Assessing the Potential Impact of Cane Toads on Australian Snakes

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Abstract: *Cane toads (*Bufo marinus*) are large, highly toxic anurans that were introduced into Australia in 1937. Anecdotal reports suggest that the invasion of toads into an area is followed by dramatic declines in the abundance of terrestrial native frog-eating predators, but quantitative studies have been restricted to nonpredator taxa or aquatic predators and have generally reported minimal impacts. Will toads substantially affect Australian snakes? Based on geographic distributions and dietary composition, we identified 49 snake taxa as potentially at risk from toads. The impact of these feral prey also depends on the snakes' ability to survive after ingesting toad toxins. Based on decrements in locomotor (swimming) performance after ingesting toxin, we estimate the LD_{50} of toad toxins for 10 of the at-risk snake species. Most species exhibited a similar low ability to tolerate toad toxins. Based on head widths relative to sizes of toads, we calculate that 7 of the 10 taxa could easily ingest a fatal dose of toxin in a single meal. The exceptions were two colubrid taxa (keelbacks [*Tropidonophis mairii*] and slatey-grey snakes [*Stegonotus cucullatus*]) with much higher resistance to toad toxins (up to 85-fold) and one elapid (swamp snakes [*Hemiaspis signata*]) with low resistance but a small relative head size and thus low maximum prey size. Overall, our analysis suggests that cane toads threaten populations of approximately 30% of terrestrial Australian snake species.*

Evaluación del Impacto Potencial de Sapos sobre Serpientes Australianas

Resumen: *Los sapos (*Bufo marinus*) son anuros grandes muy tóxicos que fueron introducidos a Australia en 1937. Reportes anecdóticos sugieren que la invasión de sapos a un área es seguida de declinaciones dramáticas en la abundancia de depredadores terrestres nativos que se alimentan de ranas, pero los estudios cuantitativos se han restringido a taxones no depredadores o a depredadores acuáticos y generalmente han indicado impactos mínimos. ¿Los sapos afectarán sustancialmente a las serpientes australianas? Basado en la distribución geográfica y la composición de la dieta, identificamos 49 taxones de serpientes como potencialmente en riesgo por los sapos. El impacto de estas presas también depende de la habilidad de las serpientes para sobrevivir después de ingerir toxinas, estimamos la LD_{50} de toxinas de sapo para 10 de las especies de serpientes "en riesgo." La mayoría de las especies presentaron la misma poca habilidad para tolerar toxinas de sapo. Tomando en cuenta la anchura del cráneo en relación al tamaño de los sapos, calculamos que 7 de las 10 especies podrían fácilmente ingerir una dosis letal en una sola comida. Las excepciones fueron dos taxones de colúbridos (*Tropidonophis mairii *y* Stegonotus cucullatus*) con mucha más resistencia (hasta 85 veces más) a toxinas de sapos y un elápido (*Hemiaspis signata*) con resistencia baja pero de tamaño cefálico relativamente pequeño (y por lo tanto, tamaño máximo de presa pequeño). En general, nuestro análisis sugiere que los sapos amenazan a 30% de las poblaciones de especies de serpientes terrestres de Australia aproximadamente.*

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Introduction

One of the most significant threatening processes for biodiversity worldwide concerns anthropogenic shifts in geographic distributions of organisms, with natural ecosystems in many parts of the world being invaded by non-native plants and animals (Williamson 1996; Mack et al. 2000). Many such invasions are likely to cause only minor and localized ecological disruption, but some feral animals cause massive degradation and in some cases widespread extinction of the local fauna and flora (Fritts & Rodda 1998; Ogutu-Ohwayo 1999). The processes and outcomes of ecological invasion vary considerably among systems, but potentially one of the most powerful effects involves the invasion of a toxic species into a fauna with no previous exposure to such toxins (Brodie & Brodie 1999). In such cases native predators may be unable to tolerate the novel toxin and thus die in large numbers when they first encounter the invader.

Although there has been extensive research on the ecological impacts of invading organisms, some major questions have attracted much less attention than others. In particular, it may often be true that the most important impact of a toxic feral taxon will be on predators, yet research on changes in the abundance of predators is fraught with logistical obstacles. Because they are relatively rare and mobile, simply quantifying the abundance of many predators, let alone detecting impacts on their abundances, poses a formidable problem. In the face of such challenges researchers have tended to focus on the impacts of invading species on smaller more abundant organisms – typically potential prey or competitors. There may thus be a real danger that studies will accumulate showing no (or minor) negative impacts from the invading organism, and the weight of negative evidence will encourage wildlife managers to afford less priority to potential impacts of the invasion. This is a dangerous path to follow in the absence of information on the effects of the invading taxon on predators. We believe the spread of cane toads in Australia reveals exactly this scenario.

The cane toad (*Bufo marinus*, Bufonidae) is a large (up to 230 mm body length) anuran native to South and Central America (Zug & Zug 1979). This species has been introduced widely throughout the Pacific, primarily by the sugar industry as an agent for biological control (Lever 2001). Cane toads were introduced into Australia in 1935 (Lever 2001). Since then, they have spread from their initial release points in eastern Queensland (QLD) to encompass more than 863,000 km^2 (50% of QLD; Sabath et al. 1981; Sutherst et al. 1995). Cane toads now extend into northern New South Wales (NSW) and the Northern Territory (NT) and are predicted to further increase their range, primarily throughout coastal and near-coastal regions of tropical Australia, to encompass an area of approximately 2 million km^2 (Sutherst et al. 1995; Fig. 1).

