PERIODICUM BIOLOGORUM
VOL. 113, No 4, 453–456, 2011

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original scientific paper

Digital thermography in analysis of temperature changes in *Pelophylax ridibundus* frog

DOMAGOJ ĐIKIĆ[1]
DARKO KOLARIĆ[2]
DUJE LISIČIĆ[1]
VESNA BENKOVIĆ[1]
ANICA HORVAT-KNEŽEVIĆ[1]
KATARINA ŠKOLNIK GADANAC[1]
ZORAN TADIĆ[1]
NADA ORŠOLIĆ[1]

[1]Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia

[2] Ruđer Bošković Institute, Centre for Informatics and Computing, Zagreb, Croatia

Correspondence:
Domagoj Đikić
Department of Animal Physiology, Faculty of Science
University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia
E-mail: magistar_djikic1@yahoo.comr

Key words: thermography, thermal scanning, cold exposure, frog, hibernation, hypometabolism

Received July 13, 2011.

Abstract

Background and Purpose: *Physiological field of metabolism manipulation tries to elucidate how tissues recuperate after ischemic reperfusion changes, how signal molecules coordinate metabolic pathways and what physiological changes are to be expected in induced artificial hypometabolism or suspended animation in biomedicine. Evolutionary developed mechanisms of lowered metabolism (torpor, hibernation and aestivation) followed by arousals to normal metabolic/thermoregulatory states present perfect models for such studies. In the light of the vast current interest in manipulating metabolism, natural behavior and adaptations of frogs, makes them among other organisms, an appropriate standard model animal for such studies. The exact measurements of thermal changes of frog's body temperature correlated with ambient temperature (T_a) changes are essential.*

Materials and Methods: *Male frogs* Pelophylax ridibundus (Eurasian Marsh Frog) *were kept for 30 days at T_a = 8°C (artificial hibernation) and then exposed to T_a = 23°C (artificial arousal). The dynamics of body temperature change over 146 minutes was analyzed with IR camera NEC Thermo tracer TH7102WL and ThermoWEB measuring system.*

Results and Conclusions: *Use of thermography allowed real time thermal measurement of changes in body temperature in frogs in a noninvasive manner. Previous attempts at thermography in hibernating frogs have not been reported, perhaps because of the lack of precision of earlier instruments. New generation cameras have the accuracy and software support to discriminate subtle differences in temperature of different body regions of analyzed frogs and the surrounding environment, as documented in this study.*

INTRODUCTION

Evolutionary developed mechanisms of lowered metabolism in animals (torpor, hibernation, aestivation) followed by arousals to normal metabolic/thermoregulatory states present perfect models for biomedical studies of manipulating metabolism. Certain animals are adapted to oscillations in food abundance, photoperiod and thermal changes in the environment by changes in metabolism and consequently body temperature *(1)*. Experiments on hypometabolic animal models explain different physiological mechanisms by which tissues recuperate after ischemic reperfusion, how signal molecules coordinate metabolic pathways, the status of oxidative stress and antioxidant defense responses. These mechanisms, that developed naturally in various animals, elucidate what physiological changes to expect in induced artificial hypometabolism or suspended animation and as such are valuable tools in explaining similar problems in biomedicine *(2, 3, 4, 5)*. The points of hypometabolism entry and arousal from it are accompanied by major changes in metabolic rate and body temperature. Measurements of both are essential in assembly of data on the status of the animals going through the processes of hypometabolism. Measurements of metabolism are usually performed by measuring the rate of oxygen consumption of the whole organism by the method of indirect calorimetry, usually combined with simultaneous measurement of body temperature recordings conducted by the use of implantable thermo coupling radio transmitters for measuring of core body temperature or by radio transmitting collars for measurement skin (surface) temperatures *(6)*. Exact and noninvasive measurements of temperature are essential in such research.

In the light of the vast current interest in manipulating metabolism, physiological and biochemical pathways and adaptation, frogs are among other organisms, valuable standard model animals for such studies. Studies on thermoregulation in amphibians have lagged behind those on reptiles, perhaps because the overt thermoregulatory behavior is less obvious in amphibians than in reptiles such as lizards. The earliest descriptions of animal thermoregulation abilities used the terms »warm-blooded« and »cold-blooded animals«. Later the terms poikilotherm and homeotherm were applied to animals according to the constancy of their body temperature. Today we use the terms »endothermic« and »ectothermic« animals, referring to the heat sources they use. Ectotherms, such as reptiles, amphibians and in this case frog, depend mostly on external heat sources, while endotherms depend basically on their inner metabolic heat.

Frogs have very limited ability to alter the rates of heat loss and gain physiologically, thus their body temperature follows the temperature of the environment. The earliest thermography systems lacked the precision and accuracy to discriminate between the two *(8)*. In this article we present the preliminary results of thermographic measurement of thermal changes in frogs used as a model animal in a larger study of physiological changes induced by low temperature exposure and upon *(9)*.

