

**Jason J. Kolbe, Michael R. Kearney, and Richard Shine. Modeling the consequences of thermal trait variation for the cane toad invasion of Australia.**

*Ecological Applications*

Appendix A: A description of previous species distribution modeling for cane toads in Australia.

The following is a brief review of species distribution models (SDM) for cane toads in Australia (also see van Beurden 1981; Floyd 1983; Sutherst et al. 1996; Urban et al. 2007; Kearney et al. 2008; reviewed in Phillips et al. 2008). A quasi-mechanistic SDM using moisture, temperature, and stress parameters estimated based on the entire native range predicted a cane toad distribution in Australia was largely consistent with its current range, except for an over-prediction into WA and under-prediction in interior QLD (Sutherst et al. 1996). Toads generally were most successful in hot, wet conditions, but sensitive to conditions too cold for development. The best-fit model had a low-temperature threshold for population growth parameter of 14°C, consistent with the 12-15°C range necessary for development and survival based on physiological studies (Floyd 1983). Stress induced by insufficient warmth for daily metabolism was suggested to be a more important limit for the range prediction than was excessive cold.

A recent correlative SDM using temporal subsets of locality data from the introduced range showed a recent expansion (after 1994) into areas exceeding a minimum

annual temperature of 12°C and maximum annual temperature of 37°C (Urban et al. 2007). Toads now occupy regions with minimum monthly temperatures less than 5°C and maximum monthly temperatures greater than 37°C. This study highlighted differences in range expansion dynamics between hotter and colder regions in Australia, and proposed evolutionary niche expansion to tolerate hotter temperatures during the rapid westward expansion in the north (NT). For southward expansion in the east (NSW), although toads inhabit colder regions than expected compared to the native range, expansion has slowed and no evidence exists for increased cold tolerance over the past 30 years. This stasis suggests a lack of an evolutionary response to colder southern conditions, and that toads may be reaching their limit in the south.

Similarly, predictions from a mechanistic SDM largely agree that thermal constraints, specifically limited breeding and adult foraging activity due to cold temperatures, limit the southern spread of cane toads in eastern Australia (Kearney et al. 2008). Toads never occupy areas where potential movement is predicted to be less than 500 m per year. Gradual decreases in temperature together with changes in the seasonal pattern of rainfall and humidity impose these constraints and may present an opportunity for evolution of cold tolerance and further range increase on the southern front (NSW). This SDM suggests that the cane toad's current range largely fills its predicted fundamental niche, and that no niche expansion is needed to explain tolerance of hotter temperatures in the north (NT), where the cane toad's range is predicted to be constrained by rainfall and evaporation.

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**Jason J. Kolbe, Michael R. Kearney, and Richard Shine. Modeling the consequences of thermal trait variation for the cane toad invasion of Australia.**

*Ecological Applications*

Appendix B: A description of the methodological details for the biophysical modeling used to predict the distribution of the cane toad in Australia.

We used a suite of biophysical/behavioral models (Niche Mapper™) to predict potential distributions (Tracy 1976, Porter et al. 1973, Porter 1989, Kearney & Porter 2004, Porter & Mitchell 2006). We briefly describe the modeling approach here; see Kearney et al. (2008) for applying this approach to the cane toad's distribution in Australia. Niche Mapper™ includes a microclimate model that uses macroclimatic data from weather stations (monthly air temperature, wind speed, cloud cover, relative humidity and rainfall derived from the ANUCLIM software package - see Kearney and Porter 2004) together with data on terrain to construct hourly estimates of microclimatic conditions directly relevant to organisms interacting with their environment. These conditions include solar and infra-red radiation, substrate temperature, soil temperature profiles, wind speed and air temperature profiles from the ground to 2 m, and relative humidity. The outputs from the microclimate model, in conjunction with morphological (body size, surface area), physiological (permeability of skin to water, respiration, thermal response curves), and behavioral (activity period, microhabitat selection, posture)

data from animals, are inputs for the ectotherm model, which solves coupled mass- and energy-balance equations to predict core body temperatures at hourly intervals throughout the day. Our modeling allowed only nocturnal activity in adult toads and assumed that toads were active whenever thermal conditions permitted (although in nature, toads may cease activity if the desiccation cost is high).

