aluminium heat sink combination we use as reference medium.

5. Conclusions

The calorimeter presented offers a portable solution for direct calorimetry on aquatic animals. Early determinations have produced reasonable results. Additions and improvements mainly to the temperature stabilization, the handling of the "missing energy" due to water evaporation inside the measuring chamber and the illumination of the inner chamber will further increase the usability of the unit.

Acknowledgements

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