

### Traumatic Injuries in Two Neotropical Frogs, *Dendrobates auratus* and *Physalaemus pustulosus*

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Traumatic injuries in amphibians have been attributed to unsuccessful predation events (Martof, 1956;

Dubois, 1979; Ouellet, 2000). Other possible sources of injury in amphibians are the result of fighting with conspecifics, mechanical accidents or scarring from infections (Dubois, 1979; Ouellet, 2000). Because most adult frogs do not have claws or teeth that could be used as weapons during combat, conspecific-inflicted injuries are unlikely. Mechanical accidents are possible in frogs, although the most likely source of such injuries are anthropogenic or acquired during the escape from predators. Scarring caused by infection is possible but has not been rigorously investigated as a source of injury in nature. The most likely source of traumatic injury is therefore through interactions with predators (Martof, 1956; Dubois, 1979). However, the majority of large-scale studies of traumatic injuries in anurans have been conducted in temperate areas (Rostand, 1949; Martof, 1956; Dubois, 1979; Ouellet, 2000). It is not known whether predation is the most likely source of traumatic injury in Neotropical anurans.

Before injury rates can be used to test hypotheses about predation, the nature of traumatic injuries and whether they reflect predation pressure in the Neotropics must be examined. If they do reflect predation, we would expect that species with differing levels of predation pressure would have correspondingly different levels of traumatic injury. To examine this, two sympatric species of similarly sized frog, the green poison frog, *Dendrobates auratus*, and the túngara frog, *Physalaemus pustulosus*, were surveyed for relative levels of traumatic injury. *Dendrobates auratus* is a diurnal terrestrial frog with toxic skin secretions containing many alkaloids (Daly and Myers, 1967; Daly et al., 1987). In contrast, *P. pustulosus* is cryptic and does not secrete alkaloids (Daly et al., 1987). Little is known of the predators of dendrobatid frogs in the wild. For *D. auratus*, predator accounts are based on single observations (a fish, *Brycon guatemalensis* in Hedstrom and Bolaños, 1986; a bird, *Baryphthengus marhi* in Master, 1998). The only well-documented predator of *D. auratus* is the theraphosid spider *Sericopelma rubronitens* (Summers, 1999), which is deterred by the frog's toxicity (Gray, 2000). In contrast, *P. pustulosus* is palatable and has a wide range of known predators (Ryan, 1985; Gray et al., 1999). In addition to differences in palatability, these two species have different reproductive strategies and differ in life span (Ryan, 1985; Summers, 1989, 1990). However, they are about the same size and are found syntopically in many of the same habitats. The Panamanian Island of Taboga is home to large populations of both species. On Isla Taboga, both *D. auratus* and *P. pustulosus* are active during the day, which contrasts with the primarily nocturnal behavior of all other studied populations of *P. pustulosus* (Jaegar and Hailman, 1981; Ryan, 1985). *Physalaemus pustulosus* were observed moving about the forest on Isla Taboga, calling and nesting throughout the day in full sunlight. Isla Taboga thus provides a perfect site to make comparisons because both species occur in high densities in the same areas and therefore are exposed to the same conditions. If there are trends in the injury levels that can be correlated with predation for these species from different anuran families, then traumatic injury may be used to understand predation pressure in a wide variety of frogs. This study represents the first population-level examination of traumatic injury in neotropical anurans.

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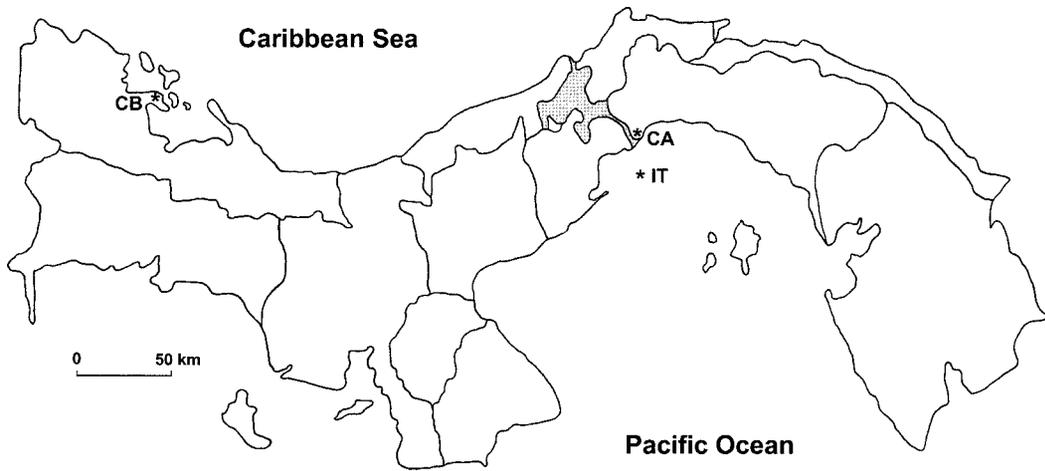


FIG. 1. Map of the Republic of Panama with sampling sites labeled (IT = Isla Taboga, CA = Cerro Ancon, CB = Cerro Bruja).

