Vietnam Journal of Marine Science and Technology; Vol. 20, No. 4A; 2020: 21–33
DOI: https://doi.org/10.15625/1859-3097/15652
http://www.vjs.ac.vn/index.php/jmst

Variation of phytoplankton community structure in Quang Ngai coastal waters during 2015–2019

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Received: 28 August 2020; Accepted: 26 October 2020

Abstract
Phytoplankton in coastal waters are important for the evaluation of either biodiversity or environmental impacts because of this highly vulnerable ecosystem. Seasonal and annual changes in the phytoplankton community structure in Quang Ngai waters during the period 2015 to 2019 were analyzed to assess the phytoplankton diversity and reveal possible causes of these changes. A total of 366 phytoplankton taxa belonging to 10 classes were identified throughout this present study. The highest species number was found in 2019 with 295 taxa, followed by those in 2015 (247), 2017 (185), and 2018 (99). The waters of Ly Son transect showed the highest diversity and most stable phytoplankton communities in both dry and rainy seasons, whereas the stations of Quang Ngai coast revealed high variability of the communities. All diversity indices including Margalef, Pielou, Shannon, Simpson did not reflect well differences in average values but a certain degree of variances, indicating possible environmental impacts. During the study time, there were blooms of certain diatom species including *Skeletonema* spp. in 2015 and *Pseudo-nitzschia* spp. in 2019. Analysis of a diatoms index, Centric/Pennate ratio, indicated that the waters were in eutrophic status with a decreasing trend from the coast area to Ly Son island in 2015 and 2019. This research built up fundamental data on phytoplankton communities for Quang Ngai province. The Centric/Pennate diatom index and diversity would be used as indicators for environmental changes and their values provided warning of eutrophication in this coastal waters including the water surrounding Ly Son island.

Keywords: Ly Son, Quang Ngai, phytoplankton, diversity index.

Citation: Huynh Thi Ngoc Duyen, Tran Thi Minh Hue, Tran Thi Le Van, Phan Tan Luom, Nguyen Ngoc Lam, Doan Nhu Hai, 2020. Variation of phytoplankton community structure in Quang Ngai coastal waters during 2015–2019. *Vietnam Journal of Marine Science and Technology*, *20*(4A), 21–33.

INTRODUCTION

Phytoplankton play an important role in primary production, nutrient cycles, water quality, and food web dynamics [1–3]. They are with small size, short generation times [4], sensitive to environmental changes [5], thus the changes in the phytoplankton community composition are important indicators of coastal ecological conditions [2, 6]. The composition of the phytoplankton community would impact on functioning of aquatic ecosystem and global climate [4], whereas recent studies revealed anthropogenic activities causing rapid change in structure of phytoplankton community [7]. Therefore, it is important to understand how nature and human impact on the phytoplankton community structure and dynamics.

Quang Ngai province is located in the Centre of Vietnam with nearly 130 km of coastline. Recently, industrial, tourism, and aquaculture activities have been developing, yet their impacts on the coastal ecosystem were inadequately evaluated in detail. As an indicator of environmental changes, understanding the structure of the phytoplankton community is essential to better assess the impacts of natural factors as well as anthropogenic activities on the aquatic ecosystem. An entire description of biota, including phytoplankton, is essential data to evaluate ecosystem changes, if any, in the future. However, the studies of the phytoplankton community were rarely conducted and lacked the coupling of climate factors (e.g., ENSO) or human activities in this water. In this study, we focused on assessment and comparison of the phytoplankton community structures using species richness, diversity indices, abundance, and biomass of data sets obtained in November 2015 (rainy season), August 2017 and August 2018, and June 2019 (dry season). These analyses aim to provide the essential data of the phytoplankton community in the Quang Ngai's coastal waters, it also helps to evaluate the environmental condition as well as possible environmental impacts.

MATERIALS AND METHODS

Time and sampling sites

The present study used 31 qualitative samples and 47 quantitative samples (table 1) of phytoplankton collected from 3 surveyed areas in Quang Ngai in November 2015 (rainy season), August 2017 and August 2018, and June 2019 (dry season). Three surveyed areas included: (1) - around Ly Son island; (2) - the Ly Son transect; and (3) - area along the coast of Quang Ngai (fig. 1). The coordinates of stations were shown in appendix 1.

