in traps) and 55 others were in associations of two to five (mean ± SE = 2.39 ± 0.84).

Our observations, as well as literature accounts (e.g., Finneran 1953), indicate that when gravid females are found together, they are associated with a "den" (hole in the ground, rock crevice, hollow log) into which they can escape from predators or unfavorably low or high temperatures. They are also in areas exposed to sun for part of the day, permitting them to bask. These associations apparently stem from mutual affinity because the snakes might be gathered tightly although nearby locations that appear to be equally suitable are unoccupied (Fitch 1999). Mutual security might lead females to associate because a predator attacking one of them would be at risk of bites from others lying within striking range. Furthermore, the association between parturient females and neonates, and that between neonates of the same or different litters, might confer some benefit in mutual security.

The association of 16 gravid females with the prospect of about 100 neonates in an area of perhaps 0.0065 ha would reduce each snake's opportunity to feed. A female could stay with her litter for several days and the neonates may remain at the birthing site even longer. Although females take little or no food during late pregnancy, and thus would not compete for food during this time, they are somewhat emaciated after the birth of their litters, and are ready to take prey. The neonates shed their skins when they are about a week old; by then they are using up their store of abdominal egg yolk and are ready for their first meal. There is usually little overlap between adults and neonates in sizes of prey animals taken and in kinds of prey (Fitch 1960, 1999), but barring rapid and long-distance dispersal, intense competition seems inevitable within each age class at such high density.

This unusually large aggregation could have resulted from a high population density in a habitat with few optimal brooding sites. Exploration of the rock outcrop revealed other sites with exposed rock, but these did not seem to receive as much sun during the day as the site occupied (ALC observation). That this locality could sustain a dense snake population is given support by comparing these females with those from two other populations located in areas that differ in food abundance. The average length and embryo count of the females from Clinton Lake were 635.8 ± 11.2 mm SVL and 6.65 ± 0.24 embryos, respectively. These values are intermediate but on the high side in comparison with females from two other locations: The University of Kansas Biotic Succession Area, a seemingly ideal habitat with a high population of prey *(Microtus ochrogaster)*, and The Natural History Reservation, an area of dense forest where prey is much less available (Fitch 1999). The average length and embryo count of 15 gravid females taken from the succession area between 1986 and 1997 were 644.8 ± 8.3 mm SVL and 7.13 ± 1.15 embryos. In comparison, a 40-yr sample (1958–1998) of Reservation snakes had an average SVL of 606.5 ± 4.8 mm and contained 5.37 ± 0.17 embryos. Although no effort was made to estimate copperhead population density at the Clinton Lake site, population density is correlated with size and fecundity of females, probably through the joint causal factor of food availability (Fitch 1999).

We cannot state conclusively why these snakes formed such a large aggregation. It is unprecedented in the literature and, in light of our current knowledge of the biology of these snakes, could have social or environmental causes.

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Burrow Use by Tiger Salamanders (*Ambystoma tigrinum*) at a Black-Tailed Prairie Dog (*Cynomys ludovicianus*) Town in Southwestern South Dakota

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Reports of amphibians utilizing the burrows of other vertebrates are common. Several species of amphibians are found in gopher tortoise burrows (Lips 1991), and ambystomatid salamanders are often observed in association with small mammal burrows (Douglas and Monroe 1981; Gordon 1968; Hamilton 1946; Loredo et al. 1996). We report the use of black-tailed prairie dog (*Cynomys ludovicianus*) burrows by a population of tiger salamanders (*Ambystoma tigrinum*) at a prairie dog town at Wind Cave National Park (WICA) in southwestern South Dakota. We also provide some evidence for commensalism and suggest future studies to determine if this type of relationship exists between these salamanders and prairie dogs.

During a herpetological survey of WICA in 1996, we observed several tiger salamanders at a prairie dog town at Bison Flats, WICA (Fig. 1). The prairie dog town at Bison Flats surrounds a temporary pond used in 1996–1998 by breeding tiger salamanders (Fig. 2). Based on these observations we designed a sampling protocol to determine 1) the number of burrows used by salamanders, 2) the total number of salamanders using burrows, and 3) the pattern of salamander activity in burrows through the spring and summer. In 1998, we also wanted to determine if salamanders used specific burrows continually through the summer and determine the spatial extent of salamanders in burrows in relation to the pond. For comparison, we also visited the Pringle prairie dog town (WICA) and the Custer prairie dog town at Custer State Park (CSP) in 1996 and 1997 (Fig. 1). In 1998, we focused only on the Bison Flats prairie dog town and expanded the area of our survey to determine if the 1996–1997 surveys were missing occupied burrows farther from the pond.

