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Project title: Thermal influence on energy assimilation in vertebrate ectotherms: a case study in a tropical lizard

Host: Michael J. Angilletta, Ph.D.
School of Life Sciences
Arizona State University
Tempe, AZ 85287, USA

Purpose of the visit:

To explore energetics of gecko eggs and embryonic development at three different constant incubation temperatures using bomb calorimetry technique

Background:

Temperature affects ectotherms at all biological levels. This single environmental variable influences many physiological and life-history parameters such as metabolic rate, growth rate, final body size, reproductive rate, egg size and quality and many others (recently reviewed e.g. in Angilletta 2009). In collaboration with my colleagues at the Faculty of Science, Charles University in Prague, I am performing a long-term experiment focusing on thermal influence on life-history and physiology in a tropical nocturnal lizard. We are trying not only to describe thermal reaction norms of body size or other life-history traits (Starostová et al. 2010), but we are searching for underlying mechanisms which could explain observed patterns in thermal reaction norms. In ectotherms, energy assimilation have been shown to be largely thermally-dependent (e.g. Angilletta 2002; Oufiero and Angilletta 2006; Storm and Angilletta 2007). Thermally-dependent ability to assimilate energy is thus an important candidate for a proximate mechanism driving thermal reaction norms in various life-history traits. For example, final body size of an individual could be influenced also by body condition or body size (both measured e.g. as body mass and structural size) at the time of hatching dependent on energy utilization during embryonic development and magnitude of maternal investment. Therefore, it is important to examine energy content of eggs laid by females at different temperatures and of hatchlings incubated under different thermal regimes.

The selected experimental organism is the tropical gecko, the Madagascar ground gecko (*Paroedura picta* (Peters, 1854)). Among reptiles, *P. picta* is an extreme fast grower maturing at the age of about four months with high fecundity and thus enormously frequent life-history decisions in females. Females lay only two hard-shelled eggs per clutch, they have extremely short interclutch intervals (as short as 7-10 days) and are able to breed in captivity continuously. Invariant clutch size, characteristic observed in *P. picta* as well as in all other gecko species, might be of particular interest in general life-history and ecological studies, because females can largely adjust only clutch frequency and egg size. More generally, *P. picta* is a tropical lizard, and thus represents majority of reptiles confined to the tropics with specific thermal requirements, which make them susceptible to climate changes (Tewksbury et al. 2008).

Description of the work carried out during the visit:

Energy content of gecko eggs and hatchlings laid and incubated under different environmental temperatures was determined using bomb calorimetry at Dr. Michael J. Angilletta's Thermal Adaptation Lab at School of Life Sciences, Arizona State University, USA. Samples originated from long-term experiment on the effect of different constant temperatures on body size and other life-history traits and physiology in Madagascar ground gecko (*P. picta*). The overall design of the experiment was as follows: freshly-laid eggs from females reared under common garden experimental design were randomly distributed into three climatic chambers differing in constant temperatures (24, 27, 30 °C). After hatching, each individual was raised separately in its respective climatic chamber till reaching final body size. During the whole experiment, snout-to-vent length and body mass were taken regularly. Lizards were fed with live crickets dusted with vitamins and minerals twice a week. We provided a superabundance of crickets during each feeding to ensure that lizards were fed to satiety. Geckos were weighed before and after each feeding. When females reached a body mass of 6 g, they were randomly assigned to males from the same temperature. The sire was always a male unrelated to the dam, with body mass larger than 4 g and prominent secondary sexual traits such as enlarged hemipenial sacs and active abdominal scent glands. Females were allowed to mate with their assigned male every month (every 14 days until the first oviposition). Laid eggs were either incubated in one of three experimental temperatures or frozen shortly after oviposition. For incubation eggs were split among temperatures according to *a priori* balanced design (females at 30 °C: eggs left at 30 °C, moved to 24 and 27 °C, similarly for females from 27 and 24 °C). Fresh hatchlings were weighed and their snout-to-vent lengths were measured. Subsets of hatchlings and eggs from each temperature were sacrificed for energy content assessment.

Preparation of samples and data analyses:

Eggs and hatchlings with known wet mass were lyophilized for 24 or 48 hours, respectively and dry mass was taken. Eggs and hatchlings were homogenized. Homogenized samples were compressed into two pellets. Mass of the pellets was within the range from 50 to 190 mg. Pellets were combusted and caloric content was determined using Parr1425 Semimicro Calorimeter (Parr Instruments Company, Moline, IL, USA). In following statistical analyses, the trends and significance of the results were the same regardless whether we analyzed results for hatchlings from eggs left in the respective temperature of their mothers (i.e. eggs laid at 30 °C incubated at 30 °C etc.) or hatchlings from eggs laid in a particular temperature transferred for incubation to other temperatures. Therefore, only results for all hatchlings pooled together are reported here.

