Report on the landing of Sharpnose guitarfish (*Glaucostegus granulatus*) and Widenose guitarfish (*Glaucostegus obtusus*) at North Goa with some insights into its reproductive and feeding biology

A Dissertation for

Course code and Course Title:ZOO-651 Dissertation

Credits: 16

Submitted in partial fulfilment of Masters Degree in Zoology

by

TANAYA SANJEEV NAIK CHOPDENKAR

Seat Number: 22P0440034

ABC ID: 781260324798

PRN: 201902944

Under the Supervision of

MS. GANDHITA V. KUNDAIKAR

School of Biological Sciences and Biotechnology

Zoology Discipline

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled **"Report on the landing of Sharpnose guitarfish (*Glaucostegus granulatus*) and Widenose guitarfish (*Glaucostegus obtusus*) at North Goa with some insights into its reproductive and feeding biology"** is based on the results of investigations carried out by me in the Zoology Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of **Ms. Gandhita Kundaikar** and the same has not been submitted elsewhere for the award of a degreeor diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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Tchopdenkar

Tanaya Naik Chopdenkar

Seat no: 22P0440034

Date: 8-4-24

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the internship report **"Report on the landing of sharpnose guitarfish** (***Glaucostegus granulatus***) **and widenose guitarfish** (***Glaucostegus obtusus***) **at North Goa with some insights into its reproductive and feeding biology "** is a bonafide work carried out by Ms Tanaya Sanjeev Naik Chopdenkar under my supervision in partial fulfilment of the requirements for the award of the degree of **M.Sc. in the Zoology** Discipline at the School of Biological Sciences and Biotechnology, Goa University.

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08/04/2024

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CONTENTS

PREFACE

This thesis is submitted for the fulfilment of the requirement for the degree of Masters in Zoology and comprises research work carried out by the author under the supervision of Miss. Gandhita Kundaikar, Assistant Professor, Zoology Discipline, School of Biological Sciences and Biotechnology, Goa University from 2023-2024.

Bycatch poses a significant threat to marine megafauna, such as elasmobranchs, with India ranks among the top three nations globally in elasmobranch landings stemming from targeted catch and bycatch, Dent and Clarke (2015).In the marine environment, elasmobranchs serve as a top predatory functional group with batoid fishes, constituting ecologically and morphologically varied clades, serving in inshore and nearshore seas as meso-predators. The ecological significance of benthic elasmobranchs is less well recognized than that of their pelagic counterparts, which massive apex predators typify, Vaudo and Heithaus (2009). As predator species at intermediate levels, batoids are expected to be crucial to the dynamics of marine ecosystems since they are an essential part of the community structure, Bizzarro et al.(2007) and Heithaus et al. (2008) and a major link in the food webs of demersal marine communities (Belleggia et al., 2008).

Giant guitarfishes, belonging to the monotypic family Glaucostegidae, encompass seven species and predominantly inhibit in shallow coastal and inshore regions of the eastern Atlantic and Indo-Pacific Oceans, Last et al.(2016). They play a significant role in the trophic dynamics of soft-sediment benthic ecosystems. Following sawfishes, guitarfishes have been recognized as the most susceptible group (Moore, 2017).

The Sharpnose guitarfish (*Glucostegus granulatus*) and Widenose guitarfish (*Glucostegus obtusus*) are benthic and coastal elasmobranchs facing significant pressures from coastal fisheries. However, their biology remains poorly understood, hindering effective management. This study aims to address this knowledge gap by providing essential data for the conservation of guitarfish species.

The introductory chapter provides an overview of guitarfish, encompassing its ecological relevance, the study's significance, its objectives, and the research hypothesis. The subsequent chapter reviews existing literature and identifies gaps in knowledge. Chapter 3 delineates the methodology employed during the study. Chapter 4 entails the data analysis and draws the conclusion from the findings.

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TABLES

FIGURES

ABBREVIATIONS USED

ENTITY	Abbreviation
Total Length	TL
Clasper Length	CL
Body Weight	BW
Gonadosomatic Index	GSI
Stomach Content Analysis	SCA
Index of Relative Importance	IRI
Length Weight Relationship	LWR

ABSTRACT

Limited knowledge exists regarding the life history of the Sharpnose guitarfish (*G.granulatus*) and Widenose guitarfish (*G.obtusus*) captured by gillnetters and shore seine in the eastern Arabian Sea off Goa. These guitarfish species face a high risk of extinction globally due to their low resilience to increasing fishing pressure, habitat loss, climate change, and growing commercial value. The lack of comprehensive information has hindered practical conservation efforts. To address this gap, this study investigates the bio-ecological characteristics, including total length-weight relationships, stomach contents, and reproductive biology, of individuals collected from landing sites in Caranzalem and Nauxim village, Tiswadi taluka, North Goa. Over the study period from August 2023 to March 2024, 32 *G.obtusus* and 3 *G.granulatus* were recorded at Caranzalem, while 16 *G.granulatus* were reported at Nauxim from July 2023 to March 2024. Female *G.obtusus* ranged from 20.1 to 92 cm TL, males from 20 to 70.4 cm, female *G.granulatus* ranged from 22.1 to 71.2 cm TL, and males from 30.35 to 60.96 cm TL. Weight ranges varied, with *G.obtusus* females from 23.3 to 1975.5 g, males from 30.2 to 1151.4 g, and *G.granulatus* females from 31.181 to 545 g, and males from 80 to 661.8 g. The length-weight relationship (LWR) for *G.obtusus* females and males was expressed as $TW= 0.0048\ TL^{2.8173}$(r^2=0.8949); $TW=0.0085TL^{2.7164}$ (r^2=0.886) respectively. For *G.granulatus*, the LWR for females and males was represented by $TW= 0.0035\ TL^{2.9279}$ ($r^2 = 0.9417$); and $TW= 0.0023\ TL^{3.0294}$ ($r^2= 0.9502$), respectively. The present study recorded a significant positive correlation between total length and weight in *G.obtusus*, observed among females ($r = 0.8427$, $p < 0.05$) and males ($r = 0.8954$, $p < 0.05$), as well as in *G.granulatus*, for both females ($r = 0.9724$, $p < 0.05$) and males ($r = 0.9381$, $p < 0.05$).

The observed maturity stages in *G.obtusus* primarily comprised immature individuals. However, exceptions included one male classified as immature stage 2 (developing), six males categorized as mature stage 3a, and only one female identified as maternal stage 3b. Conversely, most individuals in *G.granulatus* were found to be immature, except for one individual identified as being in immature stage 2. Development of claspers and testes indicated that all *G.obtusus* (males) larger than 56.2 cm TL were mature and showed calcified claspers with sizes between 11 and 14.3 cm. Both the female species exhibit aplacental viviparity with functional ovaries. The mean GSI value for female and male *G.obtusus* peaked in March 2024. In contrast, for *G.granulatus*, the peak GSI value was observed in October 2023 for females and in February 2024 for males. Dietary analysis of stomach contents (%IRI) revealed that Crustacea and Teleostei were both species' most important prey items.The spiral intestines of four specimens of *G.obtusus* and five specimens of *G.granulatus* reported the presence of cestodes from December 2023 to March 2024. Such studies also provide a platform for essential discussion on the various ways in which effective monitoring strategies for the species could be implemented and managed.

Keywords: *Glaucostegus granulatus*, *Glaucostegus obtusus*, total-length weight relationship, sexual maturity, stomach contents, conservation

CHAPTER 1: INTRODUCTION

1.1 Background

Living fish are classified into two basic taxonomic groups: (i) Chondrichthyes (cartilaginous fish) and (ii)Osteichthyes (bony fish). Chondrichthyes are classified into two subclasses: (i) Elasmobranchii, which contains skates, rays, and sharks; and (ii) Holocephali, which includes chimaera, Ebert et al. (2013) and differs from Osteichthyes in possessing placoid scales and a skeleton comprised of cartilage with surface calcification rather than bone. Sharks and rays (Batoids) belong to Elasmobranchii (elasmo=plate, branchii=gills), collectively known as Elasmobranchs.

Elasmobranchs are among the oldest vertebrate groups, having lasted for almost 400 million years since the Devonian period. They are also one of the most successful groups to have ever existed on Earth. Elasmobranchs are characterized by life history characteristics such as slow growth, late maturity, low fecundity and productivity, long gestation period, high natural; survivorship, and long life span, Dulvy and Forrest (2009) as a result of which makes them highly vulnerable to overfishing and habitat degradation (Cortés, 2016).

Elasmobranchs have adopted varied life history strategies and inhabited a range of ecological niches within aquatic ecosystems, often playing the role of apex predators, Compagno (1990). However, as a result of severe overfishing, recent studies have documented significant decreases in shark and ray populations around the world, Stevens et al.(2000). Over 50% of elasmobranch species in the Arabian Sea region are classified as threatened primarily due to fishing pressures from both artisanal and industrial sectors, with bycatch being identified as the most significant threat, Jabado et al.(2017).Unfortunately, a lack of species-specific information, along with an increase in fishing activity in this region, raises concerns for fishery management, Jabado et al. (2017). Limited species-specific reporting, along with unregulated fishing, has resulted in the depletion of numerous chondrichthyan fish throughout the world's oceans, Dulvy and Reynolds (2002) and continues to impede the advancement of management strategies, Munk (2001). Detailed reports on bycatch mortality rates are necessary to improve management plans as an integral part of conservation.

Megabenthic predators occupy higher trophic levels within the marine benthic food web and exert significant influence on benthic populations, Ebert and Bizzarro (2007). Elasmobranchs fulfill a critical role as megabenthic predators in shallow, soft-bottom-shelf habitats (Flowers et al., 2021).

The ecological significance of benthic elasmobranchs is not as well recognized as that of their pelagic counterparts, which are mostly represented by huge apex predators,Vaudo and Heithaus (2009). Batoids are predicted to be important predator species in the dynamics of marine ecosystems at intermediate levels, Ritchie and Johnson (2009). They are an important part of the community structure, Bizzarro et al.(2007) and a significant link in food webs in demersal marine communities Belleggia et al. (2008). Therefore, quantitative research on their trophic ecology and diet may help to clarify their ecological importance in marine ecosystems and pave the way for the creation of ecosystem-based management strategies (Heithaus et al. 2008).

The rhino rays are ‘shark-like rays’ and refer to five families in the order Rhinopristiformes: the sawfishes (Pristidae),Wedgefishes (Rhinidae), giant guitarfishes (Glaucostegidae), guitarfishes (Rhinobatidae), and banjo rays (Trygonorrhinidae) (Jabado, 2019).

Giant guitarfishes are medium-sized to large rays that mainly live in the subtropical and tropical inshore continental and insular seas throughout the Indo-Pacific and eastern Atlantic, including the Mediterranean Sea, Jabado (2019). Guitarfish, *G.granulatus* is a medium-sized northern Indian Ocean chondrichthyan species and possibly has a limited distribution from the Persian Gulf to southern India. *G.obtusus* is a small-sized guitarfish distributed in the Northern Indian Ocean; from Pakistan to Thailand, with eastern limits unclear. The species was abundant on the Indian coast; however, currently caught occasionally and does not form a fishery.

Guitarfishes are significant components of coastal socio-ecological systems, serving as benthic mesopredators that are relatively large and occasionally abundant. They play a crucial role in the trophic dynamics of soft sediment ecosystems, Kyne and Bennett (2002) and possess high intrinsic value as a distinctive example of biodiversity, adaptation, and extensive evolutionary lineage (Wueringer et al., 2009).

In the July 2019 release of the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species, all giant guitarfish species were categorized as Critically Endangered. Additionally, all these species were listed under Appendix II of The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), reflecting a significant decline in population attributed to intense fishing pressure and the high demand for their valuable fins in the global market.

