Artificial incubation of yellow-headed sideneck turtle *Podocnemis unifilis* eggs to reduce losses to flooding and predation, Cojedes and Manapire Rivers, southern Venezuela

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SUMMARY

Although widespread in South America, the yellow-headed sideneck turtle *Podocnemis unifilis* is considered 'Vulnerable' in Venezuela. A large proportion of eggs of this riverine species may be lost due to predation (including collection by humans) and flooding. As a technique to enhance reproductive success, transfer of wild-laid eggs to protected zones for incubation has been successfully carried out. This study undertaken in 2009, evaluated the hatch success of clutches transferred to artificial nest chambers at protected locations compared with natural clutches left *in situ* along stretches of the Cojedes and Manapire rivers (Venezuela). Along the Cojedes River, 78 turtle nests were located, eggs from 13 of which were transferred for incubation. In the Manapire River, 87 nests were located, eggs from 13 of which were transferred for incubation. In the Cojedes River, 28.2% of study clutches (n=22) were lost due to predation and flooding; in the Manapire River, 85% of nests (n=74) were lost due to predation (humans and other animals). At Cojedes River, hatching success of eggs in artificial nests was 88.2% and 63.2% in natural nests.

BACKGROUND

Podocnemis (Testudines: Podocnemididae) is a genus comprising six species of riverine freshwater turtles commonly known as South American river turtles. Typically females lay their eggs at night on sandbanks which are exposed only in the dry season. There is much evidence that points to nest predation and flooding as the main causes of low reproductive success of these and other South American freshwater turtles (Fachin & von Mülhen 2009).

In 1965, Ojasti and Rutkis estimated that 25% of giant South American turtle *Podocnemis expansa* clutches were lost each year due to

flooding in the Middle Orinoco (Venezuela): subsequently Ojasti (1967) estimated that observed losses to flooding in this region ranged between 25% and 80% annually. In addition, Soini (1986) found that along a 15 km of the Pacaya River (Brazil), human predation of P. expansa nests accounted for 22 of 30 clutches (73%) laid, although it could have risen to 100% if the remaining eight nests had not been transferred to a protected location for incubation. In 2007, in the Bita River (Colombia), Echeverri-Alcendra (2008) found that 41% of P. *expansa* clutches were taken by people and 9% were lost to flooding and, again as in the previous example, it was considered that this loss could have reached 100% had the remaining clutches not been transferred to a protected area for incubation.

Daxa (2004) determined a loss of 59 of 62 natural nests (95%) of mata mata *Chelus fimbratus*, located along 40.85 km of transects within three rivers in Cojedes state (northern Venezuela). These high losses were caused by flooding, animal predation and egg-collection by people. During a red-headed river turtle *Podocnemis erythrocephala* nesting ecology study in 2002, Batistela and Vogt (2008) located 117 nests of this species on the Ayuanã River (Brazil). Nest loss reached 100% for the season: 70% due to flash floods, 25% due to predation by wild animal and 5% due to collection by humans.

By transferring clutches of yellow-headed sideneck turtle Podocnemis unifilis along the Caqueta River (Colombia) to a safe incubation site, Paez and Bock (1998) prevented loss due to flooding of 19 of 27 nests (70%) in 1993, and 18 of 32 nests (56%) in 1994. P. unifilis (the subject of this present study) is a widespread species of rivers and lakes of the Orinoco and Amazon River basins. Adults can reach 68 cm in curved carapace length (Ernst & Barbour 1989). Females excavate their nest chamber in sandy areas along river banks, and clutches usually contain four to 35 eggs. Eggs are usually laid at the peak of the dry season thus reducing the risk of clutch loss due to flooding. P. unifilis is listed as 'Vulnerable' in the Red Book of Venezuelan Fauna and by the IUCN (2010). Human pressure on this species has increased; main threats are the capture (by fishing nets and hooks) of adults for their meat and egg collection (Ojasti et al. 2008).

This present study evaluated the success of transferring vulnerable yellow-headed sideneck turtle clutches and reburying them in nearby locations protected by fencing or wire mesh enclosures to exclude vertebrate predators. Hatching success was compared with natural clutches in two areas along the Cojedes and Manapire Rivers (tributaries of the Orinoco), southern Venezuela.

