MORPHOLOGICAL STUDY OF BLUE SPOTTED MASKRAY, *Neotrygon kuhlii* (RAJIFORMES: DASYATIDAE) FROM SABAH WATERS.

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Thank you.
This is a preliminary study about the morphological features of blue spotted maskray, *Neotrygon kuhlii* from Sabah waters. This study is aimed at determining the intraspecific morphological variations of *N. kuhlii* relative to its gender and variant life stages. A total of 40 specimens which have disc width range between 174.0 mm to 375.0 mm were examined. Nine specimens were collected from fish trawlers in Pitas (Kudat Zone); ten were collected from fishermen through the Sabah Fish Marketing (SAFMA) wharf in Kota Kinabalu (West Coast Zone); and 21 were collected from Sandakan (East Coast Zone) through trawling. Specimens were collected from July 2009 to January 2010. Out of the 40 specimens, 16 were spot types (40.0%); 17 were ring types (42.5%) and seven combination patterns (17.5%). Spot type *N. kuhlii* consists mostly of specimens with disc width of more than 300.0 mm (75.0%) whereas majority of the ring type *N. kuhlii* had disc width ranging from 200.0 mm to 300.0 mm (76.5%). There was no fuzzy spot type found in this study. Rays with combination pattern were evenly distributed in all ranges of disc width. In this study, combination pattern indicates transition stage of rays between different life stages. Rays with different spotting pattern did not show significant morphological variation. Female and male rays shared a similar spotting pattern. This study showed that the variation of colour patterns occurs depending on life stage instead of gender and species variation. This is the first study to describe the change of colour patterns relative to their life stage.
ABSTRAK

Kajian ini merupakan satu kajian asas untuk mengenalpasti morfologi pari lalat, Neotrygon kuhlii di perairan Sabah. Objektif kajian ini adalah untuk mengkaji perbezaan morfologi N. kuhlii relatif kepada jantina dan peringkat kehidupan mereka. Empat puluh spesimen yang bersaiz antara 174.0 mm ke 375.0 mm dikaji dalam kajian ini. Sembilan spesimen didapati daripada kapal pukat tunda di Pitas (Zon Kudat); sepuluh didapati daripada nelayan di Pasar Ikan Sabah (SAFMA) di Kota Kinabalu (Zon Pantai Barat) dan 21 adalah didapati daripada Sandakan (Zon Pantai Timur) melalui pukat tunda. Pengumpulan spesimen dilakukan daripada Julai 2009 hingga Januari 2010. Daripada 40 spesimen ikan pari, 16 berbintik-bintik (40.0%); 17 spesimen bercorak tompok cincin (42.5%) dan tujuh mempunyai corak kombinasi (17.5%). N. kuhlii yang berbintik-bintik terdiri terutamanya daripada ikan yang bersaiz melebihi 300.0 mm (75.0%) manakala majoriti N. kuhlii yang bercorak tompok cincin adalah ikan yang bersaiz antara 200.0 mm hingga 300.0 mm (76.5%). N. kuhlii yang bercorak tompok kabur tidak dijumpai dalam kajian ini. N. kuhlii yang mempunyai bercorak kombinasi dijumpai dengan serata di semua saiz kelas dan corak ini dipercayai hanya wujud dalam tempoh pertukaran antara satu peringkat ke peringkat kehidupan yang lain. Tidak ada perbezaan dari segi morfologi bagi N. kuhlii yang mempunyai corak warna yang berbeza. Ikan pari betina dan jantan mempunyai corak warna yang sama. Kajian ini membuktikan bahawa perubahan corak warna adalah berdasarkan perubahan peringkat kehidupan dan bukan berdasarkan perbezaan jantina dan spesis. Kajian ini merupakan kajian pertama untuk menerangkan hubungan secara relatif antara corak warna dengan peringkat kehidupan N. kuhlii.
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°   Degree
°C  Degree Celsius
%   Percentage
mm  Millimeters
cm  Centimeters
m   Meters
km  Kilometers
km² Square kilometer
CHAPTER 1

INTRODUCTION

1.1 Stingrays of Indo-West Pacific

Stingrays belong to the same subclass as sharks which are elasmobranch. Several distinct morphological characteristics can be found in elasmobranch such as their cartilaginous skeleton and denticle scales.

The scientific name of blue spotted maskray (locally known as Pari Lalat, Pari Macan, Pari Riman, and Pari Tanjung) is Neotrygon kuhlii (Muller and Henle, 1841). It is a member of the family Dasyatidae which consists of seven genera namely Dasyatis, Himantura, Neotrygon, Pastinachus, Pteroplatytrygon, Taeniura, and Urogymnus (Nelson, 2006; Last and white, 2008).

