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Original Article Diversity of Green Macroalgae Genus *Ulva* (Chlorophyta) in Hai Phong

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Abstract: The knowledge of marine algae species composition is imperative not only for understanding the structure, function and biogeographical affinities but also for planning strategies for marine algae conservation and management. This study was carried out to determine the species diversity of genus green macroalgae Ulva in Hai Phong, where many different habitats such as estuarine floodplains, lagoons or small bays and diverse in the bottom (hard, sandy, sandy-mud bottom). Ulva samples were collected at 11 sites in 5 areas in 2021. After the research process, 11 species of genus Ulva has been identified. The species composition of the genus Ulva in Hai Phong accounted for 73.3% of the total species recorded in Vietnam; accounting for 19.6% of the total number of Asian species, and 12.9% of the total number of Ulva species in the world. The Cluster and MDS (Multi-Dimensional Scaling analysis) on the similarity in vertical distribution and geographical distribution among the studied species.

Keywords: Ulva, Green macroalgae, Hai Phong, MDS.

1. Introduction

Genus *Ulva* Linnaeus, C. (1753) also known as the Sea Lettuce [1] Family Ulvaceae, Order Ulvales (Chlorophyta), has a global geographic distribution from the tropics to subtropics, with some species extending into the Mediterranean Sea and temperate regions of Australia [2]. With a lamellar leaf structure (leaf blade is lobed, has natural holes, margin is thorny, wavy, or non,...) with two layers of cells or hollow cylinders (previously *Enteromorpha*, the pyrenoid ranging from 1 or 2 or more [3]), hapter (holdfast) with developed rhizoid that helps them adapt to a variety of habitats, especially in coastal areas with high levels of eutrophication such as in marine environments,

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brackish estuaries and sometimes found in freshwater [4-6].

On the global algal database (https://www.algaebase.org/) 2021 [7] there are currently 85 species as accepted taxonomically on the base morphological and molecular. In Vietnam, research on the genus *Ulva* has been carried out by Dawson since 1954 [8], hitherto, a total of 15 species and 1 subspecies have been recorded [9], with some species having an economic value such as *Ulva lactuca, Ulva prolifera, Ulva reticulata, Ulva papenfussii, Ulva intestinalis* [5].

The coastal area of Hai Phong is characterized by many different habitats such as estuarine floodplains, lagoons or small bays, diverse in the bottom (hard, sandy, sandy-mud bottom) with a typical subtropical climate. With four distinct seasons, this place is a favorable condition for the growth and development of the genus *Ulva* [10].

However, at present, research on green macroalgae in Hai Phong coastal area is limited, the documents are scattered and it is difficult to update information, there are no in-depth studies on the diversity and distribution of the genus *Ulva*, while the number, location, nomenclature and description of some species of the genus *Ulva* need to be supplemented. Therefore, there is a need for a comprehensive,

complete and systematic study. Parallel with that, the affair provision of data and scientific basis for conservation, resource development or taxonomy some species of the genus *Ulva* is necessary and important.

2. Experimental

2.1. Material

The green macroalgae studies was followed by the "Marine Resources and Environment (Biological Procedure" Surveys and environmental survey procedure) [17]. All species of the Ulva genus samples were collected by snorkeling from shallow waters (0 - 2 m in depth) along the subtidal by SCUBA diving equipment at the depth of 3-15m [18], underwater digital camera OLYMPUS (Tokyo, Japan) for taking pictures. The freshly collected marine macroalgal samples were soaked in a solution of formaldehyde 5%, the specimens were then put on Croki paper, compressed into blotting papers, dried naturally and identified.

A total of 11 sites representing typical ecosystems in 5 areas in Hai Phong include: Cat Ba (Cai Beo, Ang Vem, Gia Luan, Phu Long); Do Son (Khu II, Khu III, Ngoc Xuyen, Hon Dau); Bach Long Vi island; Long Chau island and Tien Lang (Fig. 1 and Tab 1).

