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PHYTOPLANKTON COMMUNITY STRUCTURE CHANGES IN THI NAI LAGOON (SOUTH - CENTRAL VIETNAM) FROM 2004 TO 2020

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ABSTRACT

Phytoplankton species composition, abundance, biodiversity indices and their influence by environmental conditions were examined in Thi Nai lagoon using data from four surveys in 2004, 2008, 2009 and 2020. A total of 367 taxa of phytoplankton belonging to ten groups was recorded, of which diatoms were dominant with over 60% of the total species. In Thi Nai lagoon, the number of phytoplankton species decreased from the upper lagoon (283 taxa) to the lagoon mouth area (224) and was lowest in river stations (139). The species number was much lower in the rainy season (<95 species) and increased from 2004 to 2020. Among the biodiversity indices, Shannon, Δ, $s\Delta^+$, Λ^+ and $s\Phi^+$ were lower in the rainy season, whereas Δ^*, Δ^+ and Φ^+ had lower values in the dry season. For long-term analysis, the differences were especially significant at upper and lower lagoon for Δ, Δ^*, Δ^+, $s\Delta^+$ and $s\Phi^+$. In the dry season, the mean density of phytoplankton was lower at the river stations and upper lagoon, but higher at the lower lagoon and the lagoon mouth area. There was a clear difference of species assemblages between the two seasons with average dissimilarities in each area ranging from 94.16% to 95.57%. During 2004–2020, the difference in ratios between the main phytoplankton groups were small over years but there was a complete change in dominance of particular species, assemblage dissimilarities were from 73.4 to 77.9, greatest between 2009 and 2020. The lagoon was low in biodiversity for the whole investigated time indicated by taxonomic index Δ^+. Among biodiversity indices, species richness (S), taxonomic indices (Δ^*, $s\Delta^+$), and phylogenetic indices (Φ^+, $s\Phi^+$) were more sensitive to the changes of the aquatic environment than other traditional indices.

Keywords: Phytoplankton, diversity index, taxonomic index, phylogenetic index, Thi Nai lagoon.

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INTRODUCTION

The lagoon is a coastal marine ecosystem and is affected by land and river (Kownacka et al., 2020). The lagoon provides essential ecosystem services due to its high productivity (Viaroli et al., 2008) which is based on high primary production. In the lagoon, phytoplankton particularly plays a key role as primary producers in food web dynamics (Field et al., 1998). Phytoplankton communities are also sensitive to alterations of aquatic habitats (Li et al., 2009) so that changes in the phytoplankton community are crucial indicators of coastal ecological conditions (Sathicq et al., 2015). The phytoplankton composition not only indicates the certain status of the waters (Bianchi et al., 2003) but also the previous status of aquatic conditions. Therefore, the patterns of phytoplankton community structure are commonly measured in many different researches. These patterns could be evaluated using different parameters including species composition, abundance, and biodiversity. In which, biodiversity could be expressed simply as a traditional index (such as species richness) or with a specific index (Duarte et al., 2006) such as taxonomic indices (Clarke & Warwick, 2001a, b).

Thi Nai is the largest lagoon of Binh Dinh province, South Central Viet Nam, with an area of over 5,000 ha at high tide. This lagoon opens to Quy Nhon bay with a narrow mouth (500–700 m wide). There are two rivers that run into the lagoon, Con and Ha Thanh. The lagoon harbors different habitats including mangrove and seagrass, which make its favorable conditions to develop aquaculture and tourism. Consequently, such anthropogenic activities may alter the lagoon ecosystem. Measuring such alterations such as environmental or biodiversity changes of Thi Nai lagoon was poorly known. As bioindicators of water quality, understanding the structure and change of the phytoplankton community plays an essential role in assessing the environmental change in the waters. Although some surveys were conducted, the publication on the phytoplankton of this lagoon is still limited. There was a study about biological resources for the development of aquaculture in the Thi Nai lagoon (Kieu et al., 2017) which focused on macrofauna but only mentioned the number of 167 phytoplankton species found in the lagoon.

In this study, data sets of 150 samples collected in November 2008 (rainy season), June 2004, April 2009, and May 2020 (dry season) were used. The assessment and comparison of phytoplankton community structures were based on species composition, diversity indices, and abundance. These analyses aim to provide the essential data of the phytoplankton community in Thi Nai lagoon as a baseline for future studies and assessing possible environmental impacts.

MATERIALS AND METHODS

Time and sampling sites

The study used 61 qualitative samples and 89 quantitative phytoplankton samples (Table 1) collected from Thi Nai lagoon, Binh Dinh province in November 2008 (rainy season), June 2004, April 2009, and May 2020 (dry season). Four areas included: river stations (TN_Ri); upper Thi Nai lagoon (TN_Up); lower Thi Nai lagoon (TN_Lo); Thi Nai lagoon mouth area (TN_Mo) (Fig. 1). The coordinates of sampling stations were shown in Appendix 1.

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

Areas	Stations	Qualitative samples	Quantitative samples
River stations (TN_Ri)	1–7	15	19
Upper Thi Nai lagoon (TN_Up)	8–22	21	27
Lower Thi Nai lagoon (TN_Lo)	23–32	15	23
Thi Nai lagoon mouth area (TN_Mo)	33–36	10	20

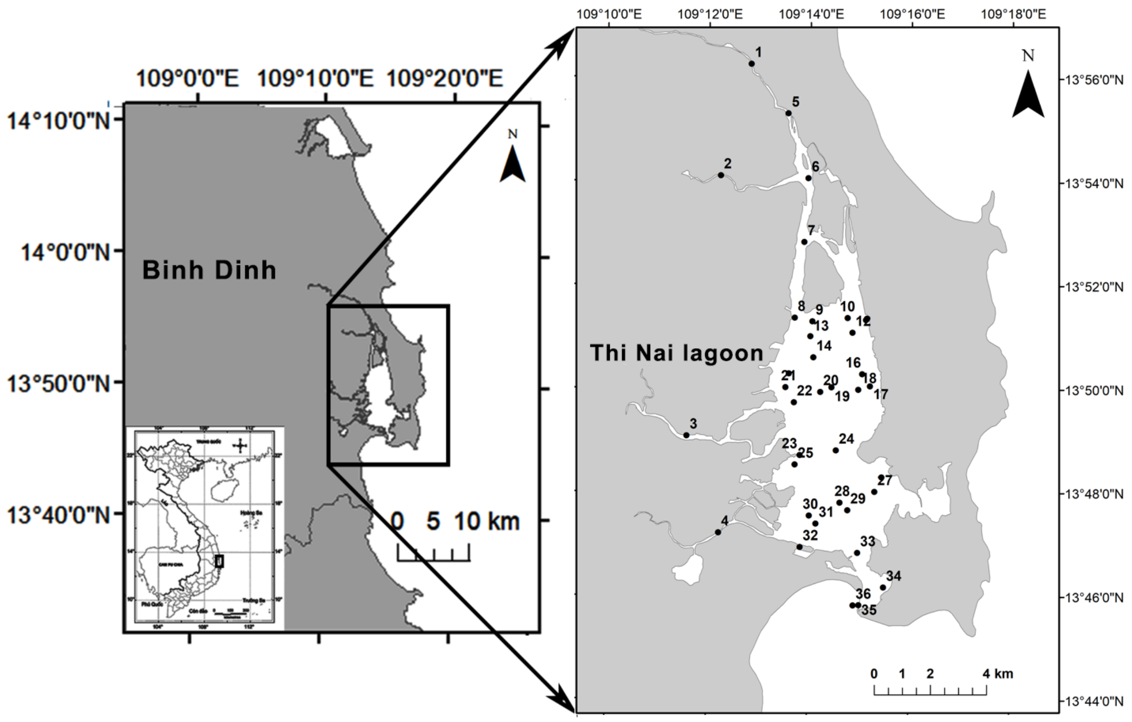

Figure 1. Maps showing studied areas and sampling stations in Thi Nai lagoon

Sampling and analysis method

Qualitative samples

Qualitative samples were collected by using a plankton net with 25 µm mesh size towing vertically at slow speed from the bottom to the surface, then fixed with formalin 5% and stored in dark for later analysis in the laboratory. Species were identified and measured under the light microscope (Leica LDMB, Germany). To identify armoured dinoflagellates Calcofluor White M2R method (Fritz & Triemer, 1985) was used, and observation was under the epifluorescence microscope (Leica LDMB, Germany).