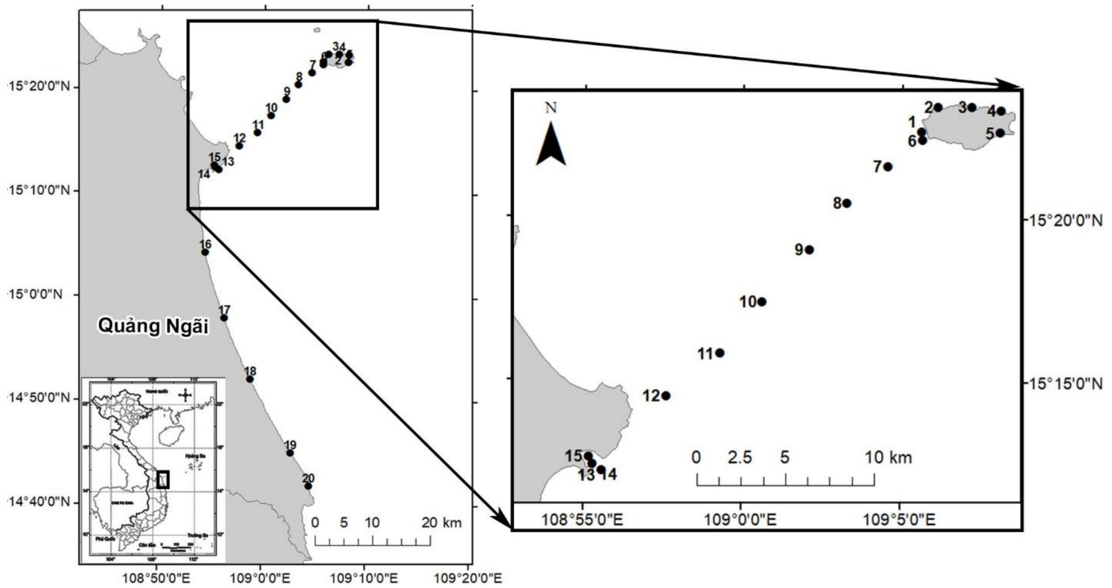

Figure 1. Studied areas and sampling stations in Quang Ngai including three areas: The coastal Quang Ngai, the Ly Son transect and the Ly Son island

Table 1. Information of stations, number of qualitative and quantitative samples

Area	Stations	Qualitative samples	Quantitative samples
Around Ly Son island (LS)	From 1 to 6	8	11
Ly Son transect (LS Trans)	From 7 to 12	12	24
Along the coastal Quang Ngai (Coast)	From 13 to 20	11	12

Sampling and analysis method

Qualitative samples

The qualitative samples were collected by using phytoplankton net with 25 µm mesh size towing vertically at slow speed from the bottom to the surface, then fixed with formalin 5% and preserved in dark for later analysis in the laboratory. Species were identified and measured under the light microscope (Leica LDMB, Germany). To identify armored dinoflagellates in the plankton samples, Calcofluor White M2R method [8] was used, and the samples were also observed under the epifluorescence microscope (Leica LDMB, Germany).

Identification of the species was based on the key literatures including Graham and Bronikovsky [9]; Hoang [10, 11]; Shirota [12]; Abé [13]; Balech [14]; Truong [15]; Licea et al., [16]; Moreno et al., [17]; Tomas [18]; Larsen and Nguyen-Ngoc [19]; Nguyen-Ngoc and Larsen [20]; Nguyen-Ngoc et al., [21]; Doan-Nhu et al., [22]; Phan-Tan et al., [23]; Phan-Tan et al., [24]. The scientific names and the nomenclature were updated according to Guiry & Guiry [25].

Quantitative samples

Quantitative samples of water (1 l) were collected using 5-litre Niskin bottle at the surface and bottom layer at each station, stored in PET plastic bottles, and fixed with neutral Lugol solution. Samples were concentrated by settling through few 48hrs-settling steps, from 1,000 ml to the final 3 ml volume, using graded cylinders. A volume of 1,000 µl of each sample was loaded onto Sedgwick-Rafter counting chamber for enumeration of phytoplankton cells following UNESCO method [26]. One drop of Calcofluor 0.5 mg/ml was added to samples for identification and enumeration of dinoflagellates [19]. Number of cell density per volume (l) of seawater is calculated from the following formula:

$$N = n\frac{1000}{A}B$$

Where: N: Cell density (cell/l); n: Number of phytoplankton cell in area A of chamber (square); A: Area of chamber (square); B: Final sedimentary volume before counting (ml).

Estimating diversity of phytoplankton community

PRIMER software version 6 (PRIMER-E Ltd., Plymouth, United Kingdom) was used for calculating diversity indices, and community analysis. Following equations were used:

Margalef index: $d = (S - 1)/\log(N)$ [27].

Pielou index: $J' = H'/\ln(S)$ [28].

Shannon-Wiener index:

$H' = -\mathrm{Sum}(P_i * \log_2(P_i))$ [29]

Simpson index:

$$(D) = \frac{1}{\sum_{i=1}^{s} p_i^2} \quad [30]$$

Bray-Curtis similarity index [31]:

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

Where: n_i: Cell number of species i; N: A total cell number in a sample; S: A total of the number of species in a sample; P_i: Frequency of species i in a sample = present probability of species i in a sample = n_i/N; C_{ij}: A total of similar species between two samples i and j; S_i and S_j: Number of species in sample i and sample j.

Data analysis

Phytoplankton data were extracted from database of PLANKTONSYS (BioConsult A/S). Excel Microsoft Office 2013 was used for calculation and plotting; R v3.6.0/RStudio was used for drawing graphs and basic statistic with package "plyrd" [32], "pgirmess" [33], ggplot2 [34] and vegan [35].

Similarity percentage (SIMPER) analysis was used for identifying the most important species in different sampling areas or groups based on Bray-Curtis similarity index. It performs pairwise comparisons of groups of sampling units and finds the average contributions of each species to the average overall Bray-Curtis similarity (dissimilarity). Non-metric multidimensional scaling (NMDS) is used for finding similarity among the phytoplankton assemblages based on abundant data. ANOVA and Kruskal-Wallis tests were used to identify the existence of significant differences from years and areas with parameter and non-parameter, respectively.

