Multi-temporal mapping and recent structures of seagrass community in Panjang Island

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Abstract. This study aims to map the multi-temporal variation of seagrass beds and describe the existing conditions of aquatic habitats and biota associated with the seagrass ecosystem in Panjang Island, Central Java Indonesia. Mapping was completed by combining RGB (red, green, blue) corrected water column with the Lyzenga formula and watershed classification using supervised classification. Field observations were carried out by snorkeling and transect squared with Line Intersect Transect (LIT), the isolation of bacteria and fungi was carried out by dilution and serial tapping methods. Analysis of multi-temporal satellite imagery Landsat 5, Landsat 7, and Sentinel 2A shows that from 2001 to 2012 the area increased by 1.30 ha and lost 0.78 ha. Observations between 2012 and 2019 resulted in an increase of 0.99 ha and a decrease of 0.65 ha. Seagrass beds near the jetty have an average coverage of 61% and are dominated by three species, namely *Cymodocea serrulata*, *Cymodocea rotundata*, and *Thalassia hemprichii*. The highest abundance of seagrass-related macro-organisms was the genus *Holothuria*, followed by the seagrass-related microorganisms obtained from the leaves, which consisted of bacteria and fungi with 49 and 17 isolates, respectively. The results showed that the decline in seagrass beds had an impact on the diversity of the species itself.
Key Words: benthic mapping, Jepara, seagrass, Sentinel 2-A.

Introduction. Panjang Islands is one of the small islands located in Jepara Regency, Central Java Province. The existence of this island becomes very important due to the seagrass beds surrounding the coastal water (Bappeda Jepara 2013). Alongside their role as the center of diversity for vertebrates and invertebrates, which includes their spawning areas, the nursery ground, the feeding ground, the shelter for fishes and shrimps, seagrass ecosystems also play a major part as the source of income for the local fishermen, the storage of organic carbon, and the natural protection from coastal abrasion (Griffiths et al 2020; Citra et al 2020).

A study in 2014 reveals the sedimentation rate in Panjang Island has influenced the density of seagrass beds. Hidayat et al (2014) found five species in their study area using quadrat transect (1 x 1 m) method which was *Thalassia* sp. (65.292%) as the dominant species, followed by *Cymodocea* sp. (18.539%), *Enhallus* sp. (6.009%), *Syringodium* sp. (5.512%) and *Halodule* sp. (4.556%) in one sampling point with three replicates. Furthermore, seagrass bed monitoring is difficult to use as a representative of overall habitat because of the limitation of sampling points, therefore only a few selected pieces of information can be taken as representative of those ecosystems (Rohmann et al 2005). Multispectral and hyperspectral satellite data have a visible infrared wave sensor, which is more effective to distinguish the basic characteristics of water. In addition, direct surveys in the waters require more time, energy, and costs, especially if the water has a broad range. Thus, the use of sensory mapping technology and the application of Geographic Information Systems (GIS) is expected to provide a higher level of efficiency and accuracy of ecosystems condition (Botha et al 2013; Zhang & Xie 2014). The seagrass-associated microorganisms were coming from the water column and trapped on to the body of seagrass. The existence of seagrass-associated microorganisms has many

functions for the plant itself such as for growth, survival and decomposition (Kirichuk & Pivkin 2015; Vandenkoornhuyse et al 2015), as well as plant defense mechanisms (Sanchez-Cañizares et al 2017). In addition, seagrass serves as foraging for organisms and as a shelter, waves buffer and sediments trap which further increase the water clarity (Larkum et al 2006).In addition to provide shelter and food, seagrass ecosystems also can reduce exposure to bacterial pathogens of fishes, invertebrates and humans (Lamb et al 2017).

To the best of our knowledge, there was no report yet concerning the mapping condition of seagrass beds in Panjang Island. This study aims to map multi temporal variation of seagrass beds area, to obtain the structure community in the field, to describe the existing conditions of aquatic habitats and the associated biota with seagrass ecosystem in Panjang Island, Jepara.

Material and Method

Materials. The materials used in this study were seagrass samples, benthic organisms such as sea cucumbers, gastropods, and crabs collected from Panjang Island's coastal waters. On the other hand, the seagrass associated microorganisms were isolated to analyze the dominant species that connected to seagrass communities. Sentinel 2A multi-spectral satellite imagery data from 2001 to 2019 was used to analyze underwater habitat and seagrass cover.

