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Michelle T. Christy

Colorado State University, Department of Fish, Wildlife, and Conservation Biology, Fort Collins, Colorado, USA

Julie A. Savidge

Colorado State University, Department of Fish, Wildlife, and Conservation Biology, Fort Collins, Colorado, USA

Richard Bischof

Norwegian University of Life Sciences, Ås, Norway

Gordon H. Rodda

U.S. Geological Survey, Fort Collins Science Center, Fort Collins Colorado, USA

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CAN TEMPERATURE BE USED AS A TOOL FOR LIMITING BROWN TREESNAKE INVASION VIA TRANSPORTATION PATHWAYS?

MICHELLE T. CHRISTY AND JULIE A. SAVIDGE, Colorado State University, Department of Fish, Wildlife, and Conservation Biology, Fort Collins, Colorado, USA

RICHARD BISCHOF, Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway

GORDON H. RODDA, U.S. Geological Survey, Fort Collins Science Center, Fort Collins Colorado, USA

Abstract: The use of extreme temperatures is one of the few chemical residue-free techniques available for control of pest species and has proven fast and effective in a variety of applications. We determined the upper and lower lethal temperatures for the brown treesnake. We also investigated whether gender, size, body condition and exposure time influenced survival at temperature extremes. Elevating snake core temperatures to 40°C and 41°C for one hour resulted in 99% mortality within seven days (40°C) and 100% mortality within one hour of exposure (41°C). Mortality decreased sharply to 51% for a one hour exposure to 39°C. Shorter, heavier snakes were more susceptible to extreme hot temperatures, but gender had no effect. The lower lethal temperature was established at a body temperature of -5°C held for one hour. Males were more susceptible to extreme cold than females, as were snakes with lower body condition. These results imply that brown treesnakes are slightly more vulnerable than many invertebrate pests, suggesting that hot thermal fumigation would not require exceptional treatments and should be feasible for shipments containing most non-biological materials and some biological ones (e.g., some live plants, grain), and cold thermal fumigation should be feasible for all materials tolerant of being frozen.

Key Words: *Boiga irregularis*, brown treesnake, control, Guam, invasive species, lethal, thermal tolerance, vertebrate pest.

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INTRODUCTION

In the five decades since its accidental introduction, the brown treesnake (*Boiga irregularis*) has had an enormous negative impact on Guam's ecology and a modest detrimental effect on its economy (Savidge 1987, Fritts and McCoid 1991, Rodda and Fritts 1992, Fritts 2002). The risk of the snake spreading to other Pacific islands (Fritts et al. 1999) and there reprising its ecological devastation (Fritts and Rodda 1998) has led to an elaborate interdiction effort targeting cargo leaving Guam (Engeman et al. 1998, Vice et al. 1999). Although the current tools used in the interdiction program (trapping, visual and dog searches) enjoy reliable, albeit limited success (Engeman et al. 2002, Rodda et al. 2007), most have high associated labor costs (Vice et al. 1999, Engeman and Vice 2001, Engeman et al. 2002) and are disrupted by the frequent typhoons that strike the region (Vice and Engeman 2000, Engeman et al. 2002). Interdiction efforts would be greatly improved if effective low cost, less labor intense

management tools could be developed to augment the current methods.

A variety of new snake control tools have been explored in recent years, such as ingested, dermal and aerosol toxicants (Brooks et al. 1998a, Brooks et al. 1998, Brooks et al. 1998c, Savarie and Bruggers 1999, Savarie et al. 2001). Chemical fumigation for cargo is effective for brown treesnakes, and several products have been registered with the United States (US) Environmental Protection Agency for this purpose (Savarie et al. 2005). However, chemical fumigation is not without problems; for example, methyl bromide is hazardous for the Earth's ozone layer (Brooks et al. 1998a, Brooks et al. 1998b, Clark and Shivik 2002). Moreover, some fumigants, such as calcium cyanide and magnesium phosphide, pose a risk to human and environmental health and safety (Savarie and Bruggers 1999, Fields and White 2001, Clark and Shivik 2002), and chemicals may react adversely with certain cargos.

The use of lowered or elevated temperatures as a non-toxic, non-reactive alternative to chemical fumigation has long been recognized as an effective strategy for invertebrate pest management (e.g., Dean 1911, Dermott and Evans 1978, Fields 1992, Dowdy and Fields 2002, Mourier and Poulsen 2000, Wright et al. 2002). However, manipulating temperature for control of vertebrates is virtually untested. Given information about thermal tolerance of *B. irregularis* and temperatures in potentially snake-infested spaces such as cargo holds or aircraft wheel wells, we can predict snake survival in that space and possibly eradicate stowaways by reducing or increasing temperature beyond their thermal tolerance.

