

## Thermal Buffering of Microhabitats is a Critical Factor Mediating Warming Vulnerability of Frogs in the Philippine Biodiversity Hotspot

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### ABSTRACT

Species may circumvent the impacts of climate warming if the habitats they use reduce ambient temperature. In this study, we identified which frog species from a tropical montane rain forest in the Philippines may be vulnerable to climate warming. To do so, we selected five anuran species that utilize four breeding habitats and identified the sensitivity and exposure of tadpoles and direct-developer eggs to heat by measuring their critical thermal maximums ( $CT_{max}$ ) and the habitat-specific temperatures they experience. Our study species included two direct-developer frogs—one species that lays its eggs on exposed leaves, and another that lays its eggs in ferns—and three species that produce aquatic free-swimming tadpoles—two stream breeders, and one phytotelm (tree hole) breeder. We compared thermal tolerances derived from microclimates of breeding habitats with tolerances derived from macroclimate (*i.e.*, non-buffered air temperature taken from the rain forest canopy). We also examined whether differences in  $CT_{max}$  existed across life-history stages (egg, metamorph/young-of-year, and adult) for the two direct-developer frog species. Habitats buffered ambient temperature and expanded thermal tolerances of all frog species. We found that direct-developers, however, are more vulnerable to increased temperatures than aquatic breeders—indicated by their high sensitivity to temperature, and exposure to high temperatures. Direct-developer eggs were more sensitive to warming than both metamorph and adult life-history stages. Thermally buffered microhabitats may represent the only protection against current and impending climate warming. Our data highlight the importance of considering sensitivity and exposure in unison when deciphering warming vulnerability of frogs.

Abstract in Spanish is available in the online version of this article.

*Key words:* amphibian; climate change; critical thermal maximum; global change; guild; life-history stage; thermal tolerance.

GLOBAL CLIMATE CHANGE WILL UNDOUBTEDLY THREATEN BIODIVERSITY (Williams *et al.* 2003, Thomas *et al.* 2004). Climate warming has triggered numerous ecological responses (Parmesan 2006) that include species range shifts (Chen *et al.* 2011), decreased fitness in adults and offspring (Derocher *et al.* 2004), and even a global reduction in species' body size (Sheridan & Bickford 2011). Also relevant are animals' physiological constraints to temperature (Bernardo *et al.* 2007, Calosi *et al.* 2008). Many species have highly defined thermal optima with limited potential to acclimate to elevated temperatures (Tewksbury *et al.* 2008, Huey *et al.* 2009). If temperatures continue to rise as predicted (Sokolov *et al.* 2009), many species, particularly ectotherms, will experience detrimental, if not fatal, physiological responses (Sinervo *et al.* 2010).

Physiological upper thermal limits are expected to increase at a slower rate than environmental temperatures (Compton *et al.* 2007, Deutsch *et al.* 2008, Huey & Tewksbury 2009). As such, many ectothermic species from tropical areas face high risks of extinction due to climate change (Compton *et al.* 2007, Deutsch

*et al.* 2008, Sinervo *et al.* 2010, Tewksbury *et al.* 2008) especially if they are unable to find refuge from extreme temperatures (Shoo *et al.* 2011b). Cool refugia serve as thermal buffers, and may allow species with relatively low thermal tolerances living in hot habitats to evade exposure to life-threatening temperatures (Kearney *et al.* 2009, Shoo *et al.* 2010) (thermal tolerances are calculated by subtracting the maximum environmental temperature that a species experiences [ $T_{max}$ ] from the temperature at which an individual loses normal motor functions *i.e.*, critical thermal maximum [ $CT_{max}$ ]). Whether or not habitats effectively buffer against hot temperatures remains relatively unknown, and a better understanding will provide important implications for conservation management and climate change mitigation strategies.

Under an integrated framework for assessing the vulnerability of species to climate change, factors that determine exposure and govern sensitivity must be identified (Williams *et al.* 2008, Shoo *et al.* 2011a). Traits that are intrinsic to a species (*e.g.*,  $CT_{max}$ ) and factors that are extrinsic to a species (*e.g.*,  $T_{max}$ ) are strong determinants of its sensitivity to climate warming. Under this premise, understanding both sensitivity and exposure in unison is critical for evaluating future warming tolerance (WT) and prioritizing conservation actions under climate change (Williams

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*et al.* 2008, Shoo *et al.* 2011a). To date, however, little research exists in way of identifying these two key components for assessing vulnerability to climate warming, especially in the understudied tropics of Southeast Asia.