Sampling site. The study site was Panjang Island, Jepara Regency, Central Java Province, Indonesia. It was located beetwen 6°35'2.70" and 6°33'48.18"S latitude and 110°35'45.10" and 110°38'29.28" E Longitude (Figure 1).
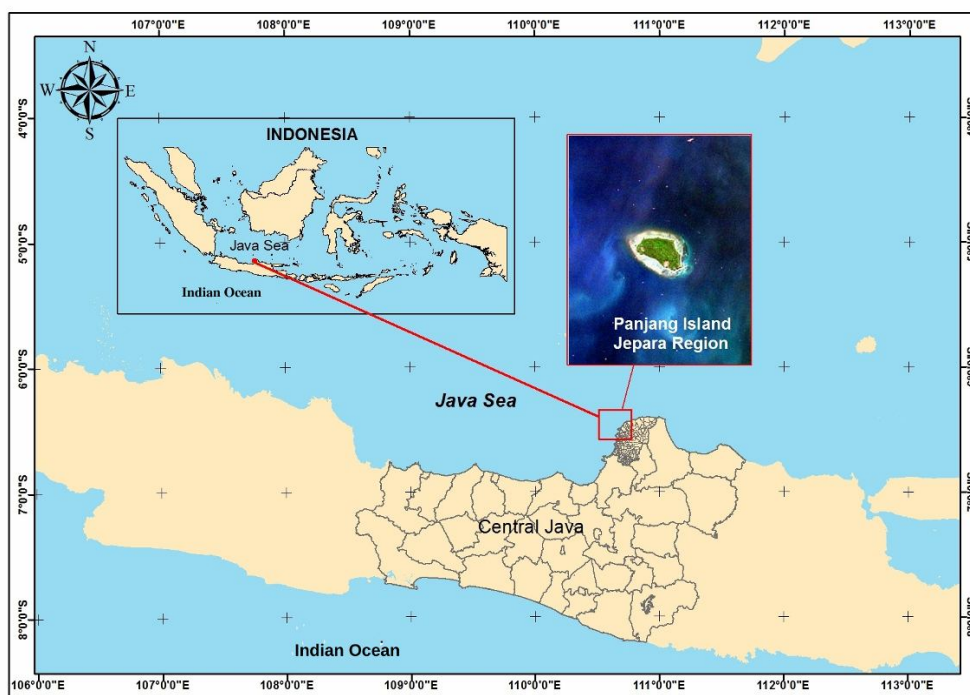

Figure 1. Map of study area in Panjang Island, Jepara Regency, Indonesia.

Field data collection. The field data was collected from 13 to 15 June 2019 based on methods stated in English et al (1997) and Delzer & McKenzie (2003). This data was used because it corresponded to the tidal period at that location. Six sampling points using line transect were used as field data for seagrasses. The length of the line transect was 30 meters, moreover, every 10 meters interval was observed with the quadrats transect (0.5 m x 0.5 m) to collect the form of seagrass samples. The study area was a small island which had sand and seagrass distribution as basic habitat in its southern part, with the distribution of patches at a depth of 0.3-1.7 meters.

Remote sensing data and image processing. Sentinel 2A/B (10 m spatial resolution) image with twelve multispectral bands obtained from Europe Space Agency (ESA) (Table 1) and an image acquisition 18 July 2018 was used in the study. The image was re-projected into a UTM zone 49S and was geometrically corrected by using 6 ground control points (GCPs) that combined with Fine Earth Indonesia map (RBI) (Roelfsema et al 2018). Land and sea masking were carried out to separate the land digital number (DN) of the land and its vegetation as well as water information, where the land DN was given a zero value and the waters had a value of 1. Image classification used the Maximum Likelihood classification algorithm with the classification parameters using supervised classification. This method has the advantage of being able to calculate variance-covariance based on the class distribution. The flowchart of this process was described in Figure 2.

