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Title

Understanding dietary differences in Indian dugongs through opportunistic gut sampling of stranded individuals

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Abstract

We analysed gut samples from stranded dugongs to understand their dietary preferences from Tamil Nadu and Gujarat, India. We quantified seagrass fragments from the gut as leaf, stem and rhizome and identified leaf fragments up t genera level by their morphological feature and epidermal cell characteristics in inverted microscope. Overall abundance of aboveground fragments (leaf, stem) was higher in all samples which may suggest dugongs use cropping mechanism to forage. The ingested seagrass generic diversity was higher in Tamil Nadu (n=5) dugong individuals than Gujarat (n=2). A total five genera were recorded from all samples viz; *Halophila* spp., *Halodule* spp., *Cymodocea* spp., *Enhalus* sp. and *Syringodium* spp. In Tamil Nadu, *Cymodocea* spp. (46.24%) was the most dominant followed by *Halophila* spp. (26.49 %), *Syringodium* spp. (14.83 %), and *Halodule* spp. (12.16%). Low occurrence of *Enhalus* spp. (0.19 %). In Gujarat, *Halodule* spp. (61.48%) followed by *Halophila* spp. (30.20%) Plastic and wood fragments recorded suggests fine spatial scale threat mapping in dugong habitats.

Keywords: Necropsy, Megaherbivore, Seagrasses, Foraging Pattern

Introduction

Dugongs (*Dugong dugon*, Müller, 1776, order Sirenia) are globally threatened marine mammals that primarily forage on seagrasses (Heinsohn & Birch, 1972; Marsh et al., 2012). Despite their vast global distribution range spanning from the East African coast to Australia (Indo-Pacific Ocean region), their population is declining due to various human-mediated drivers (Marsh et al., 2012). These drivers include mortalities from incidental capture in fishing nets, boat strikes, hunting for meat and seagrass habitat loss due to increased sedimentation and pollution (Marsh & Sobztick, 2019), which have led to local extinction of the species (Hines et al., 2005; Pusineri et al., 2013; Srinivas et al., 2020).

For species monitoring and conservation, it is crucial to understand dugong distribution, and the factors influencing the same. Availability of seagrasses is one of the limiting factors for dugong distribution, thus, understanding dugong foraging patterns is crucial in mapping their critical habitats. Dugongs use two major feeding techniques viz; cropping and excavation of seagrasses, depending on species morphology and substratum (Wirsing, 2007; Rasheed et al., 2016; Marsh et al., 2018). So far, dugong foraging preferences are known through direct observations of feeding or by analysis of stomach contents (Preen, 1992; Andre et al., 2005; De longh et al., 2007; D'Souza et al., 2015).

Indian dugong populations are imperiled due to various threats with an estimated population of less than 300 individuals left in the wild (Pandey et al, 2010; Sivakumar & Nair, 2013). Recent efforts across isolated pockets of their distribution along the Indian coastline (Gulf of Kutch in Gujarat; Gulf of Mannar and Palk Bay in Tamil Nadu; and Andaman and Nicobar Islands) have helped generate some crucial ecological data on their distribution, habitats, genetic diversity, connectivity, and threats (Sivakumar & Nair, 2013; D'Souza et al., 2015; Rajpurkar et al., 2021; Srinivas et. al, 2020). Limited studies exist on dugong feeding biology from India (D'Souza et al., 2015; Nair & Mohan, 1975), given the difficulty to observe them in the wild. Thus, stranded dugongs provide a critical opportunity to understand their dietary composition through gut sampling. In this study, we utilize the gut contents opportunistically collected from stranded dugongs to understand differences in the dugong foraging pattern within study sites. Our work fills the research gap on dugong feeding behaviour from the Indian waters and presents the first gut content analysis report to supplement existing knowledge on dugong feeding biology on a regional scale.

In 2018, we sampled gut contents of dead stranded dugongs from the coasts of Tamil Nadu (n=3 individuals) and Gujarat (n=2 individuals) (Figure 1). Strandings were informed by the local dugong volunteer network involving fishers and personnel from the Coastal Security Police and State Forest Department (Table 1). Gut samples were collected, secured in ethanol in air tight containers and preserved at -20˚C until further processing. Dugong carcasses were sexed, measured and necropsies were conducted based on carcass condition using standard protocols (reference, Table 1).

