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Tonic Immobility in Newly Emerged Sea Turtle Hatchlings

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Tonic Immobility in Newly Emerged Sea Turtle Hatchlings

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ABSTRACT. – The ability for animals to become temporarily immobile via external stimulus is known as tonic immobility (TI) and has been widely described among different taxa. However, TI is poorly documented in turtles. We demonstrate TI in newly emerged green turtle (*Chelonia mydas*) hatchlings in relation to potential practical applications and discuss the methodology of how to induce TI. On average, combining all clutches sampled, TI induced green turtle hatchlings to remain immobile for 25 ± 12 sec; thus, we argue that in this state of immobility, researchers and conservationist can safely obtain quantitative variables (e.g., animals' weight and morphological measurements without forceful stressing or harming the animal).

Animals have a wide variety of defensive behaviors that are adaptive in certain situations. Thanatopsis or tonic immobility (TI) is a distinctive behavior defined as a temporary loss of muscle and/or neurological function (partial paralysis) in response to a threat. The human equivalent is known as “hypnosis”, which dates back to the Old Testament (Ratner 1967). This phenomenon may last for a few seconds to over several hours (Gallup 1974). Although animals in a TI state seem to be unresponsive to external stimulus, evidence indicates some animals can continue to process information about the environments that surround them (Sigman and Prestrude 1981). Recent evidence suggests that TI is not associated with any suspension of consciousness (Marx et al. 2008). Indeed, Mauk et al. (1981) noted that the lizard *Anolis carolinensis* in TI can exhibit hyperalgesia (increased sensitivity to pain).

Tonic immobility has been observed in a variety of animals including fish (Tobler 2005, Wells et al. 2005), amphibians (Toledo et al. 2010), reptiles (Gehlbach 1970; Edson and Gallup 1972; Hennig et al. 1979; Santos et al. 2010) birds (Sargeant and Eberhardt 1975), and mammals (Fraser 1960; Francq 1969; Carli 1974), and TI in invertebrates also appears to be common (as reviewed in Coutinho et al. 2013). However, as far as we know, TI has not been documented in sea turtle hatchlings.

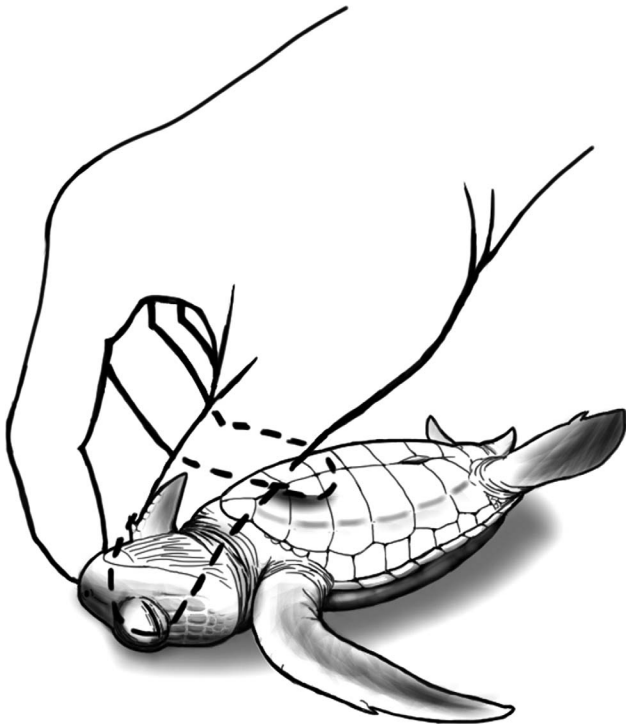


Figure 1. Diagrammatic representation of inducing tonic immobility (TI) in a green sea turtle (*Chelonia mydas*) hatchling. Observer's fingers are positioned as shown to induce TI. Image by N. Wu.

