

OCCURRENCES OF POTENTIALLY HARMFUL MICROALGAE (PHM) ASSOCIATED WITH ENVIRONMENTAL VARIABLES IN SONGKHLA LAGOON, THAILAND

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Abstract. The spatial and temporal distributions of potentially harmful microalgae (PHM) within Songkhla Lagoon were investigated in relation to environmental parameters. Samples were collected from three stations over a year-long period from October 2021 to September 2022, encompassing the northeast monsoon, intermediate dry, and southwest monsoon seasons. A total of 20 PHM species, comprising 12 dinoflagellates and 8 diatoms, were identified. While cell densities were significantly higher at stations A and B ($p < 0.001$), seasonal variations were not observed ($p > 0.05$). Five dominant species emerged throughout the year, including three diatoms; *Skeletonema* sp., *Chaetoceros* sp., *Pleurosigma* sp., and two dinoflagellates, *Alexandrium* sp. and *Blixaea quinquecornis*. *Skeletonema* sp., common red tide species, indicated peak abundance (1.37×10^6 cells/L) at site A. Salinity, silicate, phosphate, and water temperature were identified as key factors influencing PHM occurrence. The study suggests that nutrient enrichment from aquaculture activities may contribute to an increased risk of harmful algal blooms in the lagoon ecosystem.

Keywords: *harmful algal blooms, eutrophication, aquaculture, coastal ecosystems, Gulf of Thailand*

Introduction

Eutrophication, a process of excessive input of nutrients such as phosphorus and nitrogen, is widely recognized as a severe environmental threat (Naylor et al., 2000). The intensification of aquaculture is a major source of eutrophication as sewage, including untreated wastewater and pond sludge, is discharged from fish and shrimp farms directly into rivers or seas (Naylor et al., 2000; Diana, 2009; Ahmed and Thompson, 2019). Several studies have reported that effluent from fish farms loads large amounts of nutrients into coastal and estuarine waters, often stimulating the rapid growth of algae and plankton (Chislock et al., 2013; Sampantamit et al., 2020). In coastal environments, eutrophication has been identified as a cause of the dominance of certain plankton species. For instance, blooms of the blue-green algae *Trichodesmium* sp. and *Noctiluca* sp. was

observed in the Gulf of Thailand caused by the disposal of untreated sewage (Cheevaporn and Menasveta, 2003). In Malaysia, enriched phytoplankton blooms, involving *Chaetoceros* spp., *Pseudo-nitzschia brasiliensis*, *Blixaea quinquecornis*, and *Skeletonema* spp. in a semi-enclosed lagoon of the Semerak River, coincided with a high abundance of macronutrients (Er et al., 2018). More recently, again in Malaysia, blooms of *Skeletonema costatum* and *Chaetoceros* spp. in Geting Lagoon were reported (Law et al., 2023).

Harmful microalgae are present naturally at low cell concentrations but in certain conditions they can create enormous blooms which may affect the ecosystem and human wellbeing (Berdalet et al., 2015; ~~Vila et al., 2024~~; Marampouti et al., 2021, Vila et al., 2021). Toxigenic genera such as *Alexandrium*, *Dinophysis*, and *Pseudo-nitzschia* can contaminate seafood (Wang, 2008; Jester et al., 2009; Anderson et al., 2012; Trainer et al., 2012; Séchet et al., 2021) or in some cases cause fish mortality (Wang, 2008). Phytoplankton can also destroy marine organisms by generating anoxia in the surrounding water (Rensel and Whyte, 2003; Oh et al., 2023). For instance, harmful algal blooms (HABs) have significantly impaired fisheries, even killing farmed fish kept in cages, either by reducing oxygen levels or by harming or fouling the gills of fish (Rensel and Whyte, 2003; Oh et al., 2023). The first report of a HAB and shellfish toxicity in Thailand was in 1983 at Pranburi, Prachuap Khiri Khan Province, on the west coast of the Gulf of Thailand (Sudara et al., 1984). Symptoms of paralytic shellfish poisoning (PSP) were reported in 63 people and a 3-year-old girl died after consuming green mussels (*Perna viridis*) contaminated with *Alexandrium* sp. Fish mortality in open waters or fish farming areas is usually associated with large-scale microalgal blooms that emit toxic bioactive substances or cause hypoxia or anoxia in their vicinity (Rensel and Whyte, 2003; Oh et al., 2023). Large fish kills were reported in Songkhla Lagoon in 2001 and 2004. In those years, the concentrations of chlorophyll *a* was 164 mg/L and 339 mg/L, respectively (La-ongsiriwong et al., 2012). More recently, the presence of several PSP producers, including *Alexandrium* spp., and six fish-killing dinoflagellate species, *Margalefidinium polykrikoides*, *M. fulvescens*, *Karenia mikimotoi*, *K. selliformis*, *Karlodinium australe*, and *K. digitatum* has been reported in the Gulf of Thailand (Fu et al., 2021). Aquaculture in Thailand has experienced significant growth over the past few decades, leading to increased seafood production (Sampantamit et al., 2020; FAO, 2024). However, this expansion has resulted in several environmental challenges, including the introduction of non-native species and alterations to ecosystem balance, particularly in ecologically sensitive areas. Despite a recent decline in the aquaculture industry, it remains a primary threat to the coastal environment (Pornpinatepong et al., 2010).

The Songkhla Lagoon, the semi-enclosed coastal system, located in southern Thailand, comprises four distinct water bodies: Thale Noi, the Inner Lake, the Middle Lake, and the Outer Lake (Songkhla Lagoon). This coastal ecosystem is a significant hub for aquaculture activities. In 2003, shrimp production accounted for approximately 31.5 million Thai Baht, while fish and crab production were valued at approximately 2.5 million and 1.1 million Thai Baht, respectively (NICA, 2003; Pornpinatepong et al., 2010). Fishing activities are subject to seasonal fluctuations, declining during the December rainy season due to heavy rainfall and flooding (Chesoh and Lim, 2008). Shrimp supply experiences a sharp decrease from December to February. Historical data from 2003 to 2016 indicate a decline in fish catch during the months of February, March, and April, preceding the annual rainy season (Hue et al., 2018). However, from 2017 to 2020, a notable shift occurred, with a 36.06% increase in fish catch and a corresponding

15.47% decrease in shrimp catch. Local aquaculture, particularly fish and shrimp farming, contributes significantly to nutrient loads in Songkhla Lagoon through discharges of untreated wastewater, organic waste, and pond sludge. These nitrogen and phosphorus rich inputs drive eutrophication, stimulating phytoplankton growth, including PHM (Naylor et al., 2000; Sampantamit et al., 2020). Farms near hydrodynamically sensitive areas exacerbate the issue by increasing nutrient runoff, depleting oxygen, and promoting PHM proliferation (Pornpinatepong et al., 2010). Seasonal runoff and precipitation further intensify conditions for harmful algal blooms (HABs), posing risks to the lagoon's ecosystem and aquaculture sustainability.

Expanding upon prior research on PHM occurrences in the Gulf of Thailand, such as blooms of *Trichodesmium erythraeum* and *Noctiluca* sp. triggered by untreated sewage discharge (Cheevaporn and Menasveta, 2003), this study focuses on the semi-enclosed coastal system of Songkhla Lagoon. Unlike previous studies in open coastal waters, it examines how aquaculture-derived nutrient loads interact with PHM dynamics in a lagoon. The presence of toxic and harmful microalgae species in areas with significant aquaculture activity poses a substantial threat to ecosystem health, seafood safety, and economic stability (NICA, 2003; Pornpinatepong et al., 2010). Given Songkhla Lagoon's importance as an aquaculture site, baseline data on harmful algae within the lagoon are crucial for understanding and mitigating potential risks. This study is aimed to investigate occurrences of potentially harmful microalgae and factors influencing their abundance and distribution in Songkhla Lagoon. This scientific background will consequently assess the potential risks of human poisoning and fish mortality events and serve as baseline data for informed decision-making and long-term environmental sustainability.

