Chelonian Conservation and Biology, 1994, 1(2):159-162

Temperature Controlled Sex Determination as a Tool for Turtle Conservation

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Temperature controlled sex determination (TSD) has been noted for many species of freshwater, marine, and terrestrial turtles throughout the world since the discovery of this sex-determining mechanism in reptiles by Charnier (1966). Reviews of the basic literature and theories behind this phenomenon can be found in Bull (1980), Vogt and Bull (1982), Vogt and Flores (1986), Ewert and Nelson (1991), Paukstis and Janzen (1990), and Janzen and Paukstis (1991). It seems that the existence of this phenomenon and the methodology for producing the desired sex ratio of hatchling turtles should be obvious to all chelonian biologists due to the widespread dissemination of this literature in English, Spanish, French, and Portuguese languages in journals, presentations in international scientific meetings, and even in the popular literature. However, it appears that some people doing conservation management continue to incubate eggs without having a working knowledge of this information, or are unable to grasp the consequences of producing all male hatchlings, or do not understand that threshold temperatures can vary among populations of wide ranging species.

Another problem arises in the misinformed notion that the ideal scenario is to produce both sexes of hatchlings at the same time from the same clutch and that the way to do this is to incubate the eggs at the threshold temperature, defined by Bull (1980) as the temperature which produces 50% of each sex. For example, eggs of *Chelonia mydas* were being incubated at 31.5°C at a commercial turtle farm, "because that way we would be producing an equal number of males and females". The fallacy behind this argument is that the managers of the farm had done no experiments with their captive population to determine the threshold temperature. Also, it may be more useful to produce more females than males to enhance the reproductive output of a population, since one male can fertilize many females and females of many species of turtles are known to store sperm (Gist and Jones, 1989).

Another more important reason **not** to incubate eggs at or near the threshold temperature is because of the higher probability of producing intersexes. Bull et al. (1982a,b) found a higher percentage of intersexes to be produced at or near the threshold temperature. Benabib (1984) also found a high percentage of intersexes (14%) in hatchlings of *Dermochelys coriacea* incubated in natural nests. What happens to these hatchlings with bisexual gonads as they develop is unknown, but hermaphrodite subadults and adults are known from the wild. Does the gonad continue to develop both ovarian and testicular regions? Or does one type of tissue eventually dominate over the other? Whichever occurs it is not the optimum condition to be producing hermaphrodites or even turtles which eventually become unisexual if they initially are wasting energy producing cells for an organ that will later degenerate. Ernst and Zug (1994) reported that an adult female *Clemmys guttata* had only one functional ovary, but did not hypothesize as to why, but maybe, since this species has TSD (Ewert and Nelson, 1991), it was an intersex. I once found an old female *Terrapene carolina* with only one functional ovary and, since this was before the discovery of TSD, assumed this to be evidence of senescence. In our studies with natural nests of *Graptemys* in Wisconsin (Vogt and Bull, 1984) we found that most nests were unisexual. Janzen (in press) also found this to predominate in nests of *Chrysemys picta.* Limpus et al. (1993) also reported this to be the case with sea turtle nests in Australia. In fact, most studies of sea turtles have found that the majority of nests sampled were unisexual (Benabib, 1984; Maxwell et al., 1988; Mrosovsky et al., 1984; Mrosovsky and Provancha, 1989). If the natural condition is to produce one sex or the other in a nest, it may be wise for conservation biologists to do the same. Consequently, it may be more appropriate to incubate eggs at two different temperatures, one of which is known to produce only females and the other to produce males. If managers do not have the resources for two incubation rooms, they could produce one sex one year and the other sex the next year.

In another instance, I was astounded to learn at the 4th Encontra de Herpetologia Brasileiro that a multimillion dollar project was incubating all sea turtle eggs (which they took off natural nesting beaches and incubated in artificial hatcheries) at 31°C. This was done under the assumption that 31°C was a female-producing temperature, even though the literature shows this to be low for some species of sea turtles, especially in tropical climates. It would be more appropriate to sex a few hatchlings resulting from their incubation treatment to ascertain what sex was being produced (Baptistotte, 1992). This way they could avoid the same problems that developed in a long term sea turtle conservation project in Central America which was producing predominantly males for many years by incubating the eggs in styrofoam boxes in shaded huts at a mean temperature of about 27.5°C (Carr, 1979). A study which I helped initiate was later undertaken and showed this to be a male producing temperature for this population (Morreale et al., 1982). This project has since changed its methodology. It is time to unite conservation biologists with scientists and familiarize them with the published literature (Morreale et al., 1982; Spotila et al., 1983; Mrosovsky, 1982; Dutton et al., 1985).

I found that similiar incubation problems exist in a management project of *Terrapene ornata*. The incubation temperature of all of the eggs was fluctuated above and below the threshold temperature "to insure that they would produce both sexes".