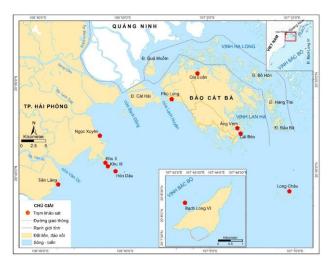


Figure 1. Locations of sampling sites

No.	Sites/ Location	Symbol	Collecting time	Latitude	Longitude	
1	Ang Vem	AV		20°44'42.56"N	107° 3'30.87"E	
2	Cai Beo	CB	March 14, 2021	20°44'5.68"N	107° 3'52.90"E	
3	Gia Luan	GL	March 14, 2021	20°50'50.67"N	106°58'52.43"E	
4	Phu Long	PL		20°48'0.92"N	106°55'47.79"E	
5	Khu II	KII		20°41'2.28"N	106°47'53.45"E	
6	Khu III	KIII	March 2, 2021	20°40'39.10"N	106°48'10.91"E	
7	Hon Dau	HD	March 3, 2021	20°40'4.96"N	106°49'4.78"E	
8	Ngoc Xuyen	NX		20°44'3.27"N	106°47'16.62"E	
9	Long Chau	LC	May 9,2021 and May 30, 2021	20°37'37.62"N	107° 9'32.99"E	
10	Bach Long Vi	BLV	May 26, 2021	20° 8'13.25"N	107°43'21.60"E	
11	Tien Lang	TL	April 13, 2021	20°38'41.73"N	106°42'17.51"E	

Table 1. Coordinates of survey sites

2.2. Methods

2.2.1. Classification

Samples were analyzed in the laboratory of the Department of Marine Botanical Ecology and Resources, Institute of Marine Environment and Resources. Vietnam Academy of Science and Technology. The classification is based on the criteria of morphology and internal structure. The studied of the internal structure is based on cross-sectional samples observed under with Motic A300 microscope in different zoom (4X, 10X, 40X, and 100X). The green macroalgae classification follows the general principles of plant classification [11]. The classification documents were consulted according to the reports of Pham Hoang Ho [12], Nguyen Van Tien [5], Hayden et al., [3].

The scientific name was corrected by comparing it to the global algal database of taxonomic, nomenclatural, and distributional information from www.algaebase.org [14].

2.2.2. Geographical Distribution

The data were calculated by using Excel MS Office 365, while multivariate were run and summarised using PRIMER V.6 [13]. The similarities of seaweed within 11 stations were summarized using the Bray - Curtis homologous index [14] as the following equation:

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j}$$

Where:

C_{ij} is the sum of the lesser values for only those species in common between both sites,

 S_i and S_j are the total number of specimens counted at both stations.

The similarity of species composition between the studied areas is displayed on the multidimensional scaling (2D-MDS).

2.2.3. Vertica Distribution

The studied of the vertical distribution of green macroalgae is based on the principle of tidal zone division of Feldmann [15], Stephenson [16] and Pham Hoang Ho [17]. Determine the tidal water level based on the tide table Hon Dau area in March, April, and May 2021[18].

3. Results and Discussion

3.1. Species Diversity of the Genus Ulva in Hai Phong

Based on the morphological classification, the studied identified 11 species of *Ulva* genus and their distribution in Hai Phong (Tab 2).

Key to species Ulva (Chlorophyta)

1a. Thallus is a leaf, cross-section consisting of 2 rows of cells......2

2a. The stem is made up of many small leaf plates from 1 to 2cm stacked on top of each other in the form of rose flowers.....1.U. conglobata

3a. The leaf blade has few natural holes, the margins are spiky or serrated.