These benthic elasmobranch predators utilize estuarine bays during their early life stages, which function as efficient nursery habitats providing abundant resources and shelter, Swift and Portnoy (2021).Despite estuarine bays being highly dynamic playgrounds of intensive prey-predator interplay, Shaw et al. (2016); and Every et al. (2019). Data is scarce, particularly concerning the Indian shelf habitat, Hegde et al. (2014); Menon et al. (2020); Sreekanth et al. (2021).On the west coast of India, the Mandovi estuary and Zuari estuary are crucial habitats for artisanal fisheries.

The study aims to reduce the information gap by providing a principle baseline on the bio-ecological features of Sharpnose guitarfish and Widenose guitarfish from landing sites in the eastern Arabian Sea.

1.2 Importance of the study

The Sharpnose guitarfish (*Glucostegus granulatus*) and Widenose guitarfish (*Glucostegus obtusus*) are both benthic and coastal elasmobranchs. They are subject to substantial impacts from coastal fisheries, yet the limited knowledge of their biology presents challenges for effective management strategies. This study aims to tackle these challenges by presenting foundational data necessary for the conservation of guitarfish species. The objective is to facilitate their recovery to a level where they can adequately perform their functional role within the ecosystem.

1.3 Objectives of the study

To our best knowledge, no study has been conducted on the bycatch analysis of guitarfish from the landing sites at Nauxim and Caranzalem and also there is a lacuna of information with regards to the diet of Widenose guitarfish. Although several studies have investigated the reproductive biology of diverse guitarfish species, there is a noticeable absence of published literature dedicated specifically to the Sharpnose and Widenose guitarfish. Considering the above scenario, the present study was undertaken to achieve the following objectives:

1. To study bycatch analysis of *G.obtusus* and *G.granulatus* at the coastal sites of North Goa (Caranzalem and Nauxim).

2. To analyze the stomach contents of *G.obtusus* and *G.granulatus*.

3. To study the reproductive biology of *G.obtusus* and *G.granulatus*.

1.4 Hypothesis of this research work

The study on feeding and reproductive biology is expected to prove the hypothesis that Mandovi and Zuari estuaries may serve as nursery and feeding grounds for *G.granulatus* and *G.obtusus*.Since estuaries provide nurseries, Heupel et al. (2007) and foraging grounds, Bethea et al. (2004) for elasmobranchs

CHAPTER 2: LITERATURE REVIEW

In Indian waters, there is a paucity of comprehensive species-specific landing data. However, data from the East Coast indicates a significant 86% decline in landings of 'guitarfishes' (including wedgefishes of the family Rhinidae and giant guitarfishes) between 2002 and 2006 (Mohanraj et al., 2009).

The dietary analysis of shortnose guitarfish (*Zapteryx brevirostri*s) revealed a predominant consumption of crustaceans, specifically carideans and amphipods, along with polychaete annelids, and occasionally small fish, sipunculids, and cephalopods. Both male and female individuals exhibited similar dietary preferences; however, variations in the proportion of prey items were observed among juveniles, subadults, and adults. Discrepancies in prey ingestion were also noted across different times of the year, likely influenced by oceanographic conditions, although overall, the species primarily feeds on crustaceans and polychaetes (Marion et al., 2011).

Dietary variations in shortnose guitarfish (*Zapteryx brevirostris*) concerning size, maturity stage, season, and bottom type selection using generalized linear models was assesed. Amphipods (39.17%), polychaetes (22.33%), and lancelets (20.33%) were identified as the most important prey based on the index of relative importance, followed by decapods (8.93%), cumaceans (5.41%), and isopods (3.41%). *Z. brevirostris* primarily consumed polychaetes and amphipods during spring and summer, exhibited higher consumption of cumaceans in winter, consumed more lancelets in spring, and showed increased predation on decapods and isopods during summer. Larger *Z. brevirostris* individuals exhibited heightened consumption of decapods, polychaetes, and isopods, whereas their consumption of amphipods decreased.

Mature *Z. brevirostris* individuals consumed more lancelets compared to juveniles. Additionally, *Z. brevirostris* demonstrated a preference for sandy bottoms over other bottom types, suggesting that diet composition may be influenced by the species' preference for this habitat (Barbini et al., 2011).

Banded guitarfish (*Zapteryx exasperata*) primarily feeds on demersal teleost fishes, with a significant preference for *P. margaritatu*s. Despite showing a high diet overlap between sexes , juveniles and adults there is a decreasing emphasis on small crustaceans in its diet as the total length of *Z. exasperata* increases (Blanco-Parra et al., 2011).

Stomach content analysis of Blackchin guitarfish (*Glaucostegus cemiculus*) revealed that 69% were classified as full, comprising 32 females and 52 males. The quantity of prey items found in the stomachs ranged from one to four, although the majority contained one or two prey items. Crustaceans emerged as the predominant taxon group (IRI % 99.48), with the green crab, *Carcinus aestuarii* Nardo, 1847, identified as the dominant species, constituting 20.56% of the diet (Bengil et al., 2013).

Stomach content analysis of Banded guitarfish (*Zapteryx xyster*) revealed that shrimp (52.3% prey-specific index of relative importance, PSIRIi) and teleosts (27.2% PSIRIi) represented the primary prey categories for *Z. xyster*. Juveniles primarily consumed smaller shrimp species (*Solenocera spp.),* whereas adults displayed a preference for larger prey. The consumption of food relative to body mass increased significantly for both juvenile and adult *Z. xyster* between 0400 and 1200 hours, accompanied by a reduction in the proportion of individuals with empty stomachs during this timeframe. These findings contradict the hypothesis suggesting that *Z. xyster* is predominantly nocturnal in its feeding behavior. Additionally, the study highlighted that *Z. xyster*, especially juveniles, exhibit foraging behavior across multiple shrimp species (Espinoza et al., 2013).

The general diet of the Shovelnose guitarfish predominantly consists of shrimp-like organisms (Penaeidae and Caridea), comprising 58.61% of the Index of Relative Importance (IRI), followed by unidentified crustaceans (14.43% IRI), brachyuran crabs (11.89% IRI), fishes (9.88% IRI), stomatopods (3.38% IRI), and cephalopods (1.47% IRI). While the prey items of females and males are similar, their proportions vary, primarily due to differences in taxonomic resolution. Shrimp-like organisms are the most important prey for females, accounting for 65.74% IRI, whereas unidentified crustaceans are more significant for males, representing 52.96% IRI. Brachyuran crabs rank second in importance for females (14.47% IRI), while they are the second most important prey group for males (34.78% IRI). Fishes occupy the third position in the diet for both sexes. Loliginid squid is more important in the diet of females compared to males, comprising 4.29% IRI. Feeding differences related to size are evident, with a general trend of reduced crustacean consumption and increased intake of fishes and squid from size class I to size class III. Crustaceans are most prevalent in size class I and II, while brachyuran crabs and fishes dominate the diet in size class III (Quiñonez et al., 2018).

In the dietary composition of sharpnose guitarfish (*Glaucostegus granulatus*), teleosts made a substantial contribution, accounting for 46.9%, closely followed by crustaceans at 44.6%. The estimated trophic level for the species was 3.9, indicating the species as a secondary consumer. With respect to feeding habits, the species displayed a generalist feeding behavior and exhibited a broader niche, as evidenced by the values of Levin’s Index (BN = 0.4) and the Shannon–Wiener Index (H’= 3.2) (Sreekanth et al., 2022).

Reproductive analyses of Banded guitarfish (*Zapteryx exasperata*) stated that females exhibited two external-type ovaries, while males displayed well-developed testes. Immature females had undeveloped ovaries, while mature females exhibited oocytes ranging from 5 to 40 mm. The smallest mature female measured 66.5 cm TL, and the largest immature female was 75 cm TL. Testis length ranged from 10 to 80 mm in immature males and from 40 to 100 mm in mature males. Claspers in males began to elongate around 50 cm TL, with rapid growth occurring between 55 and 65 cm TL. Males larger than 68 cm TL were mature, displaying calcified claspers ranging from 15 to 19 cm. Semen was observed in mature males during late May and June (Márquez-Farías, 2009).

In shortnose guitarfish (*Zapteryx brevirostris*), male lengths ranged from 196 to 647 mm LT, while females ranged from 200 to 674 mm LT, with the majority falling within the ranges of 450 to 620 mm LT for males and 500 to 640 mm LT for females. The relationship between LT and MT displayed significant disparities between sexes ($F=275$, d.f.=1,632, $P<0{\cdot}001$). The smallest mature male measured 462 mm LT, and the largest juvenile measured 572 mm LT. Clasper length exhibited a sharp increase between 470 and 550 mm LT, with overlaps in clasper length observed among different mature stages between 500 and 600 mm LT (Colonello et al., 2011).

The lengths of the Blackchin guitarfish (*Glaucostegus cemiculus*) varied from 30.5 to 148.1 cm, with the majority falling within the range of 35 to 70 cm. The predominant stage observed among individuals was determined to be immature-1, with the exception of two specimens collected in July 2015. These two individuals consisted of a 74.4 cm female categorized as immature 2-developing and a 148.1 cm male identified as mature 3b-active. Additionally, the smallest specimens were captured in July 2015 (30.5 cm) and again in September 2015 (31.0 cm) (Bengil et al., 2013).

Maturity in males of the Brazilian guitarfish (*Pseudobatos horkelii*) is indicated by the development of claspers and testes, typically occurring at a total length (TL) of 53 cm. For females, maturity is signified by measurements of the oviducal gland, largest ovum diameter, and uterus width, with maturity generally attained at a TL exceeding 57 cm. It is noteworthy that this species possesses two functional ovaries, with the ovarian cycle and gestation occurring concurrently (Martins et al., 2018).

In Bengal guitarfish (*Rhinobatos annandalei*) length-weight relationships (LWR) varied significantly between sexes ($p<0.001$), leading to the derivation of combined sex LWR. Female and male length at maturity (TL50) was estimated at 61.0 and 63.3 cm TL, respectively. In individual females, embryo count ranged from 2 to 11, with birth size estimated between 25.0 to 30.0 cm TL. Overall, the sex ratio favored females at a rate of 1.6:1 (Purushottama et al., 2020).

Bowmouth guitarfish (*Rhina ancylostomus*) ranging in total length (TL) from 44.0 to 295 cm and total weight (TW) from 0.2 to 127 kg. The length-weight relationship (LWR) did not exhibit significant differences between females and males, leading to the derivation of a common equation: $TW = 0.006604TL^{3.027504}$ ($r^2 = 0.979$). Length-at-maturity (Lm50%) for females and males were estimated at 183.0 and 164.0 cm TL, respectively. R. ancylostoma possesses two functional ovaries, and both the ovarian cycle and gestation occur concurrently (Purushottama et al, 2022).

2.1 Lacunae in study

In the Indian marine ecosystem, comprehensive landing data specific to individual species is lacking. Information regarding the feeding biology of Widenose guitarfish is scarce, and despite numerous studies on the reproductive biology of various guitarfish species, there is a notable absence of published literature specifically focusing on Sharpnose and Widenose guitarfish. Additionally, research investigating the relationship between total length and weight for both species is non-existent. Therefore, this study seeks to address these literature gaps by conducting a thorough assessment of the bycatch landing of guitarfish from the North Goa (Caranzalem and Nauxim) and examining the reproductive and feeding biology of these guitarfish species.

