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Metabolic compensation and behavioral thermoregulation of subtropical rhacophorid (*Polypedates megacephalus*) tadpoles in container habitats

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Abstract

We examined the relative importance of behavioral thermoregulation and metabolic compensation used by a subtropical rhacophorid (*Polypedates megacephalus*) tadpoles living in man-made container habitats to cope with thermal stress. We collected foam nests of *P. megacephalus* from man-made container habitats, and hatchlings were raised in 150 or 15 L of water (LWB and SWB containers, respectively). Water and air temperatures of containers were monitored using a datalogger. Tadpoles from both types of containers were acclimated at 22 and 32 °C for 10 d before measuring oxygen consumption ($\dot{V}_{O2}$) in a closed-system at 22, 27, and 32 °C. Thermal selection of tadpoles from two containers was determined using an aquatic thermal gradient. We observed daily stratification of temperature in the water column of LWB containers but not SWB containers. Tadpoles from LWB and SWB containers exhibited metabolic compensation so that tadpoles acclimated to 22 °C had significantly higher $\dot{V}_{O2}$ than those acclimated to 32 °C. This was probably related to the variation of environmental temperature experienced by the tadpoles. Tadpoles of LWB and SWB containers selected similar water temperatures with low coefficient of variation, suggesting they are good thermal selectors. Results of this study suggest that *P. megacephalus* tadpoles use both behavioral thermoregulation and metabolic compensation to cope with the environmental temperature fluctuation, and this is, in part, due to the heterogeneity in the thermal regimes of breeding habitats. Even though metabolic compensation of tadpoles incurs a cost, *P. megacephalus* tadpoles that experience no daily thermal gradient in the man-made water bodies and/or seasonal variations in temperature over tadpole period evolve metabolic compensation to maintain physiological homeostasis under different thermal regimes.

Keywords: Anura; Amphibian; Metabolic adaptation; Metabolic acclimation; Thermal physiology; Thermal selectors; Tadpole

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1. Introduction

Aquatic ectotherms have evolved various behavioral, biochemical, and physiological strategies to minimize the effect of ambient temperature fluctuations on the rate at which their life functions proceed (Johnston and Bennett, 1996; Willmer et al., 2005). For example, animals living in cold environments remain active by acclimatizing biochemical processes to minimize the effects of ambient temperature fluctuations on physiological homeostasis (Rome et al., 1992; Thibault et al., 1997; St. Pierre et al., 1998; Roger et al., 2004; Berner and Bessay, 2006). In addition, aquatic ectotherms may use behavioral thermoregulation to cope with environmental temperature fluctuations when they often experience a wide range of thermal gradient in their habitats (Hutchison and Hill, 1977; Marshall and Grigg, 1980; Dupre and Petranka, 1985; Wollmuth et al., 1987; Wollmuth and Crawshaw, 1988; Ultsch et al., 1999). By selecting preferred water temperatures at different times of the day or in different developmental stages, animals can maximize their growth and development.

Most amphibians have complex life cycles in which larvae can often be found in varied aquatic habitats such as ponds, streams, road side ditches, ephemeral water puddles, tree holes, bamboo stumps, geothermal hotsprings and others (Kam et al., 1996; Ultsch et al., 1999; Chen et al., 2001). Results of earlier

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studies have showed that metabolic compensation and behavioral thermoregulation are the physiological strategies commonly used by larvae to minimize the temperature effects on physiological homeostasis (Feder, 1985; Wollmuth et al., 1987; Ultsch et al., 1999; Chen et al., 2001; Wu and Kam, 2005). However, amphibian larvae have different larval periods and experience various thermal regimes daily or seasonally, it is unclear whether they use both physiological strategies to cope with thermal stress. Chen et al. (2001) and Wu and Kam (2005) reported that *Buergeria japonica* tadpoles living in geothermal hotsprings thermoregulate behaviorally but do not exhibit metabolic compensation. Marshall and Grigg (1980) contend that the metabolic compensation incurs a cost and confers little advantage to tadpoles with a short larval period that are usually contained within a single season. However, Feder (1982a, 1985) contends that the ability of a species to modify its metabolic rate through thermal acclimation is correlated with variation in environmental temperature experienced over a time scale of weeks to months. Thus, metabolic compensation is beneficial to species experiencing thermal fluctuation regardless of the length of larval period. Furthermore, anuran larvae living in thermally homogenous habitats such as phytotelmata (Kam et al., 2001) experience a narrow range of temperatures in their habitats. Ultsch et al. (1999) speculate that anuran species in these habitats may not behaviorally regulate body temperatures, like some tropical forest-dwelling lizards (Huey and Webster, 1975).