RESULTS AND DISCUSSION

Phytoplankton species composition

A total of 366 phytoplankton taxa belonging to 10 classes were identified in Quang Ngai waters during the surveys from 2015 to 2019. The highest species number belonged to dinoflagellates - Dinophyceae class (with 146 taxa recorded; accounting for 39.89% in total species), followed by three diatom classes Mediophyceae (90 taxa; 24.59%), Bacillariophyceae (68 taxa; 18.58%), and Coscinodiscophyceae (50 taxa; 13.66%). Cyanophyceae had 4 species (1.09%) and the remaining 8 taxa (2.19%) were from different classes including Bacillariophyta classis incertae sedis (2), Dictyochophyceae (2), Chlorophyceae (2), Euglenophyceae (1), and Chrysophyceae (1) (fig. 2).

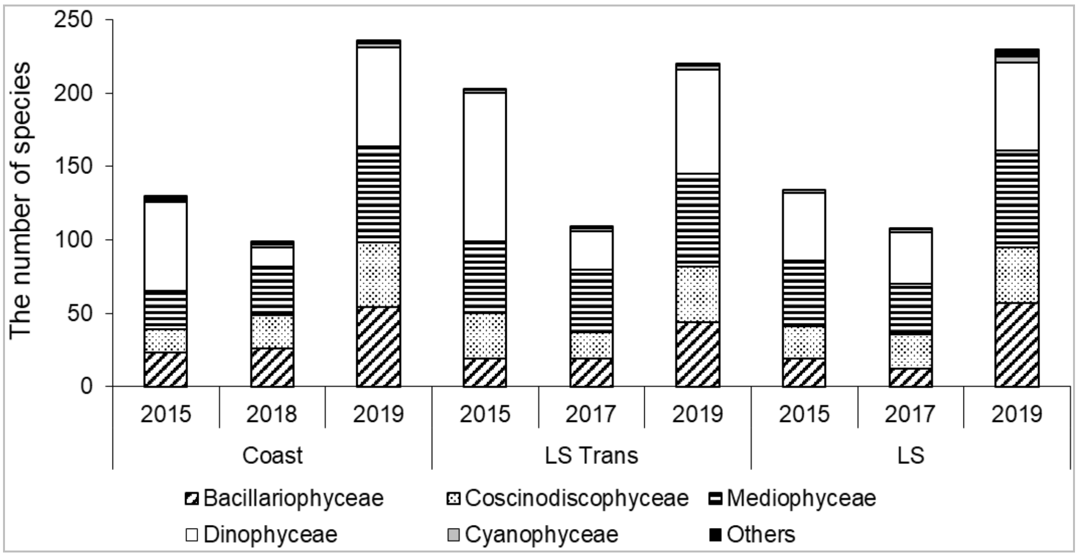

Figure 2. The number of phytoplankton species in three areas: the coastline, the Ly Son transect (LS Trans) and the Ly Son Island (LS) in 2015, 2017, 2018 and 2019 in Quang Ngai

In general, species number was considerably varied among the areas and within 4 years. Species number was most recorded in 2019 with 295 taxa, followed by 2015, 2017 and 2018 with 247, 185 and 99 taxa, respectively (table 2). In 2015, species number varied strongly (between 130 and 203 taxa) among the three sampling areas (coastal sites, Ly Son transect and Ly Son island), which is not the status in other years (108–109 taxa in 2017 and 220–236 taxa in 2019). There were significantly higher numbers of dinoflagellates recorded in 2015 and 2019, with 46.15% and 33.22% of total taxa respectively, compared to the remaining years (fig. 2, table 2). In the dry season, species composition highly varied among the years 2017, 2018 and 2019. In comparison with adjacent and other coastal waters, the species number in the study area was higher than in Da Nang (316 taxa) [36] and Quang Nam (364 taxa) [37].

Table 2. The number of species in Quang Ngai from 2015 to 2019.

Classes	Species (taxa) number				
	2015	2017	2018	2019	Total
Bacillariophyceae	31	24	26	65	68
Coscinodiscophyceae	34	33	23	46	50
Mediophyceae	62	64	33	77	90
Dinophyceae	114	59	13	98	146
Cyanophyceae	2	2	2	4	4
Others	4	3	2	5	8
Total	247	185	99	295	366

Changes in biodiversity and community structure

Biodiversity indices of phytoplankton community were varied among the study years. Average values of Margalef, Pielou, Shannon and Simpson indices in 2017 were lower than those in 2015 and 2019, but variation of diversity indices in 2015 and 2019 was much higher than in 2017 (fig. 3). This variation may indicate wide ranges of diversity indices from really low to high, reflecting rapid environmental changes among the sampling sites. However, there was no significant difference among the average indices from the three surveys in 2015, 2017 and 2019 (ANOVA, $p > 0.05$).