Description of the main results obtained:

Preliminary results

1) Energetic content of eggs laid by females at different temperatures

Females from different temperatures differed neither in body mass nor in snout-vent-length during the period of egg collecting (ANOVA: mass: $F(2, 55) = 0.00348$, $p = 0.996$; snout-vent-length: $F(2, 55) = 1.6459$, $p = 0.202$). Surprisingly, females from different temperatures laid eggs of different size, females from the highest temperature laid significantly smaller eggs (ANOVA: $F(2, 113) = 5.8875$, $p = 0.004$; Fig. 1).

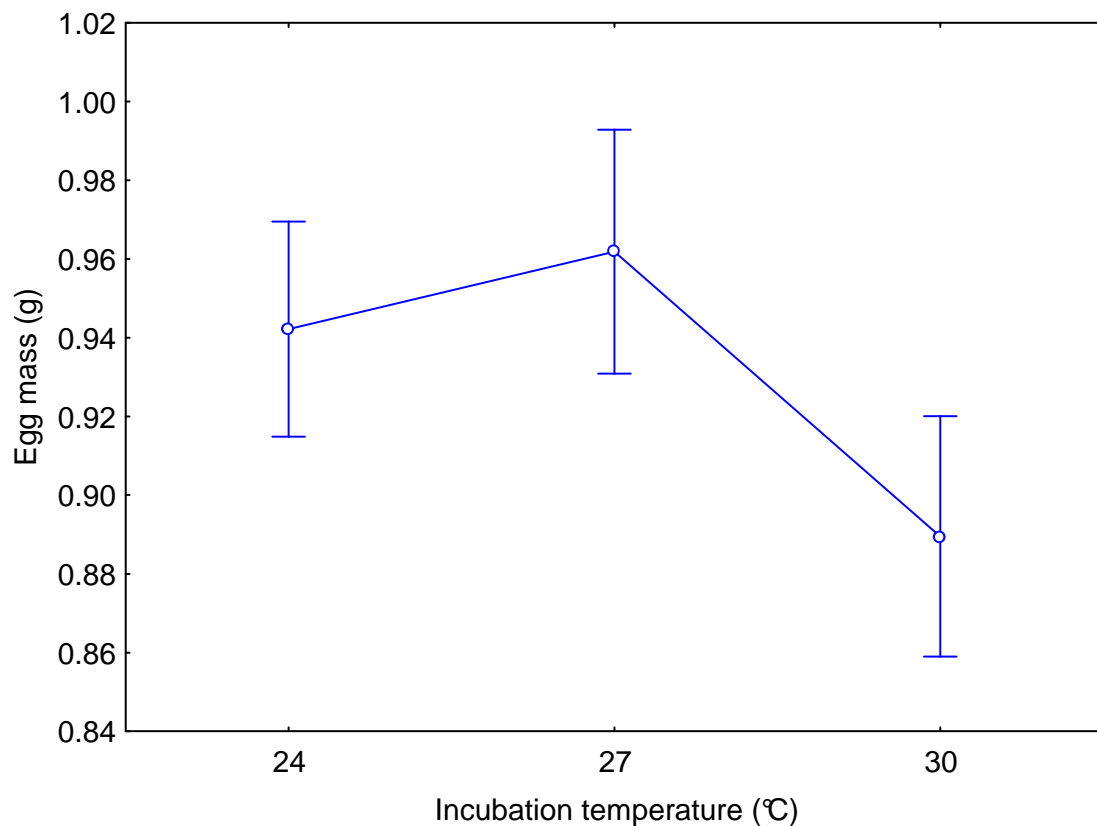


Fig.1: Mass of eggs laid by females at different temperatures. Females from the highest temperature laid significantly smaller eggs even though females from different temperatures did not differ in body mass.

Dry mass of eggs scaled negatively-allometrically with wet egg mass (scaling coefficient of log-log relationship was 0.836 ± 0.053 , 95% - CI: 0.731- 0.941), larger eggs hence contained proportionally more water at the time of laying (Fig. 2). However, dry egg mass relatively to fresh egg mass did not differ among temperatures (full-factorial ANCOVA: factor temperature and its interaction with wet egg mass n.s., p in both cases > 0.22).