Materials and methods

Study site

The Songkhla Lagoon System, situated in southern Thailand, encompasses four shallow coastal water bodies formed by the deposition of sandbars. The basin covers an area of 8,729 square kilometers, with a water surface area of 1,042 square kilometers. Extending southward from Phatthalung Province for over 150 kilometers along Thailand's eastern coast, the system comprises Thale Noi (27 square kilometers) in the north, followed by the Inner Lake (473 square kilometers), Middle Lake (360 square kilometers), and Outer Lake (182 square kilometers) in the south (NESDB and NEB, 1985). The system exhibits a salinity gradient, ranging from freshwater to saline water. Water depths typically range from 1 to 2 meters, with the outer portion connecting to the Gulf of Thailand through a narrow channel approximately 380 meters wide (Lesaca, 1977). The water quality within the system is characterized by its complexity and variability, influenced by seawater from the South China Sea, freshwater from twelve major rivers, and water from various streams and man-made drainage systems (Chesoh and Choonpradub, 2011).

Field sampling was conducted at three sites. Site A, closest to the lagoon opening to the sea, comprised four stations (A1–A4). This site is strongly influenced by saline water inflow, resulting in higher salinity and nutrient enrichment. Pollution from oil and grease due to port activities in Songkhla city further altered its nutrient profile. Site B, located further away from the lagoon opening, comprised four stations (B1–B4) and is characterized by intermediate salinity. Intense seabass and shrimp aquaculture in the vicinity contributes significant organic waste and nutrients, elevating the risk of

eutrophication and PHM blooms. Both sites had active fish and shrimp aquaculture and fishing communities. Site C, furthest from the lagoon opening, consists of three stations (C1–C3) and is influenced by freshwater inflows from agricultural runoff, livestock waste, and shrimp farming effluents. These inputs reduce salinity while increasing phosphate and silicate levels, creating favorable conditions for the growth of toxic diatoms and dinoflagellates (Fig. 1).

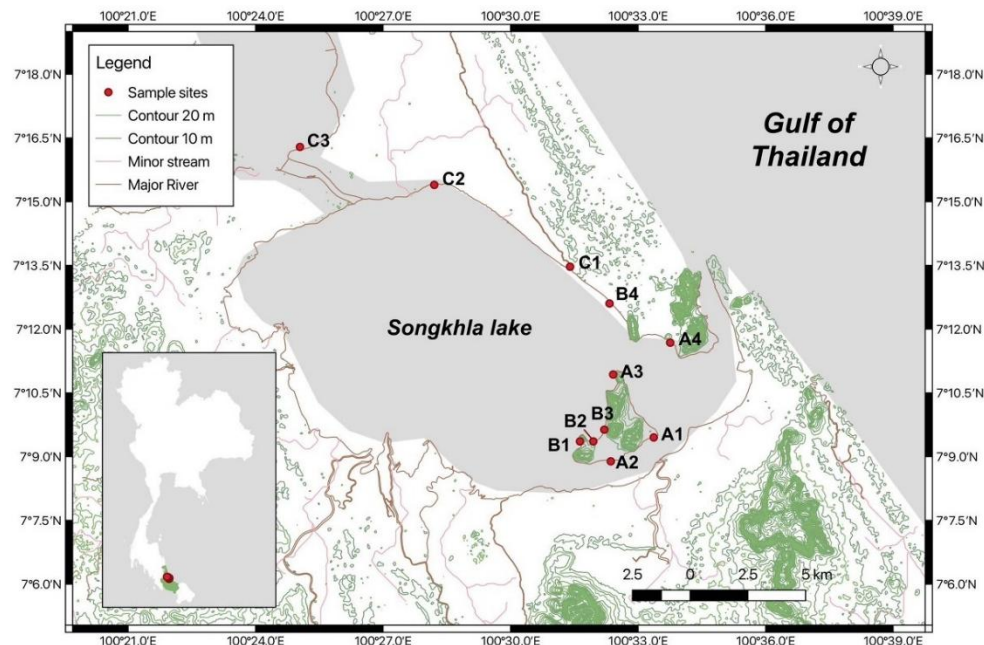


Figure 1. Sampling sites in Songkhla Lagoon: Site A, (A1–A4), Site B (B1–B4), Site C, (C1–C3)

Sample collection and laboratory analysis

Altogether, 23 field investigations were carried out every two weeks, from October 2021 to September 2022, to monitor fluctuations and seasonal patterns, covering all three seasons: northeast monsoon (October to January), intermediate dry season (February to April) and southwest monsoon (May to September) (Noppradit et al., 2021; Southern-East Coast Meteorological Center, 2024). This sampling frequency is consistent with the life cycle of PHM, enabling effective capture of short-term fluctuations and dynamic population shifts, which are essential for comprehensive temporal analysis. Water samples were collected from surface water about 50 cm deep, filtered 20L of water samples through a 20 µm pore size phytoplankton net. Then this subsample was fixed with a 2 % final concentration of formaldehyde. For nutrient analysis, collected water samples were passed through a 1.2 µm pore size glass microfiber filter. The colorimetric, Cadmium Reduction method for nitrite and nitrate analysis in water samples was analyzed. The silicomolybdate for silicate and ascorbic acid for phosphate were analyzed.

Water temperature, conductivity and salinity were measured in situ with a YSI Model 30 handheld meter and water pH was measured with a PH200 HM digital pH meter.

In the laboratory, phytoplankton were identified and counted using a 0.1 mL phytoplankton counting chamber under a light microscope (Olympus CX21LED, Olympus Corporation, Tokyo, Japan). Morphologically, phytoplankton were identified at genus or species level, according to the World Register of Marine Species (WoRMS

Editorial Board, 2024) and the AlgaeBase (Guiry and Guiry, 2022). In the acetone method, chlorophyll *a* in samples is extracted with 90% aqueous acetone. For nutrient analysis, the selection of nitrite (NO_2^-), nitrate (NO_3^-), silicate (SiO_3^{2-}), and phosphate (PO_4^{3-}) is based on their critical roles in phytoplankton growth and HABs, which are essential for understanding nutrient dynamics and the occurrence of PHM in Songkhla Lagoon (Glibert et al., 2005; Sedyaw et al., 2024). Concentrations of chlorophyll *a*, nitrite (NO_2^-) and nitrate (NO_3^-), silicate (SiO_3^{2-}) and phosphate (PO_4^{3-}) were measured and analyzed with the Thermo Scientific SPECTRONIC™ 20 spectrophotometer (APHA-AWWA-WPCF, 1990).

Data analysis

Two-way ANOVA was applied to test for differences in cell density of phytoplankton taxa, PHM, and environmental factors between sites and seasons by RStudio 4.3.2. (Wickham et al., 2020). Post hoc analysis using Tukey's Honestly Significant Difference (TukeyHSD) test was conducted to identify significant pairwise differences in PHM distributions across seasons and sites following the two-way ANOVA. One hundred and fourteen phytoplankton taxa, twenty PHMs, and seven environmental factors (temperature, salinity, pH, NO_2^- , NO_3^- , SiO_2^{-3} and PO_3^{-4}) were transformed using the transformation $\log(1+\text{abundance})$, where abundance was scaled to satisfy the statistical normality assumption for residuals. The analysis of twenty PHM species data was performed using the “ggplot2” package in RStudio 4.3.2. (Wickham et al., 2020).

Patterns of PHM in the lagoon, based on sites and seasons, were assessed from a Ward's cluster analysis, with a Bray-Curtis similarity index. The raw data were used prior to analyses.