Identification of the species was based on published descriptions of Graham & Bronikovsky (1944), Hoang (1962, 1963), Shirota (1966), Abé (1981), Balech (1988), Truong (1993), Licea et al. (1995), Moreno et al. (1996), Tomas (1997), Larsen & Nguyen-Ngoc (2004), Nguyen-Ngoc & Larsen (2008), Nguyen-Ngoc et al. (2012), Doan-Nhu et al. (2014), Phan-Tan et al. (2016), Phan-Tan et al. (2017). The scientific names and the nomenclature were updated according to Guiry & Guiry (2021).

Quantitative samples

Quantitative samples of water (1 L) were collected using a 5-liter Niskin bottle at the surface and bottom layers at each station, stored in PET plastic bottles and fixed with neutral Lugol solution. Samples were concentrated by settling through a few 48 hrs-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 the UNESCO method (Sournia, 1978). One drop of Calcofluor 0.5 mg/mL was added to samples for identification and enumeration of dinoflagellates (Larsen & Nguyen-Ngoc, 2004).

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:

$d = (S - 1)/\log(N)$ (Margalef, 1958)

Pielou index:

$J' = H'/\text{loge}(S)$ (Pielou, 1966)

Shannon - Wiener index:

$H' = -\text{sum}(P_i * \log_2(P_i))$ (Shannon, 1948)

Simpson index:

$$1 - D = \frac{1}{\sum_{i=1}^{s} p_i^2} \text{ (Simpson, 1949)}$$

Bray-Curtis similarity index (Bray & Curtis, 1957):

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

Where: i and *j*: The two sites; n_i: Cell number of species counted on site *i*; *N* is a total cell number in a sample; *S*: A total of the number of species in a sample; P_i: Frequency of the i^{th} species in a sample = present probability of the i^{th} species in a sample = n_i/N; C_{ij}: A total of similar species found in both sites; S_i and S_j: The number of species counted on each site.

A master list of all species was recorded in the study waters and arranged all taxa hierarchically into species, genera, families, orders, classes, and phylum to determined indices below:

Taxonomic diversity (Δ) (Clarke & Warwick, 2001a, b):

$$\Delta = \frac{\sum\sum_{i<j} \omega_{ij} n_i n_j}{N(N-1)/2}$$

Taxonomic distinctness (Δ^*) (Clarke & Warwick, 2001a, b):

$$\Delta^* = \frac{\sum\sum_{i<j} \omega_{ij} n_i n_j}{\sum\sum_{i<j} n_i n_j}$$

Where: $n_i = Np_i$ denotes abundance of the i^{th} species in the sample ($\sum_{i=1}^{S} n_i = N$ = total number of individuals in the sample) and ω_{ij} is the 'distinctness weight' given to the path length (usually the number of steps in the dendrogram) linking species *i* and *j* in the hierarchical classification. Summations over *i*, *j* are from 1 to *S* with $i < j$ because the path length for two individuals of the same species is zero.

Average taxonomic distinctness (AvTD) (Δ^+) (Clarke & Warwick, 2001a, b):

$$\Delta^+ = \frac{\sum\sum_{i<j} \omega_{ij}}{s(s-1)/2}$$

Total taxonomic distinctness (TTD) ($s\Delta^+$) (Clarke & Warwick, 2001a, b):

$$s\Delta^+ = \sum \frac{\sum_{i \neq j} \omega_{ij}}{s-1}$$

Variation in taxonomic distinctness (VarTD) Λ^+ (Clarke & Warwick, 2001a, b):

$$\Lambda^+ = \frac{\sum\sum_{i \neq j} \left(\omega_{ij} - \varpi\right)^2}{s(s-1)/2}$$

Where: s is the number of species present; x_{ij} is the 'distinctness weight' given to the path length linking species *i* and *j* in the taxonomy.

Average phylogenetic diversity (AvPD) Φ^+ (Clarke & Warwick, 2001a, b):

$$\Phi^+ = \frac{\sum n_i}{s}$$

(Total) phylogenetic diversity $s\Phi^+$ (Clarke & Warwick, 2001a, b):

$$s\Phi^+ = \sum n_i$$

Water environmental parameters

The study also used the water environmental parameters in November 2008 and April 2009 at 17 sampling samples in the Thi Nai lagoon. All the samples were kept in the dark at a cool temperature (4 ^{o}C) before transportation to the laboratory of the

Department of Hydro-Geochemistry, Institute of Oceanography. Salinity, total suspended solids (TSS), phosphate (PO_4^{3-}), nitrite (NO_2^-), nitrate (NO_3^{2-}), ammonia ($NH_{3,4}$), silicate (SiO_3^{2-}) were measured following standard methods (APHA, 2005) - Total Suspended Solids: dried at 103–105 ^oC (2540-D); Phosphate: Ascorbic Acid Method (4500-P); Nitrate: Cadmium Reduction Method (4500-NO_3); Nitrite: Colorimetric Method (4500-NO_2); Ammonia: Phenate Method (4500-NH_3); Silicate: Molybdosilicate Method (4500-SiO_2).

Data analysis

Phytoplankton data were extracted from the database of PLANKTONSYS (BioConsult A/S). Excel Microsoft Office 2013 was used for data treatment and plotting, R v3.6.0/RStudio was used for drawing graphs and basic statistics with package "pgirmess" (Giraudoux, 2017), ggplot2 (Wickham, 2009), and vegan (Oksanen, 2019).

Similarity percentage analysis (SIMPER) was used to identify the most important species in different sampling areas or groups based on the 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) was used for finding similarity among the phytoplankton assemblages based on abundant data. ANOVA and Kruskal-Wallis tests were used to identify whether the existence of significant differences from years and areas with parameter and non-parameter, respectively.

Funnel plot (PRIMER software v.6) identified sites with Δ^+ values by using 1,000 simulation subsamples for expected Δ^+ from a master list of phytoplankton. The funnel plot performed uncertainty 95% probability intervals based on differences between observed and expected Δ^+ values against species number (Clarke & Warwick, 2001a, b).

RESULT AND DISCUSSION

Species composition

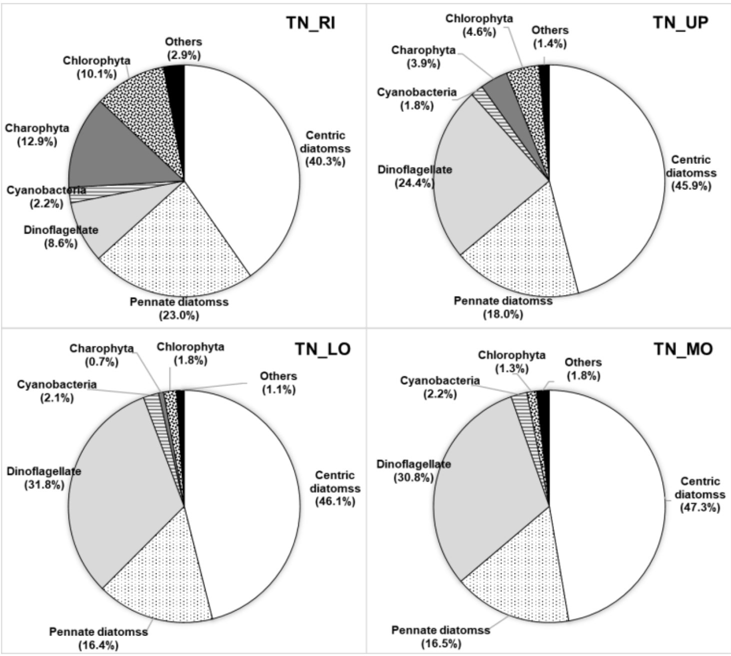

Figure 2. Phytoplankton species composition (%) of Thi Nai lagoon during 2004–2020.
Note: TN_Ri: River stations; TN_Up: Upper Thi Nai lagoon; TN_Lo: Lower Thi Nai lagoon, TN_Mo: Thi Nai lagoon mouth area

