

**DNA Barcoding and molecular phylogeny of**

**Torpediniformes (electric ray)**

Submitted in partial fulfillment of the requirements for the award  
of bachelor of science in biotechnology

by

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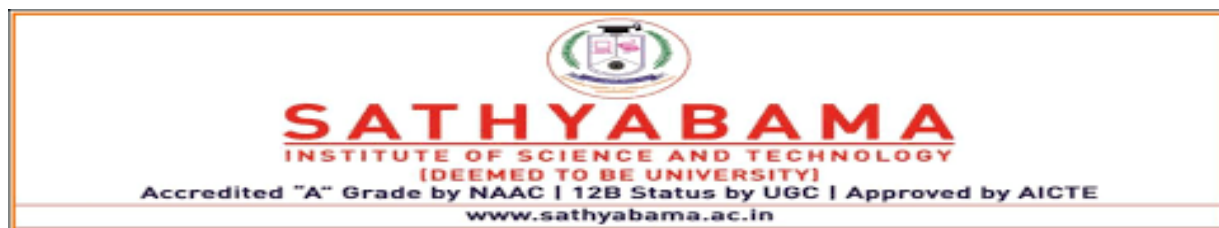
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## DEPARTMENT OF BIOTECHNOLOGY

### BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bona fide work of M.Nishanthkannan .(Reg. No. 40770035), who carried out the project entitled DNA BARCODING AND MOLECULAR PHYLOGENY OF TORPEDINIFORMES ( ELECTRIC RAY) under my / our January to April .

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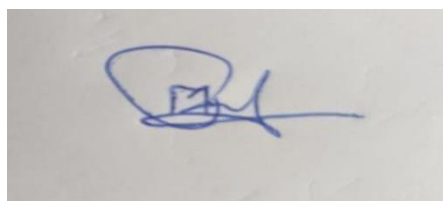
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## DECLARATION

M.Nishanthkannan(40770035) hereby declare that the Project Report entitled DNA BARCODING AND MOLECULAR PHYLOGENY OF TORPEDINIFORMES (ELECTRIC RAY) Done by me under the guidance of DR. INDUMATHI S M ,and DR. LEEBA BALAN at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of The requirements for the award of Bachelor of Science degree in biotechnology.

**DATE:**  
**12/3/23**

**PLACE: CHENNAI.**

A handwritten signature in blue ink, consisting of a large, stylized 'R' followed by a horizontal line and a small flourish.

**SIGNATURE OF CANDIDATE**

## ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. V Ramesh Kumar**, Head of the Department, Dept. Of Biotechnology for providing me necessary support and details at the right time During the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr. INDUMATHI S M AND Dr. LEEBA BALAN** for their valuable guidance, suggestions, and constant Encouragement that paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion

## **ABSTRACT**

The preservation of genetic resources has become an important element of conservation. Electric rays of the Order Torpediniformes represent a complex group of species with rather restricted distribution susceptible to capture in gillnets, shore seine and trawl fisheries, which are generally discarded as by-catch. Concise knowledge of taxonomy and biology of torpedinoid electric ray fishes is prerequisite for future studies on conservation and management of these resources. Moreover traditional taxonomic practices are insufficient to identify all the species. The task of recognizing new species and new distributional records has certain urgency. DNA sequencing and DNA barcoding is used as an additional aid to traditional taxonomy for accurate identification of species. Reliable identification is the basis for addressing many ecological and evolutionary issues and is fundamental to the management and conservation. This is particularly useful in confirming field identifications, resolving species complexes, distinguishing morphologically similar congeners and identifying cryptic species. Various species may be at risk from overfishing (as a target species or as by-catch) or habitat destruction. In general, this by-catch is not regulated or even reported, especially in developing countries. Together, the lack of biological information and the unreliability or non existence of landing statistics have limited the development of sustainable management practices and conservation strategies for these torpedinoid electric rays. Studies to date have focused mainly on their electrogenic potential or on the development and physiology of the electric organs per se. The species level systematics of electric rays has been neglected. These are known from only a few published records across a very patchy geographical distribution and as such little is known of its biology. Given its size, rarity, patchy and relatively restricted distribution, trawl fisheries may prove to be of conservation concern.

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## LIST OF ABBREVIATIONS

- ★ gms - grams
- ★ % - percentage
- ★ Cm - centimeter
- ★ TL - total length
- ★ TW - total width
- ★ mM - millimolar
- ★ M - molar
- ★ EOL - electric organ length
- ★ EOW - electric organ width
- ★ F\M - female \ male

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# CHAPTER-1

## INTRODUCTION

Chondrichthyans include cartilaginous fishes commonly called sharks, rays, skates, and chimeras which are widely distributed in all the world's Oceans, occupying a variety of habitats, from near the shoreline to the deep abyss. These are distributed from poles to tropical waters and from but most are restricted to a specific geographical area (McEachran and Aschliman, 2004). They constitute one of the most vulnerable groups due to their unique biological characteristics. They play a significant role in maintaining the ecological balance and have high economic value. The meat, skin and fins of elasmobranchs have good market value due to their use in food processing, cosmetics, medicine and leather industry (Raje et al., 2007). Although the chondrichthyans represent only 3.5% of the modern fish fauna (McEachran and Aschliman, 2004), they are a derived lineage of highly diverse, excellently adapted and wide – ranging fishes (Compagno et al., 2005a). In the last decade, chondrichthyan taxonomy has undergone a scientific renaissance with >180 new species formally described. This effort encompasses c. 15% of the global chondrichthyan fauna, which consists of 1185 currently recognized species (White and Last, 2012). Among these chondrichthyans, batoid rays (batoids) include four orders: torpediniformes (electric rays), rajiformes (skates), pristiformes (guitarfishes, sawfishes) and myliobatiformes includes two sub orders: platyrhinoidei and myliobatodei (Nelson et al., 2016). These are primarily marine, but many species can tolerate fluctuating salinities in estuaries and shallow, enclosed bays and lagoons and in rivers and lakes to the lower continental slopes and the abyssal plains. Most batoids are found in or near the bottom in proximity to land masses. Batoids are diverse in shallow continental waters of tropical and warm temperate seas, cold temperate to boreal waters and on the upper continental and to a lesser extent in insular slopes, but are less diverse at great depths (below 1,500 to 2,000m), off oceanic islands and in the open ocean, the richest batoids faunas occur in the Indo-west Pacific from south Africa and the Red Sea to Australia and Japan. Many

batoids are social animals, occurring in small to huge groups, and some species are migratory (carvalho et al.,1999).

Data on gear used for catching torpedinoid electric rays In the area is sketchy. Torpedo electric rays, numbfishes( Naricinidae), sleeper rays (Narkidae), and a few other families are minor components of local catches off kovalam coast , tamilnadu(12°47'25.8"N 80°15'10.6"E), electric rays are represented albeit in small numbers in the catches of line gear , fixed and floating hull nets, bottom trawls, and fixed fish traps, and purse seines that are used to target batoids and other teleostean fishes. The lack of taxonomic clarity in torpedo, narcines, heteronarce and narke Species of this region constrains the advancement of even basic research on the distribution, biology and population structure of these rays.