To understand the proportion of seagrasses consumed by dugongs from the gut samples, we categorized seagrass fragments as leaf, stem and rhizome based on their morphological features (Figure 2) (Adulyanukosol & Poovachiranon, 2003). We used point-intercept method (Schuette et al., 1988) to calculate the abundance of fragments of leaves, rhizomes and stems. Samples were further divided into 10 subsamples of 1 gram each and later homogeneously spread on petri plate of size 100 X 15 mm. The petri plate was divided into 24 quadrates of $1cm^2$ each and the gut material was observed under stereo-microscope (10x magnification).

We identified seagrass leaf fragments based on apex structure, visible venation and stem fragments from its fibroid structure. Rhizomes were identified by their hard structure with nodes and presence of leaf scars (Adulyanukosol & Poovachiranon, 2003).

Further, to assess the preferred seagrasses by dugongs, we identified seagrass fragments only up to genus level. Species identification of different seagrass species belonging to same genera was not possible due to similarity in their epidermal cell shapes and sizes (Channells & Morrissey,1981; Marsh at al, 1982; Adulyanukosol & Poovachiranon, 2003) Only leaf fragments was considered for genera level identification, as stem and rhizome fragments are conserved in appearance through most genera (Channells & Morrissey, 1981). Validation of genus was done referring to prepared reference slides of seagrass species collected from Tamil Nadu, Gujarat and Andaman & Nicobar Islands; present distribution range of dugongs in Indian waters. Morphology of leaf and epidermal cells' features were studied and photographed under stereo (10x) and inverted microscopes in various magnifications (4x, 10x, 20x and 40x) (Supplementary Figure 1).

Epidermal cell characteristics and tannin cell arrangements were used for identification of macerated seagrass to the level of genus, using a quadrat method (Channells & Morrissey, 1981), where one-quarter of the petri plate (25% of grids) was chosen randomly to count the leaf fragments. Gross morphological features (venation, size, apex structure and shape of leaves) were taken into consideration for intact leaves (Adulyanukosol & Poovachiranon, 2003). Fragments were identified at different magnifications (4x, 10x and 40x) using seagrasses identification keys (Channells & Morrissey, 1981; Lanyon, 1986; El Shaffai, 2016; Pande et al., 2021).

Since gut material constituted of seagrasses and algae, to independently quantify both, we identified algal fragments (Figure l) on the basis of their cellular structure (40x) using peer reviewed published literature (Channells & Morrissey, 1981).

Mann-Whitney *U* test was performed for *Halophila* spp. and *Halodule* spp. to check differences in their occurrence between sites i.e., Tamil Nadu and Gujarat as well as between fresh and decomposed carcass. This test was possible only for these two genera, as these commonly occurred in all the gut samples.

A total of five genera were recorded from the gut samples across sites viz; *Halophila* spp., *Halodule* spp., *Cymodocea* spp., *Enhalus* sp. and *Syringodium* spp. Overall proportion of seagrass leaf fragments (>40%) was higher in all the samples analysed. Percentage of above ground fragments (leaf, stem) was higher than the below ground fragments (rhizome) (Table 1). Of the two genera recorded from Gujarat samples, *Halophila* spp. and *Halodule* spp. (Figure 2), the latter was more abundant (61.48 %) than *Halophila* spp. (30.20 %) along with little algal fragments (8.30%) (Table1).

Five seagrass genera namely *Halophila* spp., *Halodule* spp., *Cymodocea* spp., *Enhalus* spp., *Syringodium* spp. and alga were recorded from Tamil Nadu dugong individuals (Figure 2). In overall samples, leaf fragments of *Cymodocea* spp. (46.24%) were dominant followed by *Halophila* spp. (26.49 %), *Syringodium* spp. (14.83 %), and *Halodule* spp. (12.16%). Low occurrence of *Enhalus* spp. (0.19 %) and algal fragments (0.069 %) were found in the samples (Table 1).