Tonic immobility can be induced in vertebrates by turning them upside down suddenly so that their dorsal side faces the ground (Edson and Gallup 1972; Carli 1974; Hennig et al. 1979; Hohtola 1981). However, turning newly emerged turtle hatchlings onto their backs does not induce TI but triggers a self-righting behavior in which the neck is used to turn the animal right side upward (Booth et al. 2013). This behavior is probably an adaptation to accidental tipping over when hatchlings rapidly crawl from the nest to the water's edge during the frantic nest emergence and off-short swim that occurs during the first 24 hrs after nest escape (Carr and Ogren 1959). Frenetic activity can be problematic to researchers when handling and restraining hatchlings. For example, measuring mass and body dimensions and counting carapace scutes are regular procedures at many sea turtle nesting beaches, and these procedures are most efficiently done when hatchlings are immobile. Hence, the inducing of TI could be a valuable tool when conducting routine research and management procedures on sea turtle hatchlings. Here, we describe a technique that can be used to induce TI in green turtle *Chelonia mydas* hatchlings.

Methods. — Newly emerged green turtle hatchlings were collected from Chagar Hutang Turtle Sanctuary, Redang Island, Malaysia in July and August 2014. Enclosures made of plastic mesh were placed on the surface of selected in situ nests to trap newly emerged hatchlings. Enclosures were checked every half hour throughout the expected emergence night to ensure

Table 1. Tonic immobility duration (sec) from 10 clutches of green turtle hatchling clutches sampled from Chagar Hutang Turtle Sanctuary, Redang Island, Malaysia. n = number of individuals sampled per clutch. Clutches were ranked to the mean value of TI. SD = standard deviation and CV = coefficient of variation.

Clutch no.	n	Mean	SD	CV (%)
9	19	9.04	6.5	72
3	19	9.37	7.3	78
10	18	10.32	6.6	64
6	22	13.99	10.2	73
8	22	14.14	10.8	76
2	33	15.30	10.7	70
5	16	21.95	16.4	75
4	10	26.77	15.8	59
7	26	28.48	22.1	78
1	24	40.24	19.4	48

hatchlings were not on the surface for longer than 30 min. Once on the surface, hatchlings were transferred to a hut (approximately 20 m from the nesting beach) to measure their TI potential. We defined TI by the cessation of any movement (suspended animation) once induced and the "TI period" as the period of time from cessation of movement until the restart of movement.

Tonic immobility was induced by placing hatchlings upside down (dorsal side on a flat surface), then gently pressing the thumb and first finger on both eyes (to eliminate visual stimulus) while the middle finger was placed on the plastron to stabilize the animal (Fig. 1). Duration of TI was recorded with a stopwatch. At least 10 hatchlings were tested from each clutch sampled. Immediately after tests, hatchlings were released back on to the beach where they completed their crawl to the sea.

Results are presented as mean \pm SD of each clutch for the duration of tonic immobility. Effect of different clutches was tested with 1-way ANOVA and statistical significance assumed if $p < 0.05$. We also investigate whether the period of time between nest emergence, and the TI trial was correlated with TI duration using Pearson correlation.

Results and Discussion. — Ten clutches were sampled, and TI duration differed between clutches ($F_{10,210} = 6.009$, $p = 0.005$). In 6 of 10 clutches, the TI duration was short, less than 16 sec, whereas in 1 clutch TI duration was approximately 40 sec (Table 1). Clutch coefficient of variation (CV) of TI varied between 48% and 78%. There was no correlation between the waiting period of hatchlings to TI trial and TI duration (Fig. 2).

The TI duration varied among clutches, and this variation could be attributable to a maternal effect. All hatchlings came from the first cohort of hatchlings to emerge from the nest and were assumed to have a similar body temperature. The CVs for TI duration are remarkably large ranging between 48% and 78%, compared with typical biological data such as morphological variables or growth, which normally ranges between 10% and 15% (Balaam 1972). Earlier studies also found much higher

