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Population structure of a newly recorded (*Halodule uninervis*) and native seagrass (*Halophila ovalis*) species from an intertidal creek ecosystem

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Abstract: 300 words

The present study documented the presence of seagrass *Halodule uninervis* for the first time along with previously documented *Halophila ovalis* at Haripur creek. The population structure of both these seagrass species is assessed. The physico-chemical parameters were similar for both seagrass species except for the sediment grain size fractions. The sand content of *H. ovalis* patches was 1.2-fold higher than *H. uninervis* beds, whereas the silt content of *H. uninervis* beds was 2-fold higher than *H. ovalis* patches. The pH levels were lower than the standard oceanic pH of 8.2. Macroalgae like *Ceramium sp.* and *Gracilaria verrucosa* were growing on the leaves of *H. uninervis* due to high nitrate and phosphate levels of the creek waters. Leaf reddening was only observed in the leaves of *H. ovalis*. Under similar environmental conditions, *H. ovalis* (5004 ± 114.51 ind. m-^{2}) had a 2-fold lower shoot density than that of the *H. uninervis* (11598 ± 187.52 ind. m-^{2}). Both above- and below-ground biomass of *H. ovalis* (96.34 ± 10.18 and 197.5 ± 18.30 g DW m^{-2}) was 2-fold lower than that of *H. uninervis* (198 ±7.45 and 456 ± 9.59 g DW m^{-2}). *H. uninervis* leaves were 9-fold longer than that of *H. ovalis*, whereas *H. ovalis* leaves were 5-fold wider than *H. uninervis*. The leaf plastochrone interval is 2.3 days for *H. ovalis* and 9.6 days for *H. uninervis*. Consequently, the leaf growth rate of *H. ovalis* is 2-fold lower than that of *H. uninervis*. *H. ovalis* had 2.6-fold longer internodes than *H. uninervis*. The root length of *H. uninervis* was longer than *H. ovalis*.

Consequently, the shorter root length of *H. ovalis* led to higher branching frequency than *H. uninervis*. The total C and N content were higher in the leaves of *H. ovalis* than *H. uninervis*.

Keywords: Seagrass, plastochrone interval, *Halodule uninervis*, Odisha, coastal ecosystems, growth rate

Introduction:

The coastal ecosystems of India are diverse, consisting of mangroves, coral reefs, salt marshes, and seagrasses. The seagrass beds of India occupy a vast area consisting of estuaries, lagoons, bays, backwaters, and open sea (Jagtap, 1991; Parthasarathy et al., 1991; Thangaradjou and Bhatt, 2018; Mishra and Apte, 2021). Seagrass ecosystems of India are part of the global bioregion model five, which has the highest biodiversity of seagrasses (Short et al., 2007). India has 16 out of the 19 seagrass species found in Southeast Asia, covering an area of 516.59 sq. km up to a depth limit of 21 m (Bayyana et al., 2020; Geevarghese et al., 2018). The genus *Halophila* is most represented in the coastal ecosystems of India, consisting of seven seagrass species, i.e., *Halophila ovalis*, *Halophila ovalis ramamurthiana*, *Halophila beccarii*, *Halophila ovata*, *Halophila decipens*, *Halophila stipulacea*, and *Halophila minor* (Jagtap, 1991; Mishra and Apte, 2021; Thangaradjou and Bhatt, 2018). However, the seagrasses such as *H. ovalis*, *H. ovata* and *H. beccarii* are present around the entire coast of India, whereas the remaining *Halophila* species are mostly restricted to the southeast coast of India and the islands of Andaman and Nicobar (ANI) and Lakshadweep (Jagtap, 1991; Mishra and Apte, 2020; Mishra and Mohanraju, 2018; Parthasarathy et al., 1991). *Halophila ovalis* has the 4th highest (with 65 nos.) and *H. uninervis* the 8th highest (with 47 nos.) number of publications when compared to various other seagrasses of India (Mishra and Apte, 2021). These studies reveal that there is a 71% chance of the presence of *H. uninervis* around the *H. ovalis* beds from the entire coast of India (Fig.2), which suggests the ecological connectivity between these two species favours each other.

Even though the presence of seagrasses around the coast of India is mostly documented through the use of remote sensing (Geevarghese et al., 2018), new areas with seagrass presence are coming into the limelight, such as the recent report of *H. beccarii* from the coast of Kerala (Prabhakaran et al., 2020). However, due to increasing human-induced disturbances and coastal developments the seagrass beds of India are under decline (Kaladharan et al., 2013; Mishra and Apte, 2020; Mishra and Apte, 2021). In these scenarios, monitoring the current status of various seagrass beds of the country is essential for the management and conservation of these ecosystems (Ramesh et al., 2019; Mishra and Apte, 2021). Consequently, the lack of these monitoring efforts has also led to a critical knowledge gap on the population trends and growth patterns of various seagrass species of India (Thangaradjou and Bhatta, 2018; Mishra and Apte, 2020; Mishra and Apte, 2021).

On the east coast of India, the bulk of seagrass species diversity is present in the Gulf of Mannar and Palk Bay of Tamil Nadu with 14 species and the ANI with 13 species, whereas the Chilika lagoon in the state of Odisha has the third-highest seagrass diversity (Jagtap, 1991; Thangaradjou and Bhatt, 2018; Mishra and Apte, 2021) A total of nine seagrass species presence have been reported by various authors in the Chilika lagoon (Fig.1). However, the current report by the state nodal agency (Chilika Development Authority, CDA) has confirmed the presence of only five seagrass species from Chilika lagoon i.e., *Halophila ovalis*, *Halophila ovata*, *Halophila beccarii*, *Halodule uninervis*, and *Halodule pinifolia* (CDA, 2021). This discrepancy in the number of species can be due to the misidentification of seagrass species by various authors, which has been observed previously (Thangaradjou and Bhatt, 2018). Out of the nine seagrass species found in the lagoon, *H. ovalis* and *H. uninervis* are most abundant (Fig.1). Studies on the seagrasses of the Chilika lagoon include distribution and abundance (Jaikumar et al., 2011; Bhatta and Patra, 2018a & b; Finlayson et al., 2020), biomass (Pati et al., 2014), molecular ecology (Kar et al., 2018; Dilipan et al., 2020), carbon sequestration (Ganguly et al., 2017), and effects of environmental variables on seagrass (Priyadarsini et al., 2014; Sahu et al., 2014). However, these studies lack proper density, biomass, and morphometric values of the various seagrass species, which are essential indicators of seagrass health (Vieira et al., 2018; Ali et al., 2018). Consequently, the only morphometric studies on

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H. ovalis are recorded from the Haripur creek area, where *H. ovalis* was first recorded away from the Chilika lagoon (Mahapatro et al., 2014). Haripur creek is situated 32 km away from the southeast part of the Chilika lagoon (Fig.3).

Considering the basic knowledge gaps on seagrass research in the state of Odisha, the objective of the present study was to assess for the first time the population structure, i.e., density, biomass, morphometrics, leaf plastochrone interval, and leaf growth rates of *H. ovalis* and *H. uninervis* at the Haripur creek. The presence of *H. uninervis* is recorded for the first time from this intertidal area.