We found occurrence *Halodule* spp. to differ between Tamil Nadu and Gujarat samples (U=987.5, $p < 0.001$) but not for *Halophila* spp. (U=1411, $p > 0.05$; Figure 3). However, occurrence of *Halophila* spp. differed according to carcass condition (U=1214, p<0.05) but not *Halodule* spp. (U=1840, p=>0.05; Figure 3).

In addition to seagrasses, plastic and wooden fragments (Figure 2) were found in the gut content of two individuals (one each from Gujarat and Tamil Nadu). Two fishing net filaments (~9.4 cm and ~ 4 cm in length), one polythene fragment (~4cm length) and one wooden fragment (~4cm in length) were obtained from Tamil Nadu dugong, while one plastic microfilament was retrieved from Gujarat dugong (Figure 2).

Globally, studies on dugong gut content have highlighted selective consumption of seagrass species like *Halophila ovalis, Halodule uninervis* (Heinsohn & Birch, 1972; Johnstone & Hudson, 1981; Aragones 1994, Adulyanukosol et al., 2010), *Enhalus* sp. (Erftemeijer et al., 1993), *Thalassia hemprichii, Syringodium isoetifolium* (Andre et al., 2005) and *Cymodocea serrulata* (Andre et al., 2005; Adulyanukosol et al., 2010). Percentage contribution of above and below ground plant material was proportionate in the gut samples of Gujarat individuals (Table 1) which possibly suggests towards dugongs excavating the whole seagrass plant. Our findings suggests that, dugongs might be exhibiting cropping mechanism over excavation in

Tamil Nadu (% of above ground seagrass fragments > % of below ground seagrass fragments) (Table 1), which needs further validation with more ecological observations and larger sample size. Limited percentage of algal material (3.58%) could be due to incidental ingestion while feeding on seagrasses. In the present study, more generic diversity of seagrasses in gut content of individuals from Tamil Nadu (n=5) than Gujarat (n=2), could be attributed to high regional generic diversity of seagrasses in Tamil Nadu (Thangaradjou & Bhatt, 2018) than in Gujarat (Thangaradjou & Bhatt, 2018).

Dominance of *Halophila* spp. in dugong gut samples from Ajad Island, Gujarat is in line with field observations of dominant *H. ovalis* and *H. beccarii* meadows in the region (Sivakumar et al., 2020). *Halodule* spp. was found to be dominant in the samples of Man Marudi Island (close to Beyt Dwarka). Thus, considering a low population size of dugongs in Gulf of Kutch (Pandey et al, 2010), locating *Halodule* spp. meadows would help in identifying critical foraging grounds in the area.

Stranded dugongs in Tamil Nadu showed a differential rate of digestion from fresh carcasses in comparison to highly decomposed state (Table 1). Fresh carcasses were recorded with higher mean occurrence of *Halophila* spp., while decomposed carcasses were diagnosed with *Cymodocea* spp. in dominance. This could be attributed to carcass condition (fresh/ decomposed) as Thayer et al. (1984) and Preen (1995) reported dugongs showing species specific differential digestion rates in their digestive tract. Further, we speculate that differential digestion is affected by carcass' condition and decomposition rate of individual species. Additionally, role of plant morphology and composition are also crucial factors affecting time required for digestion and in turn occurrence in the gut (Heinsohn & Birch., 1972). More fibrous species like *Cymodocea* spp. take longer time for decomposition (Marsh et al., 2018) and digestion as compared to smaller leaved, less fibroid plants like *Halophila* spp. Thus, differences in *Halophila* spp. fragments in both fresh and decomposed carcasses can be attributed to its availability and differential digestion rate (Lanyon & Sanson, 2006).

Our work, is a first report on dietary preferences of dugongs on seagrasses, from Indian waters, which generates key baseline from two important dugong distribution ranges in the Indian sub-continent. Occurrence of plastic, fishing net fragment and wood debris indicates towards a potential risk to dugongs at their foraging grounds. Thus, we recommend enhanced monitoring of seagrass habitats and fine spatial scale threat mapping in entire dugong distribution range in India.

