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Effect of Red Imported Fire Ant Envenomization on Neonatal American Alligators

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Alligator populations in the southeastern United States have substantially recovered since the late 1960s when they were placed on the federal Endangered Species List (Joanen and McNease, 1987). In Florida, both early age class and adult animals currently are harvested for commercial and recreational purposes (Hines and Abercrombie, 1987). In addition, the alligator is ecologically important as a keystone species and as an indicator of wetland health (Mazzotti and Brandt, 1994).

Recently, the nonnative ant *Solenopsis invicta* (the red imported fire ant) has surfaced as a potential threat to alligator populations. *Solenopsis invicta* is a relatively new (approx. 65 yr) non-indigenous addition to the invertebrate fauna of the United States. *Solenopsis invicta* is an opportunistic generalist; its main food items are other invertebrates (Wilson and Eads, 1949), but vertebrates also may serve as prey (Allen et al., 1994). Pipping young of oviparous vertebrate species may be especially vulnerable to direct mortality (Allen et al., 1994 and references therein), but indirect effects also are possible (Allen et al., 1995; Guiliano et al., 1996; Pedersen et al., 1996). Indirect effects have been documented for juvenile northern bobwhite (*Colinus virginianus*), including lower survival rates and body mass gain (Giuliano et al., 1996) and behavioral changes including reduced time spent foraging and resting (Pedersen et al., 1996). Anecdotal reports of *S. invicta* impacting herpetofauna are plentiful, but published evidence is limited (e.g., Landers et al., 1980; Mount, 1981; Mount et al., 1981; Freed and Neitman, 1988; Donaldson et al., 1994; Montgomery, 1996).

Since its introduction to Mobile, Alabama, *S. invicta* has spread throughout the southeastern United States (Vinson and Sorensen, 1986), and its range now completely overlaps the range of the American alligator. *Solenopsis invicta* prefers disturbed sites with full to partial sun exposure for nesting (Tschinkel, 1988). In inundated marsh systems, alligator nests appear to meet the nesting requirements for *S. invicta*. Alligators generally nest in fairly open microhabitats, and their clutch cavities are raised above the substrate (Deitz and Hines, 1980). In habitats that are saturated with water, alligator nests may provide sufficient exposure and disturbance to become the preferred nesting location for fire ants. Surveys of central Florida lakes by the authors indicate that up to 20% of alligator nests in marsh habitats contain colonies of *S. invicta*.

We tested the hypothesis that envenomization by *S. invicta* has an impact on hatchling alligator survival and body mass. Additionally, we tested whether eggs of the American alligator were attractive food sources for *S. invicta*. Whereas our tests were restricted to eggs and young of alligators, our results may be more generally applicable to oviparous herpetofauna.

Alligator eggs were collected as part of an ongoing long term study from Lake Apopka in central Florida (see Woodward et al., 1993). Eggs used in experiments with *S. invicta* were collected from two partially flooded nests at risk of total loss due to inundation. The remaining viable eggs from these nests (18 and 12, respectively) were incubated separately from all other collected eggs, and monitored closely when they approached full term (see Woodward et al., 1989 for collection and incubation techniques). When initial pipping was noted, eggs were transferred to facilities at the United States Department of Agriculture (USDA) Imported Fire Ant Laboratory, Gainesville, Florida. The clutches pipped approximately two weeks apart, and each clutch was divided randomly into a control and a treatment group.

Two days before the estimated hatch date of viable eggs from clutch A, we collected ten entire *S. invicta* colonies from Alachua County, Florida. Colonies were placed in 19-L plastic buckets simulating alligator nests with natural nesting material. Colonies were not collected directly from alligator nests because of logistical difficulties. It was important to collect entire colonies of *S. invicta* because worker behavior changes when queen(s) and brood are not present (Stringer et al., 1976). *Solenopsis invicta* colonies were maintained at the USDA Imported Fire Ant Laboratory, Gainesville, Florida.

After transfer to USDA facilities, treatment eggs were placed in alligator nesting material in 19-L buckets containing the viable colonies of *S. invicta*. Control eggs were transferred to identical 19-L buckets with nesting material but with no *S. invicta*. Each clutch was divided into three control and three treatment groups. For clutch A, three eggs were placed in each bucket. For clutch B, five eggs were placed in each bucket. Eggs were monitored closely during pipping. When a hatchling liberated itself from its egg, the alligator was removed from the bucket and placed in water, simulating liberation from the nest by the adult female alligator.

After all eggs in a clutch pipped, the hatchlings were removed from USDA facilities and transferred to Florida Cooperative Unit alligator incubation/rearing facilities located at the Florida Game and Fresh Water Fish Commission (GFC) Gainesville Research Laboratory. Alligators were weighed on the day of hatching and web tagged in both hind feet with sequentially numbered #1 Monel tags (National Band and Tag Co., Newport, KY). Treated and untreated members of a clutch were combined and maintained in Unit facilities. Hatchling alligators were fed extruded pelletized alligator feed ad libitum. Alligators were weighed at four weekly intervals. During each weighing period, alligators were visually inspected for scarring or other effects of *S. invicta* envenomization. Handling of alligators was limited to weekly weighing and was minimized as much as possible during those periods. Body mass was determined with a hand-held 200 g spring scale accurate to 0.1 g. After cessation of the experiment, surviving alligators were released at their original nest sites. We pooled clutches and used a repeated measures analysis of variance on body mass with body mass at time of treatment as a covariate (SAS Institute Inc., 1989).