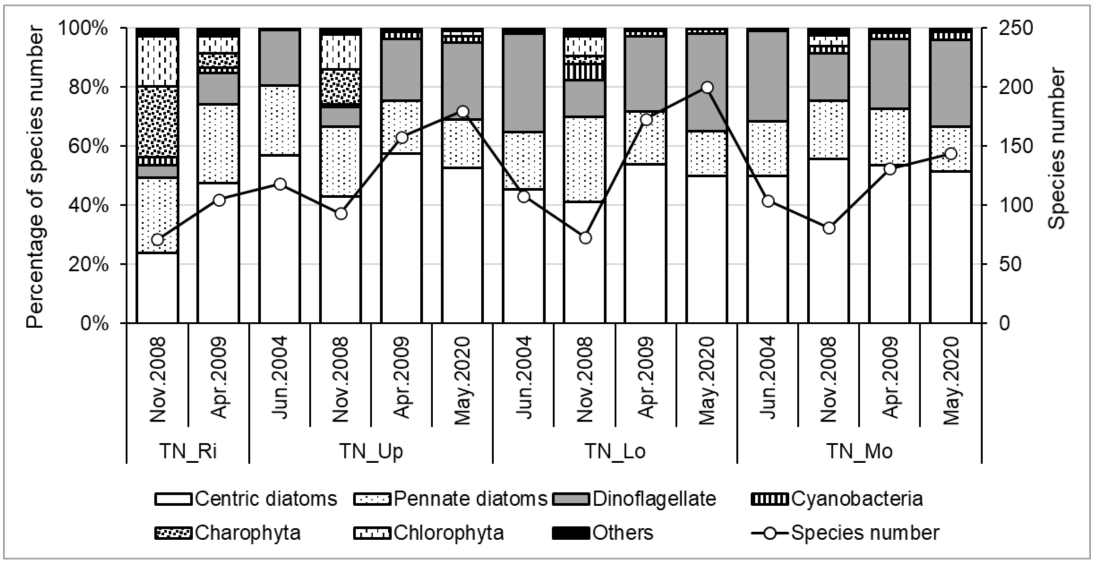

Figure 3. The species composition of phytoplankton in Thi Nai lagoon from 2008 to 2020

A total of 367 phytoplankton species and subspecies was recorded in the Thi Nai lagoon, including nine groups: Diatoms (208): centric diatoms (149), pennate diatoms (59), dinoflagellates (106), Cyanobacteria (7), Charophyta (21), Chlorophyta (18), Euglenozoa (3), Ochrophyta (2), Cercozoa (1) and Haptophyta (1). In that, phytoplankton species number in TN_Up was highest (283 taxa), followed by TN_Lo (280), TN_Mo (224), and lowest in TN_Ri (139). Diatoms were the most dominant group in all areas (over 60% of total species). Dinoflagellates were the second dominance in TN_Up, TN_Lo, and TN_Mo with 24.4%, 31.8%, and 30.8%, respectively. In TN_Ri, following diatoms, Charophyta and Chlorophyta were dominant with 12.9% and 10.1%, respectively (Fig. 2). In general, two representative groups for freshwaters (Charophyta and Chlorophyta) were decreasing from TN_Ri to TN_Mo even Charophyta be absent in TN_Mo where was connected to the open sea.

There was a significant difference in the number of species between the rainy (2008) and dry seasons (2004, 2009, and 2020). The number of species was much lower in the rainy season (< 95 species) in all areas, and Charophyta and Chlorophyta were found more common in this season (11–17 species). In the dry season, the number of species of TN_Up, TN_Lo, and TN_Mo were increasing from 2004 to 2020 (Fig. 3), which ranged from 118–180 taxa in TN_Up, 108–200 taxa in TN_Lo, and 104–144 taxa in TN_Mo. Cyanobacteria was almost absent in the dry season in 2004 but more prominent in 2009 and 2020.

Phytoplankton diversity

Some characteristics of phytoplankton communities in four areas of Thi Nai lagoon are presented in Table 2. We chose eight indices, including H', Δ, Δ^*, Δ^+, sΔ^+, Λ^+, Φ^+ and sΦ^+, to estimate their variation of two seasons and the years in the dry season.

Seasonal changes of phytoplankton diversity

In Thi Nai lagoon, mean values of phytoplankton diversity indices including Shannon, Δ, sΔ^+, Λ^+ and sΦ^+ in all areas were lower in the rainy season, while rest indices were lower in the dry season (Fig. 4). Each index was statistically different between the two seasons in a specific area such as Shannon in the mouth of the lagoon, Δ^*, Λ^+ and Φ^+ in both upper and lower lagoon, Δ^+ in the upper lagoon, and sΔ^+ and sΦ^+ in the lower lagoon.

Table 2. Mean and standard deviation (SD) values of phytoplankton diversity indices in four areas and two seasons

Area	Season	S	d	J’	H’(ln)	1-D	Δ	Δ^*	Δ^+	$s\Delta^+$	Λ^+	Φ^+	$s\Phi^+$
TN_Ri	all data	**35.16**	**4.23**	**0.35**	**1.17**	**0.50**	**44.88**	**89.37**	**87.60**	**3,051**	**283.74**	**58.76**	**1,966**
	SD	14.43	2.48	0.19	0.60	0.26	24.11	7.24	3.51	1,154	46.06	7.94	542
TN_Up	all data	**66.85**	**7.50**	**0.44**	**1.85**	**0.68**	**50.89**	**77.83**	**82.54**	**5,461**	**390.98**	**45.57**	**2,798**
	SD	29.96	3.72	0.19	0.83	0.27	21.32	15.96	3.95	2,398	104.35	9.59	842
TN_Lo	all data	**76.00**	**7.59**	**0.34**	**1.44**	**0.54**	**38.79**	**72.87**	**83.65**	**6,264**	**395.48**	**46.77**	**3,027**
	SD	44.55	4.56	0.20	0.90	0.31	24.82	22.47	3.18	3,603	95.13	13.06	1,299
TN_Mo	all data	**86.55**	**8.26**	**0.42**	**1.90**	**0.70**	**48.32**	**70.27**	**83.27**	**7,199**	**412.20**	**39.08**	**3,299**
	SD	23.77	2.29	0.17	0.78	0.27	20.07	11.09	1.12	1,958	36.25	4.63	688
TN_Ri	Dry	**39.89**	**5.56**	**0.41**	**1.40**	**0.62**	**55.56**	**89.58**	**84.79**	**3,347**	**283.38**	**56.26**	**2,094**
	SD	19.00	3.14	0.14	0.38	0.16	14.27	6.44	3.23	1,527	45.04	9.32	716
TN_Up	Dry	**83.17**	**9.52**	**0.53**	**2.31**	**0.82**	**58.26**	**70.68**	**80.28**	**6,705**	**451.44**	**39.33**	**3,207**
	SD	22.74	2.80	0.10	0.38	0.07	13.43	14.42	2.50	1,955	67.44	3.65	727
TN_Lo	Dry	**108.79**	**10.84**	**0.39**	**1.82**	**0.66**	**43.05**	**60.42**	**81.84**	**8,906**	**460.08**	**36.86**	**3,983**
	SD	19.63	2.39	0.16	0.76	0.26	23.46	20.13	1.71	1,633	40.61	2.03	552
TN_Mo	Dry	**95.31**	**9.04**	**0.48**	**2.18**	**0.81**	**54.72**	**67.93**	**83.13**	**7,921**	**419.68**	**36.89**	**3,509**
	SD	17.09	1.75	0.07	0.38	0.07	10.06	11.14	1.08	1,412	36.18	1.02	581
TN_Ri	Rainy	**30.90**	**3.04**	**0.29**	**0.96**	**0.40**	**35.26**	**89.19**	**90.13**	**2,784**	**284.06**	**61.01**	**1,850**
	SD	7.28	0.49	0.22	0.70	0.30	27.66	8.23	0.61	652	49.38	6.09	315
TN_Up	Rainy	**34.22**	**3.44**	**0.26**	**0.93**	**0.40**	**36.17**	**92.11**	**87.06**	**2,973**	**270.04**	**58.06**	**1,980**
	SD	4.63	0.71	0.20	0.73	0.31	26.99	6.41	1.76	350	32.34	2.72	218
TN_Lo	Rainy	**25.00**	**2.53**	**0.26**	**0.85**	**0.36**	**32.17**	**92.23**	**86.45**	**2,154**	**294.98**	**62.18**	**1,539**
	SD	4.90	0.79	0.23	0.78	0.32	26.81	6.12	2.90	387	59.53	4.82	235
TN_Mo	Rainy	**51.50**	**5.16**	**0.20**	**0.79**	**0.28**	**22.74**	**79.62**	**83.86**	**4,314**	**382.30**	**47.83**	**2,458**
	SD	8.35	1.44	0.25	1.02	0.38	30.89	3.56	1.25	662	16.97	1.71	363

