

Composition and Distributions of Nitrogen and Phosphorus and Assessment of Eutrophication Status in the Maowei Sea

LU Dongliang^{1), 3)}, HUANG Xueren²⁾, YANG Bin^{1), 3), 4), *}, DAN Solomon Felix^{1), 3), 5)}, KANG Zhenjun^{1), 3), 4)}, ZHOU Jiaodi^{1), 3)}, LAO Yanling¹⁾, ZHONG Qiuping^{1), 3)}, and WU Heng³⁾

1) Guangxi Key Laboratory of Marine Disaster in the Beibu Gulf, Qinzhou 535011, China

2) School of Petrochemical Engineering, Beibu Gulf University, Qinzhou 535011, China

3) School of Marine Sciences, Beibu Gulf University, Qinzhou 535011, China

4) Key Laboratory of Coastal Science and Engineering, Beibu Gulf, Qinzhou 535011, China

5) Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Institute for Advanced Ocean Study, Ocean University of China, Qingdao 266100, China

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Abstract Maowei Sea (MWS) is the largest oyster mariculture bay in Southwest China. Surface water from 12 sampling sites were collected during the dry season to study the composition and distribution of different forms of nitrogen (N) and phosphorus (P) beginning from the inner bay to the bay mouth in the MWS. An improved multi-parameter eutrophication index was developed and applied for the evaluation of the water quality in the MWS. Dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), and particulate nitrogen (PN) averagely accounted for 11.28%, 65.32%, and 23.39% of total N (TN) pool, dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP), and particulate phosphorus (PP) averagely accounted for 54.58%, 30.31%, and 15.12% of total P (TP) pool, respectively. DON and DIP were respectively the dominant forms of N and P in surface water of the MWS, during the study period. Concentration trends of DIN, DIP, DOP, PN, dissolved silicate (DSi), total organic carbon (TOC), chemical oxygen demand (COD) decreased from the inner bay to the bay mouth, suggesting that the MWS may be largely influenced by land-based inputs. Based on nutrient contents and stoichiometry, it likely that phytoplankton growth in the MWS is strongly limited by DIP during the dry season. Results from the improved eutrophication index revealed that the water quality in the MWS is in a state of moderate to poor eutrophication (average EI=0.953). The eutrophication state decreased from the inner bay to the bay mouth. Nitrate (NO₃-N), DON, DIP, DOP, and DSi showed significant positive correlations with eutrophication index ($r^2=0.52-0.78$, $P<0.05$), implying that these nutrients are among the significant factors, which could be used in evaluating the eutrophication status of the MWS.

Key words nutrient forms; eutrophication assessment; water quality; Maowei Sea

1 Introduction

Eutrophication has become a significant worldwide problem (Meyer *et al.*, 2000; Camargo *et al.*, 2006; Smith *et al.*, 2009), which has led to unusual algal blooms, environment deterioration and reduced biodiversity in many coastal waters during the past few decades (Codd *et al.*, 2000; Rodríguez *et al.*, 2005; Ferreira *et al.*, 2011). Reports show that nearly 70%–80% of nitrogen (N) and phosphorus (P) have been discharged from land-based sources into coastal water bodies on a global scale (Tuncer *et al.*, 1998; Páez *et al.*, 1999; Lu *et al.*, 2016). Several studies have demonstrated that eutrophication results from unusual load of mineral nutrients, primarily

N and P from the watershed (Alongi *et al.*, 1992; Howarth *et al.*, 2006; Kitsiou *et al.*, 2011). Since N and P are the major macronutrients for algal growth, thus, they are among the major describing factors of eutrophication status in coastal marine ecosystem (Howarth *et al.*, 2006; Rabalais *et al.*, 2009; Nguyen *et al.*, 2019).

The Redfield C:N:P:Si ratio of 106:16:1:1 has been used to understand which of the principal nutrient element is the limiting factor for algal growth, blooms and eutrophication levels (Redfield *et al.*, 1963; Billen *et al.*, 2007; Howarth *et al.*, 2008; Wang *et al.*, 2009). On the other hand, significant transformation may occur between the different forms, especially N and P by biogeochemical processes occasioned by microorganism and photochemistry (Jickells, 1998; Cotner and Biddanda, 2002; Luo *et al.*, 2017). For example, dissolved organic N (DON) and dissolved organic P (DOP) can be transformed into dissolved

* Corresponding author. E-mail: binyang@bbgu.edu.cn

inorganic N (DIN) and dissolved organic P (DIP), respectively by microorganism and photochemistry, and such changes can affect the eutrophication status of a water body (Officer *et al.*, 1980; Anderson *et al.*, 2002). Thus, studying the composition and distribution of various forms of N and P has a practical significance for eutrophication assessment in coastal marine systems, and may help in the management of seawater quality.