We tested whether eggs of the American alligator were attractive food sources for *S. invicta* prior to pipping by utilizing nonviable eggs collected from nests on Lake Apopka. These eggs (N = 20) were placed in colonies of *S. invicta* maintained in 61 cm × 36 cm × 13 cm bus trays at USDA facilities. Eggs were observed daily to see if they were breached by *S. invicta*.

On average, hatchling alligators took about 15 min to complete the hatching process. Two animals exposed to *S. invicta* died. The first death occurred within five minutes of hatching. In that instance, the alligator was slow in pipping and had difficulty escaping from its egg. As a result, that alligator received numerous fire ant stings (>30). The second death occurred six days after treatment, just prior to the second weighing. That specimen was cannibalized before discovery, and the proximate cause of death could not be determined. All animals in the control group survived to release. Non-lethal injuries as a result of *S. invicta* stings were apparent in approximately 50% of experimental animals, and included swelling of extremities, especially digits, and visible pustules on the digits and around the eyes, where most stings occurred.

On the day of treatments, body mass did not differ between treated and control groups ($P > 0.50$). One week post-treatment, body mass differed between these two groups ($P = 0.013$). This difference in body mass remained consistent (week 2, $P = 0.09$; week 3, $P = 0.006$) for the three week period of this study (Fig. 1). An overall treatment effect was present ($P = 0.002$), but there were no significant time effects or interactions ($P > 0.10$).

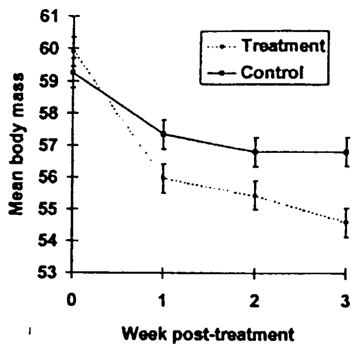

FIG. 1. Mean alligator body mass (±1 standard error) in grams over time. Treatment animals hatched in the presence of red imported fire ant colonies.

Non-viable eggs placed in *S. invicta* colonies were not breached if the eggs were completely intact. Eggs with any type of irregularities on their surface or any hairline fractures were breached and consumed by *S. invicta*. The contents appeared to be an attractive food source for *S. invicta*.

Our results should be viewed as conservative. During incubation the outer shell of most alligator eggs begins to crack because of swelling and degradation as early as two weeks before pipping (Joanen, 1969), allowing fire ants prolonged access to egg contents. Cracked eggs are supported only by the inner egg membrane, a soft covering that is readily breached by *S. invicta*. Embryos and albumen of alligator eggs were an attractive food source for *S. invicta* in our experiments with non-viable eggs. Additionally, the necrotic action of *S. invicta* venom could lead to secondary infections affecting survival.

Pipping alligators were invariably stung when *S. invicta* colonies were present. Whether this was a defensive behavior because of the disturbance of the *S. invicta* colonies during pipping or whether the response was oriented towards resource acquisition could not be determined. That vertebrates may be preyed upon by *S. invicta* has been well documented (Allen et al., 1994). Population level impacts on vertebrates also have been demonstrated (Allen et al., 1995), but much less evidence is available.

Past research concerning the impacts of *S. invicta* on vertebrates has centered on predation of newly hatched young and pipping birds. Giuliano et al. (1996) demonstrated indirect impacts of stings. In that study, four day old northern bobwhite chicks exposed to *S. invicta* had reduced survival and weight gain. Our results suggest that non-lethal impacts from *S. invicta* envenomization may occur in other wildlife species. Many species of oviparous herpetofauna laying eggs on land may be vulnerable to *S. invicta* (e.g., Landers et al., 1980; Mount, 1981; Mount et al., 1981).

Other research has centered on the direct mortality of young animals because of *S. invicta*. While also documenting this direct mortality, our research documents indirect effects of *S. invicta* envenomization. Indirect impacts may be equally or more important than direct impacts in some species. In alligators, the two gram (~5%) average difference in body mass may or may not affect long-term survival. However, reduced weight gain of juvenile animals can result in reduced survival in the wild (Brockelman, 1975; Congdon and Gibbons, 1985; Parker and Plummer, 1987; Grant, 1991). We suggest that effects may be more pronounced in smaller and less robust species.

This is the first experimental evidence documenting indirect impacts by *S. invicta* on herpetofauna. The results are probably applicable to other egg-laying reptile species. Of particular concern is the impact of *S. invicta* on hatchling sea turtles and other endangered species. The authors have documented *S. invicta* on sea turtle nesting beaches and in sea turtle nests even on remote islands in the Lower Florida Keys, Marquesas Keys, and Ten Thousand Islands in south and southwest Florida.

Anthropogenic land use change has decreased habitat for many reptile and amphibian species (Richards, 1993). Increasingly, remaining habitat is disturbed and fragmented increasing the attractiveness of these sites to *S. invicta* (Tschinkel, 1988). Furthermore, at both local (Wojcik, 1993) and regional (Cokendolpher and Phillips, 1989) scales, *S. invicta* populations and range are increasing. We believe that the population-level impact of *S. invicta* on many vertebrates is chronic and incremental, and, as such, not readily obvious.

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