4a. The leaf blade is not lobed, the leaf margin is thorny...... 2. *U. spinulosa*

4b. The macroalgae are lobed, the leaf margins are serrated and wavy...... 3. U. lactuca

3b. The leaf blade has many natural holes, the edge of the leaf has no spines4. U. fenestrata

6a. Stem single, unbranched or with only a few small hairy branches 1 row of cells at the base, twisted apex.....5. U. intestinalis

6b. Branched trunk, untwisted apex.....7

7a. Branching mainly at the base, crosssection of cells with irregular sharp angle polygons, arranged in no order.....6. U. compressa

7b. Branching throughout the stem, symmetrically as feathery, viewed from the surface of the cells rectangular or square arranged vertically at the base of the branches......7. *U. prolifera*

8a. Unbranched seaweed or very little branched mainly at the base......9

8b. The macroalgae branched a lot......10

9a. The thallus (tubular) is made up of 2-3 rows of cells, arranged in order..... 8. *U. ralfsii*

9b. Thallus (tubular) long and narrow, with 4-8 rows of cells, with 2-4 pyrenoids granules.....9. *U. kylinii*

Table 2. Diversity and distribution of the Ulva genus in Hai Phong

	Name species		Geography distribution										Vertical distribution			
No.		Cat Ba island			Do Son				BLV I	LC	intertidal zone		subtidal			
		CB	AV	PL	GL		NX	KII	KIII	HD	DLV	Le	Н	М	L	zone
1	Ulva clathrata (Roth) C.Agardh 1811			+		+						+		+		
2	Ulva compressa Linnaeus 1753*			+			+	+	+	+					+	
3	<i>Ulva conglobata</i> Kjellman 1897	+	+					+	+	+	+	+	+	+		
4	Ulva lactuca Linnaeus 1753	+	+		+			+	+	+	+	+		+		
5	Ulva fenestrata Postels & Ruprecht 1840	+	+								+			+	+	+
6	Ulva flexuosa Wulfen 1803			+		+	+					+		+	+	
7	<i>Ulva intestinalis</i> Linnaeus 1753						+	+	+					+	+	
8	Ulva kylinii (Bliding) HSHayden et al., 2003			+		+	+						+			
9	<i>Ulva prolifera</i> O.F.Müller 1778			+				+	+	+				+	+	
10	Ulva ralfsii (Harvey) Le Jolis 1863*		+	+		+	+						+			
11	Ulva spinulosa Okamura and Segawa 1936	+			+								+			

Notes: CB-Cai Beo; AV-Ang Vem; PL-Phu Long; GL-Gia LuanTL-Tien Lang; NX-Ngoc Xuyen; KII-Khu II; KIII-Khu III; HD-Hon Dau; BLV-Bach Long Vi; LC-Long Chau; H-High; M-Middle; L-Low; *- new records in Hai Phong.

10a. The macroalgae is only branched at the base, the body is fat, twisted, due to 8-15 rows of cells, there are 4-8 pyrenoids......10. *U. Flexuosa*.

10b. The macroalgae are branched all over the body, the cells are round or polygonal with edges, evenly arranged, with 2-10 pyrenoids.....11. U. Clathrata. A total of 11 species of the genus *Ulva* have been classified. The number of species at the studied sites ranges from 2 (Gia Luan) to 6 (Phu Long) (Fig 2). The number of species distributed by the studied area ranges from 3 (Bach Long Vi) to 11 (Cat Ba).

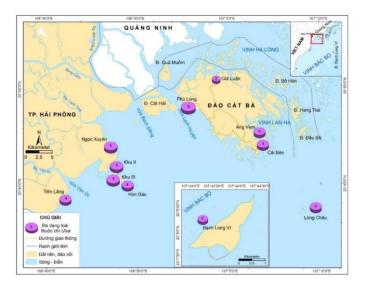


Figure 2. Number of species-genus Ulva distributed at studied sites.

3.2. Species Similarity

3.2.1. Geography Distribution

	AV	BLV	СВ	GL	HD	KII	KIII	LC	NX	PL
BLV	66.67									
CB	57.14	85.71								
GL	-	40.00	66.67							
HD	28.57	57.14	50.00	33.33						
KII	25.00	50.00	44.44	28.57	88.89					
KIII	25.00	50.00	44.44	28.57	88.89	100.00				
LC	28.57	57.14	50.00	33.33	50.00	44.44	44.44			
NX	25.00	-	-	-	22.22	40.00	40.00	22.22		
PL	22.22	-	-	-	40.00	36.36	36.36	40.00	72.73	
TL	28.57	-	-	-	-	-	-	50.00	66.67	80.00

Table 3. The similarity of species composition between studied sites

Notes: CB-Cai Beo; AV-Ang Vem; PL-Phu Long; GL-Gia Luan; TL-Tien Lang; NX-Ngoc Xuyen; KII-Khu II; KIII-Khu III; HD-Hon Dau; BLV-Bach Long Vi; LC-Long Chau.