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CHAPTER 3: METHODOLOGY

3.1 Study animal

Batoids in the Arabian Sea region face a significant risk of extinction, Jabado et al. (2018), with guitarfishes identified as one of the most vulnerable groups after sawfishes, Moore (2017). The vulnerability of this fauna may be underestimated due to limited available information. Therefore, this study was conducted on the vulnerable batoid species *Glaucostegus granulatus*, (Cuvier, 1829) and *Glaucostegus obtusus*, (Müller & Henle, 1841).

Fig.3.1 Species of study animal A) *Glaucostegus granulatus* B) *Glaucostegus obtusus*

3.2 Classification of the study animal

1.Sharpnose guitarfish (*Glaucostegus granulatus*)

Kingdom: Animalia

Phylum: Chordata

Class: Chondrichthyes

Subclass: Elasmobranchii

Superorder: Batoidea

Order: Rhinopristiformes

Family: Glaucostegidae

Genus : *Glaucostegus*

Species: *granulatus*

The sharpnose guitarfish displays a large size with a flattened, narrow, wedge-shaped disc characterized by a strongly depressed trunk. Its snout is elongated, narrowly triangular, and terminates in a bluntly pointed tip. The skin is rough, featuring enlarged denticles on the back and top of the head, most of which are joined along their entire length in the midline. The dorsal fins are closely located, with an interspace approximately 1.3-1.6 times the base of the first dorsal fin. The tail measures 1-1.4 times the length of the disc. Dorsally, it is uniformly yellow or brown with pale fin margins, while the snout is translucent except for the rostral cartilage. Ventrally, it is white. This species is found in the Northern Indian Ocean, ranging from the Persian Gulf to Myanmar.

2.Widenose guitarfish (*Glaucostegus obtusus*)

Kingdom: Animalia

Phylum: Chordata

Class: Chondrichthyes

Subclass: Elasmobranchii

Superorder: Batoidea

Order: Rhinopristiformes

Family: Glaucostegidae

Genus : *Glaucostegus*

Species: *obtusus*

The widenose guitarfish is small-sized with a flattened, broad shovel-shaped disc and a strongly depressed trunk. Its snout is short and broadly triangular, with enlarged denticles on the back and thorns present on the snout tip and around the orbit. The dorsal fins are short and closely located. Dorsally, it is uniformly grey or grey-brown, while ventrally it is white with pale fin margins. The snout is translucent.This species is found in Northern Indian Ocean; Pakistan to Thailand, eastern limits unclear.

3.3 Study area

This study was conducted from July 2023 to March 2024 at two landing sites

(Site 1= Caranzalem & Site 2= Nauxim) located at Tiswadi taluka, North-Goa.

Site 1

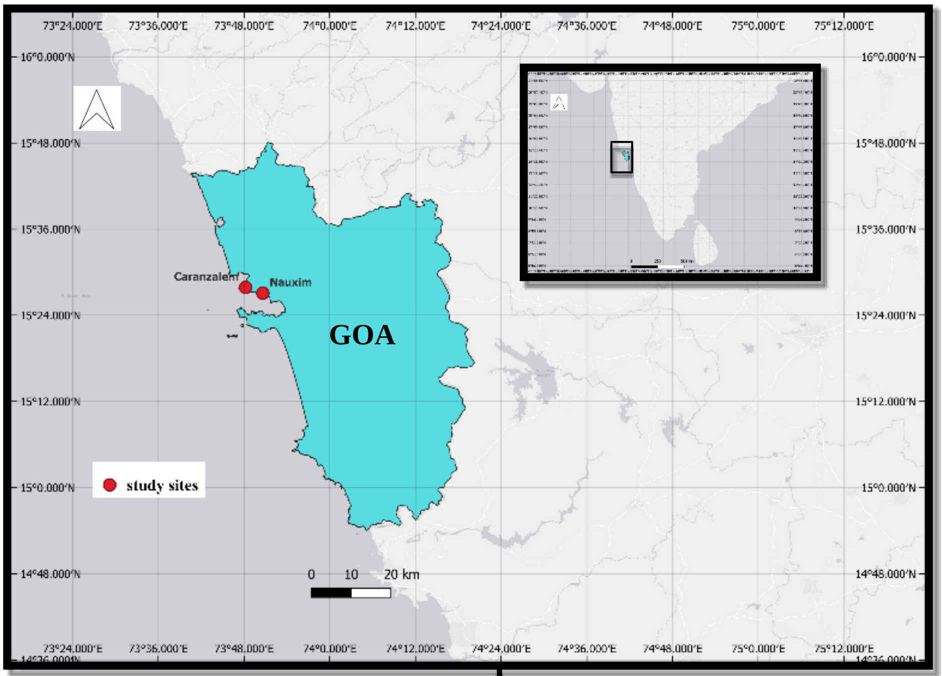
The study site was situated along the central west coast of India, specifically at the Caranzalem-Miramar coast, covering a 3.5 km stretch (Latitude: 15º28’09.75”N; Longitude: 73º47’40.01”E), at the mouth of the Mandovi estuary. This region constitutes an estuarine zone where freshwater from the Mandovi River flows in. The influx of nutrients driven by the southwest monsoon results in increased productivity. This enhanced productivity supports abundant fisheries in the area, particularly during the post-monsoon period, Sreekanth et al. (2021).Caranzalem Bay serves as an established fishing site for the local community, where catches exhibit variability from minor quantities to substantial quantities of fish, often accompanied by notable bycatch. Consequently, the dynamic characteristics of this estuarine bay demonstrate abundant productivity and fishery output, which in turn attracts numerous adult megabenthic predators along with juveniles and sub-adults that utilize this habitat as a refuge and feeding area, Sreekanth et al. (2021).The local practice of artisanal fishing techniques, specifically employing beach seines, is carried out in this region.

Site 2

This study site, Nauxim was located at latitude 15°45’16.91”N and longitude 73° 84’36.08”E. The Zuari estuary, a prominent estuarine system in Goa situated along the southwest coast of

India, serves as a highly productive coastal ecosystem for fishery resources, linking to the Arabian Sea through Mormugao Bay, Sreekanth et al. (2015).The Zuari estuary's margins feature dense mangrove vegetation and sediment comprising silt, clay, and detritus carried from upstream river sources. These mudflats and mangroves enhance productivity, supporting a diverse array of economically important species. Heavy precipitation, especially during the southwest monsoon, with stormy weather, contrasts with relatively calm conditions during other times of the year. The estuary's mouth reflects typical river estuarine influences, serving as vital nursery grounds for marine and euryhaline coastal species, with abundant juveniles in bays and estuaries. The coastal zone's rocky patches preclude trawling, making gillnet fishery the predominant method for catch, contributing significantly to the region's landed catches (Sreekanth et al., 2015).

Study Area Map



Study Site 1

Study Site 2

Fig. 3.2 Study Area

Fig.:3.3: A Shore seine operation at study site (Caranzalem-Goa), B: The evening fish auction at Caranzalem

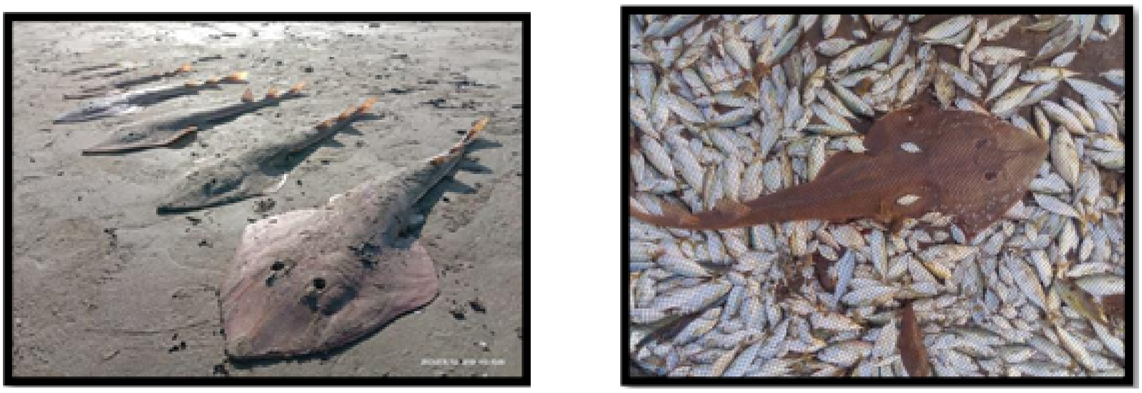


Fig.3.4:Bycatch of giant guitarfish caught in shore seine operation at Caranzalem- Goa

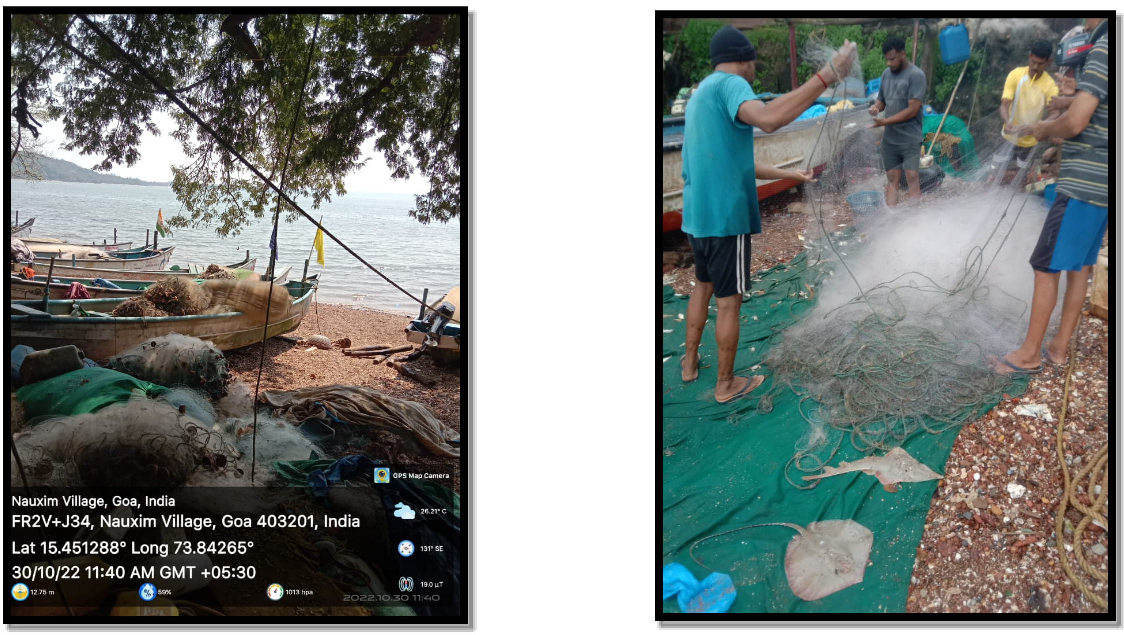


Fig.3.5: View of traditional fish landing site at Nauxim-Goa

3.4 Sample collection

Sampling was conducted at the study sites 3 days per week on alternative days, starting on different days to avoid any bias in sampling the same 3 days.

For this study, only dead samples discarded by the fishermen during their operations were examined, while live organisms were released back to the sea.

3.5 Sample processing

In the laboratory setting, fish samples underwent washing, counting, photographing, and identification to the species level using keys (Last et al. 2016). For each individual , total length (TL) (cm) from the tip of the snout to the extreme tip of the caudal fin , clasper length (CL) (cm) from posterior edge of cloaca to the tip of clasper and body weight (W) (g) were measured and sex were recorded. Sex determinations were made macroscopically by the absence (in females) or presence (in males) of a clasper (copulatory organ) on the pelvic fins.The diameter of mature ova was measured using vernier caliper with least count 0.1mm.

Later sampled fish were immediately dissected out, stomachs and gonads were removed, weighed, measured and preserved in 70% ethanol and 10% formaldehyde respectively for further analysis.