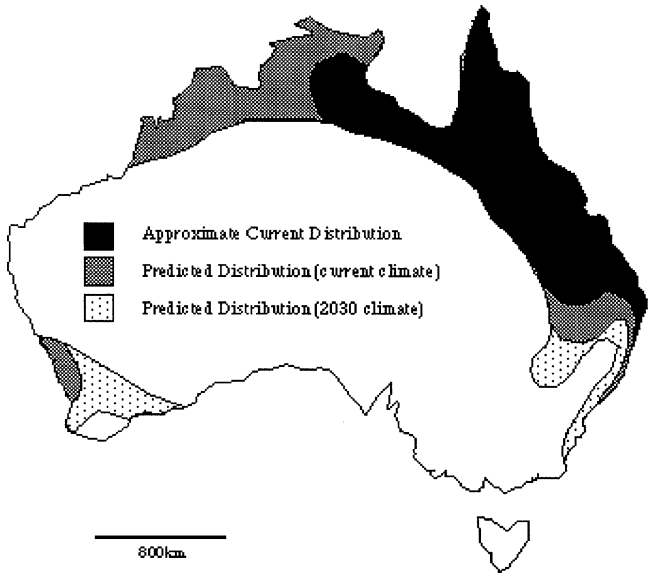

*Figure 1. Approximate current and predicted distribution of the cane toad (*Bufo marinus*) in Australia. Predicted distribution is shown under current climatic and 2030 global warming scenarios (after Sutherst et al. 1995).*

These amphibians can reach astounding densities in suitable habitat (up to 2138 individuals/ha; Freeland 1986). In addition, the toad possesses a formidable chemical defense system: all life stages are toxic (Flier et al. 1980; Lawler & Hero 1997; Crossland 1998; Crossland & Alford 1998). The active principles of the toxin (bufogenins) are extremely powerful (Chen & Kovarikova 1967) and unique to toads (Daly et al. 1987). Toads are not native to Australia (Lutz 1971) and therefore are both novel and toxic to Australian predators.

Although intuition suggests that the toad invasion may have a major ecological impact in Australia, study of this topic has been limited. The first actual impact to be noticed was that toads were becoming significant predators of the European honey bee (*Apis mellifera*), causing some economic loss for apiarists (Goodacre 1947; Hewitt 1956). It was not until the early 1960s, however, that anecdotal reports of population declines in native species became apparent: Breeden (1963) reported observations of declines in snakes, monitors (*Varanus* spp.), frilled lizards (*Chlamydosaurus kingii*), and quolls (a marsupial carnivore, *Dasyurus* spp.) following the appearance of toads. This was followed by observations of declines in snakes, monitors, and birds following the arrival of toads in southeastern Queensland and northern New South Wales (Pockley 1965; Rayward 1974). Covacevich and Archer (1975) provided further evidence for the potential impact of toads on predators by collecting numerous anecdotal reports of terrestrial predators (snakes, monitors, and marsupial

carnivores) dying as a consequence of attempting to ingest toads.

Quantitative data on interactions between native species and toads have become available in the ensuing years (e.g., Freeland & Kerin 1990; Lawler & Hero 1997; Crossland 1998; Crossland & Alford 1998; Catling et al. 1999; Crossland & Azevedo-Ramos 1999; Williamson 1999; Crossland 2000, 2001). Primarily for logistical reasons, these studies have focused on interactions between toads and relatively small, abundant native organisms (primarily fish, frogs, and aquatic invertebrates). Several of these studies have concluded that the ecological impact of toads may be less extreme than might be supposed from intuition or public concern (e.g., Freeland & Kerin 1990; Catling et al. 1999; Williamson 1999). Despite all these studies, however, there are no published quantitative analyses of the potential or realized effects of cane toads on the subset of native taxa identified anecdotally more than 30 years ago as most likely to be at risk: the terrestrial predators of frogs (the one possible exception being the work of Burnett [1997], which focused on varanids and marsupial predators, although this study too, relied on anecdotal information). Even if competition between toads and small vertebrates is minor and their role as predators on invertebrates is modest, they might still impose a massive ecological impact if they kill a high proportion of the anurophagous predators that attempt to ingest them.

Despite the fact that snakes represent the largest vertebrate group likely to be affected, there are almost no data describing interactions between toads and native snakes. Knowledge in this area comes entirely from single observations (Covacevich & Archer 1975; Ingram & Covacevich 1990; Shine 1991*c*); for example, an individual of species X survived and an individual of species Y died after eating a toad. Snakes are potentially at considerable risk from toads because many Australian snakes prey on frogs (Shine 1991*a*) and, unlike birds or mammals, have few options for prey capture. They must use their mouths to capture and consume the toad entirely and hence cannot avoid direct exposure to toxins in the toad's body.