During the spring and summer 1996–1998, we conducted a total of 17 surveys of prairie dog towns (Table 1). Surveys were conducted between 2045–2400 h and varied in duration from 19–156 minutes. Night air temperatures to the nearest 0.5°C were taken within the prairie dog town at the beginning of each survey using a hand-held thermometer one meter above the ground. During night surveys at each prairie dog town, we systematically checked from 100–187 (five surveys, mean = 161.6) burrows in 1996, 90–113 (five surveys, mean = 106.4) burrows in 1997, and 117–491 (seven surveys, mean = 264.4) burrows in 1998. We shined a light into the entrance of each burrow, and recorded whether tiger salamanders were present. Because the surveys were conducted at night, it is possible that we missed burrows or checked the same burrow twice. When salamanders were found in a burrow, the light caused them to retreat quickly into the burrow and out of view. Therefore, if a burrow was checked a second time, a false unoccupied burrow would be recorded. Although we do not believe

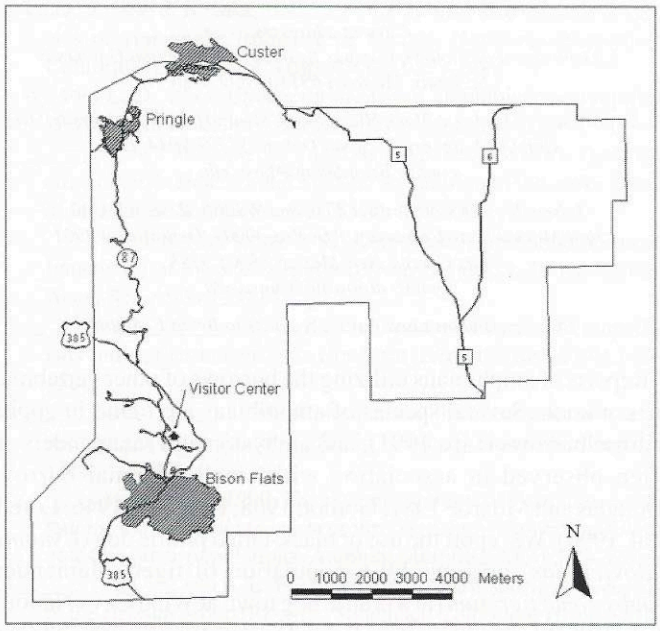

FIG. 1. Map of Wind Cave National Park indicating the location of the Bison Flats, Pringle, and Custer prairie dog towns surveyed in 1996–1998.

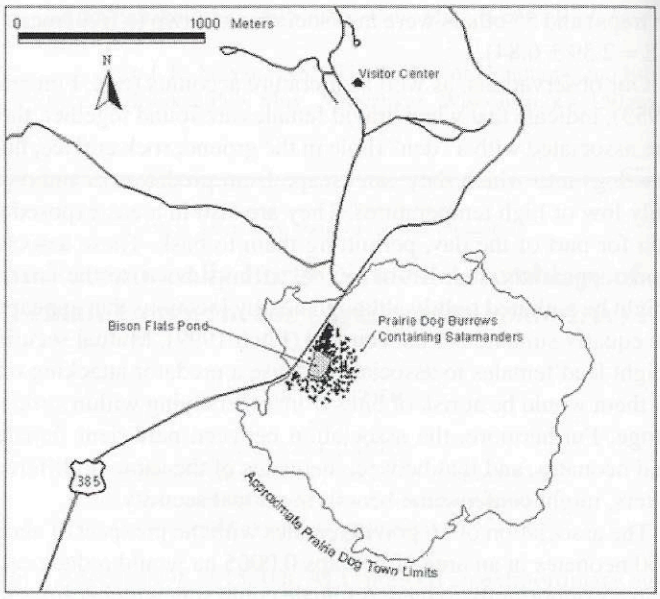

FIG. 2. Map of the Bison Flats prairie dog town showing the location of the pond, burrows occupied by salamanders in 1998, and the approximate extent of the prairie dog town limits.

that this occurred often, it would serve to underestimate the number of burrows occupied. Bison Flats prairie dog town became the focus of this study after we noticed high levels of salamander activity at this prairie dog town during studies of the adjacent pond.