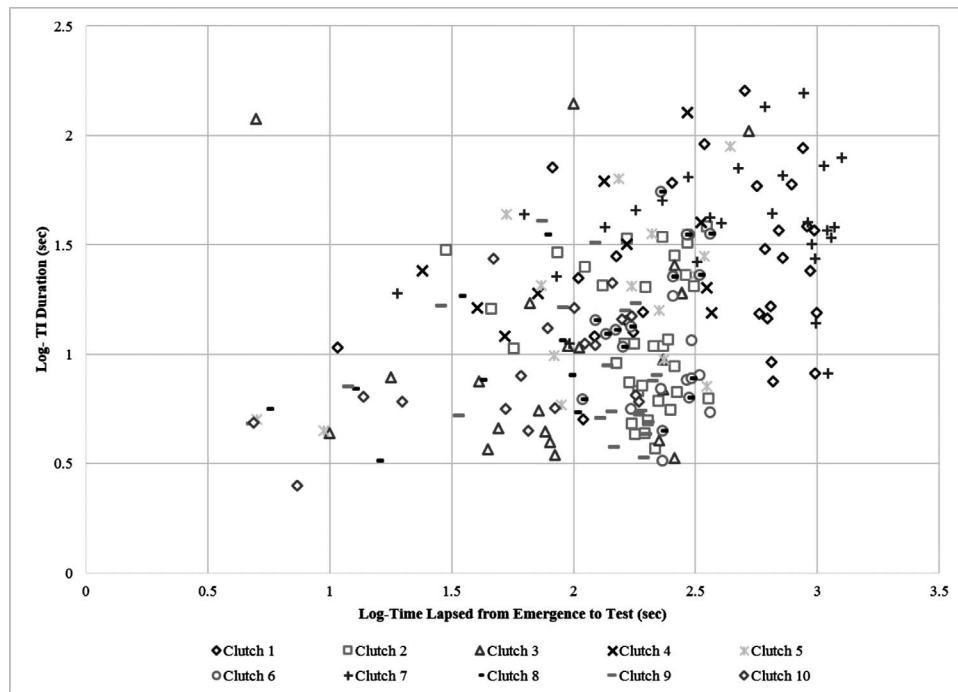


Figure 2. TI duration for each hatchling within the clutches to the retention time before test taken. Variables have been log transformed because actual data were centered at the bottom left in the graph. There was no significant correlation between tonic immobility (TI) duration and time elapsed between nest emergence and TI trial. Regression values for each clutch were Clutch 1: $r = 0.054$, $p = 0.785$, $n = 24$; Clutch 2: $r = 0.007$, $p = 0.969$, $n = 33$; Clutch 3: $r = 0.201$, $p = 0.371$, $n = 19$; Clutch 4: $r = 0.287$, $p = 0.422$, $n = 10$; Clutch 5: $r = 0.445$, $p = 0.073$, $n = 16$; Clutch 6: $r = 0.210$, $p = 0.336$, $n = 22$; Clutch 7: $r = 0.147$, $p = 0.473$, $n = 26$; Clutch 8: $r = 0.190$, $p = 0.342$, $n = 22$; Clutch 9: $r = -0.234$, $p = 0.321$, $n = 19$; Clutch 10: $r = 0.290$, $p = 0.243$, $n = 18$.

variables in locomotor performance of animals such as sprint velocity on swimming of Atlantic cod (35.4%; Reidy et al. 2000), crawling of green turtle hatchlings (31.6%; Ischer et al. 2009), and sprint speed of tree lizard (24.8%; Robson and Miles 2000). Essentially, the immense interindividual variability in physiological traits is believed to be associated with the natural selection if found to be repeatable (Bennett 1987). However, in the current study, we did not measure the repeatability of TI duration within an individual.

During the waiting period between nest emergence and measurement of TI, hatchlings were kept in a bucket, and they continuously crawled around inside the bucket for up to 30 min. This is an energetically demanding process, and we propose that there might be a relationship between depletion of energy reserves and TI duration. However, there was no relationship between TI duration and length of time between nest emergence and measurement of TI, suggesting that energy reserves are not related to TI duration. Alternatively, the amount of energy used during the holding period may not have been enough to significantly deplete energy reserves and thus influence TI duration.