In the only study to date that addresses *B. irregularis* thermal lethal tolerance limits, Anderson and Hetherington (2000) found that anesthetized *B. irregularis* < 170 g died when exposed to 40°C for one hour. Typically, wild-caught *B. irregularis* tolerate substantial variation in environmental temperature (T_e) (Perry 1999, Anderson 2002, Anderson et al. 2005), but have little ability to regulate body temperature (T_b) when heat stressed, except to seek shade (Johnson 1975). Anderson (2002) reported mean T_b of 26°C for free-ranging *B. irregularis* on Guam and suggested that the species prefers to maintain T_b below 35 - 36°C in the wild. *Boiga irregularis* in the laboratory has survived temperatures up to 40°C (G. Perry, Ohio State University, unpublished data). Few studies have addressed lower lethal temperatures for reptiles other than those that hibernate through sub-freezing winters (McConnachie et al. 2007). Therefore, the minimum temperature tolerance of a tropical species like *B. irregularis* is unknown.

The role of gender, size or body condition in tolerance or recovery from extreme thermal stress is also untested in *B. irregularis*. Small sample size limited the ability of Anderson and Hetherington (2000) to determine whether mass, gender, or other related factors affected thermal resistance at high temperature. Voluntary thermal maxima of *B. irregularis* in a gradient ranged from 34.1°C to 38.5°C over a 90-min period for two snakes (860 mm SVL and 1406 mm SVL); the larger snake maintained a higher voluntary temperature than the smaller (Johnson 1975). Studies on other snakes have found conflicting results in terms of body size and thermal tolerance (e.g., Spellerberg 1972, Sanders and Jacob 1981, Doughty 1994).

Exposure time to high or low temperatures also influences the likelihood of survival (Kour and

Hutchison 1970, Mahroof et al. 2003). For example, insect pests died if exposed to 40°C for more than 24 h, 45°C for 12 h, 50°C for 5 min or 55°C for 1 min (Fields 1992). Even if cargo containers and aircraft wheel wells reach temperatures that are potentially lethal to *B. irregularis*, they may not maintain such temperatures for an adequate time period. Several studies have speculated that most species of snake will not survive exposure to temperatures between 40 and 43°C for more than 0.5 h (Cowles and Bogert 1944, Brattstrom 1965, Johnson 1975), and the ability of most vertebrates to tolerate T_b above 45°C is limited due to the biochemical constraint of protein denaturing (Bartholomew 1982). Anderson and Hetherington (2000) suggested that at temperatures above 41°C, *B. irregularis* died after exposure times between 17 and 103 min, although results were variable.

Here we determine the upper (T_{UL}) and lower (T_{LL}) lethal temperatures for *B. irregularis* and discuss their utility for controlling stow-away brown treesnakes in shipping containers, aircraft cargo holds and aircraft wheel wells. We also investigate whether gender, body size and body condition can be used to predict the differential mortality of brown treesnakes exposed to high and low temperature extremes.

METHODS

We conducted thermal trials at the US Geological Survey Laboratory at Ritidian Point, Guam, between August 2005 and September 2006. Snakes were captured in traps throughout Guam and housed individually in plastic cages (400 × 250 × 100 mm) prior to and following thermal trials. We placed cages in a covered outdoor area to provide typical ambient Guam weather conditions prior to testing, and to avoid acclimatization to the air-conditioned lab (Hutchison and Rowlan 1975, Ludderschmidt and Hutchison 1997). We furnished cages with water and refugia, checked snakes daily and fed them weekly prior to thermal trials. We maintained individuals in captivity for no longer than three weeks pre-trial. On arrival at the lab, we weighed, measured (snout-vent length [SVL] and total length) and checked each snake for general health (such as cuts, abrasions, cloudy eyes, lethargy and general body condition). Any *B. irregularis* in ecdysis, possessing palpable follicles or considered unhealthy was rejected for use in trials. We determined gender by probing or evertng hemipenes.