Energetic content of eggs in calories per gram of dry mass did not correlate with dry egg mass ($r = -0.01$, $p = 0.85$) and did not differ among temperatures (ANOVA: $F(2, 113) = 1.55$, $p = 0.22$). We can conclude that females laid differently sized eggs across temperatures, but that these differences cannot be attributed to mechanistic constraints imposed for instance by the width of pelvic opening. Differently sized eggs differ in water content, but the scaling of relative water content does not differ among females from different temperatures. Females at all three constant temperatures

produced eggs of the same quality in the sense of energetic content per gram of dry egg mass. Females at different temperatures hence can change allocation to reproduction by alteration in egg mass by changing dry mass of eggs and egg water content, but energy quality of dry eggs stays the same at different temperatures.

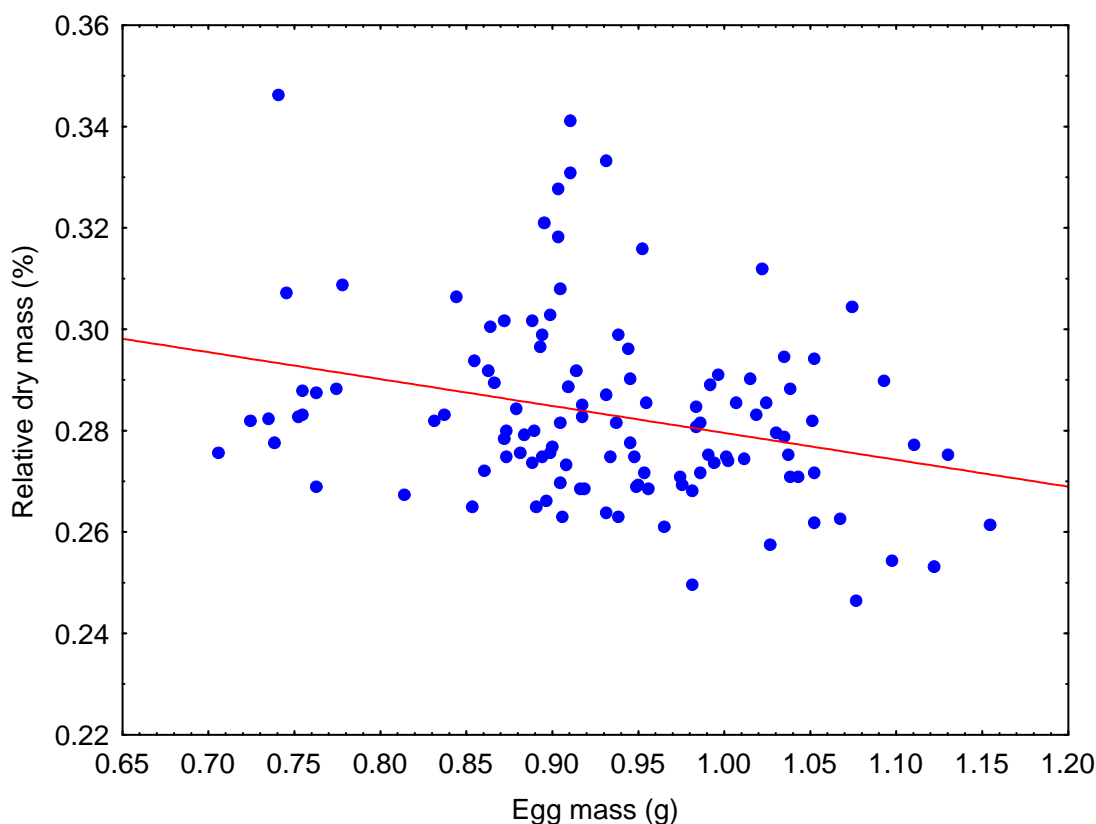


Fig.2: Negative relationship between total egg mass and percentage of dry mass in eggs across all temperatures.

2) Energy utilization during ontogenetic development

Developmental time in geckos is strongly dependent on incubation temperature (ANOVA: $F(2, 169) = 6559.3$, $p < 0.00001$; mean developmental time is 106.16 days at 24 °C, 68.72 days at 27 °C and 51.16 days at 30 °C, respectively). Neither wet nor dry body mass of hatchlings differ among temperatures when it is controlled for fresh egg mass (ANCOVA: wet body mass: $F(2, 168) = 1.375$, $p=0.256$, dry mass: $F(2, 168) = 0.442$, $p = 0.644$). On the other hand, hatchlings significantly differ in snout-vent length relative to fresh egg mass (ANCOVA: $F(2, 168) = 10.300$, $p = 0.00006$) and in energy content (ANOVA: $F(2,169) = 5.288$, $p = 0.006$; Fig 3) among temperatures. Hatchlings from 24 °C were shorter relatively to fresh egg mass than those from the other temperatures. Energy content (in calories per gram of dry mass) was significantly lower in hatchlings from 24 °C in comparison to hatchlings from 30 °C (Fig.3). The results demonstrate that neither wet body mass nor dry body mass of hatchlings is a good measure of energy utilization during embryonic development and that it is necessary to estimate energy content by methods such as a bomb calorimetry. The results demonstrate that ectothermic hatchlings from different temperatures might differ in subtle ways that might be overlooked when just their wet or dry mass is taken during a study. Different snout-vent length of hatchlings from different temperatures suggests that aspects of structural growth in embryos are thermally-dependent in lizards.