Methods:

Study site

Haripur creek is part of the Gopalpur beach area situated in the district of Ganjam in the state of Odisha and is 30 km away from Chilika lagoon (Fig.3). This creek is connected to the Bay of Bengal through a small channel, with a low tidal influence and shallow depth of 0.2 m. This creek is a unique ecosystem as the upper part of this creek is used for prawn aquaculture, while the lower part is used for boat parking that is used for fishing (Fig.4f). This creek area with seagrass is mostly used for fishing boat parking that uses the creek-sea mouth opening for venturing into the sea for fishing activities. The number of fishing boats anchored here is about ~75. This creek area is heavily polluted with various plastics and boat manufacturing debris and aquaculture waste (Fig.4d, e & f). Much of the creek area is modified by human activities such as platforms for fish drying along with a recent establishment of few prawn seedling factories that collect and rears prawn seeds and a permanent bridge (Fig.4c). The wastewater from this seedling factory is directly released into this creek area, where both seagrass species occur. There is a heavy growth of *Gracilaria verrucosa* on the *H. uninervis* meadows close to the shrimp seedling factory, that covers the seagrass meadows (Fig.4b).

However, there was no such algal growth on the *H. ovalis* beds. The *H. ovalis* patches were 10 m away from the *H. uninervis* patches towards the low tide line.

Sediment cores (n=10) were collected from each quadrat where seagrass was sampled using a 5cm diameter and 10 cm long plastic core. Sediments were collected in plastic bags and brought to the laboratory. In the laboratory, sediment samples and were oven-dried at 60°C for 72 hours before being sieved for grain size fractions (500µm, 150 µm, 75 µm, and 63 µm). Physical parameters such as pH, salinity, and temperature were measured in situ for the water column. For measuring salinity handheld probe (Salintest, HI98203, Hanna Instruments, the accuracy of ± 0.02) was used. For pH, conductivity, and temperature, a pH sensor (pH sensor, HI991300, Hanna Instruments, the accuracy of ± 0.02) was used. The nutrients such as inorganic nitrite, nitrate, and phosphate were analysed, following the method by Grasshoff et al., (2005).

Seagrass sampling and morphometric measurements

The sampling of seagrass was conducted by using the line and transect method. A quadrat of 20 cm x 20 cm and a hand shovel was used to dig out seagrass samples up to 10 cm depth in December 2020. Both seagrass species were mono-specific, with occasional growth of macroalgae. Ten quadrats of *H. uninervis* and *H. ovalis* were collected during low tide within a depth of 0.2m from a transect of 10 m x 15 m perpendicular to the beach. From each quadrat (n=10) both seagrass leaves, rhizomes and roots were collected in plastic bags and brought to the laboratory for further analysis. In the laboratory, the plant samples were washed again with double distilled water and the leaf epiphytes were scraped off by a plastic razor. Seagrass leaves were observed under a simple microscope to find out their unique identification features (Kuo and den Hartog, 2001). Shoot density (individual shoots per m^{-2}) was calculated by counting the total number of shoots per quadrat. The number of apex shoots for quadrats was counted to derive the apex density. Total density was calculated by adding the shoot and apex density. Horizontal rhizome length (n=15/quadrat) was measured for the rhizomes with the apex shoot attached. Leaf length (cm), and width (mm) from the shoot (n=20/quadrat) were measured using a Vernier Calliper (accuracy-0.02mm). The canopy height (cm) of *H. uninervis* and *H. ovalis*, i.e., the leaf length of the longest leaf from the sediment to the leaf tip was measured using a ruler (McKenzie, 2007). Root length (cm) and diameter (mm) were measured using the Vernier Calliper. From each quadrat (n=15) roots were selected at random to estimate the maximum root length and the number of primary and secondary ramifications were counted for each root. Then, the root branching frequency index (BFI) was calculated as the total number of ramifications (primary + secondary) divided by the total root length. After initial measurements, the plant parts were separated and oven-dried at 60°C for 48 hours to get the dry weight biomass (g DW m^{-2}). The above-ground (leaf biomass) and below-ground (rhizome + root) biomass were used to estimate the biomass ratios. Two mg of both seagrass leaves were analyzed using a CHNS analyzer (CHNS Analyzer, UNIQUBE, Elemental, Germany) for total carbon (C) and nitrogen (N) content.

Leaf Plastochrone interval and growth rate measurements

For both the seagrass the leaf plastochrone intervals were determined using the leaf marking techniques described by Short and Duarte, (2007). Two different techniques were used as *H. ovalis* belongs to the mono-meristematic non-leaf replacing seagrass species, where old leaves are not shed and *H. uninervis* belongs to di-meristematic leaf replacing form, where older leaves are shed (Short and Duarte, 2007).

H. ovalis leaf plastochrone interval

For obtaining the leaf growth rate, 20 shoots were marked for *H. ovalis* by clipping the end of the youngest pair of leaf blades with a scissor at a radical angle (Short and Duarte, 2007).

The number of leaves or apical meristems produced after 15 days of leaf marking was counted. The plastochrone interval of the leaf (PL) was counted using the following equation

$$PL = No. of\ days/No. of\ leaves\ produced$$

H. uninervis leaf plastochrone interval

The lower part of the outer leaf sheath of *H. uninervis* was pinched with a needle and a red tag was fixed near the rhizome to identify the marked leaf (n=20). The initial number of leaves on the vertical rhizome was counted. After 20 days the number of newly formed leaves without the pinched holes (with stems and adventitious roots) were counted on the existing shoots from the marked meristem. The plastochrone interval of the leaf (PL) was counted using the following equation

$$PL = \frac{N}{T1}$$

Where T_1 is the number of leaves produced in the marked rhizome along with the whole meristem and N is the number of days used for marking.

The leaf growth rate for *H. ovalis* and *H. uninervis* was calculated by dividing the average leaf length by leaf plastochrone interval of that species.

Statistics

One-way ANOVA was used to test the significant differences between *H. uninervis* and *H. ovalis* density, biomass, and morphometric features. Holm-Sidak posthoc test was used to derive the significance between the two groups. All data were pre-checked for normality and homogeneity of variance. Data were log-transformed when normality and homogeneity of variance were not achieved for raw data. Data are presented as mean and standard error (S.E.). *SIGMAPLOT* ver. 11 was used for the statistical analysis.

Results

The physico-chemical parameters of the Haripur creek area were similar for both seagrass species with no significant variation except for the sediment grain size fractions (Table 1). The pH values (7.81 ± 0.06) were lower than the standard oceanic condition of pH 8.2 (Table 1. The sand content of *H. ovalis* patches was 1.2-fold higher than *H. uninervis* beds, whereas the silt content of *H. uninervis* beds was 2-fold higher than *H. ovalis* patches (Table 1). The total carbon (C) and nitrogen (N) values in the leaves of *H. ovalis* and *H. uninervis* were not significantly different (Table 1). However, there were 1.6-fold higher N levels in the leaves of *H. ovalis* (N; 0.77%) than *H. uninervis* (N; 0.46%).