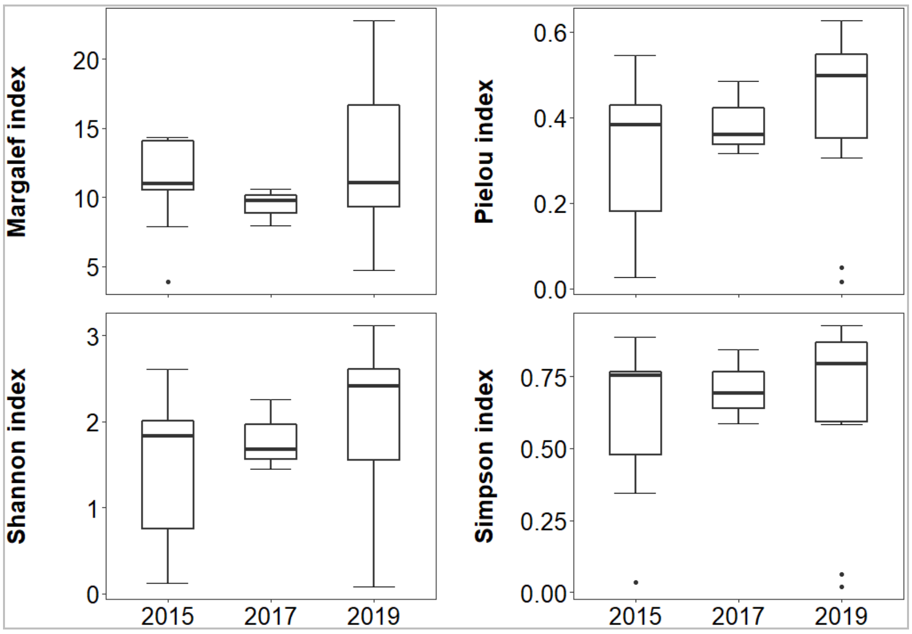

Figure 3. Box plots showing diversity indices from 3 surveys of 2015, 2017 and 2019 in Quang Ngai

The variation of diversity indices was more distinguishable when comparing different areas (fig. 4). In 2015, there was an increase of all indices from the coast to Ly Son island, but in the later years (2017, 2019) the highest values were in Ly Son transect (fig. 4). Among the years, the Margalef richness index in Ly Son transect in 2019 was much higher than that in 2015, 2017, and in other areas (ANOVA test, p-value < 0.001 and Tukey test, $\alpha = 0.05$). There was no significant difference of Pielou, Shannon and Simpson indices among the areas in all years (ANOVA test for Pielou and Shannon indices, and Kruskal-Wallis test for Simpson index, $p > 0.05$).

Generally, phytoplankton communities of Ly Son transect seemed to be less varied with higher diversity than in other areas in both dry (2017, 2019) and rainy (2015) seasons. Strong changes of the phytoplankton community in the Quang Ngai coast and Ly Son island may reflect that they would be more affected by human activities than Ly Son transect that may be stronger in water exchange. A previous study [38] on impacts of both ENSO and anthropogenic activities on phytoplankton diversity in tropical coastal waters suggested that the coastal areas under more human effects would have lower diversity indices. Comparing diversity indices with neighboring waters (Da Nang and Quang Ngai), the diversity indices (Shannon and Simpson) were not different (Kruskal-Wallis test, p-value > 0.05), except that Margalef richness index in Quang Ngai coastal waters was higher (Kruskal-Wallis test, p-value < 0.05 and Kruskal-Wallis post hoc, $\alpha = 0.05$) [36, 37].