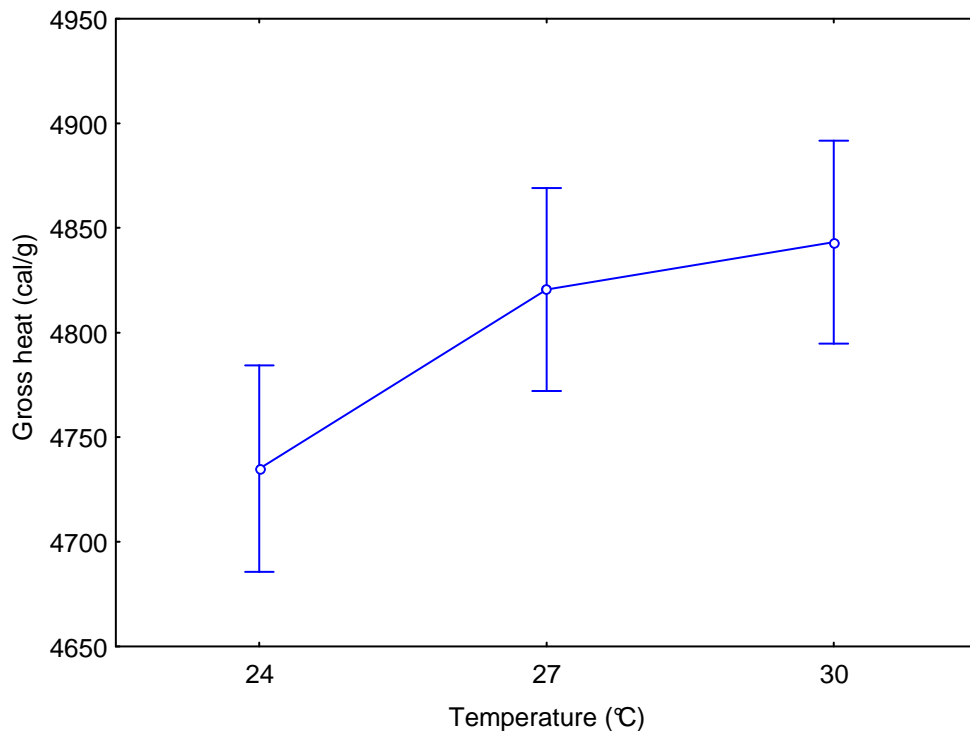


Fig. 3. Energetic content (cal/g) in samples of dry mass of hatchling incubated under different constant temperatures significantly differs. Energy content was significantly lower in hatchlings from 24 °C in comparison to hatchlings from 30 °C.

List of planned publications and planned further collaboration:

We plan to collaborate on writing at least two publications. The first focusing on egg energetics and female investment into reproduction and the second on energetic cost connected to embryonic development and incubation under different temperatures.

Other activities

Presented a talk entitled “Body size in reptiles: proximate mechanisms and ecophysiological correlates.” at School of Life Sciences, Arizona State University, SOLS Special Seminar, 10.11.2010

Attended the weekly Physiology Reading Group meetings and SOLS seminars, School of Life Sciences, Arizona State University

New skills learnt: prepare samples for bomb calorimetry, operate Parr1425 Semimicro Calorimeter

References:

- Angilletta M.J. (2002): Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* 82: 3044-3056.
- Angilletta M.J. (2009): *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford.
- Oufiero C. E. and M. J. Angilletta. 2006. Convergent evolution of growth and development in lizard embryos. *Evolution* 60: 1066-1075.
- Starostová Z., Kubička L. and Kratochvíl L. (2010): Macroevolutionary pattern of sexual size dimorphism in geckos corresponds to intraspecific temperature-induced variation. *Journal of Evolutionary Biology* 23: 670-677.
- Storm M. A. and M. J. Angilletta. 2007. Rapid assimilation of yolk enhances growth and development of lizard embryos from a cold environment. *Journal of Experimental Biology* 210: 3415-3421.
- Tewksbury J. J., Huey R.B. and Deutsch C.A. (2008): Putting the heat on tropical animals. *Science* 320: 1296 – 1297.