Unpublished studies of stingray under family Dasyatidae of the Indo-Pacific by senior researchers have found that the taxonomy of dasyatids is complex (Last and White, 2008). The species was once thought to be ubiquitous but are now known to consist of species complexes (Last and White, 2008). For example, confusions occurred between Dasyatis microphthalmus and Dasyatis acutirostra described by Manjaji and Last (2006). There are at least three new stingray species discovered at Indo-Malay region including Himantura lobistoma (Manjaji and Last, 2006), H. hortlei (Last et al., 2006) and Pastinachus solocirostris (Last et al., 2005).
Besides that, a project to survey the Elasmobranch species from Sabah had been done from January 1996 to June 1997 (18 months survey) to assess the Elasmobranch biodiversity which included both marine and freshwater species (Manjaji, 1997). It was a preliminary field survey of the Labuk bay and was carried out by members of the IUCN Shark Specialist Group (SSG) with cooperation from the Department of Fisheries (DoF) Sabah. For Family Dasyatidae, Thickspine giant stingray (*Dasyatis microps*), Giant freshwater stingray (*Himantura chaophraya*), Pink whipray (*Himantura fai*), Ocellate whipray (*Himantura fava*), and Leopard whipray (*Himantura undulate*) are marine species that were first found in Sabah (Manjaji, 2004).

A review of internet queries from Fish channel.com - *the website for fish lover* (online material accessed on 08/06/2009) and other websites (such as animal-world.com, travelblog.com and peteducation.com accessed on 16/10/2009) showed that there were some confusions with the term 'Blue spotted stingray’ between *Taeniura lyamma* and *Neotrygon kuhlii* based on their similar outlook which is the distribution of blue spots on the dorsal surface. However, the fact is they are two different species because of the differences in the shape of the disc and the stripe pattern found on their tails. *T. lyamma*, which should be known as blue spotted ribbontail ray, has an oval-shaped disc meanwhile *N. kuhlii* has a diamond-shaped disc. The tip of *T. lyamma*’s tail is lightly rounded with blue stripes along the tail. *N. kuhlii* has a pointed tip tail with alternative white and black bands (Appendix A).

1.2 Biological overview of Blue Spotted Maskray (*Neotrygon kuhlii*)

*Neotrygon kuhlii* is a bottom feeder. When hunting by speculation, the ray searches an area that is expected to have prey or it will follow another organism and expects that organism to flush prey out by its presence (Curio, 1976). Electrical stimuli and olfaction are important in prey detection (Tillett, 2005). However, *N. kuhlii* is unable to estimate the prey depth and size. *N. kuhlii* will position itself at higher tidal water movement area and wait for preys to sweep by (Motta, 2004). Then it will feed on small crustaceans such as crabs and shrimps. Sometimes *N. kuhlii* will use its pectoral fin to sweep away sand that is covering small crustaceans so that they can be exposed and be eaten.
*Neotrygon kuhlii* flaps its pectoral fin to move through the water body. It will increase fin beat frequency, wave speed, and stride length to increase swimming speed, while the amplitude remains constant (Rosenberger and Westneat, 1999; Rosenberger, 2001).

*Neotrygon kuhlii* is ovoviviparous (Dulvy and Reynold, 1997). In ovoviviparous there are two reproductive strategies. It is either the eggs develop in their endogenous yolk reserves so that the young is lighter than the original eggs or, the developing young directly receive nutrient from the uterus through its spiracles. For *N. kuhlii*, nutrient will pass into the oesophagus of the young from the thread-like extensions of the uterine wall (trophonemata) through the spiracle. The new born pup is approximately 50.0 times heavier than unfertilized eggs (Marshall *et al.*, 2004).

Blue spotted maskray, *Neotrygon kuhlii* is widely spread in South East Asia, mainly at Indo-West Pacific range from Red Sea (Last and Compagno, 1999) and East Africa to Samoa, and Tonga (Randall *et al.*, 2003), north to Japan, south to Australia (Last and Compagno, 1999). It prefers tropical areas (Gunther, 2006).

Generally, *Neotrygon kuhlii* lives on sandy bottom near rocks or coral reefs found in deeper waters. However, sometimes they can also be found at reef flat and shallow lagoon during high tide.

