Chelonian Conservation and Biology, 1997, 2(3):409–414

Radiographic Determination of Fecundity: is the Technique Safe for Developing Turtle Embryos?

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Conservation biology requires thorough knowledge of an animal's life history characteristics, as do scientifically sound ecological risk assessments. Successful management of endangered species, or determination of effects from human impacts, is difficult without fundamental demographic data. Data such as prolonged decreased fecundity, for example, can be a foreboding endpoint indicative of declining populations. Obtaining adequate samples to detect changes in fecundity, however, is a challenging task for the research biologist. Herpetologists have largely overcome the problem in the study of oviparous species by using radiography as a tool to obtain critical reproductive information. Radiographs disclose the number of eggs in the oviducts (Fig. 1). Such information is important when predicting ecological effects or examining long-term demographic trends. In addition to clutch size, information about reproductive frequency, age at sexual maturity, and egg size can be gleaned — if not totally, at least in part — from radiographs. Despite the slight enlargement of actual egg dimensions (Graham and Petokas, 1989), egg widths taken from radiographs are strongly correlated with egg wet mass, dry mass, lipid content, and size of hatchling (Congdon et al., 1983). Radiographs have provided key data on how life history characteristics may constrain population responses, information that has implications for conservation and management of long-lived organisms (Congdon et al., 1993, 1994).

Radiographic techniques are well developed for turtles (Gibbons and Greene, 1979) and have been used on many species including the federally protected desert tortoise (e.g., *Gopherus agassizii* [Turner et al., 1986]; *Kinosternon flavescens* [Iverson, 1991]; *Kinosternon sonoriense* [van Loben Sels et al., 1997]; *Chrysemys picta* [Iverson and Smith, 1993; Lindeman, 1996; Rowe, 1994a, 1994b]; *Deirochelys reticularia* [Congdon et al., 1983]; *Emydoidea blandingii* [Congdon et al., 1993]; and *Chelydra serpentina* [Congdon et al., 1994]).

Ultrasound has also been used to assess ovarian status, but this technique is not accurate when used on females carrying large numbers of eggs (Kuchling, 1989; Penninck et al., 1991).

At a recent symposium on the ecology of North American tortoises (Aguirre et al., 1995), there was significant discussion among the participants regarding the potential effects of radiographic techniques on tortoises and their developing embryos. Specific questions included: How much radiation does the egg absorb during irradiation of the adult female, and is the dose sufficient to warrant concern about the viability of the offspring? Could the very technique being used to gauge a population's health be jeopardizing the population's future? The questions are pertinent; marked effects to embryos, in terms of lethality and in the production of abnormalities, have been repeatedly observed as a consequence of large doses of ionizing radiation (Casarett, 1968). As early as 1906 the French radiobiologists Bergonie and Tribondeau (1906) recognized that cells undergoing rapid division are the most susceptible to radiation damage. Thus, the stages from gametogenesis through embryonic development are the most sensitive to irradiation, and reduced natality is likely the most limiting life history component in terms of population survival (IAEA, 1992).

Is radiography a sound scientific technique in which the benefits exceed the risks, or are the doses sufficient to warrant concern, suggesting that the technique be discontinued? We addressed these questions by: 1) measuring the dose turtle embryos receive when adult females are X-rayed for determination of clutch size, and 2) comparing the

