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Metabolic investigations of aquatic organisms with a new twin heat conduction calorimeter

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Abstract

A twin heat conduction calorimeter is presented that was specially developed as a modular design to allow for easy adaptation to changing experimental conditions. The instrument was built at the Institute of Oceanography, University of Gdansk, Poland for metabolic investigations and locomotor monitoring of aquatic organisms under different environmental conditions (e.g., salinity, temperature, oxygen tension, and pollutants) as well as physiological ones (age, sex, pregnancy, and adaptation to changing external parameters). This calorimeter has flatter and broader 24-ml vessels (height 50 mm and diameter 28 mm) than usual instruments to allow larger aquatic invertebrates to swim around during the experiment. The sensitivity is 99.1 $\mu V\,mW^{-1}$, and the time constant is 217 s with 15 ml water and 146 s without water in the vessels. Construction details are presented in this paper and the first results are given for three aquatic crustaceans of different taxonomic groups: the isopod *Idotea chelipes*, the amphipod *Gammarus tigrinus* and the decapod *Rhithropanopeus harrisii*.

Keywords: Aquatic animals; Crustaceans; Instrumental design; Heat conduction calorimetry; Metabolic rate; Energy flow

1. Introduction

Measurements of metabolic rates are regarded as an important element in studies of energy flow in marine organisms and populations [1]. Among different methods, direct calorimetry is the most valuable, because it allows simultaneous monitoring of both aerobic and anaerobic heat production [2,3]. This is especially important in studies of some aquatic organisms like bivalve molluscs for which respiration is only one component of the total metabolism, even during fully aerobic periods [4]. It is also important for benthic fauna at greater depths, for organisms temporarily exposed to oxygen depletion or even to a complete lack of oxygen [5–7], and for animals inhabiting intertidal areas [8,9]. The first response of such organisms to a stress caused by fluctuations in environmental factors (e.g. oxygen concentration, salinity, and temperature) is manifested in changes of the rate or type of metabolism [10–12]. In such cases indirect calorimetry does not provide reliable information on the total metabolic rate of the organisms. On the other hand, direct calorimetry has its drawbacks also [13]. Heat dissipation measurements of aquatic organisms at lower temperatures may be technically complicated due to strongly reduced metabolic rates and the high heat capacity of water [14,15]. In many studies commercially available calorimeters cannot be applied due to inadequate technical parameters, mainly the vessel size and form.

Against this background, it was interesting to construct a customer friendly simple calorimeter of the Calvet type combining a number of specific advantages in the above mentioned research direction. It should be of (i) smaller total size and lower weight than most instruments on the market so that it can be easily transported to the off-university research stations at the seaside and to be placed there in slightly modified commercial refrigerators if cool-chambers are lacking. (It has to be kept in mind that the calorimeter surrounding has always to be a few degrees lower than the calorimeter to avoid condensation within the instrument, a danger especially high with aquatic samples at low temperatures.) (ii) Because preliminary experimental results with the calorimeter may alter its construction details a modular form was intended which could be easily changed and adapted to new conditions and tasks. (iii) Typical "Calvet vessels" of all

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volumes are slim and high and thus restrict the movement of aquatic animals. Flatter and broader vessels are favourable for free locomotion. (iv) Under most of the envisaged experimental conditions heat production rates of 0.1–1.0 mW can be expected corresponding to 6–60 μV at a medium calorimeter sensitivity of 60 μV mW^{-1}, so that the extreme baseline stabilities of commercial instruments are not necessary. (v) The intended temperature range of the calorimeter corresponds approximately to that of the biotope: 0–50 °C are sufficient for all investigations.

A biological object is a highly complex system with a manifold of reactions running in parallel; biological calorimeters are "black boxes" with difficulties to analyze the global calorimetric signal for special effects of interest. Electrical sensors for oxygen, pH or special ions may help in liquid biochemical systems; an optical control would be advantageous with animals. For this end, combinations of calorimeters with optical devices like borescopes (rigid endoscopes) were applied, sometimes coupled to a long-term video registration of the optical signal [16–19]. With the progress of electronic miniaturization and sensitivity, bullet CCD (charge coupled device) cameras became available that are small enough to be incorporated into the calorimeter. They were and will be tested with the new instrument.