We ran separate simulations that either forced toads to remain on the surface in the shade (e.g., in dense vegetation) or allowed toads to use underground burrows if surface conditions were unsuitable. Cane toads often use shelter sites that buffer their exposure to ambient conditions (Schwarzkopf & Alford 1996; Seebacher & Alford 1999, 2002). The shelter microclimate for surface-restricted toads was assumed to be at the local air temperature with deep (90%) shade, high (90%) relative humidity, and minimal (0.1 m/s) wind. Burrow-using toads were assumed to initially retreat to 2.5 cm below ground and were simulated to move deeper if they experienced conditions at or below their CTMin. In these simulations, temperatures from the surface to 50 cm depth were calculated by the model, temperatures at 60 cm depth were taken from monthly means, and temperatures at 200 cm depth were taken from annual means. We also determined the maximum depth (up to 200 cm) needed to avoid the CTMin. Humidity underground was assumed to be 99% and wind speed was assumed to be 0.01 m/s. To conduct the ‘virtual transplants,’ we used daily measures of maximum and minimum air temperature, humidity, wind speed, and cloud cover for 2006 (the year prior to CTMin data collection) to drive the microclimate model.

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Jason J. Kolbe, Michael R. Kearney, and Richard Shine. Modeling the consequences of thermal trait variation for the cane toad invasion of Australia.

*Ecological Applications*

Appendix C: Figures showing population means for critical thermal minimum temperatures measured in the field and during the acclimation experiment, and hopping speeds at different temperatures for cane toads in Australia.

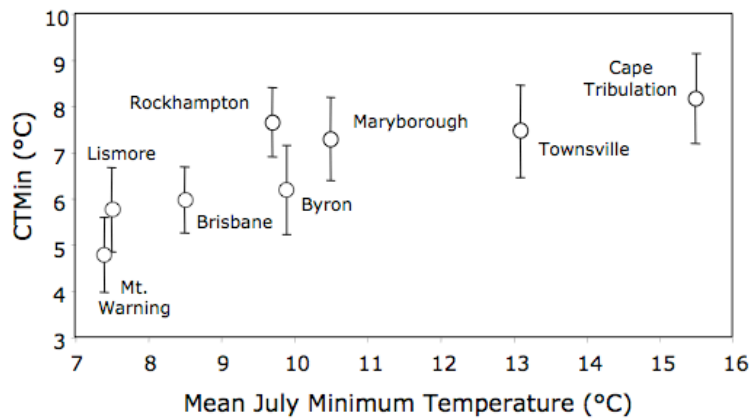


Fig. C1. Mean critical thermal minimums (CTMin) for cane toads sampled from eight sites along the eastern coast of Australia plotted against the mean July minimum temperature for each location. Bars indicate  $\pm 1$  SD.



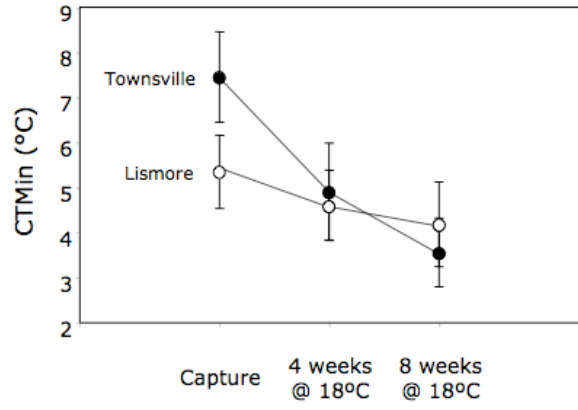


Fig. C2. Population means ( $\pm 1$  SD) for the critical thermal minimum (CTMin) in the Townsville ( $n = 22$ ; black circles) and Lismore ( $n = 27$ ; white circles) cane toad populations at capture and different acclimation times.

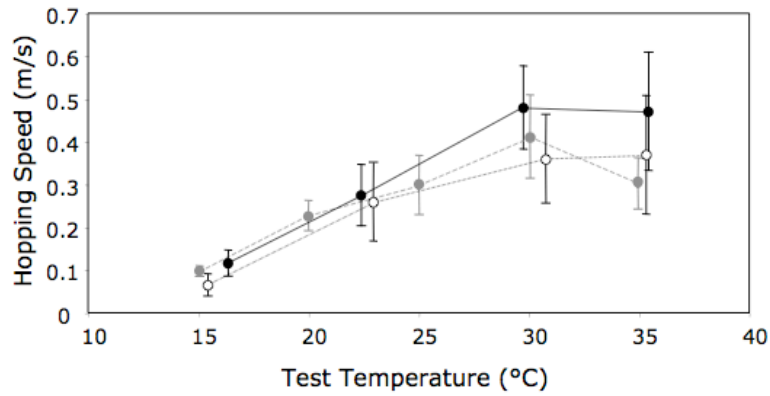


Fig. C3. Population means ( $\pm 1$  SD) for hopping speed (m/s) over 2 m at test temperatures ranging from 15 to 35°C for cane toads from Townsville ( $n = 22$ ; black circles), Lismore ( $n = 26$ ; white circles), and QLD and NT from Kearney et al. (2008;  $n = 44$ ; grey circles). Townsville and Lismore toads were acclimated at 18°C for 8 weeks prior to performance trials.