The species of the order torpediniformes which includes the families torpedinidae, naricinidae, and Narkidae, are sluggish bottom dwelling rays, feeble swimmers, spending most of their time lying on the bottom partially buried in the mud or sand, generally preferring shallow depths and are known for being capable of producing a strong electric discharge , ranging from little as 8 volts up to 220 volts depending on species, possibly as a defence from predators and to stun prey by paralysing and capturing any small creature including fishes which accidentally touches (carvalho et al., 2002b). The electric rays recorded from Indian waters are vulnerable (VU), near threatened (NT), data deficient (DD) and not evaluated (NE) species. Species are assessed as data deficient due to lack of authentic distributional records and biological information. This is often from uncertainty over their ambiguous identifications. The IUCN specialist group (IUCN,2017-3) stated that most of the Species were data poor or deficient and research to address this issue is on-going that help to govern the degree of threat faced by this group. Immediate focus on gathering more data on such species especially in south east Asia is the need of the hour.

The taxonomic status and correct identification of many of these species are so problematical. The important features for identifying electric ray species include colour pattern and markings on the dorsal surface of the body, disc, and tail shape, structure of the oronasal region and first dorsal- fin position (8. last and stevens, 2009). Dorsally the colour tone (eg. Brownish, reddish, yellowish etc.), whether it is

plain or marked (eg. Spotted, blotched, banded, reticulated etc.) and position of these markings is very informative and particularly important for identifying and distinguishing these species (Last et al., 2016). The electric rays are morphologically very distinctive and easily separated from other batoids, displaying well developed pectoral electric organs derived from branchial musculature, smooth skin devoid of dermal denticles or spines and a highly modified posteriorly arched shoulder girdle, among other characters (Bigelow and Schroeder, 1953; Compagno, 1973; Carvalho et al., 2002a). In recent years, various revision studies on electric rays revealed that some genera are more diverse than previously understood (Carvalho et al., 1999).

These rays are dorsoventrally flattened. Snout moderately elongated, broadly rounded and is supported by a rostral cartilage and anteriorly expanded antorbital cartilages. Head broad and depressed. They have eyes and well-developed spiracles on the dorsal surface of the head. The mouth and nostrils are on the ventral surface. The teeth are numerous on both the jaws and characterized by prominent single cusps. These are set in transverse rows, being constantly replaced from inside the mouth and are laterally fused to form tooth plates (Carvalho et al., 1999). They have enlarged pectoral fins originating lateral to snout, attached to the head as an adaptation for life on the bottom. The pectoral fins and the body together form the 'disc'. Locomotion is achieved by vertical muscle waves passing along the enlarged pectoral fins. Torpedinoid rays have five pairs of ventral gill openings or gill slits. A pair of kidney-shaped electric organs present on either side of the head which are not clearly visible dorsally but can be easily observed ventrally occupying the whole thickness of the disc. An oval Cloaca lies midway to pelvic fins ventrally. Paired cylindrical claspers present in males which originate lateral to the end of cloaca and extend beyond the pelvic fins in adults. Lateral tail folds present on either side of the tail originating near the posterior end of the first dorsal fin and extend till the caudal fin origin. The tail and caudal fin are well developed and serve to propel the animal by lateral undulations. Caudal fin with vertebral axis hardly rose above the body axis. Liver is bilobed, grayish brown in colour (Plate III Fig. 3, 7; Plate IV Fig. 6). Previous studies on torpedinoid fish faunas, distribution, field guides and checklists are – Cantor (1849), Annandale (1909; 1910), Whitley (1940), Fowler (1941); Bigelow and Schroeder (1948), Compagno (1984a; 1990; 1997; 1999; 2002;

2005), Masuda et al. (1984), Monkolprasit (1984), Smith and Heemstra (1986), Compagno et al. (1989; 2005a; 2005c), Quero et al. (1990), Michael (1993), Last and Stevens (1994), Bonfil (1994, 1997), Compagno and Didier (1997), Compagno et al. (1997), Manjaji (1997 a and b), Last (1997), Froese and Garilao (1997), Mansor et al. (1998), Vidthayanon et al. (1997), Anderson and Hafiz (1997), (Cox and Francis, 1997), Last and Seret (1999), Carpenter (2002), Cavanagh et al. (2003), Ebert (2003), Bonfil and Abdallah (2004), Baino et al. (2008), Weigmann (2011), Bradai et al. (2012), Aschliman et al. (2012a), Ebert and Stehmann (2013), Ebert et al. (2013b; 2013c), Clarke et al. (2015), Goren and Galil (2015), Last et al. (2016), Eschmeyer (2017). Though several studies were carried out on diversity of torpedinoids in Indian waters and several species are continuously being added to local faunistic lists due to extension of fishing operations to deeper waters and exploring new fishing grounds detailed studies on taxonomy with descriptions of external morphology and information on distribution and biology are relatively scarce.

Earliest compilations of chondrichthyan diversity in Indian waters was done by Day (1878) who has given descriptions of two species *Narcinetimlei* and *Narkeimpennis* only from Indian waters. Taxonomic details and systematic accounts were given by Prashad (1920) for torpedo rays, Setna and Sarangdhar (1949 a) for *Torpedo zugmayeri*, Munro (1956) for three species *Narcinebrunnea*, *N. timlei* and *Narkedipterygia*, Misra (1969) for eight species *Torpedo panthera*, *T. sinuspersici*, *Narcinebrunnea*, *N. maculata*, *N. timlei*, *Benthobatismoeresbyi*, *Narkedipterygia* and *Bengalichthysimpennis* (= *Narkedipterygia*). After Day's compilation of earlier works, studies on this group were carried out by: Setna and Sarangdhar (1946) reported elasmobranchs from Bombay waters, Misra (1947, 1952) recorded 52 species, Munro (1956) reported three species, Misra (1969) reported 114 species of elasmobranchs, of these he described eight species in five genera in the family Torpedinidae. Dutta and Roy (1977) from Sunderbans, Jones and Kumaran (1980) reported single species *Torpedo marmorata* from Laccadive waters. Some recent taxonomic descriptions of species that are considered valid were of *Heteronarceprabhui* by Talwar (1981 a) off Kollam, west coast of India. Talwar and Kacker (1984) reported *Narcine* and *Narke* genera of 76 chondrichthyans, Ramaiyan and Sivakumar (1991) in their elasmobranch identification guide reported four

species of electric rays – *Narcine brunnea*, *N. timlei*, *Narkedipterygia* and *N. impennis* from Parangipettai. Krishnan and Mishra (1993) reported three species – *Narcine brunnea*, *N. timlei* and *Narkedipterygia* from the Kakinada-Gopalpur sector of the east coast of India. Hanfee (2001) reported 98 elasmobranchs, Raje et al. (2002) reported three species – *Narcine brunnea*, *N. indica*, *N. timlei*. Sujatha (2002) provided taxonomic key for identification of batoids off Visakhapatnam which includes seven species – *Torpedo fuscomaculata*, *T. panthera*, *T. sinuspersici*, *Narcine brunnea*, *N. maculata*, *N. timlei* and *Narkedipterygia*. The recent guide for commercially important elasmobranchs of India by Raje et al. (2007) listed 84 species including two species – *Torpedo marmorata* and *Narkedipterygia* with their distribution pattern and importance to fishery. Few studies reported elasmobranch species in fishery - Joshi et al. (2008), Borrell et al. (2011), Akhilesh et al. (2011a; 2014 – 14 species, *Torpedo fuscomaculata*, *T. marmorata*, *T. panthera*, *T. sinuspersici*, *T. zugmayeri*, *Benthobatis moresbyi*, *Narcine brunnea*, *N. lingula*, *N. maculata*, *N. oculifera*, *N. prodorsalis*, *N. timlei*, *Heteronarce prabhui* and *Narkedipterygia*) from Kochi, Wafar et al. (2011), Mohanraj et al. (2009) from Chennai and Hanfee (1999) from Tamilnadu and Kerala. Many genera and families of electric rays are poorly known and require further taxonomic study. Many species are endemic to the area and have restricted ranges within it, while several species are known from a few museum specimens only. A wealth of new species has been revealed in deep-water, offshore Continental and even inshore habitats in the past 40 years (many of which are still undescribed) (Carvalho et al., 1999). Undoubtedly more new species and many records of described species will be discovered with further collection in poorly known parts of the area. Basic knowledge of the biology of many species is often very deficient or entirely lacking, and can be supplemented by new information gathered by fisheries workers in the area.

