

## A NOVEL FACULTATIVE MUTUALISTIC RELATIONSHIP BETWEEN BUFONID TADPOLES AND FLAGELLATED GREEN ALGAE

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**Abstract.**—Variable environments can produce intolerable conditions for certain species. In some cases, survival is assured by fortuitous mutualistic interactions. We show that the critical thermal maximum (CTM) for *Bufo* tadpoles is increased when the green alga *Chlorogonium* aggregates on their skins in warm ephemeral pools. The congregation of *Chlorogonium* may have been a response toward a source of otherwise limiting CO<sub>2</sub>, reciprocally providing O<sub>2</sub> to the stressed tadpoles. Such a relationship has not been reported previously for these organisms.

**Key words.**—*Bufo*; *Chlorogonium*; mutualism; symbiosis; tadpole; toad

### INTRODUCTION

Many ecologically important mutualisms are conditional and occur because they provide partner species with novel options for adjusting to changing environments (Hay 2004). For example, organisms reaching their critical thermal maximum (CTM) (Hutchison 1961) are incapable of escaping the lethal conditions. Aquatic organisms in thermally uniform systems have no refuge from heat stress; further, temperature increases within such systems decreases the concentration of the necessary gases oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) (Randall 1997). Aquatic organisms that are stressed for these gases for respiration and photosynthesis would benefit from fortuitous mutualistic interactions in which the “by-product” gases evolved by metabolism can be absorbed reciprocally (Hay 2004; Connor 1995).

A field observation allowed us to evaluate a hypothesis of such a fortuitous mutualistic interaction. We discovered numerous tadpoles of the Dwarf American Toad (*Bufo americanus charlesmithi*) in a shallow temporary pool subjected to extended exposure to solar radiation. The water became very warm by mid-afternoon, and some of the tadpoles possessed an atypical greenish coloration (Fig. 1). The tadpoles were late stage, and some of them exhibited well-developed legs. The pool, located in ruts of a logging road near Crossett, Ashley County, Arkansas, USA, was examined from 5-10 June 2000. Microscopic examination (60X) of live tadpoles from the pool revealed clusters of biflagellated green organisms, identified as *Chlorogonium*, scattered as greenish blotches over the skin. Individuals of this alga were observed actively flagellating to maintain a position oriented to the skin of the tadpole (Fig. 2). The distribution of *Chlorogonium* generally followed the pattern of cutaneous blood vessels on the dorsal surfaces of the legs, tail, and lateral body wall.

Rates of cellular processes are temperature dependent (Q<sub>10</sub> effects) to maximal values between 25-40°C; therefore, CO<sub>2</sub> uptake from warmer water may occur faster than atmospheric replenishment and cause carbon depletion (Reynolds 1984). In consideration of the warmth of the pool and the likely stresses faced by both organisms, we hypothesized that the algae and tadpoles were in a relationship whereby metabolic gases evolved by each were being reciprocally absorbed.



**Figure 1.** Tadpoles of the Dwarf American Toad (*Bufo americanus charlesmithi*) with symbiotic algae visible as a green haze on the surface of the skin. Photograph by Stanley E. Trauth.

### MATERIALS AND METHODS

If increased temperatures cause respiratory stress on tadpoles and coincidentally stresses algae starved for CO<sub>2</sub>, a relationship beneficial to both organisms would be a reasonable strategy for the continued survival of each species. At the field site, temperatures were measured during the afternoon (after the ambient temperature peak), and observations were made on the behavior of tadpoles at that time. The ruts forming the pool produced four deeper areas located essentially in quadrants, with the northwest and southwest pools somewhat shaded during most of the morning and the northeast and southeast pools fully exposed to the sun to test the CTM of tadpoles without *Chlorogonium* as likely suppliers of O<sub>2</sub>, we placed 30 tadpoles of normal grayish-brown coloration (with little or no *Chlorogonium*) into an aquarium containing a thermometer, mud substrate, and 3 cm depth of water (obtained from and simulating the pools). The aquarium then was exposed to direct sunlight to evaluate behavior of the tadpoles with increasing temperature. Behavior was observed in four separate trials of this experiment.