The similarity of species composition between the studied sites ranged from 0% (Gia Luan - Ang Vem; Ngoc Xuyen - Bach Long Vi, Cai Beo, Gia Luan; Phu Long - Bach Long Vi, Cai Beo, Gia Luan; Tien Lang - Bach Long Vi, Cai Beo, Gia Luan, Hon Dau, Khu II, Khu III) to 100% (Khu II- Khu III Do Son), and an average of 36.2%. To show more clearly the similarity of species composition between the studied sites, the results of Cluster analysis are applied and presented in Figure 3.

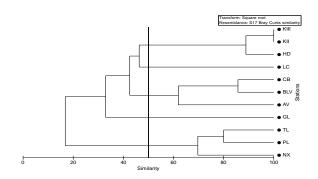


Figure 3. Cluster analysis compares the similarity of species composition between studied sites. Notes: CB-Cai Beo; AV-Ang Vem; PL-Phu Long; GL-Gia Luan; TL-Tien Lang; NX-Ngoc Xuyen; KII-Khu II; KIII-Khu III; HD-Hon Dau; BLV-Bach Long Vi; LC-Long Chau.

Cluster results using Bray-curtis similarity index are shown by the Dendrogram chart showing the branching among 11 studied sites. Accordingly, the similarity of species composition between the studied sites is divided into two major groups.

In which, Group (i) includes coastal points of Ngoc Xuyen, Phu Long and Tien Lang, typical of mangrove ecosystems and brackish water ponds with low salinity and eutrophic environment and soft bottom (sandy-mud), there is no difference in species composition (no species with leaf shape in blade form) are placed in the same clade with similarity value > 65%; Group (ii) includes sites of marine ecosystems (coral reefs, small bays or estuaries), with characteristics of hard bottom. In group (ii) is subdivided into 3 sub-clade, including clade (*iia*) representing the Seagrass ecosystem, Gia Luan, (iib) including sites of Small bay ecosystem (Cai Beo) - Saltwater lake ecosystem (Ang Vem)- Coral reef ecosystem (Bach Long Vi), (*iic*) includes two main ecosystems: Sandbank ecosystem (Khu II, Khu III), and Karst ecosystem (Long Chau, Hon Dau).

When comparing the similarity of species composition between the studied areas is shown by 2D-MDS chart (Figure 4). It was found that the species composition in the studied areas tends to group together on a large scale with two distinct groups at 40% similarity, including: Group (a) with two offshore islands, Long Chau and Bach Long Vi; Group (b) includes the nearshore areas Cat Ba, Do Son and Tien Lang. At 60% of the studied sites tend to expand, most notably the two areas of Do Son and Cat Ba because the studied sites are mainly in the above two areas with the diversity of ecosystems, are the intermediate place of many benthic components leads to diversity in the number of species components, but not concentrated at a certain site, but distributed across the studied sites.

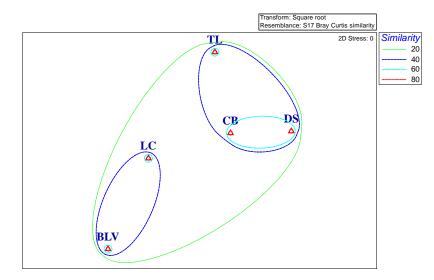


Figure 4. Analysis on the 2D-MDS chart on the similarity of species composition between the studied areas. *Notes:* BLV-Bach Long Vi; CB-Cat Ba; DS- Do Son; LC-Long Chau; TL-Tien Lang

3.2.2. Vertical Distribution

Regions	Tidal belt	Featured species	Depth
	High	Ulva conglobata, Ulva kylinii, Ulva ralfsii, Ulva spinulosa.	1,8 m
Intertidal zone	Middle	Ulva clathrata, Ulva conglobata, Ulva lactuca, Ulva fenestrata, Ulva flexuosa, Ulva intestinalis, Ulva prolifera.	0,5 m
Zone	Low	Ulva compressa, Ulva fenestrata, Ulva flexuosa, Ulva intestinalis, Ulva prolifera	0 m
Subtidal zone		Ulva fenestrata.	-8 m

Table 4. The distribution of Ulva genus by depth in Hai Phong

Source: Hon Dau regime: in March, April, and May 2021.