The article describe preliminary results of the use of thermography in recording the dynamics of thermal change over 146 minutes in body surface of frogs kept for 30 days at T_a = 8°C (artificial hibernation) and then returned to T_a = 23°C (artificial arousal) and allowed to re warm.

MATERIALS AND METHODS

Experimental animals

Male Eurasian Marsh Frogs, *Pelophylax ridibundus* (Figure 1), mean weight of 35 ± 1.3 g were used in the experiment (N=3). The animals were collected at the end of summer at the commercial fishery pond Crna Mlaka, Croatia. All experimental animals were collected on the same day. During collection of animals at the location, the environmental air temperature (T_aA) was in the range of 20°C and ambient water temperature (T_aW) was between 17–19 degrees. The collection period was selected for the end of summer the fattening period which precedes the preparation for entry into hibernation during autumn temperature drop. Animals were transferred to laboratory, acclimated and gradually (over 2 days) exposed to temperatures of 8°C in artificial hibernaculum. The aim was that the experimental exposure to the cold under laboratory conditions follows natural seasonal rhythm of animals as authentically as possible. Noninvasive catching methods were used and all handling procedures were held to minimum to prevent further stressing of animals. Animals were collected and treated according to the current laws on Animal welfare and nature protection and the study was approved by the Bioethical Committee of the Faculty of Science, University of Zagreb.

Adaptation of frogs to laboratory conditions and experimental procedures

Animals were acclimatized to laboratory conditions over the period of 15 days prior to further experimental procedure. During the time of acclimation to the laboratory conditions, the animals were kept in square terrariums (110 × 50 × 50 cm, n=6/terrarium), water depth 20 cm with 2 floating platforms in each terrarium. Standard 12:12 light-dark regime, with and T_aA = 23 ± 0.1°C and T_aW = 21 ± 0.1°C. During acclimation animals were fed only on the 2nd and 3rd day after arrival with standard commercial fishing earthworms (*Eichenia sp*.) and laboratory raised cockroaches (1 of each per every frog). Further feeding after the 3rd day was omitted to empty gastrointestinal system before entering induced hibernation in further experimental procedures. After 15 day acclimation period, the frogs were exposed to temperature of T_aA = 8°C, gradually over 2 days, placed in the same square terrariums (110 × 50 × 50 cm, n=6/terrarium). Instead of water within the terrariums, frogs were provided with several layers of wet thick cotton fabric. They were left in total darkness under these conditions for 30 days. On the 30th day frogs were taken out of the hibernaculum and exposed to T_aA = 23 ± 0.7°C and allowed to re-warm gradually. Thermal dynamics over 146 min was recorded by ThermoWEB system.

The ThermoWEB system

The Thermo WEB measuring system comprised a thermovision camera NEC Thermo tracer TH7102WL, with the following functional components added: Electromechanical components of the measuring system (Hardware components). The hardware components of the measuring system include a video CCD color camera (640 × 480 pixels) set in an appropriately shaped housing (IP54), with a mechanical adapter for assembly onto the housing of the IR camera and a circuit for choosing and accepting video signals from the camera to the computer (USB2). Operation of the functions of the IR camera is done by using a computer with RS232 interface. The software support for the measuring system comprised: ThermoCAM, software support for control of the IR camera (TH7102WL) and ThermoWORK, software support for measuring temperature and for remote control and data viewing over the WEB. The room in which the thermographic imaging is done has to be air conditioned, and the temperature of the measuring area must not oscillate more than ±1@C. The cage with the frog was protected from air flow. All thermographic imaging were done by the same thermovision camera, in the same room, by the same computer program, and by the same examiner using the ThermoMED program *(10)*. Images were analyzed by marked circle on the various body regions of the frog.

RESULTS

Thermographic measuremenst of thermal changes in a frog exposed to 23°C room temperature after 30 days of 8°C exposure are presented in Figure 2. The period of measurement was 146 minutes. The thermal scale in Figure 1 J is valid for all presented pictures (A-J).

The analysis of surface body temperature was done by marking the specific areas of the body by circles or polygon areas and recording by ThermoWEB system. For each individual animal, the temperature of specific body region (°C) was expressed as mean value of similar body parts (left and right eye, etc.), and the mean temperature change over time (min) of all three animals is presented in Figure 3. Between 76th and 89th minute there was a noticeable slowdown of thermal change in all body regions, and a certain plateau was reached. Various body regions had their individual dynamics of re-warming and there were slight differences in average temperature between them (Figure 3, 4).