**Jason J. Kolbe, Michael R. Kearney, and Richard Shine. Modeling the consequences of thermal trait variation for the cane toad invasion of Australia.**

*Ecological Applications*

Appendix D: Table and figures showing results of biophysical modeling for the cane toad in Australia.

Table D1. Results of statistical tests comparing cold stress accumulation, the cumulative hours at or below the CTMin (non burrow-using toads) and required burrow depth for cold protection (burrow-using toads) between unacclimated and acclimated toads from Townsville and Lismore across the latitudinal extent of the eastern portion of the invasion (see Fig. 4). The analyses are paired t-tests for cold stress accumulation and Wilcoxon Signed Rank tests for burrow depth. Comparisons between unacclimated and acclimated stages are 1-tailed p-values.

	t	df	P
Non-burrowing simulations:			
QLD, acclimated vs. unacclimated	-1.84	8	0.051
NSW, acclimated vs. unacclimated	-1.63	8	0.071
QLD vs. NSW, both unacclimated	1.94	8	0.044
QLD vs. NSW, both acclimated	-1.11	8	0.851
	Z	df	P

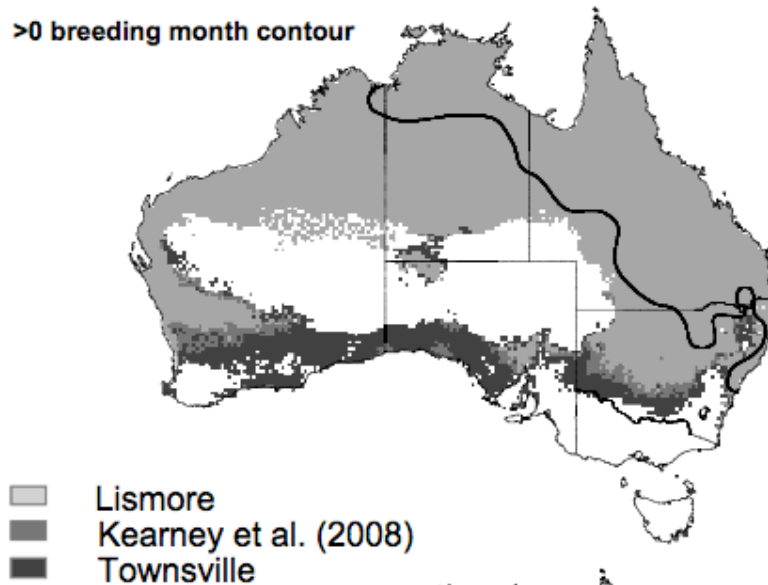
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Burrowing simulations:

QLD, acclimated vs. unacclimated	2.37	8	0.001
NSW, acclimated vs. unacclimated	1.82	8	0.033
QLD vs. NSW, both unacclimated	1.82	8	0.033
QLD vs. NSW, both acclimated	1.82	8	0.033

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**>0 breeding month contour**



■ Lismore  
■ Kearney et al. (2008)  
■ Townsville

**>2 breeding months contour**

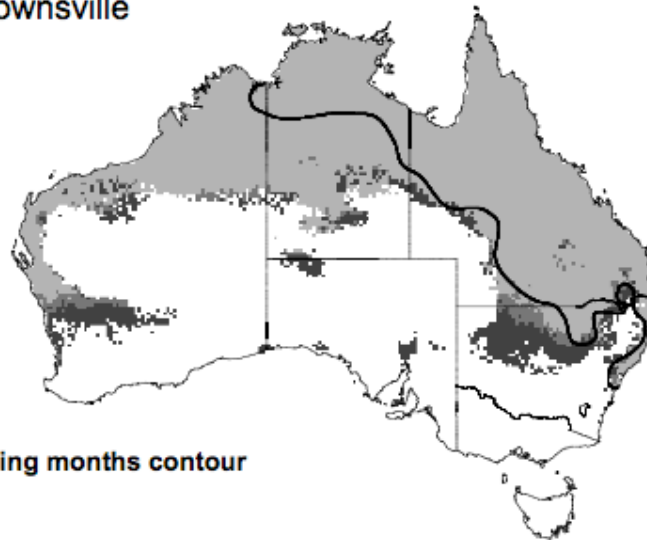


Fig. D1. Biophysical model predictions for the number of breeding months (>0 in the top panel and >2 in the bottom panel) using thermal performance curves for Lismore, Townsville, and QLD and NT populations from Kearney et al. (2008).