On the leaves of *H. uninervis* there was a growth of macroalgae of *Ceramium sp.* and *Gracilaria verrucosa* (Fig.4a), which was not observed on the leaves of *H. ovalis*. The identification of *H. uninervis* was confirmed due to the presence of a trident-like structure at the leaf tip, prominent central midrib, and a blacktip (Fig.5b). Leaf reddening was observed in the leaves of *H. ovalis* leaves, but not on the leaves of *H. uninervis* (Fig.5d).

The shoot density of *H. ovalis* (5004 ± 114.51 ind. m^{-2}) was significantly and 2-fold lower than that of the *H. uninervis* (11598± 187.52 ind. m-^{2}), whereas there were no significant differences in apex density between the two species (Fig.6A). Both above-ground (AG) and below-ground (BG) biomass of both species were significantly different (Fig.6B). The AG biomass of *H. ovalis* (96.34 ± 10.18 g DW m^{-2}) was 2-fold lower than that of *H. uninervis* (198 ±7.45 g DW m^{-2}), whereas the BG biomass of *H. ovalis* (197.55 ± 18.30 g DW m^{-2}) was 2.3-fold lower than that of *H. uninervis* (456 ± 9.59 g DW m^{-2}).

The morphometrics features of both the species were significantly different except few features such as horizontal rhizome length and root branching frequency index (Table 2). The number of leaves per ramet was higher in *H. uninervis* (4 nos.) than *H. ovalis* (2 nos.). The leaf length of *H. uninervis* (15.06 ± 0.32 cm) was 9-fold higher than *H. ovalis* (1.64 ± 0.05 cm), whereas the leaves of *H. ovalis* (0.67± 0.02 mm) were 5-fold wider than *H. uninervis* (0.13 ± 0.01 mm). The canopy height of *H. uninervis* (15.50 ± 0.21 cm) was 5-fold higher than *H. ovalis* (3.34 ± 0.12 mc). The plastochrone interval, i.e., the number of days required to produce a single leaf is 4-fold higher in *H. ovalis* (2.36 days) than that of *H. uninervis* (9.63 days). However, the leaf growth rate of *H. ovalis* (0.69 ± 0.01 cm day^{-1}) is lower than *H. uninervis* (1.53 ± 0.12 cm day^{-1}). Though the horizontal rhizome length was not significantly different between both species, it was longer in *H. uninervis* (18.67± 1.11 cm) than *H. ovalis* (14.31 ± 0.52 cm). Morphometric features like petiole length were only recorded for *H. ovalis* (3.34 ± 0.12 cm) and vertical rhizome length only for *H. uninervis* (1.11 ± 0.12 cm). The internode lengths of *H. ovalis* (4.21 ± 0.13 cm) were 2.6-fold longer than *H. uninervis* (1.81 ± 0.09 cm). The root length of *H. uninervis* (7.04 ± 0.17 cm) was longer than *H. ovalis* (5.33 ± 0.53 cm), whereas the root width was almost similar between the two species. Consequently, shorter root length of *H. ovalis* led to higher branching frequency than *H. uninervis* (Table 2)

Discussion

Seagrass ecosystems are keystone marine plant species that support a wide range of ecological functions and provides ecosystem stability. The documentation of seagrass presence in new coastal niches provides evidence that the seagrass ecosystems of India are yet to be properly explored. The present study recorded the presence of *H. uninervis* for the first time from Haripur creek of Odisha along with the previously recorded *H. ovalis* population (Mahapatro et al., 2014). *Halodule uninervis* may have migrated through plant fragmentation or seed dispersal to this creek area in recent times from the Chilika lagoon, where both these seagrass species are present (Pati et al., 2014; Pattnaik et al., 2020). The literature review of seagrass research in India indicates there is a 71% chance of the presence of *H. uninervis* beds, where *H. ovalis* is present (Fig.2). However, there is also a 71% chance of the presence of

Cymodocea serrulata along with *H. ovalis*, but we did not observe the presence of *C. serrulata* in our field survey. Interestingly, this ecological association was also observed in the islands of Lakshadweep (Jagtap, 1998) and ANI (Sachithanandam et al., 2014; Savurirajan et al., 2018a; Mishra et al., 2021) which differs in sediment dynamics from the mainland coast of India. This unique association between *H. ovalis* and *H. uninervis* sustains on *H. ovalis* changing the redox potential of sediments and making them more suitable for colonization of other seagrass species (Kaewsrikhaw et al., 2016). The ecological relevance of this association can be further explored for seagrass research in India.

The nitrate and phosphate concentration of the Gopalpur creek was higher than the nutrient-limited adjacent Bay of Bengal coastal waters (Paul et al., 2008). This increase in nutrient concentration can be related to the anthropogenic discharge of wastewater from the prawn seedling factories directly to the creek water (Sahu et al., 2013). These high nutrient levels lead to the rapid growth of *G. verrucosa* on the rhizome and leaf surface of *H. uninervis*, as the nitrate levels of (1.64 mg L^{-1}) observed in our study area is 1.3-fold higher than the cultivation grounds of *G. verrucosa* in the Chilika lagoon (Padhi et al., 2011). Along with nutrient levels, the vertical rhizome network and longer leaves of *H. uninervis* also provide a suitable habitat for this macroalgae to grow better (Han and Liu, 2014). This growth of macroalgae can lead to inter-specific competition resulting in depleted levels of nitrogen in seagrass leaves, which was evident in the leaves of *H. uninervis* (N; 0.46%). This N concentration is below the threshold level (<1.8% N) for nutrient limitation in seagrass (Duarte, 1990). Consequently, no such algal growth was observed on the leaves of *H. ovalis*, probably due to shorter leaves and lack of vertical rhizomes providing suitable habitat for the algae to grow. However, this high nutrient input may provide limited benefits to *H. ovalis* as the leaves of *H. ovalis* are losing chlorophyll to leaf reddening as an adaptation to high ultraviolet light.

This phenomenon of nutrient enrichment providing fewer benefits to *H. ovalis* was observed by Kilminster et al., (2006).

High silt content favours the growth of *H. uninervis* than *H. ovalis*, as higher silt content than 15% of the sediment fraction leads to a decrease in *H. ovalis* leaf biomass probably due to burial of the shorter leaves of *H. ovalis*. This phenomenon has been observed for the *H. ovalis* population of Havelock islands of ANI (Mishra et al., 2021). Secondly, the *H. ovalis* population at Haripur creek was present close to the low tide line, whereas *H. uninervis* was close to the high tide line, where the impact of land run-off may be higher leading to high silt content. Consequently, there was continuous dredging of the nearby area in the last few years for building a bridge (Fig.4c), which could have also altered the local sediment dynamics.

