Tonic immobility induction and duration on halmahera walking shark (*Hemiscyllium halmahera*)

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Abstract. Tonic immobility is an induce method to achieve a temporary condition of cataleptic-like, which is a sudden loss of muscle tone, as a result of various procedures such as situate the object in an “unnatural” position or situation. This method has been widely applied on elasmobranch species, except for species of *Hemiscyllidae*. This study was intended to assess the application of tonic immobility on *Hemiscyllium halmahera* - an endemic shark to Halmahera Islands, Indonesia. Our study has shown that the average induction time ranged from 11.49 to 40.12 seconds, while the hypnosis duration ranged from 6.51 to 28.36 seconds. Based on our results, tonic immobility can be applied as non-lethal method to *Hemiscyllium halmahera* during capture and physical examination such as morphometric and weight measurement. However, we suggested to investigate further physiology examination such as heart rate, and breathing frequency during tonic immobility phase on Halmahera walking shark or other reef shark species.

Keywords: physical examination, tonic immobility, walking shark

1. Introduction

Tonic immobility (TI) or animal hypnosis is an induce method to achieve a temporary cataleptic-like condition that can occur from less than one minute to several hours (Gallup 1974). This technique has been applied to handle elasmobranch shark in the field for various purpose, for example research, medical and tourism (Henningsen 1994, Kessel *et al* 2015). This method offers benefits as an anesthetic technique during live fish transport, and recovery (Wells *et al* 2005), as a method in capturing shark (Williamson 2018), and handling stress of shark (Hoffmayer 2001). In terms of animal warfare, this method provides lower risk compare to chemical anesthetic, include risk of overdose, minimalize negative impacts that influence post release behavior and fast recovery on the specimen. Despite it has been widely applied on elasmobranchs species, but none of these were applied for *Hemiscyllidae* family in Indonesia (Henningsen 1994).

TI can be initiated by various methods which includes placing the subject in a rapid dorso-ventral inversion (Watsky and Gruber 1990), pumping water into branchial chamber (Wells *et al* 2005), interfere their Lorenzini Ampullae (Fisher *et al* 2006), or applying pressure to tail’s distal end
Indonesia has recorded 116 species of sharks (Dharmadi 2013), including five walking shark species (Family Hemiscylliidae), that are *Hemiscyllium freycineti*, *H. galei*, *H. henryi*, *H. halmahera*, and *H. trispeculare* (Allen et al. 2016).

Indonesia is famous marine tourism and shark tourism is a growing industry. The recently described species Halmahera walking shark (*H. halmahera*), is an endemic to Halmahera islands. This species has maximum total length (TL) of 79.0 cm (Jutan 2018) and usually live in shallow waters near coral reef, seagrass beds or sand substrate and have small range and the habitat do not overlap between similar species (Allen et al. 2013). Its endemicity makes this species highly prone to habitat degradation and unsustainable fisheries (Allen and Dudgeon 2010, Compagno 1984). Further, this species has a very unique coloration that makes it a favourable target for aquarium display (Bennett et al. 2015).

North Maluku Province, Indonesia became famous for marine research and tourism in the last few years. Shark-watching is the main tourist underwater attraction in this region, such as Morotai, Ternate and Tidore (Ichsan 2016). The black-tip reef shark (*Carcharhinus melanopterus*), white-tip reef shark (*Triaenodon obesus*), and grey reef shark (*Carcharhinus amblyrhynchos*) were the three species from the North Maluku Province that have been regularly studied (Ichsan et al. 2016, Pridina 2015, Mukharror et al 2018b). However, no studies on Halmahera walking shark and mobula ray have been available to date (Mukharror et al 2018a). This study aims to assess TI induction and duration as a non-lethal method using halmahera walking shark as an object study during physical examination such as morphometric. The results of this study would be beneficial to support animal handling for research.

2. Materials and methods

This study was conducted between June, 8th to 10th, 2019 at the Tidore Island, North Maluku Province, Indonesia. The specimen was caught using small nets (40 cm height, 40 cm width, and 100 cm length) from the coastal area of the island. In this study, we used only one specimen to test the TI approach, therefore we cannot compare the TI response between male and female individual, although it was hypothesized that female is more susceptible and induction time will increase in size and age in sandbar shark and smooth dogfish (Whitman 1984, Whitman et al 1986). Each TI trial was conducted by restraining the subject as quickly and as gently as possible. The investigator gently rubbed and massaged both side of the snout, where Lorenzini Ampullae pores of the *H. halmahera* were densely located. In the specimen where TI was induced, a deep rhythmic respiration was observed, as a deeper and more constant ventilation of the gills and decreased respiration rate (figure 1).

![Figure 1. Inverting the walking shark (H. halmahera) for tonic immobility.](image-url)
The individual was weighted in kilogram and measured total length (TL) in centimeters. The shark was held inverted until the onset of the TI response. Each response was timing in second with the accuracy of two decimals, using handheld stopwatch (Casio HS-3). A change in muscle tone was used as the criterion for the onset of TI that correspondence to the limb response. Once the response was induced, the subject was not restrained until it regained the mobility.