ACTION

Field surveys: In February (low-water breeding season) 2009, a 5.9 km length of the Cojedes River (CR) and a 13 km length of the Manapire

River (MR) were surveyed for nesting *P. unifilis*. All beaches with turtle nesting potential were investigated for characteristic signs of turtle nesting activity, including the tracks of female turtles in the sand, or presence of eggshells and excavated nests (indicative of nest predation). Intact nest chambers were detected by sinking a wooden rod into the sand and digging in those places where the rod penetrated easily. Thirty six potentially suitable beaches (23 in the CR and 13 in the MR) were found and searched for nests.

Egg collection and transfer: In the CR, 78 nests were located. From 27 of these, five eggs were removed for artificial incubation. The remaining eggs were left in the nest which was covered back with sand. The five collected eggs of each nest were placed (maintaining their original orientation) onto a moist bed of sand (taken from the nest beach) in a small plastic container (plastic cup) and then carefully covered completely with sand. These containers were then transferred to an expanded polystyrene container (again sand filled) for transport to the incubation site at the Merecure Ranch located about 5 km away. Here, a trench (3.0 x 0.4 x 0.3 m deep) was dug in an area open to direct sunlight and protected from vertebrate predators by a metal mesh fence (1.5 m high, fixed to the ground with metal anchor pegs to prevent predators from entering under it). The trench was filled with sand from the nesting beaches. Plastic cups with eggs were transferred to the trench and buried in the sand at a depth comparable with the natural nests (5-10 cm deep). The area was monitored by two trained employees of the Merecure Ranch; sand moisture was maintained by pouring 5 litres of water each week over the surface of the incubation trench.

In May 2009, the same beaches of the CR were resurveyed to determine hatching success of the natural nests left *in situ*. Using the wooden rod method, nests were relocated, excavated and the number of shells of eggs, neonates, and dead embryos were counted.

In the MR, 87 nests were located, of which only 13 contained eggs. Eggs from the other 74 nests had been looted by local people. All eggs in these 13 nests were taken (it was considered that if any were left that these too might also be lost to egg collectors) for artificial incubation. Each clutch was transferred into a sand-filled polystyrene container (rather than reburying in an open air artificial nest chamber as at CR). Eggs were incubated in a room. without registering temperature and moisture, at the Rabanal Abajo Ranch, about 7 km from the nesting areas. Again, eggs were carefully handled in order to avoid rotation and direct exposure to the sun, and eggs were buried in the sand in the incubation containers at a depth similar to that of natural nests. In May, hatching success was evaluated.

Clutch sizes: The size of clutches (number of eggs per nest) in both locations was also recorded. Soini and Soini (1986) indicate that, for *P. unifilis*, clutch size is related to the size of the female; small (younger) females lay fewer eggs than larger (older) females. Thus, the size of clutches can indirectly indicate the size/age structure of the nesting turtle population. This information in turn may provide a good longer-term indicator of any changes in the population age structure of adult females in a stretch of river. A population comprising mostly of large females could indicate low recruitment of young, potentially attributable to constant high loss of eggs and/or hatchlings over the years.

CONSEQUENCES

A summary of field survey results and hatch success of *P. unifilis* clutches in each area is presented in Table 1. The area of the MR sampled in terms of nesting beaches, was smaller than that of the CR, but it supported a higher number of nests. Loss of nests in the CR was considerably lower (28.2%) than in the MR (85.0%). In the CR, flooding accounted for the loss of 15.4% and predation for 12.8%. In the MR the losses were caused exclusively by predation (most or all attributable to collection by local communities who take eggs both for their own consumption and to sell in nearby towns). No evidence of egg collection by people was apparent in the CR.

In May, hatching success of eggs in the CR was evaluated; greater success was achieved for eggs in the incubation trench (88.2%) than those in natural nests (63.2%). In the MR, the hatch success of eggs in artificial nests was 42.0%; no natural nests were available for comparison. The lower hatching success for artificially incubated eggs of the MR compared to CR may be attributed to less suitable incubation conditions (e.g. not being open to direct sunlight and hence incubation temperature). However, lower considering the 100% nest predation in the MR. at least some hatchlings were successfully produced under the artificial conditions. Unfortunately no analysable data regards incubation periods (hence giving an indication of possible influence of incubation temperature on hatch success) are available, as it was unknown how old eggs were when collected.