Shannon index was higher in the dry season in all areas and was highest in the upper lagoon. Variation of Shannon index between the two seasons was smallest in the river stations. In the dry season, the Shannon index was a statistically significant difference between TN_Ri and TN_Up (Kruskal-Wallis post-hoc, $\alpha = 0.05$). Taxonomic diversity, Δ, had a similar pattern with the Shannon index and was slightly higher in the dry season (Fig. 4).

The difference of Δ^* between the two seasons was clearly in the upper and lower lagoon. In the dry season, Δ^* at the river stations was much higher than in the lower and mouth areas of the lagoon (Kruskal-Wallis post-hoc, $\alpha = 0.05$.) There was no difference in this index among areas in the rainy season. Average taxonomic distinctness, Δ^+, was highest at the river stations, and the variation of Δ^+ between the seasons was higher in the upper lagoon (Fig. 4).

Variation of $s\Delta^+$ and $s\Phi^+$ was similar with a highly positive correlation ($r = 0.99$), and the means were considerably higher in the dry season (Fig. 4). The statistical differences of these indices between the two seasons were in the lower lagoon. In the dry season, these indices were lowest at the river stations.

A significant difference of Λ^+ between the two seasons was in the upper and lower lagoon (Fig. 4). In the dry season, the means were higher in such two areas. However, mean phylogenetic diversity (Φ^+) was lower in the dry season in both upper and lower lagoon

(Kruskal-Wallis post-hoc, α = 0.05). Among the areas, Φ^+ at river stations were much higher than others in the dry season, while in the rainy season all areas had similar values.

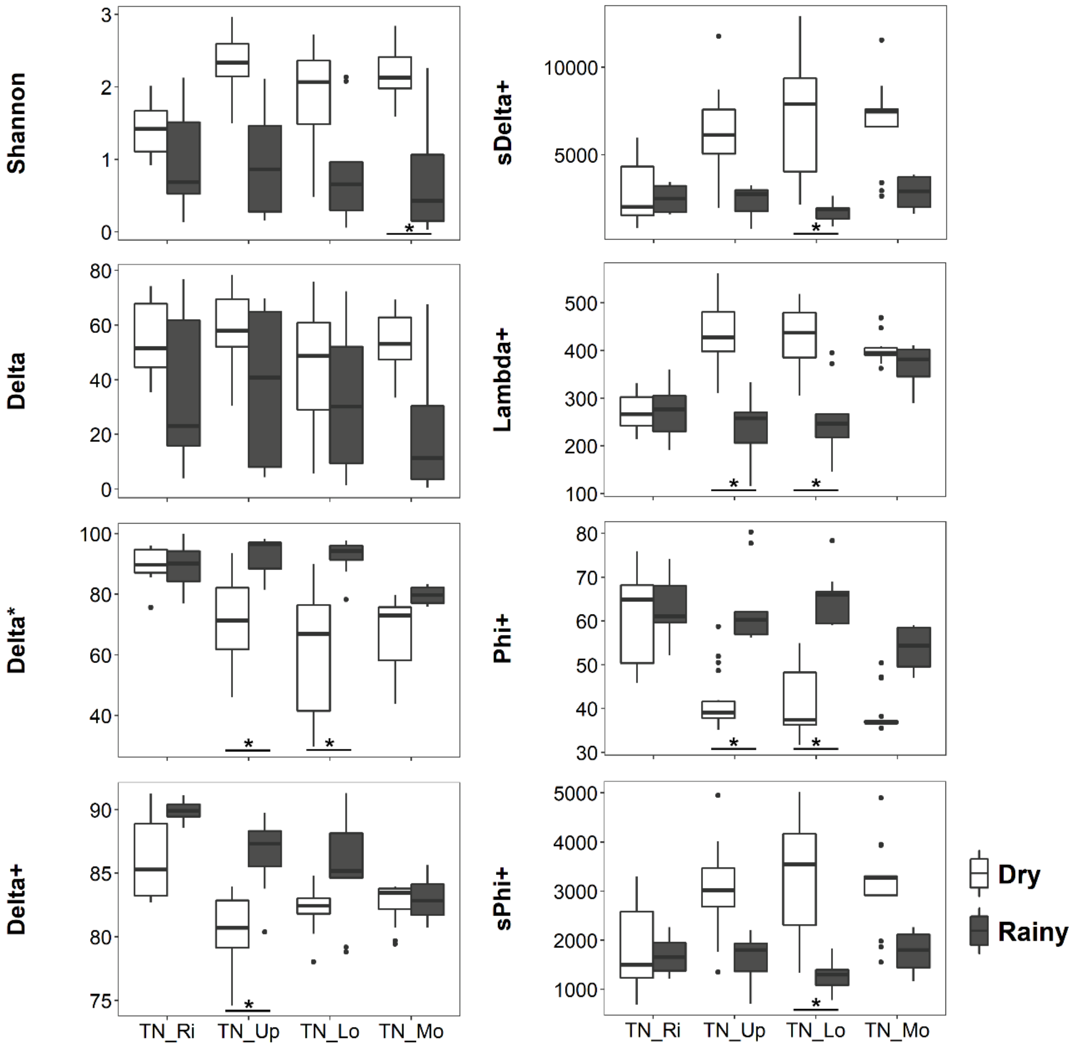

Figure 4. Seasonal variation of diversity indices in four areas of Thi Nai lagoon: TN_Ri, TN_Up, TN_Lo, TN_Mo

Note: * Indicates a significant difference (Kruskal Wallis post-hoc, α = 0.05)

In general, all these diversity indices showed a clear pattern of seasonal variations, especially in the upper and lower lagoon areas. The results of Francé et al. (2021) demonstrated that the indices of the phytoplankton community sensitively responded to seasonal fluctuations in all sampling sites of Mediterranean sub-regions (Adriatic, Ionian, and Aegean Seas). The wide seasonal variations of biodiversity indices were also observed in other coastal lagoons (e.g., Santo Andre lagoon (Duarte et al., 2006), Mediterranean coastal lagoons (Bellino et al., 2019; Stefanidou et al., 2020), etc.). This reflected seasonal variation of environmental factors, such as freshwater input, nutrients, salinity, total suspended solids. Environmental data of 2008 and 2009 in the Thi Nai lagoon highly indicated a difference between the rainy and the dry seasons in every area (Fig. 5).