**Study Sites.**—Sampling was conducted in a forested nature reserve on Isla Taboga ( $8^{\circ}47'N$ ,  $79^{\circ}34'W$ ) in the Republic of Panama from April through June of 1998 and May through August 1999. To ensure that trends seen on Isla Taboga were not site-specific, sampling was also conducted at two additional sites, Cerro Ancon, a protected area in Panama City ( $8^{\circ}56'N$ ,  $79^{\circ}34'W$ ), and a cacao plantation on Cerro Bruja in Bocas del Toro ( $9^{\circ}9'N$ ,  $82^{\circ}14'W$ ; Fig. 1). Adult *D. auratus* were sampled both years at all three sites, whereas adult *P. pustulosus* were sampled only in 1999 and primarily on Isla Taboga. A small nocturnal population of *P. pustulosus* was sampled on Cerro Ancon to determine whether there were any differences between a nocturnal and a diurnal population. A total of 592 adult *D. auratus* were surveyed over the two years; 231 on Isla Taboga, 274 on Cerro Ancon and 87 on Cerro Bruja. A total of 230 adult *P. pustulosus* were examined; 188 on Isla Taboga and 42 on Cerro Ancon. Recently metamorphosed *D. auratus* ( $N = 10$ ) and *P. pustulosus* ( $N = 111$ ) were also surveyed on Isla Taboga in 1999.

**Survey Methods.**—Frogs were hand-caught and systematically examined for evidence of injuries. Injury

was visually differentiated from developmental abnormality by ascertaining the presence of scar tissue or a regenerative spike (Ouellet, 2000). All animals were measured (snout-vent length) to the nearest 0.1 mm using dial calipers and marked by clipping the terminal phalange of the fourth digit on the right hind limb. A sketch of the color pattern was made for each *D. auratus*, which, in conjunction with the clipped toe, allowed identification of recaptured animals.

**Analytical Methods.**—The sexes were considered together. Chi-square analyses were used to test for differences in injury occurrence within and between species (Zar, 1999). Snout-vent length data for the different species and sites were analyzed using one-way analyses of variance (SYSTAT vers. 9.0, SPSS, Inc., 1998).

High levels of traumatic injury were found in *D. auratus* (Table 1), the majority of which involved the partial or total amputation of digits (89.9%), but loss of forelimbs, hindlimbs, and eyes also occurred. For the three populations surveyed, Isla Taboga, Cerro Ancon, and Cerro Bruja, the rates of trauma were 14.7, 10.2, and 19.5%, respectively. There were no significant differences between rates of trauma in these pop-

TABLE 1. Number and type of traumatic injuries in Panamanian populations of adult *Dendrobates auratus* and *Physalaemus pustulosus*. Percentages are given in parentheses following the actual number of injuries encountered. (IT = Isla Taboga, CA = Cerro Ancon, CB = Cerro Bruja).

	<i>D. auratus</i>				<i>P. pustulosus</i>		
	IT	CA	CB	Total	IT	CA	Total
Number examined	231	274	87	592	188	42	230
Digit amputation	31	27	13	71 (12.0)	7	2	9 (3.9)
Limb amputation	—	1	1	2 (0.3)	—	—	—
Foot amputation	1	—	1	2 (0.3)	—	—	—
Hand amputation	—	—	2	2 (0.3)	—	—	—
Eye enucleation	1	—	—	1 (0.2)	1	—	1 (0.4)
Limb fracture	1	—	—	1 (0.2)	—	—	—
Total trauma	34 (14.7)	28 (10.2)	17 (19.5)	79 (13.3)	8 (4.3)	2 (4.8)	10 (4.3)
Age controlled				(4.4)			(4.3)

ulations ( $N = 592$ ,  $\chi^2$  contingency test,  $\chi^2 = 5.58$ ,  $df = 2$ ,  $0.05 < P < 0.10$ ), allowing the data to be combined to yield an overall trauma level of 13.3%. Eight *P. pustulosus* adults from Isla Taboga had traumatic injuries (4.3%), with the majority of injuries being to digits (87.5%). The level of traumatic injuries in the nocturnal Cerro Ancon population (4.8%; two adults with traumatic injury to digits) did not differ significantly from the Isla Taboga frogs ( $N = 230$ ,  $\chi^2$  contingency test,  $\chi^2 = 0.07$ ,  $df = 1$ ,  $0.75 < P < 0.90$ ) yielding a combined total injury level for *P. pustulosus* of 4.3%. When *P. pustulosus* from both sites were considered together, 90.0% of all their injuries were to digits.

