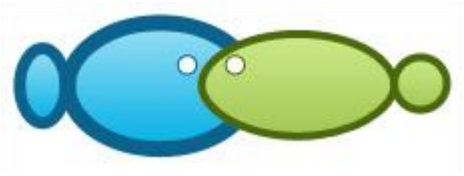

Eco-biology and spatial distribution of endemic *Hemiscyllium halmahera* at Halmahera Bay, Indonesia

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Abstract. The study explores biological characters of morphology, morphometry, reproductive, and spatial distribution of endemic walking-shark at Halmahera Bay of Halmahera Island, together with the ecosystems characteristics such as bathymetry, chlorophyll-a content, type of bottom habitat. A comprehensive database is required for a management strategy, to ensure sustainability of the protected fish. The study analyzed 28 specimens of *Hemiscyllium halmahera*. The morphometrics of mature *H. halmahera* shark consists of a total length (TL) of 16.5-79.0 cm and an average weight of 541.85 g, while the juvenile's TL range is of 15-30 cm. The skin morphology is carpet-like. The length-weight equation is $W = 0.1837\ L^{1.9645}$; the allometric exponent value of 1.964 indicates a negative allometry. *H. halmahera* inhabits the shallow waters between 8-25 m, mainly amidst coral reef boulders. Its main natural diet consists of benthic organisms such as small coral reef fishes, small-shrimps, and small crabs. Its distribution consists of small groups of 1-3 individuals and large groups of 3-6 individuals. At Gonad Maturity Index (GMI) I and II, it usually bears 2-4 immature internal eggs; at GMI-III and IV (external egg-capsules) it bears 2 egg-capsules of a length of 8-9.5 cm and a width of 3.5-4 cm, which are expelled into the seawater. Sentinel-2A data analysis shows that the outer bay mouth width is of 45.79 km, the narrowest width of the bay is of 7.35 km and the bay length is of 101.51 km. GEBCO bathymetric data reveals that the shallowest point is at 25 m and the deepest is at 1118 m. Halmahera Bay is a semi-enclosed ecosystem with a chlorophyll-a content of 0.117-0.374 mg m^{-3}, which represents a fertile seawater. The ecosystem is highly dependent on tidal changes, bringing both planktonic and small benthic organisms.

Key Words: Halmahera Bay, endemic, *Hemiscyllium halmahera*, semi-enclosed.

Introduction. Halmahera Bay is a semi-enclosed water body located in the northern part of Halmahera Island. The outer bay width is of 45.79 km, with two sections of the inner part and the outer part, separated by a narrow mouth with a width of 7.35 km and a depth of shallow coast of 25 meters, with the deepest point reaching up to 1,118 meters. The outer part of Halmahera Bay faces the Pacific Ocean and Morotai Island to the north. Halmahera Bay, in general, exhibits oceanographic, bathymetric, and ecosystem characteristics that differ from other waters. The potential for endemic resources, both discovered and undiscovered, is still high. A comprehensive database is required to support management policies for ensuring the sustainability and continuity of resources in Halmahera Bay, especially the endangered endemic ones. Efforts should be directed towards maintaining a level of utilization that provides integrated benefits to the local community and to the other stakeholders in Halmahera Bay. The pattern of currents and the movement of water masses play a crucial role in determining the nutrient flow that

supports ecosystem's health of Halmahera Bay. Understanding this is essential for assessing the distribution of natural feed and possible pollutants entering the waters from mining activities around the bay's coastal areas (Simbolon et al 2010; Lessy 2015; Simange 2015; Husen 2016; Edward 2017; Sulistijo et al 2018), which could pose a threat to the bay's ecosystem, including the habitat of the endemic species like *Hemiscyllium halmahera* (Allen et al 2013; Dudgeon et al 2022; Anderson 2013). Earlier studies (Jutan et al 2017; Jutan et al 2018) have identified the presence of *H. halmahera* in the waters of Halmahera Bay.