Coastal marine ecosystems undergo continuous changes in the rates of productivity, nutrition and community structure. These changes may be reflected the impact of eutrophication levels on water quality (Tyson *et al.*, 2011; Lee *et al.*, 2019). Because eutrophication is a complex processes, the difficulty in its assessment is because of continuous complexities in anthropogenic activities in coastal marine environments (Andersen *et al.*, 2006; Kitsiou *et al.*, 2011). To bridge this gap, several authors have considered several parameters such as seawater transparency, chemical oxygen demand (COD) and dissolved oxygen (DO) as variables for eutrophication assessment (Kitsiou *et al.*, 2011) while others tend to consider seawater nutrient concentration and phytoplankton community structure (Lundberg *et al.*, 2005; Sun *et al.*, 2016). Ignatiades *et al.* (1992) used four nutrients ($\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$) variables and Chl-*a* concentration to evaluate the eutrophication status, and many studies have shown that the stability and veracity of eutrophication index method depend on the nutrition variables (Kelly *et al.*, 1995; Anderson *et al.*, 2002; Ignatiades *et al.*, 2002; Kitsiou *et al.*, 2011). Although these variables are important for eutrophication assessment, they are not widely applied in many study areas (Krom *et al.*, 1991; Bu *et al.*, 2010). The aforementioned parameters have been used in several eutrophication assessment methods such as the integrated approaches (Kitsiou *et al.*, 2011; Wang *et al.*, 2019), multiple criteria analysis (Malczewski *et al.*, 2006; Janssen *et al.*, 2012) and remote sensing (Garcia *et al.*, 2005; Maritorena *et al.*, 2005). Nevertheless, spatial analysis (Legendre *et al.*, 1993; Dormann *et al.*, 2007), mapping (Lam *et al.*, 1983; Burger *et al.*, 2006), modeling (Rast *et al.*, 1983; Zaldívar *et al.*, 2009), and

multi-dimensional statistical analysis (Vassiliou *et al.*, 1989; Ignatiades, 2002) have also been used to evaluate the eutrophication of many coastal waters. Compared to these methods, the principal component analysis (PCA) has an advantage of limiting the number of variables related to eutrophication, easy to apply, and the outcome is well understood (Lundberg *et al.*, 2005; Kitsiou *et al.*, 2011; Primpas *et al.*, 2011). Therefore, application of multi parameters including various N and P forms in PCA may assist in the effective evaluation of eutrophication status of coastal ecosystems.

The Maowei Sea (MWS) is a typical subtropical bay in the northern Beibu Gulf. The MWS is increasingly impacted by anthropogenic activities. Large amounts of land-based pollutants enter the MWS mainly through the rivers and wastewater treatment plants, which may result in nutrient structure changes. Previous studies have shown that total N (TN) and total P (TP) pools are dominated by DON (DON/TN=35%–72%) and DOP (DOP/TP = 27%–51%), respectively in the MWS (Qin *et al.*, 2017; Yang *et al.*, 2019). On a global scale, few studies have also shown that DON and DOP are among the potential eutrophication causing nutrients (*e.g.*, Stepanauskas *et al.*, 2002; Oneil *et al.*, 2012). Given the importance of the MWS and its ecological functions, it is quite essential to correct the variables of eutrophication index by using improved indices to evaluate the eutrophication state of the MWS. The objectives of this study were as follows: 1) to reveal the composition and distribution of different N and P forms (species) in the MWS. 2) To build and calibrate the structure and variables of the eutrophication index and improve the simulation accuracy. 3) To evaluate plans for water quality management and lay a foundation for water quality improvement in the MWS.

2 Materials and Methods

2.1 Study Area

The MWS is located within the coordinates (21.55°–21.95°N, 108.4°–108.75°E), and connects with the northern

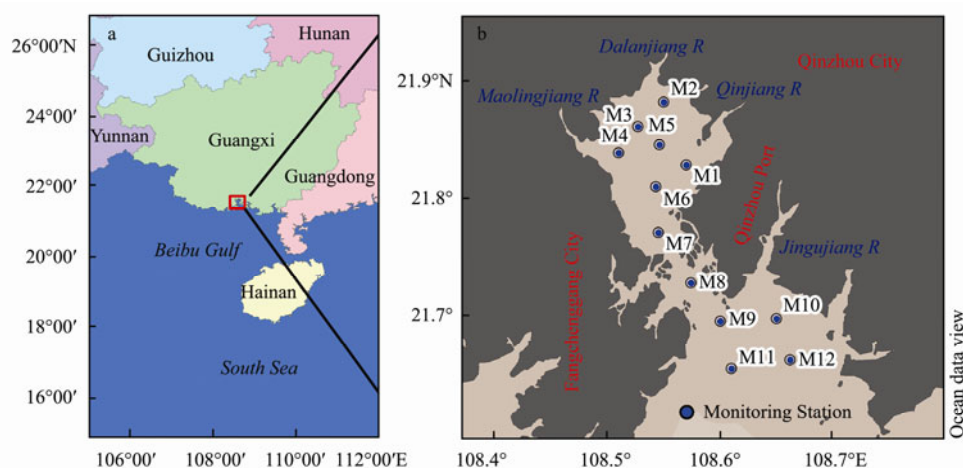


Fig.1 (a) Map of the study area (inserted) showing, (b) the sampling stations in the Maowei Sea.