The depth distribution limit of *H. ovalis* and *H. uninervis* from the coast of India ranges from the intertidal region to a depth of 3 m (Table 3). However, both *H. ovalis* and *H. uninervis* are present at >1 m depth only in the waters of Lakshadweep islands, where the water clarity and light penetration is higher than the mainland coast (Ansari et al., 1991; Jagtap, 1998; Jagtap et al., 2003). In the present study, both the seagrass was present at a depth of 0.2 m, which is similar to the depth limit of *H. ovalis* and *H. uninervis* around the Chilika lagoon and also other areas of the east coast of India like Palk Bay and the Gulf of Mannar (Jagtap, 1996; Mathews et al., 2010) and ANI (Mishra et al., 2021). However, the southern sector of the Chilika lagoon has low turbidity where *H. ovalis* and *H. uninervis* are found at 1.4 m and 1.8 m depth respectively (Ganguly et al., 2018). Being present at such low depths of 0.2 m, leads to exposure of *H. ovalis* leaf surface to prolonged ultraviolet light, resulting in leaf reddening, which has been observed for *H. ovalis* in our study and also from the islands of ANI, where the seagrass is present in shallow intertidal depth (Ragavan et al., 2013; Mishra et al., 2021).

Density and biomass are important indicators of seagrass health and ecosystem status (Ali et al., 2018; Vieira et al., 2019). Though there are 71 publications from the coast of India for *H. ovalis* and 51 nos. for *H. uninervis* (Mishra and Apte, 2021), there is a significant lack of density and biomass values of both seagrasses (Table 3). The density of *H. ovalis* from the coast of India ranges from 15 -3950 shoots m^{-2} (Table 3). However, the density of *H. ovalis* from the Haripur creek area is 1.5-fold higher than the highest density (3950 shoots m^{-2}) values recorded for India (Jagtap, 1996). This could be due to the high availability of nutrients and low impact of physical disturbance on the species at Haripur creek location, whereas the Palk Bay region has higher pollution and physical disturbances.

The density of *H. uninervis* at the Haripur creek area is similar to the density of *H. uninervis* around the Indian coast (Table 3). The density of *H. uninervis* at Palk Bay (10500 shoots m^{-2}) is similar to our results, whereas the density of *H. uninervis* at ANI (12,245-16,325 shoots m^{-2}) is 1.4-fold higher than Haripur creek (Table 3). The higher density of *H. uninervis* in the ANI is a direct result of low turbidity and high light penetration with tropical climatic conditions (Savurirajan et al., 2018), whereas the Haripur creek has a seasonal influence of monsoon and its associated changes along with algal growth on *H. uninervis* leaf surface.

The total biomass (AB + BG) of *H. ovalis* at the Haripur creek is 1.7-fold higher than the previous record biomass of *H. ovalis* from the Chilika lagoon, whereas it is 1.3-fold lower than the biomass of *H. ovalis* from the Palk Bay region (Table 3). However, different areas of Palk Bay and GOM have 19-fold and 79-fold (only for GOM) lower biomass of *H. ovalis* than that of the Haripur creek area (Mathews et al., 2010a, 2008; Manikandan et al., 2011c). Consequently, in the islands of ANI, the biomass of *H. ovalis* is 29-fold lower than that of the biomass of *H. ovalis* at the Haripur creek. This suggests there is a spatial variation of *H. ovalis* total biomass around the coast of India. The biomass of *H. ovalis* is dependent on the growth and density of the patch which positively correlates with the higher density and biomass at the

Haripur creek. Secondly in India, higher seagrass biomass for the smaller seagrass (like *H. ovalis*) species is mostly restricted to the upper intertidal zone (Jagtap et al., 2003) and our results agree with this finding.

The total biomass of *H. uninervis* in our study is 4.3-fold higher than the total biomass of *H. uninervis* in the Lakshadweep islands (Jagtap et al., 1998). Interestingly, the BG-biomass of *H. uninervis* in the Lakshadweep islands were 80-fold lower than our studies (Table 3). However, the AB-biomass is similar without studies (Ansari et al.,1991; Jagtap et al., 1998). On the other hand, the total biomass of *H. uninervis* reported by Nobi et al., (2011) is 20-fold lower than our biomass values, which suggests that the *H. uninervis* biomass of the Lakshadweep islands has a huge variation resulting from the seasonal influence and herbivore grazing. The total biomass of *H. uninervis* from Palk Bay and the GOM region are 15 to 300-fold lower than our studies, (Mathews et al., 2008; Mathews et al., 2010a; Manikandan et al., 2011c). These differences may be due to the significantly lower density of *H. uninervis* at these locations (Table 3). The total biomass of *H. uninervis* from the Chilika lagoon is 13-fold lower than our results (Pati et al., 2014) suggesting the Haripur creek area has a dense patch of *H. uninervis* even though these patches are smaller in size.

In the case of the morphometric features of both *H. ovalis* and *H. uninervis*, there are two reports on *H. ovalis* morphometric features, where there is no data on *H. uninervis* morphometric features (Table 3). The leaf length and width of *H. ovalis* in our current study are 2.5-fold and 1.3-fold lower than previously reported from the Haripur creek (Mahapatro et al., 2014). However, Mahapatro et al., (2014) reported that the leaf length of *H. ovalis* was 4-12.6 cm long, which seems to be impossible considering the small size of *H. ovalis* leaves. We think this was an error in measurement or unit selection. However, for *H. ovalis* a change in leaf length (ca.2-4 cm) is expected, as *H. ovalis* patches move up and down the intertidal zone depending on the seasonal and light influence (Kaewsrikhaw and Prathep, 2014; Kaewsrikhaw et al., 2016) . When *H. ovalis* is present in the upper intertidal zone, where high light intensity and desiccation is higher, leaves tend to be smaller in size but with higher plant densities for plant protection, which is considered as an adaptation of the *H. ovalis* population growing in the upper intertidal zone (Kaewsrikhaw and Prathep, 2014; Kaewsrikhaw et al., 2016) . However, the previous authors mentioned the presence of this seagrass at lower intertidal regions (Mahapatro et al., 2014), which we did not observe in our current study because of the habitat modifications caused during the last 7 years that have restricted the *H. ovalis* patches to certain areas. The morphological adaptation (smaller leaves and high density) observed in our results was also reported for the *H. ovalis* population from the coast of Thailand (Kaewsrikhaw et al., 2016; Kaewsrikhaw and Prathep, 2014). Consequently, the presence of leaf reddening in the *H. ovalis* population at our study locations indicates the adaptation of *H. ovalis* to high ultraviolet light stress, where the production of anthocyanins (resulting in leaf reddening) not only protects the seagrass leaves from high irradiance but also maintains high levels of plant photosynthesis(Novak and Short, 2011)

The plastochrone interval of *H. ovalis* (2.3 days) and *H. uninervis* (9.82 days) is similar to the global leaf plastochrone interval values for both *H. ovalis* (2.2 days) and of 9.6 days for *H. uninervis* (Hemminga and Duarte, 2000). The plastochrone interval of *H. ovalis* from our studies was 1.2-fold higher than previously reported from Thailand for *H. ovalis* (4.4 days) for production of a single leaf (Kaewsrikhaw et al., 2016). This high growth rate can be a result of adequate nutrient levels at the Haripur creek area. The leaf growth rate of *H. ovalis* (0.69 cm day-1) and *H. uninervis* (1.53 cm day-1) are reported for the first time in India.