The new approach in this study is focused on the eco-biology parameters, such as the morphological, morphometric, and meristic description and the spatial distribution of the endemic *H. halmahera*, but also on the bathymetry and chlorophyll-a content of Halmahera Bay. The study is based on a spatial database of oceanographic characteristics and ecosystem parameters in Halmahera Bay, intended for the development of policies and management strategies related to the habitat of the walking-shark *H. halmahera*. Also, its purpose is to explore the species morphology and recruitment, feeding habits and the habitat's depth preference of the endemic walking shark *H. halmahera* at the investigated site. The results of the study can be applied for the development of management strategies and, possibly, of policies regarding the habitat of this protected walking-shark.

Material and Method

Description of the study sites. The study was conducted in Halmahera Bay at Halmahera Island Indonesia. Halmahera Island is a remote area of the Eastern part of Indonesia, relatively less affected by environmental pressure of heavy industrial activity. Halmahera Island is highly influenced oceanographically by seawater from the Pacific. In general, the seawater ecosystem is in a good condition, mainly due to a low population and to less human activity in comparation to the densest population of Java Island (Figure 1).

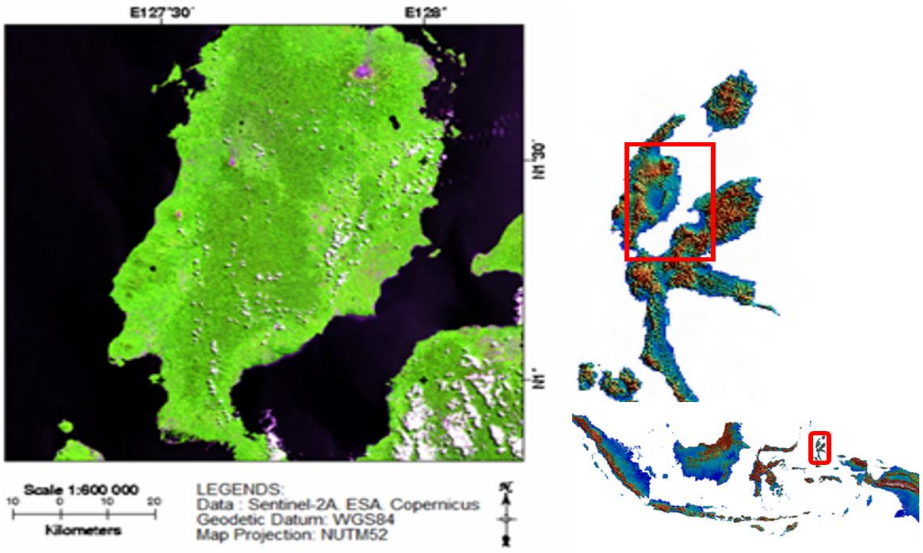

Figure 1. The location of Halmahera Bay, Halmahera Island, Indonesia (Sentinel-2A).

Underwater visual appearance sampling. Underwater visual sampling was carried out by means of diving during the night, to depths of 1-30 m and using underwater camera.

Morphology of H. halmahera. Morphology and morphometric measurement involve the main taxonomical measurements, such as: total length, standard length, fin length, distance between dorsal fin-I and II, caudal fin and ventral fin, and length ratios (Hartoko et al 2020; Dharmadi & Simeon 2019). Fish morphology can serve as a reference for identification by examining the body structure of the fish. However, relying solely on

morphological characteristics has limitations due to environmental factors, leading to species' similarity. Morphological characteristics include morphometric and meristic features, to differentiate species. Morphometric features play a role in distinguishing groups of fish from each other and include measurements such as standard length, head length, and body width. On the other hand, meristic observations involve counting features like the number of spines from front to back, on the dorsal fin, and the number of dorsal fin rays. Morphometrics involve ratios of various body part lengths, such as total length and weight, and can be used as taxonomic features during fish identification (Hartoko et al 2020).