There is no clear evidence for an adaptive advantage of TI behavior, but two likely hypotheses for its evolution have been proposed. The first is “playing possum” in response to the presence of a predator to decrease the probability of being detected by the predator (Gallup et al. 1980), and the second is immobility associated with

reproductive activities (Whitman et al. 1986). The majority of TI research has focused on the predator avoidance hypothesis. TI may have evolved as an alternative to fight-or-flight response to increase the chance of survival (Alboni et al. 2008), and 5 predator-induced TI hypothesis have been suggested by Miyatake et al. (2009); for example, to reduce the attention of predators because some rely on visual movement cues to detect prey or trigger an attack (Heinen 1995; Gregory 2008), or escape when a predator prey is left unguarded (Gallup 1974), or making it physically difficult to swallow prey (Honma et al. 2006). Experimental studies suggest that this behavior can increase the survival rate (Hoagland 1928; Sargeant and Eberhardt 1975; Thompson et al. 1981) and is passed on to future generations (Miyatake et al. 2004; Nakayama and Miyatake 2009b). Interestingly, there seems to be a genetic trade-off for TI in red flour beetles (*Tribolium castaneum*) where males with a long TI duration had increased survival against predation but had lower mating success even in predator-free environments (Nakayama and Miyatake 2009a).

In terms of antipredator defense, hatchling sea turtles can actively flee from predators, freeze, or take no perceivable action. Observations of hatchling turtles swimming away from their natal beach in Florida show that, when threatened or attacked by an aquatic predator, green turtle hatchlings continue swimming, but loggerhead turtle hatchlings (*Caretta caretta*) often become immobile, assuming a “tuck” position (Wyneken et al. 1994). Experimentally, when hatchlings were given a simulated

predation experience, loggerhead turtles and hawksbills turtles (*Eretmochelys imbricata*) became immobile (325.4 sec and 89.5 sec, respectively), but the green turtles continued to actively swim within 1.8 sec (Mellgren et al. 2003). Hence, different sea turtle species hatchlings respond differently to encounters with predators; thus, the technique of inducing TI described for green turtle hatchlings may differ or be ineffective on the other sea turtle species.

There are different methods to induce TI, most involve some sort of restraint such as stroking an animal's ventral surface, forcing it to fixate its gaze on a chalk line, or placing a hood over its head. In particular, many animals become calm and inactive when in an upside down position. However, newly emerged sea turtle hatchlings struggle to self-right when turned upside down. Thus, the first step of flipping them on to their back alone does not induce TI. We had to also gently press their plastron at the same time with 1 finger. This stimulus may imitate the pressure placed on individuals by other hatchling turtles while they are still within their nest. The third step was closing their eyes with 2 fingers as shown in Fig. 1. This technique was instigated because sea turtle hatchlings placed in total darkness soon stop moving presumably because the visual cues they use in sea finding behavior are absent in complete dark. Therefore, closing the eyes also shuts off these visual cues making body movements less likely. Hence 3 steps appear to be necessary to induce TI in green turtle hatchlings: 1) flip them onto their back; 2) gently press their plastron; and 3) close their eyes.

The ability to induce TI in animals can provide practical applications for researchers attempting to obtain quantitative variables. Specifically for sea turtles hatchlings, inducing a state of TI may allow researchers to measure the animals' body mass accurately and in other studies where a still animal is necessary such as x-ray studies and photographic studies where fine details are necessary (e.g., scute pattern or flipper morphology). All of these methods require the animal to be still for at least a short period. It is advisable to perform TI on one hatchling at a time when using this method to take measurements because TI duration is not consistent among hatchlings and typically of short duration. As soon as TI is broken, hatchlings attempt to self-right and immediately start moving again. TI does not appear to harm the sea turtle hatchlings; they assume their normal frenzied behavior as soon as TI is broken and, in our study, rapidly crawled without distraction to the sea when placed on the beach. In conclusion, inducing TI could be a valuable tool in reducing the risk of injury to the struggling hatchlings when undertaking body measurements.

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Insect Infestation of Hawksbill Sea Turtle Eggs in Rio Grande do Norte, Brazil

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ABSTRACT. – We describe infestation of hawksbill turtle (*Eretmochelys imbricata*) nests by insects on Pipa beach in the municipality of Tibau do Sul, Rio Grande do Norte, Brazil in January and June 2011. The mean number of live hatchlings (83.96 ± 43.31) was higher in nests unassociated with vegetation, although it is important to consider that a number of parameters other than proximity to vegetation may also affect nest success.