Conclusion

Halophila ovalis and *H. uninervis* are two of the widely distributed seagrasses of the coast of India. Our study reported the presence of *H. uninervis* for the first time from the

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intertidal creek of Haripur from the east coast of India. Our results provide a comprehensive overview of the density, biomass, morphometrics, and leaf plastochrone interval and growth rates for both seagrass species. There is a clear indication that the seagrass patches of *H. ovalis* and *H. uninervis* of the Haripur creek area have high density and biomass compared to other locations of India and the adjacent Chilika lagoon. Even though the Haripur creek area has undergone significant human-altered ecosystem modifications in the last decade, the seagrass ecosystems are in good health. However, proper management and monitoring of these seagrass ecosystems are essential for maintaining the ecosystem services these seagrasses provide to the local commercially important bivalves of the Haripur creek and for the livelihood of the local communities that depend on the prawn and bivalve seedlings.

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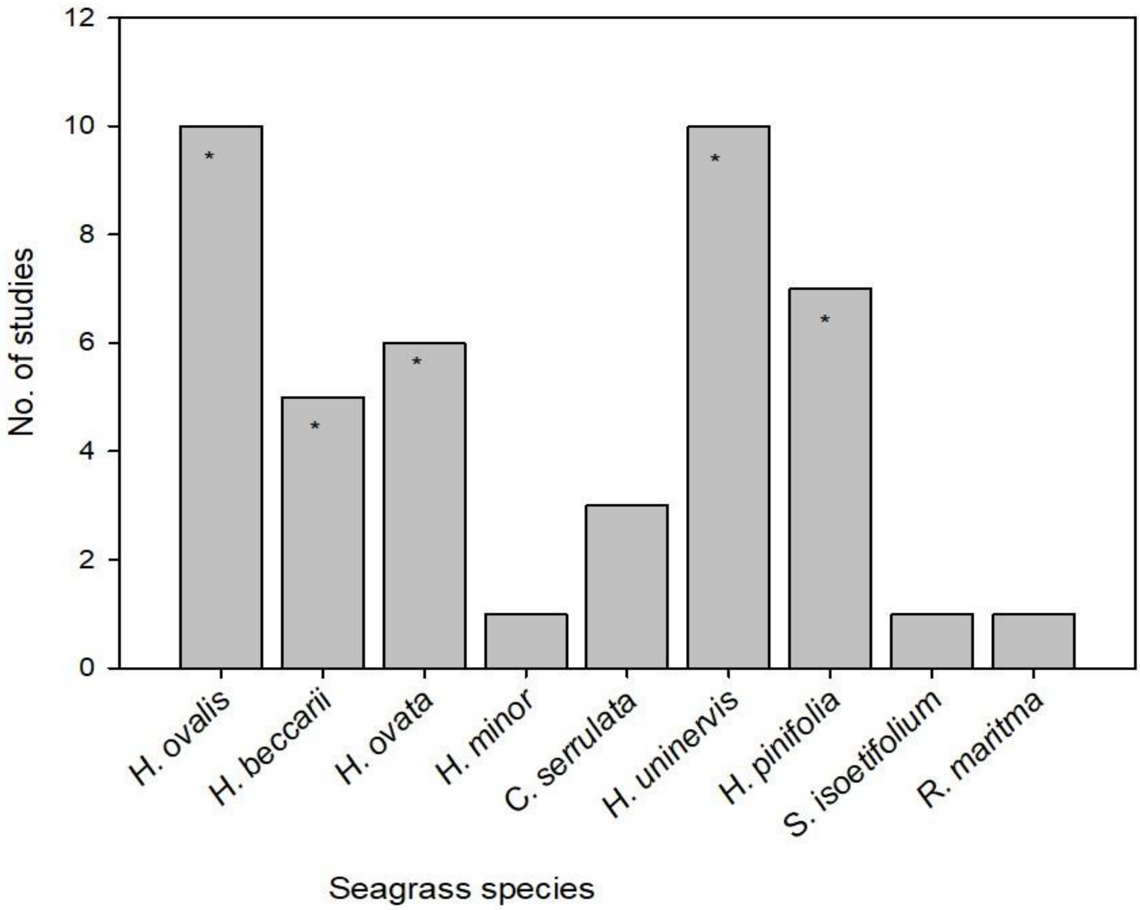

Fig.1. Various seagrass species reported from Chilika lagoon, Odisha derived from literature review. (*) sign indicates the five seagrass species that was reported from Chilika lagoon in 2021 by the nodal agency; Chilika Development Authority of the state Odisha.

