Temperature regulation in the Balkan spadefoot (*Pelobates balcanicus* Karaman, 1928) during the start of the nocturnal activity

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On land, the amphibians interact with the environment in a complex way - even small changes in the physiological condition may significantly impact the behaviour and vice versa. In ectothermic tetrapods, the transition from inactive to active phase may be related to important change in their thermal status. In the range of our study, we performed temperature recordings in a total of 204 adult Balkan spadefoot (*Pelobates balcanicus* Karaman, 1928) at the beginning of their nocturnal activity. We performed measurements of the temperature of the substrate (at a depth of 11-12 cm), the surface body temperature of the toads and provided also thermo-profiles of the animals and the surface of the substrate in their microhabitats. The adult in *P. balcanicus* spend the daytime buried in sandy substrates at depths between 10 and 15 cm and emerge at the surface after sunset. On the substrate, their thermal energy exchange is defined by the absence of heat flow from the sun. Secondary heat sources like stored heat and infrared radiation from the soil play important role for the thermal balance of the active spadefoot toads. On the basis of our measurements and additional data, we discuss on the eventual role of the air humidity and the effects of surface and skin water evaporation on the water balance and activity of the investigated toads.

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Abstract

On land, the amphibians interact with the environment in a complex way - even small changes in the physiological condition may significantly impact the behaviour and vice versa. In ectothermic tetrapods, the transition from inactive to active phase may be related to important change in their thermal status. In the range of our study, we performed temperature recordings in a total of 204 adult Balkan spadefoot (*Pelobates balcanicus* Karaman, 1928) at the beginning of their nocturnal activity. We performed measurements of the temperature of the substrate (at a depth of 11-12 cm), the surface body temperature of the toads and provided also thermo-profiles of the animals and the surface of the substrate in their microhabitats. The adult in *P. balcanicus* spend the daytime buried in sandy substrates at depths between 10 and 15 cm and emerge at the surface after sunset. On the substrate, their thermal energy exchange is defined by the absence of heat flow from the sun. Secondary heat sources like stored heat and infrared radiation from the soil play important role for the thermal balance of the active spadefoot toads. On the basis of our measurements and additional data, we discuss on the eventual role of the air humidity and the effects of surface and skin water evaporation on the water balance and activity of the investigated toads.

Introduction

According to Brattstrom (1979), for studying thermoregulation in amphibians, it is crucial to relate changes in the body temperature to the changes in the behavior of the animals – “watching for emergence, and then recording body temperature of emerging animals”. The ideas concerning the thermoregulation capacity within the amphibians have changed dramatically with the development of physiological science. According to modern concepts, body temperature crucially impacts the biochemical and physiological processes in ectothermic organisms (see Hutchinson and Dupre, 1992). These animals invest very little in heat production, so the amphibians are regarded to have a low potential to increase body temperature even at extreme levels of muscle activity. However, many species have a capacity at least for behavioral thermoregulation (see Brattstrom, 1970; Lillywhite et al., 1973; Feder, 1992). Any kind of thermoregulation may be related to very high costs (water lost, energy cost, predator pressure, etc.). However, small changes in the thermoregulatory behavior may have a great impact on the mobility and the habitat selection of the ectotherms tetrapods (Bartelt and Peterson, 2005; Kearney et al., 2009).

Most amphibians tend to retain thermal equilibrium with the surroundings. Terrestrial species like frogs and salamanders are rather small in size and are respectively limited in their potential for heat storage. Theoretically, such species should have an increased potential to thermoregulate by water evaporation when air temperature is high and may reach a thermal equilibrium rather rapidly. The energy flow into the body should equal that which leaves the body: Energy In = Energy Out (see Spotila et al., 1992). Actually, in amphibians, metabolism and thermoregulation are controlled by external factors such as water collection (Lillywhite, 1970) and the environmental temperature (see Mokhatla et al., 2019). Temperature transfer between the body and the environment may occur in different ways concerning the microhabitat (Kreith, 1973). Underwater, the most important processes for heat exchange in amphibians are convection and conduction. Temperature differences rarely increase by 2°C (Erskine and Spotila, 1978). From a thermodynamic perspective, on land, the amphibians interact with the habitat in a more complex way. The body exchanges thermo-radiation with the ground and the surrounding air. It interacts also with the floating air due to convection, conducts heat from/to the ground, but also heats from different types of sun rays (direct, diffuse, reflected, or scattered). The body may cool by the evaporative water loss. Water evaporation plays a major role in keeping lower and comfortable body temperature at the cost of massive water loss (see Tracy, 1976; Spotila et al., 1992). In fact, water equilibrium may play an even more important role in the fitness of amphibians than thermoregulation (see Bartelt and Peterson, 2005; Titon and Gomez, 2015; Roznik et al., 2018).

In the present investigation, we collected data on the thermoregulation in *Pelobates balcanicus* - a highly terrestrial and strictly thigmothermic anuran species. We studied the thermal status of adult spadefoot toads during the change in their activity mode. As anurans possess a low potential to thermoregulate (Spotila et al., 1992), we focused on the thermal conditions in which the Balkan spadefoots are located at the transition between two behavioural phases – rest and locomotion/hunting. The investigated toads spend the day buried into the substrate and do not interact directly with solar radiation - their body temperature is dependent on the thermal conditions within the sand. During the active phase, *P. balcanicus* operates in a system, which usually includes: the night sky, vapour rich atmosphere, temperate sandy ground, and air with a relatively constant temperature. We propose that the combined effect of all these components may impact complexly the thermal biology and also the activity patterns of *P. balcanicus.*

Materials & Methods

Ecological remarks on the studied species

The species belongs to a phylogenetically very old toad clade - a sister clade to the modern group Neobatrachia. This clade includes the families Scaphiopodidae, Pelodytidae, Megophryidae, and Pelobatidae (Pyron and Wiens, 2011). The close relationship of Pelobatidae to Scaphiopodidae (morphologically very similar toads, living in deserts of Northern America) suggests an old evolutionary association to fossorial life with a preference for lower body temperatures. The Balkan spadefoot inhabits the Balkan Peninsula from the sea level up to 920 m a. s. l. (Džukić et al., 2008). In Bulgaria, the species distribution follows the Danube River and coastal areas of the valleys of Maritsa and Struma rivers. There are numerous populations along the Bulgarian Black sea coasts. The adults are active from February until November and live in open lowland areas. It is a strictly nocturnal species that remains hidden in burrows (in the depth of 10 to 15 cm) or under stones during the day (Stojanov et al., 2011). The structure of the ground is a crucial factor in the habitat preferences of most Pelobatidae species, as most of them inhabit exclusively sandy soils (see Eggert, 2002; Scali and Gentilli, as wel; Yermokhin et al., 2013).