Electric rays are either harmless or minimally harmful to people. They can shock fishermen, bathers, swimmers, divers, or unwary beachcombers. *Torpedo* electric rays are unique from other rays such as sting rays in that they possess massive and highly specialized electric organs situated at the base of pectoral fins. They will wait patiently in the dark hours of the night for their prey to swim pass. The electric

organs are presumably used to paralyse and capture any small creature which accidentally touches them and possibly as a defence from predators; nevertheless the larger sharks frequently feed on electric rays (Carcasson, 1977). Electric rays stun both small and large fishes on bottom or in midwater with shocks, and swallow whole through their distensible (snake-like) mouths and flexible jaws (Compagno and Heemstra, 2007). All these are predators, but usually have small grasping or crushing teeth and tend to take their prey ranging from planktonic animals and benthic invertebrates to small to moderately large bony fishes, other chondrichthyans, and cephalopods. In recent years there has been renewed interest in the systematics and taxonomy of the electric rays represented in the coastal waters of India, but considering the large area and the numerous species involved there is no doubt that the scope for such work is vast. It is not only a question of recording and describing species, but ultimately also a question of recognizing possible sub- species and individual stocks which later form the basis of the increasing exploitation. Although some of the recent studies have helped to clear the status of some nominal species, confusion still surrounds the identification of many common Indo-West Pacific species. Identification is not easy because of several overlapping characters of related species. Taking all the factors into consideration, it was felt that it would be worthwhile to carry out a taxonomic study of species of two families of electric rays represented in the fishery of kovalam coast. The purpose of the study is to clarify confusion in identification and distribution of species. Due to their wide distribution, the species are obvious targets of molecular genetic studies and it is reasonable to expect that some taxa that are currently, considered to represent a single, widespread species will be sub- divided once genetic studies are undertaken. As it is important to lay some ground work, the present study includes alpha taxonomy of 20 species of torpedinoid electric rays of this region along with molecular taxonomy using DNA barcodes as an additional identification tool.

## CHAPTER-2

### LITERATURE AND SURVEY

The fishes of the family Torpedinidae popularly known as torpedo electric rays are small to moderately large, heavy-bodied batoids, typically soft and flabby. Torpedo electric rays occur in tropical and temperate waters, circumglobally from the shoreline down to about 600 m on the continental slope (Carvalho et al., 2002b). These are found all along the Indian coasts. The flesh of torpedo is edible but not generally preferred. Talwar (1981b) stated that the genus *Torpedo* is largest in the family. The large pectoral fins are not used for swimming, but the fish is slowly propelled by caudal peduncle and caudal fin (Smith and Heemstra, 1986). Based on the recent works of Compagno and Last (1999), Carvalho et al. (2002b), Last and Stevens (2009), Ebert and Stehmann (2013), Ebert (2014, 2015), Last et al. (2016), Nelson et al. (2016) and on the basis of specimens of six species belonging to a single genus examined during the present study. The species of this family can be distinguished from species of other two families Narcinidae and Narkidae by the following characters: disc circular, thick and fleshy. Disc broadly rounded in outline with relatively straight anterior margin and a small median protuberance anteriorly. Eyes prominent, protruding and oval. Spiracles with knob-like papillae on posterior and lateral margins. Pseudobranchial fold present inside the spiracle, lining anterior spiracular wall. Five pairs of crescent-shaped gill arches present on the ventral side originating lateral to the outer corners of mouth and nostrils. They have a pair of large and powerful kidney – shaped electric organs. These originate very close to the disc outline and ends at a level of middle of last gill opening. Electric organs are with concave inner margins and convex outer margins; widest in the region between mouth and first gill slits. A pair of circular nostrils present just anterior to mouth, connected by broad nasoral grooves and with a prominent fold surrounding their outer margins. The outer margins situated just lateral to level of mouth corners. Posterior contour of nostrils confluent with conspicuous median lobes that contact nasal curtain at its corners and separate nostrils from mouth. The nasal curtain slightly overlaps with the mouth. Mouth curved, broad with strong grooves at distal ends, slightly protractile and its width greater than internarial width. Tooth base

slightly arched at centre. Few sensory pores are present in the snout region both dorsally and ventrally. Pectoral fins large. A pair of sub angular pelvic fins present, with straight anterior and posterior margins. Tail noticeably shorter than the disc, broadest near its origin. Two dorsal fins present, the first situated partly over the pelvic fin base and larger than the second. Lateral tail folds in the form of a narrow white line. Caudal fin larger than dorsal fins, nearly symmetrical and sub angular. Dorsal surface of the body and tail brown or amber or yellowish brown in colour which is covered with small spots of cream colour, some isolated or some fused forming clusters or vermiculations. Ventral surface uniform creamish-white in colour with a thick line of brown margins along with spots present on disc, pelvic fins and tail. The electric shocks are generated by highly specialized electric organs which are composed of stacks of striated muscle in torpedo electric rays (Barnes, 2008). On contact, these can produce an electric current up to 200 volts and 600.Hz. According to Last et al. (2016) the electric rays have developed a specific region of the brain (lobuselectricus) associated with the cerebellum that integrates stimuli associated with the use of their electricity producing (electrogenic) organs. A review on the evolution and function of electric organs in these fishes was provided by Bass (1986). Studies on behaviour and electric organ discharge in various species of the genus *Torpedo* were reported by Coates and Cox (1942), Bigelow and Schroeder (1953), Belbenoit (1979; 1986), Bray and Hixon (1986), Mellinger et al. (1978), Michaelson et al. (1979). *Torpedo* electric rays are capable of delivering a dangerous electric shock. These discharges are very strong and employed as a paralyzing weapon in large pelagic torpedoes (*Tetronarce*) that wrap their prey using their discs before ingesting them whole (Last et al., 2016). They are often almost entirely buried in sand and difficult to spot, so care should be taken where there is a sandy bottom. They should be treated with caution, especially at night when they are active as they are known to charge at divers with their mouth agape, if harassed. Though they are not known to be responsible for any fatalities, they might have been involved in several unexplained, fatal diving accidents. The shock generated by the electric rays is enough to knock down an adult human. If shocked, divers should leave the water and treat the person concerned for shock (Venkataraman et al., 2012).