Spatial data analysis. The research utilizes a combination of field surveys and satellite data analysis to generate spatial databases for the habitat (oceanographic characteristics, ecosystem parameters) of the endemic resources in Halmahera Bay, Halmahera Island. The study area base-map approach, using Sentinel-2A (2023) satellite data) involved producing data for spatial oceanographic characteristics and the ecosystem's database, through the analysis of bathymetry and chlorophyll-a in Halmahera Bay. The spatial bathymetry database analysis used numeric ASCII data of GEBCO (2023), which were then processed by the interpolation geostatistical method, with ERMapper software into spatial data. The data for chlorophyll-a to indicate the seawater and were extracted from Copernicus Marine Service (2023). The field underwater survey used an underwater camera to capture images of spatial distribution along the Halmahera Bay North Halmahera Island. The goal is to establish a comprehensive understanding of the oceanographic and ecological features of Halmahera Bay spatial data, providing a foundation for effective policy management information system for the endemic sustainable management. Satellite data is validated using in-situ data from field surveys to perform the spatial distribution of *H. halmahera* overlaid on the bathymetry and the chlorophyll-a data (Hartoko & Helmi 2004; Hartoko et al 2022; Prieto et al 2018).

Results and Discussion

Morphology and morphometry. Morphology of the *H. halmahera* is a small species usually under 1 m long, the epaulette shark has a slender body with a short head and broad, paddle-shaped paired fins. The caudal peduncle (to which the tail fin is attached) comprises over half the shark's length. *H. halmahera* is known as walking shark, first discovered with two specimens at Halmahera Island, North Maluku, Indonesia (Figure 2). This species resembles *Hemiscyllium galei* found in West Papua. *H. halmahera* moves along the seafloor using its pectoral fins and is characterized by dark spots covering its skin. The size of *H. halmahera* is relatively small, with captured specimens measuring around 70 cm. These sharks move by "walking" on the seafloor using their pectoral and pelvic fins. Generally, their skin color is brown with polygonal dark spots. White spots are also found in relatively few numbers (less than 10), spaced between the dark polygonal spots. Notably, relatively large black spots are found on the snout, a pair of dark marks on the ventral side of the head, and a "U"-shaped stripe dorsally. Additionally, there are 7 to 8 dark horizontal stripes between the abdomen and the base of the caudal fin. An individual *H. halmahera* typically has 25 black spots on the upper surface of its head. Their diet consists of small fish, crustaceans (such as crabs and shrimp), and mollusks during the night. Taxonomy - Kingdom: Animalia, Phyllum: Chordata, Sub Class: Elasmobranchii, Super Ordo: Selachimorpha, Ordo: Orectolobiformes, Family: Hemiscyliidae, Genus: *Hemiscyllium*, Species: *Hemiscyllium halmahera* (Allen et al 2013; Madduppa et al 2020).

This species serves as the indicator for monitoring coral reef health in consideration to the close association of the walking shark to coral reef. The study had counted of 28 individuals of walking shark *H. halmahera* found in the Halmahera Bay waters comprises with 17 females and 11 males. The morphological characters of the walking shark *Hemiscyllium ocellatum* found in Halmahera Bay are: Total-Length (TL) ranging from 16.5-79.0 cm, average TL of 16.5-79.0 cm and average weight of 541.85

grams. The specific growth pattern as described based in the equation W = 0.1837 $L^{1.9645}$ with the allometric exponent of 1.9645 which indicates that this *H. halmahera* has a negative allometry (slim shape).

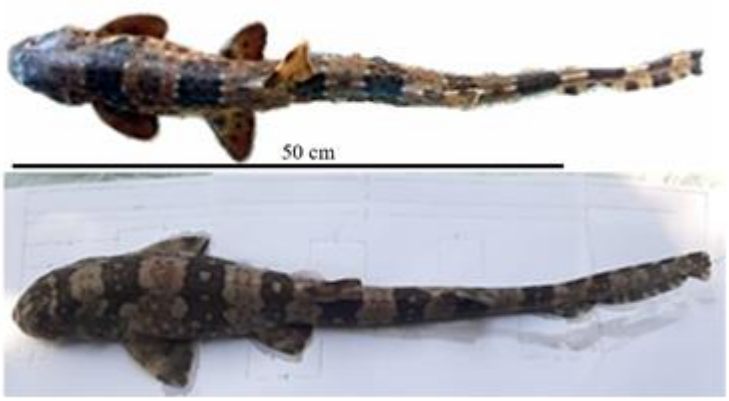

Figure 2. Morphology of *Hemiscyllium halmahera*.

