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NOTE

Some Like it Hot: Heat Tolerance of the Crab-Eating Frog, *Fejervarya cancrivora.*

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Abstract—We used Critical Thermal Methodology to quantify thermal tolerance of the crab-eating frog, *Fejervarya cancrivora*, from Hoga Island, southeast Sulawesi, Indonesia. The critical thermal maximum of *F. cancrivora* was 42.1 ± 0.76 C, and is perhaps the highest thermal tolerance value known in an anuran acclimatized to 25 C. The frogs' ability to tolerate extreme high temperatures allows them to exploit abiotically harsh mangal habitats and accounts for their wide distribution across the tropical Pacific.

Introduction

The crab-eating frog, *Fejervarya cancrivora* (formerly *Rana cancrivora*) inhabits coastal scrub zones, marshes, disturbed forest areas and mangal swamps across much of southeastern Asia (Zhigang et al. 2004) and west to the Western Ghats in India (Ranjit Daniels 1992). The frog is known to withstand full-strength seawater, and while other anurans including the green toad, *Pseudepidalea viridis,* (Gordon et al. 1961), cane toad, *Rhinella marina,* (Liggins & Grigg 1985), and African clawed frog, *Xenopus laevis,* (Romspert 1976) may tolerate modest salinity increases, *F. cancrivora* is considered to be the only truly marine amphibian

(Gordon et al. 1961, Degania et al. 1984, Jørgensen 1997). The frogs forage in seawater at high tide, sheltering in mangrove roots or shallow burrows at low tide (Gordon et al. 1961, Schmidt-Nielsen & Lee 1962). Saltwater tolerance is accomplished by eliminating the osmotic gradient between body fluids and ambient seawater by increasing tissue urea levels (Schmidt-Nielsen & Lee 1962, Wright et al. 2004). Not surprisingly, kidney function and ion balance are the best studied aspects of the frogs' physiology (Chikashi et al. 2004, Wright et al. 2004).

In July 2010 we discovered *F. cancrivora* on Hoga island, southeast Sulawesi, Indonesia (5° 28' 0.91" S, 123° 45'33.2" E). A strong El Niño that year produced unusually wet conditions and frogs remained active long into the dry season. Typically frogs at this time of year are limited to saltwater pools where temperatures may reach 40 C or more (Dunson 1977, Taylor et al. 2005). With little opportunity to escape constant high temperatures, it is reasonable to assume that crab-eating frogs are physiologically rather than behaviorally suited to tolerate temperature extremes. The only thermal study on *F. cancrivora* to date estimates exposure times of tadpoles plunged into lethal temperatures between 41 and 45 C (Dunson 1977). Our study quantifies heat tolerance of field acclimatized adult frogs from Hoga Island via standard Critical Thermal Methodology and compares the results to the previous tadpole work as well as data from other frog species.

Thermal tolerance of amphibians is typically quantified using Critical Thermal Methodology or CTM (Hutchison 1961). The method is fast, non-lethal, and produces extremely accurate results with small sample sizes (Lutterschmidt & Hutchison 1997, Beitinger et al. 2000). In their classic study of desert reptiles, Cowles and Bogart (1944) introduced the term Critical Thermal Maximum (CTmax). Since 1944, critical thermal methodology has been adapted for use in several animal groups including amphibians (Lutterschmidt & Hutchison 1997). During critical thermal trials, animals are subjected to dynamic temperature increases at a rate fast enough to prevent acclimation but slow enough to track internal body temperature (Lowe & Vance 1955). Near lethal, but non-lethal endpoints are typically used, such as loss of righting response (Brattstrom & Lawrence 1962, Claussen 1973) or onset of muscle spasms (Heatwole et al. 1965, Brattstrom 1970, Hutchison & Ferrance 1970). The arithmetic mean of the collective endpoints is taken as the CTmax of the population (Lowe & Vance 1955, Beitinger et al. 2000).

Methods

Five frogs ranging in length from 3.28 – 8.00 cm and mass from 4.53 – 70.90 g were collected at night and transported to the Hoga Island Research Center. Frogs were held at field collection temperatures (25 C) for one week in a 60×40×50 cm plastic bin containing moist compost and bowls of clean water at 5 ‰ salinity. To reduce photoperiod and diel variation in tolerance endpoints (Mahoney & Hutchison 1969), frogs were maintained under a 12 h:12 h light:dark photoperiod and all trials run between 0900 and 1200 local time. Experimental animals were fed house crickets during the holding period but were not fed 48 hours prior to heat tolerance trials (Brattstrom 1970). Frogs were released at their site of capture upon completion of experiments.