### 1.3 Significance of Study

*Neotrygon kuhlii* may show different colour patterns on the body surface. This includes the degree of spotting such as full spotted type, few spotted type and plain type; and spotting patterns include spot type, ring type and fuzzy spot type (Yano *et al.*, 2005; Manjaji personal communication). My study was carried out by measuring and identifying the morphological features and meristic counting of *N. kuhlii*. This can help in resolving the taxonomic problem of *N. kuhlii* which is currently ambiguous by increasing the understanding about the fish's diagnostic features and simultaneously encode cryptic species of *N. kuhlii*. The encoding of cryptic species of *N. kuhlii* could help fisheries department to provide a better management towards *N. kuhlii* to maintain its sustainability. This will aid in its fisheries conservation.
1.4 Aim and Objectives

The aim of this study is to determine the morphological variations of Neotrygon kuhlii relative to its species, gender and life stage. Specifically, the objectives include:

1) To determine morphological features of N. kuhlii.
2) To study the morphological variations of N. kuhlii according to their gender and life stage.
3) To clarify the existence of cryptic species of N. kuhlii in Sabah waters.

1.5 Hypothesis

The hypothesis of this study is different colour patterns (degree of spotting and spotting pattern on the dorsal surface) may indicate separate species.

In the animal world, differentiating species under same genus might be difficult (Feline Conservation Federation, 2009). However, minus different characteristics can still be observed among members under same genus. The species morphological variations include the degree of spotting on the body.

For marine fishes, bull trout (Salvelinus confluentus), brook trout (Salvelinus fontinlis), lake trout (Salvelinus namaycush), and brown trout (Salmta truta) are members of trout family which generally have light spots on a dark background. However, variations of colour pattern on the body surface do exist among species (Dundee Sportman’s Club, 2003). Bull trout do not have spots on dorsal surface and its spots are range from yellow to cream colour. The spot pattern is non-wavy on the back and non-halo on the side of the body. Lake trout has light colour or no spots on the dorsal fin. The spots are white or cream colour distribute on the side and back of the body. Brown trout has black spots on the dorsal fin. Its body is full with black, brown or sometimes red spots in light halo or ring shape. Brook trout has black marks on dorsal fin. Its spot patterns are yellow, cream, wavy ‘worm tracks’ on back and yellow, red spots/ring/halo on side (Dundee Spotman’s Club, 2003).

For terrestrial animals, Southern European lynx (Lynx lynx dinnikl) has heavy spotting on the body. Siberian lynx (Lynx lynx wrangelli), Northern European lynx (Lynx lynx lynx) and Mongolian lynx (Lynx lynx isabellina) have no spots on the body. Instead they have different coat or body ground colour (Feline Conservation Federation, 2009).
CHAPTER 2

LITERATURE REVIEW

2.1 Morphology of *Neotrygon kuhlii*

*Neotrygon kuhlii* is a maskray that has diamond-shaped disc with slightly convex anterior and posterior margin. Its snout is broad, rounded and short. There is no rostral cartilage found at the snout tip. This means that the snout is entirely supported by pectoral fin skeleton. The dorsal colour of *N. kuhlii* ranges from reddish-brown to olive drab. Commonly, *N. kuhlii* has grey or blue spots that are about the size of their eyes and also smaller black spots. These spots serve as camouflage or visual communication (Theiss *et al.*, 2006). The maximum width of *N. kuhlii* can reach up to 500.0 mm and their maximum length can reach 660.0 mm (Compagno *et al.*, 1989).

Five small gill openings can be found at the front half of pectoral disc with no gill sieves or rakers on internal gill slits. Mouth is broadly arched and sometimes upper part of mouth might be covered by elongated nasal curtain. Inside the mouth, oral teeth are small with less than 50 rows in either jaw. A transverse curtain can be found on the roof of the mouth near the upper teeth row. Two tooth bands can be found within the upper teeth row for both female and male fishes (Last and Compagno, 1999).
Neotrygon kuhlii has a broad based tail that is almost as long as its body width with black and white rings. A short dorsal skin fold and longer ventral skin fold can be found along the tail (Compagno et al., 1989). Ventral skin fold is almost uniform in height along the tail except for slightly tapering posteriorly near tail tip. Ventral skin fold is longer than dorsal skin fold but from the aspect of the height, it is only slightly taller than dorsal skin fold (Last and White, 2008). Two stinging spines can be found on its tail that is used for defense. N. kuhlii do not have dorsal or caudal fins (Last and Compagno, 1999).

According to Last and Steven (1994), one of the diagnostic characters of Neotrygon kuhlii is the rhomboidal disc with a thickening trunk. Disc width is about 1.1 to 1.3 times of the disc length. From the dorsal view, N. kuhlii has large eyes in which the orbital and spiracle length is 1.4 to 1.8 times smaller than the direct snout to preorbital length. There is a row of denticles confined along the midline of the disc. These denticles are short and thorn-like (Last and Stevens, 1994).