Table 1

Information on depth invariant bands calculated from the three Sentinel 2A

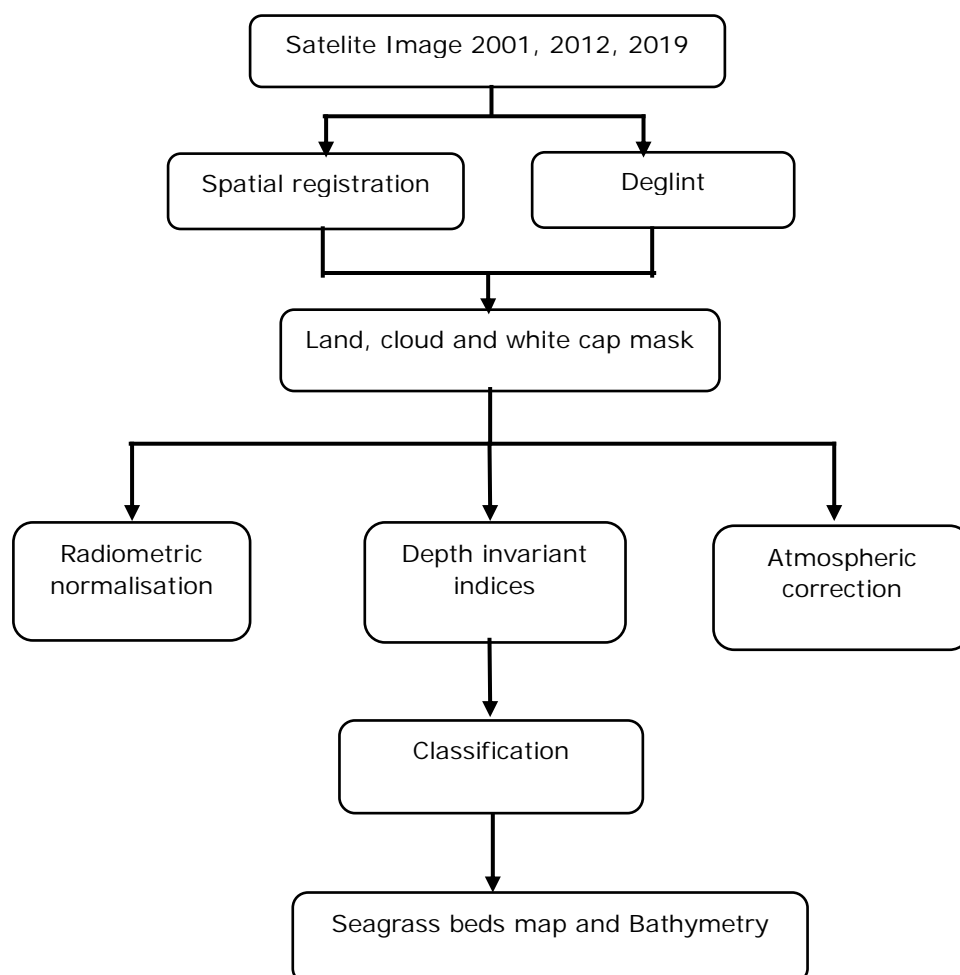| Depth invariant bands	Determination coefficient (R^2)	Equation of sunglint correction
B23	0.8688	B2-(1.0241*(B8-115))
B32	0.8773	B3-(1.0851*(B8-115))
B42	0.8630	B4-(0.9186*(B8-115))

Figure 2. Flow chart processing satelite.

Bathymetry mapping. The bathymetry map was made based on single beam echosounder data and a combination with the Sentinel 2A inversion method (Hedley et al 2009). The employed method was SWAM (Shallow Water Semi Analytical Model), which was processed using ESA SNAP software. In processing the bathymetry data from the

echosounder results, it was necessary to make corrections with the mean sea level (MSL) based on the tides issued by the Semarang Maritime Station as part of Meteorological, Climatological, and Geophysical Agency (BMKG), Indonesia. To obtain leveraging results, a linear regression was carried out between the echosounder germination data and the sentinel 2A image results.

Analysis data of community structure. The samples were collected using quadrats transect method to estimate the coverage area. The diversity of seagrass species of each sites was calculated using Shannon-Wiener diversity index.

Diversity assessment was expressed by Shannon index as:

$$H = -\sum_{i=1}^{n} \frac{n_i}{N} log2 \frac{n_i}{N}$$

where: N = total individuals of all species; n = species number; ni = number of each species; H = Shannon diversity index.

The evennes index is calculated using Simpson's Index as:

$$E = \frac{H'}{H_{max}} \quad H_{max} = log\ S$$

where : E = evennes index; H': Shannon diversity index; $H_{max} = log\ S$ (S = total species)

Dominance index was calculated using the following formula:

$$D = \sum_{i=1}^{n}(pi)^2$$

where: D = Simpson dominance index; S = total species.