For each heat tolerance trial an individual frog was placed into a 24×15×15 cm acrylic CTM chamber. Frogs were prevented direct contact with the chamber by 4 mm mesh nylon screen positioned 2 cm above the chamber floor. Twenty milliliters of water added to the space between the chamber floor and screen kept air saturation at 100% and prevented frog desiccation (Heatwole et al. 1965, Dunlap 1968, Krakauer 1970). The CTM chamber was then suspended 5 cm above the water line within a covered, 45 (dia) x 25 cm reservoir containing 6 L of water. The water served as a radiant heat source for increasing chamber air temperatures, but did not contact the CTM chamber directly. Reservoir water was heated by an AZoo™, 300-W, submersible heater while a small powerhead provided circulation to insure even heat distribution. With the heater on, CTM chamber temperatures increased at a consistent 0.30 C per minute, a rate shown to meet the CTM criteria for frogs (Dunlap 1968, Krakauer 1970). Air temperatures in the chamber were monitored continuously with a Precision® BAT-12 thermocouple meter equipped with an IT-18, tissue implantable microprobe. Air temperatures were increased until frogs exhibited onset of muscle spasms (OS). Onset of muscle spasms is a reliable indicator of upper temperature tolerance in frogs (Heatwole et al. 1965, Brattstrom 1970, Hutchison & Ferrance 1970), and is defined as spasmodic contractions of body musculature accompanied by extension of the hind legs (Heatwole et al. 1965, Hutchison & Ferrance 1970). Final OS temperatures were determined by inserting the microprobe into the frog's cloaca within 5 sec of OS. Frogs were then immediately placed into cool water where they recovered rapidly.

Results and Discussion

The CTmax of *Fejervarya cancrivora* was 42.1 ± 0.76 C, perhaps the highest reported value for an anuran acclimatized at 25 C. The value was precise resulting in a coefficient of variation of 1.8% even with only five replicate observations. A low coefficient of variation in OS temperatures for frogs ranging in size from 4.53 to 70.9 g suggests thermal tolerance in this species is not size dependent. Although methodology, chamber design and CTM endpoints vary among studies, comparisons with other frogs exhibiting high CTmax values support the contention that *F. cancrivoria* is notably heat tolerant. The CTmax for *F. cancrivoria* measured at OS is higher than LRR values (which occur at temperatures lower than OS; Lutterschmidt & Hutchison, 1997) reported for the green tree frog, *Litoria caerulea,* Great Plains toad, *Anaxyrus cognatus*, green toad, *Anaxyrus debilis,* and western spadefoot toad, *Spea hammondii* (Brattstrom 1963) of 39.4, 39.7, 40.0, and 40.0 C, respectively. Likewise, CTmax estimates using cessation of movement as an endpoint (which occurs at higher temperatures than OS; Dunlap 1968) were 41.2, 41.1 and 41.1 C for the northern cricket frog, *Acris crepitans* (Dunlap 1968), cane toad, *Rhinella marina* (Stuart 1951), and Fowler's toad, *Anaxyrus fowleri* (Sherman 1980), respectively. It is less certain how other nontraditional CTM endpoints such as an abrupt increase in evaporative water loss seen in the reed frog *Hyperolius* *viridiflavus* at 40.5-42.3 C (Geise & Linsenmair 1988) or loss of nictitating membrane response in *R. marina* at 40.0 C (Krakauer 1970) may compare with our data. High thermal tolerance likely benefits frogs whose geographic distributions encompass a range of climatic conditions (Krakauer 1970). The more extreme tolerance values in crab-eating frogs on the other hand, may be a necessary adaptation to survive harsh thermal conditions common to mangal habitats.

Thermal tolerance of adult crab-eating frogs has not been previously investigated; however, Dunson (1977) documented time to 50% mortality in tadpoles acclimatized at 22 to 25 C. Survival time was inversely related to plunge temperature in that the 19.1 hour survival time in 41 C water fell to less than 2 minutes for tadpoles plunged into 45 C water. Tadpoles survived at adult CTmax temperatures (42 C) for 72 minutes, suggesting tadpoles are more heat tolerant than adults. This finding is consistent with most CTM life stage studies, where tadpoles typically show greater heat tolerance than adult frogs (Krakauer 1970, Hoppe 1978, Cupp 1980, Sherman 1980, Dupre & Petranka 1985). Presumably adults can leave pools if temperatures become extreme and thus have less need to maintain heat tolerance physiology after metamorphosis (Krakauer 1970, Hoppe 1978). Tadpoles, however, may benefit from living in hyperthermic pools that are inhospitable to predators and competitors.

Over the past 20 years amphibians have experienced dramatic population declines as well as an increase in extinctions (Blaustein & Wake 1990, Houlahan et al. 2000, Beebee & Griffiths 2005), some of which have been directly linked to adverse habitat temperatures (Carey & Alexander 2003, Pounds 2001). Current climate models predict that temperatures across the Indo-Pacific will likely rise by 1 to 2 C over the next 50 years (IPPC 2007), fueling concerns that some species may be extirpated from their historical ranges. High temperature and salinity tolerance of larva and adult *F. cancrivora* have likely played a key role in determining the species' wide geographic distribution across the tropical Pacific and may safeguard them against direct effects should temperatures increase across their range.

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