Thermal Chamber

We used an Envirotronics™ (Model SH8) environmental chamber to test *B. irregularis* thermal tolerance. The chamber (internal size: 0.11 m³) had an accuracy of $\pm 0.5^{\circ}\text{C}$ at -10°C to 50°C , and $\pm 2\%$ relative humidity (RH) at 40 - 90% RH, above and below which accuracy decreased ($\pm 1.0^{\circ}\text{C}$ or $\pm 5\%$ RH). During trials, each snake was individually housed in galvanized metal mesh and clear plastic snake compartments ($300 \times 150 \times 150$ mm for snakes $< 1,500$ mm SVL and $445 \times 385 \times 175$ mm for snakes $> 1,500$ mm SVL) in the chamber. We tested four (if $< 1,500$ mm SVL) or two (if $> 1,500$ mm SVL) snakes per trial. For T_{UL} trials, absolute humidity in the chamber was held constant from a starting point of 25°C and 75% RH. RH was set to 0% at the target temperature for T_{LL} trials, although this value is not realistically achievable for very low temperatures by the chamber. Hence, we recorded actual RH attained during trials.

Critical Thermal Maximum and Minimum or Lethal Temperature

Critical thermal maxima (CT_{Max}) or minima (CT_{Min}) are commonly used in thermal studies and determined by heating or cooling an animal at a standard rate that does not allow for thermal acclimation (Lutterschmidt and Hutchison 1997). The end point is usually defined as a temperature at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will imminently lead to its death (Cowles and Bogert 1944). Disorganized locomotion is typically defined by the loss of righting response or the sudden onset of muscular spasms (Lutterschmidt and Hutchison 1997). The lethal temperature for an animal is generally obtained by placing an animal in a constant temperature environment and duration of exposure until death is recorded (Hutchison and Rowlan 1975, Huey 1982, Eckert and Randall 1983). Our experiment draws from both methods in that we heated or cooled snakes at a standard rate up to the focal temperature and ran trials over a set time period after core body temperature equilibration. Our dependent variable was survival, but the duration of exposure was fixed, not contingent on snake behavior. Our research goal was to identify a management prescription that did not require fumigators to observe snake behavior, as the intended application was to kill *unseen* snakes (detected snakes would have already been removed from the cargo). It was not possible to predict

T_{UL}/T_{LL} from published CT_{Max}/CT_{Min} values or vice versa, as a variety of methods and end points have been applied in different studies, those end points have not always been defined consistently or unequivocally, and because of the variability in other factors such as size and gender that could affect thermal robustness after CT is reached (Hutchison and Rowlan 1975, Lutterschmidt and Hutchison 1997, McConnachie et al. 2007).

Due to unknown variability in the thermal inertia of materials that would be operationally exposed to thermal fumigation, we chose to concentrate on snake tolerance to critical temperature for a fixed exposure time after a snake had equilibrated to the treatment temperature. Operational application will thus require use of thermal sensors to monitor the heating or cooling rate of the treated materials and “start the treatment clock” only after equilibration is complete. The proposed management prescription is based on one hour of exposure after the treated material has reached (equilibrated to) treatment temperature. Because cargos are vastly larger (and therefore have more thermal inertia) than snakes, the time required for cargo equilibration is expected to be uniformly sufficient to ensure that any enclosed snakes have also equilibrated to the target temperature before the fixed treatment period begins.

Our experimental heat treatment sequence involved four periods: (1) ramp up, (2) equilibration, (3) heat treatment, and (4) ramp down. Experimental cold treatments were the converse: (1) ramp down, (2) equilibration, (3) cold treatment, and (4) ramp up. The starting and ending points were always 25°C at 75% RH. Ramp-up or ramp-down rates were fixed at a standard rate of $1^{\circ}\text{C min}^{-1}$ (Lutterschmidt and Hutchison 1997). At the conclusion of each trial the animals were left in the chamber at 25°C for five minutes prior to removal. The animals treated in any given trial were of approximately the same size, so that they would equilibrate at about the same rate; the duration of the equilibration period was set to ensure that each snake would reach the target temperature shortly before the beginning of the treatment period.

Estimating Requisite Equilibration Duration

Heat transfer is dependent on body size and shape (Lutterschmidt and Hutchison 1997, Seebacher and Shine 2004). Copper tube models were initially used to estimate the time required for T_b and T_e to equilibrate. We constructed models from hollow copper pipes of three different diameters (12.7, 19.05 and 25.4 mm) that most closely represented the circumference of snakes tested. We cut pipes into 500 mm lengths which were filled with water and sealed with copper caps. When filled with water, the small, medium and large models weighed 64, 145 and 257 g respectively. A small hole was drilled into the side of each model through which the thermocouple probe from a pre-calibrated temperature Hobo™ data logger (Onset Computer Corporation, accuracy $\pm 1.0^\circ\text{C}$ from -30°C to 50°C) was threaded. Data-loggers recorded temperature every 60 sec throughout each trial.