The torpedinids are capable of ingesting the prey whole due to their flexible jaw cartilages and enormous gapes. They can distend their jaws allowing them to catch and consume all sizes of prey including gobies, mullet, mackerel, bogue, Pandora, damselfish and crustaceans (Shark Trust, 2012). The diet and feeding habits were previously studied by Belbenoit (1970), Belbenoit and Bauer (1972) and Capapé et al. (2007) in *T. Marmorata* and Romanelli et al. (2006) in *T. Torpedo* (Linnaeus, 1758) and *T. Marmorata*. Lowe et al. (1994) demonstrated that *T. Californica* Ayres, 1855 involves an ambush strategy from the substrate during the day, coupled with a more vigorous attack in the water column at night. According to Rudloe (1989) there has been a growing use of electric rays of the family Torpedinidae in neurophysiological and biochemical research but keeping them in the laboratory is difficult. Some of the earlier studies on taxonomy and biology in family Torpedinidae were carried out by Bleeker (1865), Lo Bianco (1888), Engelhardt (1912), Garman (1913), Fraser-Brunner (1949), Smith (1961), Collenot (1969), Mellinger (1971, 1974), Bass et al. (1975), Mellinger et al. (1978), Capapé (1979, 2001), Dor (1984), Yano and Tanaka (1988), Stevens and Lyle (1989), Mellinger and Wriese (1989), van der Elst (1993), Møller (1995), Compagno (1995), McEachran et al. (1996), Compagno and Last (1999), Capapé et al. (2000), Carvalho et al. (2002b), Consalvo et al. (2007), Compagno and Heemstra (2007) and Duman and Basusta (2013).

Many recent ichthyologists reported occurrence of various species of family Torpedinidae and provided key to species from different regions of the world. Smith and Merriner (1978) provided key to 11 species of rays belonging to seven genera (*Torpedo*, *Dasyatis* Rafinesque, 1810, *Gymnura* van Hasselt, 1823, *Myliobatis* Cuvier, 1816, *Aetobatis* Blainville, 1816, *Rhinoptera* Cuvier, 1829 and *Manta* Bancroft, 1828) from Chesapeake Bay, Virginia. From world waters, Wallace (1967c), Compagno (1995) and Sommer et al. (1996) reported *Torpedo fuscomaculata* and *T. Sinuspersici* from South Africa and Somalia respectively, Bianchi (1985) reported *T. Fuscomaculata*, *T. Panthera* and *T. Sinuspersici* from Pakistan, Kwang-sao et al. (2008) reported *T. Formosa* Haas & Ebert, 2006 and *T. Tokionis* (Tanaka, 1908) from northern south China Sea, Ebert et al. (2013c) reported *Tetronarceformosa* (Haas & Ebert, 2006) and *T. Tokionis* (Tanaka, 1908) from Taiwan. Psomadakis et al. (2015), Randall (1995), Carpenter et al. (1997), Field

and Field (1998) and Bonfil and Abdallah (2004) reported *T. Panthera* and *T. Sinuspersici* from Pakistan, Gulf of Oman, Persian Gulf, Red Sea and Gulf of Aden respectively. A perusal of literature reveals that Playfair and Günther (1866), Fraser-Brunner (1949), Smith and Smith (1963), Fourmanoir (1963), Carcasson (1977), and Compagno et al. (1989) reported the occurrence of *Torpedo fuscomaculata* from Zanzibar, Mauritius, Seychelles, Madagascar, western Indian Ocean and South Africa respectively. Günther (1870), von Bonde and Swart (1923), Smith (1961) from western Indian Ocean, Herre (1953), Smith (1965a) and Qureshi (1972) reported *T. Marmorata* from Philippines, South Africa and Arabian Sea respectively. Fowler (1934) from South Africa, Rüppell (1835), Klunzinger (1871), Bamber (1915), Fowler (1956), Gohar and Mazhar (1964), Dor (1984), Baranes and Golani (1993) from Red Sea, Blegvad (1944), Kuronuma and Abe (1986) from Persian Gulf reported the occurrence of *Torpedo panthera*. Fowler (1941), van der Elst (1993), Compagno and Randall (1985), Debelius (1993, 1999), Kuitert and Debelius (1997) reported the occurrence of *T. Sinuspersici* from Philippine Islands, South Africa, Red Sea, Gulf of Oman, Sri Lanka and Andaman Sea respectively. However, Carvalho et al. (2002b) stated that some specimens of *T. Fuscomaculata* reported by Fourmanoir (1963), Smith and Smith (1963), *T. Marmorata* reported by Günther (1870), Qureshi (1972), von Bonde and Swart (1923) and Smith (1961), *T. Panthera* reported by Fowler (1934), Fowler (1956), Blegvad (1944), Kuronuma and Abe (1986) may be misidentifications of/ may include *T. Sinuspersici*. In this family, 24 species were given in Fishbase (Froese and Pauly, 2019). Detailed descriptions on various species of the family Torpedinidae were given by Garman (1913) on 10 species of *Torpedo*, Fowler (1941) on *T. Fairchildi* Hutton, 1872, *T. Marmorata*, *T. Nobiliana* Bonaparte, 1835, *T. Sinuspersici*, *T. Tokionis*, Setna and Sarangdhar (1949a) on *T. Zugmayeri* from India, Smith (1961) on *T. Marmorata* and *T. Nobiliana* from South Africa, Carcasson (1977) on *T. Fuscomaculata*, Smith and Heemstra (1986) on *T. Fuscomaculata*, *T. Nobiliana* and *T. Sinuspersici*, Moron et al. (1998) on *T. Marmorata*, Barman et al. (2004) on *T. Panthera*, Last and Stevens (2009) on *T. Macneilli* and *T. Tokionis* from Australia, Ebert and Stehmann (2013) on *T. Marmorata*, *T. Nobiliana* and *T. torpedo* and Ebert (2014, 2015) on *Tetronarcetokionis* and *T. Cowleyi* respectively. Some new records of the species of family Torpedinidae were *Torpedo macneilli* by Duhamel (1989), *T. Adenensis* by

Carvalho et al. (2002b), T. Andersoni Bullis, 1962 by Navia et al. (2006a), T. Puelcha Lahille, 1926 by Belleggia et al. (2008), T. Californica (Ayres, 1855) by Escobar-Sánchez et al.

## **CHAPTER -3**

### **3.1 . AIM**

- To identify different species of electric rays (Torpediniformes) in Kovalam Coast.

### **2. . OBJECTIVE**

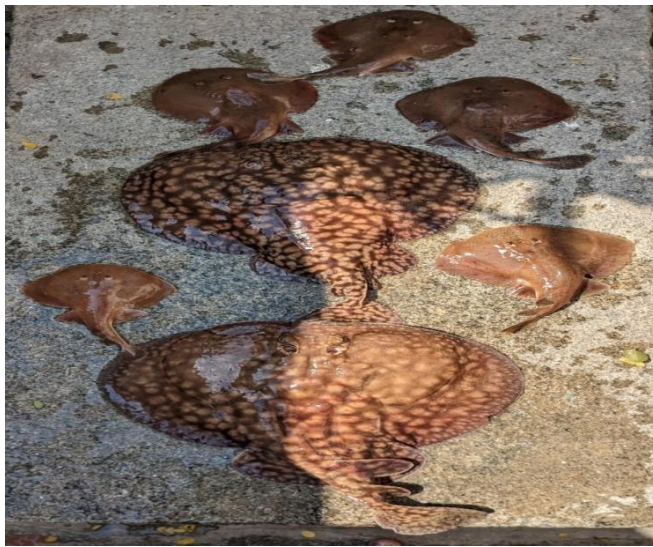
- To collect ray samples and measure their lengths and weight, total length (TL) , total weight (TW), disc length (DL), disc width (DW) , and determine their sex.
- To create awareness campaign among fishermen about the available species and endangered species of ray fishes ( Torpediniformes).