**Figure 2.** Scanning electron micrographs of *Chlorogonium* on the skin of tadpoles. Left photo shows a dense aggregation of *Chlorogonium* (scale bar = 10  $\mu\text{m}$ ); right photo shows an isolated individual (scale bar = 5  $\mu\text{m}$ ). Photomicrographs by S.E. Trauth.

In a separate experiment, 30 tadpoles with clearly evident and extensive patches of *Chlorogonium* were collected and placed into the same aquarium setup. The aquarium was positioned to be half in direct sun and half in shade to investigate behavior of the tadpoles within a thermally variable environment. Tadpoles initially were placed on the sunny side of the tank.

### RESULTS

The deepest portion of any pool was 8 cm (southeast pool). On 7 June (1700 hr) the maximum temperature was 38°C, and all tadpoles showed normal flight responses to disturbance (a slow approach to within 1 m of the pools caused evasive behavior by the tadpoles.)

On 8 June (1430 hr), previously green tadpoles in the southwest pool had died when the temperature had reached 44°C. Those tadpoles no longer were green, indicating that the *Chlorogonium* had either died or had left the carcasses. Tadpoles in the better-shaded northwest pool behaved normally at 38°C, whereas those in the unshaded northeast pool were slower to move (i.e., a touch of the water was required to elicit evasive action). None of the tadpoles in this pool was dead or even exhibited difficulty with balance at 42.5°C. In the unshaded southeast pool at 41°C, the tadpoles moved only upon disturbance of the surface of the water. Moreover, in the southeast pool, a cluster of about 50 tadpoles scattered upon direct disturbance (tadpoles that were slowly touched), but they quickly returned to the 8 cm deepest portion of the pool.

In the aquarium experiment with brown tadpoles, normal behavior was observed up to a temperature of 38°C, but at 39°C a reduced flight response to approach was observed. At 39.5°C, tadpoles demonstrated difficulty with righting behavior, their locomotor activity appeared to be compromised, and their mobility was decreased. Some tadpoles appeared to be dying at this temperature, and death was certain at 40°C. Results were consistent in each of four replications of this experiment.

After water temperature in the insulated portion of the aquarium with green tadpoles had reached 40°C, just above the previously determined CTM temperature of 39.5°, only 18 of 30 individuals (60%) had adjusted their orientation into the shaded area, but at 44°C all tadpoles moved to the shaded microhabitat of the aquarium (36°C). Field observations already had shown that 44°C exceeded the critical maximum for tadpoles supported by a coat of *Chlorogonium*.

### DISCUSSION

Adult amphibians tend to seek temperatures within the range of 10-30°C (Brattstrom 1963), but tadpoles may develop more rapidly when in warmer temporary pools (for CTMs of various anuran larvae, see Ultsch et al. 1999). The high CTMs of toads help them survive in warmer conditions and shortens the time required for development, thereby promoting metamorphosis prior to desiccation of the habitat (Noland and Ultsch 1981). Tadpoles demonstrate plasticity in development rate (Tejedo and Reques 1994; see Altig and McDiarmid 1999) and can accelerate

metamorphosis after detecting reduction of water volume (Denver et al. 1998).

Vernal pools exposed to direct sunlight and elevated temperatures promotes faster development of tolerant species such as toads (*Bufo* sp.) by increasing metabolic rate (Noland and Ultsch 1981) based on  $Q_{10}$  effects (Parker 1967; Ultsch et al. 1999; Gatten et al. 1992). The thermal optimum for locomotion in toads hypothetically evolved in response to selective pressures on juveniles (Tracy et al. 1993).