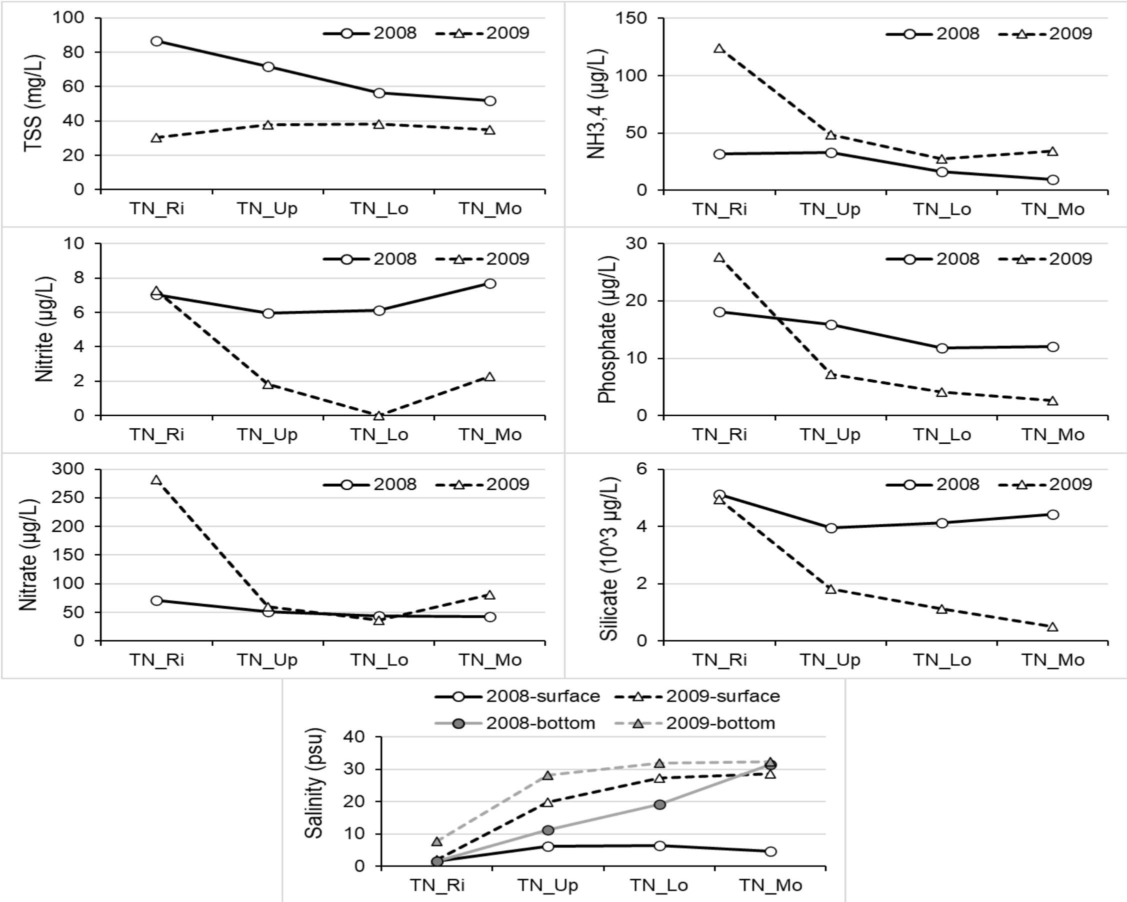

Figure 5. Variation of some environmental factors between 2008 (rainy season) and 2009 (dry season) in four areas of Thi Nai lagoon

Changes of phytoplankton diversity in the upper and lower lagoon in the dry season during 2004-2020

During 2004–2020, the Shannon index showed little difference among these years in the lagoon. Meanwhile, Δ and Δ^* indices presented notable differences between 2009 and 2020 with the highest values in 2020 (Kruskal-Wallis post-hoc, α = 0.05). The mean value of Δ^+ in 2020 was higher than that in 2004 and 2009 (Kruskal-Wallis post-hoc, α = 0.05). The patterns of $s\Delta^+$ and $s\Phi^+$ indices were similar. In particular, the mean values were increasing from 2004 to 2020, and there were statistical differences between 2004 and other years (Kruskal-Wallis post-hoc, α = 0.05). Other indices, Λ^+ and Φ^+, were less different among the years (Fig. 6).

Generally, changing of phytoplankton diversity during 2004 to 2020 in the dry season at upper and lower Thi Nai lagoon was well reflected in taxonomic indices (Δ, Δ^*, Δ^+, $s\Delta^+$) and total phylogenetic diversity ($s\Phi^+$).

The Spearman correlations between diversity indices and environment parameters based on data in 2008 and 2009 were presented in Table 3. Most of the indices indicated a certain correlation with salinity, nitrite, phosphate, and silicate. Particularly, S (species richness/the number of species), Δ^*, Δ^+, $s\Delta^+$, Λ^+, Φ^+ and $s\Phi^+$ had relatively strong correlations with these nutrients. In which, S, $s\Delta^+$, Λ^+, $s\Phi^+$ showed negative correlations, and Δ^*, Δ^+, and Φ^+ positively relates to nutrients. Results of the present study indicated that species richness, taxonomic diversity indices, and phylogenetic indices were likely more sensitive to the environmental factors in the Thi Nai lagoon. Cardinale (2011) demonstrated that nitrogen uptake rates increased linearly with species richness by a model system of stream biofilms with experiments in the stream flume facility. In a study at different locations along the coast of Viet Nam, Doan-Nhu et al. (2016) also indicated the taxonomic diversity indices better described impacts of ENSO than the traditional ones. Besides, the results of the present study were similar to the study of Filstrup et al. (2014) that richness and J' of the phytoplankton community in the lake had negative relation to the total phosphorus concentration.

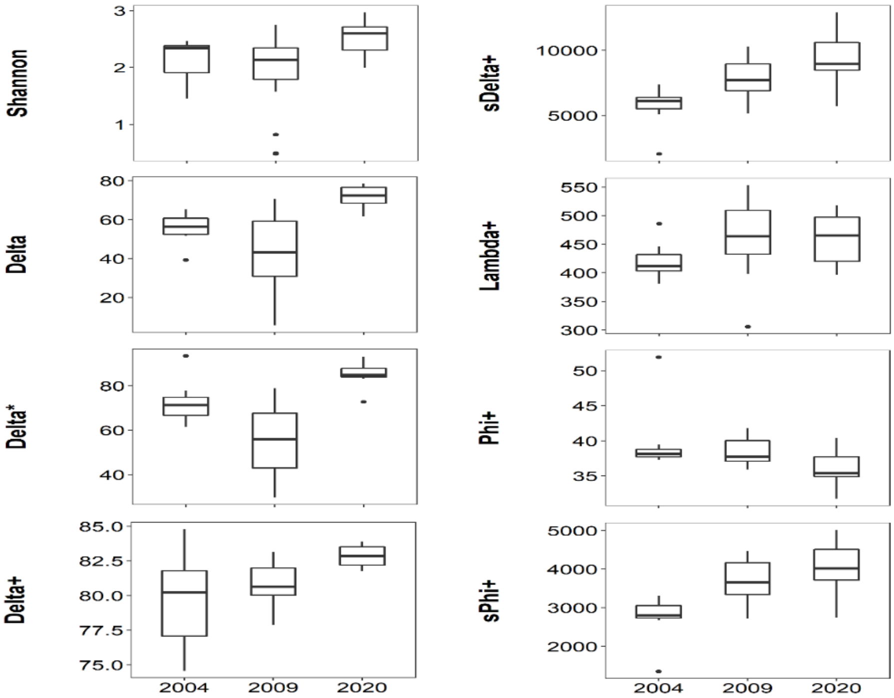

Figure 6. Variation of diversity indices in 2004, 2009 and 2020 in two areas: TN_Up, TN_Lo