## CHAPTER – 4

### MATERIALS AND METHODS

#### 4.1. sample collection

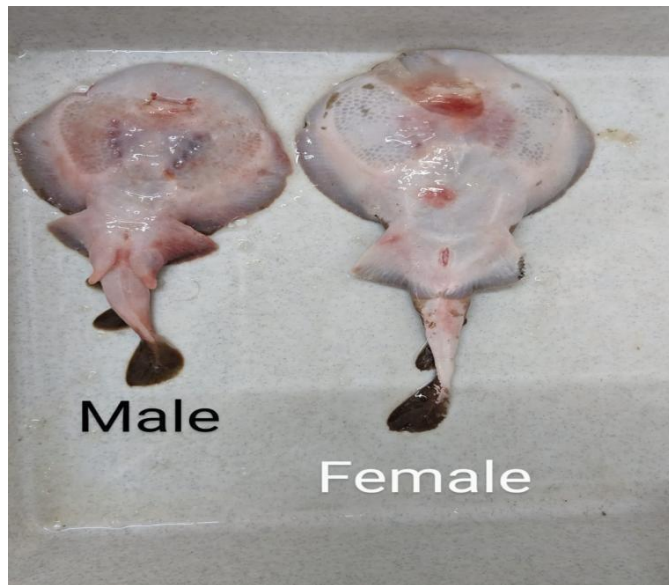
Torpediniformes( electric rays) fish samples are collected from kovalam coast  
varying different sizes and different sex, different length and weight.



**Fig 4.1.1 Torpedo**



**Fig 4.2.1 Narcine**



**Fig 4.2.2 Male and female characteristics of torpediniformes**

#### **4.2 Data's of the species from collected samples.**

We have collected 9 different samples of electric rays ( Torpediniformes ) varying different weights and lengths.

**Table 4.2.1 comparison data of 7 species of genus *narcine* and 2 species of genus *torpedo*.**

| <b>S.N<br/>o</b> | <b>species</b>        | <b>TL</b> | <b>DW</b> | <b>DL</b> | <b>EOL</b> | <b>EOW</b> | <b>SEX(M\F)</b> | <b>WEIGHT(g)</b> |
|------------------|-----------------------|-----------|-----------|-----------|------------|------------|-----------------|------------------|
| 1.               | Torpedo.<br>marmorata | 33cm      | 29cm      | 18cm      | —          | —          | F               | 792              |
| 2.               | Narcine .timeli       | 19cm      | 11.5cm    | 9.3cm     | —          | —          | M               | 108g             |
| 3.               | Narcine. timeli       | 19.7cm    | 10cm      | 10cm      |            |            | F               | 121g             |
| 4.               | Narcine. timeli       | 17cm      | 9.5cm     | 7.6cm     |            |            | M               | 75g              |
| 5.               | Narcine. Timeli       | 17.5cm    | 10.5cm    | 8cm       |            |            | M               | 83g              |
| 6.               | Narcine. timeli       | 17.9cm    | 9cm       | 8.5cm     |            |            | F               | 84g              |
| 7.               | Narcine. timeli       | 16.7cm    | 8.8cm     | 7.5cm     |            |            | F               | 74g              |
| 8.               | Narcine. timeli       | 18.3cm    | 10.3cm    | 8.3cm     |            |            | M               | 92g              |
| 9.               | Torpedo.<br>marmorata | 34.3cm    | 30cm      | 20cm      |            |            | F               | 830g             |

### 4.3 extraction of DNA using modern phenol - chloroform (PC) method

A modified phenol -chloroform method is a common laboratory technique used for DNA extraction and purification. Its is a modification of the original phenol - chloroform extraction method which is widely used protocol for isolating DNA from biological samples. The modified method has been optimized to reduce the amount of organic solvents used and to improve the efficiency and yield of DNA extraction.

#### **Procedure .**

- ★ Take minced fish muscle sample of electric ray , weighing (100-120gm).
- ★ After mincing it add 500ul of STE buffer .
- ★ Components to make STE buffer

Take 50mM of Tris - HCL ,ph 8.0: 10mM EDTA , ph 8.0 , 0.1M NaCL

Add 15ul , 20% (M\V) SDS and 30ul of 10mg ml<sup>-1</sup> proteniase K (if needed ).

- ★ After adding 500ul of STE buffer, mince the fish sample again .
- ★ Transfer the fish sample into 2ml of sterile microcentrifuge tubes.
- ★ Following with adding in micro centrifuge tube , samples were mixed briefly using a vortex.
- ★ After vortexing , samples were incubated at 50°C for 1hr.
- ★ After incubation centrifuge at 10,000g for 5min.
- ★ After centrifuging DNA was purified using successive extractions with 250ul of pure phenol , phenol : chloroform : isoamyl alcohol in the ratio of ( 25:24:1) and chloroform: isoamyl alcohol ( 24:1) respectively.
- ★ After this process DNA was precipitated with 750ul ice cold absolute ethanol.
- ★ Following centrifugation at 10,000g for 10min .
- ★ The DNA pellet is washed with 70% ethanol.
- ★ Then it is air dried and resuspended in 100ul of 10T0.1E buffer .

- ★ Components for making 10T0.1E buffer is  
(10mM Tris- HCL , pH 8.0, 0.1mM EDTA, pH 8.0)

This method is used for remaining samples to extract DNA and isolate it. Remaining 8 different samples were stored and kept in micro centrifugal tubes.

#### **4.4 PCR amplification**

PCR amplification (Polymerase Chain Reaction) is a laboratory technique used to amplify or copy a specific segment of DNA. The technique involves a series of heating and cooling cycles that are used to denature, anneal, and extend DNA strands.

The basic steps of PCR amplification are as follows:

**Denaturation:** The double-stranded DNA template is heated to a high temperature (usually around 95°C) to break the hydrogen bonds between the two strands and separate them into single strands.

**Annealing:** The temperature is lowered to allow short, synthetic DNA primers to bind specifically to the complementary ends of the target sequence. These primers define the boundaries of the target DNA segment to be amplified.

**Extension:** The temperature is raised to around 72°C, and a heat-resistant DNA polymerase enzyme extends the primers by adding nucleotides to the 3' end of each primer. This polymerization reaction generates a new double-stranded DNA molecule, complementary to the template DNA between the primers.

Repeat cycles: These three steps (denaturation, annealing, and extension) are repeated multiple times to exponentially increase the amount of the target DNA segment.

restriction enzyme digestion. PCR amplification has a wide range of applications in research, medicine, forensics, and biotechnology, such as genetic testing, diagnosis of diseases, and production of recombinant proteins.