3.6 Length-weight relationship

The relationship between weight and total length was assessed for both male and female individuals (Le Cren, 1951).

3.7 Reproductive biology

The relationship between the total length and clasper length of *G.obtusus* (male) samples was examined. Maturity stages were classified following the maturity scale proposed by (Valetta 2010). The monthly mean gonadosomatic index (GSI) was calculated for both species.

3.8 Stomach content analysis (SCA)

To analyze the stomach contents, the stomach was opened and their contents were emptied into a petri dish. Prey items were then sorted, photographed, and identified under a stereomicroscope to the lowest taxonomic level using standard taxonomic keys. Which were then measured, counted, weighed, and categorized into the following groups; Molluscs, crustaceans, and fishes.

3.9 Data Analysis

The TL–W relationships were determined using Pearson correlation and the allometric equations: $W = aTL^b$ (Le Cren, 1951) after logarithmic transformation, TW being the weight of fish in g and TL the total length in cm; *a* the intercept *b*, the regression coefficient.

The gonadosomatic index (GSI) male and female was calculated using the following formula: GSI= [Gonad weight (g)/BW (g)] × 100 ((Lara-Mendoza & Márquez-Farías,2021)

To assess the contribution of prey items to the diet, three relative measures of prey quantity were computed: percent numerical importance (%N), percent frequency of occurrence (%FO), and percent composition by weight (%W), following the methods described by (Hyslop,1980)

Analysis of diet was done to estimate the percent index of relative importance (%IRI) for each prey item was calculated using the formula (Liao et al. 2001): %IRI = (%W + %N) × %FO. Subsequently, the percentage of IRI (%IRI) was derived by dividing the IRI of each prey taxon by the total IRI, then multiplying by 100.

The program PAST was used to test dietary variation between sexes.Analysis of similarity (ANOSIM) was conducted to identify dietary variances between male and female samples of *G. obtusus* and *G.granulatus*, utilizing a Bray-Curtis similarity matrix based on %W for the prey groups. The global R statistic values of ANOSIM range from -1 to +1, with values near 0 indicating a lack of differences between the sexes. Values approaching -1 to +1 denote significant separation between groups.Similarity of percentages (SIMPER) was used to estimate the contribution of each prey category to male and female differences in diet.

CHAPTER 4: ANALYSIS AND CONCLUSION

4.1 Result

4.1.1 Bycatch Data Analysis

During the study period of August 2023 to March 2024, a total of 32 (Females:13; Males;19) individuals of *G.obtusus* and 3 (all males) individuals of *G.granulatus* were sampled at Caranzalem site; While the study conducted at Nauxim site during the study period of July 2023 to March 2024, total of 16 (Females:12; Males:4) individuals of *G.granulatus* were reported (Fig.4.1).

At site 1 largest landing of *G.obtusus* was on December 23 (8) followed by March 24 (7) and September 23 (6) and were least on October 23 (3). No bycatches were recorded on February 24. Whereas *G.granulatus* was only recorded on August 23 (3) (Fig. 4.2). And at site 2 largest bycatch of *G.granulatus* was on July 23 (4) followed by December 23 and January 24 (3) each and the least were recorded on August 23, October 23, November 23, and March 24 (1) each. No samples were recorded in September 2023 (Fig.4.3).

At site 1, *G.obtusus* (Males) dominated the landings during October 2023, January 2024, and March 2024, but females and males otherwise occurred in the same relative proportions during the entire sampling period. While the *G.granulatus* (females) dominated the landings during August 2023 and December 2023 at site 2.

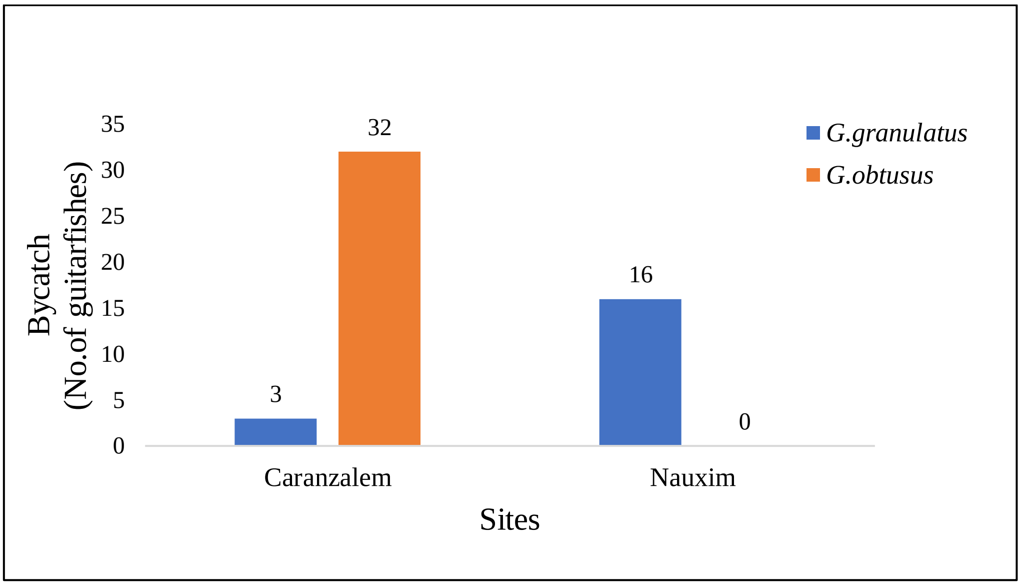


Fig.4.1Total landing of *G.obtusus* and *G.granulatus* at landing sites (Caranzalem and Nauxim) from July 2023 to March 2024.

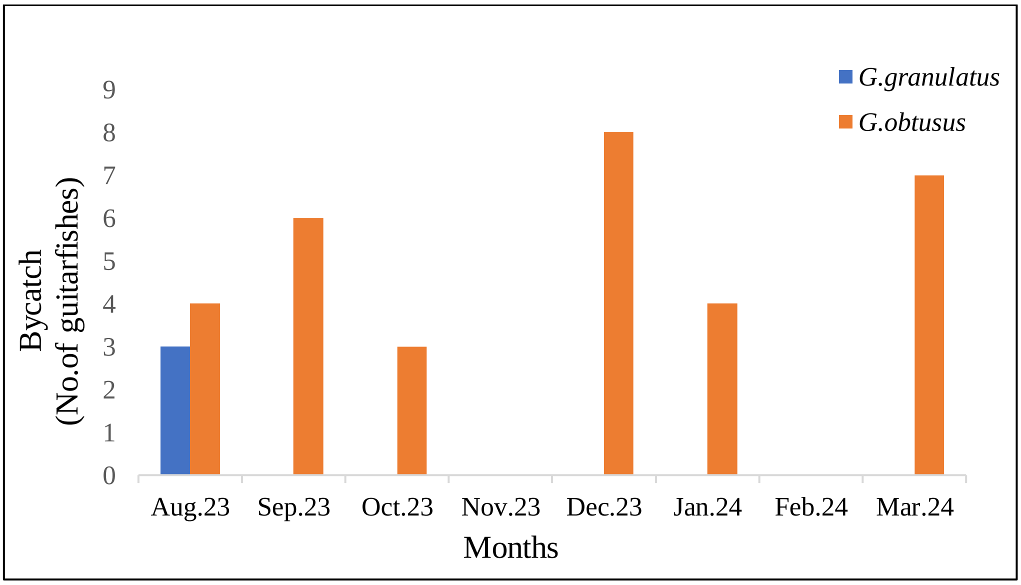


Fig.4.2 Monthly bycatch of *G.obtusus* and *G.granulatus* from landing Site-1 from August 2023 to March 2024.

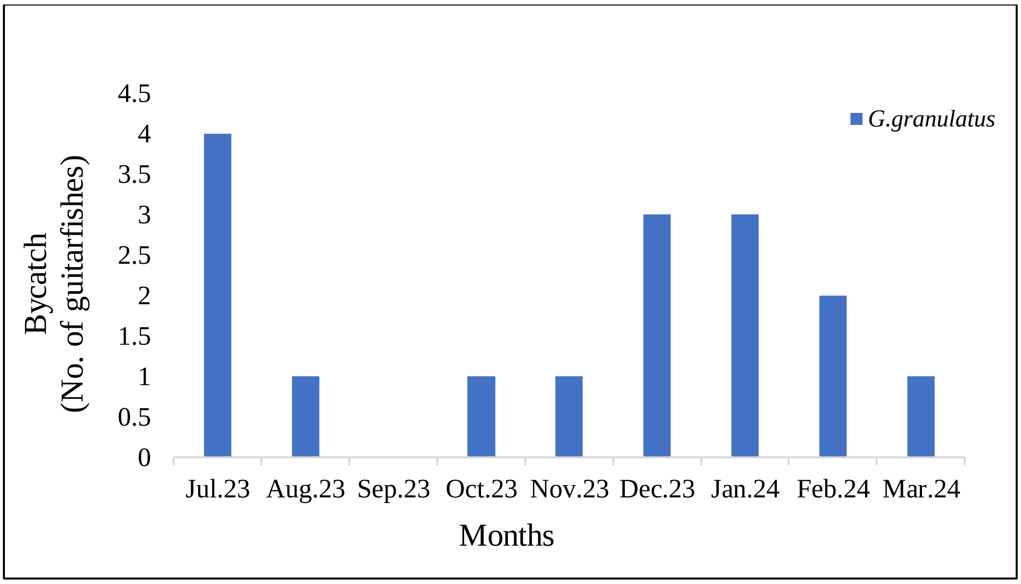


Fig.4.3 Monthly bycatch of *G.granulatus* from landing Site-2 from July 2023 to March 2024.

G.obtusus, females ranged from 20.1– 92 cm TL with a mean (43.39± 22.60 cm), n=13; whereas for males ranged from 20-70.4cm TL with a mean (47.69±18.28cm), n=19.

G.granulatus, females ranged from 22.1-71.2cm TL with a mean (43.38±14.62 cm), n=12; whereas males ranged from 30.35-60.96cm TL with a mean (45.75±11.32 cm), n=7.

The maximum total lengths (TL) observed for *G.obtusus* were 92 cm for females and 70.4 cm for males. While the maximum TL for *G.granulatus* for females and male is 71.2cm and 60.96cm respectively.

Regarding weight, *G.obtusus* females ranged from 23.3-1975.5g with a mean (340.54±572.43g),n=11, and males ranged from 30.2-1151.4g with a mean (440.4316±*416.3085*g), n=19. Whereas *G.granulatus* female ranged 31.181-545g with a mean (214.3351± 206.9947g), n=8 and male ranged 80-661.8g with mean (244.78± 238.23g), n=5.