Field surveys, documentation, and measurements

The investigated population near Durankulak (NE Bulgaria) was visited in five evenings within 33 days in the late Spring and the early Summer of 2020 (14.06; 25.06; 27.06; 28.06; 17.07). We started our field investigations always at sunset before the adult spadefoot toads appeared on the surface. We followed always the same track and worked in one pair. Pursuant to Stojanov et al. (2011), the investigated locality is inhabited by two species of spadefoot toads - *P. fuscus* and *P. syriacus balcanicus.* (*P. balcanicus,* according to Dufresnes et al., 2019 a,b). In the range of the present study, we registered only adults of the species *P. balcanicus*. None *P. fuscus* were located. On 28.06 (3) and 17.07.2020 (1), we were able to detect a total of four active specimens in the twilight after the sunset, but before the full darkness. On all of our working evenings, we spent two hours at the beginning of the night in an attempt to register the temperatures of the toads shortly after they became active. We were able to collect data from a total of 204 specimens. Each detected toad was immediately photographed by using “Panasonic Lumix FZ 200” (Panasonic Corporation, Kadoma, Osaka, Japan) and “Sony RX 10 III” (Sony Electronics Corporation, Minato, Tokyo, Japan). On spot, we measured the temperature of the substrate at a depth of 11-12 cm by using “Ebro TLC 720” (-EBRO- Xylem Analytics Germany Sales GmbH & Co. KG, -ebro-, Ingolstadt, Germany). This device is equipped with a solid and sharp metal spike (with a length of 11.5 cm) on which is located a highly sensitive contact probe thermo-couple with accuracy of ± 0,8 °C in the range of -18,0 to +119,9°C.

For measurement of the body temperature of the spadefoot toads, we used a combination of several devices (see Navas et al., 2013). First, we measured the body temperature by using the Infrared measurement unit of “Ebro TLC 720” (further referred to as IRT). We used the factory set emissivity of 0.95. The device is producing an IR beam with ratio Distance/Spot diameter = 5/1. According to the producer, the accuracy of the thermometer is +2% in the range of -33 to +220°C. The unit was oriented longitudinally to the midline of the toad as the laser pointer was aimed between the eyes (see Fig. 1) and the IRT beam was projected on the dorsal surface of the specimen (similar to Mitchell and Bergmann, 2016). At a distance of 125 mm between the beam source and the animal, the diameter of the measurement circle on the skin of the toad was 25 mm (see Fig. 1).

Additional measurements of the skin surface temperature were performed by using a Thermometer “Therma Elite 221-061” (ETI ltd, Easting Close, Worthing, West Sussex, UK). The accuracy of the unite is ±0.4°C, or ±0.1% in the range of -99.9 to 299.9°C. As a K-thermocouple for the Thermometer, we used a “Fluke Electronics 80PK-1 K-Type Thermocouple Bead Probe” (Fluke Corporation, Everett, Washington, USA). The combination of these two devices allowed for extremely fast temperature measurements of under 3 sec. In accordance with previous protocols (see Navas et al., 2013), the temperature was measured with the beaded probe positioned in the inguinal area of the body (Fig. 1). We used this set also to measure the air temperature 12 cm above the substrate. Special attention was paid to the lack of any contact between the investigator and the toads to avoid any thermal exchange (see Navas and Araujo, 2000). The only contact with the toads was performed by the tip of the K-Probe. In case the toads were prone to move, they were immobilized by gently pressing them with a piece of the plastic net against the substrate. All experiments were conducted in accordance with national animal welfare regulations - Permit number 767/21.01.2019 of the Ministry of Environment and Water, Bulgaria.

The thermo-profiles (thermal images on which every pixel is loaded with thermal value) of the toads in dorsal projection and the surface of the substrate were recorded by the use of a Thermal camera “FLIR C2” with an MSX Thermosystem (FLIR® Systems, Inc. Wilsonville, Oregon, USA). The emissivity settings were adjusted for the measurement of the frog’s body temperature (according to Rowley and Alford, 2007). The accuracy of the system is ± 1.5 °C. The radiometric images were analysed by the use of “FLIR Tools 6.X” software (FLIR® Systems, Inc. Wilsonville, Oregon, USA). The images were used for the calculation of the minimal, maximal, and average temperature of the substrate surface in the closest possible vicinity to the toad. The minimum, maximum and average temperatures of the body and the minimum, maximum, and average temperatures of the head were recorded (see Fig. 2). In one of our measurements, the toad defecated immediately before the production of the thermo-profile. In this case we measured additionally the temperature of the excrements, to compare it to the other measurements gained from the dorsal profile of the toad.

Statistical analysis

The measurements of all parameters were analyzed by descriptive statistics (see Appendix). For our values, we performed a Paired Two Sample for Means t-test, as this is the nature of the measurements. Several measurements in different body sections, as well as the environmental temperatures for each individual were compared. The confidence interval was calculated as a product of the statistical error and the critical value of the statistics t ($\bar{x} \pm t_{\alpha}\frac{\sigma}{\sqrt{n}}$) for a significance level of 0.05. The two mean values, which showed no statistical significance and are assumed to represent the null hypothesis are marked with an asterisk.

Results

Before the first evening of the investigation (14.6.2020), the weather was rainy and the substrate was rather wet, both on the surface, as well as in-depth. This was the evening with the lowest temperatures measured for the substrate, but also in the 39 measured animals (see Appendix). During the other working visits, the weather conditions were dry. The highest number of the Balkan spadefoot toad with a total of 60 investigated animals was registered on 28.6.2020. The lowest number of toads was detected on the last evening (17.7.2020) of our study – on that night we found 22 active animals. On 17.7.2020 we registered the highest temperatures of the air (24.6°C) and also the highest average maximal temperatures of the substrate surface (22.59°C). However, the average body surface temperature of the measured toads was relatively low (Appendix 1).

As represented in Table 1, the average temperature of the substrate in depth was relatively high, but on the surface, the maximal and minimal temperatures were measured to be about 9°C colder (20.86°C and 18.42°C accordingly). The difference between measurements by the use of TC and IRT was in average 0.84°C and between the Thermal camera images and IRT was 0.53°C. No statistically significant differences in the data provided by the measurements by the TC and the Thermal camera were detected - the values were in the average of 0.31°C (Table 1; Fig. 3, 4, 5).