The morphometry of *H. halmahera* is presented in Table 1.

Table 1

Morphometry of *Hemiscyllium halmahera*

Characteristic	*Value*	*Characteristic*	*Value*
Total length (TL)	58 (cm)	Eye diameter (SL)	1/50
Standard length (SL)	50 (cm)	Pre-dorsal length (SL)	7/50
Head length (SL)	4/50	First dorsal fin base (SL)	4/50
Body depth (SL)	8/50	Caudal fin length (SL)	8/50
Snouth length (SL)	2/50	Pectoral fin length (SL)	4/50

Habitat preference. Based on one-year underwater survey, had confirmed that the main preference habitat of *H. halmahera* is in a very close association with the coral-reef habitat (Figure 3), generally found in the depth range of 8-15 m. Most of live coral reefs are in a good condition. The substrate is mainly calcareous sand mixed with macro-algae. Seawater is very calm, with very small wave disturbances due to the geomorphology of the deep, semi-enclosed bay of the Halmahera Bay.

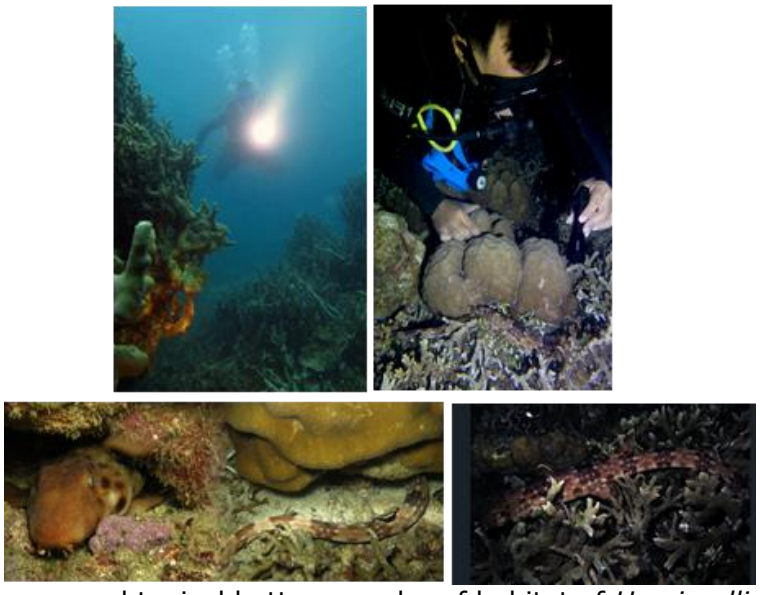

Figure 3. Appearance and typical bottom coral reef habitat of *Hemiscyllium halmahera* in Halmahera Bay.

Reproduction, fecundity, and recruitment. Fecundity of the walking shark *H. halmahera* is understood after the stomach dissection and gonad maturity observation of female samples. Data of the gonad maturity index (GMI) were found during the period from November 2017 to January 2018 and consisted of four maturity levels: 1. The first underdeveloped state as GMI-I, known as the early state of internal eggs; 2. The start of GMI-II was indicated by premature internal eggs (Figure 4), both with a yellowish-brown color; 3. Medium gonad maturity index of GMI-III when starts the development of encapsulated eggs or external capsule, expelled into the seawater; and 4. The GMI-IV, with the most mature external capsule, ready to spawn. In most cases, the walking shark specimens are found with two egg-capsules, of a length of 8-9.5 cm and a width of 3.5-4 cm. Samples of mature external egg-capsules, covered with a sticky layer for the attachment on a surface of coral-reef, were found at the north region of Halmahera Bay.