Length-weight relationship (LWR)

The values of the constants in the equation TW = aTL^b that describes the length-weight relationship were as follows:

G.obtusus

Females:TW= 0.0048 $TL^{2.8173}$ (r^2 = 0.8949, n=11)

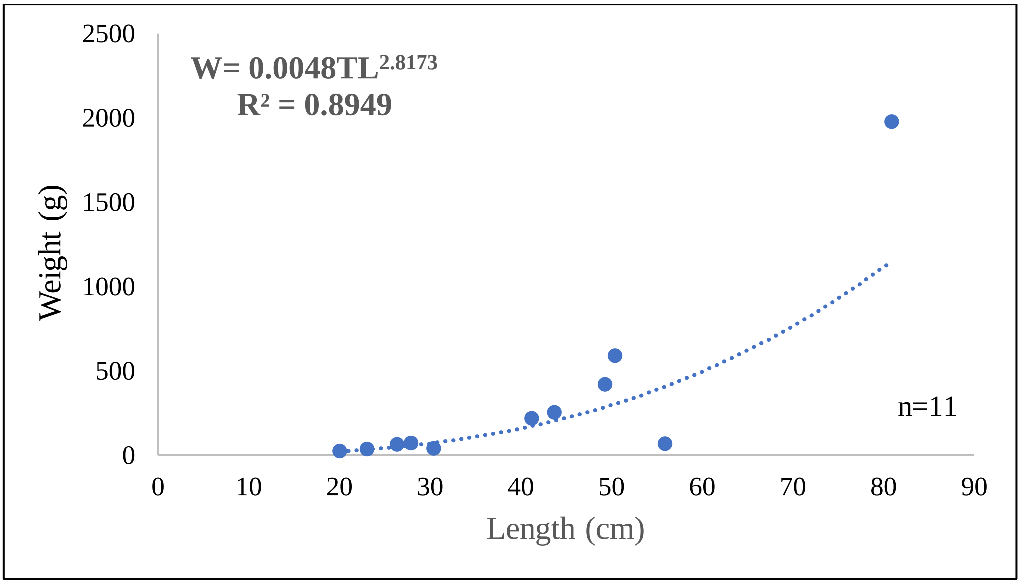
Males: TW= 0.0085 $TL^{2.7164}$ (r^2 =0.886, n= 19)

G.granulatus

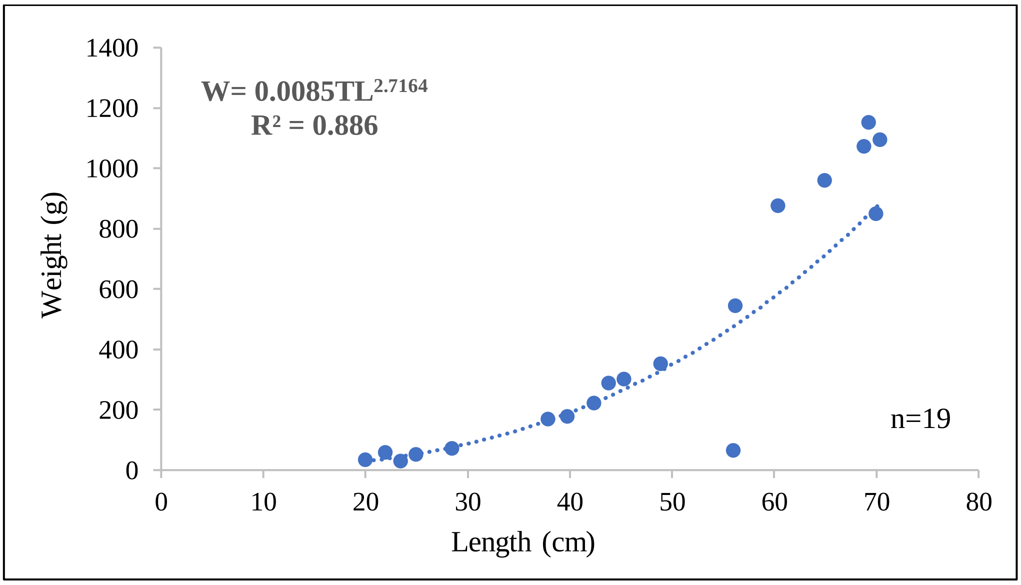
Females: TW= 0.0035 $TL^{2.9279}$ (r^2 = 0.9417, n=8)

Males: TW= 0.0023 $TL^{3.0294}$ (r^2= 0.9502, n=5)

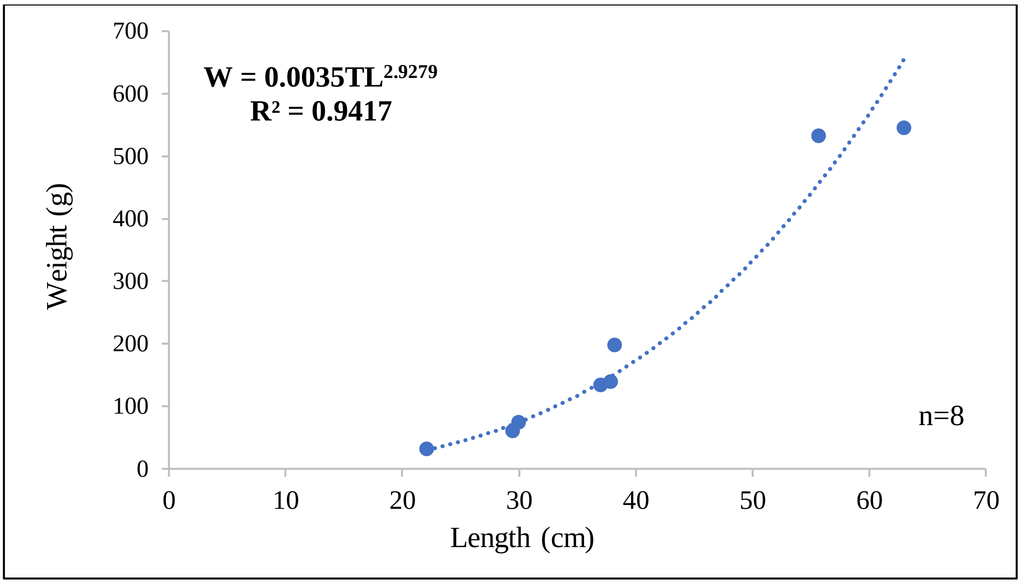
In the length-weight regression equation for *G.obtusus* and *G.granulatus,* a b-value below 3 suggests negative allometric growth. Specifically, the b-values for female *G.obtusus* and *G.granulatus* are 2.8173 and 2.9279 respectively, while for male *G.obtusus* it was 2.7164. Whereas *G.granulatus* male shows positive allometric growth i.e. 3.0294 (Fig.4.4)There was a significant positive correlation between total length and weight in both the species (Table 4.1).



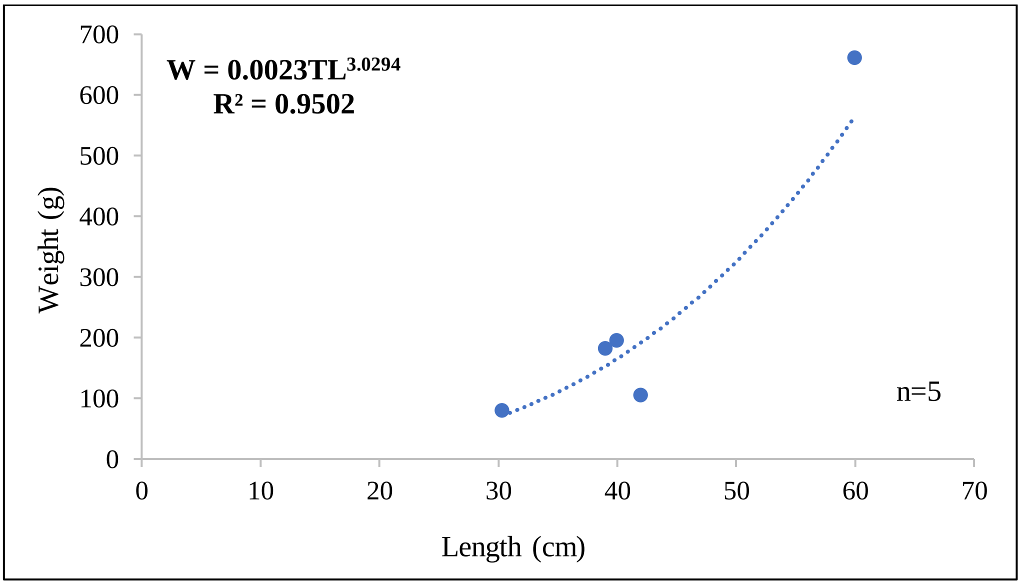
A) *G.obtusus* (female)



B) *G.obtusus* (male)



C) *G.granulatus* (female)



D) *G.granulatus* (male)

Fig. 4.4 Length-weight relationship

Table 4.1 Pearson's correlation between total length and weight of male and female

A)*G.obtusus*

	Length-Female	Weight-Male
Length- Male		0.8954****
Weight- Female	0.8427**	

B) *G.granulatus*

	Length-Female	Weight-Male
Length- Male		0.9381*
Weight- Female	0.9724****	

The total length and weight relationship in both the species was found to be positively significantly correlated ($p<0.05$).

4.1.2 Reproductive biology

In this study, 23 specimens of *G.obtusus* (females:7; males:16) and 11 specimens of *G.granulatus* (females:8; males=3) were examined to assess their reproductive biology.

Male

For the reproductive study of males data were obtained from 7 and 3 samples of *G.obtusus* and *G.granulatus* respectively.

Testes. - The testes in male *G.obtusus* and *G.granulatus* are paired, elongated, dorsoventrally flattened organs that are attached along either side of the vertebral column by a mesorchium. Both testes are well-developed and functional in adult males. The epigonal organs entirely envelop the ventral surface of the testis as a thin layer.

Reproductive ducts. - The epididymis lies ventral to the slender anterior part of the kidney. In mature males, these tubules of the epididymis can be seen greatly coiled through the peritoneum. Each duct of the paired ductus deferens opens into the anterior end of the urogenital sinus, respectively.

Clasper

The male possesses a pair of claspers which are dorsoventrally flattened, elongate structures extending caudad from the medial portion of each pelvic fin. The clasper length of *G.obtusus* ranged from 2-14.3 cm with a mean 7.16$\pm$ 4.68cm. The ICL was found to increase with body size; for *G.obtusus*, the smallest male measured at 22.0 cm TL had an ICL of 2.0 cm, whereas the largest male at 70.4cm TL exhibited an ICL of 14cm. Similarly, in *G.granulatus*, the smallest male at 40.0 cm TL displayed an ICL of 2.8 cm, while the largest male at 60 cm TL had an ICL of 5 cm.

Total length-Clasper relationship

Growth of clasper is gradual with a positive trend of the scatter diagram as a function of TL (Fig.4.5). Clasper growth presented a sigmoid pattern, showing three phases: the first, representing slow growth; the second, showing fast growth and the third phase with slow growth. Development of claspers is gradual; they appear flaccid in juvenile specimens and begin to grow rapidly once *G.obtusus* males reach 48.9 cm TL. All *G.obtusus* (males) larger than 56.2 cm TL were mature and showed calcified claspers with sizes between 11 and 14.3 cm. The smallest mature male with calcified claspers measured 56.2 cm TL.

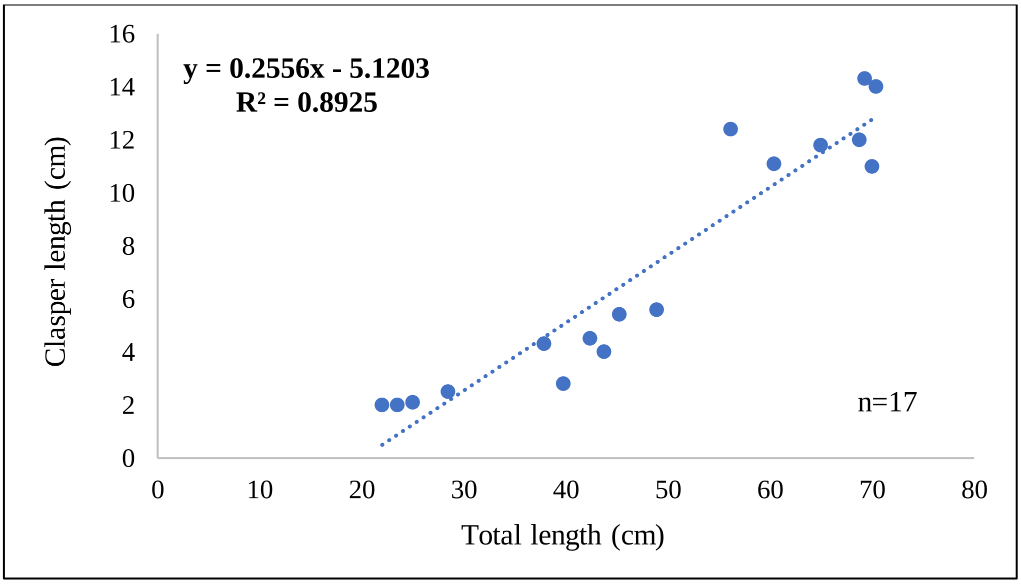


Fig.4.5 Total length-clasper length relationship of *G.obtusus* (male).