We compared the number of burrows occupied and percentage of burrows occupied per survey with the date, temperature at the start of each survey, and the number of days since the last rain using linear regression (SAS Institute Inc. 1997). Percentages were arcsine transformed prior to analyses. Date was used to determine if the pattern of burrow occupancy changed during the spring and summer. Temperature and number of days since the last rain were used because salamander activity could be associated with these environmental variables. Because of the intentional increase in the number of burrows checked at Bison Flats in 1998, that is, an average of greater than 100 more burrows were checked in 1998 than the previous years, percentages of burrows occupied are lower for 1998. Therefore, we present analyses of both the number of burrows occupied and percentage of burrows occupied at Bison Flats from 1996–1998. Finally, we used ANOVA to determine if the number of burrows occupied was different among the three years.

In 1998, we marked burrows that were occupied by salamanders with a stake for future identification and measured the distance from the center of the pond to each of these burrows. To evaluate the spatial extent of burrow use, we used a chi-square test to determine if the number of occupied burrows in each 30 m-wide concentric ring around the pond was different (Sokal and Rohlf 1995). The expected number of occupied burrows was calculated based on the area of each successively larger concentric ring (i.e., 30–60 m, 60–90 m, 90–120 m, and 120–150 m) divided by the total area. The 0–30 m area was not included because the pond inundated it. The zero point was defined as the deepest point of the pond. We also monitored marked burrows in subsequent surveys to determine if salamanders used the same burrows.

Night air temperatures during surveys ranged from 15–25°C

TABLE 1. Results of tiger salamander surveys at three prairie dog towns in Wind Cave National Park and Custer State Park 1996–1998.

Prairie Dog Town	Date	Temp. (°C)	Number of Burrows with 0–5 Salamanders Per Burrow						Number of Burrows Occupied	Total Number of Salamanders	% of Burrows Occupied
			0	1	2	3	4	5			
Bison Flats	17 May 96	16.0	134	36	10	1	0	0	47	59	26.0
Bison Flats	10 Jun 96	17.0	124	35	6	3	0	0	44	56	26.2
Bison Flats	4 Aug 96	21.0	169	3	0	0	0	0	3	3	1.7
Pringle	24 Jun 96	23.5	181	6	0	0	0	0	6	6	3.2
Custer	26 Jun 96	25.0	100	0	0	0	0	0	0	0	0.0
Bison Flats	16 Jun 97	19.5	64	21	2	3	0	0	26	34	28.9
Bison Flats	18 Jun 97	17.0	81	24	3	3	0	0	30	39	27.0
Bison Flats	19 Jul 97	23.5	85	17	4	1	0	0	22	28	20.6
Bison Flats	4 Aug 97	24.5	88	21	1	1	0	0	23	26	20.7
Pringle	17 Jun 97	15.0	111	2	0	0	0	0	2	2	1.8
Bison Flats	20 Jun 98	—	275	35	2	1	0	0	38	42	12.1
Bison Flats	11 Jul 98	—	437	46	6	2	0	0	54	64	11.0
Bison Flats	23 Jul 98	16.0	335	19	2	0	1	0	22	27	6.2
Bison Flats	3 Aug 98	16.0	207	34	2	3	0	0	39	47	15.9
Bison Flats	13 Aug 98	15.5	161	4	0	0	0	0	4	4	2.4
Bison Flats	26 Aug 98	18.5	104	12	0	0	0	1	13	17	11.1
Bison Flats	18 Sep 98	21.0	159	3	0	0	0	0	3	3	1.9