The realization of the above mentioned wishes, a lower price and an integration of graduate students and younger scientists into this project were the main reasons to construct this twin calorimeter of the Calvet type at the Institute of Oceanography, University of Gdansk, Poland. Technical details of the new instrument as well as some first results are presented in this paper.

2. Experimental

2.1. Calorimeter

The calorimeter consists of an aluminium block of 150 mm diameter, 200 mm height and a mass of 9000 g, housing 2 vessels (Fig. 1). The block is covered by a Polyamide 6 insulation and surrounded by a heater (200 W). The whole unit is covered by an additional insulating layer (20 mm) and enclosed in an aluminium shield. Four Peltier elements of 30 mm × 30 mm × 4.7 mm (Pt100 P72 A20; Dreisen-Kern GmbH, Germany), connected in series, are placed around each vessel. The new calorimeter proper is of rather small size. Its height and diameter without insulation are 370 and 230 mm, respectively. Two identical vessels (Fig. 2), for the sample and the reference, are made from titanium steel alloy with an active volume of 24 ml (external dimensions: diameter 28 mm and height 50 mm). The Teflon stoppers of the vessels are connected to Plexiglas rods operating the vessels. Because the vessels are filled with 15 ml water for the experiments, a headspace of 9 ml air remains with enough oxygen for long-term experiments. Moreover, there are two steel pipes with an internal diameter of 1.2 mm inserted into the vessel plugs which allow maintaining air exchange. They can be also used to introduce a needle oxygen sensor or for the flow-through system. The oxygen consumption rates of the aquatic organisms of interest are small, so that the low diffusion rate of oxygen into the water is without significance. Moreover, moving activities of the animals will provide a stirring effect that enhances the gas exchange. Control

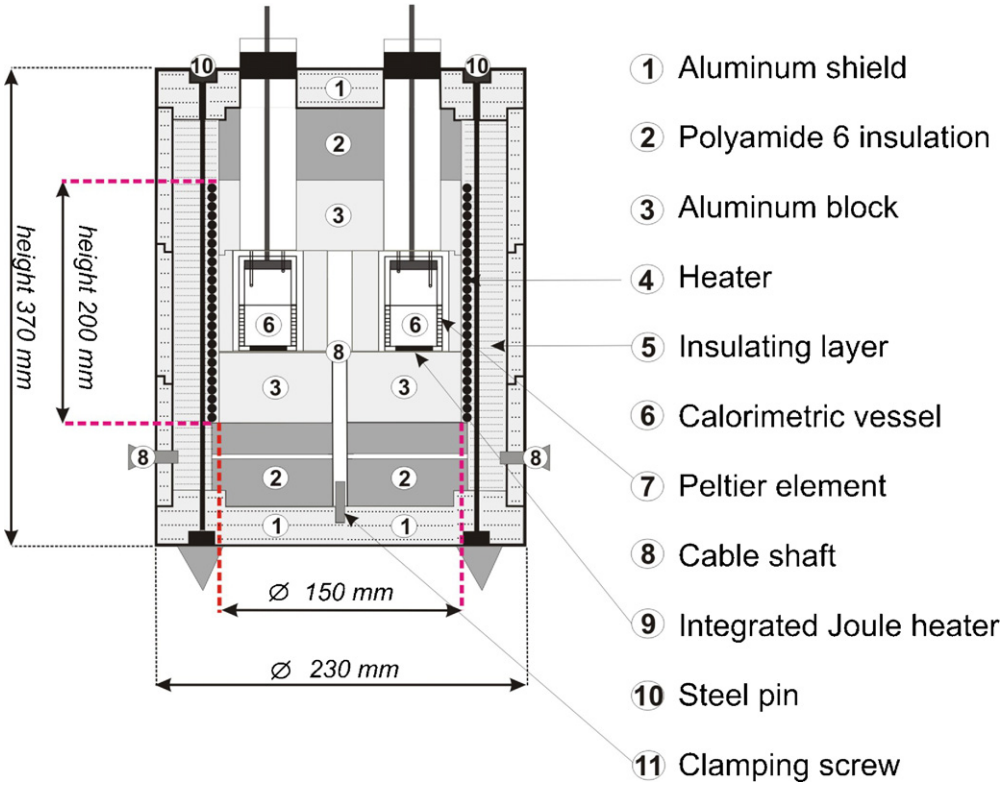

Fig. 1. Principal sketch of the calorimetric setup.