Table 1. Summary of field survey results and hatch success of *Podocnemis unifilis* clutches located along the Cojedes and Manapire rivers in 2009.

Variable	Cojedes River	Manapire River
Km of river sampled	5.9	13.0
Number of evaluated nesting beaches	23	13
Number of nests found	78	87
Number of nests transferred for artificial incubation	27*	13
Total number incubated eggs	136	401
Nest abundance (nests/km surveyed)	13.2	6.7
Abundance (nests/beach)**	3.4	6.7
Clutches lost to predation (%)	12.8	85.0
Clutches lost to flooding (%)	15.4	0.0
Total clutch loss (%)	28.2	85.0
Hatch success of eggs in natural nests (%)	63.2	0.0
Hatch success of eggs in artificial nests (%)	88.2	42.0

^{*} only five eggs of each nest taken for artificial incubation.

** this index will help determine whether populations of nesting females are increasing or decreasing in the study areas.

The sex of the turtles is determined by the incubation temperature in the early stages of gonad development (Hulin et al. 2008). Alho et al. (1985) could significantly change the sex ratio in natural nests of *Podocnemis expansa* by lowering the incubation temperature through preventing direct exposure to sun during the incubation period, producing more males. Conversely, Valenzuela et al. (1997) showed that recently laid P. expansa eggs transferred to artificial nests with the same characteristics of natural nests (i.e. depth and exposure to light), produce the same proportion of sexes as those of natural nests. These examples indicate that although incubation temperature determines the sexes, clutches can be successfully managed without risk of duly altering the sex ratio. In this present study, hatchlings produced under artificial conditions in the MR may thus be expected to exhibit a skewed sex ratio. In order to evaluate this, all hatchlings from both rivers are being raised in captivity so that sex can be determined.

Clutch sizes: Size structure of *P. unifilis* clutches in the CR (n=50) was represented by six size classes (Fig. 1), the majority (60%) comprised 21-25 egg/clutches. In the MR, only three classes of clutch size were observed but the

sample size (n=13) was small (thus results interpreted taking this into should be consideration). In MR the clutch size was heavily skewed towards larger clutch size, the most abundant class being 26-30 eggs/clutch (69%). A potential reason for this could be the heavy human predation of P. unifilis eggs in the MR that has existed for many years, with a concurrent decrease of hatchling recruitment that could now be manifesting itself by an absence of young mature adults. In addition, there may be hunting pressure regarding larger (older) adult turtles, which might account for the bigger clutch-size classes not being represented. However, due to the small sample size, these hypotheses should be viewed with caution. In the CR no human predation of eggs was detected. and even though this river system is under heavy anthropogenic pressure, being polluted, some areas are deforested, dammed and water taken for irrigation (Mendoza & Seijas 2007), it retains high biological importance. For example it supports the most important Orinoco crocodile Crocodylus intermedius population in the world, their abundance perhaps due to the fact that the river is not very navigable and is transited by few people. Hence it has served as a refugee for the crocodile and this may also explain the absence of human collection of *P*. unifilis eggs.

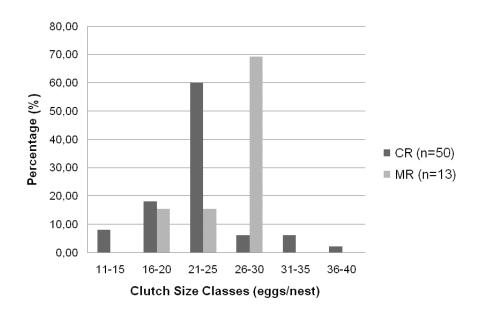


Figure 1. Clutch sizes of Podocnemis unifilis nests in the Cojedes (CR) and Manapire (MR) rivers, February 2009.

Conclusions: These results overall, demonstrate the effectiveness and potential of these lowtechnology *P. unifilis* nest management schemes for increasing the number of hatchlings. We conclude that it would be a pertinent conservation measure to regularly transfer turtle clutches (especially those that are particularly vulnerable to predation or flooding) to safe sites to enhance incubation success with subsequent release of hatchlings back into the wild. In the context of this study, this should be undertaken in both the Cojedes and Manapire rivers but particularly so in the Manapire where heavy egg losses due to human collection was identified as a serious problem.

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