Beibu Gulf (south coast of Guangxi, China) through a narrow channel (width: about 0.5 km), which limits rapid water exchange between the two water bodies. The three major rivers, which play a fundamental role in the transport of nutrients from land to the MWS, are the Qinjiang River (QJR), Maolingjiang River (MLJR) and Dalanjiang River (DLJR), (Fig. 1). The basin has an area of about 134 km² and an average water depth of about 5.4 m (range, 2–7 m) with a coastline of about 58 km (Chen *et al.*, 2018; Gu *et al.*, 2018). The MWS is rich in natural resources, including oyster aquaculture, and mangrove forest reserve. Land use in the catchment areas is dominated by urban settlements and shrimp ponds in the north. The eastern catchment areas are dominated by shrimp ponds, including agricultural activities and mangrove forest to the east, while the western catchment areas are clustered by factories. Human population in this area is about 0.45 million within 1–15 km as at 2016, which represents about 10% of the Qinzhou total population (data from Guangxi Statistical Yearbook 2017). The MWS is dominated by an irregular diurnal tide with average and maximum tide ranges of 2.5 and about 5.7 m, respectively (Yang *et al.*, 2019). The area is also influenced by the East Asian monsoon (Zhang *et al.*, 2019). Monthly average rainfall is 25–105 mm (Guangxi Statistical Bureau, 2017), while the total annual average rainfall is about 1600 mm. More than 80% of freshwater from the adjoining rivers is discharged during the flood season (March to October), and the remaining 20% of freshwater is discharged during the dry season (April to September). A large amount of wastewater from the urban areas is transported to the sea *via* riverine discharge.

2.2 Sampling and Analysis

Twelve (12) sites, starting from the inner bay to the mouth of the bay were selected along the watercourse at an interval of about 0.2–1 km for surface water sampling. This sampling design provides the basis for comprehensive evaluation of the effects of N and P on the eutrophication status and water quality of the MWS. Water temperature (T), dissolved oxygen (DO) and salinity were measured at each station using a multi-parameter analyzer (AP-700, Aquaread Ltd., England, UK). Seawater samples were collected at 0.5 m below the sea surface using a 5-L Niskin bottle water sampler (General Ocean Ltd., USA) during the dry season month (November, 2017). Exactly 500 mL-samples were filtered through acetate cellulose membrane (0.45 µm), which were presoaked in diluted hydrochloric acid (pH = 2) for 24 h, and rinsed with Milli-Q water (pH = 7) before use. The filtered water samples and the filters were kept cool with ice in the dark, and were immediately transported to the laboratory and stored at –20 °C. The analyses were completed within two weeks after sampling.

Concentrations of ammonium (NH₄-N), nitrate (NO₃-N), nitrite (NO₂-N), dissolved inorganic P (DIP) and dissolved silicate (DSi) were analyzed in the filtered water samples using an automatic nutrient analyzer (Model

QuAAtro AA3, Bran + Luebbe, Germany). The standard error was 0.5% for NH₄-N (at 3.5 µmol L⁻¹), 0.4% for NO₃-N, 0.3% for NO₂-N, 0.3% for DIP and 0.2% for DSi. DIN concentrations were calculated as the sum of NH₄-N, NO₃-N and NO₂-N. The concentrations of DON and DOP were operationally calculated as the difference between total dissolved nitrogen (TDN) and DIN, and between TDP and DIP, respectively. The suspended solids retained on the filters were used to measure particulate N (PN) and particulate P (PP). Total dissolved phosphorus (TDP) and TDN were measured using the molybdate colorimetry and ultraviolet spectroscopy method after digesting the samples with potassium persulfate. Exactly 0.3 L water samples were filtered through a 0.45 µm glass fiber filter, and the suspended solids retained on the membrane were used to measure chlorophyll-*a* (Chl-*a*). The content of Chl-*a* was extracted with 90% acetone in the dark at 4 °C for 12 h, and then centrifuged for 10 min, and measured using a fluorescence spectrophotometer (F-4500, Hitachi Co, Japan) (Welschmeyer *et al.*, 1994). Surface seawater samples were collected and mixed in 250 mL acid-washed bottles, and 2–3 drops of concentrated sulfuric acid were added and kept cool on ice at 4 °C while in the field. The samples were then transported to the laboratory for the measurements of chemical oxygen demand (COD_{Mn}) through titration with acidic potassium permanganate (State Environment Protection Administration of China, 2002).

2.3 Data Analysis

The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS Ver.19.0; IBM, Armonk, NY, US) for Spearman's correlation and t-test comparison. The acceptable values were set at $P < 0.05$, the Kaiser-Meyer-Olkin (KMO) value must be > 0.5 (Cohen *et al.*, 2013). Origin Version 8.0 software was used to fit the equation and making of concentration plots. Eutrophication assessment was carried out using nutrient parameters (PO₄, NH₄-N, NO₃-N, and NO₂-N) and Chl-*a*, which were analyzed with the PCA, as widely reported in other study areas (*e.g.*, Parinet *et al.*, 2004; Lundberg *et al.*, 2005; Primpas *et al.*, 2010). The eutrophication index (EI) was assigned five grades: (a) < 0.04 , (b) 0.04–0.38, (c) 0.38–0.85, (d) 0.85–1.51 and (e) > 1.51 . These five classes of EI water quality corresponds to the High, Good, Moderate, Poor and Bad water qualities, respectively. The EI equation is expressed as follows:

$$EI = aC_{\text{NO}_2\text{-N}} + bC_{\text{NO}_3\text{-N}} + cC_{\text{NH}_4\text{-N}} + dC_{\text{DON}} + eC_{\text{DIP}} + fC_{\text{DOP}} + gC_{\text{Si}} + hC_{\text{Chl-a}}, \quad (1)$$

where the $C_{\text{NO}_2\text{-N}}$, $C_{\text{NO}_3\text{-N}}$, $C_{\text{NH}_4\text{-N}}$, C_{DON} , C_{DIP} , C_{DOP} , C_{Si} , and $C_{\text{Chl-a}}$ represents concentrations of NO₂-N, NO₃-N, NH₄-N, DON, DIP, DOP, DSi and Chl-*a*; a , b , c , d , e , f , g and h are the coefficients derived from the PCA analysis for the first principal component after standardization of the variables.