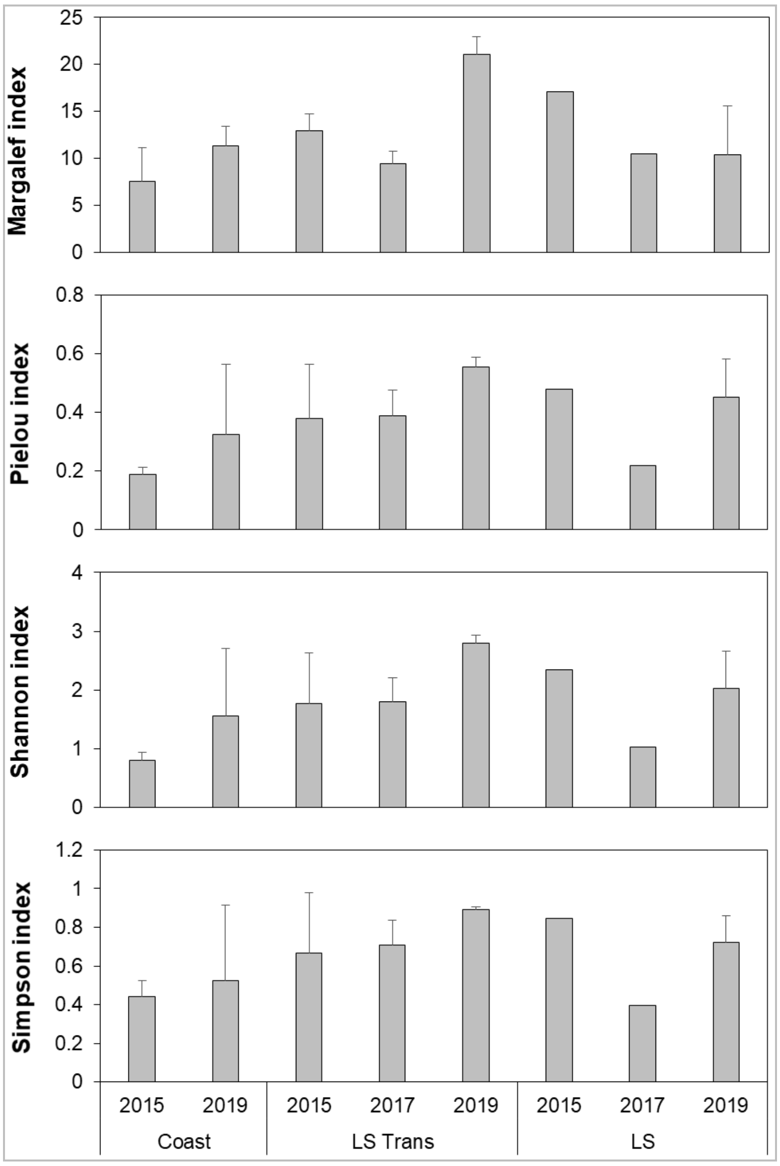

Figure 4. Variation of Margalef, Pielou, Shannon, Simpson indices in 3 surveyed areas (Coast = coastal Quang Ngai, LS Trans = Ly Son transect, LS = Ly Son island) in 2015, 2017 and 2019

There were changes in dominant species and this varied in each period and each area (table 3, SIMPER analysis). Among years, the changes in phytoplankton communities, presented by dissimilarity analysis, were very high, ranging from 90.74 (between 2015 and 2017) to 95.25% (between 2017 and 2019). *Skeletonema* species was most dominant with 65.41% of abundance in the coastal area in 2015. The group of small dinoflagellate (< 20 µm), centric diatom *Chaetoceros* spp., and *Thalassionema frauenfeldii* dominated at Ly Son transect, contributing 20.16, 19.40 and 16.49% of total abundance, respectively. In 2019, dominant genus at the coast was *Pseudo-nitzschia* (accounting for 53.42%) and it caused a bloom. Species dominance changed in other areas (Ly Son transect and Ly Son island). Both *Pseudo-nitzschia* (30.56%) and *Chaetoceros* (19.68%) genera were dominant in the Ly Son transect, while the medium-sized pennate diatom (20–50 µm) and *Cylindrotheca closterium* were dominant at Ly Son island with 28.54% and 18.29% of the total abundance, respectively. In 2017, two genera of centric diatom *Chaetoceros* and *Bacteriastrum* were highly abundant with 36.48% and 25.70%, respectively (table 2).

At the coastal area, comparison was only possible between 2015 and 2019, the phytoplankton community changed dramatically between the two years with 98.73% difference. The average species number was higher in 2019 than that in 2015, with 125 and 71 species (per sample), respectively. In this coastal area, the domination of species was changing from centric diatom *Skeletonema* in 2015 to *Pseudo-nitzschia* in 2019 (table 3).

In the Ly Son transect, three phytoplankton groups, dinoflagellates with size < 20 µm, pennate diatom (*Pseudo-nitzschia*) and centric diatom (*Chaetoceros*), were dominant in 2015, 2019 and 2017, respectively. The values of average dominance (% of total abundance) in these three years at Ly Son transect were relatively varied with the highest value in the dry season in 2017 (34.30%), followed by the rainy season in 2015 (28.77%) and the lowest in the dry season in 2019 (21.63%) (table 3).

Table 3. Abundant contribution (%) of dominant species among the years and study areas. Higher values were in bold.

Abundant species	Coast		LS Trans			LS
	2015	2019	2015	2017	2019	2019
Bacteriastrum spp.				25.70	2.54	
Chaetoceros spp.		9.24	19.40	36.48	19.68	
Dinoflagellate (< 20 µm)	3.32		20.16	3.79		
Thalassionema frauenfeldii	7.41		16.49	10.42	4.68	
Chaetoceros diversus	4.45		11.04	4.33		
Pseudo-nitzschia spp.	4.45	53.42	7.41	4.10	30.56	7.63
Cylindrotheca closterium		5.68			8.16	18.29
Trichodesmium erythraeum		2.61	8.18		2.29	7.40
Guinardia striata					2.38	
Pleurosigma sp.	7.61	4.10			4.96	5.92
Skeletonema spp.	65.41					
Pennate diatom (20–50 µm)						28.54
Nitzschia sigma					2.85	7.82

The result of NMDS based on the density of phytoplankton data revealed some differences in the species composition of phytoplankton from the different areas and years. Especially, most of 2019's samples are distinct from those of other years. In 2019, phytoplankton communities of three study areas, including the coast, Ly Son transect and Ly Son island, were separated with 40% of similarity. In the rainy season in 2015, samples from Ly Son transect were distributed with a clear cluster, and also separated clearly with those of the coast and the Ly Son transect. In 2015 and 2017, the points from Ly

Son transect were distributed within a cluster. These two phytoplankton communities of two years (2015 and 2017) at Ly Son transect shared about 40% of similarity despite the seasonal differences (the dry season in 2015 and the rainy season in 2017) (fig. 5). The coastal phytoplankton community in the rainy season in 2015 was distinctly separated with all other groups (fig. 5). The group of 2015 also shared low similarity (20%), indicating strong variation of the community.