Canonical correspondence analysis (CCA) was used to describe correlations between environmental factors and the PHM by PC-ORD version 7.11 (Grandin, 2006). Seven environmental factors, including water temperature, salinity, pH, NO_2^- , NO_3^- , SiO_2^{-3} , and PO_3^{-4} , were used as explanatory variables. The raw data were transformed using the transformation $\log(1+\text{abundance})$. This transformation can enhance statistical assumptions by improving normality and linearity. It also simplifies the interpretation of ecological data by converting multiplicative relationships into additive ones, facilitating direct comparisons of relative changes in PHM abundance across samples. The Monte Carlo permutation test was used to assess the importance of environmental variables in relation to the organization of species communities.

Results

Species composition, spatial and seasonal variation of phytoplankton

A total of 114 phytoplankton taxa were identified, with *Skeletonema* sp., *Chaetoceros* sp., *Pleurosigma* sp., *Nitzschia reversa*, *Odontella* sp., and *Surirella* sp. dominating the community. Spatially, sites A, B, and C exhibited 107, 105, and 88 taxa, respectively. *Skeletonema* sp., *Chaetoceros* sp., and *Pleurosigma* sp. predominated at sites A and B, while *Skeletonema* sp., *Pleurosigma* sp., and *Thalassiosira* sp. were most abundant at site C. Seasonally, 62, 106, and 93 taxa recorded during the northeast monsoon, intermediate dry season, and southwest monsoon, respectively with the dominations of *Skeletonema* sp., *Chaetoceros* sp., and *Pleurosigma* sp. (Table 1). Species richness during the northeast monsoon differed significantly ($p < 0.05$) (Table 2).

Table 1. Spatially dominant species of phytoplankton, diatoms and dinoflagellates collected in three sites in aquaculture areas of Songkhla Lagoon

| Sites | Dominant phytoplankton | Average±SE (cells/L) | Min-Max (cells/L) | Dominant diatoms | Average±SE (cells/L) | Dominant dinoflagellates | Average±SE (cells/L) |
|---------|-----------------------------|----------------------|-------------------|-----------------------------|----------------------|------------------------------------|----------------------|
| Overall | <i>Skeletonema</i> sp. | 700,066±330,325 | 14-66,211,200 | <i>Skeletonema</i> sp. | 700,066±330,325 | <i>Alexandrium</i> sp. | 71±14 |
| | <i>Chaetoceros</i> sp. | 5,499±1,507 | 14-205,628 | <i>Chaetoceros</i> sp. | 5,499±1507 | <i>Blixaea quinquecornis</i> | 47±21 |
| | <i>Pleurosigma</i> sp. | 4,097±807 | 4-103,009 | <i>Pleurosigma</i> sp. | 4,097±807 | <i>Gymnodinium</i> sp. | 31±10 |
| | <i>Nitzschia reversa</i> | 459±156 | 7-22,825 | <i>Nitzschia reversa</i> | 459±156 | <i>Noctiluca scintillans</i> | 26±13 |
| | <i>Odontella</i> sp. | 447±441 | 10-111,626 | <i>Surirella</i> sp. | 432±161 | <i>Scrippsiella</i> sp. | 19±6 |
| | <i>Surirella</i> sp. | 432±161 | 7-34,744 | <i>Pseudo-nitzschia</i> sp. | 412±170 | <i>Protoperidinium quarnerense</i> | 14±5 |
| | Total | 713,309 | | | 712,946 | | 363 |
| | | | | | | | |
| Site A | <i>Skeletonema</i> sp. | 1,368,010±833,328 | 16-66,211,200 | <i>Skeletonema</i> sp. | 1,368,010±833,328 | <i>Blixaea quinquecornis</i> | 57±45 |
| | <i>Chaetoceros</i> sp. | 5,668±2,118 | 24-150,408 | <i>Chaetoceros</i> sp. | 5,668±2,118 | <i>Alexandrium</i> sp. | 49±21 |
| | <i>Pleurosigma</i> sp. | 4,362±1,298 | 9-70,646 | <i>Pleurosigma</i> sp. | 4,362±1,298 | <i>Scrippsiella</i> sp. | 36±15 |
| | <i>Nitzschia reversa</i> | 900±413 | 10- 22,825 | <i>Nitzschia reversa</i> | 900±413 | <i>Gymnodinium</i> sp. | 29±15 |
| | <i>Pseudo-nitzschia</i> sp. | 743±422 | 10- 33,958 | <i>Pseudo-nitzschia</i> sp. | 743±422 | <i>Noctiluca scintillans</i> | 21±18 |
| | <i>Surirella</i> sp. | 510±228 | 9- 17,033 | <i>Surirella</i> sp. | 510±228 | <i>Protoperidinium quarnerense</i> | 11±7 |
| | Total | 1,381,981 | | | 1,381,647 | | 334 |
| | | | | | | | |
| Site B | <i>Skeletonema</i> sp. | 545,420±356,957 | 14- 31,556,800 | <i>Skeletonema</i> sp. | 545,420±356,957 | <i>Blixaea quinquecornis</i> | 56±33 |
| | <i>Chaetoceros</i> sp. | 9,216±3,523 | 14-205,628 | <i>Chaetoceros</i> sp. | 9,216±3,523 | <i>Alexandrium</i> sp. | 48±11 |
| | <i>Pleurosigma</i> sp. | 5,761±1,561 | 11-103,009 | <i>Pleurosigma</i> sp. | 5,761±1,561 | <i>Noctiluca scintillans</i> | 47±37 |
| | <i>Odontella</i> sp. | 1,216±1,213 | 10-111,626 | <i>Surirella</i> sp. | 521±379 | <i>Gymnodinium</i> sp. | 28±15 |
| | <i>Surirella</i> sp. | 521±379 | 7-34,744 | <i>Thalassiosira</i> sp. | 403±158 | <i>Protoperidinium quarnerense</i> | 24±12 |
| | <i>Thalassiosira</i> sp. | 403±158 | 8-14,344 | <i>Pseudo-nitzschia</i> sp. | 352±197 | <i>Scrippsiella</i> sp. | 12±4 |
| | Total | 564,879 | | | 564,424 | | 455 |
| | | | | | | | |
| Site C | <i>Skeletonema</i> sp. | 15,668± 11,032 | 20- 755,595 | <i>Skeletonema</i> sp. | 15,668±11,032 | <i>Alexandrium</i> sp. | 130±38 |
| | <i>Pleurosigma</i> sp. | 1,526±1,159 | 4-80,054 | <i>Pleurosigma</i> sp. | 1,526±1,159 | <i>Gymnodinium</i> sp. | 37±23 |
| | <i>Thalassiosira</i> sp. | 532±283 | 11-16,269 | <i>Thalassiosira</i> sp. | 532±283 | <i>Blixaea quinquecornis</i> | 20±9 |
| | <i>Chaetoceros</i> sp. | 319±179 | 30-11,135 | <i>Chaetoceros</i> sp. | 319±179 | <i>Tripos furca</i> | 10±7 |
| | <i>Cyclotella</i> sp. | 273±173 | 10-9,770 | <i>Surirella</i> sp. | 207±173 | <i>Scrippsiella</i> sp. | 7±3 |
| | <i>Surirella</i> sp. | 207±47 | 9-1,781 | <i>Pseudo-nitzschia</i> sp. | 50±30 | <i>Dinophysis caudata</i> | 6±6 |
| | Total | 19,652 | | | 19,372 | | 280 |
| | | | | | | | |