Table 3. Correlation matrix of diversity indices and environment factors in 2008 and 2009

	Salinity	NO_2^{2-}	NO_3^-	$NH_{3,4}$	PO_4^{3-}	SiO_3^-
S	0.60	-0.72	-0.17	0.18	-0.57	-0.70
d	0.47	-0.60	-0.01	0.34	-0.33	-0.54
J'	0.52	-0.51	-0.10	0.28	-0.42	-0.50
H'(ln)	0.58	-0.58	-0.09	0.29	-0.47	-0.55
1-D	0.54	-0.55	-0.07	0.32	-0.41	-0.54
Δ	0.20	-0.17	0.21	0.42	0.05	-0.08
Δ^*	-0.66	0.72	0.31	-0.01	0.72	0.76
Δ^+	-0.49	0.64	0.21	-0.14	0.51	0.64
$s\Delta^+$	0.60	-0.72	-0.15	0.19	-0.56	-0.71
Λ^+	0.54	-0.62	-0.32	-0.12	-0.63	-0.63
Φ^+	-0.57	0.70	0.20	-0.16	0.54	0.69
$s\Phi^+$	0.61	-0.71	-0.13	0.19	-0.56	-0.69

The 95% funnels (or 95% probability limit of funnels) for the simulated distribution of average taxonomic distinctness (Δ^+) and variation in taxonomic distinctness (Λ^+) for random subsamples of 367 species in the studied lagoon were presented in Fig. 7a, b. Most Δ^+ values in the upper, lower and the mouth area were below the 95% funnel with a higher taxa number, while Δ^+ values in the river stations were within the 95% funnel with a low taxa number (Fig. 7a). In contrast, some stations in the lagoon (TN_Up, TN_Lo and TN_Mo) were higher 95% of Λ^+ funnel within higher taxa number (Fig. 7b). The ellipse of Δ^+ and Λ^+ also showed that the taxa number of most samples in the lagoon were relatively low (Fig. 7c), especially with samples from river stations. The values of Δ^+ below the lower limit of the funnel indicated that they were lower than the expected taxonomic distinctness. At the river stations (TN_Ri), most of the samples were in the 95% funnels, and only a few points in 2009 (dry season) were below the lower limit. The lagoon mouth area (TN_Mo), however, had a reverse pattern, that most samples were below the 95% limit, and only one in 2008 (rainy season) was within the funnel. Upper (TN_Up) and lower lagoon (TN_Lo) were similar, in which most samples of 2008 and one of 2009 were within the 95% funnel, while the remaining samples fell below the lower limit. Generally, except TN_Ri, other areas revealed the strongly skewed Δ^+ distribution and were lower than expected Δ^+. Warwick & Clarke (1998) pointed out the tendency for Δ^+ to be lower than expected values for the ‘polluted’ sites, in some cases falling below the lower limit of the funnel. The results of this analysis indicate the main body water of Thi Nai lagoon (upper and lower lagoon, and the mouth of lagoon area) were under negative impacts of environmental condition, possibly with eutrophication.

However, the recent study of Jog & Bried (2021) suggested that native species richness (S) has the potential to predict the values take lower than expected Δ^+. In our study, the funnels seemed to have a higher Δ^+ in the rainy season and at the stations with lower species richness (of the river stations). We also found a negative correlation between Δ^+ and richness (r = -0.6). It seems that the Δ^+ at the river stations, in our case, was biased somehow. To check this situation, some individual samples were used to compare the number of scales in a taxonomic hierarchy (Table. 4). For example, although sites 1 and 2 had an incredibly low species (19–29) with 14–17 orders or 5–8 classes, they were in the funnel with high Δ^+ (> 89). Meanwhile, in some cases, such as sites 7 and 8, there were in high species (91–110) with 22–25 orders or only 4–7 classes, consequently, they were below the funnel because of Δ^+ lower than 84 (Table 4). The sites with lower species numbers were with higher numbers of classes and phylum and vice versa. Jog & Bried (2021) suggested that, in evolution, this is caused because of phylogenetic clumping which was often observed in predominantly anthropogenic systems. The bias on Δ^+ is also due to its calculation was not based on species relatedness (Jog & Bried, 2021) or if the species number was too low for simulation (Bevilacqua et al., 2011). Previously, few of the researchers in freshwater ecosystems found that there was a negative correlation between Δ^+ and species richness (Alahuhta et al., 2017) or insensitive to human impacts but water quality (Zhang et al., 2020) and that the use of this index needs to be carefully interpreted and more data is needed. On the other hand, the result from a correlation between Δ^+ and nutrients in the lagoon is likely to support the suggestion of Warwick & Clarke (1998) and Zhang et al. (2020) that Δ^+ positively related to nitrite, phosphate, and silicate with r = 0.64, 0.51, 0.64, respectively. In our analysis, however, the combination between species richness and average taxonomic distinctness or average phylogenetic diversity presented with $s\Delta^+$ or $s\Phi^+$ reflected well diversity of different samples/areas (Table 2) as well as a single sample (Table 4).

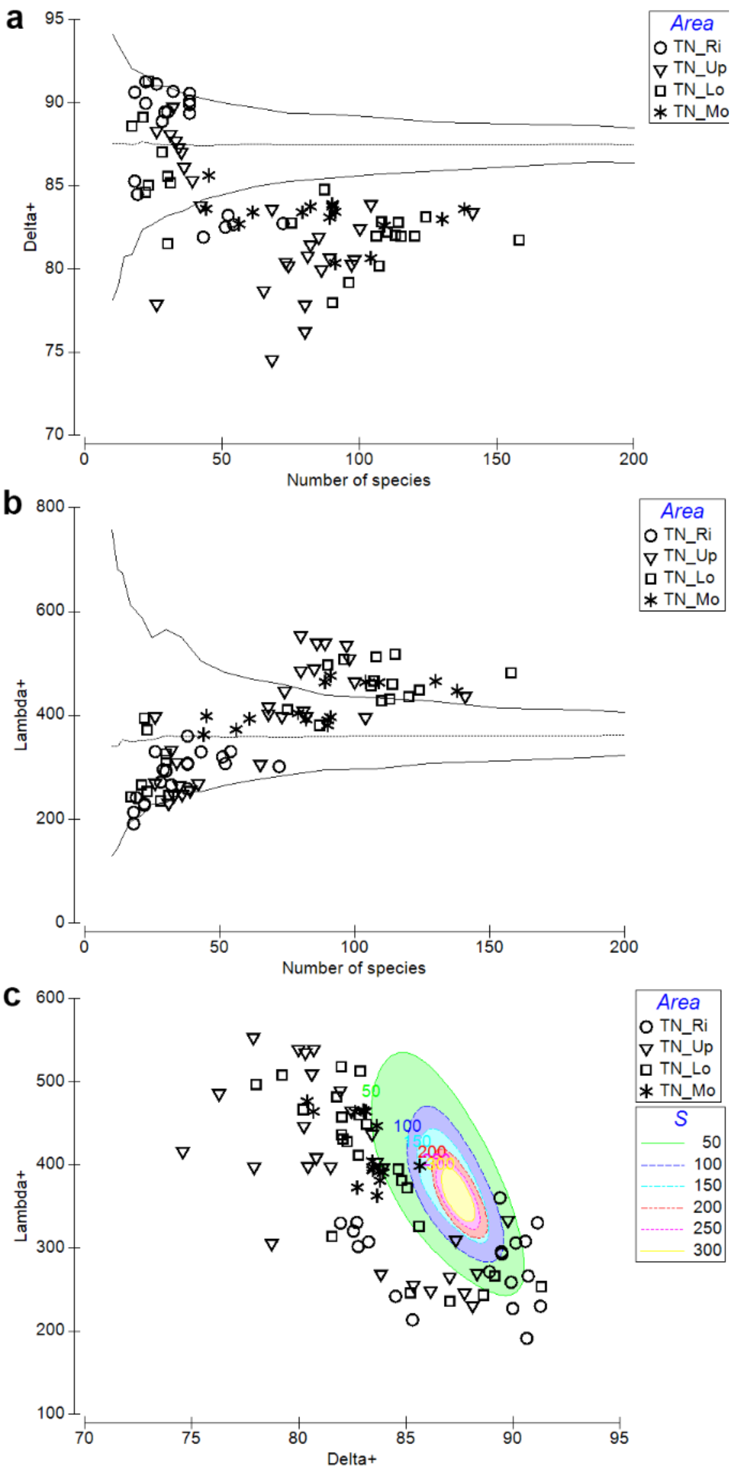

Figure 7. Funnel plots indicating average taxonomic distinctness (a), variation in taxonomic distinctness (b) of phytoplankton and ellipse plot (c) during 2004 to 2020 in river stations (TN_Ri), upper lagoon (TN_Up), lower lagoon (TN_Lo), and the lagoon mouth area (TN_Mo)