Under the premise that the vulnerability of a species to climate warming is directly tied to its sensitivity and the exposure it experiences in its habitat (Williams *et al.* 2008), we derived two primary goals for our study: (1) identify the critical thermal maximum of frog larvae from four distinct breeding habitats (*i.e.*, sensitivity), and (2) identify the extent to which breeding habitats used by frogs (specifically the larval life-history stage) buffer ambient temperature (*i.e.*, exposure). On the basis of these two metrics, we can deduce the vulnerability of specific life-history stages to future warming, which we hereafter term ‘warming vulnerability’. We conducted our study in the Philippines—an archipelago with some of the highest species richness and endemism per area on Earth. We chose an isolated mountain site, Mt. Banahaw (approx. 10,000 ha) in Luzon, to examine the thermal tolerance of larvae for five endemic frog species. No study to date, however, has examined potential sensitivities of amphibians to climate warming in the Philippines. Our study location is completely isolated from other contiguous mountain ranges (*e.g.*, Sierra Madres in the northern Philippines). Consequently, species at this mountain site have limited options for evading climate warming via dispersal.

Lastly, the majority of literature regarding thermal tolerances of ectotherms is derived from adult life-history stages. As amphibians undergo multiple life-history stages, threats (*e.g.*, hot temperatures) that may not threaten one life-history stage might dramatically affect another (Becker *et al.* 2007). We therefore examined whether warming vulnerability varied by life-history stage for select species. [This article was corrected on 20 June 2013. The previous sentence was moved from the abstract.]

## METHODS

**STUDY REGION.**—The Philippines is recognized as an important global biodiversity hotspot, due to its exceptionally rich endemic fauna (Diesmos & Brown 2011). Almost 80 percent of its amphibians and reptiles are found nowhere else in the world. Because the majority of these species are forest-dependent, however, they are highly threatened by loss of primary forest cover, which has been reduced by 63 percent during the 20th century (Heaney & Ragalado 1998). Due to extensive clearing of lowland forest, the little forested habitat that remains is confined to mountains (Peh *et al.* 2011).

We conducted our study on Mt. Banahaw in southern Luzon, the largest island in the Philippines. The site is characterized by lowland dipterocarp forest up to 800 m, dipterocarp and montane forest from 900 to 1700 m, and mossy and *Pinus* forest above 1700 m (Mallari *et al.* 2001). The topography of our study area in the Philippines is characterized by moderate to steep terrain and sandy clay loam soil (Banaticla & Buot 2005). The climate is marked by the absence of a distinct dry season with annual rainfall around 3100 mm and 85 percent relative humidity (PAGASA 2000).

**STUDY SPECIES AND LARVAE TYPE.**—Amphibians undergo varying modes of reproduction (Duellman & Trueb 1994); depending on the species, one mode may be more or less susceptible to climate warming. The two dominant modes of development in our study area are reproduction via eggs deposited in water that develop through multiple tadpole stages (*e.g.*, see Gosner 1960) and direct development, whereby frogs lay eggs in terrestrial jelly-like clutches with no free-living larval stage (*e.g.*, see Townsend & Stewart 1985). In our study area, nine stream and two phytotelm-breeding frog species reproduce via aquatic tadpoles, and six terrestrial frog species reproduce via direct-developing eggs. Of the 17 species available, we chose to use five species that used the four unique breeding habitats, represented both reproductive methods and were reliably encountered in our study area.

For this study, we identified the  $CT_{max}$  for larvae of these five species of frogs collected in tropical montane forest (900–1300 m asl) between the months of May and September, 2011. We identified four unique breeding habitats and chose one or more species that utilize each habitat: (1) Bird’s nest fern (BNF) breeders: direct-developing eggs laid by *Platymantis banahaw* in *Asplenium* bird’s nest ferns (BNFs). BNFs collect canopy debris due to their circular frond orientation and thus serve as a large area of aboveground humic soil. This fern-dependent frog species is endemic to Mt. Banahaw and occurs from 1100 m to a maximum of 1600 m asl within our study area; (2) Leaf breeders: direct-developing eggs laid on exposed leaves by *P. montanus*. This is a parental care species, whereby males guard and incubate eggs at night. Clutches are typically laid on the surface of a broad leaf at approximately 1 m aboveground. This species is endemic to Mt. Banahaw and occurs from 1250 m to 1900 m asl; (3) Phytotelm breeders: *Kaloula kalingensis* tadpoles found in phytotelmata. The phytotelm environment is typically a tree hole that forms a small pocket of water of varying depths, ranging from almost dry to several centimeters deep, depending on rainfall. This species is endemic to the Philippines and occurs from 50 m to 1600 m asl; and (4) Stream breeders: tadpoles of two species (*Sanguirana luzonensis* and *Kaloula walteri*). The stream environment is characterized by aquatic pools that vary greatly in depth (from 12 cm to >200 cm), size (from 0.5 m to ~8 m wide), and flow depending on recent rainfall. Deep pools maintain relatively constant water depths throughout the year. *Sanguirana luzonensis* occurs from 0 m to 2000 m and *Kaloula walteri* occurs from 50 m to 900 m; both species are endemic to the Philippines (Diesmos & Brown 2011). We collected larvae of each species from six BNFs, two phytotelmata, two exposed leaves, and four stream pools.