3 Results

3.1 Physicochemical Characteristics of Seawater

The COD_{Mn} and DO levels in surface water of the MWS are shown in Fig.2. COD_{Mn} ranged from 3.07 to 4.52 mg L^{-1} (average, 3.96 mg L^{-1}), and exceeded the grade II water quality standard (Standard of $\leq 3 \text{ mg L}^{-1}$) (National standard of the People Republic of China, Environmental Quality Standard for surface Seawater, GB3097-1997) in all the 12 sampling stations. Concentrations of DO ranged from 6.34 to 7.77 mg L^{-1} (average,

7.04 mg L^{-1}), and were higher than the water quality standard (5 mg L^{-1}) (National standard of the People Republic of China, Environmental Quality Standard for Surface Seawater, GB3097-1997). There were differences in the distributional trends of COD_{Mn} and DO from the bay head to the mouth of the bay (Fig.2); while COD_{Mn} showed a gradual decreasing trend from stations M1 to M12, the opposite trend was observed for DO. The concentrations of TOC and Chl-*a* ranged from 23.73 to 7.1743 mg L^{-1} (average, 20.12 mg L^{-1}) and 7.10 for $3.96 \mu\text{g L}^{-1}$ (average, $5.63 \mu\text{g L}^{-1}$), respectively, and exhibited significant variation tendencies ($P < 0.05$) between the sampling stations.

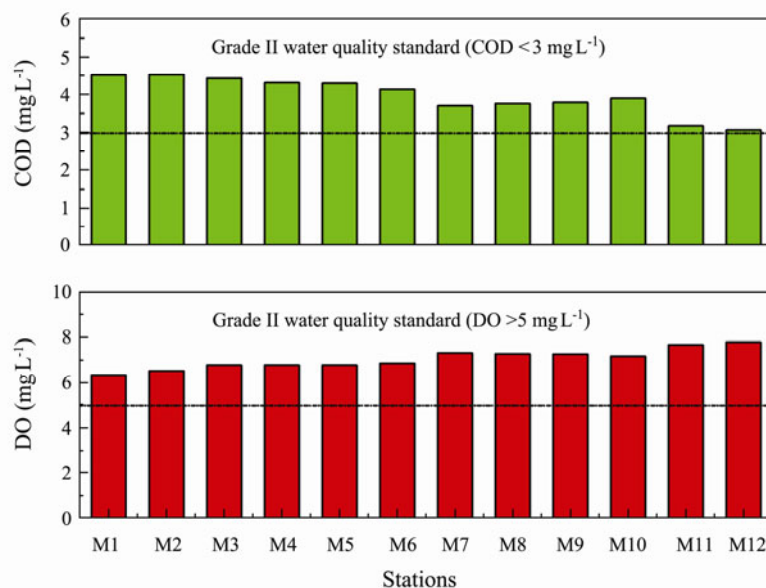


Fig.2 Spatial variation of chemical oxygen demand and dissolved oxygen in surface water of the Maowei Sea.

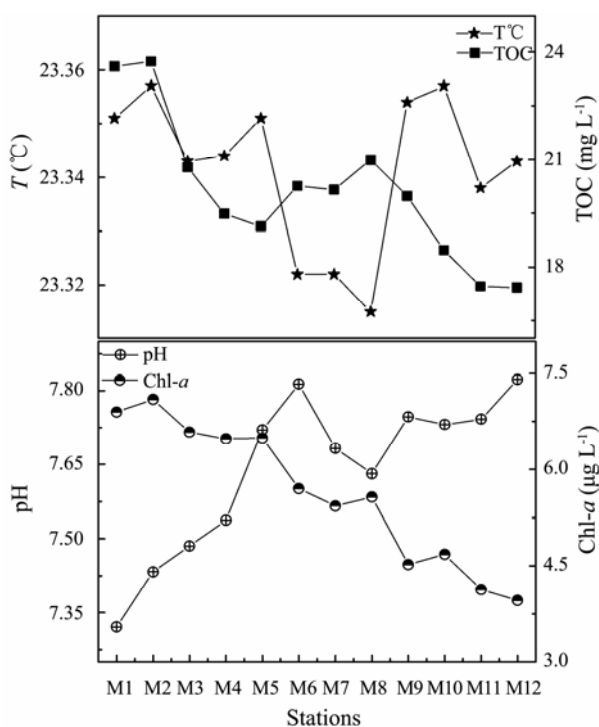


Fig.3 Spatial variation in temperature (T), pH, total organic carbon (TOC) and chlorophyll *a* (Chl-*a*) in surface water of the Maowei Sea.