The average body temperature was higher than the average surface temperature only in the first, second and fourth animals detected on 28.06 and in the first animal on 17.07. In all other cases, the temperature values measured in the different sections of the dorsal skin of *P. balcanicus* were lower than the average substrate temperature. The differences ranged from 0.6°C to about 7.4°C. All temperature measures taken in different areas of the dorsum of these toads were lower than air temperature (Fig. 4). During the first two evenings, the temperature differences in all measurements, except the deep substrate temperature, were relatively small (between 1 and 3 degrees). After these two initial evenings, the surface temperatures of the substrate were significantly different from the measured temperatures from the animal's surface (Table 2). Substrate surface temperature was about 4-5 degrees higher than the measured body temperatures and even exceeded air temperature in two of the nights. Body temperature differences remained close in all nights of observation (Fig. 5).

The temperature values of the different section of the toad's dorsal profile had a dynamic character. In animals which were detected early after the start of our work (presumably freshly emerging from the substrate), the temperature was relatively higher and was closer to that of the subsoil on the spot. Shortly after that, body temperature presumably decreased rapidly and continued to change slowly, along with air temperature. Exponential decrease in temperature as a function of air temperature could be observed in all measured areas of the toad's body.

Discussion

According to our results, in over 98% of the measured toads the body temperature was lower than the surrounding air and the substrate surface. However, the differences were within the range of 2-3°C. In frogs, a strong correlation between the substrate surface and body temperature was reported (Carvajalino-Fernández et al., 2011; Gómez-Hoyos et al., 2016). However, these authors did not used methods that allow for discrete measurements of maximal and minimal temperatures. In most model studies, the aim was to define core temperature or body temperature which can be included in the energy balance (see Sousa et al., 2010). However, careful examination of our thermograms showed that the body in *P. balcanicus* cools unevenly and on the dorsal surface there is a thermal gradient (Fig. 2). In our analysis, the temperature values obtained by the IRT seem to be always lower compared to those measured by the use of the thermocouple system. For both measurements, we used high-quality instruments and both methods were used largely in temperature investigations in anurans (for an overview see Rowley and Alford, 2007). We propose that the differences in the measured values are related to the section of the body surface from which the thermal information was obtained. The coldest spot in the dorsal thermal profile of *P. balcanicus* was always the head. On the dorsal body surface, we detected that the positive temperature gradient is increasing (the differences may reach over 1.5°C) toward the posterior-lateral body sections. We gained the temperature data from the TC from the inguinal section and this may explain the higher values. That practically means, that the instruments work precise, deliver reliable information and this information reflects the actual distribution of the temperatures on the dorsal skin.

The thermo-profile represented in Fig. 6 demonstrates, that the temperature of the body and the temperature of the excrements during defecation in *P. balcanicus* were within the range of 1.1°C. For small-sized amphibians, Rowley and Arnold (2007) report differences between skin surface temperature and cloacal temperature in the range of 0.5°C. Actually, the combined use of measurement instruments used in the range of this study demonstrated that the data collected by all three systems are reliable and all differences in the values can be logically explained. The IRT, the penetration probe thermocouple, the K-type beaded thermocouple thermometer, and the thermal camera complement each other and their parallel use allows for a fast collection of big amounts of thermal data, both from the biological objects, as well as the substrate of their microhabitat. The measurements were performed in several second and do not demand any contact between the researcher and the anurans. The field method we applied is noninvasive, and should be considered for similar studies as it dramatically reduces the need to capture and handle small sized ectotherm tetrapods.

The mechanism which allows the Balkan spadefoot to remain slightly cooler compare to the surrounding cannot be fully understood by direct measurements. Under controlled conditions, the processes of thermoregulation in amphibians are well studied, but data from field investigations are rather scarce (see Feder, 1992). Most amphibians possess a very low potential for thermoregulation (Brattstrom, 1979). According to Mokhatla et al. (2019), both thermoregulation and metabolic rate are not only related, but fully controlled by the environmental factors. In the framework of the thermal inertia concept, Carey (1978) demonstrated that larger toads cool down more slowly than smaller specimens. According to Tracy et al. (1993) however, the most relevant factors were considered temperature, body water balance and the way the animals deal with water evaporation. During hot days, for most amphibians it is critical to keep their body temperature under 40°C (Brattstrom, 1963, 1968). They may search for cooler spots by implementing migratory behavior (Young et al., 2005), or may rely on cutaneous evaporation water loss to regulate their temperature (Duellman and Trueb, 1986; Mitchell and Bergmann, 2016). This fits the first scenario proposed by Spotila et al. (1992) for the impact of the surrounding factors on the anuran’s body temperature. In this case, the sun will directly warm the system, the humidity will evaporate by contact to the skin and will cause the effect of water evaporation cooling. From a thermo-physical perspective, the cooling effect of the water evaporation from the body surface may be crucial for the amphibians to keep low temperature when in the sun (Brattstrom, 1963). However, intensive water evaporation may lead to water imbalance in frogs. Body hydro-regulation is even more challenging than thermoregulation in some amphibians (see Lillywhite and Navas, 2006; Mitchell and Bergmann, 2016). For two aquatic and one terrestrial anurans, Mokhatla et al. (2019) reported that at high air temperatures, the body temperature remained lower than the surrounding air. The authors report differences of 4-7°C and propose that all three species rely on evaporative cooling at higher temperatures. According to Spotila et al. (1992), under nocturnal conditions, the humidity contained in the air could condense on the surface of the toad forming a “dew layer” and simultaneously (if in open) the toad will lose also heat against the sky (especially by lack of clouds). These authors stressed, that such phenomena were not an object of direct investigation. However, such a scenario would be only possible in case of very dramatic difference in the temperature of the air and the anurans. In all of our observations, the highest temperature was measured deep within the substrate. It exceeded the air temperature by 3.5 to 8.8 degrees. In three of the nights, the soil surface temperature was lower, and in two it was close (1-2 degrees higher) to the air temperature. After sunset, any surface can only be warmed by ground heat and cooled by air exchange and (mostly) by evaporation (Spotila et al., 1992). The surface temperature should depend on both the air humidity and the humidity within the soil. According to Eggert (2002), the spadefoot toads spend several days in a row buried underground without any activity at night - in our case in warm and moist sandy soil. Actually, after sunset, only few of the toads appear on the surface and this might be related to thermoregulation and water balance regulation. At the beginning of the night, the body temperature is determined by the heat and moisture accumulated from the soil. The body temperature is practically independent from absorption of solar radiation (see also the conclusions of Tracy, 1976). Above ground, the toads engage in heat exchange with the air and the surface of the substrate through heat conduction and convection (see Spotila et al., 1992). These processes can lower the body temperature to equalize it with the ambient temperature. The further decrease in body temperature is due to the evaporation of a part of the absorbed moisture. We propose that the described heat exchange processes take place quickly (presumably in the initial 10 to 20 minutes of the night activity) and the evaporation rate is low. In the following stages, the changes are small and synchronised with the air temperature variation. Unlike water frogs, the skin of *Pelobates* is not covered with a layer of fluid (Stojanov et al., 2011). It has a non-uniform structure - in some section, it is rough and warty (with a large surface for evaporation), and in other sections, it is smoother (and perhaps with higher permeability). We propose that this inhomogeneity causes uneven surface humidity and due to uneven evaporation, temperature differences occur. These are visible in the thermal images (Fig. 2 and 6).