Thus, there is a critical need to evaluate the severity of the probable impact of cane toads on Australian snakes. To do so, we require information on two separate topics: (1) how many Australian snake species are potentially vulnerable to toads, based on their geographic distributions and dietary habits (i.e., how many species eat frogs and live in areas that toads will occupy) and (2) how many Australian snake species can tolerate a quantity of toxins equivalent to ingesting a small toad.

To answer these questions we reviewed published information on the distributions and dietary habits of Australian snakes and tested the tolerance of 10 at-risk snake taxa to toad toxins.

Methods

Numbers of Snake Species Potentially at Risk

To identify which Australian snake species might potentially be affected by the invasion of the toad, we used ecoclimatic predictions of the likely eventual distribution of toads within Australia (Sutherst et al. 1995) and published and unpublished data on the dietary composition (Shine 1991*a*; references in Greer 1997; J. Webb & G. Brown, personal communication) and geographic distribution (Cogger 2000) of Australian snakes.

Sutherst et al. (1995) generated two maps of the likely final distribution of cane toads in Australia, one under the present climate and one under a conservative 2030 climate-change scenario. The latter method produced a slightly larger predicted distribution of the toad. We used both maps for our analysis (Fig. 1), but even the larger potential range might be a conservative estimate because adaptation by the toad or lack of competition from congeners may increase its range outside the ecoclimatic envelope of its native range (used by Sutherst et al. [1995] to generate predictions for the Australian invasion). Thus, our estimates of the snake species affected may be conservative.

As well as species recorded as eating frogs, we also included some species that are likely to consume frogs but for which detailed dietary information is not available. For these species, we assessed their likelihood of consuming frogs based on the dietary habits of their congeners. For each species of snake recorded or likely to include frogs in their diet, we estimated the proportion of the species' range likely to overlap with that of the toad. Multiplying this percentage by the proportion of frogs in the diet of each species yielded an index (between 0 and 100) of the potential impact from the toad (Table 1).

Snakes' Tolerance to Toad Toxins

We tested 10 species of native snakes for their susceptibility to toad toxin. Because populations of snakes that are sympatric with toads might have already adapted to this novel prey type, snakes were collected from areas where toads were either absent or had been present for <15 years. Table 2 lists the species studied, with information on their body sizes and localities of collection. The study taxa included four species of snakes from the family Colubridae, one species from the Pythonidae, and four from the Elapidae. These species were chosen because they were all identified as at risk and were sufficiently common at our study sites to enable collection. We excluded animals that were obviously ill, in poor condition, or contained large prey items.

We obtained toad toxin from skins of 78 freshly killed cane toads collected from the Lismore area (northern NSW). We killed toads by freezing. We made a single

Table 1. Australian snake species potenially affected by the invasion of the cane toad (*Bufo marinas*).

Species	Frogs in diet (%)	Percent overlap[a] current	potential (current climate)	potential 2030 climate	Potential impact index[b]	Conservation status[c]
Boidae						
Antaresia childreni	33	70	100	100	33	
Antaresia maculosus	6	95	100	100	6	
Antaresia stimsoni	8	7	10	10	0.8	
Morelia spilota	1	43	55	64	0.64	
Morelia carinata	?	0	100	100	?	R
Morelia oenpelliensis	?	20	100	100	?	R
Colubridae						
Boiga irregularis	6	65	91	100	6	
Dendrelaphis calligastra	50	100	100	100	50	
Dendrelaphis punctulatus	78	63	87	95	74.1	
Enhydris polylepis	30	83	100	100	30	
Stegonotus cucculatus	50	79	100	100	50	
Stegonotus parvus	?	?	100	100	?	R
Tropidonophis mairii	97	70	100	100	97	
Elapidae						
Acanthophis antarcticus	6	43	51	58	3.48	R
Acanthophis praelongus	27	65	100	100	27	
Cacophis churchilli	?	100	100	100	?	
Cacophis squamulosus	6	63	100	100	6	
Demansia papuensis	?	45	100	100	?	
Demansia psammophis	7	16	21	23	1.61	
Demansia simplex	?	0	100	100	?	
Demansia vestigiata	27	87	100	100	27	
Denisonia devisii	88	45	55	60	52.8	
Denisonia maculata	95	100	100	100	95	V
Drysdalia coronata	53	0	38	88	46.64	
Drysdalia coronoides	5	0	0	16	0.8	
Echiopsis atriceps	?	0	100	100	?	V
Echiopsis curta	31	0	14	32	9.92	V
Elapognathus minor	66	0	0	50	33	V
Hemiaspis damelii	95	60	80	100	95	
Hemiaspis signata	22	75	92	100	22	
Hoplocephalus bitorquatus	77	72	83	94	72.38	V
Hoplocephalus stephensi	11	50	75	100	11	V
Notechis ater	%?	0	22	56	?	V*
Notechis scutatus	92	5	5	20	18.4	
Pseudechis australis	20	20	31	32	6.4	
Pseudechis colletti	25	77	77	77	19.25	
Pseudechis guttatus	40	64	86	100	40	
Pseudechis papuanus	?	?	100	100	?	
Pseudechis porphyriacus	60	32	41	53	31.8	
Pseudonaja affinis	2	0	14	36	0.72	
Pseudonaja guttata	41	44	47	47	19.27	
Pseudonaja nuchalis	4	19	27	27	1.08	
Pseudonaja textilis	9	48	52	57	5.13	
Rhinoplocephalus incredibilis	?	?	100	100	?	
Rhinoplocephalus nigrescens	1	61	69	77	0.77	
Rhinoplocephalus pallidiceps	6	45	100	100	6	
Suta ordensis	?	0	100	100	?	
Suta suta	3	24	30	31	0.93	
Tropidechis carinatus	41	71	86	100	41	