Surface water temperature ranged from 23.57 to 23.22°C (average, 23.34°C), while pH ranged from 7.32 to 7.82 (average, 7.64), and both showed no significant difference ($P > 0.05$) between the sampling stations. The distributions of surface water temperature, pH, total organic carbon (TOC) and Chl-*a* in the MWS are shown in Fig.3. While the trends of TOC and Chl-*a* gradually decreases from stations M1 to M12, the opposite trend was found for pH. However, surface water temperature showed a decreasing trend from stations M1 to M8, and then rapidly increased to station M12 (Fig.3).

3.2 Composition and Distribution of Nitrogen and Phosphorus Forms

The composition and spatial variations in the different forms of N and P in surface water of the MWS are shown in Figs.4a-d). The concentrations of TN, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$ and ranged from 4.46 to 5.64 mg L^{-1} (average, 5.02 mg L^{-1}), 0.03– 0.11 mg L^{-1} (average, 0.07 mg L^{-1}), 0.06– 0.11 mg L^{-1} (average, 0.08 mg L^{-1}) and 1.19– 1.56 mg L^{-1} (average, 1.17 mg L^{-1}), respectively, with no significant variations in their distributional trends from stations M1 to M12. The concentrations of $\text{NO}_3\text{-N}$ and DON ranged from 0.24– 0.63 mg L^{-1} (average, 0.41 mg L^{-1}) and 2.71– 3.91 mg L^{-1} (average, 3.29 mg L^{-1}), respectively. The con-

centrations trend of $\text{NO}_3\text{-N}$ decreased from stations M1 to M12, while that of DON increased from station M1 to M12, respectively. DIP, DOP and PP concentration ranged from 0.04 to 0.12 mg L^{-1} (average, 0.09 mg L^{-1}), 0.03–0.14 mg L^{-1} (average, 0.05 mg L^{-1}) and 0.01–0.04 mg L^{-1}

(average, 0.02 mg L^{-1}), respectively, with no significant variations in their distributional trends from station M1 to M12. The concentrations of DIN, DON, and PN averagely accounted for 11.28%, 65.32%, and 23.39% of TN pool, respectively. Concentrations of $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, and

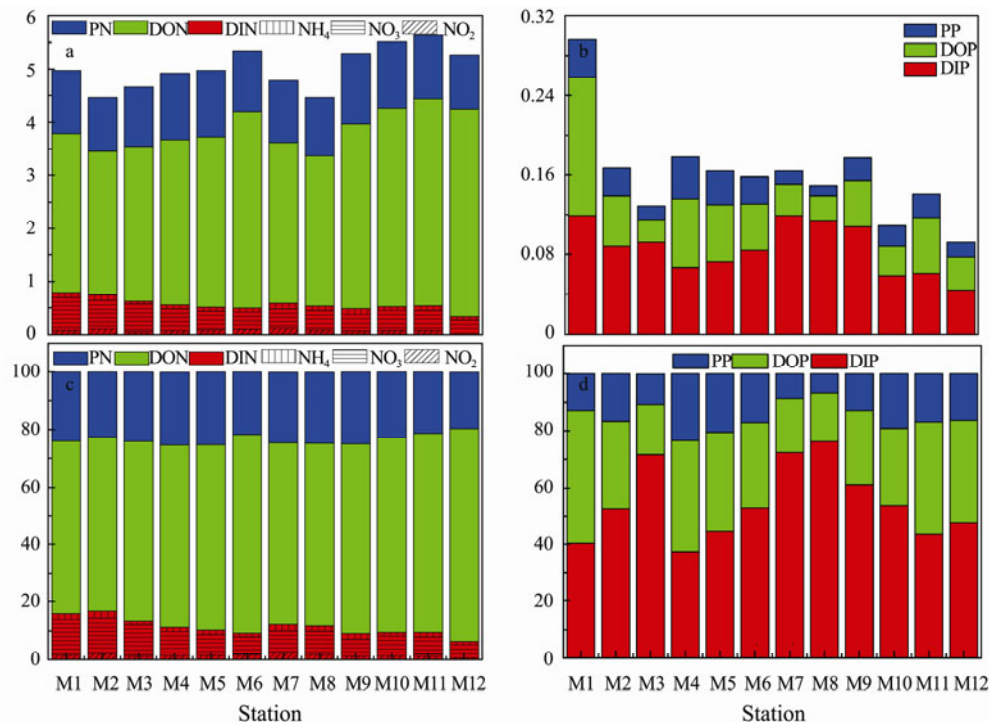


Fig.4 Concentrations (mg L^{-1}) of (a) N and (b) P forms, and (c) ratios (%) of different N and P forms to TN and (d) TP, respectively in surface water of the Maowei Sea.