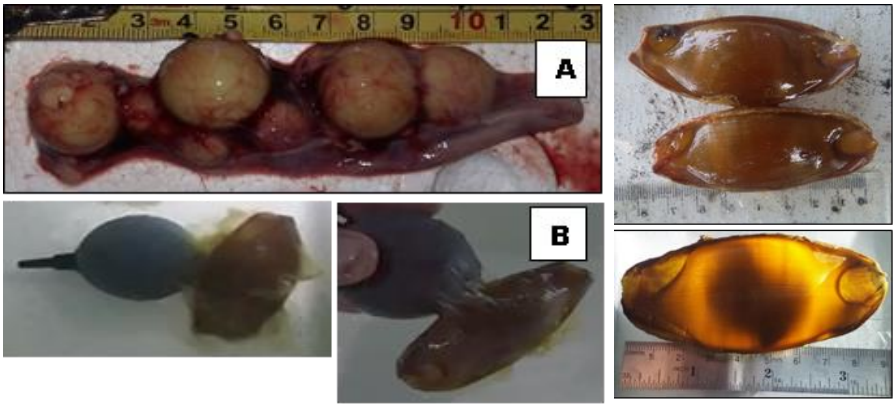

Figure 4. Earlier internal eggs of *Hemiscellyum halmahera* (A and B Left) and mature external egg-capsules.

The fecundity represents the number of eggs produced over the lifespan. In this study, most mature walking sharks were found with 8-10 immature internal yellowish eggs; only two mature egg-capsules were expelled. According to an earlier study, the factor of fertility of female fish, spawning frequency, parental care, size of eggs, population density and its environmental conditions affect the number of the produced eggs (Jutan et al 2019). During the egg spawning, only one egg-capsule is expelled at once, and the other egg-capsule is expelled one day later. During the period of egg spawning, the female fish is protected by the male fish swimming above or beside of the female one. This kind of behavior is found in low fecundity fish to protect from other predator groups. After the spawning phase, the couple of sharks still protect the egg-capsule attached on the substrate during the incubation period. Then follows the development of the embryo into the larval stage, in the egg-capsule, until the spawn of the young soft juvenile from the egg-capsule. During this study, most of the empty egg-capsules were found attached on the coral, meaning that these coral reefs functioned as living habitat, feeding area as well as the spawning and nursery ground for these sharks. The dark brownish color of these egg-capsules is a perfect camouflage. The egg-capsule shells are hard, to protect the development of the embryonic phase during the incubation period of 3-4 months. This is known as a characteristic of the low fecundity species for the protection of the eggs until the spawning into young juvenile joint into the parental populations (Jutan et al 2019).

Specifically, the recruitment of the walking sharks at Halmahera Bay, North Halmahera occurred over two periods. The minor population recruitment period happened during January to April, with 2.88%, and the major population recruitment from May to November, with 15.40% from the total 604 samples counted over a year, during the 296 diving trips with 3 divers in the depth range of 8-25 m. A total of 20 *H. halmahera* young juvenile were found during the study within a range of a total length of 16.9-26.0 cm (Jutan et al 2018) as in Figure 5.

Figure 5. Juvenile of *Hemiscellyum halmahera*, TL of 16.9 cm (above) and January 2018, TL of 26.0 cm (below).

The oviparous walking sharks will reach the mature gonads after 3 years, at a TL of 62.13 cm. Among 604 individuals found during the one-year underwater survey, only 56 of them had mature gonads and were ready to spawn. The walking shark of *H. halmahera* is known as a slow-recruiting species, in comparation to *Hemiscyllium ocelatum* reared in the Tinesse Aquarium Australia, whose incubation from egg-capsule to juvenile takes 120 days, and which reaches a TL of 20 cm after 4 months, 30 cm after 14 months and 45-51 cm after 2 years (Allen et al 2013). On the other side, there are issues of mercury pollution in the Halmahera Bay since 2020, due the gold mining operations (Jutan et al 2019; Mu'min et al 2021). In a similar case was *Hemiscyllium michaeli* in the Milne Bay Papua New Guinea, with the operation of gold mining and sedimentation from the palm plantation (Allen et al 2013).