[a] *Percentage of the species' range encompassed by the toad currently and under the predicted distribution of toads (under present climate and a 2030 predicted scenario).*

[b] *Index of potential impact is based on the proportion of frogs in the diet and the predicted percent overlap with toads (under 2030 climate scenario).*

[c] *Conservation status as listed in the action plan for Australian reptiles (Cogger et al. 1993): V, vulnerable; R, rare or insufficiently known; ∗, conservation status refers to a South Australian population that is unlikely to come into contact with toads.*

Table 2. Data gathered on snakes and tolerance of snakes to cane toad toxin.

Species	*Location*[a]	*Total* n[b]	*Snout-vent length (mm)*[c]	*Weight (g)*[c]	*Gape width (mm)*[c]	LD_{50}^{d} *dose (μL/g)*	LD_{50}^{d} *absolute (mg)*	LD_{50}^{d} *percentage of gape*
Antaresia childreni	Humpty Doo, NT	5	892.5 (33)	227.6 (34)	15.5 (0.6)	0.816	19.25	64.44
Boiga irregularis	Casino, NSW	1	1210 (–)	299 (–)	21.9 (–)	—	—	—
Dendrelaphis punctulatus	Lismore, NSW	10	984 (122)	218 (61)	19.7 (2.9)	0.744	17.51	48.83
Enhydris polylepsis	Humpty Doo, NT	20	611 (19)	113 (12)	13.2 (0.3)	0.448	10.56	65.17
Stegonotus cucullatus	Humpty Doo, NT	18	1041 (45)	303 (37)	18.6 (0.8)	15.016	353.49	111.32
Tropidonophis mairii	Humpty Doo, NT	27	550 (20)	84 (8)	12.5 (0.5)	37.992	894.35	185.54
Acanthophis praelongus	Humpty Doo, NT	20	418.5 (22)	117.7 (20)	20.1 (1.1)	0.774	18.24	48.91
Hemiaspis signata	Casino, NSW	3	455 (7)	32 (2)	9.4 (0.1)	0.768	18.07	107.7
Pseudechis porphyriacus	Casino, NSW	28	900 (41)	347 (51)	22.0 (0.8)	0.692	16.29	42.99
Pseudonaja textilis	Lismore, NSW	3	1225 (115)	592 (220)	24.9 (1.6)	0.572	13.46	36.58

[a] *Abbreviations: NT, Northern Territory; NSW, New South Wales.*
[b] *Number of individuals tested for toxin resistance (numbers for morphological measures were different in some instances).*
[c] *Numbers in parentheses represent standard errors. Gape width is the distance across the head at the hinge of the jaw.*
[d] *The LD_{50} for each species is expressed as (1) dose of toxin per body mass of snake (μL/g); (2) absolute dose based on the weight of an average individual, expressed in milligrams of dried toad skin equivalent; and (3) percentage of the average snake's head width that a toad's head width represents, whose size is sufficient to provide the absolute dose (see text for details).*

extraction of toad toxin for the entire study to remove among-toad variance in toxicity and to accurately control dosing. We measured freshly killed toads for snout/urostyle length, head width, and mass. We then removed the dorsal skin (from the back of the head to the knees), including the parotoid glands. We dried this tissue at room temperature for several days and then weighed each skin. We blended the dried skins with 10x v/w of 40% ethanol. This mixture was strained and the solids discarded. At room temperature we evaporated the resulting liquid to 50% of its initial volume. We recorded the final volume and then dispensed the extract into 25-mL containers and froze it. Bufogenins are stable, partially water-soluble compounds with a very high evaporation temperature (Meyer & Linde 1971). Our crude extract thus contained the bufogenins, although it is possible some were lost as a result of saturation.

We tested the resistance of individual snakes to toad toxin using the decrement in swimming speed following a dose of toxin (methodology modified from that of Brodie & Brodie 1990). Each snake was encouraged to swim around a circular pool 3 m in diameter. The circumference of the pool was divided into quarters. We recorded each snake's speed (with an electronic stopwatch) as the time it took to traverse one-quarter of the way around the pool. Before dosing, we subjected each snake to two swimming trials one hour apart. In each trial we recorded the snake's time to traverse eight quarters of the pool. The fastest speed from each trial was taken and the resulting times averaged over the two trials. This yielded an estimate of maximum swimming speed before dosing (b). We also measured the snake's mass, snout-vent length (SVL), and head width at this time.