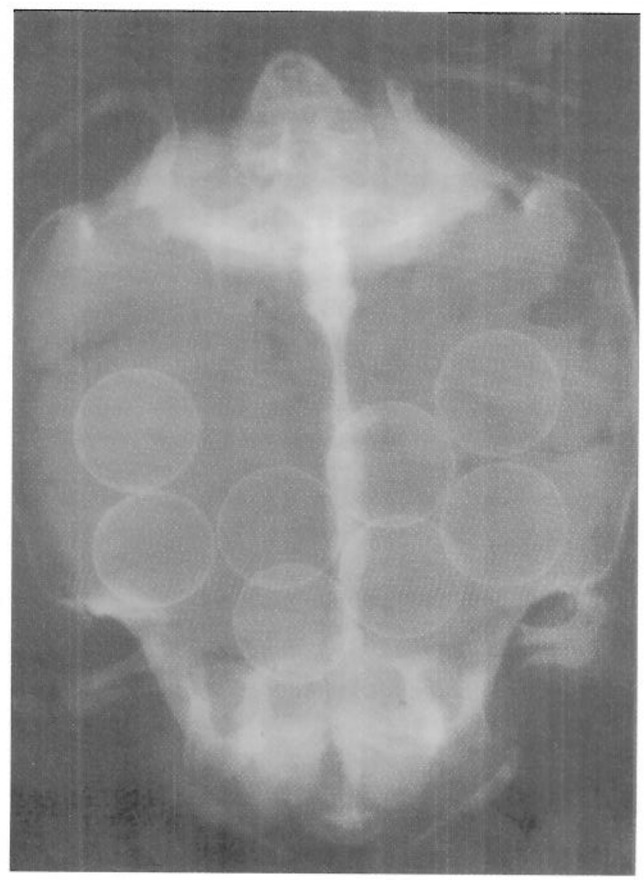

Figure 1. Radiograph of a female gopher tortoise (*Gopherus polyphemus*) with 8 hard-shelled eggs ready for oviposition.

magnitude of our measured dose to levels known to have caused damage.

Materials and Methods. — We used thermoluminescent dosimeters (TLD) to estimate the dose absorbed by developing embryos during the X-ray procedure. A TLD is designed to absorb energy proportional to the radiation exposure. The absorbed energy causes electrons within the TLD to be elevated and trapped in higher energy states, where they remain until the TLD chip is heated. Heat, produced by the instrument used to analyze the TLD, causes the electrons to return to their previous state and to give off a light that is quantifiable and proportional to the energy the TLD received. We used Panasonic UD-802 TLDs containing two lithium borate and two calcium sulfate chips with specific amounts of filtration associated with each. Characteristics of the TLDs are found in Table 1. A plastic housing containing all four chips measured 23 x 49 mm. The entire package is subsequently referred to as a single TLD. We exposed TLDs to X-rays generated from a MinXray 903 Type B-85 unit, containing a Toshiba D-183BS X-ray tube. The unit is used routinely to perform research radiography at the Savannah River Ecology Laboratory (SREL).

Two exposure scenarios were used, both at a distance of 73 cm from the X-ray tube. For the first we taped three TLDs to the inside plastron of an empty yellow-bellied slider (*Trachemys scripta*) shell (plastron length 25 cm) and then followed the standard procedures established by SREL scientists for X-raying turtles when using Ready Pack film (Gibbons and Greene, 1979). Our technique approximated the dose an egg would receive, partially shielded from the X-rays by the female's shell. The plastron of the *T. scripta* shell faced up towards the X-ray beam during exposure. Exposures were repeated with a different set of three TLDs, then again with two more sets of three TLDs on a different day. Exposure times and voltage were altered to encompass the range normally used (Table 2). A similar methodology was employed using the shell of a desert tortoise (*Gopherus agassizii*; plastron length 21 cm).

The second exposure scenario was made by placing TLDs directly in the X-ray beam without a turtle shell. Measuring the dose from the naked beam represents the worst case scenario because no shielding or attenuation of the beam is provided by the adult's shell, flesh, or shell of the egg. This scenario was replicated with 16 TLDs (Table 2). An unexposed group of TLDs was used to subtract natural background dose from the test TLDs.

Units of Measure. — Dose, as used in the radiation sciences, is a measure of the energy absorbed by the organism from the radioactivity. The international unit for absorbed dose is the Gray (Gy = 1 joule/kg); previously, the unit of radiation absorbed dose was used (rad, where 100 rad = 1 Gy). Standard prefixes can be used to alter the magnitude of the units (i.e., mGy = 10^{-3} Gy).

Results. — Readings from the TLDs are presented in Table 2. Averaged over all exposure conditions (disregarding species differences), the mean (± SE) estimate of dose to developing embryos shielded behind an adult's plastron was 1.17 ± 0.04 mGy (n = 21). The TLDs exposed to the naked beam received 1.99 ± 0.08 mGy (n = 16). The adult *T. scripta* shell attenuated 48% of the X-ray beam when exposed for 2.5 sec at a 90 kilovolt peak (kvp) compared to a 28% attenuation by the *G. agassizii* shell. Increasing the kvp by 33% (from 90 to 120) increased the dose by 11% within the *T. scripta* shell. Increasing the exposure time by 20% (from 2.5 to 3.0 sec at 90 kvp) caused an 8% increase in dose to the TLDs exposed within the naked beam.