Satellite image data analysis. Image processing of the Sentinel-2A provided an overview of the outer mouth width of Halamhera Bay at 45.79 km (A), the narrowest part of the bay at 7.35 km (B), and the length from the outer mouth to the deepest part of Halmahera Bay at 101.51 km (C) as in Figure 6, left. It can be considered that Halmahera Bay is a semi-enclosed bay that significantly extends inward, forming a semi-enclosed condition. The shallowest part reaches a depth of 5 m, and the deepest part of Halmahera Bay is 1,311 m (Figure 6) based on the GEBCO spatial database.

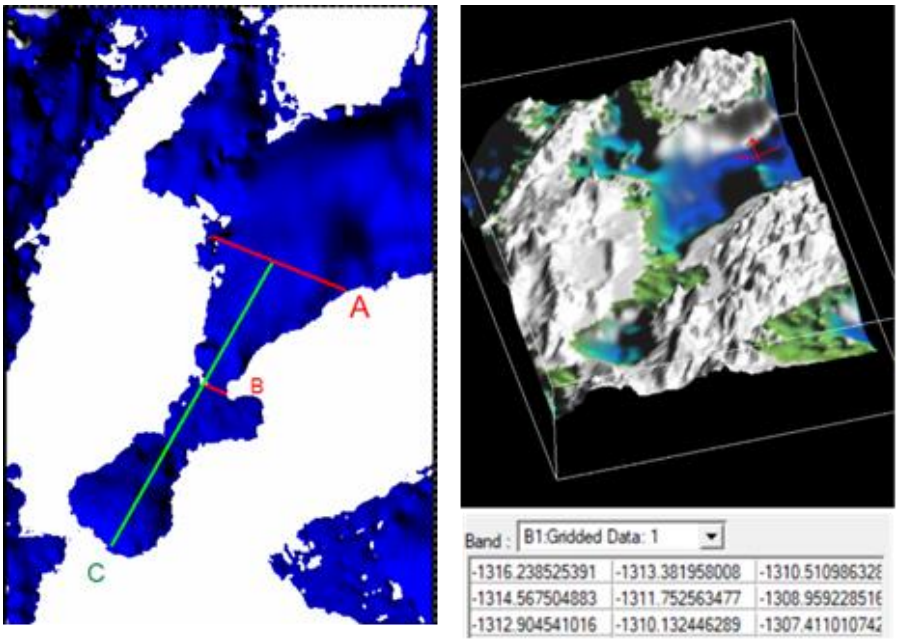

Figure 6. The geomorphology, bathymetry (Left) and the deepest site (Right) of Halmahera Bay, Halmahera Island. (GEBCO data).

Distribution of H. halmahera over bathymetry and chlorophyll-a data. The distribution of *H. halmahera* shows two grouping patterns, that is with 1-3 individuals in