The following day we gave each snake a specific dose of toxin through a feeding tube attached to a syringe or calibrated micropipette. We inserted the tube into the snake's stomach to a depth of 30–50% of its SVL. Swimming trials commenced one hour after dosing. We swam each snake twice: 1 hour and 2 hours after dosing. Maximum swimming speed was calculated as before to yield an estimate of maximum swimming speed after dosing (a). We then calculated the percent reduction in swim speed (%redn) following dosing for each snake ($\%\text{redn} = 100 \times (1-b/a)$). Experiments on neonate snakes confirmed that reduction in swimming speed following this methodology is due to the toad toxin and not the carrier fluid (B.L.P., unpublished data).

Because we collected most of the data in the field, temperature could not be rigorously controlled across trials. We kept temperature differences between before and after trials within 2° C by running the postdose trial at a time when the water temperature was similar to that of the predose trial. Although maximum speed may vary with temperature, the repeatability of speed assays in snakes is consistent across temperatures (Brodie & Russell 1999). Thus, we expected our percent reduction measure to be unaffected by temperature differences across sets of before and after trials. To allow animals to adjust to the water temperature, we placed them in plastic boxes floated in the pool for a minimum of 30 minutes before trials commenced.

We subjected each species to a range of toxin doses, with the exact range based on the effects observed. A weak or zero effect in a trial meant the dose was doubled for the next snake; a lethal effect meant the dose to the next snake was quartered. To minimize mortality, we initially tested snakes within each species on low doses. We tested each snake only once. Where sample size permitted, we tested multiple snakes at each dosage level. Dosage rates were calculated on a volume-to-mass ratio

for each individual (0.002 mL/g of body mass). Different dosages were achieved by dilution of the original toxin extract. We used six initial dilution levels (0.025x, 0.05x, 0.1x, 0.2x, 0.5x, and 1x), with some species later given intermediate doses. Higher doses were achieved by successively increasing the dose per mass of undiluted extract (thus, 2x = 0.004 mL/g, 4x = 0.008 mL/g, etc.).

This process yielded data on reduction in speed as a function of dose for each species. In all cases there was a strong positive relationship between dose and percent reduction in speed, within the range of doses that elicited an effect. Percent reduction scores were transformed according to the following formula, modified from that of Brodie et al. (2002):

$$y' = \ln(2/y - 1),$$

where y is the proportional reduction in speed (%redn/100). There were three instances where the proportion reduction was <0. Because these values do not transform correctly, they were entered as a proportional reduction of 0.01 for the purposes of transformation (following Brodie et al. 2002). This transformation makes it simple to estimate the dose giving a 100% reduction in speed (the LD_{50}, $y = 1$). The reason for this is that when $y = 1$, $y' = 0$. Thus, the LD_{50} is the x-intercept of the regression of y' on dose, which can be estimated as $-\alpha/\beta$, where α is the intercept and β is the gradient of the line. Least-squares regressions of y' on dose were conducted and LD_{50} estimates made for each species.

We performed analysis of covariance (ANCOVA) on transformed mean proportional speed-reduction data with species as the factor and dose as the covariate to test for differences in resistance between species. Because of the large discrepancy in doses between some species, the data violated the assumptions of ANCOVA. We thus performed the ANCOVA on the two natural groups of resistance (low and high) to test for differences within each group. To test whether different species required different doses to achieve a similar decrement in swimming speed across all species, we performed an analysis of variance (ANOVA) with species as the factor and dose as the dependent variable. We conducted this analysis on all individual data where the decrement in speed was >20%. Selecting the data in this way ensured that we were comparing doses only within the effect range for each species.

A snake species' vulnerability to toads will be determined not only by the amount of toxin that it can tolerate, but also by the size of anurans that it consumes relative to its own body mass. A snake that eats only very small toads might thus be able to survive ingestion, whereas a snake that takes larger prey relative to its own body size might exceed the lethal dose. Because snakes are gape-limited predators, a snake's head size offers an index of the maximum size of prey that it can consume (Shine 1991*d*). We thus calculated the head width of a toad large enough to contain a potentially lethal dose of toxin for an average-sized specimen of each snake species, and compared that prey size to the head width of this average snake.

For species with sufficient data, we calculated the LD_{50} (in terms of absolute dose) for a snake of average body size. We then converted this dose into the equivalent mass of toad skin and used unpublished data on the relationship between toad body size and skin mass to calculate the size of toad that would constitute this LD_{50}. To compare this potentially lethal minimum toad size to the size of toad that a given snake species could physically ingest, we calculated the average mass and gape width for each snake species. We then divided the LD_{50} toad size (expressed as toad head width) by the mean snake head width to provide an index of lethal prey size relative to the snake's physical ability to ingest a prey item of that size. That is, the head width of a toad of a size sufficient to provide the LD_{50} to an average-sized snake was expressed as a percentage of mean gape width for snakes of each species. Percentages of <100% mean the snake could easily ingest a lethal-sized toad, whereas higher values make it increasingly unlikely that the snake could ingest a toad large enough to kill it.