Table 1. Characteristics of the Panasonic UD-802 thermoluminescent dosimeter containing four TLD elements. Lower limits of measurements (LLM) are given, the upper limit for each element is 10,000 mGy (WSRC, 1993).

Element	Purpose	Filtration	Radiation Type	LLM (mGy)
$Li2B_4O_7$:Cu	skin dose	plastic/mylar 17 mg cm^{-2}	gamma, x-ray, beta	0.10 0.30
$Li_2B_4O_7$:Cu	skin and whole-body	plastic 320 mg cm^{-2}	gamma, x-ray, beta	0.10 0.30
$CaSO_4$:Tm	indicates low energy photons	plastic 320 mg cm^{-2}	gamma, x-ray	0.10
$CaSO_4$:Tm	whole-body	lead/plastic 1020 mg/cm^{-2}	gamma, x-ray	0.10

Discussion. — When considering the risk to turtles from an increased radiation exposure, it is important to know how sensitive reptiles are to radiation. Several reviews have been conducted on the response of organisms to radiation exposure (Templeton et al., 1971; Turner, 1975; Blaylock and Trabalka, 1978; NRCC, 1983; NCRP, 1991; IAEA, 1992). Evaluating the comparative sensitivity of organisms to radiation has been attempted by establishing the dose at which 50% of the organisms die within a specified time frame, the "median lethal dose," or LD_{50}. LD_{50} values suggest that turtles do not appear to have a greater sensitivity to radiation than other reptiles, amphibians, or fish (Table 3). The scant data available on reptiles give no indication that turtles are more radiosensitive than other organisms.

Radiation is one of the most studied carcinogens. General references on the effects of radiation include books by Bacq and Alexander (1961), Casarett (1968), and Arena (1971). Notable reviews have been prepared on radiation effects on humans (NAS, 1972; UN, 1972), as well as on

Table 2. Doses to turtle embryos received during radiography of gravid females, as estimated by placing thermoluminescent dosimeters within the body cavities of empty shells of *Trachemys scripta* and *Gopherus agassizii* during the X-ray procedure. Unshielded doses from the naked X-ray beam are also included. Voltage, exposure times, number of samples, and mean dose (± SE) are presented. Settings are based on requirements for Ready Pack film.

Location	kvp	Time (sec)	n	Dose (mGy)
Naked beam	120	2.5	4	2.16 ± 0.07
Naked beam	90	2.5	9	1.89 ± 0.13
Naked beam	90	3.0	3	2.04 ± 0.09
Inside *T. scripta* shell	120	2.5	6	1.10 ± 0.02
Inside *T. scripta* shell	90	2.5	6	0.98 ± 0.02
Inside *G. agassizii* shell	90	2.5	9	1.35 ± 0.03

Table 3. A comparison of the LD_{50} for reptiles to other organisms, adapted from Hinton and Scott (1990). Time periods over which effects were measured were generally 30 days for birds and mammals and 60–90 days for poikilotherms.

Organism	LD_{50} (mGy)	Organism	LD_{50} (mGy)
Turtles		**Fish**	
Gopherus	10,000–15,000	*Carassius*	8000
Chelydra	< 8000	**Ducks**	
Chrysemys	< 10,000	*Anas discors*	12,600
Testudinidae	8500	*Anas carolinensis*	4800
Lizards		*Spatula clypeata*	8900
Sceloporus	15,000	**Rodents**	
Uta	10,000–22,000	*Citellus*	12,600
Snakes		*Peromyscus*	9200–11,500
Elaphe	3000–4000	*Ochotoma*	3800–5600
Frogs		*Rattus*	3000–6000
Hyla	11,200	**Humans**	3000–6000
Rana	7000		
Salamanders			
Desmognathus	5200		
Necturus	800		
Notophthalmus	4,700		

specific groups of organisms: protozoa (Wichterman, 1972), brine shrimp (Metalli and Ballardin, 1972), insects (O'Brien and Wolfe, 1964), amphibians (Brunst, 1965), reptiles (Cosgrove, 1971), birds (Mellinger and Schultz, 1975), plants (Sparrow et al., 1958), plant communities (Whicker and Fraley, 1974), and terrestrial and aquatic animal populations (Turner, 1975; Blaylock and Trabalka, 1978). The National Council on Radiation Protection and Measurements has examined the effects of ionizing radiation on aquatic organisms (NCRP, 1991), the International Atomic Energy Agency has considered whether or not non-human species are adequately protected by radiation standards designed for humans (IAEA, 1992), and the lower limits of radiosensitivity in non-human species have been reviewed (Rose, 1992).