Seagrass associated-macroorganisms. The seagrass associated-macroorganisms were observed during field data collection. The organisms were found in every line of sampling site (noted as sites A, B, C, D, E and F respectively), then counted and listed in the logbook without taking it out from seagrass ecosystem.

Samples of healthy seagrass were collected on June 2019 from Panjang Island waters. The isolation method was employed using protocols from Supaphon and team with modifications according to Ayuningrum et al (2019b) and Sabdaningsih et al (2019). The seagrass isolation process was carried out at the Aquatic Resources Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro. Seagrass leaves, stems and roots were washed by sterile natural seawater and surface sterilized in 10% alcohol for 3 min, 3% sodium hypochlorite for 1 min, then in 10% alcohol for another 3 min and rinsed in sterile natural seawater. Samples were dried into sterile paper towels, then cut into 4 small pieces and placed on malt extract agar (MEA) for fungi diluted by natural seawater, containing 50 mg L^{-1} chloramphenicol. Isolation of bacteria was conducted using serial dilution method, 1 g of samples were cleaned by sterile natural seawater and grinded by a mortar. The 30 µL aliquot from 10^{-3} dilution was spread on the M1 agar sterile plate (Supaphon et al 2013). A litter M1 agar medium contains starch (10 g), yeast extract (4 g), peptone (2 g), agar (15 g). Plates were incubated at room temperature (29±2°C) until the growth of fungi and bacteria. The purification of both fungi and bacteria were done according to the morphological characterization. The morphological characterization of seagrass-associated bacterial isolates includes observation of the shape, size, colour, margin and elevation of the bacterial colonies (Ayuningrum et al 2019a). The seagrass-associated bacteria were tested to know the potential of antibacterial activity. The employed method was agar plug method (Sabdaningsih et al 2017). All the plates were made in triplicates. The antibacterial activity was indicated by the formation of clear zone surrounded the seagrass-associated bacterial isolates.

Results and Discussion

Multi temporal seagrass analysis. Based on the results of the analysis of Landsat 5, Landsat 7, and Sentinel 2A satellite imagery with water column correction using the particularly band 3,2,1 for Landsat and 4,3,8 for Sentinel sensors, it can be seen that the area of seagrass for 18 years has experienced changes in area dynamics, where there are new area, growing area, disappearing area, and remaining area. There was an increase in seagrass beds from 2001 to 2012 by 1.30 ha and a loss of 0.78 ha, which increased by 19.18%. In 2012-2019 there was a decrease in the area of seagrass beds by 13.49% from 0.34 ha covering area, where the lost and increase seagrass was 0.99 ha and 0.65 ha, respectively (Figure 3).

Figure 3. Seagrass beds distribution in Panjang Island: A) 2001; B) 2012; C) 2019.