**CRITICAL THERMAL MAXIMUMS.**—In order to stabilize  $CT_{max}$ , all field sampled tadpoles and direct-developer eggs were acclimated to a constant 22°C at our field base camp at 1100 m for a minimum of 4 d. This was a conservative time period for stabilizing  $CT_{max}$  (Hutchison 1961, Brattstrom 1968). Because  $CT_{max}$  estimates may vary as a function of methodology, we standardized  $CT_{max}$  estimates for all species experimented on in this study. Tadpoles were housed in an aquarium and fed lettuce leaf and crickets *ad libitum*. We maintained eggs by separating them in

shared containers by clutch and sprayed eggs with water on a daily basis to avoid desiccation.

The critical thermal maximum of ectothermic vertebrates provides a useful index for the thermal constraints of animals (Hutchison 1961, Hutchinson & Dupré 1992). We obtained upper critical thermal tolerances via Hutchinson dynamic methods (Lutterschmidt & Hutchison 1997b), whereby each individual was exposed to a constant temperature increase of 0.5°C/min until muscular spasms were observed. A spasm was defined as the combination of head over tail movement and/or lateral rotations while swimming or while being suspended in the egg. Heating experiments were conducted using a generator-run incubator.

Tadpoles were placed in individual containers filled with 60 mL of water and were gradually heated inside the incubator. We ended the experiment as soon as we observed muscular spasms. A k-type thermocouple temperature sensor (model #421502; Extech, Nashua, New Hampshire, U.S.A.) was placed inside the container to record the water temperature. Tadpoles were placed in a water-filled container at ambient temperature immediately following  $CT_{max}$  to enable recovery. Placed in individual containers, direct-developing eggs were gradually heated in the incubator until the onset of spasms. A k-type thermocouple temperature sensor was placed just beneath the jelly coating of the egg to record temperature. Following muscular spasms, we removed eggs from the incubator, sprayed them with water, and allowed them to cool at ambient temperature. Mass was recorded for all tadpoles and eggs prior to each experiment. We only included tadpoles between Gosner stages 26 and 38 (with the only variation being slight differences in limb development) (Gosner 1960) and direct-developer eggs between stage 9 and 12 (Townsend & Stewart 1985). Our staging criteria were equivalent for tadpoles and direct-developer eggs. Each individual was tested only once, and data from individuals that died ( $N = 7$ ) during experimentation were not included in analyses. Following heating experiments, all individuals were housed in a holding tank for 1–2 d and released.

**METAMORPH AND ADULT LIFE-HISTORY STAGES.**—To determine whether warming vulnerability varies by life-history stage, we repeated our heating experiments for metamorph and adult individuals for the two direct-developer species, *P. banabao* and *P. montanus*, as: (1) they are locally endemic to Mt. Banahaw and their ranges are therefore constrained to high elevations; (2) we observed that these two species were likely most vulnerable to increased temperature based on initial observations; and (3) behaviors such as parental care in direct-developer species may help circumvent high temperatures (e.g., reduce ambient air temperature by sitting on eggs). Thus, to determine if the adults will be able to provide such care for their eggs as temperatures increase, we must better understand their vulnerability in conjunction with their larvae. We applied the same heating and response criteria to both metamorph and adult life-history stages; each individual was exposed to a temperature increase of 0.5°C/min until muscular spasms were observed. We defined a spasm as the combination of erratic and uncontrolled body spasms. Onset of body spasms is an accurate method for identifying a

definitive  $CT_{max}$  in terrestrial amphibians (Lutterschmidt & Hutchison 1997a).