The effects of radiation on reproduction have been most extensively studied in mammals with the majority of results suggesting that natality is a more radiosensitive parameter than mortality (Carlson and Gassner, 1964). Data for poikilotherms are not as extensive as for mammals. However, among the vertebrates, mammals are generally more radiosensitive than birds, fishes, amphibians, or reptiles (Casarett, 1968). Therefore, a review of doses at which effects have been observed in radiosensitive vertebrates (Table 4) will help put into perspective the 1 mGy dose that we estimated turtle embryos received during the X-ray procedure. Rose (1992) reviewed the literature for lower limits of radiosensitivity in organisms and found that the lowest dose from an acute exposure that caused changes was 10 mGy; an exposure that, when delivered to pregnant rats, impaired the reflexes of their offspring (Semagin, 1986). Studies on radiation effects to non-mammalian organisms have indicated higher radioresistence and, like studies conducted on mammals, that the early life cycle stages are the most radiosensitive (NCRP, 1991). The lowest dose reported to have an impact on amphibians is 20 mGy, a level that damaged newt eggs (*Triturus alpestris*; Peters, 1960). Anderson and Harrison (1986) found that radiation doses in excess of 10 mGy were necessary to damage the most sensitive stages of fish development.

These doses are well above the approximately 1 mGy dose we estimated turtle embryos received during radiography procedures, suggesting that the probability of deleterious effects from irradiation is small. Pertinent, supporting data from Gibbons and Greene (1979), using the same radiography technique reported herein, revealed that the hatching success of X-rayed turtle eggs (22 of 56) was statistically equal to that of a control group (18 of 57). An unusual number of carapacial shields was observed in one hatchling from the irradiated group; however, it is not possible to state whether this frequently observed anomaly in natural populations (Zangerl, 1969) was X-ray induced.

A particularly important consideration when discussing radiation effects to turtles is that an additional measure of protection is afforded by the timing of embryonic development. Because development is arrested in the late gastrula stage, while the egg is within the female, embryonic development occurs largely in the nest (Ewert, 1985; Miller, 1985). The very premise of concern — higher radiosensitivity during rapid cell division of embryogenesis — is partially negated because the eggs are X-rayed while in a quiescent developmental period.

The National Council on Radiation Protection and Measurements (NCRP, 1991) recently established criteria for the protection of populations of aquatic organisms. They concluded that limiting the maximum chronic dose rate to 10 mGy/d would provide adequate protection for endemic populations of aquatic organisms in environments receiving discharges of radioactive effluent. The NCRP recognized that other environmental stresses might act in combination

Table 4. Effects of exposure to radiation on various organisms. Doses at which effects have been documented can be compared to the estimated dose received by turtle eggs during radiography procedures (approximately 1 mGy).

Organism	Observation	Reference
Mice	Reproduction impaired in females at doses above 200 mGy; permanent sterility occurred at 1000 mGy	(1)
Mice	Reproduction in males impaired at 3200 mGy	(2)
Mice	3000 mGy did not affect longevity of first or second generation offspring	(3)
Rats	150 mGy to fetuses did not impair maze-learning abilities	(4)
Frogs	200 mGy to eggs affected frogs	(5)
Ambystoma	200 mGy affected axolotl larvae	(6)
Toads	Adults maintained their population level at 3000 mGy	(7)
Japanese quail, bobwhite quail, leghorn chickens	Egg production was reduced for 10 days following exposure to 4000 mGy; reproductive performance of the progeny was not affected	(8, 9)
Swallows	Doses > 1600 mGy caused increased incubation times and decreased growth; doses up to 3200 mGy did not affect hatching or fledgling success	(10)