When *D. auratus* that were originally captured in 1998 were recaptured in 1999, seven of 34 (20.6%) recaptured animals had new injuries. On Isla Taboga, three of the 20 recaptured animals had new injuries (15.0%), whereas four of 14 (28.6%) recaptured animals had new injuries on Cerro Ancon. Only Isla Taboga and Cerro Ancon populations were considered as these sites had the greatest sampling efforts with 19.7% of animals caught in 1998 recaptured in 1999. The rate of new injury (20.6%) was not significantly higher than the total average traumatic injury level (12.3%) for Isla Taboga and Cerro Ancon ( $N = 539$ ,  $\chi^2$  contingency test,  $\chi^2 = 1.30$ ,  $df = 1$ ,  $0.25 < P < 0.50$ ).

The two frog species overlapped in size on Isla Taboga (*D. auratus* ranged in snout-vent length (SVL) from 24.4–33.4 mm, mean = 29.1 mm,  $N = 163$ , whereas *P. pustulosus* range from 27.8–48.2 mm, mean = 31.8 mm,  $N = 180$ ), although *P. pustulosus* was significantly larger (one-way analysis of variance for SVL data from 1999,  $F = 132.5$ ,  $df = 1$ ,  $P \ll 0.001$ ). There were significant interpopulation differences in size within both species. Isla Taboga *P. pustulosus* were significantly larger than the nocturnal *P. pustulosus* from Cerro Ancon (one-way analysis of variance for SVL,  $F = 142$ ,  $df = 1$ ,  $P \ll 0.001$ ). For both years, there were significant differences in size between the three populations of *D. auratus* (one-way analysis of variance for SVL in 1998,  $F = 96.3$ ,  $df = 2$ ,  $P \ll 0.001$  and 1999,  $F = 59.5$ ,  $df = 2$ ,  $P \ll 0.001$ ). Overall, *D. auratus* from Cerro Bruja were larger than those from Cerro Ancon, which were larger than those from Isla Taboga. Although there were significant differences in the sizes of frogs between species and between populations of the same species, most of the known predators of *P. pustulosus* and the potential predators of *D. auratus* are much larger than either species of frog so the size differences detected here should not restrict predation (Ryan, 1985; Gray et al., 1999; Summers, 1999).

Of 111 recently metamorphosed juvenile *P. pustulosus* captured on Isla Taboga, three had traumatic injuries (one digit amputation, two foot amputations). Although the percentage of metamorphs with injuries (2.7%) was lower than that found in adults (4.3%), it is not significantly so ( $N = 111$ ,  $\chi^2$  contingency test,  $\chi^2 = 0.14$ ,  $df = 1$ ,  $0.50 < P < 0.75$ ). Only 10 *D. auratus* juveniles were captured. Although one juvenile was missing two digits, the sample size is insufficient for statistical treatment.

To take life span into account, the level of injury for each species was divided by the life span of the frog. Based on mark-recapture data of frogs in the wild, *P. pustulosus* usually lives less than one year, whereas *D. auratus* lives about three years (D. Marsh, K. Summers,

pers. comm.). If one crudely divides overall trauma rate by life span, the rate of traumatic injury is 4.4% for *D. auratus* and 4.3% for *P. pustulosus* (Table 1).

Traumatic injury rates in large-scale studies of anurans generally fall below 5% (Rostand, 1949; Dubois, 1979; Read and Tyler, 1994; Ouellet, 2000). In French populations of *Rana esculenta* complex, Dubois (1979) found 4.6% of adults ( $N = 1040$ ) had traumatic injuries, whereas in populations of *Bufo bufo* 1.0% of males ( $N = 44000$ ) captured had digit abnormalities (Rostand, 1949). The rate of trauma for *P. pustulosus* at 4.3% falls within this range. The rate of injury for *D. auratus* adults at 13.3% is high compared to the rate for *P. pustulosus* as well as those rates previously documented.