To determine the actual duration of T_b/T_e equilibration and T_b throughout the trials, we fed temperature loggers (i-button™; Maxim Integrated Products, 16×5 mm; $\pm 1.0^\circ\text{C}$ from 30°C - 70°C) to at least 25% of *B. irregularis* tested at each temperature. We also attached an additional i-button inside each snake compartment to record T_e and placed one Hobo™ temperature and humidity logger (accuracy $\pm 3\%$ RH from 0 - 50°C) in the chamber.

Trial Set Up

We weighed to the nearest gram (g), measured SVL (mm) and recorded heart rate (bpm) of each snake immediately prior to placement in the chamber. Snakes were used in one trial only. At the completion of each trial we recorded heart rate, breathing rate (breaths per five min period), mass and pupil constriction (1 = constricted, 2 = normal, 3 = dilated). If snakes were alive, we recorded whether they were able to right themselves within 5 min. Live snakes were returned to their cages and observed daily for seven days. We euthanized all snakes that survived seven days post trial and confirmed gender and presence/absence of food in the gut via dissection. We determined T_{UL} and T_{LL} had been reached when a snake's T_b attained the test temperature for one hour and the snake died within seven days post trial.

Sequence of Test Temperatures

We initially tested T_{UL} at 39°C , based on existing literature (Anderson and Hetherington 2000) and unpublished data. Following the 39°C

trial, our protocol was to home in on the lethal threshold by increasing or decreasing temperature by 1°C increments depending on the outcome of the initial trial. Our protocol for determining T_{LL} was similar, although setting our initial T_{LL} was more difficult because of lack of available data. However, based on results for other reptiles (Claussen et al. 1990, McConnachie et al. 2007), anecdotal information and informed estimates, we initiated our T_{LL} test at -3°C .

Individual Snake Attributes

Our initial trials aimed to test a group of snakes we considered most likely to survive exposure to extreme temperatures. Based on literature including but not limited to Johnson (1975), Huey (1982), Gibson et al. (1989), Lutterschmidt and Reinert (1990), Peterson et al. (1993), Madsen and Shine (1996), Anderson and Hetherington (2000), Anderson (2002), and Ladyman and Bradshaw (2003), we hypothesized that large ($>1,000$ mm SVL), sexually mature, non-shedding, non-follicular (if female), fully hydrated and satiated *B. irregularis* with a high (>1.0) body condition index were the most likely to survive extreme temperatures. Because snakes better tolerate cold air when it is dry (McConnachie et al. 2007), we did not provide water during trials. We countered the potential drying effect of the hot temperature trials with elevated humidity (see above). Snakes entered the chamber having consumed food item(s) of a size approximately 10% of their body weight between 48-72 h prior to the trial (e.g., Secor and Diamond 1997).

Following the initial trials to identify critical temperature ranges, we tested T_{UL} and T_{LL} for *B. irregularis* of different size, gender and body condition. We tested the effect of gender of snakes assigned to one of five size classes for each trial (≥ 50 , 50–100, 100–150, 150–200, and 200–250 g). To balance potential gender effects, we maintained roughly an equal sex ratio for each size class. Unless a snake was a neonate or juvenile (i.e., <600 mm SVL), individuals of known sex were tested. We also tested an additional group of *B. irregularis* that were exposed to T_{UL} for 0.5 h (instead of 1 h) following equilibration.

Analysis

We evaluated the relationship of gender and three body metrics (condition index, SVL, and mass) to survival. Condition index was calculated as the ratio of a snake's individual mass to its expected mass given its length. Expected mass was

estimated by linear regression on a logarithmic scale, and based on the 286 snakes used in this study. We calculated the means, standard deviation, range, and 95% confidence limits (CL) for snakes of each thermal treatment and used 95% CL to assess differences between groups. Unless otherwise stated, values expressed are mean \pm standard deviation. The Chi-square statistic was used to compare temperature tolerance between genders. Statistical significance was set at $\alpha \leq 0.05$.

RESULTS

Validation of Chamber Temperature

We placed thermocouples (hobo loggers and i-buttons) within the chamber to verify accuracy of the device. At set temperatures above 38°C during 97 trials, the actual chamber temperature was on average 0.67°C below the set temperature ($\xi_{\text{set}} = 38.99 \pm 0.05^\circ\text{C}$, $\xi_{\text{actual}} = 38.32 \pm 0.19^\circ\text{C}$; Figure 1). To account for the difference, we set the chamber temperature 0.5°C higher for all trials.