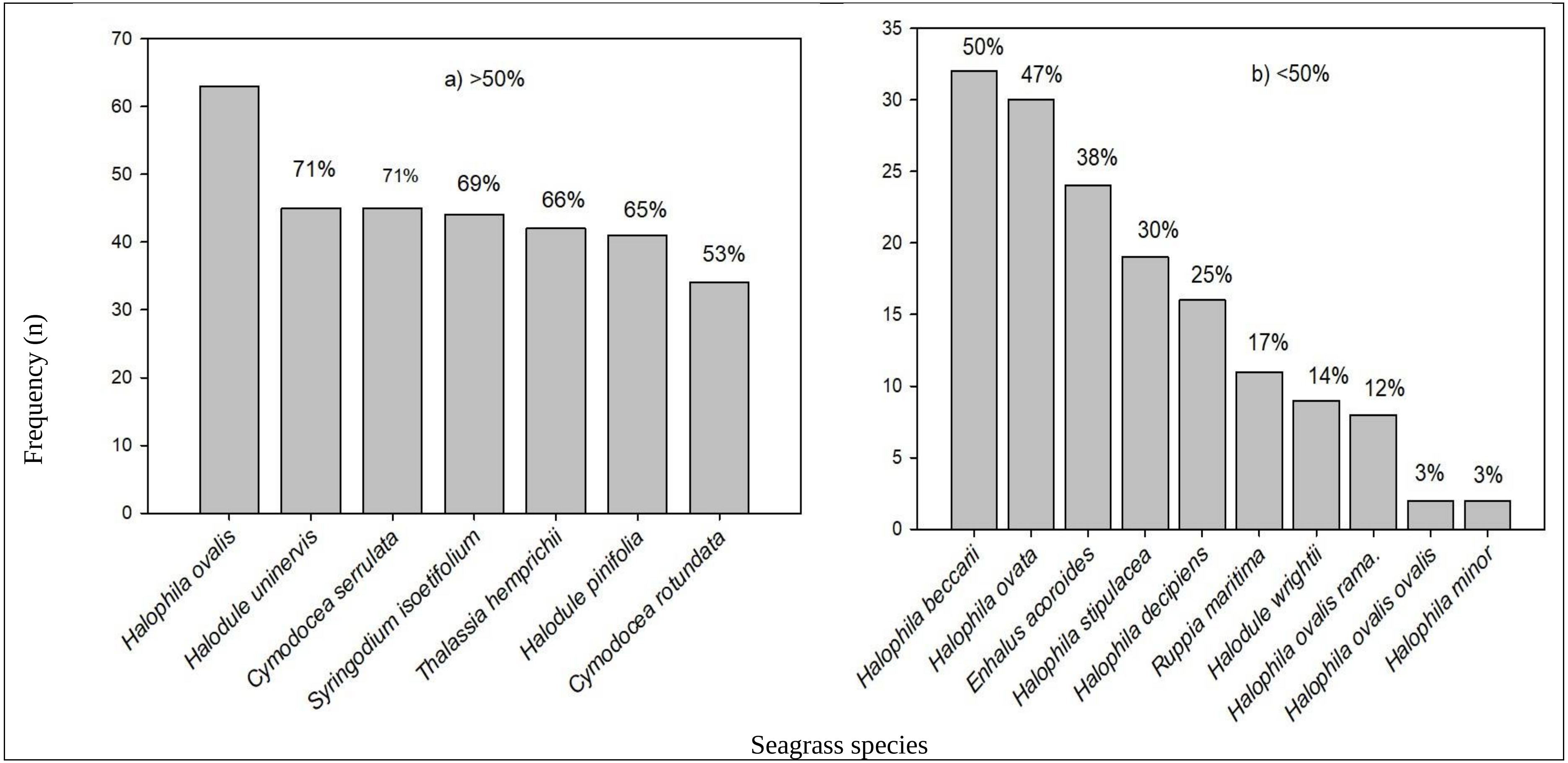

Fig.2. The percentage chance of presence *H. uninervis* and other seagrass species adjacent to *H. ovalis* beds from the coast of India derived from literature review. The percentage value on top of each bar represents the chance of that seagrass species found to be associated with *H. ovalis* beds from the coast of India.

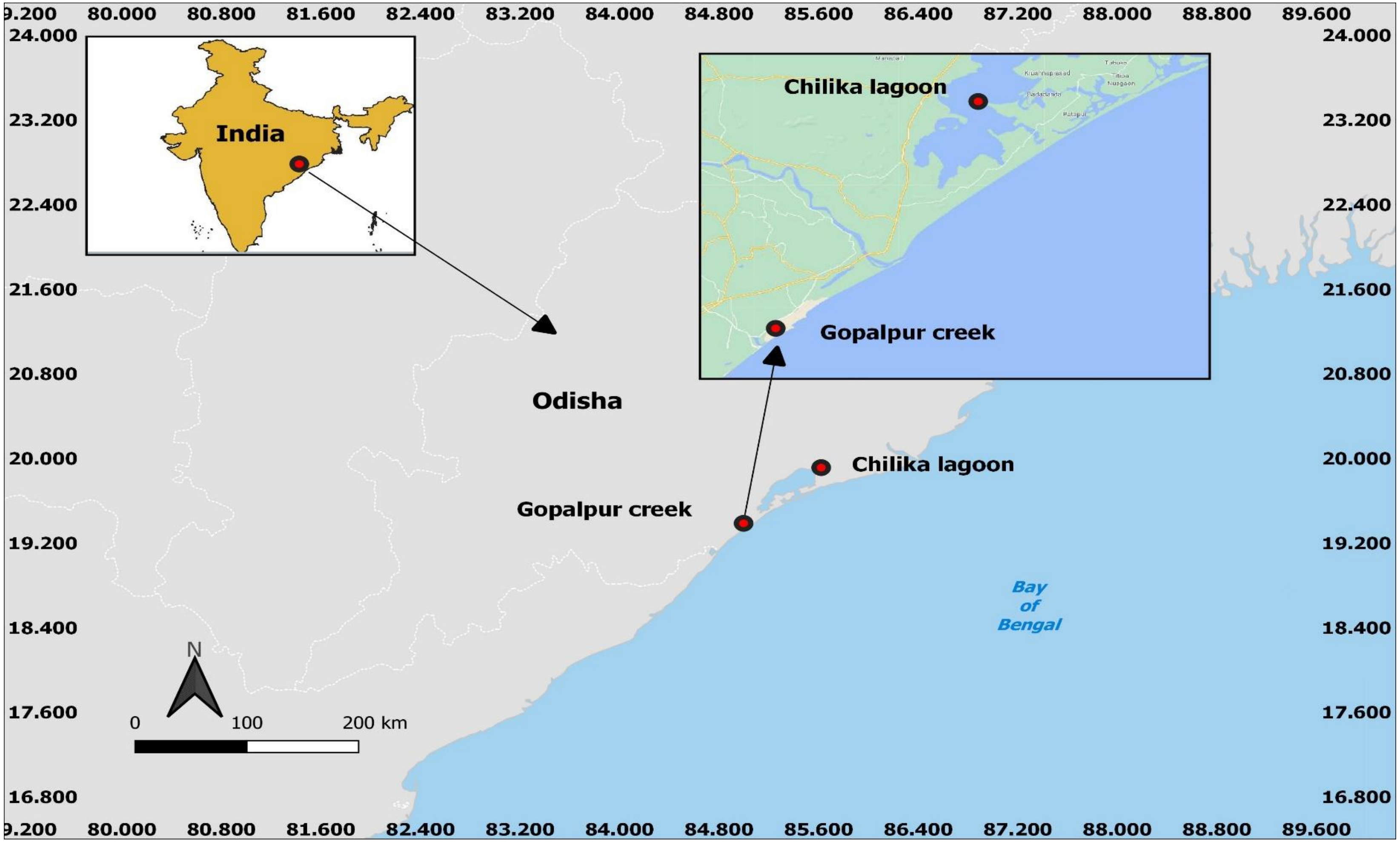

Fig. 3. Map showing the study area of Gopalpur creek area in the state of Odisha.


a
b
c
d

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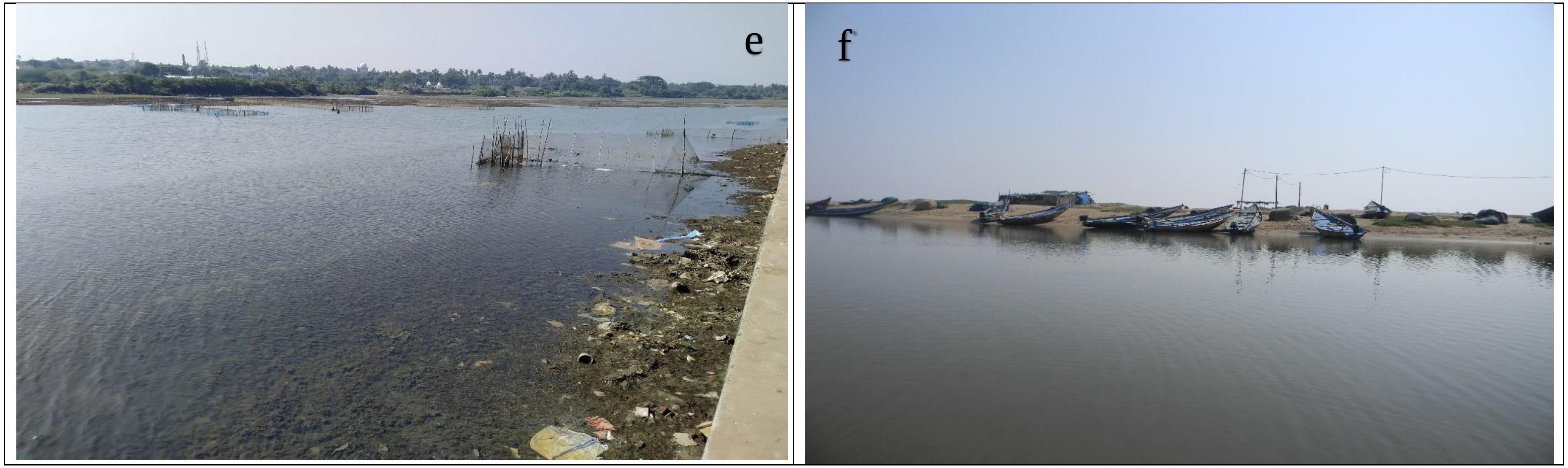