Table 2. The Two-way ANOVA analysis results for the difference of cell density between phytoplankton taxa, harmful microalgae taxa, and environmental factors between sites and seasons

| Parameters | The two-way ANOVA Test P value (sig) | | | | | |
|-----------------------------------|---|--------------|-----------------------|--------|--------------|--|
| | Seasons | | | Sites | | |
| | F | P | Seasons | F | P | Sites |
| Phytoplankton richness | 14.099 | 1.73e-06 *** | NE-IMD, NE-SW | 1.044 | 0.407 | A1-C1, A1-C2, A1-C3, A2-C1, A2-C2, A2-C3, A3-C1, A3-C2, A3-C3, A4-C2, A4-C3 B1-C2, B1-C3, B2-C2, B2-C3, B3-C1, B3-C2, B3-C3 |
| phytoplankton abundance (cells/L) | 2.217 | 0.111 | | 9.100 | 1.55e-12 *** | |
| HAB abundance (cells/L) | 2.008 | 0.137 | | 10.200 | 4.61e-14 *** | A1-C1, A1-C2, A1-C3, A2-C1, A2-C2, A2-C3, A3-C1, A3-C2, A3-C3, A4-C2, A4-C3 B1-C2, B1-C3, B2-C1, B2-C2, B2-C3, B3-C1, B3-C2, B3-C3, B4-C3 |
| Conductivity (uS/cm) | 16.379 | 2.34e-07 *** | NE-IMD, NE-SW | 3.694 | 0.000137 *** | A1-C2, A1-C3, A3-C2, A3-C3, A4-C2, A4-C3 |
| Salinity (psu) | 11.799 | 1.36e-05 *** | NE-IMD, NE-SW | 3.152 | 0.000861 *** | A1-C2, A1-C3, A3-C2, A3-C3, A4-C3 |
| Water temperature (°C) | 43.211 | < 2e-16 *** | NE-IMD, NE-SW, SW-IMD | 2.460 | 0.00832 ** | B1-C3 |
| pH | 24.384 | 2.72e-10 *** | NE-SW, SW-IMD | 0.745 | 0.681 | |
| Nitrite (µM) | 1.245 | 0.290 | | 4.591 | 6.27e-06 *** | A3-C1 B1-C2, B1-C3, B2-C1, B4-C2, B4-C3 C1-C2, C1-C3 |
| Nitrate (µM) | 3.005 | 0.052 | | 2.708 | 0.00375 ** | A2-B3 |
| Silicate (µM) | 68.478 | <2e-16 *** | NE-IMD, NE-SW | 1.654 | 0.093 | |
| Phosphate (µM) | 37.963 | 6.86e-15 *** | NE-IMD, NE-SW, SW-IMD | 13.751 | < 2e-16 *** | A1-B4, A1-C1, A2-B4, A2-C1, A3-B4, A3-C1, A4-C1 B1-B4, B1-C1, B2-B4, B2-C1, B3-B4, B3-C1, B4-C1, B4-C3 C1-C2, C1-C3 |
| Chlorophyll a (µM) | 68.322 | <2e-16 *** | NE-IMD, NE-SW | 0.185 | 0.997 | |

Note; NE: Northeast monsoon, IMD: Intermediate dry, SW: Southwest monsoon

Phytoplankton cell density was highest at site A, averaging 138.20×10^4 cells/L, followed by site B at 56.49×10^4 cells/L and site C at 1.96×10^4 cells/L (Table 1). Among all taxa, *Skeletonema* sp. exhibited the highest abundances, comprising 98.98%, 96.55%, and 79.72% of the total cell count at sites A, B, and C, respectively. The abundance of *Skeletonema* sp. varied significantly among sites (ANOVA, $df = 2$, $F = 9.100$, $p < 0.001$) but showed no significant differences between seasons ($p > 0.05$) (Table 2).

Spatial and seasonal distribution of the potential harmful microalgae (PHM)

Among the total phytoplankton, twenty potentially harmful microalgae (PHM) species were identified, including twelve dinoflagellates and eight diatoms. Four of these species were classified as potentially toxic, encompassing three dinoflagellate genera, namely *Akashiwo sanguinea*, *Alexandrium minutum*, *Dinophysis caudata*, and *D. miles*. Sixteen red tide species were recorded, comprising eight dinoflagellates viz., *Alexandrium* sp., *Blixaea quinquecornis*, *Gonyaulax polygramma*, *Gymnodinium* sp., *Noctiluca scintillans*, *Protoperidinium quarnerense*, *Scrippsiella* sp., and *Tripos furca* and eight species of diatoms viz., *Chaetoceros* sp., *Nitzschia reversa*, *N. sigma*, *Pleurosigma* sp., *Pseudo-nitzschia* sp., *Surirella* sp., *Skeletonema* sp., and *Thalassiosira* sp.

Spatially, the cell density of the PHM was significantly higher at sites A and B than C (ANOVA, $df=10$, $F=10.200$, $p<0.001$) (Table 2). There were 17 species of the PHM with a high frequency of occurrence at each site (>6 stations), namely *Nitzschia reversa*, *N. sigma*, *Pseudo-nitzschia* sp., *Pleurosigma* sp., *Surirella* sp., *Chaetoceros* sp., *Skeletonema* sp., *Thalassiosira* sp., *Tripos furca*, *Alexandrium* sp., *A. minutum*, *Noctiluca scintillans*, *Protoperidinium quarnerense*, *Blixaea quinquecornis*, *Scrippsiella* sp., *Dinophysis caudata*, and *Gymnodinium* sp. Fourteen species including *Nitzschia reversa*, *N. sigma*, *Pseudo-nitzschia* sp., *Pleurosigma* sp., *Surirella* sp., *Chaetoceros* sp., *Skeletonema* sp., *Thalassiosira* sp., *Tripos furca*, *Alexandrium* sp., *Protoperidinium quarnerense*, *Blixaea quinquecornis*, *Scrippsiella* sp., and *Gymnodinium* sp. were encountered at all stations (Fig. 2).

Species richness of potentially harmful microalgae (PHM) exhibited significant seasonal variations (ANOVA, $df = 2$, $F = 11.86$, $p < 0.001$) (Table 2), while cell density remained relatively consistent ($p > 0.05$). Five species, including three diatoms (*Skeletonema* sp., *Chaetoceros* sp., and *Pleurosigma* sp.) and two dinoflagellates (*Alexandrium* sp. and *Blixaea quinquecornis*), were observed at all sites throughout the year. Among these, *Skeletonema* sp., a common red tide organism, reached peak cell densities of 1.37×10^6 cells/L at site A and 2.15×10^6 cells/L during the northeast monsoon. Spatial and seasonal cell densities of *Alexandrium* sp. peaked at 130 cells/L at site C and 144 cells/L during the northeast monsoon season (Table 3). A notable shift occurred during the intermediate dry season, with a transition from non-toxic species to toxic diatom species, including *Pseudo-nitzschia* sp., *Noctiluca scintillans*, and *Alexandrium minutum*.

Cluster dendrogram indicated the formation of three distinct cluster groups (Fig. 3), based on seasonal variables. Group I comprised of all samples collected during northeast monsoon season and a few samples collected during southwest monsoon (SWC2 and SWC3) and intermediate dry seasons (IMDC3). Prominent phytoplankton in this group included *Skeletonema* sp., *Protoperidinium quarnerense*, *Scrippsiella* sp., and *Alexandrium* sp. Most of the samples collected during intermediate dry season formed Group II together with two samples collected in southwest monsoon season. A diverse range of phytoplankton species in this cluster was observed.