**ENVIRONMENTAL TEMPERATURES.**—We used Maxim ibutton (<http://www.maxim-ic.com/>) temperature loggers to determine the thermal profiles of each breeding habitat. We deployed temperature loggers: (1) under the fronds of four BNFs; (2) suspended approximately 1 m from the ground at two locations for leaf breeders; (3) at the deepest bottom of two stream pools where tadpoles were collected; and (4) at the bottom of two phytotelm tree holes where tadpoles were collected. Loggers recorded temperature data every 20 min. Duration of sampling temperature varied by breeding habitat: approximately 2 mo for BNFs (6/29–8/25), approximately two and a half months for leaf habitats (7/5–9/23), and approximately 1 mo for phytotelm and stream habitats (8/27–9/25). To identify the maximum potential ambient air temperature for our study area, we placed data loggers in the upper canopy of five trees at 1100 m (specific to phytotelm, stream, and BNF habitats) and five trees at 1300 m (specific to leaf breeders). The locations of canopy loggers were randomly selected within our study area. We suspended canopy loggers and leaf habitat loggers under a plastic funnel to shelter them from direct solar radiation and precipitation.

**ANALYSIS.**—We examined the relationship between habitat-specific temperatures and ambient air temperatures taken from the forest canopy. To achieve this objective, we created two scatter plots to compare: (1) the minimum temperature recorded daily in each habitat (e.g., minimum recorded of all 20 min observations) to the daily minimum ambient air temperature recorded from the adjacent forest canopy; and (2) the maximum temperature recorded daily in each habitat to the daily maximum ambient air temperature recorded from the adjacent forest canopy. If habitats fail to buffer temperature, points will align along a line of equivalency between the x and y axis—a line with a y-intercept of 0 and a slope of 1 (herein ‘equivalency line’). If habitats reduce temperature, points will occur below the equivalency line; if they are warmer than air temperature, they will occur above the equivalency line.

We calculated WT of a species by subtracting the maximum environmental temperature that it experiences ( $T_{max}$ ) from its  $CT_{max}$ . A positive thermal tolerance indicates the number of degrees in temperature that the climate must warm before a species begins to fail physiologically. A negative thermal tolerance indicates that the environmental temperature has surpassed a species’  $CT_{max}$  and should cause physiological failure and/or death. In other words, a small WT predicts a low tolerance for warming; a large WT predicts a high tolerance.

We calculated a single overall naïve thermal tolerance for each species. Naïve warming tolerance ( $WT_n$ ) reveals the thermal tolerance of animals in the absence of habitat that buffers ambient air temperatures, while habitat-specific thermal tolerance ( $WT_h$ ) indicates realized thermal tolerance. Naïve thermal tolerances were derived by subtracting the average maximum temperature from the average  $CT_{max}$  for each species. The average maximum temperature was derived from loggers placed in five

local canopy trees at 1100 m (to correspond to phytotelm, stream, and BNF breeders), and in five canopy trees at 1300 m (to correspond with leaf breeders). We derived a habitat-specific thermal tolerance by subtracting the average maximum temperature across loggers in species-specific breeding habitats from the average  $CT_{max}$  for each species (see Table 1). We conducted four sets of analyses of variance (ANOVA) models to test for statistical differences in  $CT_{max}$  among species and among life-history stages, thermal tolerance derived from naïve air temperatures, and thermal tolerances derived from habitat-specific temperatures among the four breeding habitats. We used the single highest value averaged across data loggers to determine thermal tolerances for each individual. To further explore differences in  $CT_{max}$ , naïve thermal tolerance, and habitat-specific thermal tolerances among breeding habitats, we used a Tukey’s ‘Honestly Significant Difference’ (HSD) method to conduct pairwise comparisons among breeding habitats in R (v. 2.12.2). This method accounts for potential inflated probabilities caused by multiple comparisons, which can cause spurious error in determining statistical significance.

To explore the relationship between animal body mass and  $CT_{max}$ , we performed a linear regression analysis with our response variable as  $CT_{max}$  and predictor variable as body mass. We conducted this analysis for each breeding habitat. Both  $CT_{max}$  and body mass were log-transformed to normalize data. We checked all models for heteroscedasticity via the studentized Breusch-Pagan test. All models were non-heteroscedastic.

**RESULTS**

**SENSITIVITY.**—Species from stream and phytotelm habitats had higher  $CT_{max}$  than species from BNF and leaf habitats (stream:  $37.8^{\circ}C \pm 0.8$  (SD throughout), phytotelm:  $38.3^{\circ}C \pm 0.8$ , BNF:  $34.8^{\circ}C \pm 1.9$ , and leaf:  $33.5^{\circ}C \pm 0.3$ ).

According to analysis of variance,  $CT_{max}$  differed for species that breed in each of the four habitats ( $F_{3,58} = 33.10, P < 0.001$ ; see Table 1 for means and Table 2 for pairwise comparisons). Specifically, our Tukey’s HSD test indicates that  $CT_{max}$  differed among all breeding habitats, except between leaf and BNF breeders and between phytotelm and stream breeders (Table 2).