(Table 1). Tiger salamanders were found in few burrows at the Pringle prairie dog town in 1996–1997 and in no burrows at the Custer prairie dog town in 1996 (Table 1). For May–July of all three years, 22–54 prairie dog burrows were occupied by salamanders at Bison Flats, clearly many more than at the Custer or Pringle prairie dog towns (Table 1). In 1996 and 1998, there were drops in occupancy of varying magnitudes in August (Fig. 3), a month when the ephemeral pond at Bison Flats usually tended to dry. However, burrow occupancy by salamanders remained consistent through early August in 1997. The number of burrows occupied was negatively correlated with date for the three years combined ($r = -0.73$, $P = 0.003$, N = 14) as was the percentage of burrows occupied ($r = -0.75$, $P = 0.002$, N = 14). A similar negative relationship was detected for each year separately with a significant result for the number of burrows occupied in 1998 ($r = -0.77$, $P = 0.04$, N = 7), but not in 1996 ($r = -0.97$, $P = 0.15$, N = 3) or 1997 ($r = -0.80$, $P = 0.20$, N = 4). There was also a negative relationship between percentage of burrows occupied and date with a significant relationship in 1997 ($r = -0.90$, $P = 0.05$, N = 4), but not in 1996 ($r = -0.95$, $P = 0.20$, N = 3) or 1998 ($r = -0.52$, $P = 0.23$, N = 7). Non-significant results in 1996 and 1997 were likely because of small sample sizes as both correlations were strong. Overall, burrow occupancy by tiger salamanders decreased through the course of the spring and summer (Fig. 3). There was no relationship between the number of burrows occupied and the temperature at the start of each survey ($P = 0.26$, N = 12) nor with the percentage of burrows occupied and temperature ($P = 0.98$, N = 12). Furthermore, there was no relationship between the number of burrows occupied and the number of days since the last rain ($P = 0.44$, N = 14) nor with the percentage of burrows occupied ($P = 0.29$, N = 14). There was no difference among years in the number of burrows occupied ($F_{2,11} = 0.15$, $P = 0.86$).

More occupied burrows than expected were found in the 60–90 m and 90–120 m areas, less burrows than expected in the 120–150 m area, and the same number as expected in the 30–60 m area ($\chi^2 = 56.46$, df = 3, $P < 0.0001$). We marked 86 occupied burrows from 11 July to 13 August 1998. Of these marked burrows, 72 were checked in subsequent surveys and 38.9% had at least one salamander present. Thus, repeated use of the same burrows existed to some degree at Bison Flats.

Our data show the spatial extent and temporal pattern of black-tailed prairie dog burrow use by tiger salamanders during the spring and summer. The pond at the center of the Bison Flats prairie dog town may explain the higher percentage of burrow occupancy compared with Pringle and Custer prairie dog towns. At Bison Flats, salamander burrow occupancy was consistent in May–August 1997 when the pond still held some water, but in 1996 and 1998 burrow occupancy dropped when the pond dried during August of each year (Fig. 3). Even though burrow occupancy numbers were low in September 1998, salamanders were still present (Table 1; Fig. 3). Additionally, an anecdotal observation on 3 October 1998 found three salamanders in burrows (D. Frankfort, WICA, pers. comm.). The negative relationship between burrow occupancy and date could represent either decreasing activity levels or migration out of the area. We do not know whether tiger salamanders were still present, but inactive, at the prairie dog town after our surveys concluded.

We cannot discern whether the drop in the number of salamanders observed in 1996 and 1998 was because of decreased activity or migration from the study area. Post-breeding dispersal distances have been reported for other species of ambystomatid salamanders. California tiger salamanders (*Ambystoma californiense*) leaving breeding ponds traveled a mean distance of 35.9 m (range = 8–129 m) and settled in ground squirrel burrows

83% of the time (Loredo et al. 1996). Two studies of post-breeding dispersal in *Ambystoma maculatum* documented mean dispersal distances of 192 m (range = 157–249 m) (Kleeberger and Werner 1983) and 150 m (range = 6–220 m) (Douglas and Monroe 1981). Migration out of the study area is unlikely because of the distance from this area to other suitable habitat (i.e., ~1.5 km to the nearest wooded cover and ~1.75 km to the nearest permanent water). Radio-tracking of a subset of individuals is needed to determine if salamanders hibernate in prairie dog burrows or if they migrate out of the area.

Almost 40% of burrows marked and subsequently rechecked had salamanders present. Though we did not mark individuals, use of the same burrow during more than one survey indicates repeated or continual use by one or more salamanders and suggests the salamanders might be residents for at least the spring and summer. However, mark and recapture studies are needed to determine if salamanders occupy one or more burrows over the course of the summer. The expansion of sampling efforts in 1998 resulted in checking nearly twice the number of burrows, yet the number of burrows occupied did not increase (Table 1). This relationship is reflected in the analysis that showed there were more occupied burrows than expected between 60–120 m and less occupied burrows than expected beyond 120 m. Salamander activity might be restricted to close proximity to the pond.