Results

Number of Snake Species Potentially at Risk from Toads

Analysis of the distribution and dietary preference of Australian snakes suggests that 49 species are potentially at risk from the invasion of the cane toad (Table 1). Of these, 26 are likely to have their range totally encompassed by that of the toad (under predicted climate change by 2030) and 3 have already had their range totally encompassed by that of the toad. Nine of the at-risk species are already recognized as being threatened on either a federal or state level (Cogger et al. 1993). Thus, the toad invasion constitutes a potential threat to 70% of the Australian colubrid snakes (7 of 10 species), 40% of the pythons (6 of 15), and 41% of the elapids (36 of 87). These at-risk taxa include 9 of the 38 terrestrial species identified as being of most concern in terms of conservation status (Cogger et al. 1993).

Snakes' Tolerance to Toad Toxins

For most species of snake we tested, the percent reduction in locomotor performance was highly associated with survival after ingestion of toxin: most animals with 100% reduction in swim speeds died 1–2 hours after dosing. Animals with <100% reduction generally recovered over the course of 8–24 hours. Common blacksnakes (*Pseudechis porphyriacus*) were an exception to this generality, with two of four individuals given a 0.3x dose exhibiting swim-speed reductions of only 36% and 65% but dying 8–24 hours later. For the purposes of our analysis,

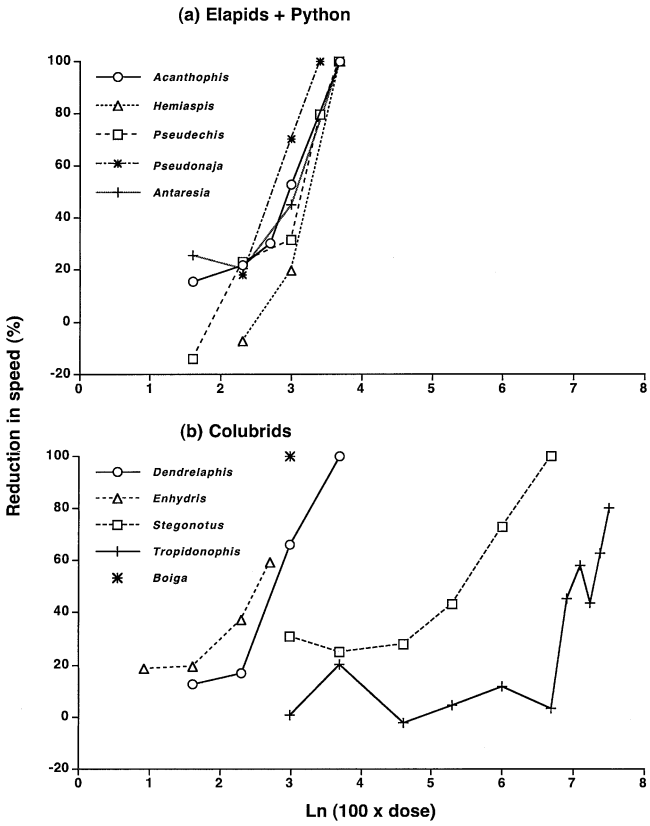

Figure 2. Percentage reduction in speed as a consequence of toad toxin dose for 10 species of Australian snake. The x-axis is ln(100 × dose), where dose is expressed as a concentration of toxin extract administered at a rate of 0.002 mL/g. The upper graph (a) shows data for elapid and pythonid species; the lower graph (b) shows data for colubrid species. Plotted points represent the mean value for all individuals tested at each dosage level (error bars omitted for clarity).

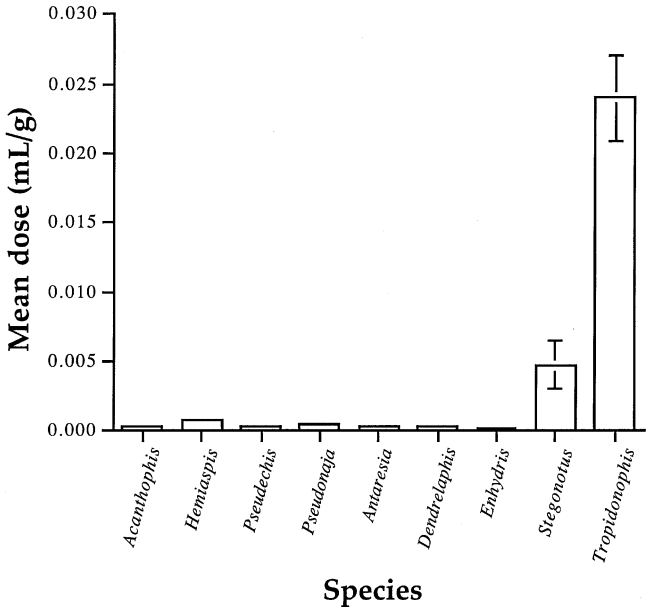

Figure 3. Average dose required to cause a reduction in speed greater than 20% for nine species of Australian snake. Mean dose is expressed as milliliters of toxin extract per gram of snake weight. Error bars represent 2 standard errors and are too small to visualize at this scale for all species except Stegonotus *and* Tropidonophis.

these individuals were scored as showing a 100% reduction in speed.

In all snake species tested, a higher dose (mL toxin/g) resulted in a greater reduction in locomotor performance (Fig. 2). The estimated LD_{50} was approximately 55 times higher for *Tropidonophis* and 22 times higher for *Stegonotus* than for the other eight taxa we tested (Table 2; Fig. 1; x-axis is ln-transformed in this figure). The LD_{50} for *Tropidonophis* was 85 times higher than the lowest LD_{50} estimate—that for *Enhydris*.