We explored whether body mass of eggs/tadpoles predicts  $CT_{max}$  in each habitat type using linear regression models. There

TABLE 2. Multiple comparisons among critical thermal maximums, naïve thermal tolerances, and habitat-specific tolerances for individuals found in four breeding habitat types (egg/tadpole life-history stage). Provided are the upper and lower confidence intervals on the differences between the means of the levels of each factor with the specified family-wise probability of coverage. The intervals are based on the Studentized range statistic, Tukey’s ‘Honestly Significant Difference’ method. P-values of  $<0.05$  are significant.

Comparison	Difference	Lower	Upper	P-value adjusted
<b><math>CT_{max}</math></b>				
Leaf – BNF	–1.27	–2.96	0.42	0.202
Phyto – BNF	3.57	2.06	5.08	<b>&lt;0.001</b>
Stream – BNF	3.06	1.99	4.12	<b>&lt;0.001</b>
Phyto – Leaf	4.84	2.82	6.83	<b>&lt;0.001</b>
Stream – Leaf	4.33	2.66	6.01	<b>&lt;0.001</b>
Stream – Phyto	–0.51	–2.01	0.99	0.805
<b>Naïve</b>				
Leaf – BNF	–0.17	–1.86	1.52	0.993
Phyto – BNF	3.57	2.06	5.08	<b>&lt;0.001</b>
Stream – BNF	3.06	1.99	4.12	<b>&lt;0.001</b>
Phyto – Leaf	3.74	1.72	5.73	<b>&lt;0.001</b>
Stream – Leaf	3.23	1.56	4.91	<b>&lt;0.001</b>
Stream – Phyto	–0.51	–2.01	0.99	0.805
<b>Habitat Specific</b>				
Leaf – BNF	–6.77	–8.46	–5.08	<b>&lt;0.001</b>
Phyto – BNF	3.12	1.61	4.63	<b>&lt;0.001</b>
Stream – BNF	5.36	4.29	6.42	<b>&lt;0.001</b>
Phyto – Leaf	9.89	7.90	11.88	<b>&lt;0.001</b>
Stream – Leaf	12.13	10.46	13.81	<b>&lt;0.001</b>
Stream – Phyto	2.24	0.75	3.74	<b>0.001</b>

were no significant relationships between body mass and  $CT_{max}$  for any of the species within each breeding habitat type: BNF ( $F_{1,21} = 1.969, r^2 = 0.042, P = 0.175$ ; Regression), leaf ( $F_{1,4} = 1.709, r^2 = 0.124, P = 0.261$ ), phytotelm ( $F_{1,6} = 0.091, r^2 = -0.149, P = 0.773$ ; Regression), or stream ( $F_{1,23} = 1.712, r^2 = 0.029, P = 0.204$ ; Regression).

**EXPOSURE.**—Temperatures for all four breeding habitats were lower than the ambient air temperatures derived from the forest canopy (Fig. 1). We compared daily habitat-specific tempera-

TABLE 1. Critical Thermal Maximum ( $CT_{max}$ ) of five frog species (egg/tadpole life-history stage) from a sub-montane rain forest in the Philippines.  $T_{max}$  indicates the maximum averaged temperature observed for each species’ habitat. Overall naïve and habitat-specific warming tolerance is  $CT_{max} - T_{max}$  of air ( $WT_n$ ) and habitat ( $WT_h$ ).  $T_{max}$  for naïve air temperature at 1100 m is  $31.1^{\circ}C$  and at 1300 is  $30.0^{\circ}C$ .

Strategy	Breeding habitat	Species	N	Mass (SD) (g)	$CT_{max}$ (SD)	$T_{max}$	$WT_n$	$WT_h$
Direct Dev.	BNF	<i>P. banabao</i>	23	0.44 (0.2)	34.8 (2.0)	22.3	3.7	12.2
Direct Dev.	Leaf	<i>P. montanus</i>	6	0.13 (0.02)	33.5 (0.3)	27.8	3.5	5.7
Aquatic Tadpole	Phytotelm	<i>K. kalingensis</i>	8	0.10 (0.2)	38.3 (0.8)	22.8	7.2	15.5
		<i>S. luzonensis</i>	23	0.31 (0.37)	37.8 (0.8)	20	6.7	17.8
Aquatic Tadpole	Stream	<i>K. walteri</i>	2	0.03 (0)	38.5 (1.3)	20	7.4	18.5
		All Species (stream)	25	0.29 (0.4)	37.8 (0.8)	20	6.7	17.8