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Table 1: Details on location, carcass condition, cause of mortality and Mean, Percentage contribution of Fragments of Seagrasses and Seagrass genera present in Gut samples of dead stranded dugong from the coast of Tamil Nadu and Gujarat

Dugong Individual	**Date**	**Location**	**Sex**	**Age class**	**Carcass condition**	**Cause of Mortality**	**Informant group**	**Body Length (m)**	**Total gut content weight (g)**	**Mean ± SD and Percentage proportion of Fragments of Seagrass in /gm samples**			**Percentage of Above ground seagrass fragments (leaf and Stem) (%)**	**Percentage of below ground seagrass fragments (rhizome) (%)**	**Mean ± SD and Percentage of Fragments (in parenthesis) of Seagrass genera in /gm of Samples**					**Algal Fragments**
										Leaf	**Stem**	**Rhizome**			***Halophila* spp.**	***Halodule* spp.**	***Cymodocea* spp.**	***Syringodium* spp.**	***Enhalus* spp.**	
Dugong 1	04-02-2018	Ajad Island	Female	Adult	Decomposed	Net Entanglement	Fisherman	2.6	64.77	12.13±8.09 (43.29%)	7.05±3.41 (25.22%)	8.80±3.80 (31.48%)	68.51	31.48	23.8±14.14 (79.20%)	3.55±4.38 (11.81%)	0	0	0	2.70±5.18 (8.98%)
Dugong 2	20-05-2018	Man Marudi Island	Male	Adult	Fresh	Net Entanglement	Fisherman	2.4	159.03	14.2±9.15 (40.86%)	9.30±5.96 (26.76%)	11.25±6.06 (32.37%)	67.62	32.37	4.2±4.81 (6.28%)	53.65±35.46 (85.36%)	0	0	0	5.00±5.12 (7.95%)

n=2 (Gujarat)	-	-	-	-	-	-	-	-	-	13.15±8.59 (52.44)	8.17±5.76 (22.08%)	10.02±5.15 (25.47%)	74.52	25.47	14±14.39 (30.20%)	25.8±35.67 (61.48%)	0	0	0	3.85±5.22 (8.30%)
Dugong 3	16-06-2018	PM Valsai	Male	Juvenile	Fresh	Boat Strike	Fisherman	1.5	76.832	7.45±7.43 (41.45%)	4.60±4.58 (25.59%)	5.92±3.77 (32.94%)	67.04	32.94	8.82±9.21 (44.08%)	5.72±6.85 (28.59%)	1.02±7.56 (5.10%)	4.35±6.25 (21.74%)	0.1±0.30 (0.50)	0
Dugong 4	20-06-2018	Thondi	Male	Adult	Fresh	Poached	Forest Department and Marine Police	2.4	25.94	16.55±1.0 (55.29%)	8.05±6.46 (26.89%)	5.30±2.55 (17.80%)	82.19	17.80	16.45±6.87 (49.76%)	1.86±1.46 (5.63%)	2.50±1.57 (7.56%)	12.08±8.51 (36.45%)	01.±0.30 (0.30)	0.1±0.30 (0.30%)
Dugong 5	07-12-2018	Vembar	Not identified	Adult	Decomposed	Na	Fisherman	2.9	30.73	21.65±7.31 (60.81%)	5.25±4.38 (14.74%)	8.70±2.61 (24.43%)	75.55	24.43	5.50±2.96 (7.68%)	4.15±2.47 (5.80%)	61.8±14.14 (86.31%)	0	0	0
n=3 (Tamil Nadu)	-	-	-	-	-	-	-	-	-	13.30±10.27 (41.95%)	5.60±5.20 (26.06%)	6.46±3.46 (31.97%)	68.01	31.97	9.50±4.89 (26.49%)	4.36±5.26 (12.16%)	16.58±27.31 (46.24%)	5.32±7.45 (14.83%)	0.07±0.26 (0.19)	0.025±0.15 (0.069%)

Figure Legends

Figure 1: Locations of stranded dugongs in Gulf of Mannar and Palk Bay, Tamil Nadu and Gulf of Kutch, Gujarat. – A) an adult female dugong found in Ajad Island, Gulf of Kutch on 4th February 2018 B) an adult male dugong found in Man Marudi Island, Gulf of Kutch on 20th May 2018 c) juvenile male dugong found in PM Valsai, Palk Bay on 16th June 2018 D) an adult male found in Thondi, Gulf of Mannar on 20th June 2018 E) adult individual found in Vembar, Palk Bay on 7th December 2018