Female

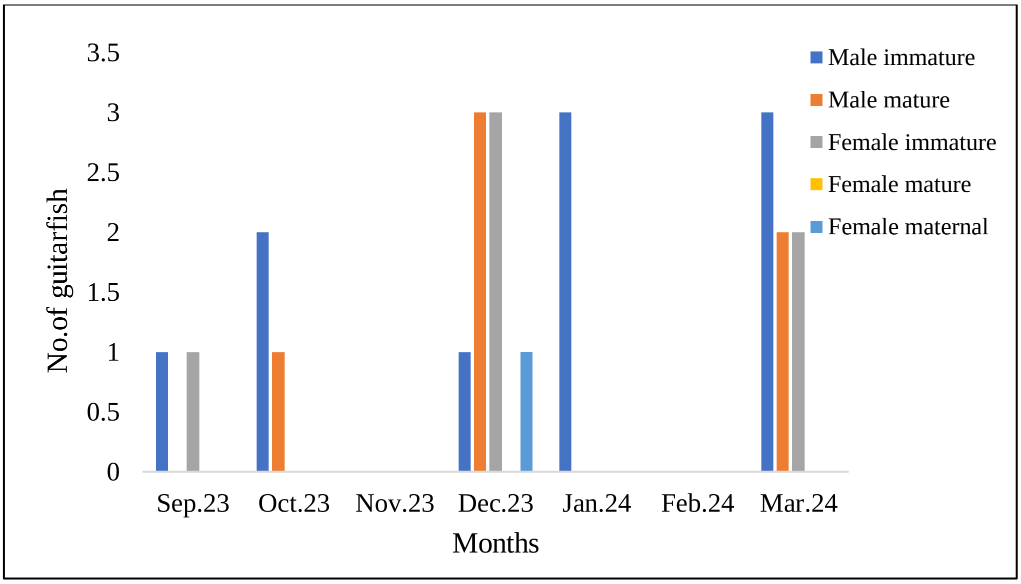
Reproductive data were obtained from 7 female samples of *G.obtusus* and 8 female samples of *G.granulatus*. Both the species are aplacental viviparous species, with each female having two ovaries. Their medial edges are attached along either side of the vertebral column by a mesorchium.

In immature specimens, ovaries were undeveloped whereas in mature specimens, ovaries contain ova of different sizes. Ova turn yellow as they ripen, increasing in size as yolk accumulates. Although both ovaries are functional, the number of developing eggs in two ovaries is not always equal. In immature samples, the uterus is a thin tube along the body cavity.

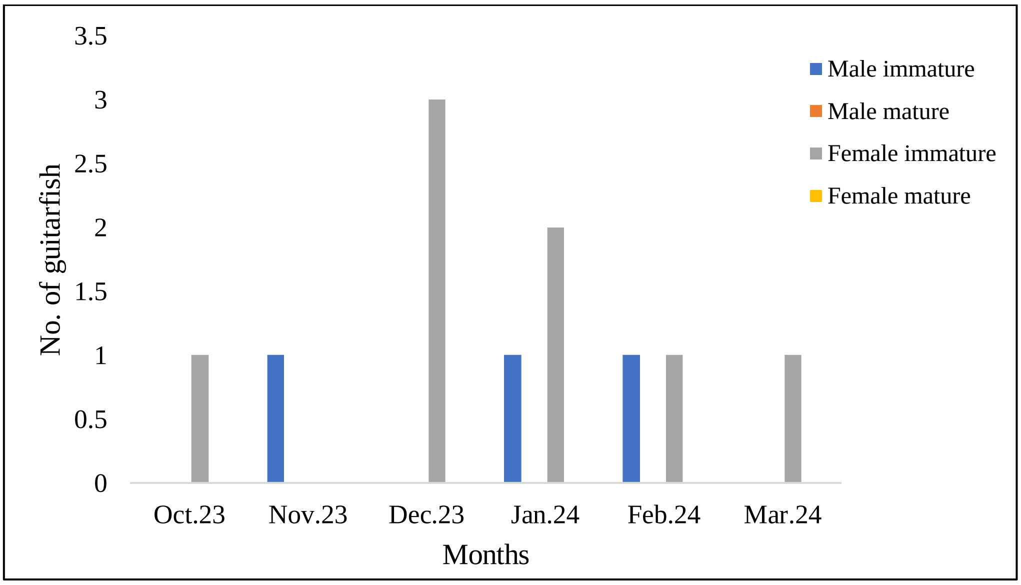
Only one mature *G.obtusus*(female)was recorded in December 2023 and presented an egg case in uteri measuring 8cm. The largest ovum was 0.719g with a diameter of 0.38cm.

Macroscopic maturity stages

At site 1, landings primarily consisted of stage immature-1 individuals of *G.obtusus* and only one male was found to be immature stage-2, whereas six 3a mature males were reported in December 2023 and March 2024. Only one female stage maternal-3b was recorded in December 2023. While at site 2, only immature individuals of stage 1 were reported throughout the study period excluding one male sample which was identified as stage immature-2, and not a single mature individuals were reported at site 2 (Fig 4.6) and (Fig.4.7).



A) *G.obtusus* (Site 1)



B) *G.granulatus* (Site 2)

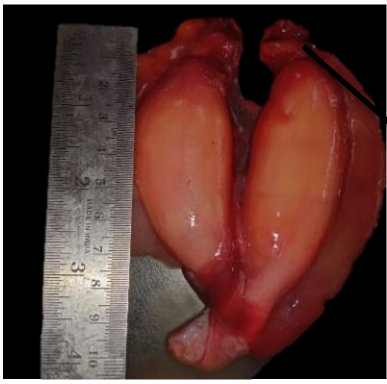
Fig.4.6 The number of immature and mature individuals of *G.obtusus* and *G.granulatus* recorded from site 1 and site 2.

A)Immature stage 1
(Male testes)

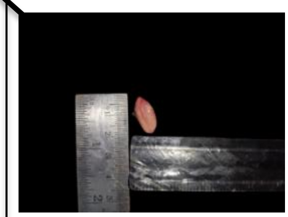
B)Immature stage 2
(Male testes)

C)Mature stage 3a
(Male testes)

D)Immature stage 1
(Female ovary)



E)Maternal stage 3b
(Female ovary)



Mature ova

A)Immature stage 1
(Male clasper)

B)Immature stage 2
(Male clasper)

C)Mature stage 3a
(Male clasper)

Coiled epididymis (EP) in mature male (*G.obtusus*)

A) *G.obtusus*

A)Immature stage 1 (Male testes)

B)Immature stage 2 (Male testes)

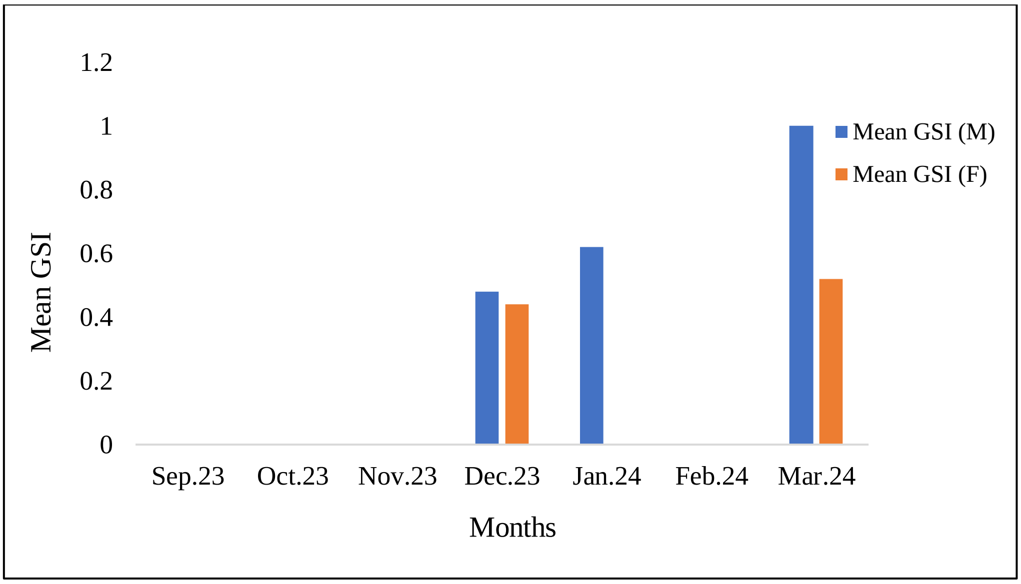
C)Immature stage 1 (Female ovary)

B)*G.granulatus*

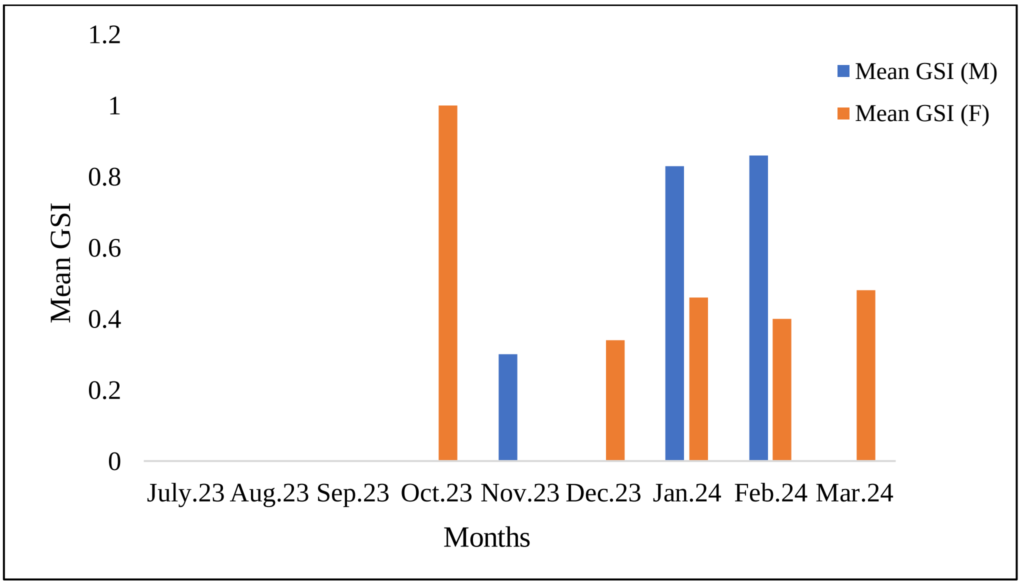
Fig.4.7 Macroscopic maturity stages

Gonadosomatic index (GSI)

The mean GSI value of female and male *G.obtusus* was highest in March 2024 and in *G.granulatus* female and male GSI value was peak during October 2023 and February 2024 respectively. The sample size was too small to provide information on their reproductive activity (Fig.4.8).



A)*G.obtusus*



B)*G.granulatus*

Fig. 4.8 Monthly average gonadosomatic index (GSI).

