Implications of Treating a Gravid Turtle: A Case Study from the Island of Crete, Greece

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In response to an ever-increasing number of sea turtles reported injured and in need of rehabilitation, ARCHELON established a Sea Turtle Rescue Centre (STRC) in Glyfada, Attica in 1994 (Margaritoulis 2024). Injured and sick turtles found all over Greece are admitted via an established stranding network, treated, and once rehabilitated, are released back to their natural environment. The STRC was launched ten years after ARCHELON's first standardized monitoring programs had begun in Zakynthos and Kyparissia Bay, with Lakonikos Bay, Rethymno, Chania and Messaras Bay following suit.

The main monitoring activities at these programs involve daily patrolling of the nesting beaches, recording the previous night's nesting activity, and protecting identified nests. During the night, trained volunteers patrol sections of the beaches in search of nesting females, tagging them and collecting biometric data. During those shifts, team members occasionally encounter female turtles bearing injuries that include healed past wounds, entanglements in fishing gear (see Margaritoulis et al. 2024) or more recently inflicted lacerations on the head, limbs, neck and/or carapace. These cases have posed the conundrum of identifying the cases where team members should intervene, intercepting the turtle and sending it to the STRC for treatment. Further considerations involve dealing with the implications of treating a turtle with fully formed eggs within its oviduct. Here we present the case of "Chloe", a reproductively active turtle found injured in Chania, Crete, during the 2000 nesting season.

Chania is one of the most important nesting sites for loggerhead turtles in Greece and has been systematically monitored since 1992. During the early morning survey of 23 July 2000, project members found an injured adult female turtle on the beach, close to the town of Kolymbari, at the westernmost edge of the nesting site. The turtle had emerged the night prior to deposit its clutch. It bore a large triangular trauma over the left eye, wounds on the neck and abrasions on the plastron and hind limbs, likely the result of a direct human action. The turtle had failed to nest, was disoriented presumably due to her injuries, and had crawled onto an asphalt road at the back of the beach. The turtle's inability to return to the water was the principal reason for the on-site decision that the turtle should be transported to the STRC.

The turtle, named Chloe by the field team, was transported by boat from Chania and admitted to the STRC the following day, 24 July 2000. At the STRC, she was placed in a tank and treatment protocols began. On 25 July 2000, Chloe deposited a full clutch of 113 eggs in its tank (Fig. 1).

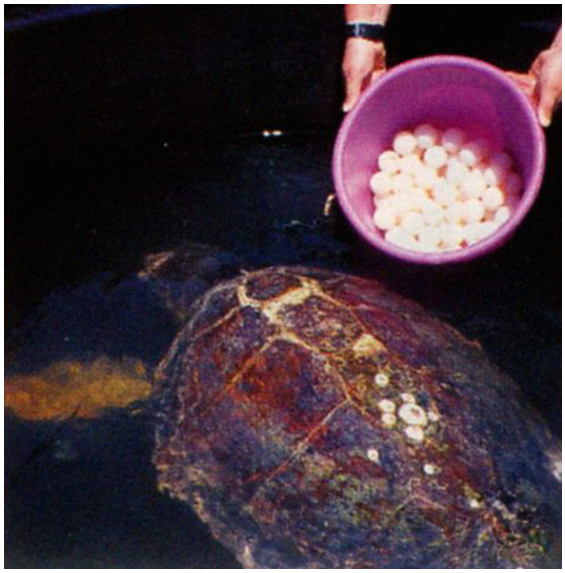

Figure 1. Chloe and the 113 eggs collected from her rehabilitation tank.

The eggs remained in the water between one and two hours, at which time they were removed and placed in a Styrofoam box using sand from a nearby beach. The warm sand, surrounded by the insulating material of the box helped keep a stable temperature of about 26°C until the clutch was ready to be relocated. That was of particular importance considering that eggs seldom hatch if outside the range of 25° – 35°C (Ackerman 1997). The same evening, the Styrofoam box containing the clutch was airlifted to Chania and was collected by the field team at approximately 2200 that night. The field team decided to place the clutch close to the location where Chloe was originally found. Following the construction of an egg chamber big enough for the clutch of 113 eggs, their removal from the Styrofoam box and their placement in the sand started at 06:50 and was completed by 07:15 the following morning, about 20 hours since they had been dropped in the STRC tank. Great care was taken not to rotate the eggs in any way. For this reason, before removal, a small dot was made on the top of each egg using a black crayon. The egg was then slowly removed from the box and placed inside the egg chamber, taking care that the black dot remained on top which would indicate that the egg had not been rotated in any way. This continued until 112 eggs were inside the artificial egg chamber, as one egg was broken.