Finding these four cases within my small sphere of activity suggests that the problem may be more widespread. Information from the scientific literature does not appear to be reaching project managers in a readily digestible form. Thus, I believe it appropriate to provide a review of the results from the scientific literature in this journal, so that project managers will stop using methods that have been discredited many years ago.

Most species of turtles studied to date have temperature controlled sex determination (TSD), as opposed to genotypic sex determination (GSD). Females are produced at higher incubation temperatures in all turtle species with TSD; although some of these species also have a low threshold temperature and produce females at extremely low, nearly lethal, temperatures. For the purposes of conservation management, low temperature females should not be produced due to low survivorship of eggs incubated at such low temperatures (Gutzke and Paukstis, 1984).

In simple terms, for turtles with TSD the sex of the hatchling is determined by incubation temperature during the middle third of development (Yntema, 1979; Bull and Vogt, 1981; Pieau and Dorizzi, 1981; Reyes et al., 1988; Yntema and Mrosovsky, 1982). Thus, temperatures in the initial and terminal thirds of development do not influence the sex of the hatchlings, so it is necessary to control the incubation temperature for sex determination only during the middle third. Also, it has been shown that the effect of temperature is additive and that at least 4 hours per day above the threshold temperature are needed during this critical period to produce females; if not, males are produced (Bull, 1985). These experiments have not been undertaken for all species of turtles with TSD, but this is the pattern for the species that have been studied. Threshold temperatures vary among species and among populations of the same species from different geographic areas (Bull et al., 1982a; McCoy et al., 1983; Schwarzkopf and Brooks, 1985; Limpus et al., 1985; Mrosovsky, 1988; Vogt and Flores Villela, 1992). Thus, it is important that conservation biologists attempting to enhance the reproductive fitness of a particular turtle population know the threshold temperature of their population, and then manipulate incubation temperatures such that they produce more females than males, if this is so desired. Across species, the upper threshold varies from as low as 27°C in some species to 34°C in others. Within species using the same techniques, we have found a 2°C difference between populations of *Chelydra serpentina* and *Trachemys scripta* (Vogt and Flores Villela, 1992).

Another problem I have found is the misguided notion that nature produces a 1:1 sex ratio and that this is the optimum way to manage populations. Following this notion, people think that incubation of eggs should be made at temperatures that simulate "nature" and produce half males and half females. Fisher (1930) stated that at equilibrium the sex ratio should stabilize at 1:1, but the key phrase is "at equilibrium"; possibly all turtle populations, perhaps all natural populations of all animals, are in a state of flux. When does one find a natural population of animals at equilibrium? In this era, for that matter, when does one find a natural population of turtles? Since one male can fertilize many females, I would recommend producing at least 6-8 females per male, maybe even as many as 20. If what we need is more egg factories, resulting in more hatchlings, then we need to produce as many females as possible. If one wants to mimic "nature", then do not move the eggs, protect the beach, and allow the population to follow its evolutionary heritage. It was brought to my attention that the director of conservation at a reserve was considering moving nests of *Podocnemis unifilis* from natural beaches and incubating them at lower temperatures because many more females than males were found in the population (Lily Rodriguez, *pers. comm.*). Even if this was correct, there would be no reason to do this unless the fecundity of the eggs was low, suggesting that not enough males were present to fertilize females. In nests of natural populations of turtles from Wisconsin, Mexico, and Brazil, fecundity of successful clutches was usually greater than 95%. Only if a large number of nests had approximately 20% or more infertile eggs would I recommend producing more males than females. I have never seen this phenomenon in nature or reported in the literature. I have found complete clutches that were inviable; some of these were known to be the eggs of hybrids, others were undersized eggs of females nesting for the first time, and others were clutches from late in the nesting season, perhaps 3rd or 4th clutches. In those populations of *Graptemys* and *Dermatemys* where I found adult sex ratios of 4 to 6 females per male in nature, fertility of the eggs was still over 90%.

Objections from conservation biologists to implementing controlled incubation techniques range from lack of funds for incubators and thermometers to inability to sex hatchling turtles or to the reluctance to kill a few hatchlings (to determine their sex) to save the species. When survivorship of hatchlings to maturity of many turtle species can perhaps be as low as 1 in 1000 at best, there is no coherent reason not to sacrifice a few hatchlings to enhance the survivorship of the species in the long run. Nest temperatures can be manipulated by shade and sun exposure, depth and substrate texture, placing black plastic above nests to warm them or palm leaves to shade them, etc. (Alho et al., 1984). Simple low cost incubators can also be constructed from ice chests and aquarium heaters and can be run off wet cell batteries if no current is available.