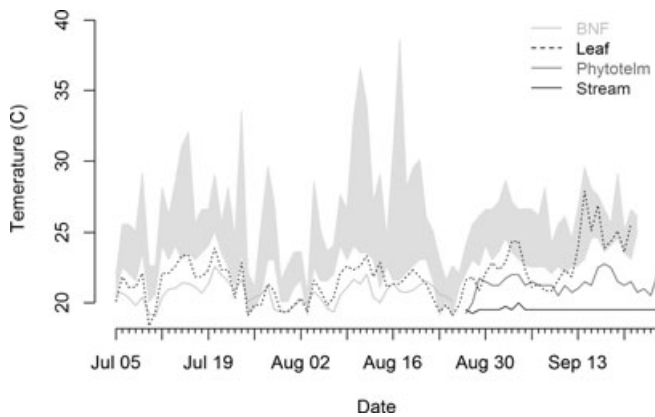


FIGURE 1. The relationship between non-buffered ambient and habitat-specific temperatures. The shaded area indicates the minimum and maximum range in daily ambient temperature taken from the canopy of 10 rain forest canopy trees at 1100 m and 1300 m asl. Lines indicate habitat-specific temperatures for BNF, leaf, phytotelm, and stream habitats. Habitat specific temperatures were derived from daily maximum temperatures averaged across locations.

tures to ambient temperatures—BNF, leaf, phytotelm, and stream habitats were cooler than minimum ambient temperatures 73 percent, 60 percent, 93 percent, and 93 percent of the time, respectively, and were cooler than maximum ambient temperatures 100 percent, 98 percent, 100 percent, and 100 percent of the time, respectively (*i.e.*, below the equivalency line of Fig. 2). On average, all four habitats were cooler than the minimum temperature: BNF habitats by  $0.37 (\pm 0.8)^\circ\text{C}$ , leaf habitats by  $0.2 (\pm 0.4)^\circ\text{C}$ , phytotelm habitats by  $0.7 (\pm 0.6)^\circ\text{C}$ , and stream habitats by  $0.7 (\pm 0.6)^\circ\text{C}$ . In comparison, on average, all four habitats were cooler than the maximum temperature: BNF habitats by  $5.1 (\pm 3.4)^\circ\text{C}$ , leaf habitats by  $3.2 (\pm 2.2)^\circ\text{C}$ , phytotelm habitats by  $3.7 (\pm 1.0)^\circ\text{C}$ , and stream habitats by  $5.9 (\pm 1.4)^\circ\text{C}$ .

**WARMING VULNERABILITY.**—We compared the thermal tolerance of frogs, derived from habitat-specific temperatures (thermal tolerance =  $CT_{\max} - T_{\max}$  of habitat), to naïve thermal tolerances, derived from ambient air temperature recorded in the rain forest canopy (thermal tolerance =  $CT_{\max} - T_{\max}$  of ambient). The naïve thermal tolerances derived from ambient air temperatures in the forest canopy were lower than tolerances derived from habitat-specific temperatures (Fig. 3). According to our ANOVAs,  $WT_n$  and  $WT_h$  significantly differed for species that breed in the four breeding habitats ( $F_{3,58} = 27.77$ ,  $P < 0.001$ ;  $F_{3,58} = 145.27$ ,  $P < 0.001$ ; respectively; see Table 1 for means and Table 2 for pairwise comparisons) (Fig. 3). Based on Tukey's HSD test, naïve thermal tolerance differed among breeding habitats, except between leaf and BNF breeders and between phytotelm and stream breeders (Table 2). Comparisons among habitat-specific tolerances revealed that  $WT_h$ s differed among breeding habitats, except between phytotelm and stream breeders.

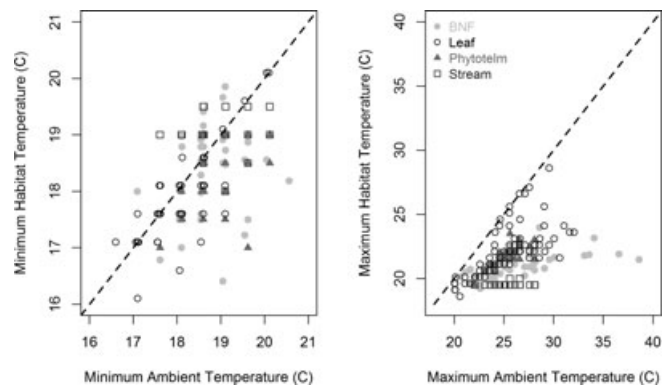


FIGURE 2. Relative difference in daily temperature extremes between ambient air temperature (macroclimate) and habitat-specific temperatures (microclimate). Daily minimums and maximums were derived from 20 min temperature readings. Ambient temperatures are derived from loggers placed in the canopy (*i.e.*, ambient air temperature) of 10 rain forest trees at 1100 and 1300 m asl. Habitat-specific temperatures are derived for bird's nest fern (BNF), leaf, phytotelm, and stream habitats. The dashed line indicates temperature equivalency between ambient and habitat-specific temperatures.