Our results indicate that the temperature differences between the environment and the toad's dorsal surface increased due to the change in the air humidity. Based on meteorological data from the nearest weather stations (Weather station WMO_ID = 15499 - link under Fig. 4), we added relevant graphs of the relative humidity in Figs. 3 and 4. During the first three nights of our investigation, the humidity was 100% and this might have reduced the possibility of evaporation from the skin of the animals (low water loss). As the relative humidity lowers, the temperature differences between the toads’ bodies and the substrate surface increased (presumably because the water evaporation through the skin increased). The spontaneous evaporation from the soil surface depends not only on the air humidity, but also on the soil moisture and this may explain that the surface temperature of the soil even exceeded that of the air on June 27 and 28. In those nights, the soil was presumably drier, so the heat absorbed during the day was released more slowly due to the lack of evaporation from the soil. On the base of that analyses we can propose that the potential water loss in hotter and dryer nights may be one of the crucial factors which constrain the activity of the investigated toads. Our work indicates on such tendency, however the data are too limited to allow for defined conclusion. The potential relation between the environmental conditions and the behaviour in pelobatids has to be proven by field investigation and in the present study we propose a theoretical frame which can be experimentally tested in the future.

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Table 1(on next page)

Table 1. Mean values of the temperatures of the soil, air and different areas of *Pelobates balcanicus* sorted by the dates of observation

t (deep) – temperature of the substrate at 12 cm depth; t (air) – temperature of the air lair at the substrate surface; t (substrate) – temperature of the substrate surface; t (TC) – temperature measured by the use of the beaded thermocouple sensor in the inguinal section of the body; t (IRT) – temperature measured by the use of IRT at the body surface of the toad; t (aver. body) – average temperature within the circle EI1 (see Fig. 2); t (aver. head) – average temperature measured in the circle EI2 (see Fig. 2); n – number of animals.

Table 1. Mean values of the temperatures of the soil, air and different areas of *Pelobates balcanicus* sorted by the dates of observation. t (deep) – temperature of the substrate at 12 cm depth; t (air) – temperature of the air lair at the substrate surface; t (substrate) – temperature of the substrate surface; t (TC) – temperature measured by the use of the beaded thermocouple sensor in the inguinal section of the body; t (IRT) – temperature measured by the use of IRT at the body surface of the toad; t (aver. body) – average temperature within the circle EI1 (see Fig. 2); t (aver. head) – average temperature measured in the circle EI2 (see Fig. 2); n – number of animals.

Date	t (deep)	t (air)	t (substrate)	t (TC)	t (IRT)	t (aver. body)	t (aver. head)	n
14.6.2020	23.39	18.02	16.90	16.27	15.82	15.11	15.18	39
25.6.2020	26.07	22.55	21.57	20.94	20.37	20.06	20.05	42
27.6.2020	28.38	19.60	20.67	19.03	17.99	17.40	17.32	41
28.6.2020	29.15	20.60	22.39	18.90	17.63	17.80	17.49	60
17.7.2020	30.62	24.60	22.59	19.31	18.82	17.55	17.24	22

Table 2(on next page)

Table 2. Average temperature differences between the different parts of the body of *Pelobates balcanicus* obtained from measurements with different instruments sorted by the dates of observation

t (TC) – temperature measured by the use of the beaded thermocouple sensor in the inguinal section of the body; t (IRT) – temperature measured by the use of IRT at the body surface of the toad; t (aver. body) – average temperature within the circle EI1 (see Fig. 2); t (aver. head) – average temperature measured in the circle EI2 (see Fig. 2).

Table 2. Average temperature differences between the different parts of the body of *Pelobates balcanicus* obtained from measurements with different instruments sorted by the dates of observation. t (TC) – temperature measured by the use of the beaded thermocouple sensor in the inguinal section of the body; t (IRT) – temperature measured by the use of IRT at the body surface of the toad; t (aver. body) – average temperature within the circle EI1 (see Fig. 2); t (aver. head) – average temperature measured in the circle EI2 (see Fig. 2).

Date	t (TC) - t (IRT)	t (TC) – t (aver. body)	t (TC) - t (aver. head)	t (aver. body) - t (aver. head)	t (IRT) - t (aver. body)
14.6.2020	0.456 ±0.053	1.167 ±0.079	1.090 ±0.130	-0.077 ±0.086	0.710 ±0.068
25.6.2020	0.564 ±0.066	0.876 ±0.273	0.886 ±0.275	0.010 ±0.028	0.312 ±0.291
27.6.2020	1.041 ±0.134	1.634 ±0.227	1.715 ±0.228	0.080 ±0.046	0.593 ±0.218
28.6.2020	1.268 ±0.109	1.098 ±0.159	1.408 ±0.176	0.310 ±0.070	-0.170 ±0.143
17.7.2020	0.491 ±0.175	1.759 ±0.292	2.068 ±0.346	0.309 ±0.130	1.268 ±0.310

Figure 1

Fig. 1. Dorsal view of a specimen of *Pelobates balcanicus* during the procedure of thermal measurements

The laser pointer of the IRT is aimed at the head of the toad; the red circle indicates the spot of measurement with the IRT; the yellow section represents the area where the temperature was measured by the use of the beaded thermocouple sensor.