4.1.3 Feeding biology

A total of 19 individuals of *G.obtusus* and 10 individuals of *G.granulatus* were examined for stomach content analysis. A total of 82 prey items were identified to the lowest taxonomic level. The diet of *G.obtusus* was composed of three main groups of prey: Molluscs, Crustaceans, and fishes, and *G.granulatus* composed of two main groups of prey: Crustaceans and fishes (Fig. 4.9).

Shrimp represents the major food group in the stomach of both the species of *G.obtusus* and *G.granulatus* by numerical abundance (43.07%); (35.29%), frequency occurrence (84.21%); (50%) and weight (29.45%); (46.42%). Teleosts were the second most important forage category by occurrence (31.57%);(40%), composition (20.79%);(2.61%), and number (29.23%);(35.29%). While Brachyurans, Pagurids, stomatopods, Bivalves, and Cephalopods came next in importance in G.obtusus.Whereas Brachyurians and Pagurids came next in importance in *G.granulatus*

In general, the most important prey of *G.obtusus* in terms of %IRI were shrimp (67.94%), followed by teleosts (17.57%). Brachyurans (7.36%), and stomatopods (5.75%) were less important in the diet. Other prey items represented less than 1% of %IRI like Pagurids, bivalves, and cephalopods. In *G.granulatus* the most important prey in terms of %IRI were shrimp (65.71%), followed by teleosts (24.38%), brachyurian (5.44%), and pagurids (4.45%) (Table 4.2).

A difference in diet was observed between males and females of *G.obtusus* and G.*granulatus* i.e. (ANOSIM: R=1,p= 0.3348) and (ANOSIM; R=1,p=0.3316) respectively (Table 4.3). Overall average dissimilarity was found to be 59.4% and 27.34% respectively (Table 4.4).

The spiral intestines of four specimens of *G. obtusus* and five specimens of *G. granulatus* reported the presence of cestodes from December 2023 to March 2024 (Fig.10).

Table.4.2 Prey composition in stomach contents of A)*G.obtusus* B)*G.granulatus* and relative measures of prey quantity; percent number (%N), percent weight (%W), frequency of occurrence (%FO), index of relative importance (%IRI).

A)

Prey categories	%N	%W	%FO	IRI	%IRI
Shrimps	43.0769	29.45316	84.2105	6107.796478	67.943
Pagurids	6.15385	1.270437	10.5263	78.15037863	0.86934
Brachyuran crab	13.8462	1.882583	42.1053	662.2623924	7.36699
Stomatopods	3.07692	46.06842	10.5263	517.3196069	5.75465
Bivalve	3.07692	0.206127	10.5263	34.55843488	0.38443
Teleosts	29.2308	20.79275	31.579	1579.690237	17.5724
Cephalopods	1.53846	0.326538	5.26316	9.81578967	0.10919

B)

Prey categories	%N	%W	%FO	IRI	%IRI
Shrimp	35.29412	46.42677356	50	4086.044678	65.71687
Pagurids	17.64706	10.02516346	10	276.7222346	4.450592
Brachyuran crab	11.76471	22.08857552	10	338.5328552	5.444708
Teleosts	35.29412	2.614626406	40	1516.349856	24.38783

Table 4.3 Dietary composition variation between male and female A) *G.obtusus* B) *G.granulatus* to percent weight of prey (% W).

A)

Prey categories	Prey group (%W) in Female	Prey group (%W) in Male
Shrimps	19.61508	36.83172
Pagurids	1.270438	0
Brachyuran crab	0.692533	2.477609
Stomatopods	0	46.06842
Bivalve	0.206127	0
Teleosts	9.796146	21.40367
Cephalopods	0.326538	0

B)

Prey categories	Prey group (%W) in Female	Prey group (%W) in Male
Shrimps	5.756347	5.158123
Pagurids	1.221467	0
Brachyuran crab	2.691275	0
Teleosts	2.258058	3.327762

Table.4.4 Similarity Percentage Analysis (SIMPER) showing the contribution of the prey items that are responsible for the dissimilarity between the diets of male and female A)*G.obtusus* B)*G.granulatus*

A)

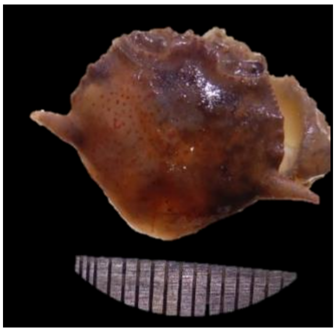
Prey Categories	Av.dissimilarity	Contribution %	Cumulative %	Male	Female
Stomatopods	31.07	52.31	52.31	46.1	0
Shrimps	11.61	19.55	71.86	36.8	19.6
Teleosts	7.828	13.18	85.04	21.4	9.8
Bivales	6.607	11.12	96.16	0	9.8
Brachyuran crab	1.204	2.027	98.19	2.48	0.693
Pagurids	0.8568	1.443	99.63	0	1.27
Cephalopods	0.2202	0.3708	100	0	0.327

B)

Prey Categories	Av.dissimilarity	Contribution %	Cumulative %	Male	Female
Brachyuran crab	13.18	48.22	48.22	0	2.59
Pagurids	5.984	21.89	70.11	0	1.22
Teleosts	5.24	19.17	89.28	3.33	2.26
Shrimp	2.931	10.72	100	5.16	5.76


Shrimp

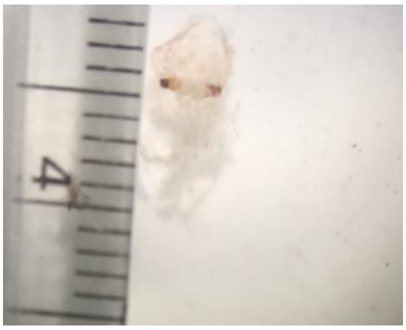
Pagurids


Brachyuran

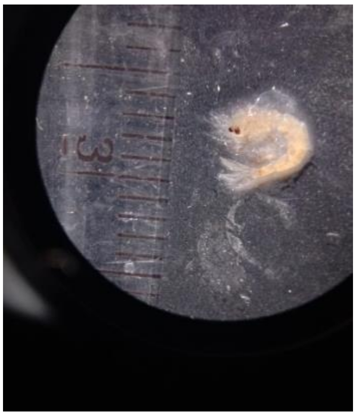

Stomatopod


Bivalve


Teleost

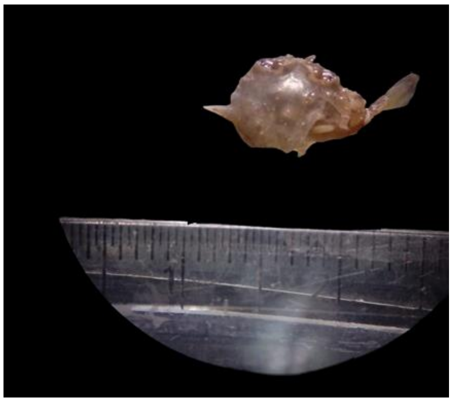

Cephalopod

A)*G.obtusus*



Shrimp

Pagurid



Brachyuran

Teleost

B)*G.granulatus*

Fig.4.9 Prey composition

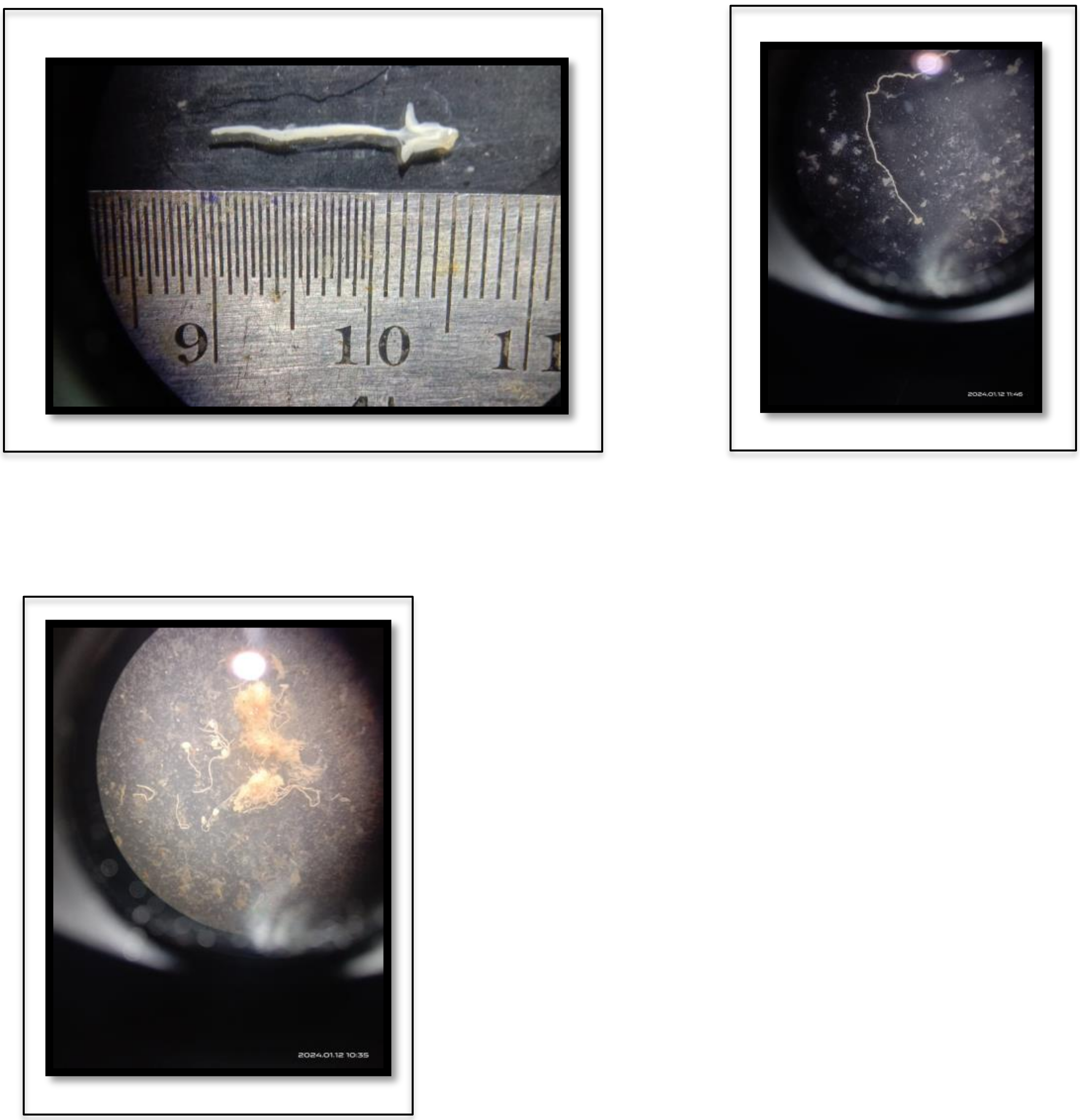


Fig. 4.10 Cestodes

4.2 Discussion

The present study provides essential information on bycatch data, length-weight relationship, stomach content analysis, and reproductive biology of *G.obtusus* and *G.granulatus*.The current observations of the two species of giant guitarfish at the two sampling sites exhibited notable distinctions. Bycatch data indicates that only *G.obtusus* species were documented at the Caranzalem site, whereas *G.granulatus* was solely recorded in August 2023. While at the Nauxim site, only *G.granulatus* species were recorded throughout the sampling period. Based on the observation of the current study, the differences in habitat selectivity between the two species may be attributed to a range of ecological factors. Each species likely evolved to occupy distinct ecological niches characterized by differences in dietary preferences, habitat requirements, and reproductive behaviors. Competition for similar resources, such as food or space, may result in habitat partitioning to alleviate competitive pressures, thereby promoting coexistence. Furthermore, variances in physiological adaptations, such as tolerance to salinity levels or temperature, could influence the suitability of estuarine environments for each species.