Fig.4. The state of the Haripur creek area with a) algal growth of *Ceramium* sp. on *H. uninervis* leaves, b) growth of *Gracilaria verrucosa* on *H. uninervis* meadows, c) newly constructed bridge on the creek that have devastated the ecosystem, d) anthropogenic pollution and algal growth upstream the seagrass meadows, e) prawn gharries and pollutants derived from boat making and f) boats parked on the opposite side of seagrass meadows.

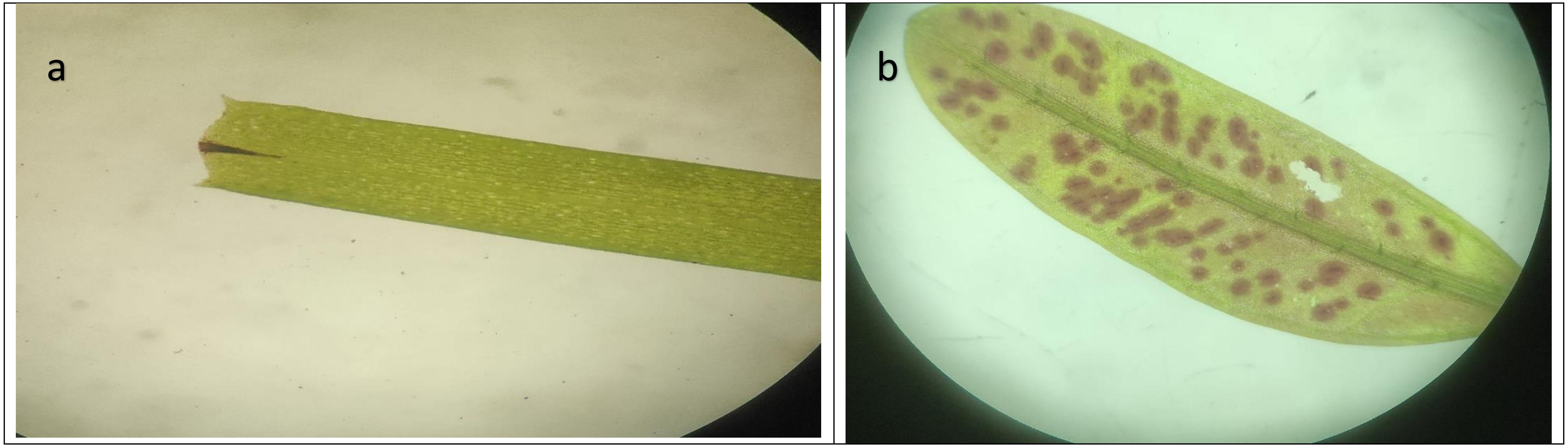

Fig.5. a) *Halodule uninervis* key identification features; trident teeth at the leaf tip; prominent central leaf midrib and b) leaf reddening in the leaves of *H. ovalis.*

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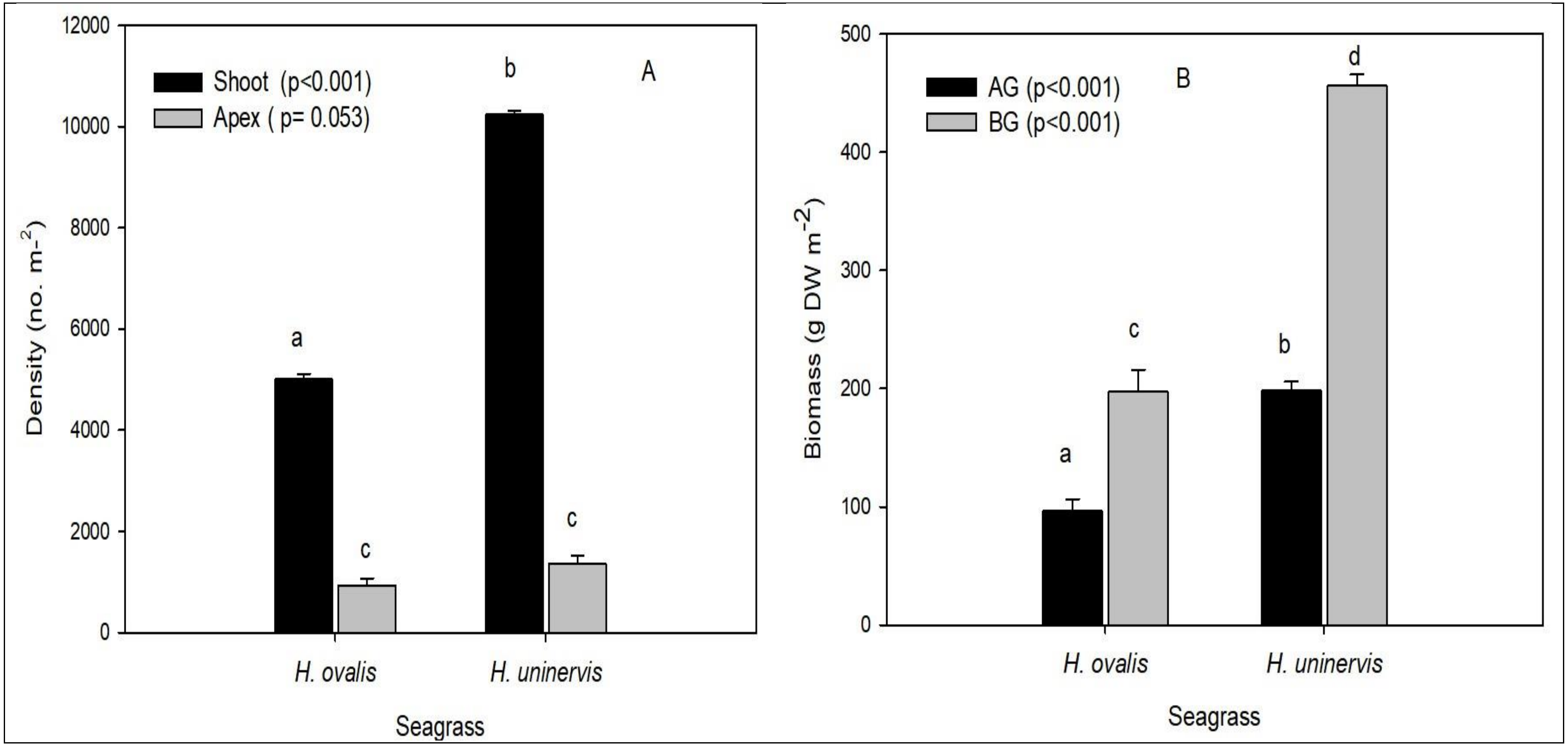

Fig.6. A) Density (shoot and apex) and B) biomass (Above ground, AG and Below ground, BG) of *H. ovalis* and *H. uninervis* at the Haripur creek area. Small letters indicate significant difference between density and biomass derived from one-way ANOVA analysis.