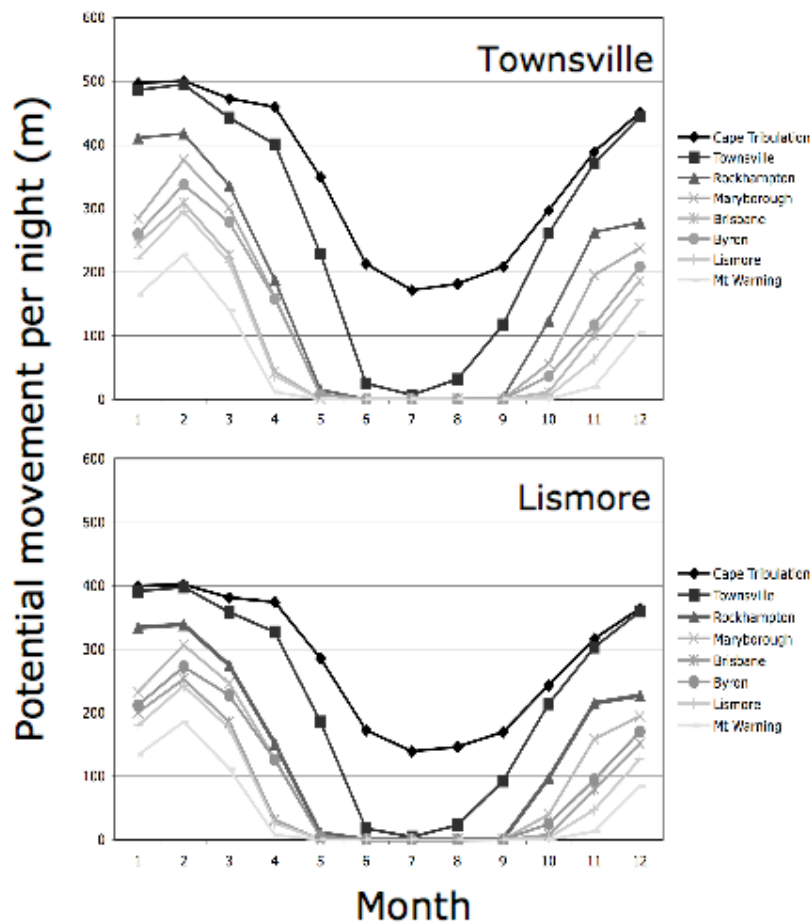


Fig. D2. Biophysical model predictions for average distance moved per night (m) by month using long-term monthly climatic averages at all eight sites sampled for field-measured CTMin. Simulations based on hopping speeds of the Townsville and Lismore populations are shown in separate panels. See Table 1 for latitude, elevation, and mean July minimum temperature for the eight sites.

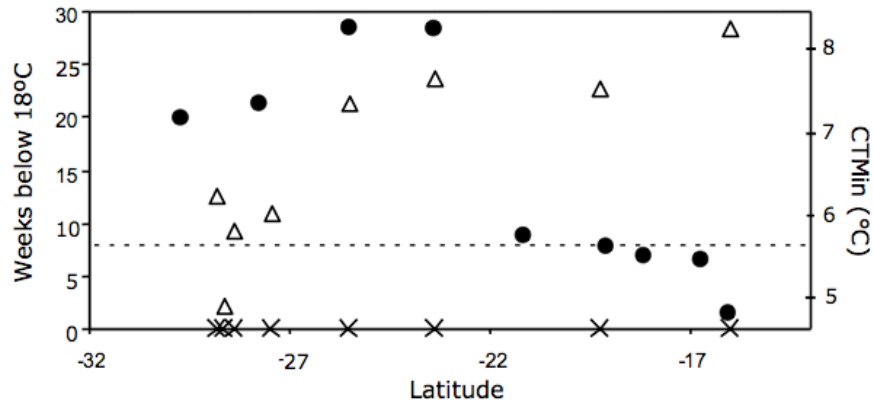


Fig. D3. Number of weeks in which body temperatures for toads predicted from biophysical models would be below 18°C using observed daily climate data from 2006 (black circles). Simulations were performed for nine weather stations along a latitudinal transect (16.9 to 29.7°S), including the six weather stations closest to our sites sampled for CTMin using field-caught toads. The dashed line indicates the 8-week mark, which corresponds to the length of low-temperature acclimation in the laboratory experiment. CTMin values sampled from eight sites along the eastern coast of Australia are also shown (open triangles). X's along the x-axis give the latitude of sites sampled for CTMin using field-caught toads (see Table 1).