Based on the water level of Hon Dau tide table for March April and May 2021, all 11 species of the genus *Ulva* have been identified and distributed in the intertidal zone, of which 1 species is distributed in both the intertidal and subtidal.

In this study, although the survey frequency at 11 sites was only once a year, it

could also represent the green macroalgae genus *Ulva* in the studied areas because the survey time coincided with the season. Compared with some previous research results the number of species in these studied sites in Hai Phong is relatively large (Tab 5).

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Table 5. The number of th	ie <i>Uiva</i> genus in Hai Phon	g 2021 compared i	previous studies in	vietnam and the world

Regions	Number species	References			
Viet Nam	15	Nguyen Van Tu et al., [9]			
Ha Long bay (1996)	5	Nguyen Van Tien [19]			
Ly Son island (2011)	5	Dam Duc Tien et al., [20]			
Dam Nai lagoon (2016)	6	Dam Duc Tien et al., [21]			
Nam Du island (2019)	2	Do Anh Duy et al., [22]			
Dong Bac bay-Con Dao island (2019)	4	Nguyen Manh Linh [23]			
Co To island (2020)	4	Dam Duc Tien et al., [24]			
Con Co island (2021)	2	Dam Duc Tien et al., [25]			
Hai Phong (2021)	11	This study			
The World	85	Algaebase [7]			
Taiwan (1987)	15	Lewis et al., [26]			
Gujarat Coast-India (2009)	10	Jha et al., [27]			
Qingdao-China (2011)	8	Du et al., [28]			
Hainan Island-China (2017)	5	Titlyanov et al., [29]			
Jeju Island-Korea (2019)	9	Kang et al., [30]			
Asia (2020)	56				
Oceania (2020)	40				
Europe (2020)	38				
North America (2020)	34	Mantri et al., [31]			
Africa (2020)	31				
South America (2020)	20]			
Antarctica (2020)	12				

This study also shows that Hai Phong has very favorable conditions for the existence and development of *Ulva* species. Compared to some other islands and areas in Vietnam, the number of genus *Ulva* in Hai Phong is more diverse than Ha Long: 5 species, Con Co: 2, Dam Nai: 6 and Ly Son: 5.

When compared with other regions in the world, it can be seen that the number of species of genus *Ulva* in Hai Phong is much more diverse than in neighboring areas such as Qingdao: 8 species, Hainan island: 5, Jeju Island: 9; and inferior to Taiwan: 15 species.

The species composition of the genus *Ulva* in Hai Phong accounted for 73.3% of the total species recorded in Vietnam; accounting for 19.6% of the total number of Asian species, and 12.9% of the total number of Ulva species in the world currently accepted taxon.

Especially in this study, *Ulva ralfsii* and *Ulva compressa* were first recorded in Hai Phong. Therefore, the research results can make a significant contribution to the database on species diversity and distribution of the green macroalgae genus *Ulva* in Vietnam.

4. Conclusion

The classification results identified 11 species of green macroalgae genus *Ulva* in Hai Phong. At 11 sites in 5 areas, the number of species from 2 (Gia Luan) to 6 (Phu Long). The number of species distributed by the studied area ranges from 3 (Bach Long Vi) to 11 (Cat Ba). All 11 species have been identified and distributed in the intertidal zone, of which 1 species is distributed in both the intertidal and subtidal.

The species composition of the genus *Ulva* in Hai Phong accounted for 73.3% of the total species recorded in Vietnam; accounting for 19.6% of the total number of Asian species, and 12.9% of the total number of *Ulva* species in the world. Can see that Hai Phong has very favorable conditions for the existence and development of green macroalgae genus *Ulva*.

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