Figure 2: Fragments and epidermal cell structures of seagrasses and non-biological materials from dugong gut samples – A) & B) Leaf fragment of *Halophila* spp. with venation under stereo-microscope and compound microscope (4x); C) fibroid structure of vertical stem under stereo-microscope; D) epidermal cell structure of stem under compound microscope (20x); E) rhizome fragment; F) Presence of leaf scars in rhizome fragment under stereo-microscope; G) epidermal cell structure of *Halophila* spp. (40X); H) epidermal cell structure of *Halodule* spp. (40x); I) epidermal cell structure of *Cymodocea* spp. (40x); J) epidermal cell structure of *Enhalus* spp. (40X); K) epidermal cell structure of *Syringodium* spp. (40X); L) epidermal cell structure of an algal fragment (40X); M) wooden fragment (4cm); N) fishing net filament (4cm); O) polythene fragment (4cm); P) fishing net fragment (9cm); Q) red-coloured microfilament (20X)

Figure 3: Mean number of seagrass fragments obtained from the gut of stranded dugongs sorted according to A) carcass condition and B) sampling site.

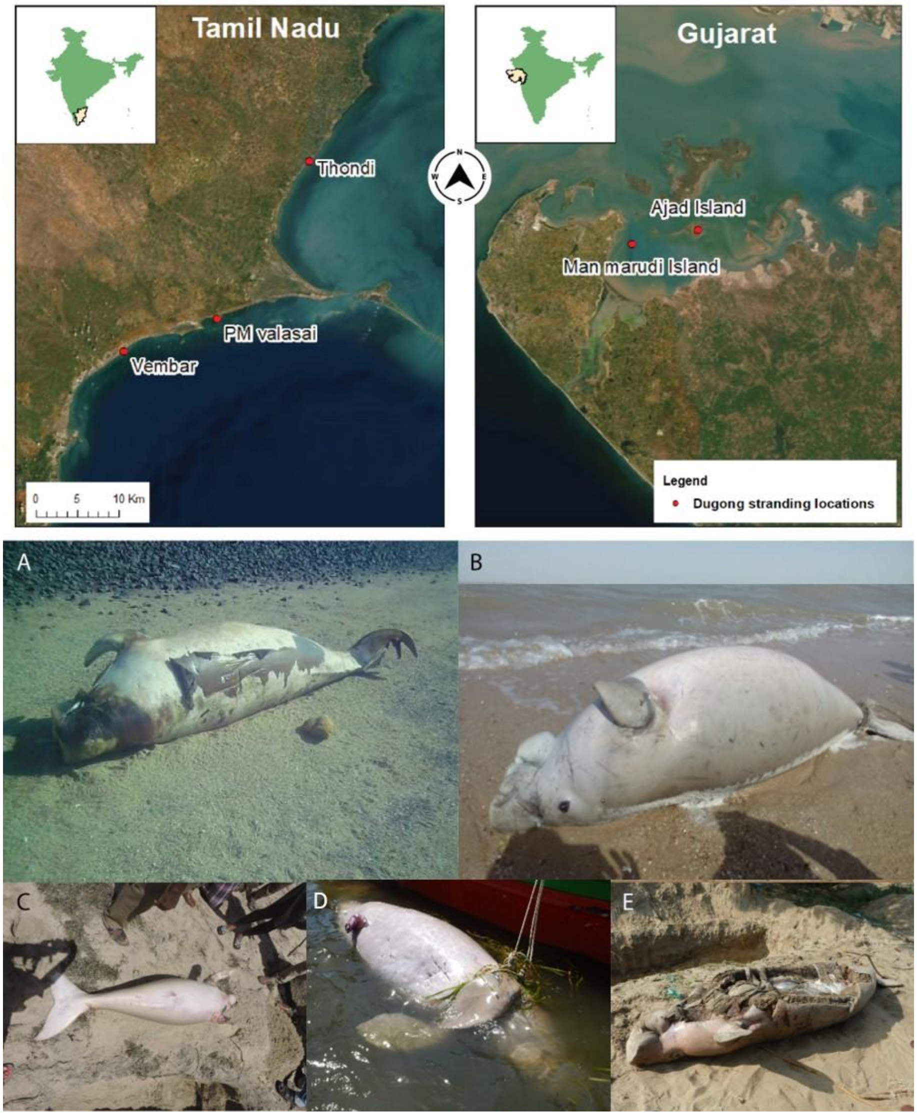
Tamil Nadu
Thondi
PM valasai
Vembar
0 5 10 Km
Gujarat
Ajad Island
Man marudi Island
Legend
Dugong stranding locations
A
B
C
D
E