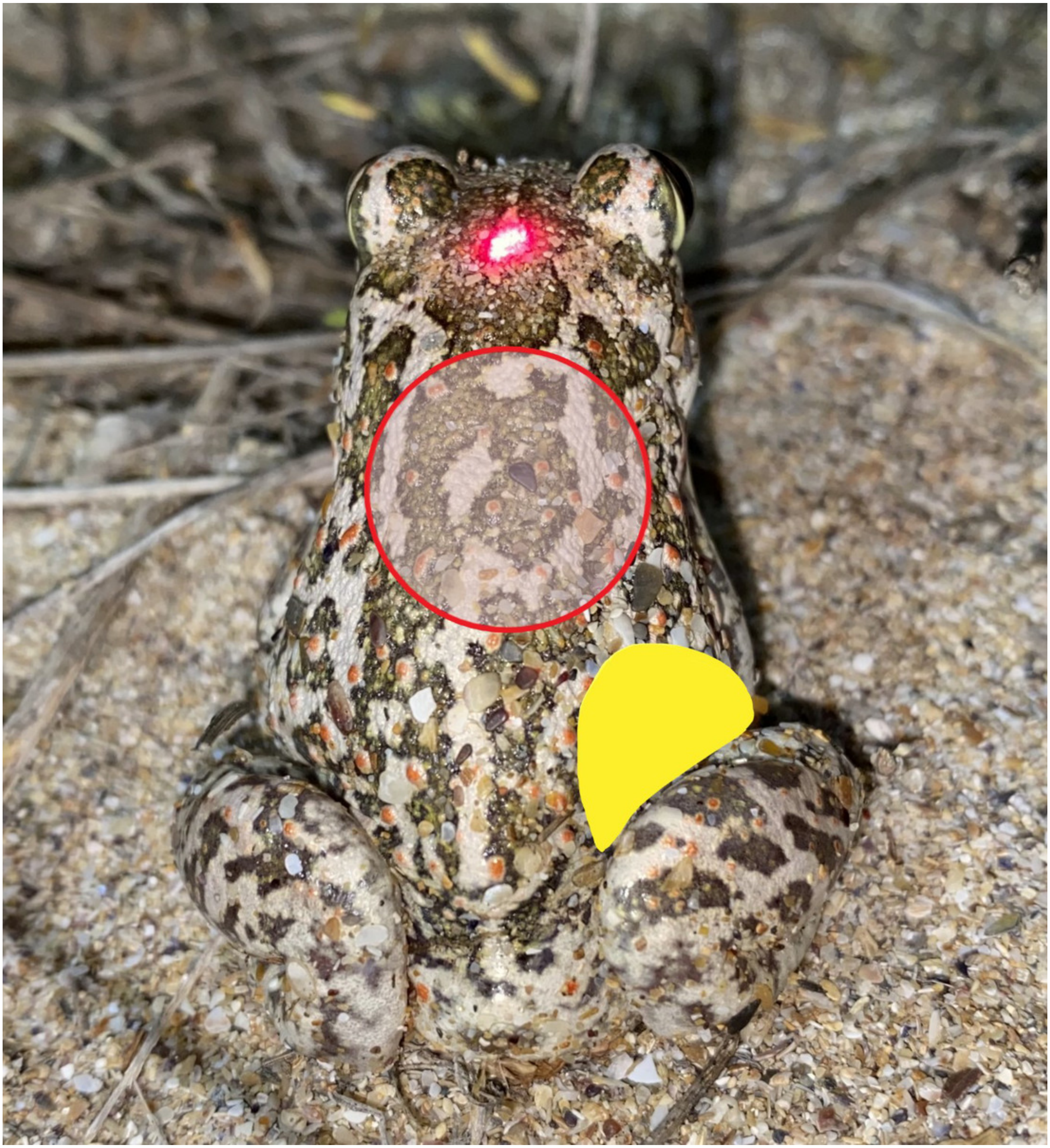
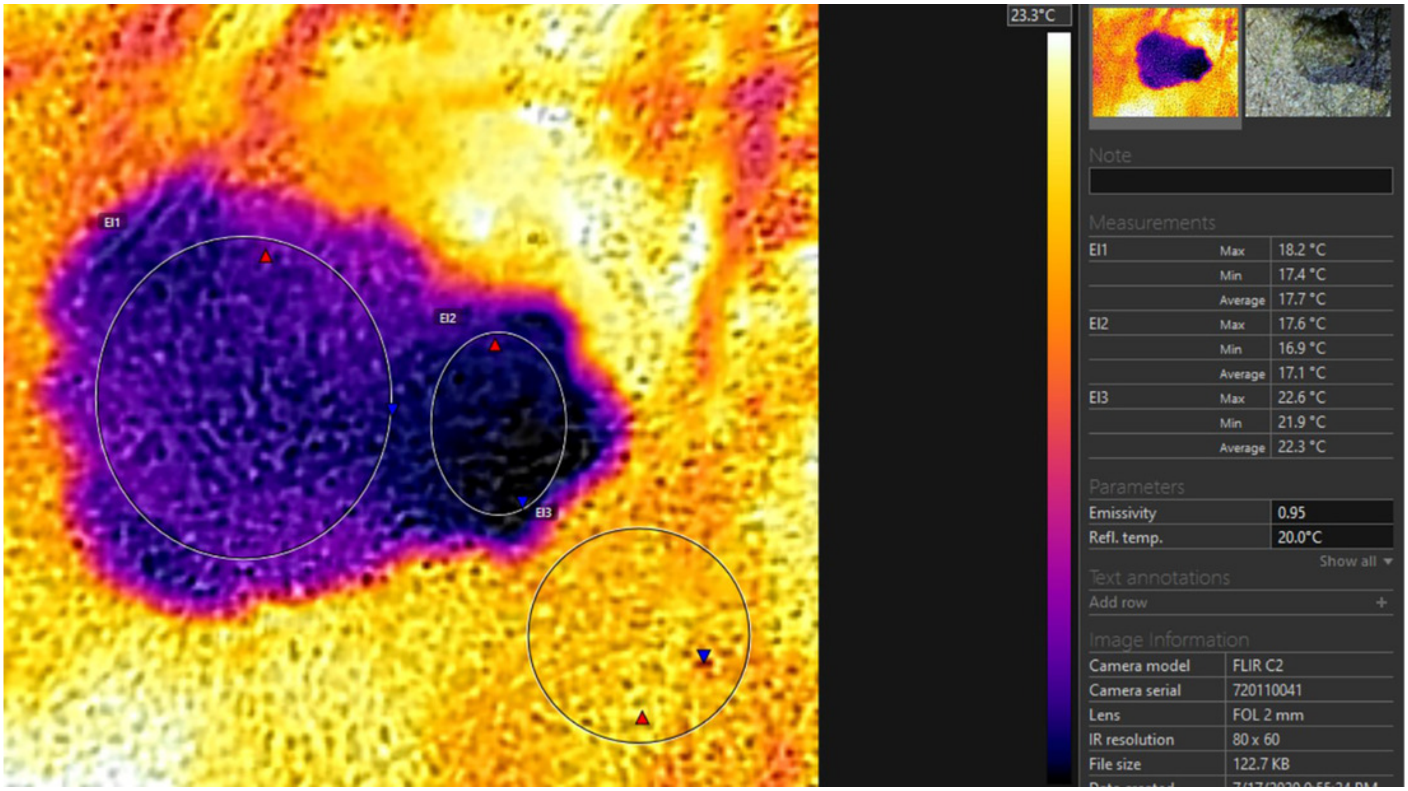