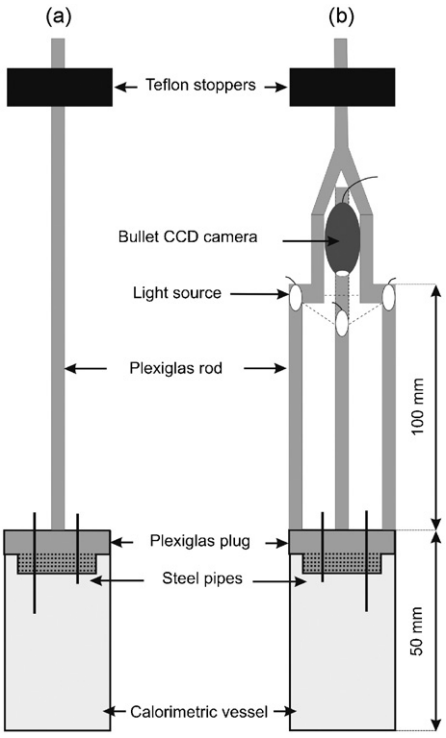

Fig. 2. Schematic design of the calorimetric vessel for heat dissipation measurements without (a) and with (b) the system for optical observations.

measurement of the oxygen tension in the water at the end of an experiment showed only small reductions. All the calorimetric experiments were performed at 15 °C.

The calorimetric signal is monitored after a 1000× amplification with a resolution of 0.01 mV by a data logger (LD-20; CIE, Taiwan) and registered on-line by a commercial PC (Amilo A7640; Fujitsu-Siemens, Poland). The temperature inside the calorimeter is regulated by an external temperature controller (LC6; Julabo, Germany) with an accuracy of ±0.01 °C.

2.2. Optical observation

Two bullet CCD cameras (B-Cam10; CONRAD, Germany) with an input voltage of 9–12 V dc, operational current of 18 mA, focal length $f = 4.9$ mm, and diaphragm of $F = 2.8$ are positioned on top of the lids outside the vessels and the heat measuring area and allow for the observation of animal activity during the measurement (Fig. 2b). Both vessels are illuminated in a twin setup by means of Plexiglas rods as light guides, two times 3 cold light sources of a diameter of 3 mm (LED, L-934MWC; Kingbright, Germany) and through an additional water filter (water layer 4 cm, not shown). Fig. 3 represents photos taken from the video monitoring.

2.3. Biological material

Three crustaceans of different taxonomic groups living in the Gulf of Gdansk entered these investigations. The isopod *Idotea chelipes* (maximal length and mass around 16 mm and 0.05 g) is a widely distributed, boreal species which occurs in different types of aquatic biotopes ranging from the brackish waters of estuaries to marine waters [20,21]. It is especially abundant at the underwater meadows of the shallow, coastal areas. The amphipod *Gammarus tigrinus* (maximal length and mass around 14 mm and 0.02 g) and the decapod *Rhithropanopeus harrisii* (maximal carapace width up to 22 mm, mass up to 4 g) are further invaders of the Baltic Sea from North America [22,23]. The latter species exhibit high tolerance towards environmental factors. All three species are widely distributed in the Polish brackish waters [20–23]. They were collected in their habitat and transferred to the laboratory, where they were held for a week before measurements ($T = 15$ °C and salinity $S = 7$‰). Animals were fed once a week with macroalgae of the genera Enteromorpha and Cladophora (*I. chelipes*) or with fish flesh (*G. tigrinus* and *R. harrisii*).