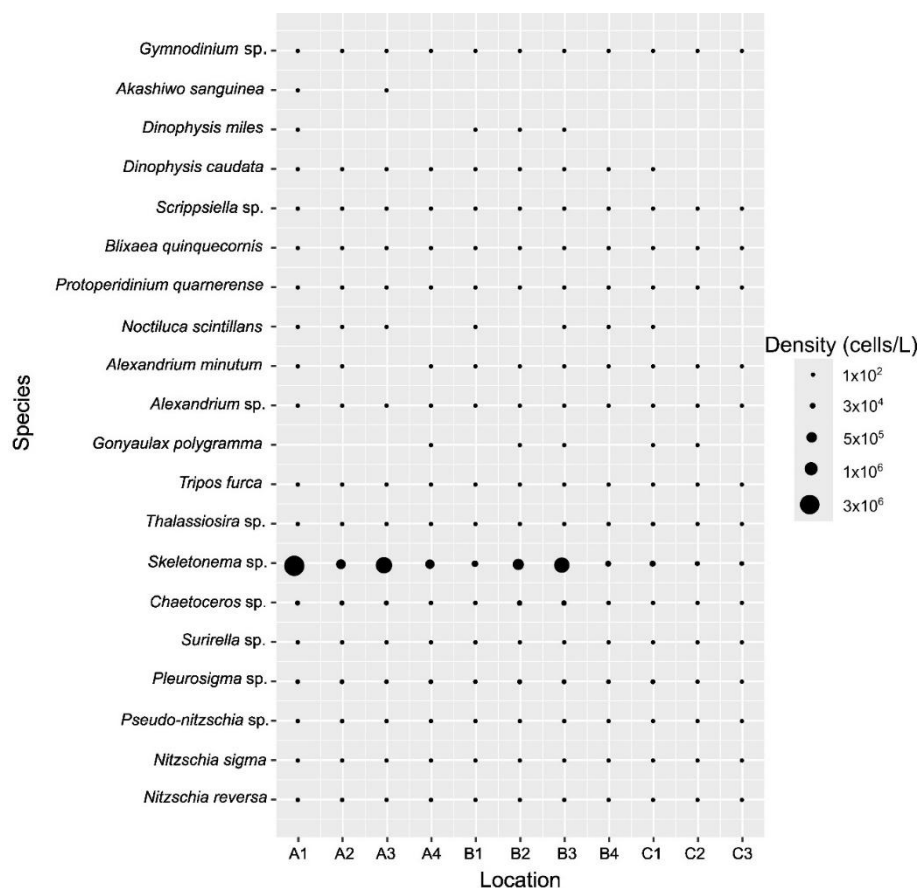


Figure 2. Spatial distributions of potential harmful microalgae (PHM) in aquaculture areas of Songkhla Lagoon. The size of the circles represents cell density

Pseudo-nitzschia sp., *Alexandrium minutum*, *Chaetoceros* sp., *Tripos furca*, *Noctiluca scintillans*, *Dinophysis miles*, and *Akashiwo sanguinea* dominated during intermediate dry season at sites A and B, which were closer to the lagoon opening than site C. During intermediate dry and southwest monsoon seasons (SWB4 and SWC1), *Thalassiosira* sp., *Gymnodinium* sp., *Nitzschia sigma*, *Gonyaulax polygramma*, *Blixaea quinquecornis*, *Alexandrium* sp., and *Dinophysis caudata* were abundant at site C, which was furthest from the lagoon opening. All samples in Group III were those collected during southwest monsoon. *Nitzschia reversa*, *Pleurosigma* sp., and *Surirella* sp. were responsible for the formation of this cluster.

Environmental factors

Spatial and seasonal variations were observed in several water parameters. Nutrient concentrations ranged from not detectable (<0.01) to 11.07 μM for nitrite, from not detectable (<0.01) to 46.84 μM for nitrate, from 3.15 to 325.00 μM for silicate, and from not detectable (<0.01) to 13.95 μM for phosphate. Surface water temperature, conductivity, salinity and pH, and chlorophyll *a* concentration ranged from 25.90 to 32.30 $^{\circ}\text{C}$, from 306.30 to 58,600 $\mu\text{S}/\text{cm}$, from 0.10 to 39.70 psu, from 4.68 to 10.45, and from not detectable (<0.01) to 1.94 μM , respectively.

Table 3. Temporally dominant species of phytoplankton, diatoms and dinoflagellates collected in three seasons in aquaculture areas of Songkhla Lagoon

| Seasons | Dominant phytoplankton | Average \pm SE (cells/L) | Min-Max (cells/L) | Dominant diatoms | Average \pm SE (cells/L) | Dominant dinoflagellates | Average \pm SE (cells/L) |
|---------|-----------------------------|----------------------------|-------------------|-----------------------------|----------------------------|------------------------------------|----------------------------|
| NE | <i>Skeletonema</i> sp. | 2,151,810 \pm 1,071,600 | 14-66,211,200 | <i>Skeletonema</i> sp. | 2,151,810 \pm 1,071,600 | <i>Alexandrium</i> sp. | 144 \pm 34 |
| | <i>Chaetoceros</i> sp. | 494 \pm 403 | 75-30,700 | <i>Chaetoceros</i> sp. | 494 \pm 403 | <i>Protoperidinium quarnerense</i> | 39 \pm 16 |
| | <i>Pleurosigma</i> sp. | 466 \pm 76 | 4-4,275 | <i>Pleurosigma</i> sp. | 466 \pm 76 | <i>Scrippsiella</i> sp. | 38 \pm 15 |
| | <i>Surirella</i> sp. | 217 \pm 83 | 9-5,644 | <i>Surirella</i> sp. | 217 \pm 83 | <i>Blixaea quinquecornis</i> | 21 \pm 16 |
| | <i>Alexandrium</i> sp. | 144 \pm 34 | 8-1,778 | <i>Nitzschia reversa</i> | 51 \pm 12 | <i>Tripos furca</i> | 3 \pm 1 |
| | <i>Navicula</i> sp. | 67 \pm 33 | 6-2,546 | <i>Thalassiosira</i> sp. | 25 \pm 8 | | |
| | Total | 2,153,678 | | | 2,153,389 | | 288 |
| IMD | <i>Skeletonema</i> sp. | 53,521 \pm 15,409 | 69-755,595 | <i>Skeletonema</i> sp. | 53,521 \pm 15,409 | <i>Blixaea quinquecornis</i> | 130 \pm 76 |
| | <i>Chaetoceros</i> sp. | 17,337 \pm 5,370 | 25-205,628 | <i>Chaetoceros</i> sp. | 17,337 \pm 5,370 | <i>Noctiluca scintillans</i> | 81 \pm 55 |
| | <i>Pleurosigma</i> sp. | 3,178 \pm 1,314 | 12-80,054 | <i>Pleurosigma</i> sp. | 3,178 \pm 1,314 | <i>Gymnodinium</i> sp. | 62 \pm 31 |
| | <i>Odontella</i> sp. | 1,693 \pm 1,691 | 10-111,626 | <i>Pseudo-nitzschia</i> sp. | 1,488 \pm 635 | <i>Alexandrium</i> sp. | 56 \pm 29 |
| | <i>Pseudo-nitzschia</i> sp. | 1,488 \pm 635 | 15-33,958 | <i>Thalassiosira</i> sp. | 540 \pm 246 | <i>Scrippsiella</i> sp. | 29 \pm 7 |
| | <i>Rhizosolenia</i> sp. | 653 \pm 291 | 9-14,869 | <i>Nitzschia reversa</i> | 104 \pm 27 | <i>Alexandrium minutum</i> | 22 \pm 9 |
| | Total | 80,190 | | | 79,647 | | 542 |
| SW | <i>Skeletonema</i> sp. | 71,772 \pm 14,060 | 40-860,855 | <i>Skeletonema</i> sp. | 71,772 \pm 14,060 | <i>Gymnodinium</i> sp. | 33 \pm 13 |
| | <i>Pleurosigma</i> sp. | 7,191 \pm 1,633 | 11-103,009 | <i>Pleurosigma</i> sp. | 7,191 \pm 1,633 | <i>Alexandrium</i> sp. | 28 \pm 8 |
| | <i>Chaetoceros</i> sp. | 1,901 \pm 791 | 14-60,993 | <i>Chaetoceros</i> sp. | 1,901 \pm 791 | <i>Blixaea quinquecornis</i> | 14 \pm 3 |
| | <i>Nitzschia reversa</i> | 958 \pm 355 | 7-22,825 | <i>Nitzschia reversa</i> | 958 \pm 355 | <i>Noctiluca scintillans</i> | 11 \pm 9 |
| | <i>Surirella</i> sp. | 802 \pm 363 | 7-34,744 | <i>Surirella</i> sp. | 802 \pm 363 | <i>Tripos furca</i> | 5 \pm 5 |
| | <i>Thalassiosira</i> sp. | 510 \pm 165 | 9-14,344 | <i>Thalassiosira</i> sp. | 510 \pm 165 | <i>Dinophysis caudata</i> | 4 \pm 4 |
| | Total | 84,922 | | | 84,614 | | 308 |