Sex of hatchling turtles of most species can be ascertained by examination of the gonads under a stereo dissecting microscope (Bull and Vogt, 1979; Yntema, 1981). For most species of turtles it is not necessary to do histology to determine the sex of the gonad, although in some species the differences are more subtle than in others. The technique developed by van der Heiden et al. (1985), where gonads are preserved in neutral buffered formalin and then cleared in a glycerin solution, has worked fine to confirm the sexes of both hatchling freshwater turtles (Vogt and Flores Villela, 1992) as well as marine turtles (Benabib, 1984). If the material is not preserved properly (M. Benabib, *pers. comm.*), as appears to be the case in the study of Mrosovsky and Benabib (1990), the technique does not work. Careful buffering is important, and the use of unknown buffering agents, or assuming that commercial formalin is sufficiently buffered, is not adequate. I am not saying that histology is not a worthwhile technique to sex turtle gonads, only that there is no reason to go to this trouble and expense under normal circumstances.

Turtle biologists also **must** preserve and deposit all of the sacrificed turtles used in their studies in a museum collection, so that the gonads can later be accessed if a controversy arises. The hatchlings studied by Bull and myself are located at the University of Wisconsin Zoology Museum, Carnegie Museum of Natural History, Instituto de Biologia of Universidad Nacional Autonoma de Mexico, and Instituto Nacional de Pesquisas da Amazônia, being deposited in the permanent museum collections of the regions where the turtles were studied. The usefulness of this arrangement can be illustrated by the following example. Confirmation of hatchling sex ratios has become a problem in several cases where the researchers did not preserve the hatchlings. Once in Mexico, two of my students did not follow my instructions to preserve all of the hatchling *Dermochelys* under study. They thought it too much bother, too many hatchlings, but they lost a year of research because they dissected out only the kidneys, thinking they had the gonads!

Also, the study on the effect of humidity lowering the threshold temperature of *Chrysemys picta* (Gutzke and Paukstis, 1983; Paukstis et al., 1984) has not been repeatable (Packard et al.,1989, 1991). Gutzke (*pers. comm.*) told me several years ago that they had misidentified the sexes due to their not knowing at that time that recently hatched males of some species may also have Müllerian ducts (oviducts). They did not deposit their material in a museum collection, so there is no way of knowing if they were right or not. Paukstis et al. (1984) however, did both histology and gross morphology with identical results. They preserved their hatchlings so their data can be verified. The problem may lie in the fact that the experimental methods of Packard et al. (1991) were not identical to those of Paukstis et al. (1984). Even if humidity does lower the threshold temperature slightly it does not cause such dramatic effects that turtle conservation managers need to worry about producing a few extra females.

If populations are so low that sacrificing even a few individuals is out of the question, then laparoscopy could serve to identify the sex of larger species of freshwater turtles without having to kill them. However, the cost or the proximity to a facility where this specialized equipment is available could be a limiting factor. Valentine Lance (Bryan Bock, *pers. comm.*) now has a technique where he can sex hatchling turtles with a few drops of blood after they have been injected with a particular hormone. This method, however, remains to be verified and would involve more sophisticated equipment and techniques than are usually available for most conservation projects. If it is really impossible to control the incubation temperatures or to record them or sex the hatchlings, estradiol painted on the eggs with a micropipet during the middle third of development has been shown to produce only female hatchlings even when the eggs are incubated at male-producing temperatures and even in species with GSD (Bull et al., 1988; Gutzke and Bull, 1986; Gutzke and Chymiy, 1988; Crews et al., 1989; Vogt, 1991). This approach is perhaps the most cost-effective way to produce all female hatchlings at any viable temperature without having to buy incubators, thermometers, or worry about power outages and worker ineptness. However, long-term studies are needed to confirm that hormonally-produced females are indeed equivalent to temperature-produced females.

By controlling incubation temperatures conservation biologists should be able to augment significantly the populations of all species of turtles being managed. During the next 50 years we should see populations of many species increase if incubation temperatures are managed to produce a higher percentage of females. Purists will say that this method is unnatural, but the natural approach is to let the populations die off. Take your pick: produce 6 to 20 females per male to ensure the survival of the population, or produce a 1:1 sex ratio because that is what happens in humans and *Drosophila* experiments in the laboratory? With humans and other animals eating or killing turtles in most populations faster than they can grow and reproduce, I see no alternative. Obviously there is much more to turtle conservation than protecting eggs and producing viable hatchlings of the desired sex, but it is a first step in the never ending journey of turtle conservation. We must remember that life history tables show that the most important parameter in turtle demography is the standing crop of mature reproducing individuals, and if the factors contributing to their demise, be it habitat degradation or predation, are not addressed, then we are only fooling ourselves if we think that releasing hatchlings is enough to solve the problem.

Acknowledgments. — This paper was written while I was on sabbatical leave at CPEC/INPA, Manaus, Brazil, with support from CNPq of Brazil and DGAPA of UNAM. Bill Magnusson is thanked for his critical review of my vanishing English. Fred Janzen is thanked for critically reviewing the manuscript, clarifying data, and providing a preprint of his article in press.

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Accepted: 24 May 1994