Figure 4 shows that the distribution of seagrass on Panjang Island is randomly distributed by clustering. The high decline in seagrass cover in Panjang Island is caused by several factors, including the construction of a new dock, the high waves from the Java Sea and from passenger ships towards Karimunjawa, as well as tourism activities such as snorkeling which often destroy seagrass ecosystem. Meanwhile, the natural location of seagrass beds itself is closely situated to the port of tourist boats, which put more pressures on the growth and distribution of seagrass beds (Figures 3, 4, 5).
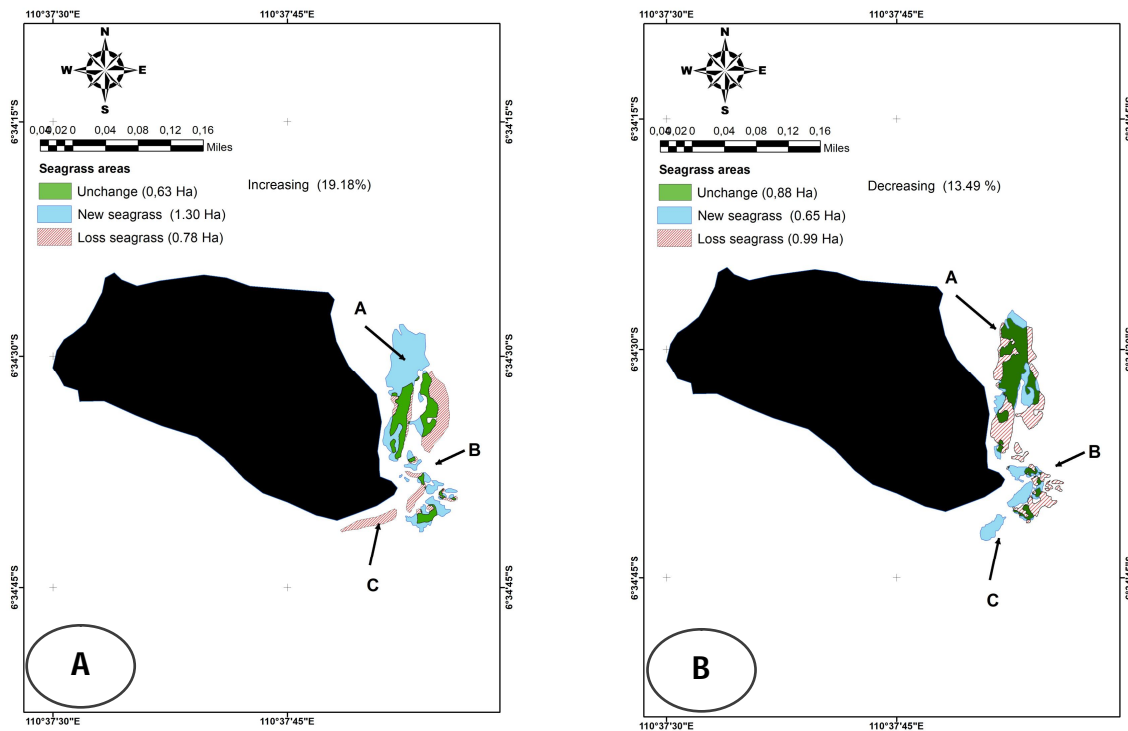

Figure 4. Change of seagrass beds in Panjang Island: A) 2001-2012; B) 2012-2019.

Figure 5. Google earth image seagrass beds trend in 2001, 2012, 2019.

Based on the results of the mapping, it is indicated that the overall confusion matrix accuracy test has an accuracy and a Kappa coefficient of 83% with the best penetration of the water body in clear conditions as deep as 5 m. The bathymetry mapping also revealed that seagrass beds in Panjang Island, Jepara Regency was found in a depth range between 0.5 and 2.5 m (Figure 6).
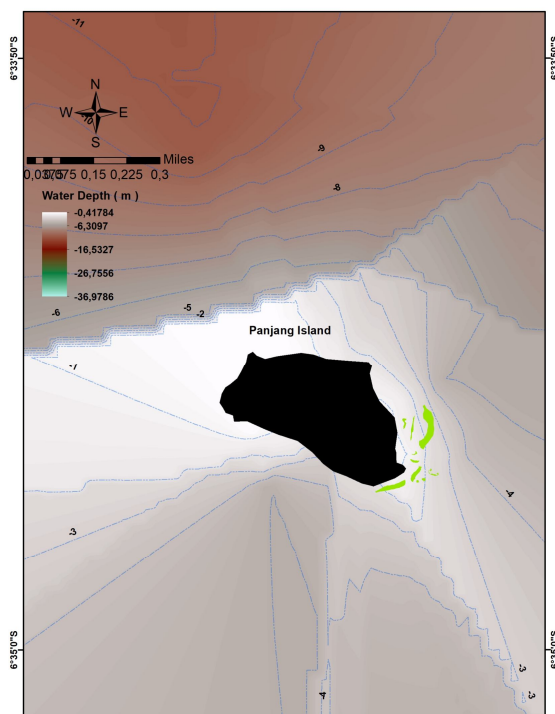

Figure 6. Bathymetry map produced from Hydrographic and Oceanographic Center, Indonesian Navy indicated seagrass beds were found in a depth range of 0.5-2.5 m.