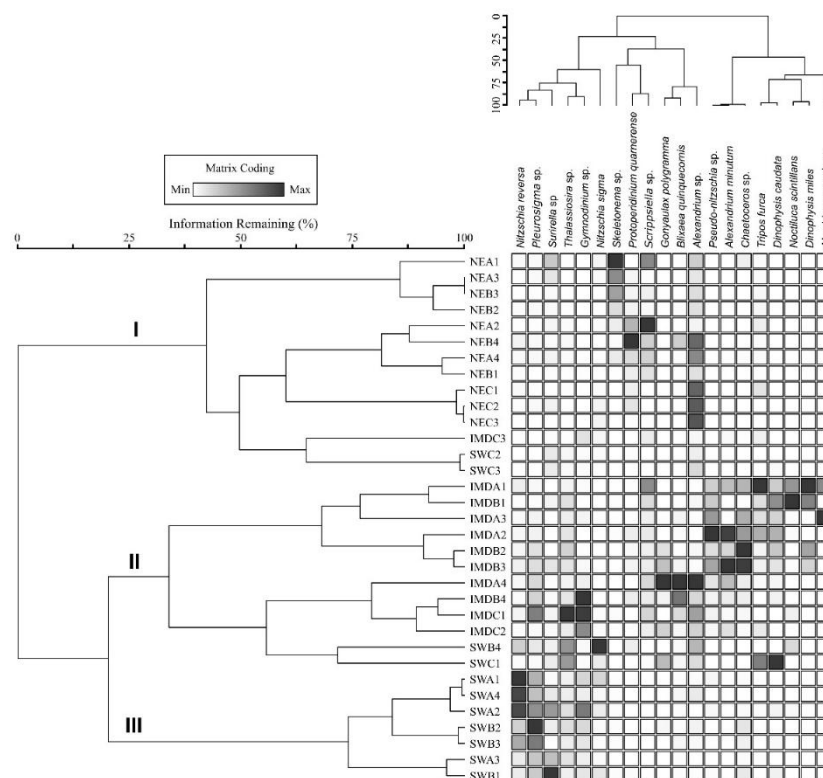


Figure 3. Dendrogram of a hierarchical cluster analysis of potential harmful microalgae (PHM) community in Songkhla Lagoon. Ward's method was used with Bray–Curtis distance measurements

Significant variations were found spatially and seasonally for conductivity, salinity, water temperature, and phosphate concentration ($p < 0.001$). Values of pH, silicate, and chlorophyll *a* significantly varied only between season ($p < 0.001$), but nitrite and nitrate concentrations differed between sites ($p < 0.01$) (Table 2).

Relationships between the PHM and environmental variables

The degree of difference between distributions of the PHM and associated optimal environmental conditions are shown in Figure 4. Variations of the PHM between sites and seasons were influenced by salinity ($r^2 = 0.925$), silicate ($r^2 = 0.653$), water temperature ($r^2 = 0.363$), and phosphate ($r^2 = 0.290$) (Figs. 4-5).

The PHM taxa, namely *Chaetoceros* sp., *Blixaea quinquecornis*, *Gymnodinium* sp., *Dinophysis caudata*, *D. miles*, *Pseudo-nitzschia* sp., *Noctiluca scintillans*, *Akashiwo sanguinea*, *Protoperdinium quarnerrense*, and *Gonyaulax polygramma*, exhibited notably high abundances during the intermediate dry and southwest monsoon seasons at sites A and B, coinciding with elevated salinity levels. In contrast, the assemblages of *Skeletonema* sp., *Nitzschia reversa*, *Alexandrium minutum*, *Tripos furca*, and *Scrippsiella* sp. during the northeast monsoon at site C were associated with elevated silicate and phosphate concentrations. The abundance of *Alexandrium* sp., *Thalassiosira* sp., and *Gymnodinium* sp. during the southwest monsoon was linked to higher water temperatures (Figs. 4-6).

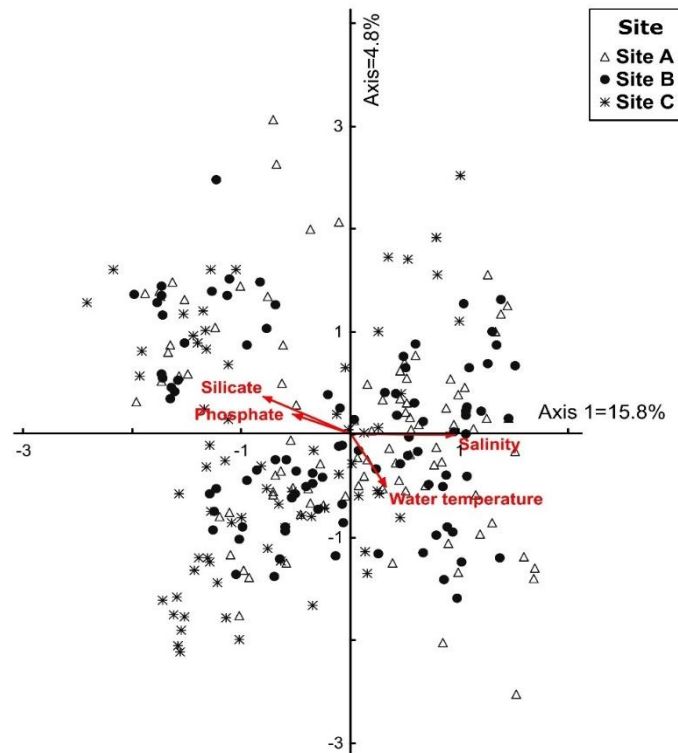


Figure 4. Bi-plots constructed from the canonical correspondence analysis (CCA) of potential harmful microalgae (PHM) distributions in aquaculture areas of Songkhla Lagoon. Sampling sites are represented by 'Δ' for site A, '●' for site B, and '*' for site C

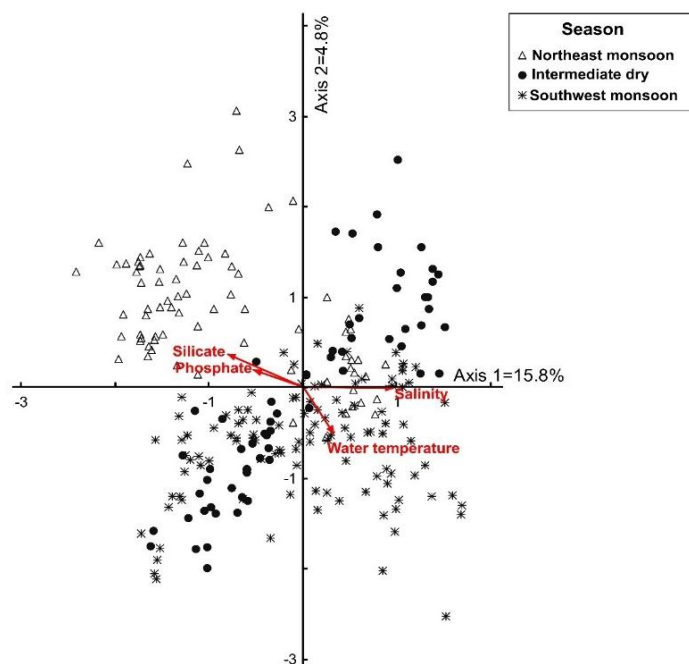


Figure 5. Bi-plots constructed from the canonical correspondence analysis (CCA) of potential harmful microalgae (PHM) distributions in aquaculture areas of Songkhla Lagoon. Seasons are represented by 'Δ' for northeast monsoon, '●' for intermediate dry, and '*' for southwest monsoon