From the ventral view, its mouth is small with two broad papillae on the mouth’s floor. The internasal curtain is skirt-shaped, moderately elongated and narrow. Another diagnostic character of Neotrygon kuhlii is the cutaneous tail folds on both side of the tail. The ventral fold is low and elongated while the dorsal fold is short and situated beyond the sting, subequal in height to ventral fold (Last and Steven, 1994). N. kuhlii has reduced placoid scales throughout its body and pectoral fin. This is to increase the flexibility of the fins for movement (Pough et al., 1996).

According to Yano et al. (2005), Neotrygon kuhlii can be divided into three types based on the spotting degree found on its dorsal surface. They are spotted type, less spotted type and non-spotted type (Photo 2.1).
A. Non-spotted type

B. Less spotted type

C. Spotted type

Photo 2.1  Degree of spotting of Blue spotted maskray (Neotrygon kuhlii). A. Non-spotted type found in Singapore. B. Less spotted type found in Sulawesi, Indonesia. C. Spotted type found in Queensland, Australia.
The spotted type and less spotted type fish can still be further divided into spot type, fuzzy spotted type and ring type. Different colour patterns can be present on *N. kuhlii* of different gender and different life stage (Yano *et al*., 2005) (Photo 2.2).

**Photo 2.2** Spotting patterns of Blue spotted maskray (*Neotrygon kuhlii*). **A.** Spot type found in Similan Island, Thailand. **B.** Fuzzy spot type found in Sulawesi, Indonesia. **C.** Ring type found in Australia.
2.2 Morphological Differences

2.2.1 Genders

Morphological differences do exist between female and male *Neotrygon kuhlii* as in many batoid species such as skates. These differences include the teeth form and body size. According to Kajiura and Tricas (1996), female has molariform teeth throughout its life whereas male *N kuhlii* will periodically shift its teeth from female-like molariform teeth to a recurved cuspitate form teeth during reproductive season to increase its grip tenacity against female and also increase feeding efficiency and sexual selection to maximize mating success. Besides that, generally, female also has larger body size compared to male *N. kuhlii*. The main reason for it is because larger body size enables female fishes to gain more energy which will be used for reproduction (Kajiura and Tricas, 1996).

2.2.2 Life stages

Morphological differences also exist for *Neotrygon kuhlii* at different life stages. According to Last and Compagno (1999) pectoral fin apex for adult *N. kuhlii* is somewhat angular where as the pectoral fin apex for juvenile *N kuhlii* is narrowly rounded. In addition, single row of thorn-like, short, lanceolate, flat topped denticles can be found along the disc midline of adult which is often absent in juvenile fish. Clasper length for immature male juvenile fish is slightly shorter than those of adult fish.
CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

Sabah is located at the east of South China Sea, northern Borneo Island. Its longitude is between 5°N to 7°N and latitude is between 115°E to 119°E. It is surrounded by South China Sea on its west, Sulu Sea on its north east, Sulawesi Sea on its south east, and by Sarawak, Brunei Darussalam and Kalimantan of Indonesia on its south. Its landmass is 73711 km$^2$ and has a coastline of approximately 1450 km (Figure 3.1).

Generally, Sabah has an average temperature of 21°C to 32°C with annual rain fall of 200 cm. The highest rainfall for Sabah falls from October to December which is more than 300 cm due to Northeastern Monsoon. Humidity of Sabah can reach 80%. Tidal range in Sabah is 1-2 m and has a wave height less than 3.5 m (WorldMark Encyclopedia of The Nations, 1998).

Sabah is connected with mainland Asia through Sunda Shelf. Sunda Shelf is flat at its central part and become slightly tilted when approaching Sabah, forming a 40 m depththourgh at the shelf edge. Sunda Shelf becomes narrow to less than 100 km along the west part of Sabah. Palawan Trough (3475 m) can be found at northwest part of Sabah (Morgan and Valencia, 1993).
Sabah contains high diversity of marine flora and fauna due to the existence of mangrove and coral reef. Sabah is covered with approximately 3600 km² of mangrove (more than 50% of total mangrove forest in Malaysia) (Morgan and Valencia, 1993). The most abundant mangrove species includes *Rhizophora mucronata, Nipa fruticans, Oncosperma vigillaria* and *Bruguiera gymnorrhiza* (Morgan and Valencia, 1993).

Due to the reason of specimens in this study only can be collected from Kota Kinabalu, Kudat and Sandakan, therefore these three areas were chosen as specific locations for specimens collection as represented *Neotrygon kuhlii* caught from whole Sabah.

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**Figure 3.1** Sabah map  
**A.** West Coast Zone: Kota Belud, Kota Kinabalu, Papar, Beaufort, Tuaran and Kuala Penyu.  
**B.** Kudat Zone: Kudat, Kota Meludu and Pitas.  
**C.** East Coast Zone: Beluran and Sandakan.
REFERENCES


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