Figure 2

Fig. 2. Thermoprofile of a *Pelobates balcanicus* specimen represented by the “Flir tools” software

The circles on the body (EI1) and on the head (EI2) represent the surface temperatures within these sections of the toad; the circle EI3 represents the surface temperatures of the substrate at the direct vicinity of the toad.



Measurements

EI1	Max	18.2 °C
	Min	17.4 °C
	Average	17.7 °C
EI2	Max	17.6 °C
	Min	16.9 °C
	Average	17.1 °C
EI3	Max	22.6 °C
	Min	21.9 °C
	Average	22.3 °C

Parameters

Emissivity	0.95
Refl. temp.	20.0°C

Image Information

Camera model	FLIR C2
Camera serial	720110041
Lens	FOL 2 mm
IR resolution	80 x 60
File size	122.7 KB

Figure 3

Fig. 3. Average temperature values in depth of the soil, on the soil surface, the ground air layer and in the different parts of the surface of the *Pelobates balcanicus*

t (deep) – temperature of the substrate at 12 cm depth; t (air) – temperature of the air lair at the substrate surface; t (substrate) – temperature of the substrate surface; t (TC) – temperature measured by the use of the beaded thermocouple sensor in the inguinal section of the body; t (IRT) – temperature measured by the use of IRT at the body surface of the toad; t (aver. body) – average temperature within the circle EI1 (see Fig. 2) ; t (aver. head) – average temperature measured in the circle EI2 (see Fig. 2).

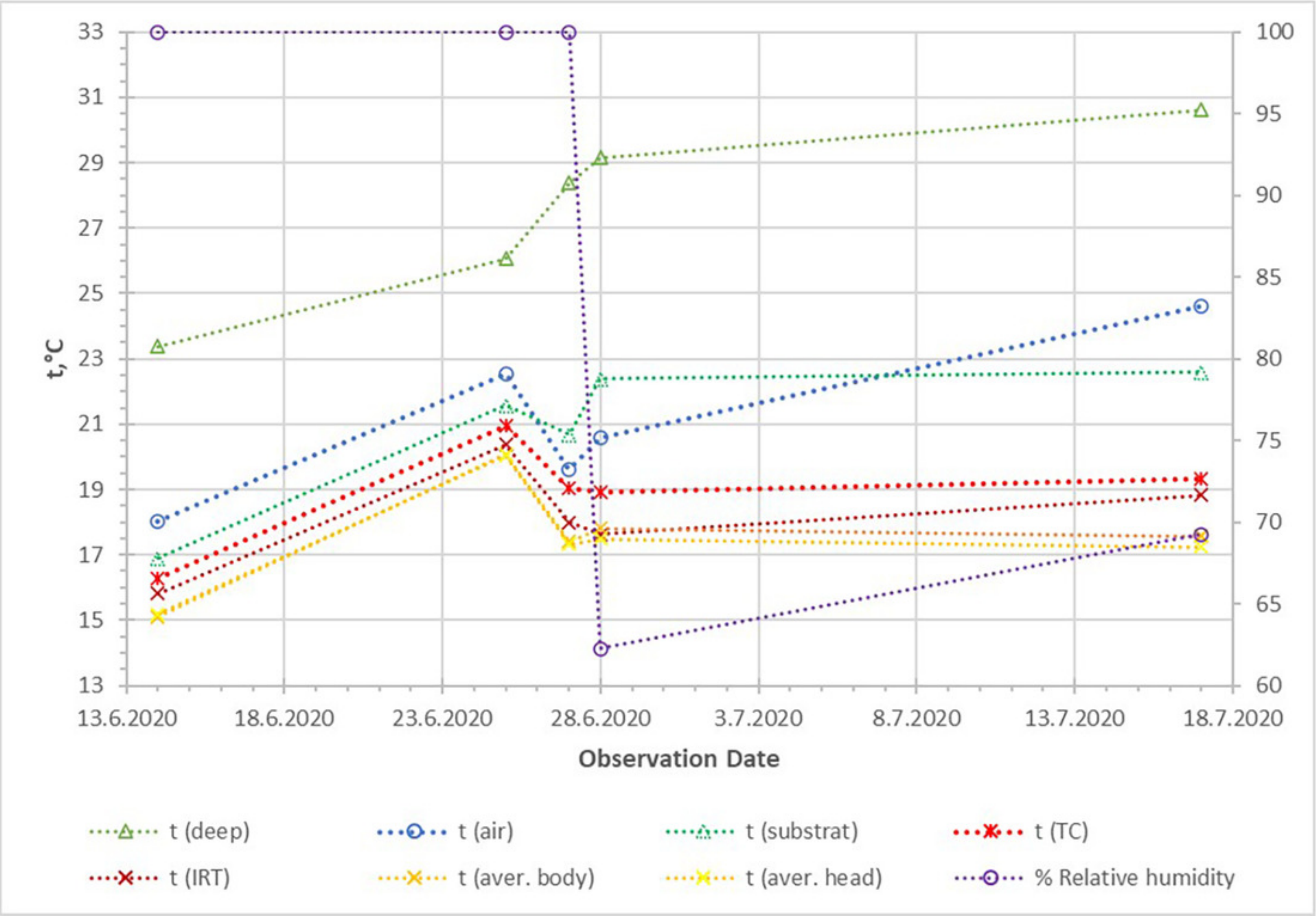


Figure 4

Fig. 4. Average temperature differences between the measured temperatures of the air, the substrate surface, the deep substrate and on the body and head surface of *Pelobates balcanicus*

t (deep) – temperature of the substrate at 12 cm depth; t (air) – temperature of the air lair at the substrate surface; t (substrate) – temperature of the substrate surface; t (TC) – temperature measured by the use of the beaded thermocouple sensor in the inguinal section of the body; t (IRT) – temperature measured by the use of IRT at the body surface of the toad; t (aver. body) – average temperature within the circle El1 (see Fig. 2); t (aver. head) – average temperature measured in the circle El2 (see Fig. 2). The relative humidity of the air is provided according to the website "Reliable Prognosis", from Weather station “Mangalia”, Romania, WMO_ID=15499 (http://rp5.ru/archive.php?wmo_id=15499&lang=en).