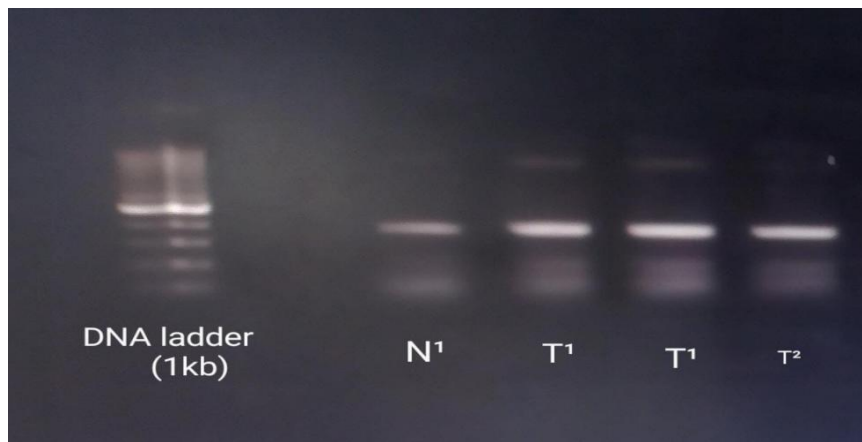
## **4.5 DNA sequencing**

DNA sequencing on torpediniformes

Torpediniformes is an order of electric rays, which are cartilaginous fish known for their ability to produce strong electrical fields. DNA sequencing can be used to study the genetics of torpediniformes species and their evolutionary relationships.

One commonly used method for DNA sequencing is called Sanger sequencing, which involves amplifying a specific region of DNA using PCR and then using a DNA polymerase to generate a series of fragments with varying lengths, each terminating with a labeled dideoxynucleotide that stops DNA synthesis. The fragments are separated by size using gel electrophoresis and the sequence can be determined by analyzing the pattern of labeled fragments.

Another sequencing technique that has become more commonly used in recent years is Next-Generation Sequencing (NGS), which allows for high-throughput sequencing of DNA. NGS involves the generation of millions of sequence reads in parallel, and these reads are then assembled into a complete genome sequence using bioinformatics tools.



**Fig 4.2.3 loaded Gel sample of Narcinetimeli and Torpedo marmorata**

- ★ **N1 = Narcinetimeli**
- ★ **T1 = Torpedo marmorata**
- ★ **T2 = Torpedo marmorata**
- ★ **T3 = Torpedo marmorata**

## CHAPTER 5

### Results and survey reports

## Narcinetimlei, complete genome

### ORIGIN

1 tatatttagagtgtaggggtaggaggcactgagcactaattatccataattttacttta  
61 ttacggatttaaaataatcctttacccatacaaaaatttttaacacttctcattaatta  
121 aaccatttaaacctgaaaaccaaccataaaaagtacctaacacatatttaacataacct  
181 cattcacccaacactcattaccaattaaaaattttaacgtactattaccttatcaaaaat  
241 tattaaatttacacttattattaactatcttaaacacaccaattaacaaccaacctca  
301 aaaagaaaagacttgaacttctatctcgactcccaaagccaaaattttaattaaactac  
361 tctttgaaatatacaccactaaaaaggcacataacaactagtgtggtcaccaacatacgtg  
421 ccaacaacaaccaactccgccacatcagtacaatcaaatactcaccccatgctcctaca  
481 gtccttaactaaaacattaaccattcaccaacctacaaaataacacaaaaaacccatag  
541 tatgccaaatcaatttctatcacgtagtgttttacacaacaatagcaatttcagtgtgag  
601 aagcaccacgccaatagtagttatataaacacactatgtataatcagcattaacagac  
661 attcccatattcattacatccctatgtataataccattaatagacattcctcatcttc  
721 attacacttaagtttaaatcctaacaaatctaatttccaaaatttcattacataattcc  
781 ataaaactacctcattcattaattaatcggtattccattccatattattctaattaata  
841 atttaacagtattatccaaccattatgtgggttggtgagaaatcagcaatgacctattct  
901 atccacctttagcaggtttagtcacagattaagattaatcccttataattgcttaaatg  
961 ccggcatttggcaaccttgaatacacatcaggttctgctcgatcacgaatttattacccc  
1021 tagttaccattattgacactttactcttacctgatgtctgtcccacgacagacggtaaga  
1081 gtactcttaattgtctcaagatttattccctcccagtttttcttcttggtatgctgag  
1141 caatcgctagccgggtcgaaggcccggtcagggtcattatcttagatttgcgttatcctcg  
1201 acatatcatatattaatccattactaattcattcatgaattatgattgtcaagtaaactt  
1261 tagtggttaggcttcctttaatattgttatcatcattgacaagcgcaacgtaggttagtt  
1321 gtcagctgaataattcactactttcactaattaattgtgaaacgagtttcacaatgaaa  
1381 tagtgaatgacaagaagatattaggtagtggaaatatactcataataacagggtattaatt  
1441 atcgtctaattgtaaattcggtatcaaatcttaggaacatttaaagatttattggacatta  
1501 ttattatatatatgccccctcttcgaaaacgaacgaaaaaaaaaaaaaacacttttaca  
1561 atttttcggaaaaacccccctccccctgcacttcgagttattctcgaaaaacccta

1621 aaacgaggactggaagtgcattttccgggcatgcaaaagcaatcaaaaacgccatttt  
1681 attgtgacatgccattttgacacatcatactgatatcaaccaaattttgggcaaaaaa  
1741 agtgaaaaatttcatttttttttttttaaaaaattttaaaatttttcattt  
1801 tttttaacaaaaaaataggaacaaaatccatatttttgaaaatgttttaaaatgatt  
1861 ttaaaaataaaaactttaactaatcttacctcaaaaacaagagcgaatattatatata  
1921 accacaaaaccaagataataagtataatggaataataaacttatattattatcttaac  
1981 tagaatggaatatacacataacaaaattaatacactaaaaacggaaatgaggtacacacg  
2041 tataatacaataaaccagcacatacactgccatacacaaatatacatgtattaaacccta  
2101 tcatataatgtttaaactcatatccttagttattaactcccatcgccatatataggacta  
2161 aatcagtacaccatgtatctctaaaaacttaaaaaataagagttacataatcattattaa  
2221 accacaatatacattatcct

# Torpedo marmorata,mRNA genome

```
1 attttttattttgagttttcaataattaaagaagtgtgcttaaggcattgac
   61 ttcgttttccaggatgccgtttcgtactagggaacacatcattatagataagaaactg
  121 accttggtactccgatatagctttccacgactaaataatataagttttgattatt
  181 accaccatcatttcttctttacttgcctcagctggagtagaagctggagcaggcacagg
  241 atgaacagtttaccaccccttgcaggcaaccttgcacatgctggagcatcagttgacct
  301 aactatcttctcacttcatctagctggcctcttctattctagcctccattaattttat
```

## **5.1 Results of collected samples from DNA sequencing .**

As we got DNA sequencing of collected samples of Narcine .Timeliand Torpedo . marmorata , we found that in bay of bengal , kovalam , tamilnadu the common species of torpediniformes are TORPEDO . MARMORATA AND NARCINE TIMELI are the species which are going to be endangered because of overfishing and over exploitation . as per the report from IUCN it is stated that research to address this issue will govern the degree of threat faced by this group is that all data deficient and least concerned species should be the immediate focus of more data - gathering .

The present study resolves taxonomic confusions in identification of closely related species Narcineprodorsalis, N. maculata and N. lingula. Narcinetimlei was originally described as Raja timlei from kovalam, tamilnadu south India by Henle (1834).