**LIFE-HISTORY STAGES.**— $CT_{\max}$  varied by life-history stage for the two direct-developer species. Notably,  $CT_{\max}$  for the egg stage was lowest for both species. The  $CT_{\max}$  of *P. montanus*, the leaf-breeding species, increased with each subsequent life-history stage (*i.e.*, adults had the highest  $CT_{\max}$ ), whereas the metamorph stage had the highest  $CT_{\max}$  for *P. banabao*, the BNF-breeding species (Table 3). The  $CT_{\max}$  significantly differed among life-history stages for *P. banabao* and *P. montanus* ( $F_{1,48} = 22.1$ ,  $P < 0.001$ ;  $F_{1,30} = 15.42$ ,  $P < 0.001$ ; respectively).

## DISCUSSION

We show that breeding habitats buffer ambient air temperature, expanding the  $WT_h$ s of frogs by  $\sim 2$ – $11^\circ\text{C}$ . Without the buffering capacity of specific habitats, direct-developer eggs laid in BNF and leaf habitats may experience temperatures close to their critical thermal maxima, with low margins for tolerating future elevated temperatures. Our findings prompt the inclusion of microclimatic (habitat-specific) variables in assessing thermal tolerances of animals when developing predictive models of climate warming (Williams *et al.* 2008).

**SENSITIVITY AND EXPOSURE.**—We considered two metrics indicative of warming vulnerability in our study—sensitivity (*i.e.*,  $CT_{\max}$ ) and exposure (*i.e.*, temperature) (Williams *et al.* 2008). In our study area, sensitive species were exposed to the warmest temperatures (high sensitivity with high exposure) and less sensitive species were exposed to the coolest temperatures (low sensitivity with low exposure). Sensitivity of frog larvae differed by species— $CT_{\max}$  was highest for stream breeding species (range:  $37.8$ – $38.5^\circ\text{C}$ ) and lowest for direct-developer species (range:  $33.5$ – $34.8^\circ\text{C}$ ). A comparison of mass and  $CT_{\max}$  for larvae of each species showed no

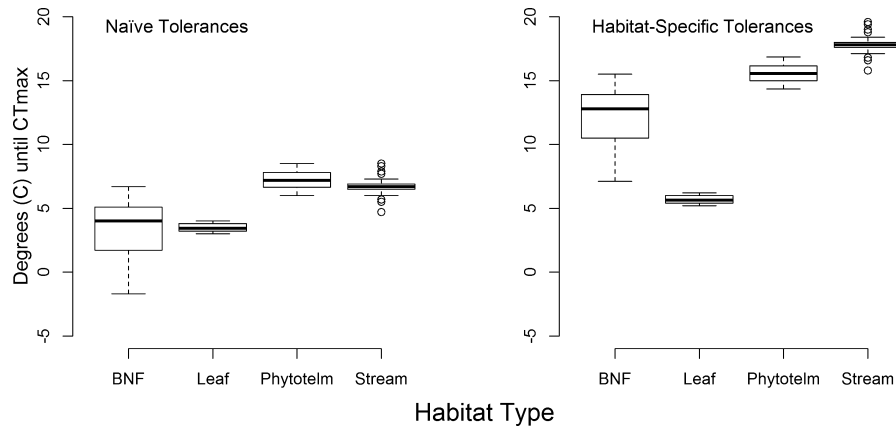


FIGURE 3. A boxplot for naïve warming tolerances (*left*) and habitat-specific warming tolerances (*right*) for frog species that breed in four different breeding habitats found on Mt. Banahaw in the Philippines. Dark horizontal lines represent the median for each habitat, boxes with dotted hashes indicate the sample minimum, lower quartile, median, upper quartile, and sample maximum. Open circles indicate outliers.

TABLE 3. Critical Thermal Maximum ( $CT_{max}$ ) for multiple life-history stages of two direct-developer frog species (BNF and Leaf habitat) collected from a sub-montane rain forest in the Philippines.  $CT_{max}$  is derived from first observation of body spasms.