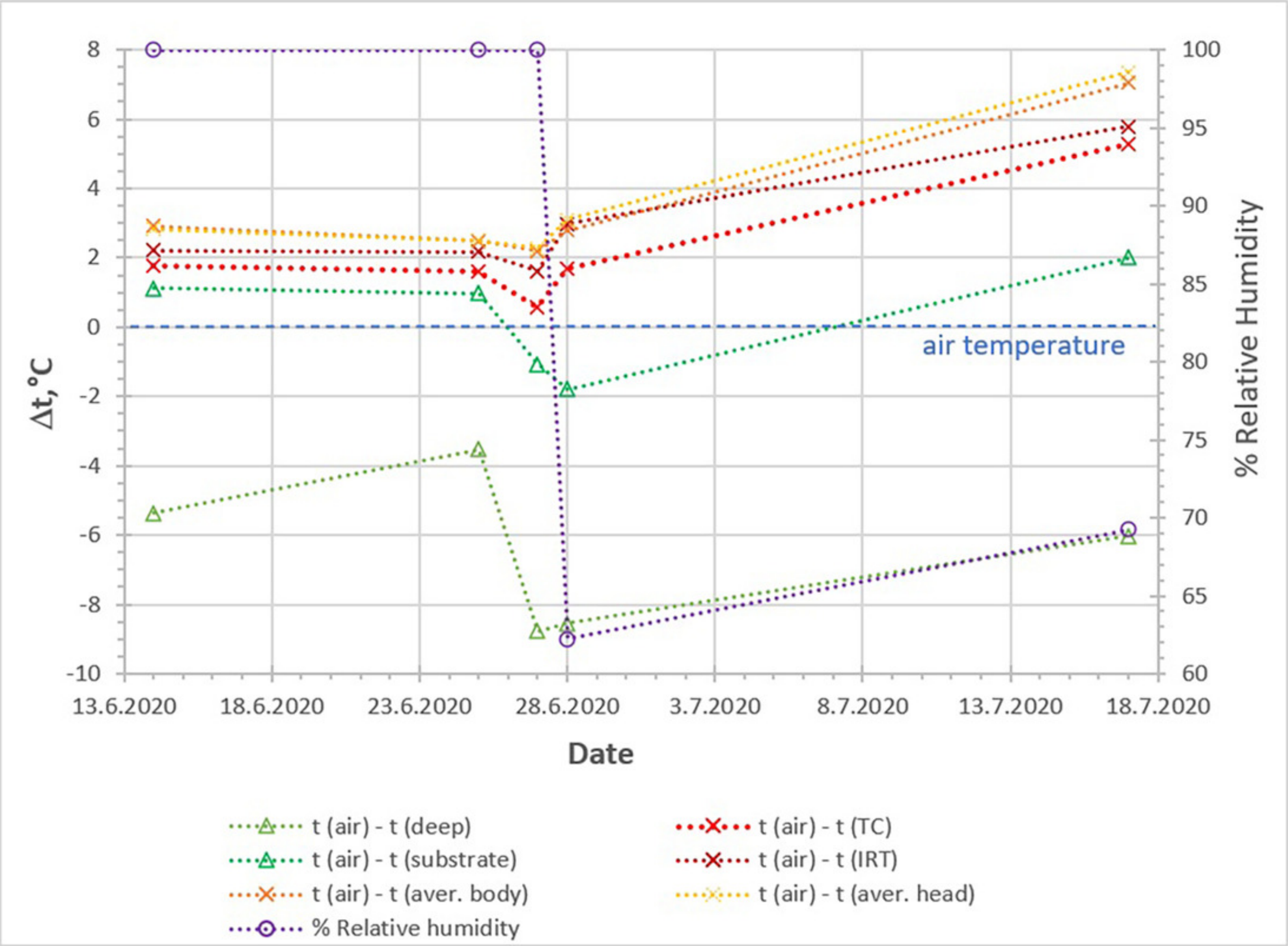

Δt, °C
8
6
4
2
0
-2
-4
-6
-8
-10
% Relative Humidity
100
95
90
85
80
75
70
65
60
air temperature
13.6.2020
18.6.2020
23.6.2020
28.6.2020
3.7.2020
8.7.2020
13.7.2020
18.7.2020
Date
t (air) - t (deep)
t (air) - t (TC)
t (air) - t (substrate)
t (air) - t (IRT)
t (air) - t (aver. body)
t (air) - t (aver. head)
% Relative humidity

Figure 5

Fig. 5. Kinetics in the measured temperature changes of the substrate, the air and the different section of the surface of *Pelobates balcanicus* measured on 28 June 2020

t (deep) – temperature at 12 cm under the substrate; t (air) – temperature of the air; t (max. substrate) – maximal temperature of the surface of the substrate; t (TC) – temperature of the toad surface measured by the use of the beaded thermocouple sensor; t (IRT) – temperature of the toad measured by the use of IRT; t (aver. body) - average temperature of the body measured by the use of the thermal camera.