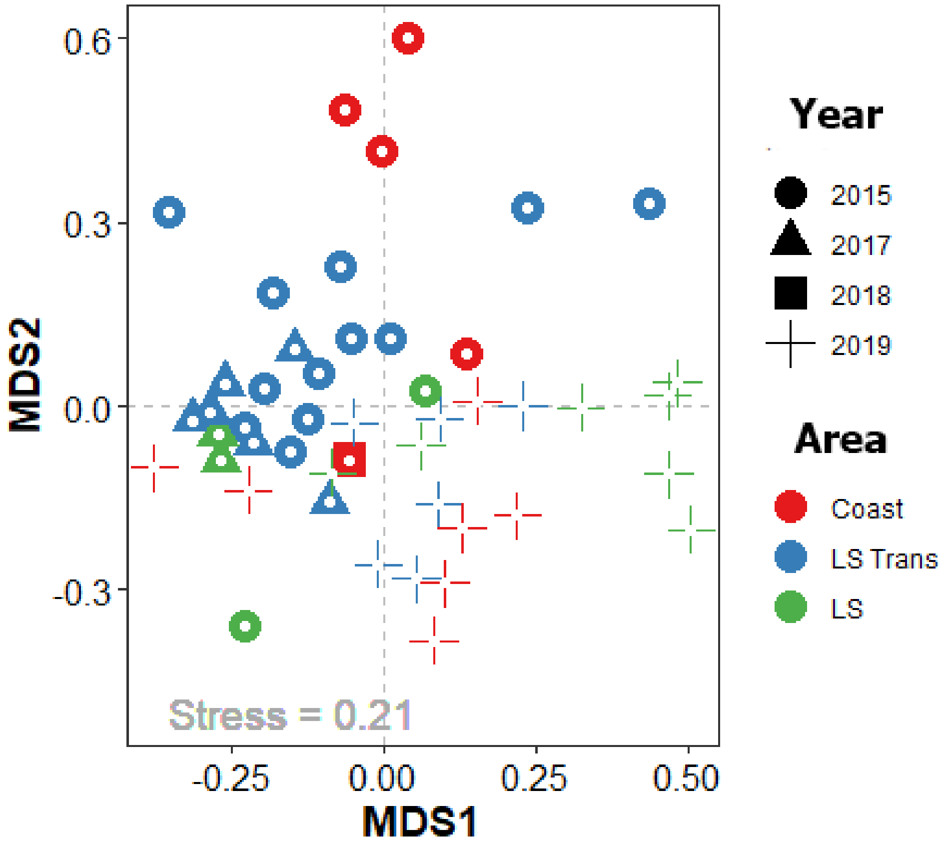

Figure 5. Non-metric multidimensional scaling (NMDS) of phytoplankton community data

Abundance and biomass

Abundance of phytoplankton in three different areas considerably varied among the years. There was a strong difference between phytoplankton density in 2015 and 2017 (Kruskal-Wallis test, p-value < 0.01, and Kruskal-Wallis post hoc test, α = 0.05) with density in 2017 as 3.5 times higher than that in 2015. In the coastal area, the average density was increasing from 2015 to 2019 (fig. 6), especially at the coast in 2019, due to the bloom of *Pseudonitzchia* spp. (with 1,045,010 cells/l) at two stations near Tra Khuc river. In Ly Son transect abundance of phytoplankton in two years 2015 and 2019 were lower than that in 2017 with 7,414 ± 8,280 cells/l, 6,432 ± 5,180 cells/l and 48,876 ± 60,822 cells/l, respectively (fig. 6). At Ly Son island, phytoplankton abundance was lower about 6 times in 2015 than those in 2017 and 2019 (fig. 6).

Biomass of phytoplankton was significantly different in 2015, 2017 and 2019 (Kruskal-Wallis post hoc, α = 0.05). The change in phytoplankton biomass was in similar pattern as change in density in the coast. However, at Ly Son island and Ly Son transect, there was an increase of biomass from 2015 to 2019 despite the different pattern of abundance in these two areas (fig. 7).