	<i>P. montanus</i>			<i>P. banabao</i>		
	Egg	Metamorph	Adult	Egg	Metamorph	Adult
N	3	9	17	12	8	17
$CT_{max}$ (SD)	33.5 (0.3)	36.2 (3.2)	37.4 (3.4)	34.8 (2.0)	41.7 (0.6)	36.2 (2.2)

statistical significance, suggesting that observed differences in sensitivity may be more influenced by physiological differences among species rather than morphology alone.

Canopy temperatures (*i.e.*, macroclimate) were almost exclusively warmer than associated habitat-specific temperatures suggesting that habitats buffer temperature and exposure based on macroclimate (*e.g.*, see Deutsch *et al.* 2008) alone may provide a misleading impression of vulnerability. For example, naïve tolerances (derived from canopy temperatures) between BNF and leaf breeders and between phytotelm and stream breeders did not differ. After accounting for habitat-specific exposure, however,  $WT_h$  (*i.e.*, warming vulnerability) of BNF breeders was significantly greater than  $WT_h$  of leaf breeders. Likewise,  $WT_h$  of stream breeders was significantly greater than for phytotelm breeders. Naïve thermal tolerances for the two direct-developer species only differed by 0.6°C, but when comparing habitat-specific thermal tolerances, the difference was much greater (6.5°C).

WARMING VULNERABILITY AND ITS CAVEATS IN THE CONTEXT OF CLIMATE CHANGE.—Although thermal tolerances for some species in our study appear to be quite large, we must consider a few factors to properly assess ‘true’ climate vulnerability: (1) We considered temperature as the primary driver of warming vulnerability in our study; however, we recognize that ‘true’ vulnerability can only be determined by complex interactions among numerous variables (Brook *et al.* 2008). For example,

water, in addition to temperature, strongly influences the physiology (*e.g.*, evaporative water and energy loss) of animals and therefore when considered in combination may provide a more holistic assessment of vulnerability (McCain & Colwell 2011). (2) The estimates in our study are conservative. Frogs may be negatively affected by environmental temperatures well before  $CT_{max}$  is realized. Animals will alter their behavior under sub-optimum temperatures and attempt to seek alternative habitats that are optimum (Vickers *et al.* 2011). This behavioral response occurs before temperatures reach  $CT_{max}$  (as discussed below, behavioral mitigation is not an option for some species in our study) and can severely impact populations (Huey & Tewksbury 2009). (3) Climate warming projections are typically based on mean temperatures. Extreme, above-average temperatures are capable of causing rapid population declines (Welbergen *et al.* 2008). Thus, extreme weather events may substantially increase the vulnerability of all species in our study area.

Vulnerability to future warming is highly dependent on a species’ behavior (Huey & Tewksbury 2009). Free-swimming tadpoles, especially stream tadpoles, are able to seek deeper, cooler temperatures within stream pools. Unlike tadpoles, direct-developing larvae are confined within eggs and habitat preferences are likely fixed as extensive surveys in our study area suggest that *P. banabao* and *P. montanus* are obligate BNF and leaf breeders (B. Scheffers, unpubl. data). Parental care of the leaf-breeding species, *P. montanus*, may circumvent hostile temperatures via cov-

ering and watering the eggs (Bickford 2004). However, daytime mitigation of hot temperatures is also unlikely, as adult frogs only guard eggs at night. The elimination of a single life-history stage from climate warming may cause substantial population declines.

Behavior aside, eggs of this direct-developing species begin body spasms at 34°C, only a few degrees above ambient  $T_{max}$ . Thus, larvae of *P. banahaw* live close to their physiological limits, with little opportunity for behavioral mitigation. This suggests that the  $CT_{max}$  of larvae may be an important determinant of range limits (Sunday *et al.* 2012), especially considering the limited scope for behavioral or evolutionary adaptation to alter the status quo (Monasterio *et al.* 2011).

According to our study, the egg life-history stage is most sensitive to hot temperatures for direct-developer species. Literature regarding thermal tolerances of ectotherms is confined to the adult life-stage even though susceptibility to threats may vary by life-history stage (Becker *et al.* 2007). Thus, without considering all life-history stages, the true vulnerability of ectotherms to climate warming may be difficult to discern.

The species in our study are globally and locally limited in distribution. All are endemic to the Philippines, and two species are endemic to Mt. Banahaw. Species with restricted geographic ranges have limited capacity to adjust physiologically (Brattstrom 1968). Furthermore, Mt. Banahaw is an isolated mountain completely surrounded by deforested lowlands (below 700 m asl.), thus limiting the dispersal potential of these species. Microhabitat temperatures should be a critical component when considering the impacts of climate change, as alternative habitats are extremely limited for range-restricted species—particularly montane species (Ohlemüller *et al.* 2008).

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