A series of morphometric analysis were then conducted on each of the TI phase of halmahera walking shark, following the induction phase. These induction and hypnosis phase were continued until all morphometric parameters completely taken place. Whitman (1986) suggested an interval in between of each trial of TI, therefore the TI induction were conducted after 10-15 minutes interval between each trial. In order to prevent habituation (Watsky and Gruber 1990), the assessment was conducted only for 8 trials. Once the response was induced, the subject was not restrained until it regained mobility. Morphometric parameters to be assessed was following the Compagno (1984) methodology. This method includes measurement of total length, head width, snout length, sub-caudal length, etc using Sellery™ Plastic Steel Caliper. After the trials, halmahera walking shark was safely brought back to the Tidore sea at 10th June 2019.

3. Results and discussion

This shark was identified as a young female with 495 mm length (maximum total length 790 mm, Jutan 2018) and 290 g weight. Complete result of morphometric measurement was shown in table 1.

| Measurement                     | Size (mm) | Measurement               | Size (mm) |
|---------------------------------|-----------|----------------------------|-----------|
| Total length                    | 495       | Mouth width                | 38        |
| Pre-caudal length               | 380       | Barbel length              | 9         |
| Head width                      | 80        | Snout-1st dorsal origin    | 377       |
| Head depth                      | 46        | Snout-pelvic origin        | 283       |
| Pre-anal body depth             | 38        | Snout-anal opening         | 300       |
| Snout-pectoral fin origin (HL)  | 88        | Anal opening-anal fin origin| 361       |
| Snout-1st gill slit             | 68        | Anal opening-tail tip      | 328       |
| 1st to 5th gill slit            | 34        | Interdorsal distance       | 75        |
| First gill slit height          | 13        | Pectoral fin length        | 51        |
| Fifth gill slit height          | 18        | Pelvic fin length          | 53        |
| Eye diameter (horizontal)       | 15        | 1WAM dorsal fin base       | 44        |
| Eye diameter (vertical)         | 7         | 1st dorsal fin height      | 34        |
| Bony interorbital width         | 30        | 1st dorsal fin free margin | 23        |
| Fleshy interorbital width       | 36        | 2nd dorsal fin base        | 45        |
| Snout to eye (snout length)     | 40        | 2nd dorsal fin height      | 42        |
| Snout to spiracle               | 49        | 2nd dorsal fin free margin | 22        |
| Snout to mouth                  | 16        | Anal fin base              | 71        |
| Lower labia furrow length       | 8         | Anal fin height            | 19        |
| Maximum width lower labial flap | 8         | Anal fin free margin       | 12        |
| Postoral fold                   | 11        | Sub-caudal length          | 114       |
The results of Halmahera walking shark’s TI induction and duration time can be seen in table 2. The result showed that average duration of TI (17 s) can be used as the window opportunity to assess the morphometric of the halmahera walking shark, without causing fatality. The morphometric measurement result on table 1 shows that all parameter of morphometric can be completely measured within the TI phase of Halmahera walking shark (which in average three measurement in each TI).

| Trial no. | Induction time (s) | TI Duration time (s) |
|-----------|-------------------|---------------------|
| Trial 1   | 20.81             | 25.37               |
| Trial 2   | 32.28             | 25.72               |
| Trial 3   | 40.12             | 9.88                |
| Trial 4   | 30.26             | 13.67               |
| Trial 5   | 11.49             | 6.51                |
| Trial 6   | 25.64             | 28.36               |
| Trial 7   | 34.45             | 6.76                |
| Trial 8   | 31.32             | 20.22               |
| Time Range | 11.49-40.12   | 6.51-28.36          |
| Time Average | 28.30           | 17.06               |

The results showed that induction time of the Halmahera walking shark (28.3 s) was relatively faster than swellshark (36 s), Carribean reef shark (30 s), but longer than blacktip reef shark (25 s), whitetip reef shark (21 s), and almost as long as leopard shark (28 s) (Heningsen 1994). For the duration of TI, Halmahera walking shark (17 s) was faster than leopard shark (37 s, Heningsen 1994), swellshark (85 s, Heningsen 1994), blacktip reef shark (92 s, Davie 1993), whitetip reef shark (177 s, Heningsen 1994), and Carribean reef shark (274 s, Heningsen 1994) (figure 2).

![Figure 2. Comparison of induction time (blue) and TI duration (orange) between several species of sharks (Heningsen 1994).](image_url)

Based on these studies, we can conclude that in general, the smaller the shark, the shorter duration of the shark was in TI condition. Thus, the size of shark might influence the duration of TI. Further
studies are needed to investigate the correlation between duration of TI and the size of the shark, and assesses whether examination within the same species and between species shown different results.

Reduced duration of anthropogenic intervention and shorter physical struggle are related with the shark capture, and restraint effort is expected to reduce physiological signs of distress (Hoffmayer 2001). Induction of TI and other nonlethal capture methods warrant further research (Williamson 2018).

4. Conclusion

Based upon number of trials of induction, TI responses were shown in H. halmahera. This TI duration can be used as the window opportunity to conduct morphometric assessment on Halmahera walking shark. The duration of TI for Hemiscyllium halmahera was the shortest compared to other sharks that has been examined. The size of sharks might influence the duration of the TI. Therefore, further studies are needed to support this hypothesis.

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