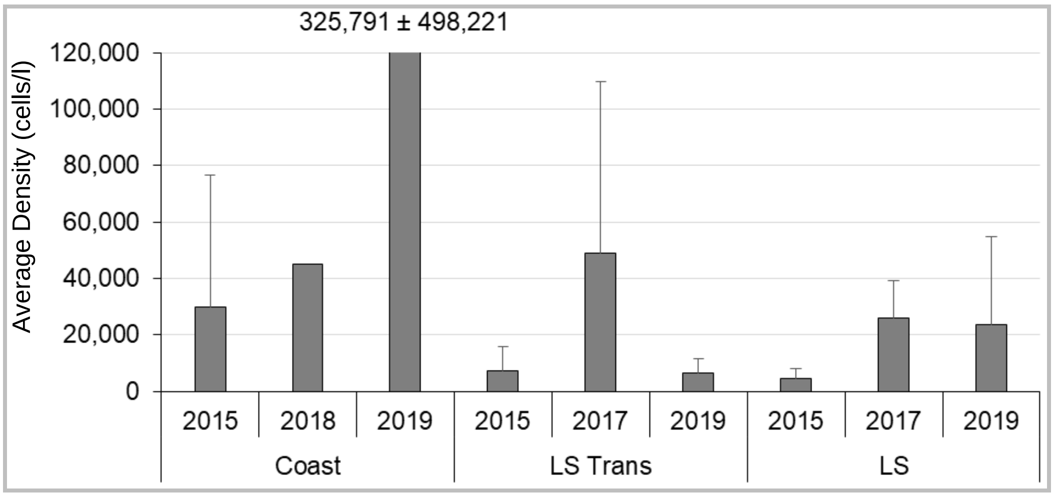

Figure 6. Variation of average cell density of the three study areas: coast, LS Trans and LS during 2015–2019 in Quang Ngai coastal waters

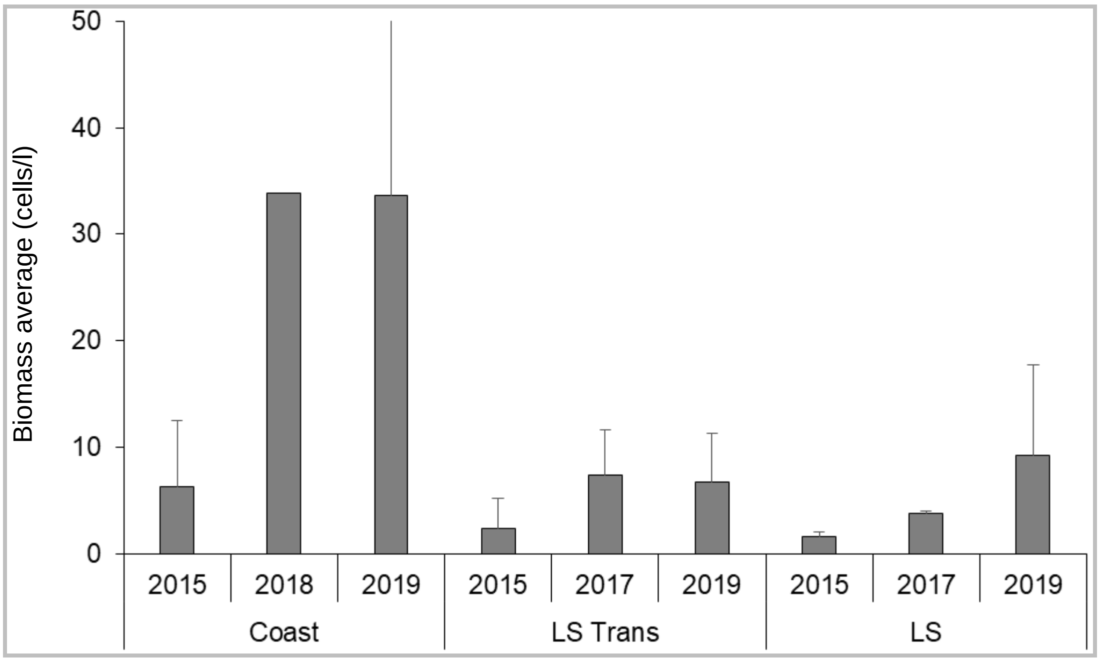

Figure 7. Variation of average biomass of three study areas: coast, LS Trans and LS during 2015–2019 in Quang Ngai coastal waters

In Ly Son transect, phytoplankton abundance and biomass in 2015 and 2019 were generally lower than those in 2017. This could be due to the El Niño influences as reported in a previous research [6], which was conducted in Río de la Plata estuary (Argentina). Besides, averages of diatom density in Ly Son transect in 2015 and 2019 (during El Niño phase) were much lower compared to 2017 (neutral phase) with 3,545 ± 3,350 cells/l (2015), 6,208 ± 5,277 cells/l (2019) and 47,057 ± 61,586 cells/l (2017). This coincided with the result of Rousseaux and Gregg (2012) [39] suggesting the concentration of diatoms lower in the El Niño phase.