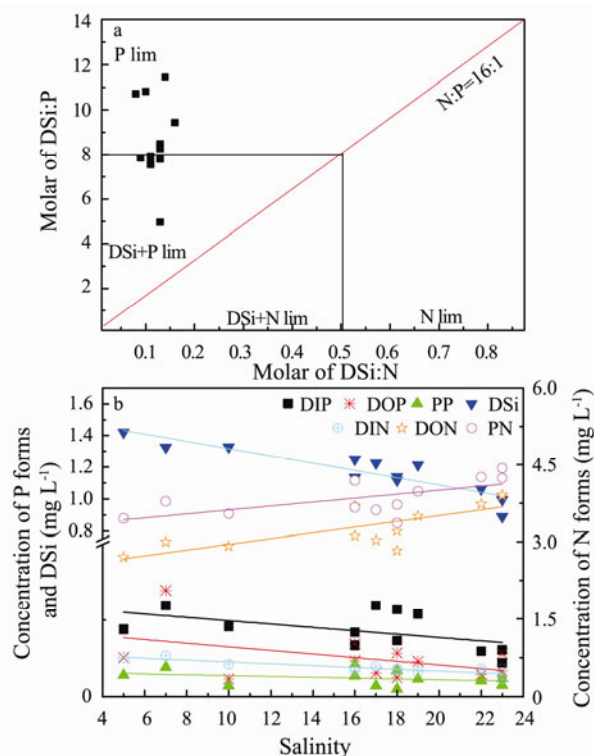


Fig.5 (a) Molar ratios of DSi:N and DSi:P at twelve sampling stations and (b) the relationships between DIN, DON, PN, DIP, DOP, PP, DSi and salinity of the Maowei Sea.

$\text{NH}_4\text{-N}$ also accounted for 13.27%, 71.28%, and 15.45% of DIN pool, respectively. $\text{NO}_3\text{-N}$ and DON were the main components of DIN and TN, respectively. The average contribution of DIP, DOP, and PP to the TP pool was 54.58%, 30.31%, and 15.12%, respectively.

3.3 Relationships Between Nutrients with Salinity and Nutrient Ratios

The relationship between different nutrients and Salinity is shown in Fig.5a. The concentrations of DIN, DIP, DOP, PP and DSi gradually decreased as salinity increases from the inner bay to the bay mouth, while DON and PN increased alongside with increasing salinity of the surface water. The concentrations of TN, TP and DSi were used to calculate the Redfield ratio. The molar ratios of DSi to N (DSi:N) and P (DSi:P) were plotted against each other as depicted in Fig.5b. The inserted lines theoretically represent nutrients ratios, which separates the plot into four compartments, each of which represents different limiting factor. As depicted, the 12 sampling stations in the MWS were distributed in the compartment characterized with higher N:P ratios (*i.e.*, above the Redfield value). Both P and P + DSi were identified as the limiting nutrients for phytoplankton growth in the MWS during the study period.

3.4 Relationship Between Eutrophication Index and Nutrient Levels

The result of the Pearson correlation matrix for the eight variables ($\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, DON, DIP, DOP, DSI and Chl-*a*) was analyzed with PCA (Table 1). A good consistency between the results was observed for all samples. Statistically significant correlations were observed for all the data ($P < 0.05$), while the KMO value was 0.68 ($\text{KMO} > 0.5$). Significant positive correlations were observed between Chl-*a* and nutrient variables ($\text{NO}_3\text{-N}$, DON, DOP and DSI) with the exceptions of $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$ and DIP. Among the nutrients, DSI correlated significantly with $\text{NO}_3\text{-N}$, DON and DIP. The correlation matrix results were further analyzed with PCA as shown in Table 2. The principal component analysis showed that the Eigen values of the first principal component (Factor 1) represent 67.33% of the total data variance. The other

seven components explain parts of the remaining variation from 19.34 to 0.19%. Therefore, information provided by Factor 1 can be characterized as the process of dimensionality reduction. The coefficients of the eight variables in the first principal component were standardized as shown Table 2, indicating that the eight variables (Section 2.3) participated with equal weights to the formation of the first principal component, and were used to calculate the proposed eutrophication index as shown in Eq. (1) (Parinet *et al.*, 2004; Primpas *et al.*, 2010). The assessment of the eutrophication level of the MWS considering the 12 sampling stations is depicted in Fig. 6. The average result for the eutrophication index in surface water of the MWS was 0.953, which suggests that the eutrophication status of the MWS is between moderate and

Table 1 The correlation matrix of the eight state variables with EI by the principal component analysis

Variable	$\text{NO}_2\text{-N}$	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$	DON	DIP	DOP	DSi	Chl- <i>a</i>	EI
$\text{NO}_2\text{-N}$	–								
$\text{NO}_3\text{-N}$	0.659*	–							
$\text{NH}_4\text{-N}$	0.654*	0.143	–						
DON	0.445	0.662	0.261	–					
DIP	0.535*	0.426	0.558*	0.656*	–				
DOP	0.053	0.585*	0.181	0.148	0.232	–			
DSi	0.470	0.794**	0.366	0.834**	0.636*	0.320	–		
Chl- <i>a</i>	0.390	0.773**	0.020	0.622*	0.399	0.422*	0.829**	–	
EI	0.265	0.879**	0.129	0.522**	0.558*	0.732**	0.758**	0.848**	–

Notes: **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level.