As soon as the procedure was complete, the nest was mapped and protected using a metal cage and continued to be monitored daily until 18 September when the first 23 hatchlings emerged from the nest and successfully made it to the water after an incubation duration of 54 days. Hatching continued for the next nine days, with a total of 70 hatchlings reaching the sea which represents 64.2% of the total number of eggs counted at the time of the excavation. It should be noted that clutch size, as estimated based on eggshell counts totalled 109 eggs, which differs from the number of eggs counted at the time of the relocation (112). This discrepancy is explained by biases due to human error when it comes to counting eggshell fragments inside the nest during excavations (Ceriani et al. 2021). Chloe remained under rehabilitation for a total of 109 days and was successfully released in Chania on 10 November 2000.

Chloe's clutch had a relatively good hatching success rate, even though all signs indicated that this would not be the case. First, the eggs are estimated to have remained between one and two hours in the water, which in principle could mean that it would not be possible for the embryos to develop inside the eggs. Second, this "emergency relocation" occurred more than 20 hours after the eggs had been deposited in Chloe's tank, indicating very low chances for the clutch to produce any hatchlings due to movement-induced mortality.

However, as is the case with many reptile species, sea turtle eggs undergo arrested embryonic development, where embryos are placed in a kind of «hibernation» inside the female's oviduct following the initial steps of development (Miller 1985). This allows the mother to hold on to the eggs until the time is right to lay them therefore having greater flexibility in her reproductive schedule (Rafferty & Reina 2012). In addition, this preovipositional arrest occurs before the embryonic membranes are attached to the egg shell membranes, therefore eggs are protected from movement-induced mortality when deposited in the egg chamber (Rafferty et al. 2013; Rings et al. 2015). It appears that as the eggs leave the hypoxic environment in the oviduct and are dropped within the egg chamber, the exposure to oxygen serves as the trigger for development of the embryos inside the nest to resume (Rafferty et al. 2013). In the case of Chloe, it appears that since her eggs were dropped directly in the water, they were not exposed to aerobic conditions while submerged, therefore embryos remained in the state of arrested embryonic development. This could explain the fact that the clutch was resilient

to the adverse environmental conditions and the eggs were still viable when they were removed from the tank and kept at stable temperature conditions until the time they were relocated to Chania.

During relocation of clutches, it is important that no movement-induced injury is inflicted on the embryos inside the egg. During the early stages of incubation inside the egg chamber, the vitelline embryonic membrane migrates through the albumen and attaches to the egg-shell membranes. The outer layer of the egg-shell dries where these membranes are fused, forming an opaque white spot on the upper surface of the egg (Thompson 1985). If a turtle egg is moved following this stage, typically starting within the first 12 hours of development, these membranes can easily rupture resulting in the death of the embryo. For this reason, the general recommendation is that nests should be relocated as soon as possible after laying, preferably within 12 hours or less (Boulon 1999). Because more than 20 hours had passed since Chloe's eggs had been dropped to the water, the relocation of her clutch to the nesting site of Chania was a painstaking process undertaken by the project's more experienced personnel. The eggs were placed inside the new egg chamber one at a time, taking great care not to rotate them. This probably contributed to the clutch having a hatching success of more than 60%.

The case of Chloe is unique and is thought of as a "happy" story within ARCHELON. First, Chloe was successfully released back to her environment following her treatment, and although she has not been encountered since, she may still be roaming the Mediterranean having deposited many more clutches since. Second, the hatchlings that emerged from the nest and successfully reached the sea represented the hope for the future of the Chania population that shows signs of stabilization and even recovery following a steep decline (ARCHELON, unpublished data).

However, it was a case with a much greater impact as it was the first time we came face to face with implications related to dealing with an injured gravid female. Following this experience, ARCHELON has shaped a more specific strategy on how to address similar cases. Specifically, when encountering an injured female during beach patrols, it has been determined that intervention is only necessary if the turtle is unable to complete the nesting process and return to the sea. In addition, this incident provided valuable experience on how to best address cases where gravid females under treatment drop their eggs in the water. On a more practical level, the relocation of Chloe's clutch at this late stage contributed to finalizing the protocol on how to conduct "emergency relocations", i.e. the relocation of clutches after the first 12 hours since oviposition, which was added to ARCHELON's standard monitoring and nest management practices.

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