Community structure. The type of seagrasses found in the six sampling sites using 17 points of observation were dominated by three species, i.e. *Cymodocea serrulata, Cymodocea rotundata* and *Thalassia hemprichii* (Figure 7a). The most dominant species was *C. rotundata* with the abundance 1054 ind m^{-2} in the station A and *C. serrulata* 778 ind m^{-2} in the station C (Figure 7b). The Shannon diversity index showed that location D has the highest value which means the number of species were found in the equilibrium (Table 2). A previous study from Hidayat et al (2014) resulted in five different species in the water of Panjang island, where *T. hemprichii* was found as the most abundant species. This distinction occurred from the differences of sampling site. Hidayat et al (2014) only used one sampling site from our study which is station C. Based on our investigation from analysis of satellite imagery in the period 2014-2020, there has been a coastal erosion event in the area near the dock so that it will affect the condition of the bottom of the waters. Therefore, at that same location we found that it was dominated by genus *Cymodocea*. The study from Abdulkadir et al (2019) confirmed that the wave is one of physical factors that influence the coastal and bottom water erosion with the average about 0.78-0.86 m wave height ranging from 4.7 to 4.9 seconds in wave periods. Hence, the dynamics of the seagrass bed area were also influenced the diversity of species.
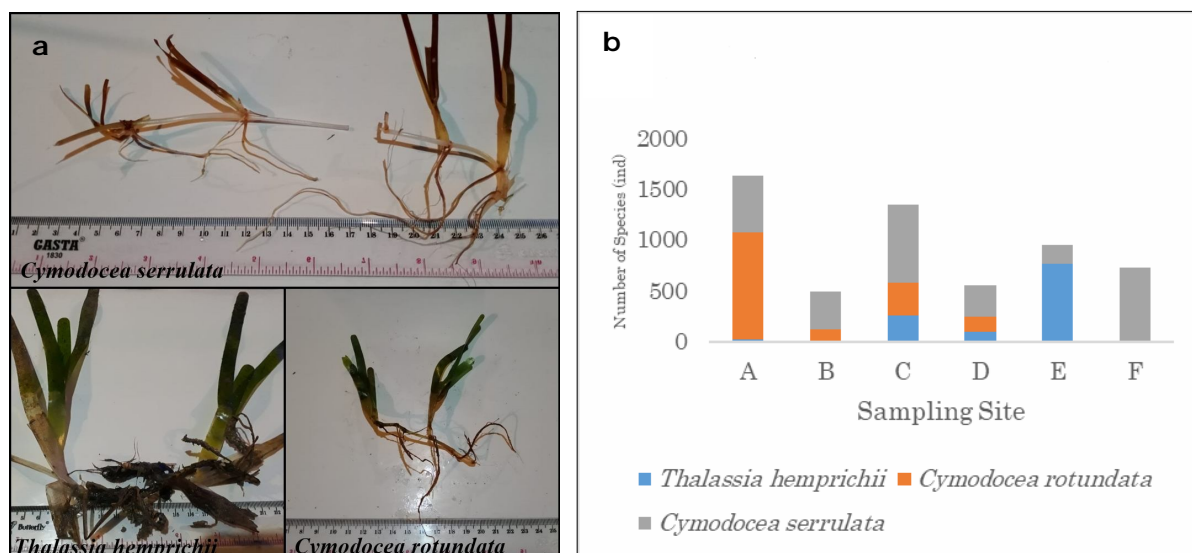
Figure 7. a. The morphology of seagrass in Panjang Island; b. seagrass beds abundance in the study area of Panjang Island.

The Shannon diversity, evenness, and dominance index of seagrass

Plots	Total of individual	S	Shannon diversity index (H)	Evenness index (H')	Dominance index
A	1645	3	0.7121	0.648	0.529
B	498	2	0.561	0.809	0.626
C	1360	3	0.976	0.888	0.420
D	559	3	0.982	0.894	0.413
E	959	2	0.487	0.703	0.691
F	728	1	0	0	0

S = species.