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Table. 1. Sediment grain size and physico-chemical parameters of the Haripur creek location. *P* value derived from one way ANOVA analysis only for sediment grain size and Total Carbon and Nitrogen values.

Variables			***H. ovalis***	***H. uninervis***	**p-value**
Grain size (%)	Sand	-	84.77 ± 0.54	68.57± 0.21	<0.001
	Silt	-	15.22 ± 0.67	31.42± 0.34	<0.001
pH		7.81 ± 0.06	-	-	-
Salinity (psu)		28.51 ± 0.31	-	-	-
Temperature (ºC)	Air	27.40 ± 0.84	-	-	-
	Water	29.10 ± 1.54	-	-	-
Dissolved Oxygen (mg l^{-1})	Water	6.20± 2.01	-	-	--
Conductivity (Ms cm^{-1})	Water	47.45 ± 2.15	-	-	-
Nitrite (mg l^{-1})	Water	0.17 ± 0.04	--	-	-
Nitrate (mg l^{-1})	Water	1.64 ± 0.14	-	-	-
Phosphate (mg l^{-1})	Water	0.76 ± 0.16	-	-	-
Total Carbon (%)	Leaf		39.31 ± 0.31	35.98± 0.12	0.493
Total Nitrogen (%)	Leaf		0.77 ± 0.01	0.46 ± 0.01	0.095
C:N ratio	Leaf		51.15 ± 0.21	71.84 ± 0.43	0.126

Table 2. Morphometric variables of *H. ovalis* and *H. uninervis* of Haripur creek of Odisha.

Root Branching Frequency Index (BFI), No values (nv), not tested (nt)

Variables		***H. ovalis***	***H. uninervis***	***p-value***
No. of leaves ramet^{-1}		2	4	<0.001
Leaf	Length (cm)	1.64 ± 0.05	15.06± 0.32	<0.001
	Width (mm)	0.67 ± 0.02	0.13 ± 0.01	<0.001
Petiole length (cm)		1.69 ± 0.09	nv	nt
Canopy height (cm)		3.34 ± 0.12	15.50± 0.21	<0.001
Horizontal rhizome length (cm)		14.31 ± 0.52	18.67 ±1.11	0.088
Vertical rhizome length (cm)		nv	1.11 ±0.12	nt
Internode length (cm)		4.21 ± 0.13	1.80 ± 0.09	<0.001
Root	Length (cm)	5.33 ± 0.53	7.04 ± 0.17	<0.001
	Width (mm)	0.12 ± 0.00	0.14±0.0	0.111
	BFI	6.14 ±0.58	5.02 ± 0.25	0.137
Plastochrone interval (days)		2.36	9.83	<0.001
Leaf growth rate (cm day-1)		0.69 ± 0.01	1.53 ± 0.12	<0.001

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Table 3. Mean or range values of density, biomass and morphometric features of *H. ovalis* and *H. uninervis* derived from the literature review and **this study**. Above ground (AB), Below ground (BG), Palk Bay and Gulf of Mannar (GOM) of Tamil Nadu, Chilika lagoon and Haripur creek of Odisha, Andaman and Nicobar Islands (ANI)

Location	**Seagrass**	**Depth (m)**	**Density (ind. m^{-2})**	**Morphometrics**				**Biomass (g DW m^{-2})**			**Reference**
				Leaf length (cm)	**Leaf width (mm)**	**Canopy height (cm)**	**Horizontal Rhizome Length (cm)**	**AB**	**BG**	**Total**	
Lakshadweep islands	*H. ovalis*	2	-	-	-	-	-	81	-	-	Ansari et al., 1991
Lakshadweep islands	*H. ovalis*	3	-	-	-	-	-	170.6	5	175.6	Jagtap et al., 1998
Palk Bay, GOM	*H. ovalis*	0.4	3950	-	-	-	-	-	-	-	Jagtap, 1996
GOM	*H. ovalis*	-	15	-	-	-	-	-	-	10	Mathews et al., 2008
Palk Bay, GOM	*H. ovalis*	0.2	29.93	-	-	-	-	-	-	10.25	Mathews et al., 2010a
ANI	*H. ovalis*	-	-	-	-	-	-	-	-	3.4	Thangaradjou et al., 2010b
GOM	*H. ovalis*	-	20	-	-	-	-	-	-	2.5	Manikandan et al., 2011c
Chilika lagoon	*H. ovalis*	-	-	-	-	-	-	-	-	113	Pati et al., 2014
Chilika lagoon	*H. ovalis*	1.4	-	-	-	-	-	-	-	-	Ganguly et al., 2017

Haripur creek	*H. ovalis*	-	-	4-12.6	0.8	-	-	-	-	-	Mahapatro et al., 2014
ANI	*H. ovalis*	0.5	391.7-451.3	1.05-2.02	0.94-1.23	1.15-2.20	9.05-15.26	0.71-1.68	2.72-4.14	3.43-6.82	Mishra et al., 2021
Palk Bay	*H. ovalis*	-	-	-	-	-	-	-	-	200-275	Thangaradjou et al., 2015
Haripur creek	***H. ovalis***	**0.2**	**5941**	**1.64**	**0.67**	**3.34**	**14.31**	**96.34**	**197.55**	**293.90**	**This study**
Lakshadweep islands	*H. uninervis*	2	-	-	-	-	-	179	-	-	Ansari et al., 1991
Palk Bay, GOM	*H. uninervis*	0.8	10500	-	-	-	-	-	-	-	Jagtap, 1996
Lakshadweep islands	*H. uninervis*	3	-	-	-	-	-	115.9	5.7	121.6	Jagtap et al., 1998
Lakshadweep Islands	*H. uninervis*	-	-	-	-	-	-	-	-	34.78	Nobi et al., 2011
GOM	*H. uninervis*	-	45	-	-	-	-	-	-	42	Mathews et al., 2008
Palk Bay, GOM	*H. uninervis*	0.5	59.06	-	-	-	-	-	-	18.28	Mathews et al., 2010a
GOM	*H. uninervis*	-	18	-	-	-	-	-	-	2	Manikandan et al., 2011c
Chilika lagoon	*H. uninervis*	1.8	-	-	-	-	-	-	-	-	Ganguly et al., 2017
Chilika lagoon	*H. uninervis*	-	-	-	-	-	-	-	-	47.4	Pati et al., 2014
ANI	*H. uninervis*	-	12,245-16,325	-	-	-	-	-	-	-	Savurirajan et al., 2018a
Haripur creek	***H. uninervis***	**0.2**	**11598**	**15.06**	**0.13**	**15.50**	**18.67**	**198**	**456**	**654**	**This study**

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