Table 4. Number of scales in taxonomic hierarchy and Δ^+ values in some sites. Bold numbers indicated two opposited values of species numbers and Δ^+ but with $s\Delta^+$ and $s\Phi^+$ supported species richness

Area	Site	ID Sample	Phylum number	Class number	Order number	Family number	Genus number	Species number	Δ^+	$s\Delta^+$	$s\Phi^+$
TN_Ri	1	1-11-2008-1	5	8	17	18	22	23	89.97	1979	1500
	2	2_1-11-2008-1	5	8	19	22	26	29	89.45	2594	1767
TN_Up	3	21-6-2004-1	3	5	22	33	45	82	80.80	6545	3067
	4	9-6-2004-1	2	4	21	30	40	75	80.22	5937	2783
TN_Lo	5	29-6-2004-1	4	6	24	34	52	88	84.79	7377	3317
	6	32-6-2004-1	2	4	22	31	42	76	82.77	6208	2800
TN_Mo	7	33-4-2009-1	4	7	25	39	60	110	82.62	9005	3983
	8	34-6-2004-1	2	4	22	35	50	91	83.77	7540	3300

Abundance

In the Thi Nai lagoon, higher phytoplankton densities were found at the lower lagoon and the mouth area, even though the patterns of density variation among the years were different in each area (Fig. 8). Seasonal difference in cell density showed clearly in the river stations (TN_Ri), higher in the rainy season (Nov2008) with chlorophyte domination. At the upper lagoon (TN_Up), overall averages of cell density were quite low (<50,000 cells.L^{-1}) compared to the others. The highest density recorded in this area in 2004 was with the domination of centric and pennate diatoms. Centric diatoms were also abundant in the other survey times as well, and Chlorophyta domination was in the rainy season. At the lower lagoon (TN_Lo), high densities were recorded in 2008 and 2009 with 66,923 ± 110,960 cells.L^{-1} and 72,777 ± 80,838 cells.L^{-1}, respectively. There were strong changes in the dominant group between 2004 and other years. The change was from pennate diatoms (2004) to centric diatoms (other years). At the lagoon mouth area (TN_Mo), high densities were recorded in 2004 (72,631 ± 83,076 cells.L^{-1}) and 2008 (89,778 ± 145,703 cells.L^{-1}). The change of the main dominant group was similar to TN_Lo. Generally, in the Thi Nai lagoon, centric diatoms were abundant in all survey times, while the pennate diatoms were dominated in 2004. In this survey, main taxa with high abundance were *Pseudo-nitzschia* spp. and *Thalassionema frauenfeldii*. In 2020 cyanobacteria were also reached higher density in the lagoon but not at the river stations. As bioindicators of water quality, Cyanobacteria are related to eutrophication processes (Mateo et al., 2015; Teta et al., 2017). Hence, the higher density of cyanobacteria in later years was able to indicate eutrophic status in the study area.

With the seasonal variation, phytoplankton densities were lower in the dry season in the river stations (TN_Ri) and upper lagoon (TN_Up), and higher in the lower lagoon (TN_Lo) and the mouth area (TN_Mo) (Fig. 9). Seasonal difference in cell density among areas was in a clearer pattern in the dry season, increasing from the river to the mouth area. Statistically, cell densities in TN_Lo and TN_Mo were much higher than in TN_Ri (Kruskal-Wallis post-hoc, $\alpha = 0.05$). A study by Cutrim et al. (2019) conducted in Jansen Lagoon (northern Brazil) demonstrated that the phytoplankton abundance was higher in the dry season. Bortolini & Bueno (2013) also revealed similar results in the São João River (Iguaçu National Park, Brazil).

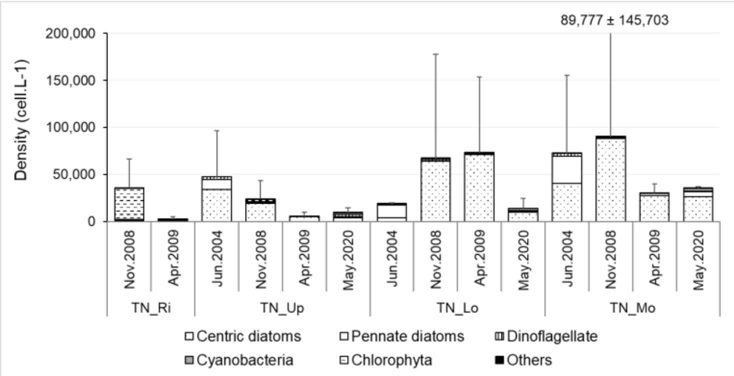

Figure 8. Variation of cell density of the four study areas: TN_Ri, TN_Up, TN_Lo, TN_Mo during 2004‒2020 in Thi Nai lagoon

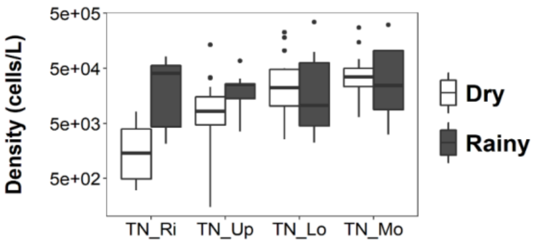

Figure 9. Variation of cell density of four areas in the dry and rainy seasons

In the Thi Nai lagoon, there was a slightly decreasing trend of phytoplankton density from 2004 to 2020 (Fig. 10), but not statistically significant (Kruskal-Wallis test, $p > 0.05$).

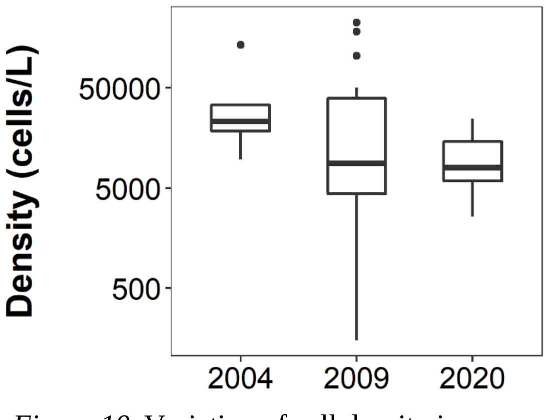

Figure 10. Variation of cell density in upper and lower Thi Nai lagoon in the dry season during 2004–2020

The Spearman correlation between densities and environmental factors (such as nutrients, salinity) revealed a weak relationship between them. It is different from many previous studies (e.g., Hlaili et al., 2007) that phytoplankton abundances are positively correlated to nutrient inputs.

Comparing similarities of phytoplankton communities among the study areas and years were performed using non-metric multidimensional scaling (NMDS) on abundant data. The areas and seasons were strongly defined phytoplankton communities in different clusters. Specifically, there were distinct clusters between the river stations in 2008 and others (Fig. 11).