Rates of oxygen consumption in tadpoles increase with higher temperatures (Parker 1967; Marshall and Grigg 1980; Ultsch et al. 1999), but water at higher temperature holds a lower concentration of gases (Randall et al. 1997). Although tadpoles are tolerant to warmer temperatures, the  $O_2$  deficits can lead to respiratory distress and death (see Ultsch et al. 1999). Under conditions of low  $O_2$ , tadpoles of some species can supplement oxygen intake by gulping air, but the late development of the lungs precludes this in *Bufo* (Duellman and Trueb 1994).

Consumption of  $O_2$  increases sharply prior to metamorphosis (Feder 1982), thus the warmer water contains less  $O_2$  at a time when more may be needed. Even after acclimation to warmer temperatures (Wilson et al. 2000), the CTM of tadpoles of most anuran species is 38-40°C (Duellman and Trueb 1994; Noland and Ultsch 1981), with a few exceptions above 41°C in species that develop in xeric or tropical habitats (Brown 1969).

The rate of photosynthesis tends to increase with increases in temperature up to an optimum temperature, after which it decreases rapidly, partly limited by the availability of inorganic carbon (Davison 1991). Growth rate of algae slows in stagnant cultures because the rate of diffusion of  $CO_2$  from the air becomes limiting (Fogg 1975), partly because  $CO_2$  diffuses  $10^4$ X faster in air than in water (Graham and Wilcox 2000). The green algae (Chlorophyceae, including *Chlorogonium*) tend to dominate in temperatures of 15-30°C, but are replaced by blue-greens (Cyanobacteria) above 30°C (DeNicola 1996). Thermophilic algae thrive best in waters rich in  $CO_2$ , where conditions necessary for maintaining high rates of photosynthesis are met (Fogg 1969). We believe the pattern of association and distribution of *Chlorogonium* over the skin of tadpoles allowed maximum potential for uptake of otherwise limiting  $CO_2$  released via cutaneous respiration by the tadpole.

The relatively small size of the *Chlorogonium* specimens also could indicate stress. The mean length of 103 individuals taken from the tadpoles was 13.4  $\mu$ m (range 7-22), and width ranged only between 1.5-3  $\mu$ m. The normal measurements from species known to occur in the United States ranges from 19-59  $\mu$ m in length and 5-18  $\mu$ m in width (Nozaki et al. 1998). Smaller cells result in a higher surface/volume ratio, which could help maximize absorption in a  $CO_2$ -limited environment (Foy 1980; Reynolds 1984).

A precedent for a mutualistic relationship between a flagellated green alga and amphibian larvae exists with *Oophila amblystomatis*, which is symbiotic within the egg jellies of spotted salamanders, *Ambystoma maculatum* (Gilbert 1942). The eggs of the salamander are surrounded by a gelatinous matrix, which impedes diffusion of  $O_2$  toward the embryo and  $CO_2$  from the embryo. *Oophila*, endogenous within the membranes of the eggs, absorbs the  $CO_2$  produced during metabolism of the embryo, and the  $O_2$  produced by the alga is available to the developing embryo (Pinder and Friet 1994). It has been argued that this arrangement leads to improved growth and survival of embryos due to decreased respiratory stress and enhances growth of the alga in a protective gelatinous medium.

Our experiments demonstrated that the CTM at which tadpoles of *Bufo americanus* could survive independently was 39.5°C. In a heat-stress-inducing environment, however, the CTM could be expanded by over 3°C (to about 43°C) in the presence of a photosynthetic mutualist, such as *Chlorogonium*. Considering these phenomena, we hypothesize that the *Chlorogonium* and tadpoles were exhibiting a facultative symbiosis in which tadpoles gained  $O_2$  produced via photosynthesis adjacent to the skin, and concomitantly the *Chlorogonium* received the metabolic  $CO_2$  evolved from the tadpoles.

We suggest that future determinations of CTMs should consider factors in addition to temperature due to interactions. Although not investigated, we found similar algal accumulations on tadpoles of gray treefrogs (*Hyla versicolor*) and cricket frogs (*Acris crepitans*) at other locations within Arkansas.

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