Multivariate analysis has been carried out to distinguish the three closely related species N. indica, N. maculata and N. timlei. This species is valued as an aquarium fish. It occurs over soft bottoms and feeds on bottom-living organisms.

## 5.2 Survey reports

We took survey and reports from several fisherman groups and conducted awareness campaign regarding on saving electric rays . This survey and awareness campaign was conducted at kovalam coast on 5 /03/ 2023 .

They stated that this species commonly conduct high voltage of electricity in order to attack prey and for defense purpose . they also stated that it contains 8 to 220 volts depending on their size , due to this conductivity of electricity most of the fishermens have been dead and some of them got paralyzed due to high voltage . in order to get rid of those attacks they used to throw it away ashore and usually waste them . they also additionally added a statement that this fishes are not usually eaten because of their electric organ which conducts poison and electric shocks , they are wasted and thrown away . as we took their statement for awareness purpose we did an awareness campaign which states not to overfish on the particular region where electric rays located . they are actually taken as incidental by - catch when fishermen target other species like tuna , sting rays and swordfish but accidentally end up with electric rays in their nets . because electric rays have little commercial value they are usually discarded dead by the fishers . however recently targeted catch has also increased .

In other hands because of climate change and habitat loss this electric rays are getting endangered due to warmer waters are affecting shark and ray behaviour , causing them to migrate to different areas . Important habitats like coral reefs which also act as nursery grounds for electric rays are also negatively impacted by climate change due to ocean acidification and severe weather events. Human activities contribute to the significant destruction of coastal habitats by trawling fishing nets over the sea bottoms and reef systems which are important nursery and feeding grounds for electric ray species. Rays losing areas that are central to their life cycle has severe consequences for ray populations around the world.



**Fig 5.1 awareness campaign conducted at kovalam coast on 05/03/2023**

## SUMMARY AND CONCLUSION

Knowledge of the relationship between length and weight of a fish species in a given geographic region is useful for at least three reasons: a) for the estimation of weight-at-age from total reported catch weight and length- frequency distributions; b) the weight-length relationship is a practical index of the condition of fish; c) for between – regions life history compositions. Fisheries management and research often require the use of biometric relationships in order to transform data collected in the field into appropriate indices. Studies on length frequency distribution, Length-Weight Relationship (LWR) have important implications for fisheries science and population dynamics. In fishery biology, these estimates can be used in studying gonad development, rate of feeding, metamorphosis, maturity and condition to compare inter specific and intra -population morphometry and to determine the index of well being of individual fish. Torpedinoid electric rays are captured, albeit in small numbers, by the trawlers operating along the inner half of the continental shelf off kovalamcoast ,tamilnadu , They represent a complex group of restricted endemics susceptible to capture in gillnets, shore seines and trawl fisheries, which are generally discarded bycatch. Most of these catches are unintentional and their habitat is being destructed heavily. As most of the by-catch is not monitored regularly, a strict plan towards monitoring on the development of sustainable management practices and conservation strategies for these torpedinoid electric rays is required in order to protect them from extinction. Hence studies on length frequency distribution and length-weight relationship are of great importance in the development of management plans. The present study which includes detailed studies on several aspects of reproductive biology such as maturity stages, description of male , female genital organs, length at first maturity, sex ratio, fecundity, size at birth, gestation period, helps us to improve our knowledge on size at first sexual maturity of both males and females, to delineate the reproductive period, assess both ovarian, uterine fecundities, thus providing information to prepare a monitoring plan for electric ray species in Indian waters. To the best of our knowledge this is the first study to report biology of these torpedinoid electric rays from world waters.

## References

- Carvalho, M. R. de., M. E. McCord, M. Manjaji, B. Samiengo, C. Vidthayanon, B. Fahmi and E. Capuli. 2009. Narcinetimeli. The IUCN Red List of Threatened Species 2009: e.T161560A5451485. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161560A5451485.en>. (Last accessed on 22 May 2018).
- Carvalho, M. R. de., M. E. McCord, M. Manjaji, B. Samiengo, C. Vidthayanon, B. Fahmi and E. Capuli. 2009. Narcinetimlei. The IUCN Red List of Threatened Species 2009: e.T161445A5425834. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161445A5425834.en>. (Last accessed on 22 May 2018).
- Carvalho, M. R. de. and M. E. McCord. 2009. Narcineatzi. The IUCN Red List of Threatened Species 2009: e.T161660A5474871. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161660A5474871.en>. (Last accessed 20 May 2018).
- Eschmeyer, W. N. 2017. Catalog of fishes Electronic version. [http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcat\\_main.asp](http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcat_main.asp). (Last accessed on 22 May 2018).
- Froese, R. and D. Pauly. 2019. Editors. Fishbase. World Wide Web electronic publication. [www.fishbase.org](http://www.fishbase.org). (Last accessed 31 March 2019).
- Ishihara, H. and Y. Wang. 2009. Narkedipterygia. The IUCN Red List of Threatened Species 2009: e.T161607A5463202. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161607A5463202.en> (Last accessed on 22 May 2018).
- IUCN 2013. International Union for the Conservation of Nature, Species Survival Commission. IUCN 2017. The IUCN Red List of Threatened Species. Version 2017-3. <http://www.iucnredlist.org>. (Downloaded on 25 May 2018).
- Notarbartolo di Sciara, G., F. Serena, N. Ungaro, F. Ferretti, S. Pheeha and B. Human. 2009. Torpedo marmorata. The IUCN Red List of Threatened Species 2009: e.T161328A5398909. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161328A5398909.en>. (Last accessed on 22 May 2018).
- Shark Trust. 2012. An Illustrated Compendium of Sharks, Skates, Rays and Chimaera. Chapter 1: The British Isles. Part 1: Skates and Rays.

Smale, M. J. 2006. *Torpedo sinuspersici*. The IUCN Red List of Threatened Species 2006: e.T60136A12311480. <http://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T60136A12311480.en>. (Last accessed on 22 May 2018). Wang, Y., C. Vidthayanon and B. Samiengo. 2009. *Narcineprodorsalis*. The IUCN Red List of Threatened Species 2009: e.T161545A 5448211. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161545A> 5448211. en. (Last accessed on 22 May 2018). [1:25 pm, 02/05/2023] +91 96888 76762: Vélez-zuazo, X. and I. Agnarsson. 2011. Shark tales: A molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). *Mol. Phylogenet. Evol.*, 58(2): 207–217. Doi: 10.1016/j.ympev.2010.11.018. Venkataraman, K., C. Raghunathan, C.R. Sreeraj and R. Raghuraman. 2012. Guide to the Dangerous and Venomous Marine Animals of India, (Published by the Director, Zool. Surv. India, Kolkata), 1-98. Vianna, G. M. S. and C. M. Vooren. 2009. Distribution and abundance of the lesser electric ray *Narcine brasiliensis* (Olfers, 1831) (Elasmobranchii: Narcinidae) in southern Brazil in relation to environmental factors. *Braz. J. Oceanogr.*, 57(2): 105-112. Vianna, M., F. E. S. Costa and C. N. Ferreira. 2004. Length-weight relationship of fish caught as by-catch by shrimp fishery in the southeastern coast of Brazil. *Bol. Inst. Pesca.*, 30(1): 81-85. Vidthayanon, C., J. Karnasuta and J. Nabhitabhata. 1997. Diversity of freshwater fishes in Thailand. Office of Environmental Policy and Planning, Bangkok. 102 pp. Villavicencio-Garayzar, C. J. 2000. Taxonomía, abundancia estacional, edad y crecimiento y biología reproductiva de *Narcine entemedor* Jordan y Starks (Chondrichthyes: narcinidae), en Bahía Almejas (in Spanish), B. C. S., Doctoral dissertation. Universidad Autónoma de Nuevo León, Mexico. von Bonde, C. and D. B. Swart. 1923. The Platosomia (skates and rays) collected by the S.S. "Pickle". Reports of the Fisheries and Marine Biological Survey of the Union of South Africa, 3(5): 1-22. Wafar, M., K. Venkataraman, B. Ingole, S. Ajmal Khan and P. LokaBharathi. 2011. State of Knowledge of Coastal and Marine Biodiversity of Indian Ocean Countries. *Plos ONE*, 6(1): e1463. Waghray, S. 1985. Olfactory organ and its sexual dimorphism in the electric ray, *Narcine timlei* (Day). *Indian J. Fish.*, 32(1): 148-151. Walker, T. I. 2005a. Management measures. In: Musick, J. A. and R. Bonfil. (Eds.), *Management Techniques for Elasmobranch Fisheries*. FAO Fish. Tech. Pap. 474, Rome, Italia,