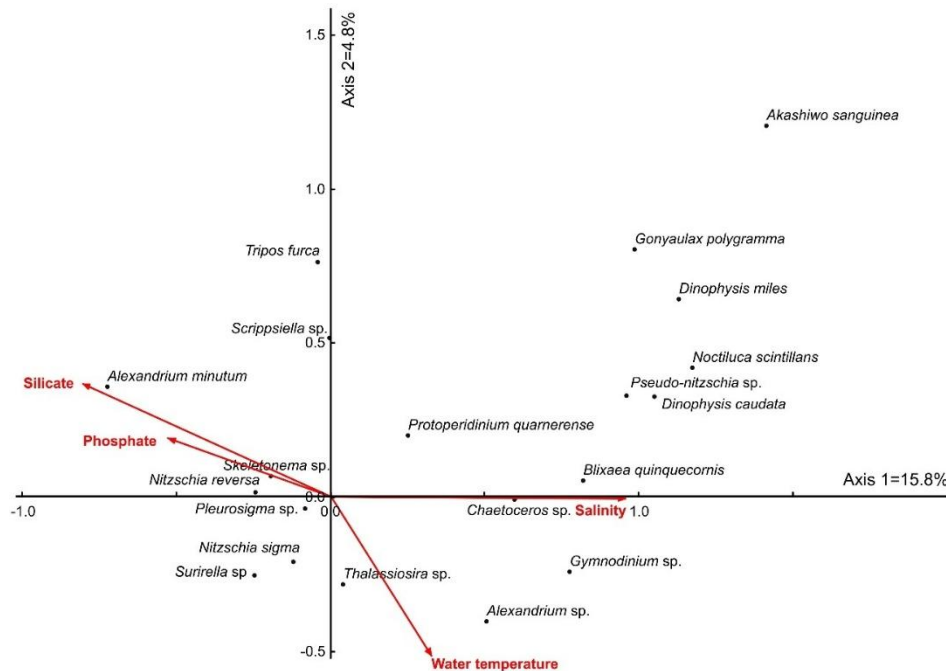


Figure 6. Bi-plots constructed from the canonical correspondence analysis (CCA) of potential harmful microalgae (PHM) distributions in aquaculture areas of Songkhla Lagoon. PHM taxa are grouped by association with environmental variables (arrows)

Discussion

Water quality in coastal areas is adversely affected by anthropogenic activities, including the release of toxic chemicals, untreated wastewater, oil pollutants, and biological agents (Zaiko et al., 2011). In Songkhla Lagoon, seabass farms located near sites A and B contribute to high organic loads from fish waste, contaminating the underlying sediment. The decomposition of this organic matter depletes dissolved oxygen in the overlying water and releases nutrients into the water column. Eutrophication and dense cage culture in the area can further exacerbate oxygen depletion, leading to anoxic or hypoxic conditions at the lake bottom during periods of low runoff (Sompongchaiyakul et al., 2004; Diana, 2009; Pornpinatepong et al., 2010).

The recorded biodiversity of potentially harmful microalgae (PHM) in Songkhla Lagoon, comprising twenty species, underscores the potential impact on the lagoon ecosystem. Fourteen of these species were distributed across all study sites. Phytoplankton and PHM cell densities exhibited a weak seasonal pattern, with the highest abundances observed during the northeast monsoon season and relatively low levels during the intermediate dry and southwest monsoon seasons. The majority of phytoplankton species identified in this study were marine-oriented, suggesting their susceptibility to seasonal salinity fluctuations. While the species composition observed in this study aligns with previous reports (Angsupanich and Rakkheaw, 1997; Puttapreecha et al., 2008), the seasonal variations differed. For instance, Angsupanich and Rakkheaw (1997) reported peak densities of blue-green and green algae during heavy rains of the northeast monsoon season and light rains of the southwest monsoon season, with minor diatom blooms occurring during the dry season. The risk of phytoplankton and PHM blooms varied spatially, with cell densities differing among study sites. The

present study highlights the influence of season on the average number of taxa for both phytoplankton and PHM species. Moreover, distinct regions within the lagoon demonstrated significant variations in cell density for both overall phytoplankton and PHM. The high density of PHM at sites A and B indicates the vulnerability of these areas to harmful algal blooms (HABs).

This may reflect relationships of various species, given the extremely high density of species observed in the vicinity of seabass farms. For instances, densities of *Alexandrium* sp. were 1,742 cells/L at A4, and *Pseudo-nitzschia* sp. was 33,958 cells/L at A2. These areas should therefore be closely monitored for PSP and Amnesic shellfish poisoning (ASP) incidences that may be harmful to aquaculture industry and local community. Elsewhere, the presence of PSP-toxin producing species at concentrations of only 20–100 cells/L triggered further toxin analysis in shellfish harvesting (Anderson et al., 2001). Widely distribution of *Pseudo-nitzschia* spp. have been reported in Malaysian coastal waters (Teng et al., 2014). Spatial variations of potentially harmful microalgal density in different locations may be influenced by water current, availability of nutrients and coastal human activities (Costa et al., 2017).

Eutrophication in Songkhla Lagoon is not a new phenomenon, as previous phytoplankton blooms within the inner, middle, and outer lagoons resulted in oxygen depletion and mass fish mortality (Sompongchaiyakul et al., 2004). It is well-established that elevated nitrogen concentrations pose significant risks to aquatic organisms (Pornpinatepong et al., 2010). The primary sources of pollution in the Outer Lake of Songkhla Lagoon include rubber and food industries, pig farms, arable farms, rubber plantations, shrimp farms, and surrounding communities (Pornpinatepong et al., 2010). Heavy precipitation during the northeast monsoon season often increases river discharge, leading to elevated nutrient loads, reduced water salinity, and enhanced water surface stability. High chlorophyll a concentrations were attributed to runoff from a small canal draining agricultural fertilizers and discharges from coastal aquaculture. In this study, the primary sources of nutrients and pollution affecting water quality and the phytoplankton community were identified as discharges from the U-Tapao River (near site B), Pawong Canal (near site A), and factories, as well as effluents from shrimp farms around the Pak-Ro Canal (near site C).