small groups indicated by small black dots and 3-6 individuals in large group, indicated by large red dots (Figure 7-Left). *H. halmahera* in Halmahera Bay is found mostly in the coastal bay, at a depth range of 8-25 meters. They perfectly hide under or among the coral-reef structures and seaweed beds. This combination of coral-reef and seaweed bed is a perfect habitat for the abundance of *H. halmahera* fish larvae and juveniles (Erzad et al 2020; Jutan et al 2019; Mu'min et al 2021), given the sheltering and feeding opportunities. As a semi-enclosed bay, the sea waves are weak, and the seawater in the inner-part of the bay is very calm, relying primarily on tidal currents flushing seawater masses during high tide period. The patterns of currents and the movement of water masses play a crucial role in determining the flow of nutrients that support the health of the ecosystem as well as the supply of planktonic organisms into the Halmahera Bay. The analysis of the chlorophyll-a content using the Marine Copernicus data in Halmahera Bay gave a range of 0.117-0.374 mg m^{-3} (Figure 7-Right). Fundamentally, this chlorophyll-a content indicates that the seawater of Kao Bay is still in the category of a good condition. The spatial pattern of the chlorophyll-a content indicates the flows from the Pacific Ocean, at the mouth of the bay (0.374 mg m^{-3}) and into the bay (0.117 mg m^{-3}). The spatial distribution of the *H. halmahera* had shown a non-synchronous pattern over the chlorophyll-a spatial data (Figure 7-Right). This fact had concluded that *H. halmahera* prefer to inhabit the shallow bays of a depth range of 8-25 meter. Based on the spatial bathymetry analysis and the highest presence of *H. halmahera* (large red-dot position in Figure 7), at the narrow part of the bay, a Marine Protected Area can be determined, in the future (Hartoko & Helmi 2004; Hartoko et al 2014; Barzehkar et al 2021; Grilli et al 2019; Rossetto et al 2015). There are some possible issues related to household organic pollutants, as well as to industrial pollutants entering the Halmahera Bay. These might have the potential to damage the bay's ecosystem, which is the natural habitat of *H. halmahera*, one of the endemic species first discovered in the waters of Halmahera (Allen et al 2013; Edward 2017; Husen 2016; Lessy 2015; Simange 2015; Simbolon et al 2010; Sulistijo et al 2018).