Table 2 The PCA results showing the component and coefficients matrices of the studied variables

Principal components	Component matrix		Coefficient matrix	
	Factor 1	Factor 2	Factor 1	Factor 2
Eigenvalue	5.387	1.548		
% Variance explained	67.333	19.345		
Accumulative%	67.333	86.678		
$\text{NO}_2\text{-N}$	0.619	0.597	0.141	0.368
$\text{NO}_3\text{-N}$	0.820	−0.420	0.186	−0.271
$\text{NH}_4\text{-N}$	0.474	0.749	0.108	0.484
DON	0.674	0.470	0.149	0.045
DIP	0.751	0.373	0.171	0.241
DOP	0.458	−0.370	0.104	−0.239
DSi	0.932	−0.096	0.212	−0.062
Chl- <i>a</i>	0.844	−0.404	0.192	−0.261

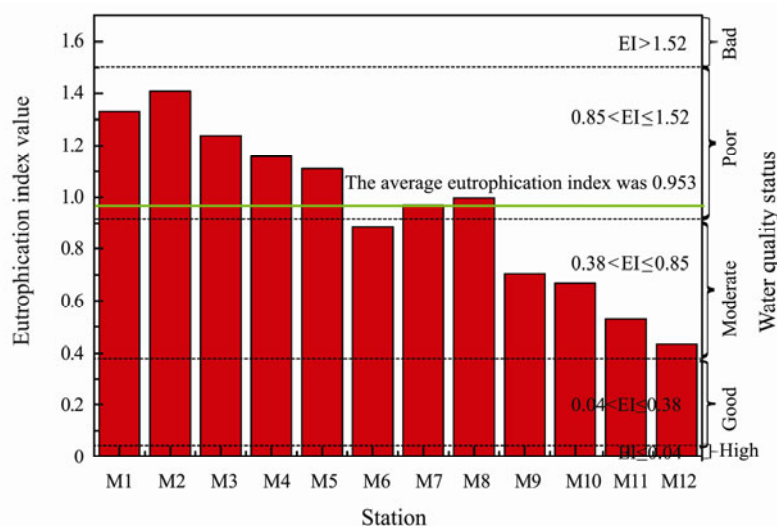


Fig. 6 Eutrophication level for different surface water stations in the Maowei Sea.

poor levels. The eutrophication level was gradually weakened from stations M1 to M12.

4 Discussion

4.1 Physicochemical Characteristics of Seawater

The COD is used to quantify organic contamination load in coastal waters (Barakat *et al.*, 2016). The higher COD levels in this study, which exceeded the grade II water quality standard ($\leq 3 \text{ mg L}^{-1}$), implies that MWS is seriously polluted by organic pollutants. Significant negative correlation ($R^2=0.67$, $P<0.05$) was found between COD_{Mn} , TOC, Chl-*a*, and salinity. The QJR and MLJR annually discharge about $64.4 \text{ m}^3 \text{ s}^{-1}$ and $49 \text{ m}^3 \text{ s}^{-1}$ freshwater, of which about 80% of the freshwater is discharged during the wet season (Li *et al.*, 2018). A large amount of land-based COD and TOC are transported to the MWS mainly through the QJR and MLJR. Li *et al.* (2014) found that the COD discharged from the MLJR and QJR was 13388 and 23352 tons, respectively, in 2012. The authors indicated that poultry feeding industry discharged about 50.5% and 79.4% of COD into the MLJR and QJR, respectively (Li *et al.*, 2014). This suggests that COD and TOC are mainly discharged from land-based sources including industrial and agricultural waste, domestic sewage, and aquaculture activities into the MWS. Both DO and pH are among the essential parameter that helps in the sustenance of an aquatic ecosystem, and commonly used to assess the quality of seawater (Barakat *et al.*, 2016). In this study, the concentrations of DO were $>5 \text{ mg L}^{-1}$ in all the investigated sites, which fits into the grade II seawater quality standard (GB3097-1997) based on DO levels in the MWS. The reason for this was oxygen-demanding waste, such as COD_{Mn} , DON, DOP and $\text{NH}_3\text{-N}$ (Li *et al.*, 2012). The relationships between DO and salinity ($R^2=0.62$, $P<0.05$) and DON ($R^2=0.54$, $P<0.05$) were positive and significant. The gradual increase in the DO trend from station M1 to M12, suggests that DO is mainly affected by hydrodynamics, probably from northeast monsoon, during the dry season (Zhang *et al.*, 2015). The pH range between 7.32 and 7.82 was found at 75% of the sampling sites. Stations M1, M2 and M3 were unfit for grade II seawater quality standard (GB3097-1997) based on pH levels. The lower pH levels at these stations may be attributed to the riverine discharge, industrial effluents, and domestic sewage effluents (Yang *et al.*, 2013).

4.2 Nutrient Levels and Structure

The concentrations of DIN in all the sample stations exceeded the grade II seawater quality standard (Standard, $\leq 0.3 \text{ mg L}^{-1}$) (GB3097-1997). The DIN concentrations in this study are slightly higher than those reported for much polluted urban Jiaozhou Bay (average: DIN 0.46 mg L^{-1} ; Lu *et al.*, 2016), but lower than those reported for major tropical bays, such as the Shenzhen Bay (average: 1.63 mg L^{-1} ; Wu *et al.*, 2016) and Lianzhou Bay (average: 0.82 mg L^{-1} ; Yang *et al.*, 2015). $\text{NO}_3\text{-N}$ occupied the