Determining T_b/T_e Relationship

The larger the copper model, the greater the time to T_b/T_e equilibration ($r^2 = 0.89$; Figure 2). Models heated to 41°C took approximately 10 min longer to reach equilibrium than those heated to 38°C regardless of size (64 g = 13–22 min; 145 g = 22–32 min; 257 g = 33–42 min). We used the regression equation derived from the copper model trials to estimate the equilibration time required for *B. irregularis* of varying sizes at 40 and 41°C and compared the estimated values with actual values from snakes with ingested i-buttons. We found that the copper models consistently overestimated the time required for a snake T_b to equilibrate by an average of 10 min (± 6.48 min, $n = 22$). However, our primary goal was to ensure that a snake's T_b was held at the test temperature for a minimum of one hour, thus slightly overestimating the time did not compromise the experimental design.

T_{UL}

We tested 233 snakes over three hot temperatures (39°C: $n = 92$, 40°C: $n = 88$ and 41°C: $n = 53$). Forty nine percent of snakes exposed to 39°C survived (Figure 3). Survival decreased to 99% at 40°C (within 7 days) and 0% at 41°C, the temperature at which no snake survived longer than 1 h post treatment.

At 39°C, there was no difference in susceptibility to death between genders ($\chi^2 = 0.70$, $DF = 1$, $P = 0.4$). Of the three body metrics assessed, SVL and mass were better indicators of susceptibility to death than condition index, although confidence intervals for both of those metrics overlapped (Table 1). On average, shorter, heavier snakes were more likely to die after 1 h exposure to 39°C than longer, lighter snakes.

When exposure time was halved from 1 h to 0.5 h, the death rate at 1 h and 1 d post-treatment decreased marginally (Figure 3). However, overall mortality was similar between the two treatments (99% for 1 h exposure, 95% for 0.5 h exposure). Snakes with better body condition exposed to 40°C for 0.5 h died just as readily as those exposed to 40°C for 1 h ($\xi_{0.5\text{ h}} = 1.12 \pm 0.16$; $\xi_{1\text{ h}} = 0.99 \pm 0.15$). Neither length ($\xi_{0.5\text{ h}} = 996 \pm 218$ mm SVL; $\xi_{1\text{ h}} = 1000 \pm 273$ mm SVL) nor mass ($\xi_{0.5\text{ h}} = 144.6 \pm 75.3$ g; $\xi_{1\text{ h}} = 160.9 \pm 168.0$ g) affected survival at either exposure period.

T_{LL}

We tested 53 snakes at two cold temperatures: -3°C ($n = 17$) and -5°C ($n = 36$). A larger sample size was initially planned; however, because of ethical considerations resulting from the unanticipated protracted time to death, we chose to reduce the number of low-temperature trials. All snakes died within 7 d of exposure to -5°C, but 29% survived past 7 d at -3°C (Figure 4). At both temperatures most deaths occurred between 24 h and 1 d post-trial. At -3°C, male snakes were more susceptible to death than females ($\chi^2 = 4.41$, $DF = 1$, $P = 0.04$). Snakes that died at -3°C had less body mass than those that survived, although confidence intervals overlapped (Table 1).

DISCUSSION

The aim of our study was to quantify the high and low lethal temperatures of the snake *B. irregularis* to evaluate possible use of thermal manipulation as an interdiction tool for snakes in cargo. The upper and lower thermal limits resulting in mortality for one hour exposure after equilibration (-5 and 41 C) were values similar to those that are already in use for thermal fumigation of insect pests (Fields 1992, Mourier and Poulsen 2000, Wright et al. 2002). Thus, thermal fumigation can be used for both