Throughout the study period, a total of 32 individuals of *G.obtusus* and 19 individuals of *G.granulatus* were documented at the landing sites. Catch trends of elasmobranchs in the inshore waters of Goa reported 5 individuals of G.obtusus and 6 samples of *G.granulatus* , Hegde et al. (2014). Comparable declines in giant guitarfish landings have been observed in Bangladesh, attributed to the consumption and trade of their meat and fins (Haque and Spaet, 2021). Elasmobranch landing from trawlers at Malvan, Maharashtra, reported 17 samples of giant guitarfish, Gupta et al. (2020). Four individuals of *G.obtusus* and one of *G.granulatus* were documented in shore seine catches of Malvan, Maharashtra, Gupta et al. (2022). Additionally, 48 individuals of *G.granulatus* were reported from the Zuari estuary, representing one of the highest abundances among the total count of elasmobranchs ,Bhavan et al. (2023).In the face of the rapid global population decline of up to 99% of giant guitarfish and wedge fish, Haque et al. (2021).Mandovi and Zuari estuaries may serve as significant habitats for both species of guitarfish.

The Guitarfishes reported during the study period *G.obtusus* TL ranged between 20 - 92cm and *G.granulatus* TL 22.1-71.2cm. A study conducted at Malvan documented that size ranged from TL 29-148cm (Gupta et al. 2020). The landing of *G.obtusus* and *G.granulatus* in Bangladesh reported TL n=22;60.96–137.17(97.40±21.82); n=137;42.67–213.36 (105.40±32.53) respectively, Haque et al. (2021). Males presented smaller TLs than females, which, in general, is common among guitarfishes and elasmobranchs, Capapé and Zaouali (1994). The average weight of *G.obtusus* female and male was recorded to be (340.54±572.43g),n=11 and (440.4316±416.3085g), n=19, while for *G.granulatus* average weight was found to be (214.3351± 206.9947g), n=8 and (244.78± 238.23g), n=5.A study by (Jit et al 2014) reported 2-2.5kg of *G.granulatus*.Similar findings have been reported in other species of guitarfish, with females reaching larger weight and size than males (Ismen et al. 2007; Márquez-Farías 2007).

Elasmobranchs exhibit notable sexual dimorphism in their morphology, growth patterns, and biology, Gladson et al. (2018). Therefore, sex-specific Length-Weight Relationships (LWRs) were calculated for both species in this study. The calculated parameter 'b' for each elasmobranch species falls within the "acceptable range of 2.5-3.5, Froese (2006)."The r^2 value for *G.obtusus* female and male were 0.8949 and 0.886 and for *G.granulatus* female and male were 0.94147 and 0.9502 respectively. The "b" value of the *G.granulatus* estimated in this study does not match the values , Gladston et al.(2018) calculated from the north-eastern

Arabian Sea, India.The total length and weight relationship in both the species was found to be positively significantly correlated ($p < 0.05$).

The findings of this study indicate that estuaries serve as habitats for Giant guitarfish species, primarily dominated by juvenile individuals. These findings align with previous reports, as demonstrated by, Bhavan et al. (2023). Moreover, the inshore fishing grounds adjacent to Goa are identified as crucial nursery areas for juvenile elasmobranchs, as documented by, Ansari et al. (1995). Hence, it should be noted that estuaries function as crucial nursery grounds for elasmobranch species. Such information contributes to conservation efforts for the species because all information relative to the breeding and nursery areas of an endangered species is imperative for strategic management plans.

The reproductive organs in both male and female individuals of giant guitarfish species demonstrate functional efficacy. As noted by Wourms (1977), it is a prevalent trait among elasmobranchs for both sexes' right and left reproductive tracts to exhibit functional symmetry.

The study demonstrates the gradual development of claspers in male *G.obtusus*. Claspers initially appear flaccid in juveniles but begin rapid growth at around 48.9 cm TL. All males over 56.2 cm TL were mature, displaying calcified claspers sized between 11 and 14.3 cm. This indicates clasper development correlates with growth, with calcification and maturity occurring at approximately 56.2 cm TL. A similar study of other species of guitarfish *R.productus* calcification of claspers in mature specimens was noted to commence at a total length (TL) of 53 cm or greater. The smallest mature male, possessing calcified claspers, measured 53 cm TL. The claspers of mature individuals exhibited a size range of 8 to 11 cm ,Márquez-Farías (2007). *Z.exasperata* recorded onset near 50 cm TL in males. Rapid clasper growth was initiated at 55 cm TL. Males over 68 cm TL displayed maturity, featuring 15-19 cm calcified claspers. The smallest mature male measured 58 cm TL, Márquez-Farías and

Galván-Magaña (2009). In *Pseudobatos horkelii*, individuals measuring 11.7 cm CL and beyond displayed calcified claspers, indicating maturity (Martins et al., 2018).

Clasper length and total length relationship of G.obtusus presented a sigmoid growth pattern, a common pattern observed in several elasmobranchs, Martins et al. (2018) This suggests that clasper growth is faster when individuals are close to attaining sexual maturity.

The mean Gonadosomatic Index (GSI) reached its highest point for both female and male *G.obtusus* in March 2024. Conversely, for *G.granulatus*, females displayed their peak GSI value in October 2023, while males reached their peak in February 2024. In comparison, in the species *Rhinobatos hynnicephalus*, male guitarfish exhibited a peak GSI in June, followed by a substantial decrease from July to August, Kumeet al. (2009). Furthermore, in *P.glaucostegmus*, GSI values exhibit a seasonal pattern, with the lowest values observed during the winter months (December to February), intermediate values during autumn (September to November), and peak values during spring to summer (May to July) (Lara-Mendoza et al., 2021).

In the present study, crustaceans contributed significantly to the diet of *G.obtusus* (IRI 81.93%) and *G.granulatus* (IRI 75.61%). A similar pattern was also observed in the diet of, *Rhinobatos leucorhynchus* (IRI 87%), *Zapteryx xyster* (43.19%), *Rhinobatos glaucostigma*, *Rhinobatos rhinobatos*, *R.percellens* (IAI 69%) and *Rhinobatos annulatus* (IRI 88.1%), Silva-Garay et al. (2018). Previously, only one report provided basic information on the diet of *G.granulatus* species. Sreekanth et al. (2022) suggested that *G.granulatus* fed on teleosts (PSIRI 46.9%) followed by crustaceans (PSIRI 44.6%).

The dietary preferences of elasmobranchs for specific prey items can be influenced by various factors, including the prey's abundance in the environment, its likelihood of evasion, the suitability of foraging habitats, and competitive interactions among predator species. This insight is supported by research conducted by (Heithaus, 2004). Fish morphology plays a vital role in determining the type of prey consumed, while morphological variation can lead to changes in foraging habits and, subsequently, differential exploitation of food resources and trophic levels (Karpouzi & Stergiou 2003)

The ANOSIM analysis conducted in this study reveals a significant difference between both species' male and female diets. This finding contrasts with the existing literature, Sreekanth et al. (2022), which generally indicates no dietary variation between male and female *G.granulatus*. Also, similar findings were recorded in other species of guitarfish (Blanco-Parra (2012); De la Rosa-Meza et al. (2013). However, it is worth considering that the observed variation in this study could be attributed to the relatively smaller sample size. Therefore, further research with a larger sample size is warranted to validate these findings.

The presence of cestodes within the spiral valves of both species may be acquired through the ingestion of infected prey such as invertebrates or fish, thereby serving as intermediate or paratenic hosts, Williams and Jones (1994). It is commonly hypothesized that elasmobranch cestodes utilize two or potentially three different intermediate host species before reaching their elasmobranch definitive host. However, the possibility of a fourth intermediate or paratenic host has been suggested in certain instances, Palm (2004). As parasites, cestodes possess the capacity to inflict harm upon their hosts. Moreover, studies have demonstrated that cestodes can impact host behavior, growth, and potentially even evolution (Taylor et al., 1998; Loot et., al)

4.3 Conclusion

To conclude, bio-ecological information obtained from this study provides an important contribution to filling the current gaps in knowledge. Our findings serve to highlight the significance of coastal waters as crucial habitats for these species, particularly during their early life stages. Priority in conservation efforts should be given to safeguarding potential nursery and feeding areas and locations with the highest populations of giant guitarfish.

Numerous coastal fisher communities in India rely on traditional fishing practices for their livelihood and sustenance. Yet, the capture of endangered elasmobranch species, particularly juveniles, suggests that this fishery could be more effectively managed to safeguard vulnerable marine fauna. Conservation initiatives could focus on the live release of endangered species, while also addressing the requirements of traditional fishers.

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APPENDIX I

Criteria used to determine the stages of maturity of *G.obtusus* and *G.granulatus* (ICES,2010)

Males

Description	Maturation state	Stage	Maturity
Claspers flexible and shorter than pelvic fins. Testes small (in rays, sometimes with visible lobules). Sperm ducts straight and thread-like	IMMATURE (Immature)	1	IMMATURE
Claspers slightly more robust but still flexible. Claspers as long as or longer than pelvic fins. Testes enlarged; in sharks testes start to segment; in rays lobules clearly visible but do not occupy the whole surface. Sperm ducts developing and beginning to coil (meander).	DEVELOPING (Immature)	2	IMMATURE
Claspers fully formed, skeleton hardened, rigid and generally longer than pelvic fins. Testes greatly enlarged; in sharks testes are fully segmented; in rays filled with developed lobules. Sperm ducts tightly coiled and filled with sperm.	SPAWNING CAPABLE (mature)	3a	MATURE
Description similar to stage 3a, however with clasper glands dilated, often swollen and reddish (occasionally open). Sperm often present in clasper groove or glans. On pressure sperm is observed flowing out of the cloaca or in the sperm ducts.	ACTIVELY SPAWNING (mature)	3b	MATURE
Claspers fully formed, similar to stage 3. Testes and sperm ducts shrunken and flaccid.	REGRESSING (mature)	4	MATURE

Females

Description	Maturation state	Stage	Maturity
Ovaries barely visible or small, whitish; undistinguishable ovarian follicles. Oviducal (nidamental) gland may be slightly visible. Uterus is thread-like and narrow.	IMMATURE (Immature)	1	IMMATURE
Ovaries enlarged with small follicles (oocytes) of different size. Some relatively larger yellow follicles may be present. Ovaries lack atretic follicles. Developing oviducal gland and uterus.	DEVELOPING (Immature	2*	IMMATURE
Large ovaries with enlarged yolk follicles all of about the same size so that they can be easily distinguished. Oviducal gland and uterus developed without yolky matter, embryos and not dilated	CAPABLE to REPRODUCE (mature)	3a	MATURE
Uteri well filled and rounded with yolk content (usually candle shape). In general segments cannot be distinguished and embryos cannot be observed.	Early pregnancy (maternal)	3b	MATERNAL
Uteri well filled and rounded, often with visible segments. Embryos are always visible, small and with a relatively large yolk sac.	Mid pregnancy (maternal)	3c	MATERNAL
Embryos fully formed, yolk sacs reduced or absent. Embryos can be easily measured and sexed.	Late pregnancy (maternal)	3d	MATERNAL
Ovaries shrunken without follicle development and with atretic (degenerating) follicles. The oviducal glands diameter may be reducing. Uterus appears much enlarged, collapsed, empty and reddish.	REGRESSING (mature)	4a	MATURE
Ovary with small follicles in different stages of development with the presence of atretic ones. Uterus enlarged with flaccid walls. Oviducal gland distinguishable.	REGENERATING (mature)	4b*	MATURE

*Be careful, these stages can be easily confused