3. Results and discussion

3.1. Baseline, calibration and time constant

The signal noise of the new instrument is still rather high and shall be reduced in the near future. Fig. 4 shows two 5-h experiments with 15 ml water in the vessels. Slope *a* with a mean of 2.95 ± 0.22 μV is in the dark, slope *b* (4.32 ± 0.16 μV) with illumination of both vessels. These scatters correspond to 2.2 and 1.6 μW, respectively. Although the signals are not zero they are perfectly constant and can be used as baselines for the experiments. It becomes obvious from Fig. 6 that the scatter is not visible in the power–time curves of the crustaceans.

Calibration experiments with water, but without animals showed a perfectly constant baseline which could be taken as reference for the real investigations. The calibration of the new

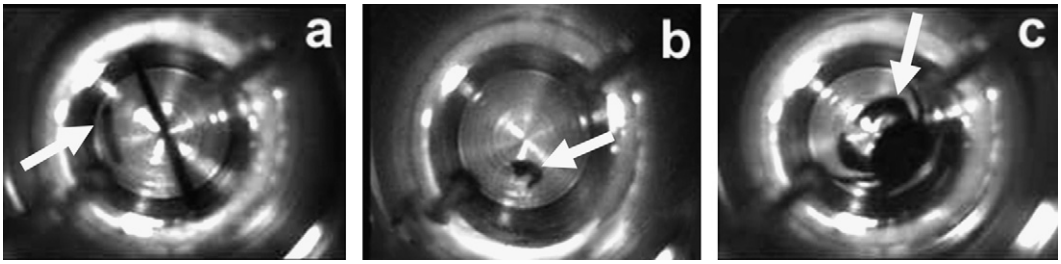

Fig. 3. Photos of the studied specimens inside the calorimetric vessel taken with the CCD camera (a) *Idotea chelipes*, (b) *Gammarus tigrinus* and (c) *Rhithropanopeus harrisii*. Arrows indicate the animal position. For an easier identification see the inserts in Fig. 6. The light grey structures at 2 o'clock and 8 o'clock in all photos are the shadows of the steel rods (Fig. 2), the black diagonal in photo (a) the artificial substratum.

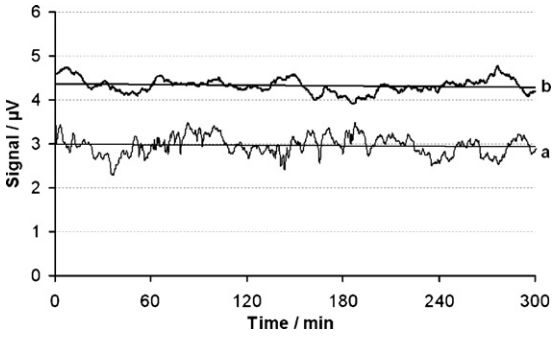

Fig. 4. Signal noise of the new instrument in the twin setup: (a) under dark conditions and (b) under light conditions with the active bullet CCD camera. Vessels were filled with 15 ml of water.

instrument was done by means of integrated electrical heaters (1000 Ω) placed under the vessels. Different power (mW) was supplied to the vessel and the signal (μV) recorded (Fig. 5). The average sensitivity ($n = 5$) under all experimental conditions amounts to $99.1 \pm 2.1\ \mu V\ mW^{-1}$. The time constant of the calorimeter with empty (dry) vessels amounts to 146 s and to 217 s with 15 ml of water.

3.2. Metabolic rates of aquatic animals

Crustaceans of the genus *Gammarus* and *Idotea* were intensively investigated in recent years, including direct calorimetric experiments [12,24–26]. Here, individual heat production rates of 3 specimens of *I. chelipes* (wet weights (ww) 23.9, 34.0 and 35.0 mg), 3 specimens of *G. tigrinus* (6.4, 6.5 and 8.2 mg ww) and of 4 specimens of *R. harrisii* (0.307, 0.384, 0.692 and 1.005 g ww) were determined at 15 °C and a salinity of 7‰. In a subsequent experiment, two *R. harrisii* males (0.307 and 0.384 g ww) were acclimated in a step-wise manner (2‰ per day) to a salinity of 23‰ and their heat production rates measured again. Only in the case of *G. tigrinus* all heat measurements mentioned above were performed under CCD control. For *I. chelipes* and *R. harrisii* additional measurements with CCD cameras were performed: two *I. chelipes* males (10.7 and 13.2 mg ww) and one *R. harrisii* male (0.46 g ww) were used at $T = 15$ °C, $S = 11$‰ and $T = 10$ °C, $S = 7$‰, respectively. Moreover, the calorimetric measurements on *I. chelipes* were conducted with a piece of artificial sea grass to reduce the stress and render more natural conditions. All experiments were performed on single males placed in a vessel containing 15 ml of filtered (0.45 μm) sea water leaving a headspace of 9 ml air above them.