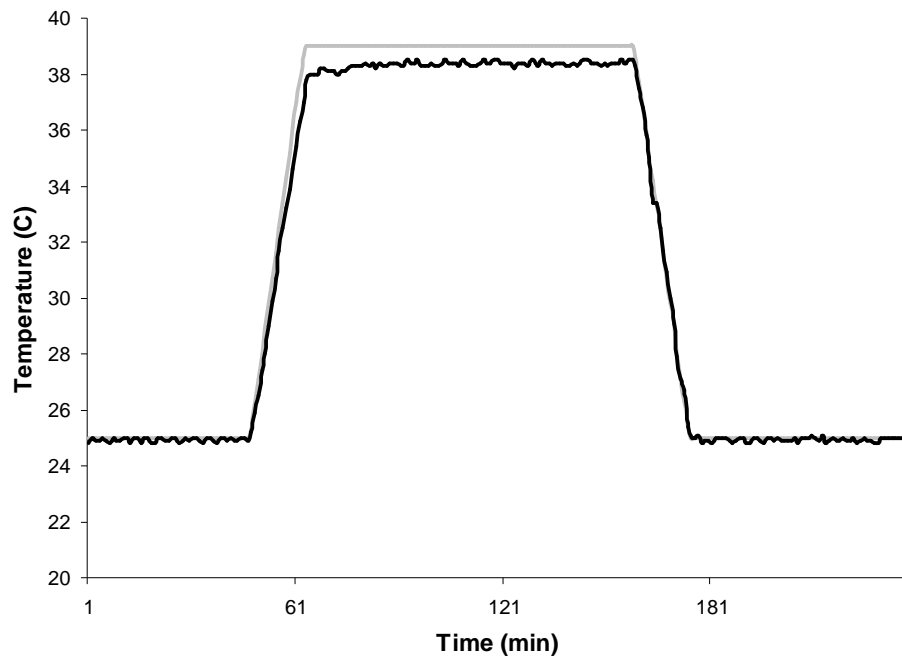


Figure 1. Comparison between the set chamber temperature (grey line) and the actual chamber temperature (black line) for a 39°C trial. The chamber was set to 25°C after which temperature increased to 39°C at $1^{\circ}\text{C} \cdot \text{min}^{-1}$ before returning to 25°C at the same cooling rate. The set temperature was subsequently adjusted upward by 0.5°C to compensate for the difference between set and actual chamber temperature.

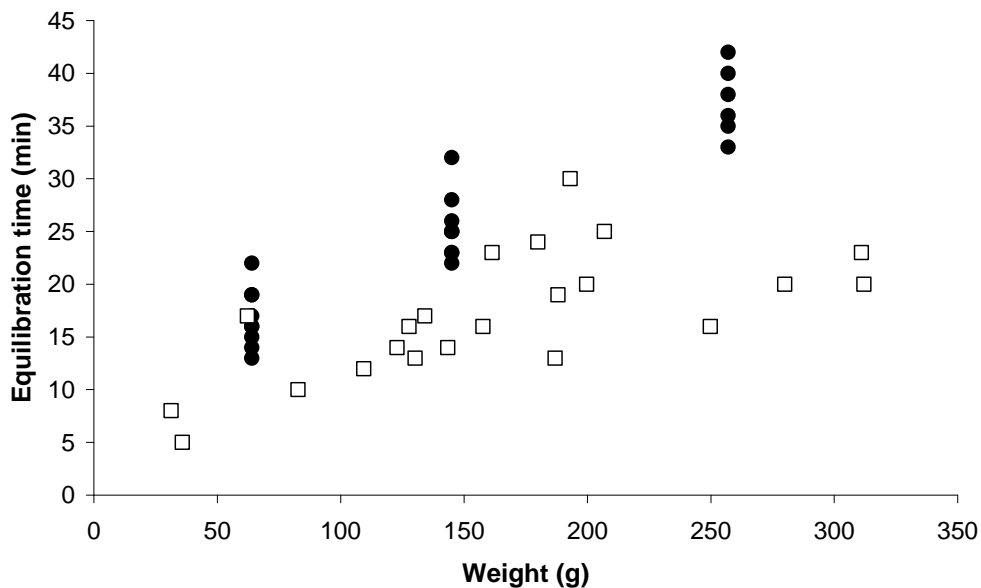


Figure 2. Copper models (●) consistently overestimated the time *Boiga irregularis* (□) required to equilibrate to T_e , as indicated by internal temperature loggers. A group of six copper models of three sizes (64, 145 and 257 g) were run twice at four temperatures (38 - 41°C). *B. irregularis* were tested at 40 - 41°C ($n = 22$ snakes).