If predator interactions are the source of injuries, a difference in traumatic injury levels between these two species is not surprising given that the species have very different antipredator strategies. *Dendrobates*, which possess potent skin toxins that would make them unpalatable to predators (Daly et al., 1978), will produce visible amounts of skin secretions when threatened by theraphosid spiders (Gray, 2000). When threatened by spiders, *P. pustulosus* will puff its body up with air making itself much larger and presumably more difficult to handle (Gray, 2000). Before differences in antipredator strategy and predator response are used to explain differences in the levels of injury, the basic assumption that traumatic injury rates accurately reflect predation pressure must be satisfied. When the source and nature of the traumatic injuries was examined, this assumption seems unjustified. When *D. auratus* were attacked and then released by the spider *Sericopelma rubronitens*, the only injuries they ever sustained were scrapes from fangs on the torso that subsequently healed (Gray, 2000). The vast majority of injuries found in both frog species are to digits, suggesting there may be a common source. There are a myriad of predators known for *P. pustulosus* (Ryan, 1985), whereas there are very few described predators of the toxic *D. auratus*, and the one predator examined does not produce injury to digits, suggesting that the common source may not be a shared predator. In addition, it is unlikely that unsuccessful predation would result in such a minor injury as the partial loss of a digit, implying that predation is not the primary cause of injury in the frogs studied.

The most likely sources of the injuries are small invertebrates, such as ants, small spiders, and small crabs, which are neither prey nor predators but share the habitat. *Dendrobates auratus* have been caught in the field with ants firmly clamped to the tips of digits (Gray, 2000). Interactions with ants were often observed while males were transporting tadpoles to waterpools, when frogs that approached ants would be chased away (Dunn, 1941; Gray, 2000). Another possible source of digit trauma, to males transporting tadpoles particularly, may be through interactions with small crabs such as *Armases angustum*. This crab was found inhabiting a treehole and readily consumed *D. auratus* tadpoles and could pose a risk to males entering the water to deposit tadpoles (Gray and Christy, 2000). Small invertebrates, such as leeches and small bivalves, have been found to be the source of digit injury to a number of temperate amphibian

species (Dubois, 1979; Davis and Gilhen, 1982; Veith and Viertel, 1993).

Traumatic injuries accumulate over time; therefore the level of trauma for juveniles is expected to be lower than that for adults. An increase in traumatic injury with age has been shown in French populations of *Rana esculenta* complex where froglets had injury rates of 2.9% and adults had injury rates of 4.6% ( $N = 2524$  froglets and 1040 adults; Dubois, 1979). This trend is seen in *P. pustulosus* as 2.7% of metamorphs had injuries which was lower than the level in adults (4.3%).

As the reproductive period of an animal's life is the most active, it is expected that more injuries would occur during this period. For *D. auratus*, the reproductive period is one of male territory defense, female search for mates, lengthy courtship for pairs, male exploration for suitable pools, and transportation of tadpoles to these pools. The reproductive period is one of greater movement for *P. pustulosus* as well. Males must move through the forest to water to call, whereas females move to the water to mate with the calling males. Both then move away from the water. For both species, the adult life stage involves extensive moving about, which would expose the frogs to many potential aggressive interactions with other forest dwellers. A trend for increased risk of injury to reproductive adults is seen for *D. auratus*. The recapture data for *D. auratus* show that the rate of new injury over a full year (20.6%) is higher than the total average traumatic injury rate on Isla Taboga and Cerro Ancon (12.3%).

Although the absolute rates of trauma are different between the two frog species, when the life span of each species is considered, the differences become less obvious. The age-controlled rate of traumatic injury is very similar between the two species (Table 1). Although this rate does not equal the observed yearly accumulation rate for *D. auratus*, as seen from the recapture data, it does imply that more work is needed on the demographics and age-specific accumulation of injury. If the proportion of animals in each life stage and the proportion of life span spent in each stage are approximately the same for the two species, then the rate of injury accumulation is a function of the time spent moving about the environment. Further testing of traumatic injury levels in ecologically similar species of frog which differ in life span is needed.

These frogs provide evidence that traumatic injury is not a result of unsuccessful predation attempts. The nature of their injuries is minor, and the types of injury are the same in the two species of frogs, although they have different predators and antipredator strategies. The toxic, brightly colored *D. auratus* has very few known predators in the wild, whereas *P. pustulosus* is palatable and cryptically colored and has a wide range of known predators. Such differences in predators would likely result in differences in the types of injuries seen if predators were their cause. The primary source of injuries for the two species of frogs is therefore not predation but is most likely the myriad of small invertebrates that interact aggressively with the frogs. When comparing levels of injury, careful consideration of life history must be exercised as traumatic injury is accumulated and therefore is a function of a species' use of time and space.

*Acknowledgments.*—We are grateful to the scientific and administrative staff at the Smithsonian Tropical

Research Institute for logistic support and advice. We thank Autoridad Nacional Del Ambiente (ANAM) of the Republic of Panama for collection and research permits. This study was funded by a Smithsonian Tropical Research Institute short-term fellowship to HMG and a NSERC research grant to DMG. We also thank J. Brinkman, J. Irwin, and two anonymous reviewers for comments on the manuscript and K. Summers and S. McRae for discussion and encouragement.

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Accepted: 19 April 2001.