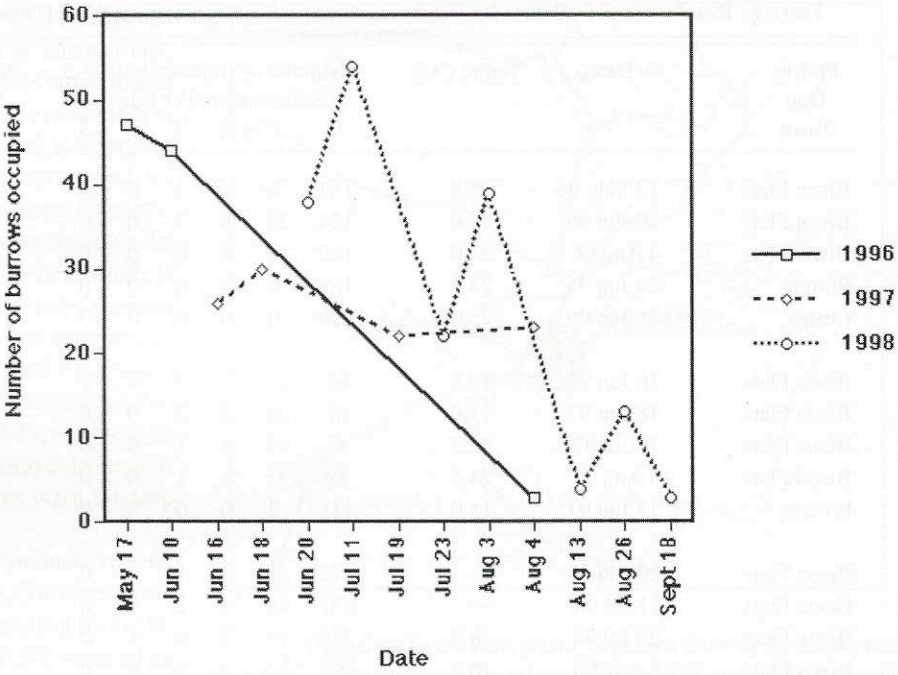

FIG. 3. Number of burrows occupied by date for surveys conducted at the Bison Flats prairie dog town in 1996–1998.

The potential benefits for tiger salamanders living in prairie dog burrows at Bison Flats include an abundance of burrows to use as shelters or hibernation sites, proximity of water for breeding, and a potential source of invertebrate prey attracted to prairie dog feces. Black-tailed prairie dog burrows are remarkably stable environments, with winter temperatures ranging from 5–10°C, summer temperatures from 15–25°C, and a relative humidity that is generally higher within burrows than on the surface (Hoogland 1995). Hoogland (1995) recorded an average relative humidity of 88% in burrows. The benefits of such stable temperatures and humidity levels are important in habitats such as the prairies of western South Dakota, where summers can be hot and dry and winters extremely cold. In summer, access to burrows would allow salamanders to remain underground during the day and become active above ground at night. This potentially decreases the risk of predation and desiccation for tiger salamanders. The use of prairie dog burrows for hibernacula would also offer advantages, such as relatively high and constant temperatures and humidity. Additionally, prairie dogs have anti-predator behaviors, such as mobbing potential predators and plugging holes (Hoogland 1995), which might benefit tiger salamanders.

Lips (1991) suggested that gopher tortoise feces retained within the burrow chamber might attract insects and subsequently insect-feeding amphibians. We observed both fecal pellets and insects around the entrances of prairie dog burrows. Salamanders might forage in close proximity to burrows, where insect abundance might be higher and there is shelter nearby. Future studies comparing the characteristics of burrows occupied by salamanders versus unoccupied burrows might provide important information concerning salamander requirements.