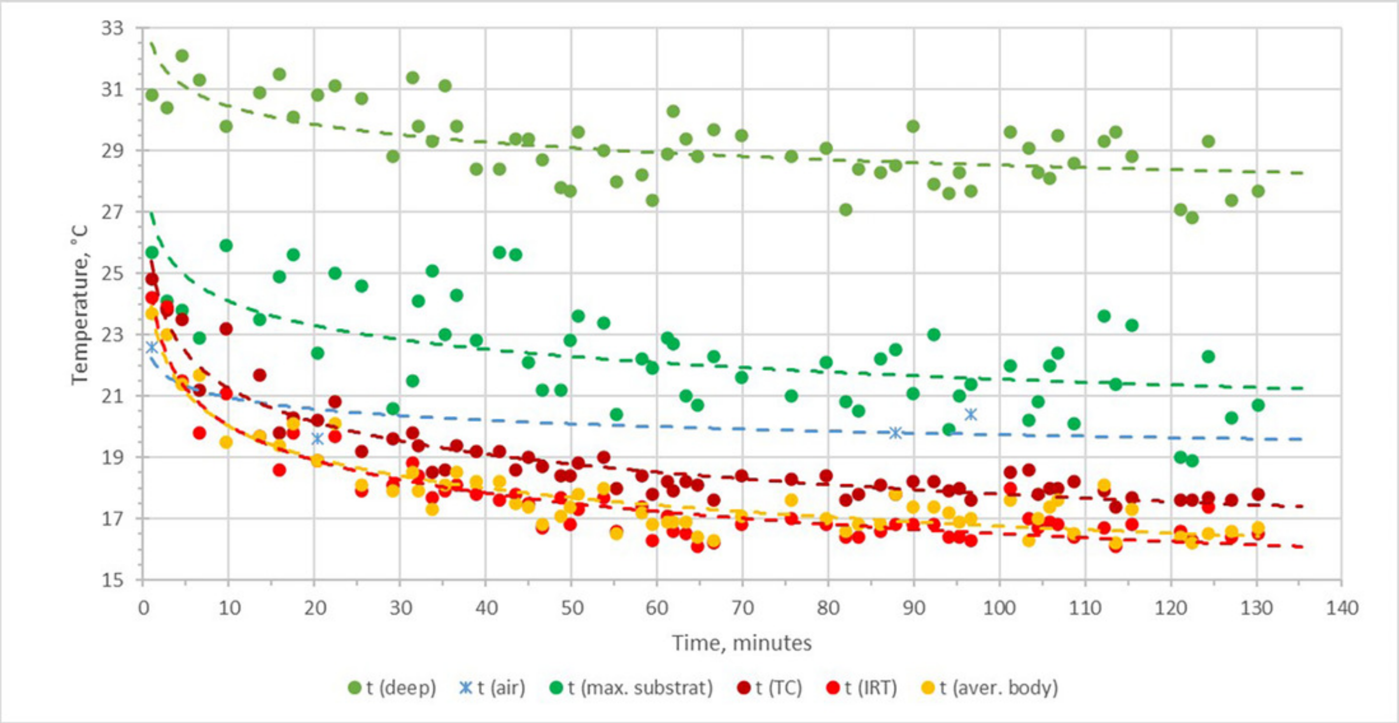
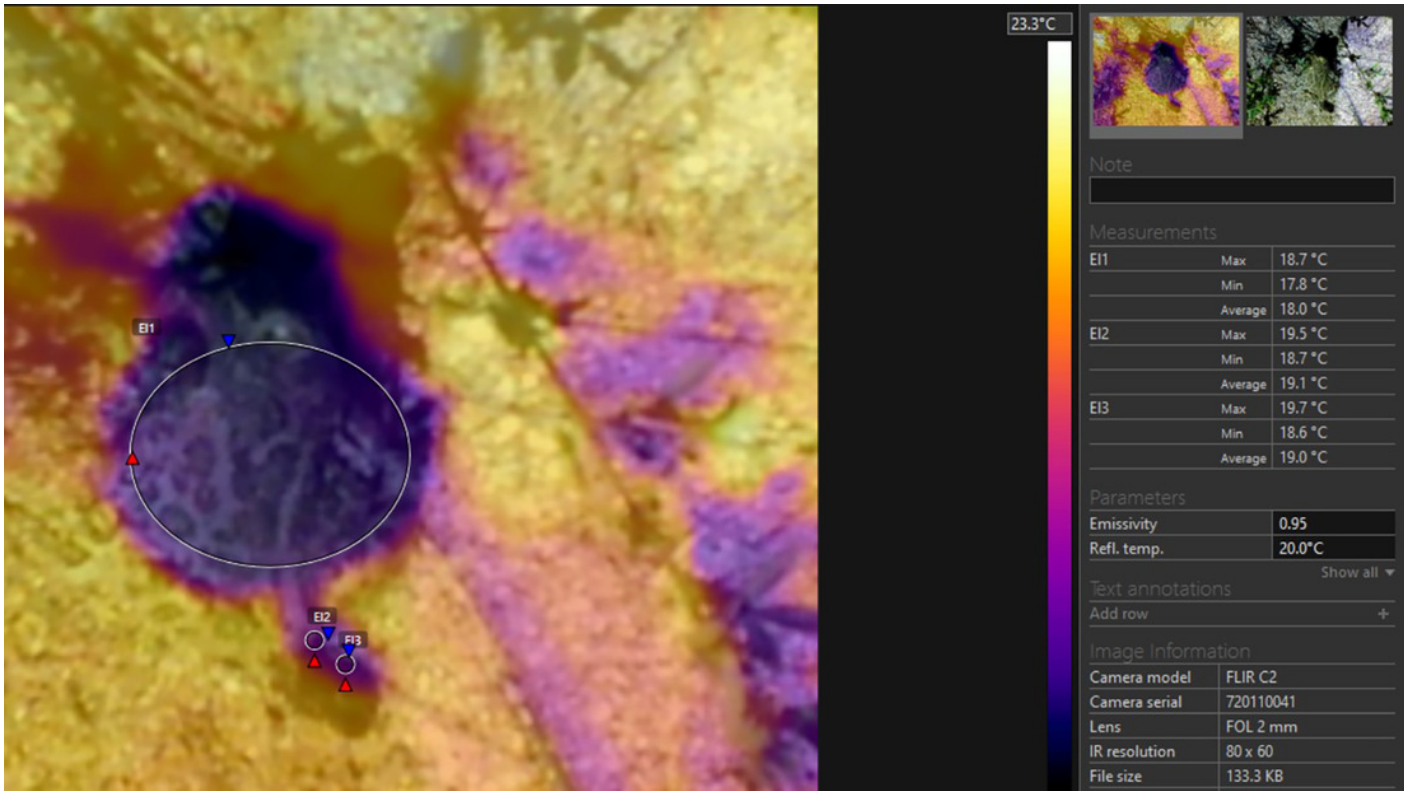


Figure 6

Fig. 6. Thermal image of a specimen of *Pelobates balcanicus* during defecation

EI1 – maximal, minimal and average temperature of the body of the toad; EI2 – maximal, minimal and average temperature of the proximal section of the excrement; EI3 – maximal, minimal and average section of the distal section of the excrement.



Measurements

EI1	Max	18.7 °C
	Min	17.8 °C
	Average	18.0 °C
EI2	Max	19.5 °C
	Min	18.7 °C
	Average	19.1 °C
EI3	Max	19.7 °C
	Min	18.6 °C
	Average	19.0 °C

Parameters

Emissivity	0.95
Refl. temp.	20.0°C

Image Information

Camera model	FLIR C2
Camera serial	720110041
Lens	FOL 2 mm
IR resolution	80 x 60
File size	133.3 KB