Our data clearly indicate two groups of taxa, those with high resistance (*Tropidonophis* and *Stegonotus*) and those with low resistance (all others). We performed ANCOVA separately on these two groups with species as the factor, dose as the covariate, and transformed proportional speed-reduction data as the dependant variable. In both cases there was no significant interaction between species and dose factors (high, $F_{1,13} = 0.019$, $p = 0.89$; low, $F_{6,14} = 2.02$, $p = 0.13$). After the interaction term was removed, both analyses suggested significant differences between species in resistance (high, $F_{1,14} = 24.58$, $p = 0.0002$; low, $F_{6,20} = 3.17$, $p = 0.023$). In the high group, *Tropidonophis* was significantly more resistant than *Stegonotus*. In the low group, this result appeared to be driven by *Hemiaspis,* which was slightly more resistant than other species, although Fisher's PLSD gave only one significant pairwise comparison (*Hemiaspis* vs. *Pseudonaja*, $p = 0.02$). After reductions of <20% were excluded, there was no significant difference in the percent reduction scores between species ($F_{8,91} = 1.81$, $p = 0.085$). The dose required to achieve these similar reduction scores differed significantly among species (Fig. 3; $F_{8,91} = 84.05$, $p < 0.0001$). Fisher's PLSD confirmed that this effect was due entirely to *Tropidonophis* and *Stegonotus*, which both required significantly higher doses than other species ($p < 0.02$ in all cases), with *Tropidonophis* requiring significantly more than *Stegonotus* ($p < 0.0001$).

Discussion

Cane toads have been spreading rapidly through Australia for more than 60 years, and warnings of their possible ecological impact on the native fauna have been voiced throughout that period (Lever 2001). Despite the clear

inference from anecdotal reports that terrestrial predators were the component of Australian ecosystems most likely to be affected by the toads' arrival, research on toad impacts has been dominated by studies of the effects of toads on potential prey items, competitors, and aquatic predators. Several such studies have suggested that toad impacts are likely to be less severe than had been predicted, and these results have been interpreted to mean that toads may pose less of a conservation disaster than anticipated by ecological doom-sayers (e.g., Freeland & Kerin 1990). Unfortunately, this conclusion is misleading: a lack of effect at lower trophic levels tells us nothing about potential impacts on predators, the component of the fauna most likely to be affected.

Why have previous workers focused on taxa other than terrestrial predators, despite anecdotal reports of major mortality events in native terrestrial predators—snakes, monitors, marsupial carnivores—following toad arrival? Logistical difficulties in quantifying the abundance of large vertebrate predators are the most likely reason, especially when combined with high levels of stochasticity in resources and therefore in predator populations in many Australian habitats (Flannery 1994). Even if we cannot measure abundances accurately in the field, however, we can study the vulnerability of predators in a laboratory setting to assess the likely result of encounters between a predator and a toad. The clear result from our analysis is that the invasion of cane toads is likely to have caused, and will continue to cause, massive mortality among snakes in Australia.

Our methods to estimate vulnerability based on geographic distributions and dietary composition are crude and subject to several sources of error (most leading to a conservative bias). Notably, there is still uncertainty about the eventual distribution of cane toads within Australia, and we do not know how a given proportion of amphibian prey items within the diet of a particular snake species will translate into prey preferences within specific populations or among individuals within any given population. Obviously, the impact of toads will be different if a 50% utilization of anuran prey is the result of 50% of individual snakes eating only frogs while the other individuals do not attempt to consume this prey type, as opposed to the (more probable) scenario where all individuals within the population prey upon anurans as well as other prey.

Nonetheless, our analysis indicates that a high proportion of Australian snake species are potentially at risk from toads (Table 1). Although it is possible that habitat differences will reduce contact with toads for some species, the fact that the toad is an extreme generalist in Australia and can be found in most habitats (Lever 2001) suggests that this factor will be relatively unimportant. Our calculations probably underestimate vulnerability for many of the taxa listed as feeding on frogs only infrequently. A low percentage of anurans in the diet does not necessarily equate to low potential impact for two reasons. First, cane toads typically attain higher population densities than most native frogs; thus, any individual snake prepared to attack an anuran prey item is likely to encounter a toad. Second, many snake species exhibit ontogenetic and/or sex-based shifts in prey preference, such that certain size and sex classes within a population consume a higher proportion of anurans than do other classes. For example, both *Pseudonaja textilis* and *Boiga irregularis* display an ontogenetic shift from ectothermic to endothermic prey (Savidge 1988; Shine 1989, 1991*c*). Sexual divergence in prey composition has been recorded in *A. praelongus*, *P. porphyriacus*, and *B. irregularis* and is likely to be widespread across many snake species (Shine 1991*b*; Pearson et al. 2002). In all these cases, the percentage of frogs in the diet (averaged across all individuals) may lead to an underestimate of the likely impact of toads on a population.