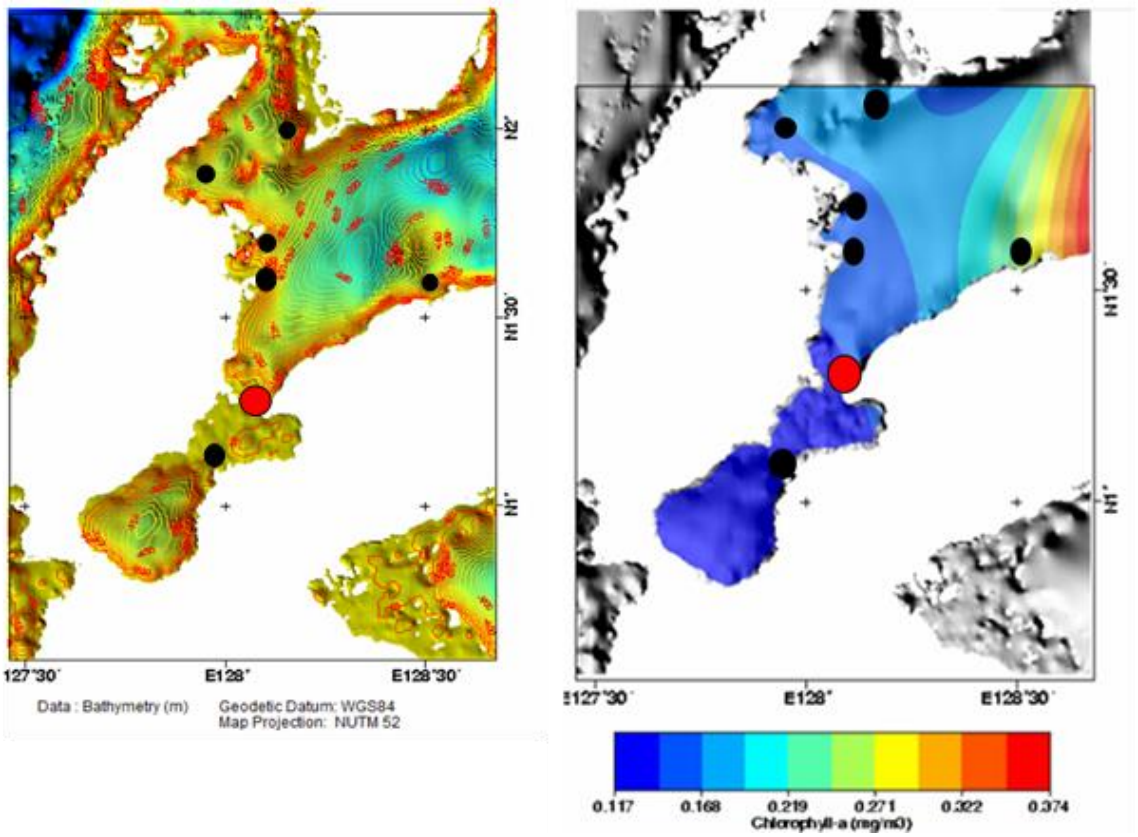

Figure 7. The spatial distribution of *Hemiscellyum halmahera* (black and red dots) overlaid on the bathymetry map (Left, source: GEBCO 2023) and the chlorophyll-a content (Right, source: Sentinel-2A 2023) in the Halmahera Bay, Halmahera Island.

Discussion. During the night diving survey, solitary *H. halmahera* specimens were spotted at a depth ranging 8-35 m. More specifically, during the dark moon nights, small and medium size of *H. halmahera* were spotted, while during the bright moon nights, bigger specimens were spotted. *H. halmahera* were distributed at Weda, Kao, Tobelo Bay, as parts of the Halmahera Bay, and at Morotai Island, Ternate Island, Bobale Island and South Halmahera. *H. halmahera*, a species from the Hemiscylliidae family, is classified in the Near Threatened (NT) category. It is a benthic carnivore; its main diet includes small fish, annelids, mollusks, crustaceans, and zooplankton.

According to Van der Wrigt (2021), adults *H. halmahera* are light brown above, with scattered darker spots and indistinct saddles. Epaulette sharks have nocturnal habits and are frequently found in shallow waters of coral reefs or in tidal pools. This shark has evolved to cope with the severe night time oxygen depletion (hypoxia), in isolated tidal pools, by increasing the blood supply to its brain and selectively shutting down non-essential neural functions. It is capable of surviving complete anoxia for two hours without damaging effects, and at a much higher temperature than most other hypoxia-tolerant animals. Rather than swimming, epaulette sharks often "walk" by wriggling their bodies and pushing with their paired fins. This species feeds on a wide range of small benthic invertebrates and bony fishes. The study reveals that *H. halmahera* is oviparous fish, and live mostly solitary. *H. halmahera* was spotted in a very close association with live coral-reef, sponges, and seagrass. The findings of the study could be used as a database information and are essential for assessing the distribution of possible pollutants entering the waters of Halmahera Bay of human activities around the bay's coastline, in support for developing an environmental policy to protect the walking shark *H. ocelatum, H. halmahera* and *Chiloscyllium plagiosum*. Law enforcement of the recently launched the Ministerial Decree No. 30 of 2023 of the Minister of Marine and Fisheries, Republic Indonesia, is also required for the protection and conservation of the walking shark *H. halmahera.* The future research should focus on the analysis of the stomach content of the walking shark *H. halmahera* as it had been studied for the stranded whale at East Nusa Tenggara by Pringgenies et al (2021), Maro et al (2021), Van der Wright et al (2021), Grilli et al (2019) and on the DNA bar coding methods of the walking shark *H. halmahera* (Pringgenies et al 2021; Maro et al 2021; Hartoko et al 2022), to confirm its eco-biology relationships of the habitat and feeding habit.

Conclusions. This study discovered two clusters of walking sharks, *H. halmahera*, consisting of small groups of 1-3 individuals and large groups of 3-6 individuals. Their preferred habitat distributes in the depth range of 8-25 m. The sampled specimens were at one of the four maturity levels of gonads: internal eggs, premature internal eggs with a yellowish-brown color, developing encapsulated eggs or external capsule expelled into the seawater, and mature external capsule ready to spawn. Most of *H. halmahera*'s natural feeds are benthic organisms generally trapped among coral-reef after high tide seawater flushing into the Halmahera Bay. The chlorophyll-a content in Halmahera Bay is in the range of 0.117-0.374 mg m^{-3}, in the category of a fertile seawater condition.

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Conflict of interest. The authors declare no conflict of interest.

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