References: (1) Gowen and Stadler, 1964; (2) Rugh and Wolff, 1957; (3) Spalding, 1964; (4) Werboff et al., 1963; (5) Shekhtman et al., 1930; (6) Sheremetjeva, 1937; (7) Blair, 1961; (8) Baumgartner, 1985; (9) Maloney and Marz, 1969; (10) Zach and Mayoh, 1986.

with those from radiation and, thus, might cause an impact at the maximum reference level of 10 mGy/d. Therefore, they conservatively recommended that a comprehensive ecological evaluation of the radiation exposure and environmental stressors be conducted when populations are exposed to 2.4 mGy/d. The International Atomic Energy Agency (IAEA, 1992) has also addressed the issue of effects of ionizing radiation on plants and animals. They concluded that "There is no convincing evidence from the scientific literature that chronic radiation dose rates below 1 mGy/d will harm animal or plant populations." It should be emphasized that the NCRP (1991) and IAEA (1992) recommendations are for organisms receiving *daily* doses, compared to the single, one-time dose turtle eggs receive from radiography. Dose limits for humans are particularly conservative, and the allowable cumulative dose to the human fetus during the gestation period is 5 mGy (CFR, 1993), a dose well above that which the turtle eggs were estimated to have received.

Summary and Recommendations. — The dictum for humans is protection of individuals. In contrast, the prevailing philosophy for non-humans is protection of the population. Seldom are we concerned with the individual fish or tree, as long as we know that the population is viable. Therefore, in situations where researchers are working with well established populations, we believe that radiography can be used routinely without fear of adverse effects for the following reasons:

1) Estimated dose to the turtle eggs was measured with TLDs to be 1.17 ± 0.04 mGy.

2) There is no evidence in the literature documenting reduced fecundity, teratogenic, or population level effects at an acute dose of 1 mGy.

3) The data available give no indication that reptiles should be especially sensitive to radiation when compared to other organisms.

4) The review by Rose (1992) indicated that the lowest dose at which harmful effects from acute irradiation have been reliably observed is 10 mGy.

5) Gibbons and Greene (1979) did not find a reduction in hatchability of eggs from X-rayed turtles compared to controls.

6) Embryogenesis in turtles is delayed while the egg is in the female and does not resume until oviposited. Thus, concern for heightened radiosensitivity to the eggs because they are undergoing rapid cell division is negated.

7) Reviews by the National Council of Radiation Protection and Measurements, the International Atomic Energy Agency, and the National Research Council of Canada have concluded that acute doses below 100 mGy, and chronic doses of 1 mGy/d, to even the more radiosensitive species are unlikely to produce persistent, measurable deleterious changes in populations or communities of plants or animals.

With endangered species a shift from concern about the population to that of individuals may be necessary, particularly if they exhibit low reproductive rates (IAEA, 1992). The desert tortoise falls into this category of requiring special consideration and extra caution — the level at which risks become acceptable must be carefully considered. We suggest that prudence be applied when considering the use of radiography on endangered species. Efforts to reduce exposures should be taken when practical, and can be achieved, in the context of this manuscript, by using cassette-type film with rare earth screens.

Researchers generally have two film options when using radiography for field studies, Ready Pack or cassette-type film. The choice depends on the required definition (sharpness), contrast, and detail of the radiograph, as well as the ease of use in field settings. Both options have positive and negative aspects associated with their use. Ready Pack film is convenient because each sheet of film is encased in an aluminum foil envelope that is light proof and does not require the film to be removed for exposure or storage after exposure. Thus, Ready Pack film does not require the use of darkroom facilities. The primary problem is that Ready Pack film requires substantial and prolonged exposures to produce an image with adequate contrast. A related problem is the difficulty of completely immobilizing the animals during the long exposures (typically 2.5 sec). In addition, although Ready Pack film is adequate for use in imaging the relatively large, well-calcified eggs of turtles and alligators, it may not be adequate in situations that require good contrast, definition, sharpness, and detail because of its graininess.