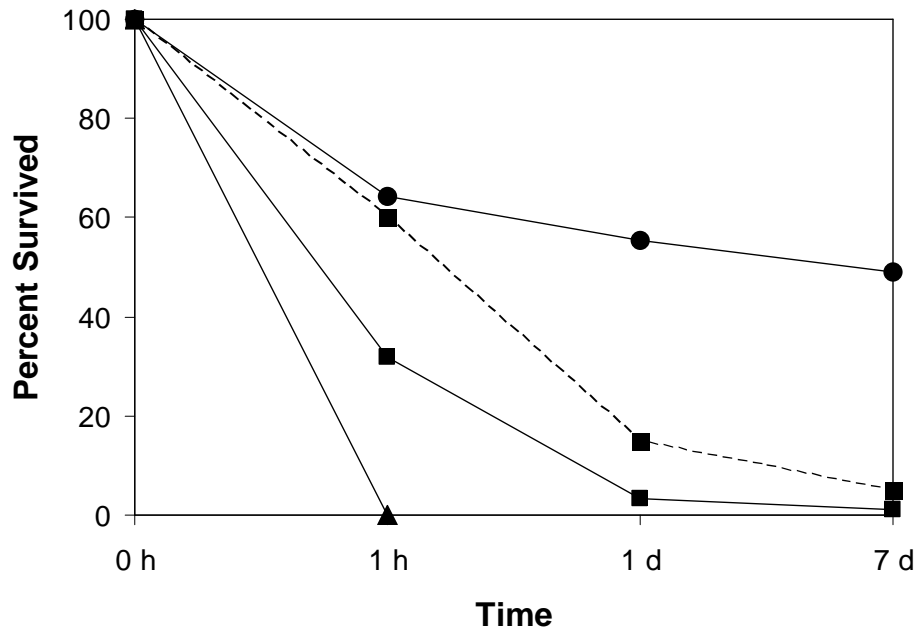


Figure 3. The number of *Boiga irregularis* that died within 1 h, 1 d or 7 d after exposure to temperatures of 39°C (●), 40°C (■) and 41°C (▲) for 1 hour plus equilibration time. The dashed line represents 0.5 h exposure to 40°C (---■---). At 1 h exposure, all snakes died within an hour at 41°C ($n = 53$ snakes), only one of 88 snakes tested at 40°C ultimately survived, but 49% survived past 7 days when the temperature was lowered to 39°C ($n = 92$ snakes). When exposure time was decreased to 0.5h, 5% survived at 40°C ($n = 20$ snakes).

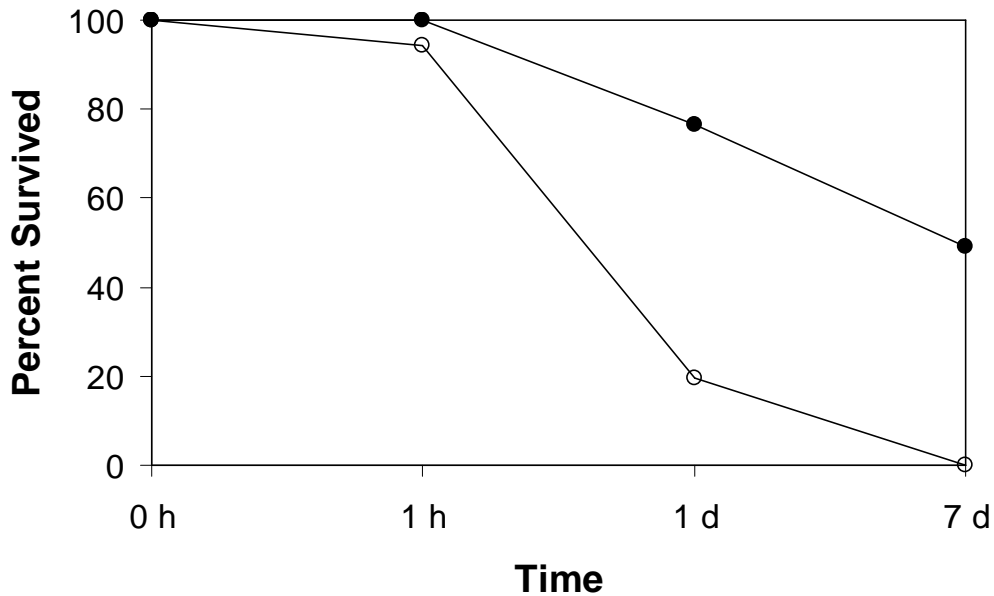


Figure 4. The number of *Boiga irregularis* that died within 1 h, 24 h or 7 days after exposure to -3°C (●) and -5°C (○) for 1 hour plus equilibration time. All snakes died within seven days of exposure to -5°C ($n = 36$ snakes) but 49% survived past 7 days when exposed to -3°C ($n = 17$ snakes).

Table 1. Mean, range and 95% confidence limits (CL) for condition index, SVL (mm) and mass (g) for 109 *Boiga irregularis* that either died or survived when exposed to 39°C or -3°C for one hour plus equilibration time. *n* = number of snakes.