Figure 1

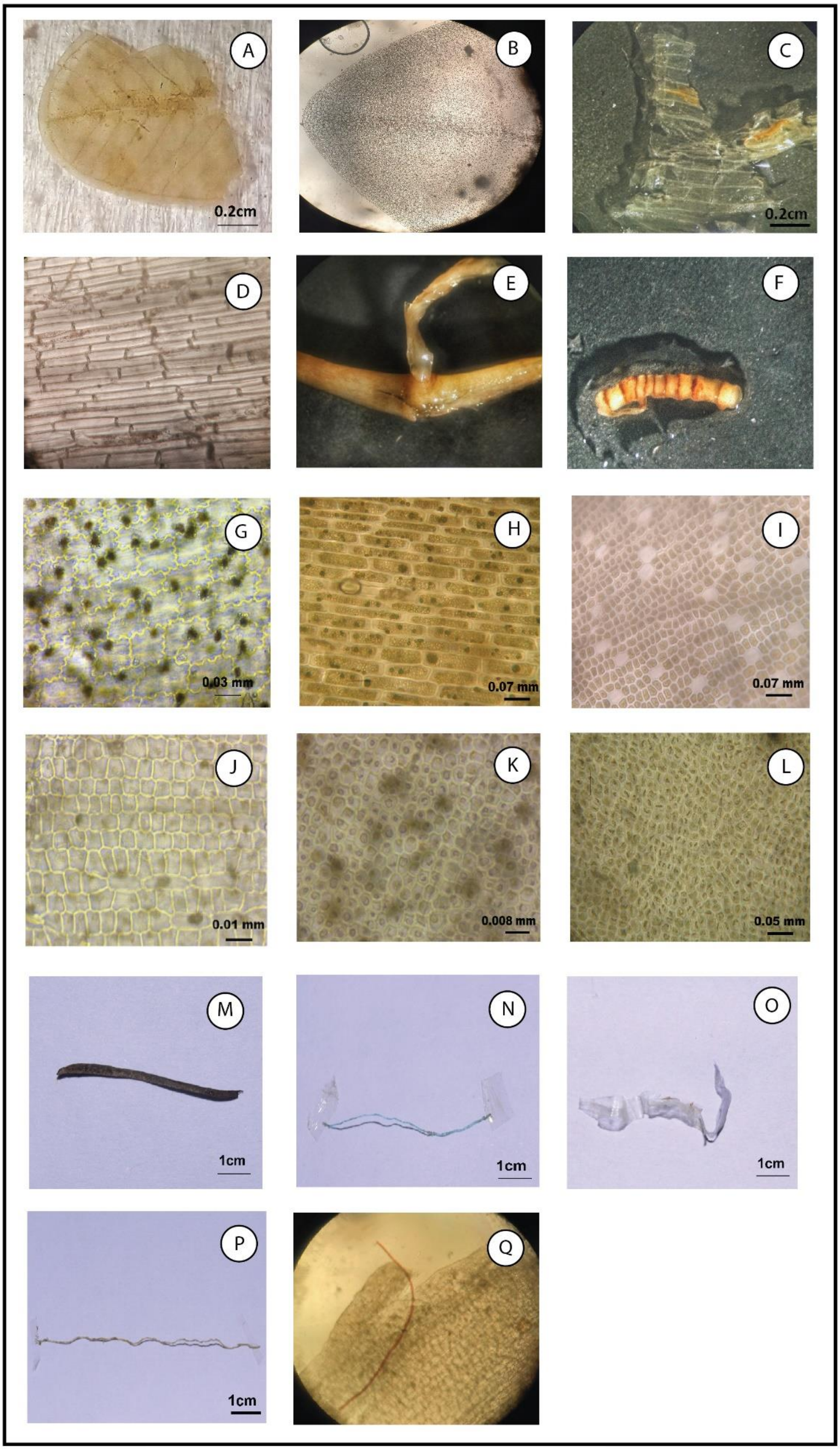
A
0.2cm
B
C
0.2cm
D
E
F
G
0.03 mm
H
0.07 mm
I
0.07 mm
J
0.01 mm
K
0.008 mm
L
0.05 mm
M
1cm
N
1cm
O
1cm
P
1cm
Q