Variations in water parameters in the lagoon were associated with sites and seasons. Generally, specific factors affecting occurrence of toxic and harmful algal blooms (Liu et al., 1999; Wang et al., 2008) include hydrological conditions, nutrients and biological factors (Chen et al., 2001). During northeast monsoon season, the influx of freshwater pushes the brackish water mass out resulting in relatively fresh water conditions in the lagoon. The tidal rhythm also plays a significant role influencing salinity fluctuation in the outer part of the lagoon. The distribution of PHM species in Songkhla Lagoon is shaped by key environmental factors, including salinity, silicate, phosphate, and temperature. Elevated salinity during the intermediate dry and southwest monsoon seasons supports higher abundances of dinoflagellates and diatoms such as *Dinophysis caudata*, *Pseudo-nitzschia* sp., and *Noctiluca scintillans*, which thrive in high-salinity, low silicate and phosphate concentration (de Azevedo Tibiriçá et al., 2015). During the northeast monsoon, increased silicate and phosphate levels from freshwater inflows and runoff favor diatom dominance, including *Skeletonema* sp. and *Nitzschia reversa* (Hildebrand et al., 2018; Thangaraj et al., 2019; Kazmi et al., 2022). Warmer temperatures in the southwest monsoon enhance the growth of *Alexandrium* sp. and *Gymnodinium* sp., highlighting the critical role of temperature in driving tropical PHM blooms (Hallegraeff

et al., 1995; Anderson et al., 2012). During the intermediate dry and southwest monsoon seasons, water temperatures rise compared to the northeast monsoon season. Temperature is known as a key factor triggering the germination of *Alexandrium* resting cysts, leading to their presence in the water column (Anderson et al., 2012). These seasonal variations in environmental conditions lead to differential responses among PHM species. *Tripes furca* exhibits sensitivity to low salinity and high phosphate concentrations, a preference also observed in Vietnamese waters (Huynh et al., 2022). This suggests its adaptability to nutrient-enriched, low-salinity environments, which may provide a competitive advantage in such conditions. This is probably due to its mixotrophic species that may have a competitive advantage over its mixotrophic species, which could have a competitive edge over only photosynthetic species (Smalley et al., 2003; Mouritsen and Richardson, 2003).

During northeast monsoon, higher density of the PHM found at sites A and B, close to the lagoon opening, than at site C was due to a great dominance of *Skeletonema*. This diatom is normally distributed in offshore and coastal waters with relatively high salinity (Castillo et al., 1995; Balzano et al., 2011), and their blooms have contributed to the occurrence of red tide events in coastal areas (Marshall and Ananda Ranasinghe, 1989; Ramaiah and Furuya, 2002; Balkis, 2003). Salinity and higher nutrient loads during northeast monsoon season were considered factors regulating the bloom in this ecosystem. During the blooming of *Skeletonema*, highest concentrations of silicate and phosphate and highest salinity were coincidentally observed. Nutrient concentrations measured during the blooming in this lagoon were higher than the concentrations measured in Jiaozhou Bay; 3.90 μM phosphate and 0.51 μM silicate (Huo and Shu, 2005).

An assemblage of another PHM species, *Noctiluca scintillans*, was strongly correlated with high surface chlorophyll *a* concentration. This phenomenon occurred even during intermediate dry season with low discharge. A succession of *N. scintillans* can be explained by its mixotrophic ability, allowing consumption of external food, such as diatoms and other dinoflagellates, while still obtaining energy from a photosynthetic endosymbiont (Lirdwitayaprasit, 2001; Goes et al., 2018). However, Lirdwitayaprasit (2001) demonstrated that, without an external food supply, a green endosymbiont of *N. scintillans* disappeared after approximately two weeks of incubation, and the *Noctiluca* host cells died about a month later. Consequently, *N. scintillans* blooms should only be caused by an increase in precipitation that loads the water with land-based nutrients. According to Luang-on et al. (2022), seasonal dynamic of green *Noctiluca* and other red tides, including their blooming frequency, were influenced by monsoon-induced components such as river flow, precipitation, and sea surface winds.

Compared to previous studies in other areas of Thailand, outbreaks of *Noctiluca* red tide were often observed during December to February in the western part of the inner Gulf of Thailand, and during March to August in the eastern part of the inner Gulf (Lirdwitayaprasit et al., 1994). Blooms of *T. furca* occurred more often in both parts of the inner Gulf of Thailand. Besides a blooming of *Noctiluca* and *Tripes*, blooms of other red tide organisms such as *Trichodesmium*, *Chaetoceros*, *Coscinodiscus*, and *Skeletonema* were also sometimes observed (Lirdwitayaprasit, 2001). Recent reports have indicated a change of major species responsible for algal blooms. Toxic diatoms and dinoflagellates have replaced non-toxic species. Among them, *Alexandrium minutum* was reported in the upper Gulf of Thailand and confirmed by high performance liquid chromatography to produce gonyautoxins (Piumsomboon et al., 2001; Fukuyo et al., 2011). Fukuyo et al. (2011) reported two toxic *Pseudo-nitzschia* species in the Gulf and,

later, Fu et al. (2021) found five potentially toxic *Pseudo-nitzschia*; three PSP-producing species, *Alexandrium tamiyavanichii*, *Alexandrium fragae*, and *Gymnodinium catenatum* and six fish-killing dinoflagellates, *Margalefidinium polykrikoides*, *Margalefidinium fulvescens*, *Karenia mikimotoi*, *Karenia selliformis* ribotype B, *Karlodinium australe*, and *Karlodinium digitatum*. Results of cluster analysis clearly indicate that seasonal factors affecting the community structure of the PHM in the lagoon.

In this study, significant blooms of *Pseudo-nitzschia* sp., *Alexandrium* spp., *Scrippsiella* sp., and *Dinophysis caudata* were observed, all of which are known toxin producers linked to shellfish poisoning and fish kills. Similarly, regions such as the Philippines and Malaysia have documented blooms of *Alexandrium* spp., *Prorocentrum minimum*, and *Karlodinium australe*, associated with paralytic shellfish poisoning (PSP) and fish mortality. These findings underscore the regional patterns of harmful algal bloom (HAB) dynamics and their significant ecological and economic impacts, highlighting the importance of monitoring and management strategies to mitigate risks (Azanza et al., 2005; Lim et al., 2014; San Diego-McGlone et al., 2024). These differences highlight variations in PHM species driven by local nutrient sources and hydrodynamic conditions. The study emphasizes the role of aquaculture-derived nutrients in PHM proliferation, consistent with findings from Malaysia and the Philippines, where nutrient discharges from aquaculture significantly contribute to eutrophication and harmful algal blooms during warm, nutrient-rich seasons (Azanza et al., 2024; San Diego-McGlone et al., 2024). Aquaculture in Songkhla Lagoon, especially fish and shrimp farming, contributes significant nitrogen and phosphorus through insufficiently treated wastewater, organic waste, and pond sludge, promoting eutrophication and PHM growth (Dalbem Barbosa et al., 2024; Sedyaw et al., 2024). Effective nutrient management strategies, including low-phosphorus feed and the use of retention ponds or recycling systems, help reduce nutrient discharges and mitigate harmful algal blooms (Sampantamit et al., 2020; Dalbem Barbosa et al., 2024). Integrated multi-trophic aquaculture (IMTA) offers a sustainable solution by cultivating species at different trophic levels, which recycles nutrients and reduces waste. By incorporating fish, shellfish, and seaweed, this method enhances nutrient utilization and reduces the environmental impact of aquaculture effluents (Sampantamit et al., 2020). These strategies align with sustainable aquaculture practices, reducing the risks of harmful algal blooms (HABs) while emphasizing optimized feed use and best management practices to balance productivity with environmental sustainability (Sampantamit et al., 2020; Dalbem Barbosa et al., 2024). More importantly, the involvement of local policymakers in regulating nutrient inputs from aquaculture and agriculture is a critical component in supporting sustainable waste management practices and ensuring long-term ecosystem health.

Conclusions

The risk of harmful algal blooms (HABs) in Songkhla Lagoon is influenced by its proximity to the sea and the presence of seabass farms. Seasonal factors drive the community structure of potentially harmful microalgae (PHM), with at least 20 species identified, 14 of which were present at all sampling sites. Variations in water parameters, such as temperature, salinity, and nutrient levels, significantly affect PHM composition, supporting the study's primary objective. The high abundance of PHM near aquaculture areas raises concerns about human poisoning and fish mortality. Effective strategies are needed to manage HABs, minimize ecological losses, and ensure public health. The

findings highlight the importance of improved nutrient management to reduce HAB risks and sustain both aquaculture and the lagoon ecosystem. Advanced mariculture practices and baseline data on PHM distribution are crucial for informed decision-making and long-term environmental sustainability.

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