Our laboratory studies of the effects of toad toxins on snake locomotor speeds are also likely to have underestimated the severity of effects from ingesting entire toads. First, we extracted toxin only from the dorsal skin of toads. Toxin that is present in the ventral skin and internal organs was not included in the extraction. Second the extraction process is unlikely to have been 100% efficient, and some toxin will have been lost. Therefore, the actual lethal dose in terms of toad size is likely to be even lower than those listed in Table 2. It is also important to note that many snakes will take multiple prey items. Our calculations are limited to the effect of a single prey item. Once again, we are underestimating the potential impact on an individual snake.

Nevertheless, it appears that most species of snakes can easily ingest a single toad large enough to be fatal (Table 2). This result reflects the facts that (1) most of the snakes we tested were severely affected even by small amounts of toad toxins; (2) even small cane toads contain considerable toxin; and (3) most snakes can swallow prey items that are relatively large compared to their own body mass. In this respect, broad-headed snakes such as *Acanthophis* and *Hoplocephalus* are at higher risk than relatively small-headed taxa. Interestingly, the slightly higher resistance of *Hemiaspis*, coupled with its small relative head width, suggest that this species will be less affected by toads (LD_{50} as percentage of gape width = 107%). Nevertheless, the clear result from these analyses is that most of the snake species we tested are likely to be at substantial risk when cane toads invade their habitat.

Most of the snake species we tested exhibited low (and relatively similar) tolerance to the toxins of the cane toad (Table 2). The most striking exception in this respect was the keelback *T. mairii*. This species has been reported previously to ingest toads without ill effects (Covacevich & Archer 1975), but other authors have reported that keelbacks have sometimes died after eating toads (Ingram & Covacevich 1990; Shine 1991*c*). A survey of wild-caught keelbacks indicated that toads constituted only a small proportion of prey items inside the alimentary tracts of

these snakes (Shine 1991*c*). Our data support the notion that *T. mairii* is extremely resistant to toad toxin and, hence, that individuals of this species are unlikely to die as a consequence of ingesting a toad. The only other snake species reported to tolerate ingestion of toads is the tree snake *Dendrelaphis punctulatus* (Covacevich & Archer 1975), but our data argue against this possibility; several individuals died after relatively small doses (Table 2).

Although both keelbacks and slatey-grey snakes are predicted to survive the ingestion of a toad (Table 2), this does not mean these species are capable of eating toads on a regular basis. Physiological costs associated with neutralizing the toxin may entirely negate any energetic benefit associated with the consumption of the prey. Alternatively, the toxin may have a chronic cumulative effect. In the laboratory, keelbacks maintained exclusively on a diet of toads lost condition and died (Shine 1991*c*). It is also important to remember that our methodology removed among-toad variance in toxicity. It is entirely likely that some toads are more toxic than others, and thus the outcome of an individual encounter may vary from predictions made here.

Both of the snake species that show high levels of resistance to toad toxin are colubrids. This family is believed to be a recent invader (post Mid-Miocene but probably as late as the Pleistocene) of the Australian continent (Cogger & Heatwole 1981; Greer 1997). Recent ancestors of Australian colubrids are likely to have been sympatric with *Bufo* in southeast Asia, raising the possibility that some Australian colubrids may be pre-adapted to bufonid toxins. *Tropidonophis* spp. in Southeast Asia prey on native bufonids (Malnate & Underwood 1988), and a genus closely related to *Stegonotus* in central China contains at least one species that preys on toads (Pope 1935; McDowell 1972). The three other colubrids we tested (*Enhydris polylepis, Boiga irregularis*, and *Dendrelaphis punctulatus*) showed low levels of resistance, dismissing the possibility of a familial-level divergence in resistance to toad toxins.

Most of the snake species we tested showed a similar response to toad toxin on a dose-per-unit mass basis (Table 2; Fig. 3). This result is made more striking by the fact that the snakes tested cover a broad phylogenetic span (three families). It thus seems likely that most Australian snake species will show a similar low level of resistance. However, the significance of this common and low resistance level must be assessed in relation to the behavior, ecology, and morphology of each snake species. Specific factors important in the interaction include foraging behavior, habitat preference, and the ability of snakes to learn or acquire resistance. Further research is currently underway to assess these factors, particularly the possibility of an adaptive response.

The maximum relative prey size of each species does appear to mediate the impact of toads. For example, *Hemiaspis signata*, despite exhibiting a similar LD_{50} estimate to susceptible species, is less likely to be affected because individuals can only ingest toads that are small relative to their body mass (Table 2). *Acanthophis praelongus*, on the other hand, is capable of eating much larger toads than is required to provide a lethal dose and is thus predicted to be badly affected.

Our data suggest that many species of Australian snake are likely to be adversely affected by the invasion of the cane toad. The exact magnitude of the effect will depend on factors specific to each species and whether or not populations can mount an effective adaptive response. It seems prudent, however, to treat the invasion of the cane toad as a serious threat to many populations of frog-eating snakes. Some of the species listed in Table 1 are already regarded as threatened, and it would be wise for wildlife managers to give serious consideration to the impact of the cane toad on these species in particular. More generally, we should not allow logistical impediments to discourage work on the components of natural systems most likely to be affected by alien organisms.

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