Seagrass-associated macro-organisms. The seagrass beds were used to be the nursery and feeding ground of some species. During the field data collection, we found several highly abundant seagrass-associated macro-organisms in the area study (Figure 8). The results showed an abundance of *Holothuria*, gastropods and crab. This happened because crabs were still categorized as tillers so they did not become predators for *Holothuria*. Ecologically, the seagrass ecosystem functions as a spowning, nursery and feeding area for benthic organisms (Jalaluddin et al 2020). The most dominant group was *Holothuria*, which was found in five different sites. Gastropods were also highly abundant in the site C, while crab was mostly found in the site C. The study from Wolkenhauer et al (2010) reported that sea cucumber associated with seagrass had important role to the growth of seagrass, they proved in the experiments with control that seagrass biomass decreased when the density of *Holothuria* was set up near zero. Morphologically, seagrass species tend to develop higher biomass under the substrate (rhizomes and roots), so that they can accumulate higher carbon. In addition, the carbon under the substrate is a place for storing photosynthetic products that will support seagrass growth if the photosynthesis process does not run optimally (Citra et al 2020).
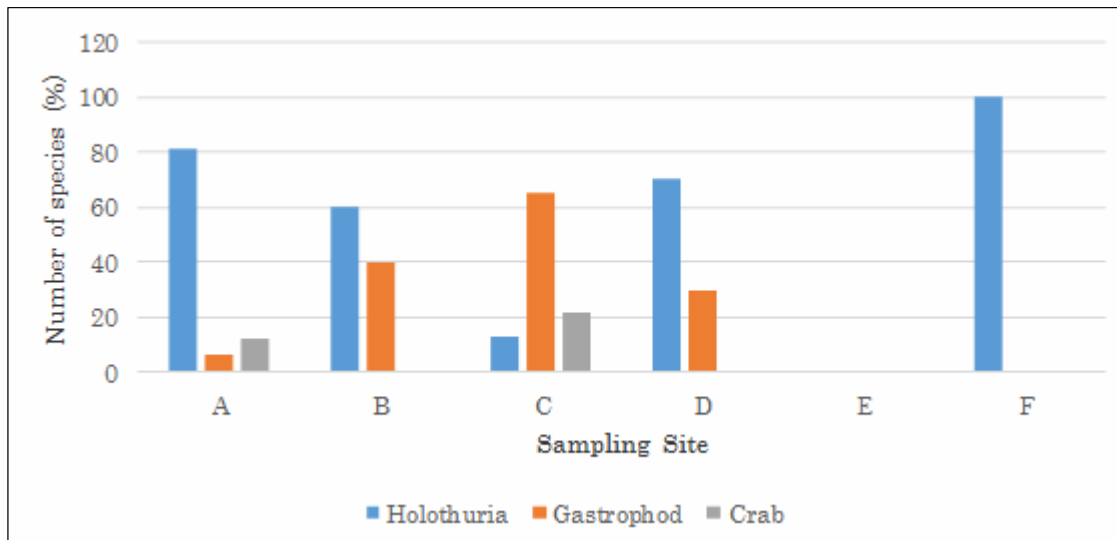
Figure 8. Distribution of seagrass-associated macroorganisms in the study area of Panjang Island.

Seagrass-associated microorganisms. A total of 49 bacterial isolates and 17 fungi isolates were successfully isolated from three seagrass samples including *C. rotundata*, *T. hemprichii*, and *C. serrulata*. The highest number of bacteria and fungi were isolated from species *C. serrulata* (40.8% and 35.3% respectively), while the least came from *T. hemprichii* (24.48% and 29.4% respectively) (Figure 9a). According to the research from Rotini et al (2020), the bacterial community composition of *T. hemprichii* consisted of 8 phyla, 16 classes, 27 orders, 32 families and 29 genera. The most dominant phylum was Proteobacteria, accounting for 94%, specifically, the class Gammaproteobacteria was the most represented from overall samples (86%). The Gammaproteobacteria was mostly found more in the subsurface (99%) rather than at the surface (49%). Furthermore, within this class, the order Vibrionales and the family Vibrionaceae (66%) were the most abundant phylotypes, followed by the family Enterobacteriaceae (12%). Supaphon et al (2013) reported that fungi were associated with *C. serrulata*, *Halophila ovalis*, and *T. hemprichii* in Thailand waters mostly isolated from the leaf pieces. Moreover, the filamentous fungi that dominant in Supaphon et al (2013) study were *Fusarium*, *Penicillium*, *Stephanonectria*, and *Trichoderma*, while in this study we found *Aspergillus* (Figure 10) and *Penicillium.*

The bacterial community which live in root, stem, and leaf of seagrass were quantitatively different in concentration. We successfully isolated the most bacteria either fungal from leaves, which the full data could be seen in Figure 9b. Since root was in the anaerobic condition and the isolation process used aerobic condition thus only aerobic bacteria were successfully isolated, meanwhile the marine-derived fungi was also obtained with aerial spore. Furthermore, the phylum of the most abundant bacteria was different between one part and another. Specifically, in the study of Kaimenyi et al (2018) the most abundant bacteria associated with leaf samples were from the phyla Proteobacteria (61.8%), Cyanobacteria (25.9%) and Bacteroidetes (8.1%). In comparison, root microbiomes contained predominately Proteobacteria (73.9%), Cyanobacteria (8.7%), Actinobacteria (3.6%), Acidobacteria (1.0%) and Fibrobacteres (1.4%).