In this study, we examined the prevalence of behavioral thermoregulation and metabolic compensation of a subtropical rhacophorid (*Polypedates megacephalus*) tadpoles living in container habitats i.e., tanks, containers, and rain-collecting reservoirs in the Bagua Terrace in central Taiwan. Specifically, we measured the temperature selection and precision of thermoregulation by tadpoles raised in containers with large and small water volumes. In addition, we measured the oxygen consumption of tadpoles raised in these containers after across three temperatures being acclimated to 22 and 32 °C to assess their ability for metabolic compensation.

2. Materials and methods

2.1. Study animals and collection sites

Polypedates megacephalus is a mid-sized rhacophorid (snout–vent length 4–6 cm) commonly found in the lowland areas of Taiwan, inhabiting woods, orchards, and bamboo groves. Breeding season is from March to September, and foam nests are deposited overhanging ponds, wetlands, rice field, and man-made water containers. Upon hatching, tadpoles drop into the water and continue to grow until metamorphosis.

Bagua Terrace, an elongated tableland (width 4–10 km and length ca. 33 km) running in a south–north direction, is located in the coastal region of Changhua County. This low elevation terrace (100–400 m in elevation) is characterized by orchards and early- and mid-succession woods (Yen et al., 2004). It is the only remaining wooded area in the western coastal plain of Taiwan and surrounded by urban areas and rice fields. The main breeding habitats of *P. megacephalus* in the Bagua Terrace are the man-made water containers such as rain-collecting reservoirs, tanks, and containers of various sizes in orchards.

2.2. Experimental design

We collected four foam nests from the tanks of orchards on the Bagua Terrace in June–August of 2002 and transported them to the woods of Changhua University campus, 2 km away from the collection sites. Upon hatching, we randomly assigned half of the tadpoles of each nest to either a container with 150 L of water (100 cm in depth; LWB container) or a container of same size containing 15 L of water (15 cm in depth; SWB container). We set up two LWB containers, and each contained tadpoles of two egg clutches. We set up four SWB containers each containing tadpoles from a single clutch, to minimize the crowding. This type of container as commonly found in the orchards and was used to collect rain water for irrigation or agrochemical spraying. Throughout the study we added water to maintain water level. We continuously monitored air and water temperatures at different water depths of a LWB and SWB container using a datalogger. Tadpoles were fed *ad libitum* with broiled spinach and tadpole chow which was produced by a local food factory.

2.3. Resting metabolism of tadpoles acclimated at 22 °C and 32 °C

For each container type, a 2 × 3 factorial design with 10 tadpoles in each treatment combination was used, and the factors were acclimation temperature (22 or 32 °C) and test temperatures (22, 27, or 32 °C). We collected 60 tadpoles (Gosner stage 26–28) from each container type and acclimated 30 tadpoles at 22 ± 1 °C and 30 tadpoles at 32 ± 1 °C for 10 d. These acclimation temperatures approximated the minimum and maximum water temperatures experienced in the field. During acclimation, we continued to feed the tadpoles with broiled spinach *ad libitum* but fasted them for a day before the $\dot{V}_{O2}$ measurements. All tadpoles were maintained under a LD 12:12 photoperiod. We measured the $\dot{V}_{O2}$ of 10 tadpoles in each group at each test temperature (22, 27, and 32 °C). Every tadpole was weighed and staged after measurements.

We measured $\dot{V}_{O2}$ of tadpoles by a closed system described in Chen et al. (2001) and Kam et al. (2001). Determination of $\dot{V}_{O2}$ was made between 1200 and 1800 h. At first, each tadpole was placed in a beaker containing water within 3 °C of the tadpole's acclimation temperature, and the beaker was put in a temperature-controlled water bath (±1 °C). Over 20–30 min, we gradually raised or lowered the water temperature to the designated temperature. Then, each tadpole was put in a metabolic chamber containing 20 mL of water. At the same time, we set up an empty metabolic chamber as a blank. Each metabolic chamber was made up of a 20-mL syringe fitted with a three-way stopcock. Just before we closed the chamber, we slowly injected 1 mL of water into an O_2 analyzer (Cameron Blood Gas Meter) to measure initial oxygen pressure (P_{IO2}, mm Hg). The time interval between initial and final gas samples was