DISCUSSION

Earlier attempts at thermography in ectotherms were hampered by the lack of precision in thermovision. One of the possible reasons was probably that the body temperature of ectothermic animals is very close to ambiental temperature. This might explain the limited amount of studies employing thermography as a method of choice for measurements of temperature in amphibians *(8)*, compared to other methods of temperature recording in

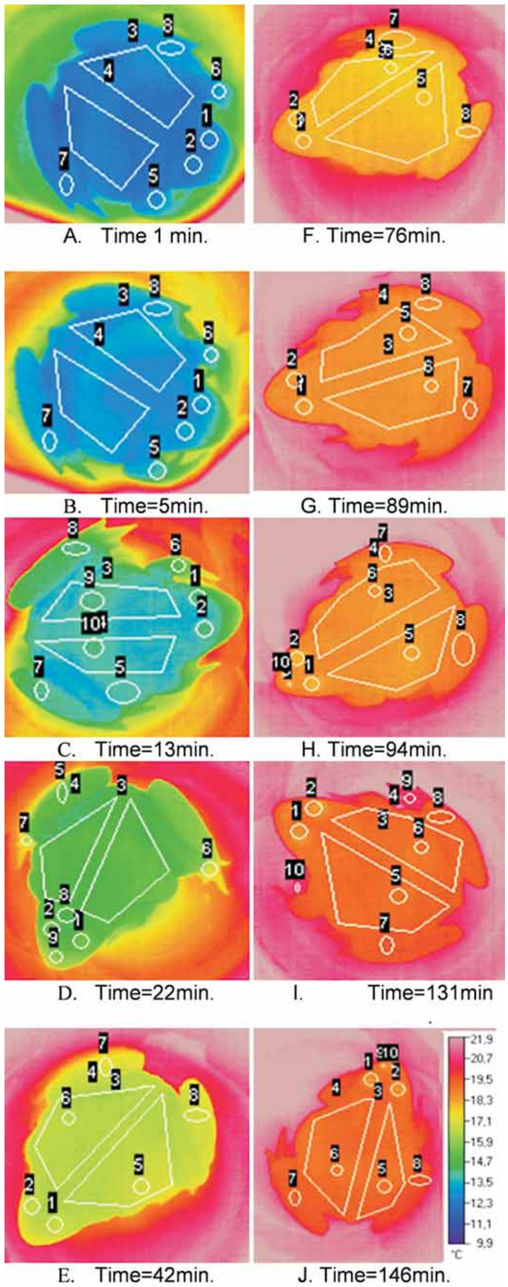

Figure 2 A-J. *Changes of temperature in the frog over a period of 146 minutes after shift from 8°C exposure for 30 days to 23°C. The thermal color scale is valid for all shown recordings from A-J.*

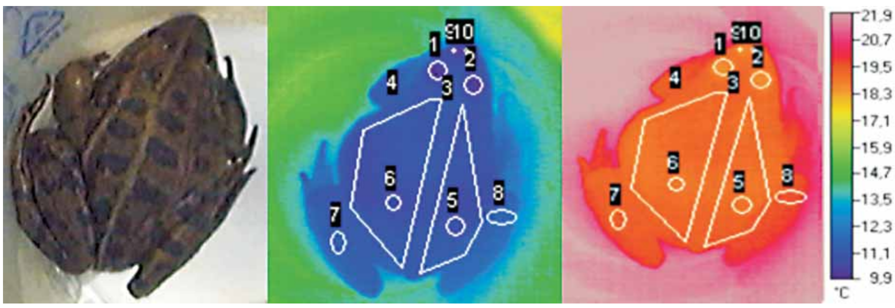

Figure 1. *Comparison of photographs taken in visual spectrum and thermographic imaging.*

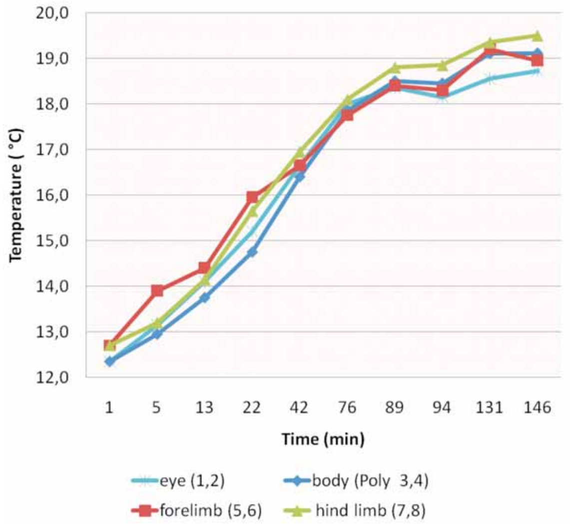

Figure 3. *Changes of temperature in the frog over the period of 146 minutes after shift from 8°C exposure for 30 days to 23°C. Each point represents the average of three frog's mean values of the same body regions.*