	39°C		-3°C	
	Survived	Died	Survived	Died
<i>n</i>	45	47	5	12
Condition Index				
Mean ± SD	1.00 ± 0.15	0.99 ± 0.17	1.10 ± 0.12	1.15 ± 0.14
Range	0.73 – 1.36	0.65 – 2.00	0.98 – 1.30	0.98 – 1.38
95% CL	0.96 – 1.04	0.95 – 1.04	0.99 – 1.20	1.07 – 1.23
Snout-Vent Length (mm)				
Mean ± SD	998.56 ± 184.80	947.91 ± 88.13	1029.00 ± 159.27	952.42 ± 24.68
Range	608.00 – 1205.00	510.00 – 1290.00	905.00 – 1305.00	852.00 – 1090.00
95% CL	832.56 – 940.55	922.72 – 973.11	889.39 – 1168.61	904.04 – 1000.80
Mass (g)				
Mean ± SD	91.61 ± 68.45	123.10 ± 88.13	151.12 ± 85.76	115.58 ± 41.18
Range	20.90 – 269.20	11.20 – 290.00	84.60 – 298.00	65.60 – 190.80
95% CL	71.61 – 111.61	97.90 – 148.29	75.94 – 226.30	92.29 – 138.88

vertebrate and invertebrate pests with only minor modification of the treatment protocol. Many cargoes can tolerate exposure to these temperatures, but especially sensitive live materials (e.g., some horticulture materials) probably can not. Those might be candidates for chemical fumigation.

Individual variation in temperature tolerance was minor, but was not fully explained by the individual covariates we measured (gender, size or body condition). However, shorter, heavier snakes were more likely to die at one hour exposure to 39°C than longer, lighter snakes. At cold temperatures death was protracted and snakes took up to 6 days to die at -5°C; thus brown treesnakes arriving in the wheel wells of aircraft (i.e., exposed to extreme cold en route) may be active but moribund on arrival, and may cease movement many days later. The most robust group of snakes at cold temperatures was somewhat heavy-bodied females.

While we expected *B. irregularis* T_{UL} to be between 40°C and 43°C, we were surprised to find that this semi-tropical snake, originating from areas

where overnight winter temperatures rarely drop below 0°C, can survive sub-freezing conditions. The mechanisms that allow *B. irregularis* to survive extreme cold depend on its ability to control or prevent ice formation (Ramløv 2000), either by adaptive freeze tolerance (Storey and Storey 1996) or freeze avoidance (Costanzo et al. 1995). Freeze tolerance in reptiles is rare, and is usually limited to species that experience regular freezing of up to 65% of body fluids (e.g., Storey 1990, Costanzo et al. 1995). The ice content in *B. irregularis* was not tested, but we did observe tissue damage in snakes exposed to -5°C that we attributed to freezing. It therefore seems unlikely that the species is freeze tolerant. It is more plausible that *B. irregularis* avoids freezing by being able to supercool (the ability to depress freezing point by the production of antifreeze compounds or cryoprotectants; McConnachie et al. 2007).

Perry and Vice (1999) documented thermal conditions within loaded and unloaded cargo shipping containers stacked on a dock, and in

loaded containers in transit from Guam to Micronesia and the US mainland. Assuming a T_{UL} of 41°C for one hour, containers consistently failed to reach adequate temperatures to ensure snake mortality while in transit. Lethal temperatures were reached dockside, but the capacity to attain sufficient temperatures was dependent upon shading, stacking and whether the container was full or empty. It is therefore unlikely that T_{UL} for *B. irregularis* could be achieved by passive heating of containers alone. However, passively increasing temperatures in shipping containers could be a cost-effective technique to augment the efficacy of forced hot-air fumigation. Moreover, by identifying low-risk cargo containers (i.e., those that have reached 41°C throughout the entire container for at least 1 hour), only the high-risk containers (those that have not reached 41°C for at least one hour) would require searching.

In contrast to hot temperature fumigation, the use of active cold temperature fumigation has limited applications based on practicality. For example, unless the shipment is of frozen food (very low risk), the cost of increasing the temperature of a container by 11°C from 25°C to 41°C is presumably less than cooling the container by 30°C from 25°C to -5°C, particularly if the container is passively warmed by solar radiation prior to fumigation.

T_{LL} may nonetheless provide useful guidelines for management. Perry (2002) found that during flight, cargo hold temperatures averaged 18.6°C (\pm 5.0°C SD), not sufficient to cause death in *B. irregularis*. On the other hand, wheel wells of aircraft flying at high altitude frequently drop well below freezing (average minimum of -18°C) on long-haul flights (>7 h), increasing the probability of death of a wheel-well stowaway (Perry 1999, 2002). In combination with knowledge of T_{LL} , this information can be used to reduce inspection effort (cost) for low risk flights.

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