In the lagoon, species assemblages of the upper, lower and mouth areas were relatively similar because of their adjacent areas. The seasonal separation was also clearly shown for all areas. In which, phytoplankton assemblages in the rainy (2008) and the dry (2004, 2009, and 2020) seasons were on the left and the right of the dimensional axis 1 (Fig. 11), respectively. Among the years, in the dry season, there were the clustering distributions, despite these clusters were quite close to each other. In general, these results indicated that the phytoplankton community in the study areas significantly changed between the seasons and obviously between the river stations and others. In the dry season, there were small changes in the community structure over the years.

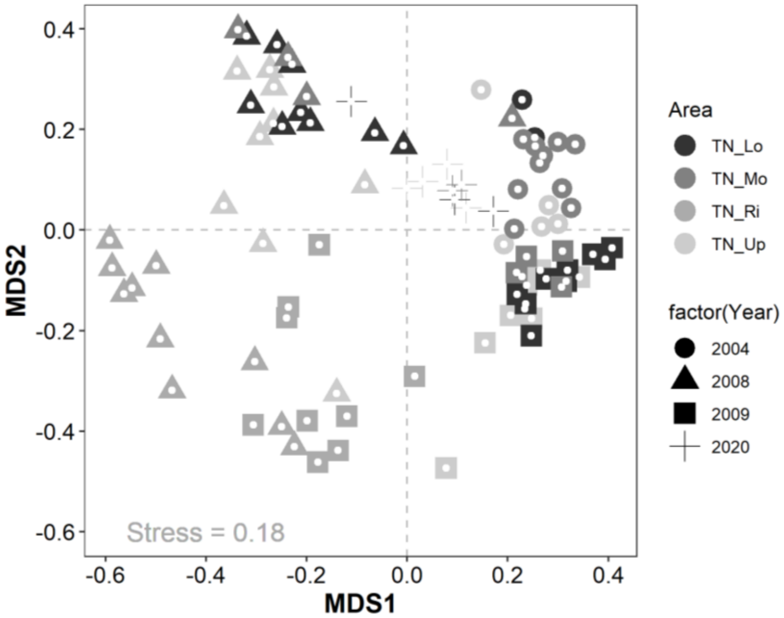

Figure 11. Non-metric multidimensional scaling (NMDS) of phytoplankton community data

Table 5. Abundant contribution (%) of dominant species during 2004−2020 in the upper and lower Thi Nai lagoon. Higher values were in bold

Species	2004	2009	2020
Chaetoceros spp.	17.32	28.49	25.70
Thalassionema frauenfeldii	27.22		
Chaetoceros compressus		22.32	
Bacteriastrum spp.	16.91	3.01	
Skeletonema sp.			14.09
Chaetoceros pseudocurvisetus		15.92	
Protoperidinium spp.	8.52	2.15	12.71
Chaetoceros lorenzianus	5.26		
Pseudo-nitzschia spp.	3.85		9.35
Guinardia striata	2.16	8.88	
Cyclotella sp.		6.96	
Trichodesmium erythraeum			4.97
Cylindrotheca closterium			4.04

Similarity percentages analysis (SIMPER) was used to determine to change of dominant taxa over the years and among the areas. Obviously, the dominant species in the river stations made this area different from other areas including chlorophyte taxa, such as *Pleodorina illinoisensis*, *Scenedesmus* sp., *Pediastrum* sp.. Between the dry and rainy seasons, average dissimilarities in each area were very high with 94.16% (TN_Ri), 94.96% (TN_Up), 95.57% (TN_Lo), and 94.77% (TN_Mo). The number of dominant taxa was lower in the rainy season, except for TN_Ri. During the years (2004−2020), in the dry season, the composition of dominant species of the Thi Nai lagoon was alternative changed (average dissimilarity about 88%) among the taxa/groups including centric diatoms (*Chaetoceros* spp.), pennate diatoms (*Pseudo-nitzschia* spp.), and dinoflagellates (*Protoperidinium* spp.) (Table 5). Specially, in 2004, *Chaetoceros* spp., *T. frauenfeldii* and *Bacteriastrum* spp. dominated with 17.32%, 27.22% and 16.91%, respectively. In 2009 and 2020, the two latter taxa were no longer dominated. Instead, in 2009, only *Chaetoceros* spp. contributed over 66% of phytoplankton density. In 2020, there were other taxa dominated including *Skeletonema* sp., *Trichodesmium erythraeum* and *Cylindrotheca Closterium* that were absent in the previous years. Besides, dinoflagellates (*Protoperidinium* spp.) increased in 2020 by 12.71%.

CONCLUSION

There were clearly seasonal variations in species number, species composition, diversity indices, and abundance of phytoplankton in the study waters. Species number was lower in the rainy season in all areas. Similarly, the biodiversity indices differed significantly between the dry and rainy seasons. Shannon, Δ, $s\Delta^+$, Λ^+ and $s\Phi^+$ indices were lower in the rainy season, whereas Δ^*, Δ^+ and Φ^+ were lower in the dry season. The river stations (TN_Ri) and upper lagoon (TN_Up) had lower densities in the dry season, while the lower lagoon (TN_Lo) and the mouth area (TN_Mo) had higher values in this season.

Among the years (2004, 2009, and 2020), dry seasons, species number increased in all areas (TN_Up, TN_Lo, and TN_Mo) of the Thi Nai lagoon. Changes in phytoplankton community structure were more knowable at the lagoon main water body (TN_Up and TN_Lo) by some taxonomic indices (Δ, Δ^*, Δ^+, $s\Delta^+$) and total phylogenetic diversity ($s\Phi^+$). The species composition, however, had some small changes over years, such as the increasing species number of Cyanobacteria from 2004 to 2020. In addition, the result of NMDS demonstrated some differences in phytoplankton communities among these years. SIMPER results presented alternative changes of dominant taxa among the years including centric diatoms (genus *Chaetoceros, Bacteriastrum, Skeletonema*), pennate diatoms (*T. frauenfeldii*, *Pseudo-nitzschia* spp.), and dinoflagellates (genus *Protoperidinium*).

Correlations between the diversity indices and environmental factors showed that most of the taxonomic or phylogenetic indices were more sensitive than traditional indices in assessing the change of environmental conditions.

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Appendix 1. The coordinates of survey stations

Station	Area	Longitude	Latitude	Station	Area	Longitude	Latitude
1	TN_Ri	109.2139	13.93772	19	TN_Up	109.241	13.83375
2	TN_Ri	109.204	13.90178	20	TN_Up	109.2373	13.83225
3	TN_Ri	109.1933	13.81798	21	TN_Up	109.2258	13.83375
4	TN_Ri	109.2039	13.78682	22	TN_Up	109.2286	13.82885
5	TN_Ri	109.2262	13.92185	23	TN_Lo	109.2306	13.81186
6	TN_Ri	109.2329	13.90105	24	TN_Lo	109.2426	13.81348
7	TN_Ri	109.2317	13.88047	25	TN_Lo	109.229	13.80887
8	TN_Up	109.2287	13.85612	26	TN_Lo	109.2577	13.80488
9	TN_Up	109.2345	13.85493	27	TN_Lo	109.2554	13.80021
10	TN_Up	109.2462	13.85612	28	TN_Lo	109.2439	13.79662
11	TN_Up	109.2522	13.85548	29	TN_Lo	109.2465	13.79423
12	TN_Up	109.2478	13.85141	30	TN_Lo	109.2338	13.79242
13	TN_Up	109.2339	13.85015	31	TN_Lo	109.2361	13.78988
14	TN_Up	109.2349	13.8434	32	TN_Lo	109.2309	13.78227
15	TN_Up	109.2269	13.83812	33	TN_Mo	109.2498	13.7805
16	TN_Up	109.2511	13.838	34	TN_Mo	109.2584	13.7694
17	TN_Up	109.2537	13.83415	35	TN_Mo	109.2503	13.76368
18	TN_Up	109.2498	13.83297	36	TN_Mo	109.2484	13.76361