Figure 2

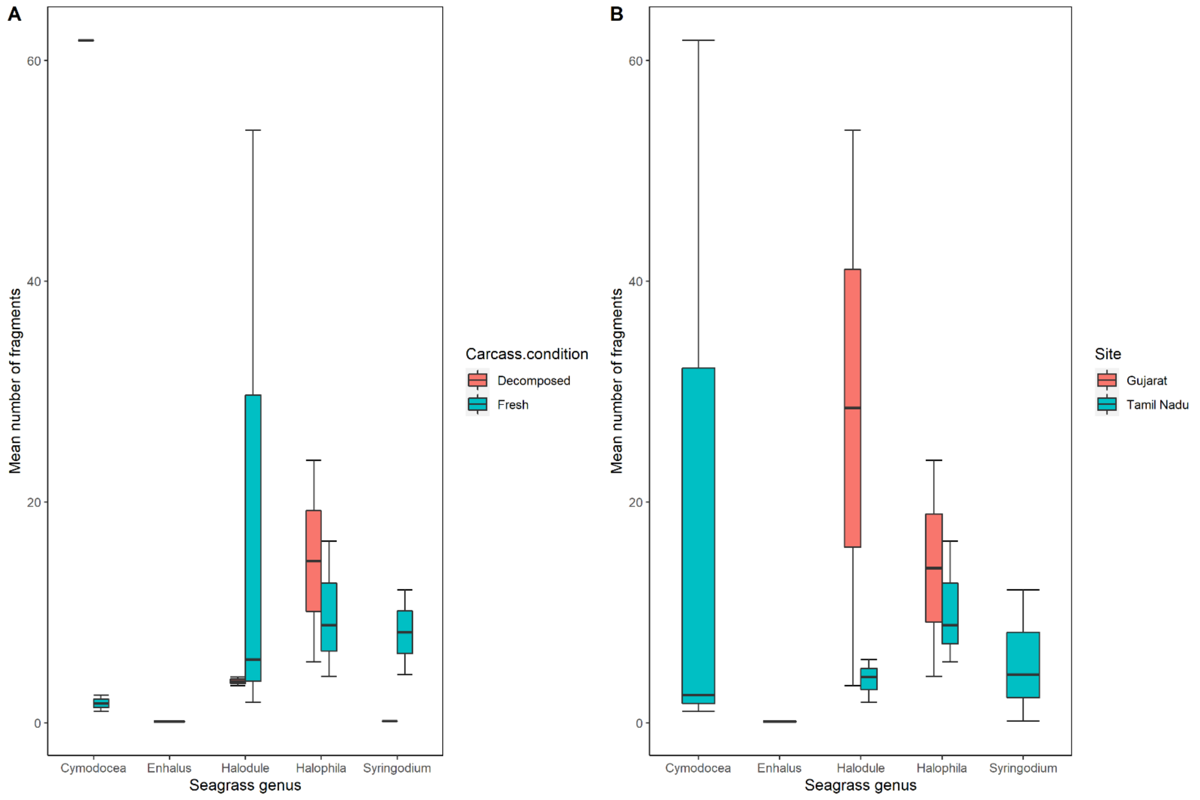
A
B
Mean number of fragments
0
20
40
60
Cymodocea
Enhalus
Halodule
Halophila
Syringodium
Seagrass genus
Carcass.condition
Decomposed
Fresh
Site
Gujarat
Tamil Nadu

Figure 3