In contrast to Ready Pack film, cassette film is placed between two rare earth screens in a light proof cassette. Rare earth screens, most containing a gadolinium oxysulfide compound, are widely used today to intensify the radiograph. The film is exposed primarily by light produced by the screens and secondarily by X-rays. Each screen consists of a thin layer of phosphor crystals that emit a light whose brightness is proportional to the intensity of the absorbed X-rays. Irradiation times can be reduced substantially because the film is very sensitive to light exposure. The problem with cassette film is that each individual sheet is not protected from light and, therefore, requires darkroom procedures when loading and unloading the film into light-proof cassettes and also while storing the film until it is developed. Whereas such problems may appear to be difficult to overcome in field situations, they have not proved to be so in situations involving turtle studies at SREL in South Carolina, in Michigan, in Arizona, and in Acadia National Park in Maine (A.G.J. Rhodin, *pers. comm.*).

Much lower exposures are possible when cassette film is used. For example, between 1978 and 1992 Ready Pack film was used to produce radiographs of gravid females of three species of turtles on the E.S. George Reserve in Michigan. The settings used were 80 kvp (all species) and exposures of 1.0 sec (*Chrysemys picta*, 500 g maximum body mass), 2.0 sec (*Emydoidea blandingii*, 1300 g maximum body mass), and 3.0 sec (*Chelydra serpentina*, 6000 g maximum body mass). In 1993 we used cassette film with rare earth screens and Kodak T-MAT G film. The T-MAT G film was selected because it has the broadest tolerance to settings (i.e., settings did not have to be changed to adjust for variation in body size within the range of each species). The settings used to expose the cassette film were reduced to 70 kvp (all species) and exposures of 0.08 sec (*C. picta*), 0.10 sec (*E. blandingii*), and 0.12 sec (*C. serpentina*). These settings represent a 12% reduction of the kvp and 92, 95, and 96% reductions in exposures previously used with Ready Pack film for each species, respectively. Radiographs of *C. picta* (up to 800 g) using rare earth cassettes have been successfully obtained at even lower exposures: A.G.J. Rhodin (*pers. comm*) used settings of 58 kvp at 0.05 sec, for reductions of 27% in kvp and 95% in exposure time as compared to Ready Pack film. Our data indicate that a significantly reduced exposure time would substantially lower the radiation dose and, thereby, reduce even further the already low probability of harmful effects. We, therefore, recommend that rare earth screens be used when radiography is applied to threatened or endangered species, and that they be preferentially used on all other species whenever feasible.

A general problem with both types of radiographic film is enlargement of actual egg dimensions (Graham and Petokas, 1989). The amount of enlargement can be minimized by being aware of two concerns when setting up an X-ray system. First, if possible, the organism should be placed ventral side down to minimize the distance between eggs and film. The difference between ventral and dorsal placement is particularly important if the organism is large and thick-bodied as in a turtle. Second, the X-ray head should be placed at the greatest possible distance from the object to be examined (within practical limits). At maximum distances radiographic definition is improved and enlargement of the object is reduced (Eastman Kodak, 1968).

An additional problem related to applying the technique in field situations is finding a power source. At the E.S. George Reserve, three different X-ray units have been powered by standard motor-generators over the past 12 years. The only problem encountered was the poor quality line voltage, which tripped voltage regulating relays on two of the units. The solution to that problem was to place the generator under a slight load to clean voltage peaks from the line, accomplished by keeping a 60 watt incandescent bulb on during operation of the unit. Strangely, operation of a fluorescent light prevented the unit from working.

If conservation biology and ecological risk analyses are to succeed, we must work with enhanced knowledge about the reproductive condition of organisms. Radiography is a powerful research tool that provides critical data about a population's reproductive status and health. It is clearly an improvement over the technique of earlier times — routine sacrifice of a large series of adult turtles to examine their ovaries. Although definitive studies on the long-term effects of radiographs on hatchling health, fecundity, and survivorship still need to be undertaken, our data strongly suggest that doses received from prudent radiography, especially when using rare earth screens, do not place adults, embryos, or populations into jeopardy.

Acknowledgments. — Funding for this project was through contract DE-AC09-76SR00819 between the U.S. Department of Energy and the University of Georgia Savannah River Ecology Laboratory. We thank J. Graves and L. Marsh for editing, and appreciate the insightful comments of C. Strojan and F.W. Whicker on an earlier version of the manuscript. We graciously recognize Joyce Klevering for assistance with all aspects of using radiography in our research, and we thank Tracey D. Tuberville for the radiograph used in Fig. 1.

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Received: 17 November 1995
Reviewed: 24 July 1996
Revised and Accepted: 18 November 1996