The obtained power–time curves differed according to the basic metabolism and the activity of the studied animals (Fig. 6) which exhibited diverse types of behavior similar to those observed in aquaria. Despite of the presence of artificial substrata *I. chelipes* (Fig. 3a) did not reduce its activity. Animals spent most of their time moving on the artificial grass (Fig. 6a, peak A; Fig. 6b) or directly on the vessel bottom (Fig. 6a, peak B). Sometimes *I. chelipes* swam in the vessel on its dorsal side (Fig. 6a, peak C). Small peaks in Fig. 6b correspond to antennae movements. The highest ratio between active and resting metabolic rate recorded in this species was 3.2:1, a value lower by 27% than that observed calorimetrically by Lapucki et al. for the same species [25]. This could be caused by differing vessel

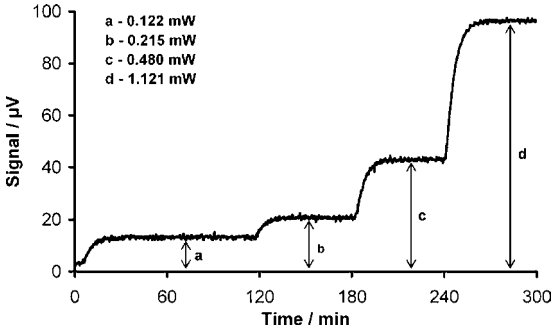

Fig. 5. Calibration curve obtained with the incorporated electrical heaters. Vessels were filled with 15 ml of water.

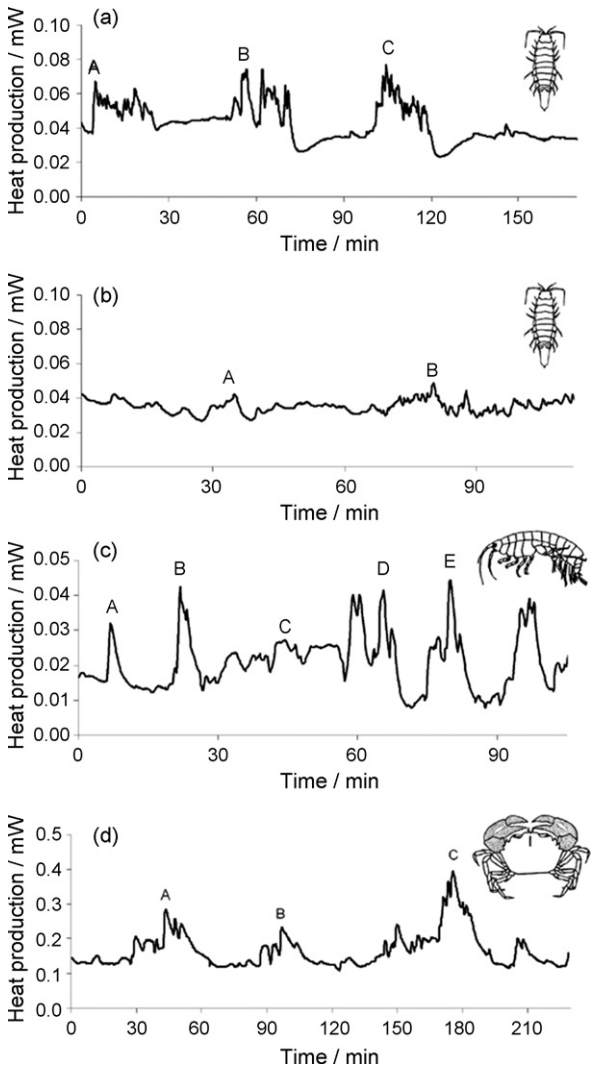

Fig. 6. Power-time curves of single specimens of (a and b) the isopod *I. chelipes* (10.7 and 13.2 mg, $T = 15$ °C, $S = 11$‰), (c) the amphipod *G. tigrinus* (6.4 mg, $T = 15$ °C, $S = 7$‰) and (d) the decapod *R. harrisii* (0.46 g, $T = 10$ °C, $S = 7$‰). Peaks indicated by different letters refer to various types of activity described in the text.

sizes and shapes as well as by smaller masses of the individuals studied by those authors.