similar studies *(11)*. Exact and precise measurements of thermal changes of frogs' body temperature, correlated with ambiental temperature (T_a) changes, are essential in metabolic studies. Use of thermography today allows precise recording of real time thermal changes of body surface temperature in frogs in experiments with different temperature exposure. Today the thermal recording systems such as the one used in this study have the precision of 0.07°C (at 30°C), and allow the real time recording. The development of new technologies and state-of--the-art thermovision cameras has enabled thermography inspection in numerous branches of science and industry. In this article we demonstrated its use in an ectothermic animal. Thermography is an efficient and simple method which successfully and reproducively records thermal images of tested areas of frog skin. A typical new generation camera, the ThermoTracer TH7102WL (NEC San-ei Instruments, 2004) with its cooled sensor elements was accurate enough to discriminate thermal differences between various body parts and between the body and the environment. In comparison to standard thermometer measurements, the advantage of this method is that there is no contact (and thus heat loss) between the instrument and the animal. Furthermore, there is no need for unnecessary handling which stresses the animal and influences the accuracy of results. Use of thermography has an advantage over implantable thermo coupling instruments as well as there is no unnecessary animal loss because of the surgical procedure of implantation and additional time of recuperation after surgical implantation is avoided. Thermography has an advantage over recording of temperature by radio transmitter collars since it allows the multiple simultaneous recording of different body areas, compared to the collar

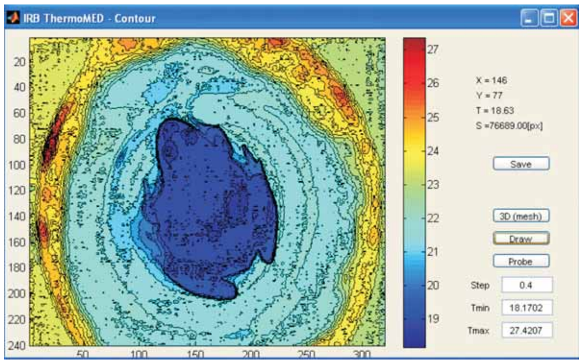

Figure 4. *Different skin regions in frog change temperature at different rate, the eyes are cooler than the rest of the body.*

thermal element which measures a single spot on the skin where it is placed.

In conclusion: The use of thermography allowed us to precisely record the real time thermal changes of body surface temperature in frogs in experiments with different temperature exposure in a non invasive manner. The imaging system used in this study could discriminate thermal changes between different skin areas of the frog's body. Previous attempts at thermography in hibernating frogs have not been reported, perhaps because of the lack of precision of the earlier instruments. New generation cameras have the accuracy and software support to discriminate between subtle differences in temperature of different body regions of examined frogs and the surrounding environment, as documented in this study.

REFERENCES

1. ĐIKIĆ D, HELDMAIER G, MEYER C W 2008 Induced torpor in different strains of laboratory mice. *In*: Lovegrove B, Mc Kechnie A E (*ed*) Hypometabolism in animals – Hibernation, torpor and cryobiology. Interpackbooks Pietermaritzburg, p 223–226
2. KAISER J 2002 New prospects for putting organs on ice. *Science 295 (5557):* 1015–1016
3. STOREY K B 1987 Organ-specific metabolism during freezing and thawing in a freeze tolerant frog. *Am J Physiol 253:* R292–297
4. COWAN K J, STOREY K B 2001 Freeze–thaw effects on metabolic enzymes in wood frog organs. *Cryobiology 43:* 32–45
5. BAGNYUKOVA T V, STOREY K B, LUSHCHAK V I 2003 Induction of oxidative stress in *Rana ridibunda* during recovery from winter hibernation. *J Therm Biol 28:* 21–28
6. HELDMAIER G, KLINGENSPOR M (*ed*) 2000 Life in the cold. Springer Verlag Berlin-Heideberg, p 546
7. DUELLMAN W E, TRUEB L (*ed*) 1994 Biology of Amphibians. The John Hopkins University Press. Baltimore USA, p 670
8. KOBAYASHI T, SHIM M, SUGI H 1998 Infrared thermography of bullfrog skeletal muscle at rest and during an isometric tetanus. *Jap J Physiol 48:* 477–482
9. BERZ R, SAUER H 2007 The Medical Use of Infrared-Thermography History and Recent Applicatons, Thermografie-Kolloquium-Vortrag 04, Berlin p12; http://www.ndt.net/article/dgzfp-irt-2007/Inhalt/v04.pdf
10. KOLARIĆ D, SKALA K, DUBRAVIĆ A 2007 ThermoWEB-remote control and measurement of temperature over the web. *Period biol 108(6):* 631–637
11. GLENN J, TATTERSALL G J, ETEROVICK P, De ANDRADE D E 2006 Tribute to R. G. Boutilier: Skin colour and body temperature changes in basking *Bokermannohyla alvarengai* (Bokermann 1956). *J exp Biol 209:* 1185–1196