In our research, we also found that seagrasss-associated bacteria show a low antibacterial activity against pathogen *Micrococcus luteus,* while in fungi the activity to several pathogen including *Bacillus subtilis*, *Escherichia coli*, and *M. luteus* was absent. A study from Cristianawati et al (2019) also reported *Bacillus flexus* and *Streptomyces lienomycini* having antibacterial activity against multidrug resistant bacteria.

Furthermore, seagrass-associated microbes also play big role in biogeochemical cycle related to the sulfur metabolism which detoxify sulfide by oxidizing it to sulfur or sulfate (Ugarelli et al 2017). Cúcio et al (2016) reported that amplicon sequencing of some seagrass species like *Zostera marina*, *Zostera noltii*, and *Cymodocea nodosa* were

dominated by bacteria involved in the sulfur cycle. Thus, due to in marine sediment which typically anoxic and enriched in organic matter, seagrass and its associated bacteria played an important evolutionary role in allowing terrestrial angiosperms to recolonize the marine environment (Fraser et al 2018). Thus, we can conclude that microorganisms-associated seagrass play a big role in the seagrass community.
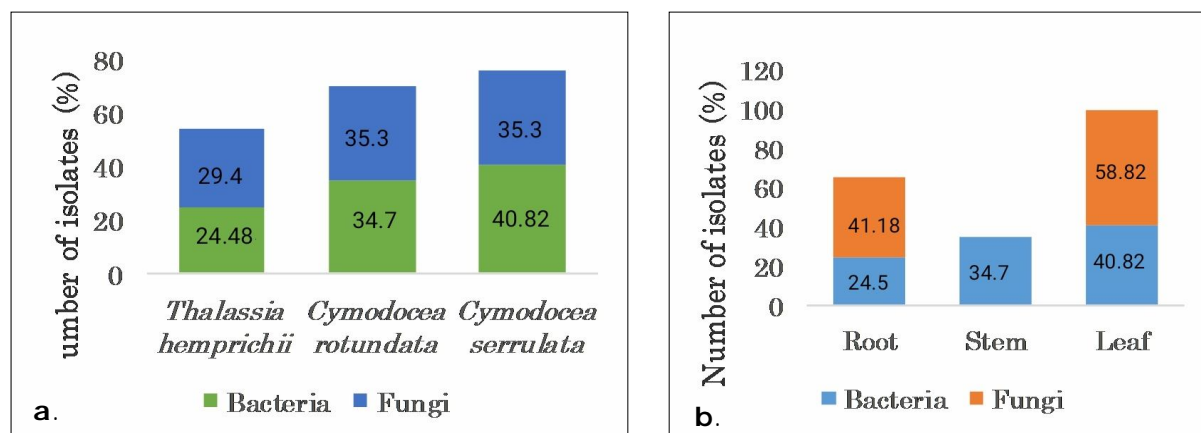

Figure 9. a. The number of seagrass-associated microorganisms in different species; b. distribution of seagrass-associated microorganisms in root, stem, and leaf.

Figure 10. The seagrass-associated fungi in Panjang Island was dominated by *Aspergillus* sp.

Conclusions. Analysis of the multi-temporal Landsat 5, Landsat 7 and Sentinel 2A satellite imagery showed that there was a fluctuation of increase and decrease in the area of seagrass beds in Panjang Island. There was an increase in the area of seagrass beds from 2001 to 2012 by 1.30 ha and a loss of 0.78 ha, which increased by 19.18%. In 2012-2019 there was a decrease in the area of seagrass beds by 13.49% covering an area of 0.34 ha, where the lost seagrass was 0.99 ha and an increase of 0.65 ha. The decrease in seagrass area might occurs due to the construction of a new dock and high coastal erosion. The observation of communities found three species of seagrass, holothurian, gastropods, crabs, as well as bacteria and fungi. This study revealed that the decreasing seagrass beds impacts on the diversity of species itself.

Conflict of interest. The authors declare that there is no conflict of interest.

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