G. tigrinus (Fig. 3b) showed two major types of activity during the measurements. Animals were sitting on the bottom of the vessel contracting their bodies and forming an O-shape (Fig. 6c, peak A). The cost of this activity was 62% of the total heat dissipated. Amphipods were also swimming inside the vessel. Both, vertical (Fig. 6c, peaks B, D, E) and horizontal movements (Fig. 6c, peak C) with different speeds were noticed. *G. tigrinus* expended 54% of the active metabolic rate for slow swimming around in the vessel (Fig. 6c, peak C), whereas the total ratio of active to resting metabolic rate of the fast swimming specimen (Fig. 6c, peak D) amounted to 3.7:1.

R. harrisii represents a walking form of Brachyura which does not swim. Its behavior was therefore different from that observed in *I. chelipes* and *G. tigrinus*. Despite of their big size, crabs had enough space to move inside the calorimetric vessel (Fig. 3c). They could walk (Fig. 6d, peak A) or move the massive claws (Fig. 6d, peak B) increasing the energy expenditure by 55 or 45%, respectively. The highest energy output was observed when crabs were climbing backwards on the vessel wall (Fig. 6d, peak C). In this case the ratio of active to resting metabolic rates was 3.1:1.

According to [27] the ratio between maximum and basal metabolic rates in invertebrates varies in a range between 2 and 10. Thus, the observed values seem to be at the lower end of the scale. But it has to be kept in mind that due to the high heat capacity of the water around the animals the calorimetric signal is considerably damped. First calculations show that a factor of 2 or 3 seems reasonable to find the true calorimetric signal. Comparing the CCD registration of *R. harrisii* with the recorded power–time curve it became clear that the curve peaks occurred less than 4 min after the locomotor activities of the crab in the vessel. This is in good agreement with the time constant of the calorimeter (217 s). Moreover, it seems justified to talk about different peak shapes for different types of movement: smaller regular peaks are linked to breathing activities, higher and sharp peaks to quick movements of the claws. In this way, a calorimetric setup with incorporated bullet CCD cameras may – like microphones, borescopes and motion detectors – shed light into the "black box" of a biological calorimeter and render further and subtle information about the ongoing biological processes. But further intensive investigations and characterizations will be necessary to establish a data bank with correlations between calorimetric signals and underlying locomotor activities.

The highest specific heat production rates (around 0.84–0.95 mW g^{-1}) were observed in *G. tigrinus*. The values were lower for *I. chelipes* (0.64–0.71 mW g^{-1}), and lowest for *R. harrisii* (0.13–0.29 mW g^{-1}). The specific metabolic rate decreased with the mass of the studied species according to a power function (Fig. 7). It is difficult to make any detailed comparison of the obtained metabolic data with those existing in the literature, mostly because of methodological differences between the various approaches (e.g. equipment type, small number of repetitions, temperature, and salinity, mass). For example, the values for *G. tigrinus* from the Baltic Sea are lower than those reported for the same species from the German rivers Werra and Weser, where the average value was 1.31 ± 0.33 mW g^{-1} ww ($n = 16$) [28]. On the other hand *G. tigrinus* is characterized by lower metabolic rates than the other gammarid, *Gammarus oceanicus,* from the Baltic Sea. Specimens of the latter species with a three-fold higher mean weight (24.5 mg) were characterized by a metabolic rate of 2.24 ± 0.57 mW g^{-1} at $T = 10\,^{\circ}$C [24]. The calorimetrically determined metabolic rates of *R. harrisii* are lower than those obtained respirometrically at $T = 24.5\,^{\circ}$C by Diamond et al. [29] who reported 102.8 μl g^{-1} ww h^{-1} (0.57 mW g^{-1} if 1 ml O_2 = 20.08 J) for specimens of 0.433 g. With an assumed Q_{10}-value of 2 and a temperature difference of 8 K this rate reduces to 0.36 mW g^{-1}, nearer to the range observed here, but 64% higher than the value calculated from the slope in Fig. 7.