We have documented the spatial extent and temporal pattern of tiger salamander burrow use in a black-tailed prairie dog town over three years. These observations provide a starting point for investigations into a commensal relationship benefiting salamanders at Bison Flats prairie dog town. Additionally, observations of multiple salamanders per burrow (Table 1), often stacked on top of one another, raise interesting questions concerning previously documented territorial behavior in ambystomatid salamanders that warrant further study (Ducey 1989; Ducey and Ritsema 1988; but see Martin et al. 1986). This tiger salamander aggregation at Bison Flats presents an opportunity to study intraspecific and interspecific interactions of *A. tigrinum* in the field.

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Epizootic Disease and High Mortality in a Population of Eastern Box Turtles

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Little is known about diseases affecting wild populations of box turtles (*Terrapene* spp.). To our knowledge, there are only three published reports. Evans (1983) diagnosed two *T. carolina* with chronic bacterial pneumonia. Both individuals died after exhibiting signs of emaciation, discharge of a cream-colored exudate from the nares, and marked respiratory distress characterized by open-mouth breathing. A mixture of gram-negative bacteria, including *Morganella morgani*, *Acinetobacter calcoaceticus*, *Serratia marcescens*, and *Pseudomonas* sp. was isolated from both turtles. The collective mixture was considered the etiological agent of the pneumonia. Schwartz et al. (1984) reported ear infections in some individuals of a population of *T. ornata*. The etiology of the infections was undetermined. Tangredi and Evans (1997) documented 19 *T. carolina* from scattered locations on Long Island, New York, with one or more of the following signs: listlessness, ocular and nasal discharge, conjunctivitis, swollen eyelids, and inflammation of the middle ear. No single etiologic agent was identified from either microbiologic or histopathologic investigations. The authors speculated, however, that the pathogenesis of the infections might have resulted from low-level exposure to organochlorines.

Recently, *Mycoplasma agassizii* was isolated from a wild *T. c. bauri* with upper respiratory tract disease (URTD) in Florida (M. Brown, pers. comm.). *M. agassizii* is known to cause URTD in wild desert tortoises (*Gopherus agassizii*, Brown et al. 1994) and gopher tortoises (*G. polyphemus*, Smith et al. 1998). Upper respiratory tract disease is contagious and may be a major source of mortality in many tortoise populations (Berry 1997). Clinical signs of URTD include intermittent serous, mucoid, or purulent nasal discharge, ocular discharge, conjunctivitis, swollen eyelids, eyes recessed into the orbits, and dull skin and scutes (Brown et al. 1994; Jacobson et al. 1991).

From May 1997 to October 2000, a population of *T. c. carolina* was monitored using radio telemetry in a southern Appalachian wetland. During the study period, high mortality occurred in this population, apparently as a result of an infectious disease. In this paper we report the general characteristics of the disease, necropsy results from two individuals that died, and blood tests that detect exposure to *Mycoplasma* sp.

The study area is a 95-ha floodplain of Tulula Creek in Graham Co., North Carolina, USA (elev. 785–800 m). A complete description of the site is in Rossell et al. (1999). Each year a maximum of ten turtles with carapace lengths > 10 cm (measured using calipers) were radio-tagged with 15 g transmitters (Wildlife Materials, Inc.). Transmitters were mounted on the rear edge of the carapace with galvanized wire and never exceeded 7% of the mass of any turtle.

Sex and age of turtles were recorded when possible, using eye color and plastron shape (Schwartz et al. 1984; Stuart and Miller 1987). Age was estimated by the mean number of growth rings on four randomly selected carapace scutes (Ewing 1939). All turtles were permanently marked by notching marginal scutes (Cagle 1939).

Turtles (N = 34) were located at least weekly from mid-May through mid-August and biweekly from mid-August until they entered their hibernacula. Twenty-two turtles (64%) were monitored ≤ 1 yr, eight turtles (24%) were monitored 1–2 yr, and four turtles (12%) were monitored 2–3 yr. Because turtles did not start dying from disease until mid-September 1997, individuals were not regularly examined for signs of disease until 1998. Transmitters were removed from six animals with no signs of disease during the study because they moved long distances outside the study area. Transmitters were placed on new individuals as they were encountered in the field.

Of the 34 *T. c. carolina* (20 M, 12 F, 2 unknown) that were radio-tagged, seven (21%) died while being monitored. Five turtles (2 M, 2 F, 1 unknown) died in 1997, one male died in 1998, and one male died in 2000. Turtles that died in 1997 and 1998 all exhibited clear ocular and nasal discharge, and swollen eyelids prior