216-242. Walker, T. I. 2005b. Reproduction in fisheries science. In: Hamlett, W. C. (Ed.), Reproductive Biology and phylogeny of chondrichthyes: sharks, rays and chimaeras. CRC Press, Boca Raton, Florida, 45-79. Wallace, J. H. 1967c. The batoid fishes of the east coast of southern Africa. Part III. Skates and electric rays. Investigational Report, Oceanographic Research Institute Durban, 17: 1-62.[1:25 pm, 02/05/2023] +91 96888 76762: Waltrick, D., S. M. Simpfendorfer and C. A. Awruch. 2014. Endocrine control of embryonic diapause in the Australian shrapnose shark *Rhizoprionodon taylori*. PLoS One., 9(7): e101234. Ward, R. D., B. H. Holmes, W. T. White and P. Last. 2008. DNA barcoding Australian chondrichthyans: results and potential uses in conservation. Mar. Freshwat. Res., 59(1): 57–71. Ward, R. D., R. Hanner and P. D. Herbert. 2009. The campaign to DNA barcode all fishes, FISH-BOL. J. Fish Biol., 74(2): 329-356. Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last and P. D. Hebert. 2005. DNA barcoding Australia's fish species. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 360(1462): 1847-1857. Weigmann, S. 2011. Contribution to the taxonomy and distribution of eight ray species (Chondrichthyes, Batoidea) from coastal waters of Thailand. Proceedings of the Society of Natural Sciences in Hamburg, 46: 249-312. Weigmann, S. 2016. Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. J. Fish. Biol., 88(3): 837-1037. White, W. T. and P. R. Last. 2012. A review of the taxonomy of Chondrichthyan fishes: a modern perspective. J. Fish. Biol., 80(5): 901-917. Doi: 10.1111/j.1095-8649.2011.03192.x.

Whitehead, P. J. P., M. L. Bauchot, J. Hureau and E. Tortonese. 1984. Fishes of the Northeastern Atlantic and the Mediterranean, Vol. I. UNESCO, Paris. 1-150.

Whitley, Gilbert. P. 1940. The fishes of Australia. Part I. The shark rays, devil-fish and other primitive fishes of Australia and New Zealand. Sydney, Royal Zoological Society of New South Wales, 170-178. Wood-Mason, J. and A. Alcock. 1891. Natural history notes from H. M. Indian Marine Survey Steamer 'Investigator', Commander R. F. Hoskyn, R. N., commanding. No. 21. Note on the results of the last season's deep-sea dredging. Ann. Mag. Nat. His., (6)7: 186-202, 258- 272. Wood-Mason, J. and A. Alcock. 1892. Illustrations of the zoology of the Royal Indian Marine Surveying Steamer Investigator, under the command of Commander

A. Carpenter RN, DSO and Commander RF Hoskyn, RN Crustacea, Part 1, 1-5.

Wootton, R. J. 1990. Ecology of teleost fishes. Fish. Fish Series 1. Chapman and Hall, London, 404 pp.

Worm, B., L. Ketteimer, C. A. Ward-Paige, D. Chapman, M. R. Heithaus, S. T. Kessel and S. H. Gruber. 2013. Global catches, exploitation rates, and rebuilding option for sharks. Mar. Policy., 40: 194-204.[1:25 pm, 02/05/2023] +91 96888 76762: Wourms, J. P. 1977. Reproduction and development in Chondrichthyan fishes. Amer. Zool., 17(2): 379-410.

Wright, J. M. 1988. Seasonal and special differences in the fish assemblage of the non-estuarine Sulaibikhat Bay, Kuwait. Mar. Biol., 100(1): 13-20.

Yamada, U., M. Tokimura, H. Horikawa and T. Nakabo. 2007. Fishes of the East China Sea and Yellow Sea. Tokai University Press, Kanagawa, 864-875.

Yamaguchi, A., I. Kawahara and S. Ito. 2005. Occurrence, growth and food of long headed eagle ray, *Aetobatus flagellum*, in Ariake Sound, Kyushu, Japan. Environ. Biol. Fishes., 74(2): 229-238.

Yano, K. and S. Tanaka. 1988. Size at maturity, reproduction cycle, fecundity and depth segregation of the deep sea squaloid shark *Centriscymnus owstoni* and *C. coelalepis*. Nippon Suisan Gakk., 54(2): 167-174.

Yano, K., A. Ahmad, A. C. Gampang, A. H. Idris, A. R. Solahuddin and Z. Aznan. 2005. Sharks and Rays of Malaysia and Brunei Darussalam, SEAFDEC-MFRDMD/SP/12., Kuala Terengganu, 557 pp.

Yigin, C. C. and A. Ismen. 2009. Length-weight relationships for seven rays from Saros Bay, North Aegean Sea. J. Appl. Ichthyol., 25(1): 106-108. Doi: 10.1111/j.1439-0426.2008.01161.x.

Zar, J. H. 1984. Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall, 718 pp.

Electronic references: Barnes, M. K. S. 2008. *Torpedo marmorata*. Marbled electric ray. In: Tyler-Walters, H. and K. Hiscock, (Eds.), Marine Life Information Network: Biology and Sensitivity Key Information Reviews (on-line). Plymouth: Marine Biological Association of the United Kingdom.

<https://www.marlin.ac.uk/species/detail/75.Carvalho>, M. R. de. and M. E. McCord. 2006. *Torpedo panthera*. The IUCN Red List of Threatened Species 2006: e.T60134A12311224. <http://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T60134A12311224.en>. (Last accessed on 22 May 2018).

Carvalho, M. R. de. and M. E. McCord. 2009. *Heteronarce garmani*. The IUCN Red List of Threatened Species 2009: e.T161746A5494167. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161746A5494167.en>. (Last

accessed on 22 May 2018).Carvalho, M. R. de., M. E. McCord, C. Vidthayanon, Fahmi, B. Samiengo, E. Capuli and M. Manjaji. 2009. *Narcine lingula*. The IUCN Red List of Threatened Species 2009: e.T161523A5443013. <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161523A5443013.en>. (Last accessedon 22 May 2018).