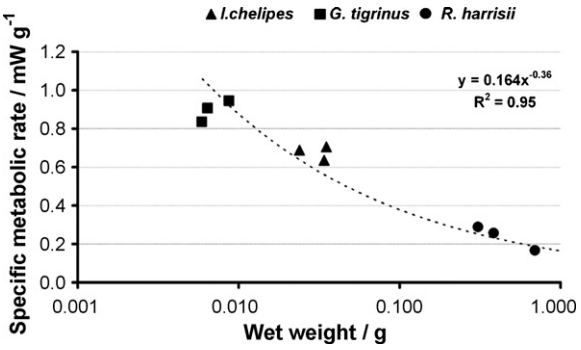

Fig. 7. Specific heat production rates as function of wet weight for *I. chelipes*, *G. tigrinus* and *R. harrisii*, determined in the present investigation.

Some experiments were performed on the influence of salinity on the heat dissipation of *R. harrisii*. Fig. 8 shows a reduction by 55% of the heat output in specimen 1 (0.385 g) and by 67% in specimen 2 (0.308 g) after an acclimation to a higher salinity of 23‰. Such a strong reduction in metabolic rates might be explained by a significant decrease in the difference between the osmotic concentrations of the internal and external medium; this difference becomes zero at 20–22‰ [29–31]. At isoosmotic conditions *R. harrisii* does not need expending energy for the ion transport against a salinity gradient, thus reducing the maintenance energy cost significantly. Thus, *R. harrisii* experiences a similar reduction with increasing salinity as *G. oceanicus* which

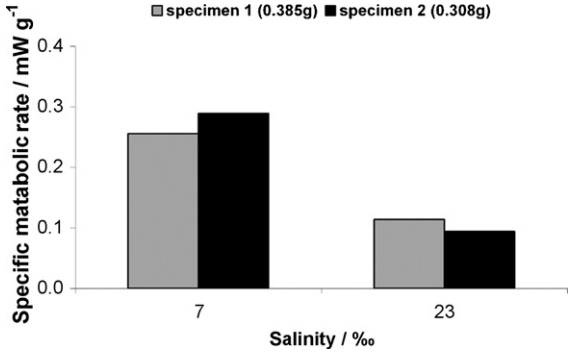

Fig. 8. Specific heat production rates of two specimens of the mud crab *R. harrisii* at a habitat salinity of 7‰ (left) and after step-wise acclimation to 23‰ (right).

lowered its metabolic rate by 53% in the salinity range 7–30‰ [12].

4. Conclusion

Heat production rates of aquatic animals at low temperatures are relatively small. Nevertheless, the chosen organisms with wet weights of some 10th of a gram produce heat flows around 100 μW which are easily monitored without too much care about the temperature stability of the air around the calorimeter. Its size permits placing it in slightly modified commercial household refrigerators (with forced air circulation and a temperature regulation of ±1 °C) when low experimental temperatures are obligatory.

The present results show that the new calorimeter works as expected and offers advantages which will help to improve the future research on aquatic organisms. Its small size allows transporting it to the external research stations of the Institute of Oceanography to make studies "in situ". The flatter and broader vessels facilitate studies of larger invertebrates at a lower stress level, e.g. for the mud crab *R. harrisii*. The sensitivity guarantees the detection of even rather small behavioral and physiological answers to changes of the experimental environment. The not yet tested flow-through system will be the most important feature of the new calorimeter. It opens the possibility to control the medium and to change the parameters like salinity, oxygen tension or pollutant concentrations inside the vessel without interrupting the monitoring. Oxygen and carbon dioxide electrodes enable simultaneous direct and indirect calorimetric studies. The mini CCD cameras allow observing the animal behavior during the measurements and especially during the transition periods of changing ambient parameters.

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