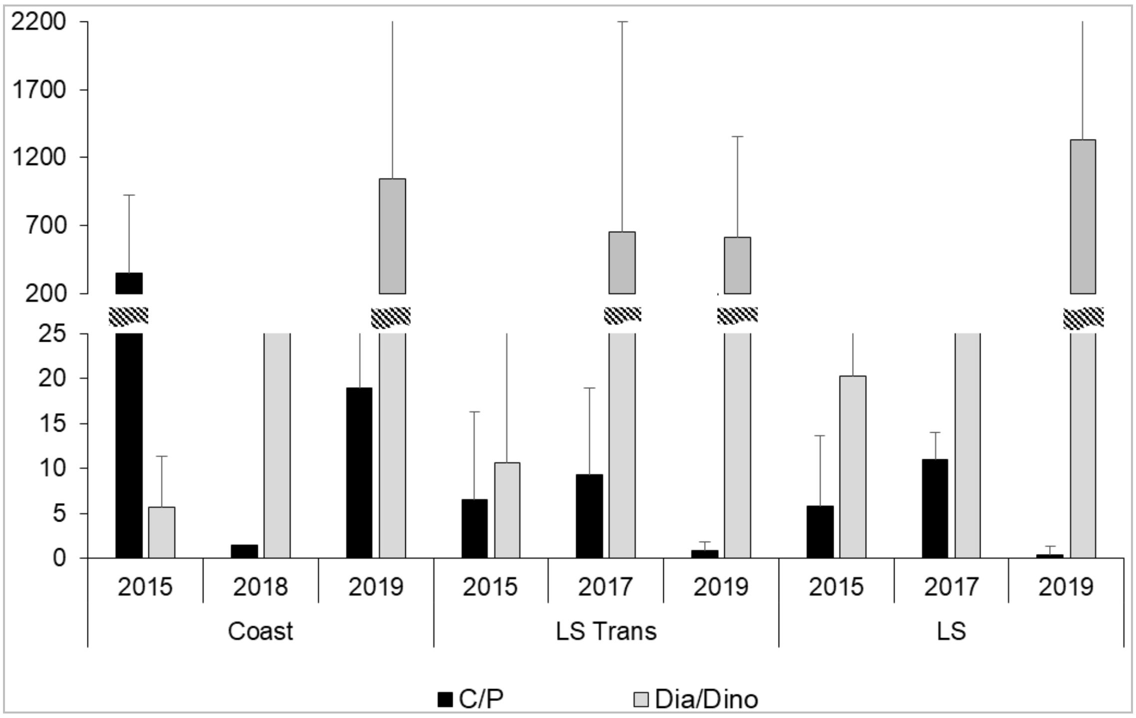

Figure 8. Ratio of centric to pennate diatoms (C/P) and of diatoms to dinoflagellates (Dia/Dino) among the three areas

Analysis of the trophic diatom index revealed that most of the C/P ratios (centric to pennate diatom density) in these areas by years were higher than 2. It indicated that these waters were in eutrophic status [40, 41]. The C/P ratio also showed a decreasing trend from the coast area to Ly Son island in 2015 and 2019 (fig. 8). The Dia/Dino ratios (diatom to dinoflagellate density) in the rainy season 2015 (approximately 5–20) were much lower than in other years in all areas (roughly 40–1,300) and showed a clear trend, increasing from the coast area to Ly Son island.

CONCLUSION

Phytoplankton community in the coastal waters of Quang Ngai Province in 2015–2019 was analyzed, revealing a list of 366 taxa belonging to 10 classes. This species richness was higher than in the neighboring waters, Da Nang (316 taxa) and Quang Nam (364 taxa). Biodiversity of the phytoplankton in the studied areas varied greatly among the years and locations, increased from coastline to Ly Son island. Among the years (2015–2019), lower biodiversity was found in 2017 but with much less variation of all indices among the sampling sites than in 2015 and 2019. This variation may indicate wide ranges of diversity indices from really low to high, reflecting rapid environmental changes among the sampling sites.

Among the three studied areas, phytoplankton communities of Ly Son transect were less varied but with higher diversity than in other areas in both dry (2017, 2019) and rainy (2015) seasons. Strong changes of the phytoplankton community in the Quang Ngai coast and Ly Son island may reflect that they would be more affected by human activities than Ly Son transect.

The abundance and biomass also showed variations by years, in which they were higher in 2017 in the Ly Son transect. Domination of phytoplankton taxa was different by years and areas. In the coast area, dominant species was changing from centric diatom *Skeletonema* in 2015 to *Pseudo-nitzschia* in 2019. In Ly Son transect, three phytoplankton groups, dinoflagellates with size < 20 μm, pennate diatom (*Pseudo-nitzschia*) and centric diatom (*Chaetoceros*), were dominant in 2015, 2019 and 2017, respectively. In Ly Son island, pennate diatom (20–50 µm) and centric diatoms *Cylindrotheca closterium* were dominant.

The trophic C/P diatom index revealed that the coastal waters of Quang Ngai province were in eutrophic status, especially along the coastline of the province. Blooms of *Pseudo-nitzchia* spp. were recorded in the coastal area that may affect shellfish aquaculture (if any).

Acknowledgements: This research is funded by the scientific research project of the Institute of Oceanography, grant number: 01/CSSVPD-2020. This paper used data from projects which were conducted by Department of Marine Plankton from 2015 to 2019.

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Appendix 1. The coordinates of surveyed stations

Station	Area	Longitude	Latitude
1	LS	109.094	15.37788
2	LS	109.1025	15.39057
3	LS	109.1202	15.39073
4	LS	109.1357	15.38901
5	LS	109.135	15.3776
6	LS	109.0944	15.37349
7	LS Trans	109.076	15.36
8	LS Trans	109.0549	15.34115
9	LS Trans	109.0352	15.31743
10	LS Trans	109.0108	15.29048
11	LS Trans	108.9888	15.26407
12	LS Trans	108.9607	15.24213
13	Coast	108.927	15.20379
14	Coast	108.922	15.2072
15	Coast	108.92	15.2111
16	Coast	108.9064	15.07047
17	Coast	108.9378	14.96508
18	Coast	108.9802	14.86765
19	Coast	109.0466	14.7494
20	Coast	109.0762	14.69616