largest portion of DIN, and may have resulted from the possible conversion of $\text{NH}_4\text{-N}$ oxide to $\text{NO}_3\text{-N}$ by ammonia-oxidizing bacteria. This may be supported by the dissimilar trends between $\text{NO}_3\text{-N}$ concentrations and DO concentration, as more DO may have been used during the process. DON was the main components of TN (Fig.4). Sources of DON may be attributed to the surrounded mangrove reserve and aquaculture industry in the MWS catchment. Mangrove forests at the interface between terrestrial and marine ecosystems represent a rich biological diversity of plants, animals and microorganisms, and can contribute a lot of DON to coastal waters (Thatoi *et al.*, 2103; Dan *et al.*, 2019). As mentioned earlier (2.1), land use in the catchment areas of the MWS is dominated by agriculture, aquaculture and industry, while the upstream regions of the QJR and MLJR are densely inhabited districts, with about 0.45 million people (Zhang *et al.*, 2019). Thus, a lot of DON can be transported from diffuse sources including run off from the urban areas. The gradual increase in the concentrations of DON in the MWS from the inner bay to the bay mouth is consistent with the DON distribution trend reported in the Jiaozhou Bay (Lu *et al.*, 2016). Here, it is believed that DIN may have been transformed to DON in the MWS during the dry season. Thus, the spatial distributions of DIN and DON may not only relate to the hydrodynamic transport mechanisms affecting the composition of DIN and DON, but also depend on the biogeochemical processes and environmental factors. The *Phaeocystis globosa* bloom occurred nine times between 2008 and 2016; seven of which occurred between October and April. This suggests that the *P. globosa* bloom can commonly occur during the dry season. Research findings suggest that light intensity of $80\text{--}120 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and seawater temperature in the range of $20\text{--}25^\circ\text{C}$ are optimum for the growth conditions of *P. globosa* (Chen *et al.*, 2011). In this study, the intensity of light and seawater temperature ranged from $80\text{--}110 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (average, $90 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and $23.31\text{--}23.35^\circ\text{C}$ (average, 23.34°C), respectively in the MWS, in November 2017, which are comparable to the ranges reported by Chen *et al.* (2018). Therefore, it is likely that the transformation of DIN to DON may have occurred during the bloom period in the MWS.

In the past few years, previous studies have reported frequent occurrences of *P. globosa* red tide in the MWS during the dry season, which may be caused by the changes in nutrient concentrations and structure (Gong *et al.*, 2018). Hu *et al.* (2010) and Zhang *et al.* (2015) have demonstrated that DON can be preferentially utilized as nitrogen source and this may enhance the competition potential for the blooms forming species at lower DIN contents (Hu *et al.*, 2010; Zhang *et al.*, 2010). In this study, we found that DON accounted for the highest proportion of TDN pool ($\text{DON/TDN}=85.47\%$) in the MWS during the dry season. Thus, the high concentration of DON may be among the main factor leading to the frequent outbreak of *Phaeocystis globosa* red tide blooms in the MWS. The concentrations of Chl-*a* gradually de-

creased from the inner bay to the bay mouth as shown in Fig.3, consistent with the concentrations of P specifi and DSi. The decreasing concentrations of P specifi and DSi are mainly attributed to land-based discharge, especially from the MLJR, consistent with the previous reports by Zhang *et al.* (2019) and Yang *et al.* (2019) during the dry season. The average contributions of DIP, DOP, and PP respectively accounted for 54.58%, 30.31%, and 15.12% of TP (Figs.4 and 5), and DIP accounted for the largest proportion of TP pool. The derived C:N:P:Si ratio in the MWS during this study was 267: 31:1.00:7 (Fig.5a), with major deviations from the Redfield ratio (C:N:P:Si = 106:16:1:1). This implies that P is the limiting nutrient in the MWS during dry season. Both P and Chl-*a* exhibited similar gradually decreasing trend from the inner bay to the bay mouth, suggesting that the growth of phytoplankton in the study area is related to P limitation.

4.3 The Level of Eutrophication and Its Influencing Factors

Accurate assessment of the degree of eutrophication in the MWS is somewhat convoluted because of many required physical, chemical and bacteriological parameters for eutrophication assessment. DON and DOP were considered among the potential eutrophication factors in this study because they can respectively transform into DIN and DIP through biogeochemical processes and photochemistry. The eutrophication assessment method for the MWS was improved based on the findings of Primpas *et al.* (2010). Therefore, the variability of DON and DOP was added to properly assess the eutrophication level in the MWS. Because the degree of seawater eutrophication is difference in different coastal zones, the influencing factors are expected to be different (Kitsuou *et al.*, 2011). In the MWS, we found that NO₃-N, DON and DOP contributed to eutrophication and phytoplankton biomass (*Phaeocystaceae*). These nutrients provide an accurate description for eutrophication in the MWS. The PCA analysis of the eight variables (Section 2.3) was reduced to one principal component, which was used for the calibration of the index. The index was found to be efficient when tested on an independent set of data from the MWS during this study. Hence, the improved eutrophication index method was successfully applied to evaluate the eutrophication level of the MWS.

As shown in Fig.2, nutrient concentrations exceeded the seawater quality standards, a potential cause of eutrophication in the MWS. Based on the correlation matrix, PCA was carried out to understand the underlying relationships between nutrient concentrations and the level of eutrophication in all the investigated sites. The average EI was 0.953, which indicates that the overall water quality in the MWS is in a state of medium to poor eutrophication. The EI was higher in the inner bay sites such as the M1 (QJR), M2 (DLJR) and M3 (MLJR), and lower in the mouth of the MWS at stations M9-M12. The relationships between the EI and nutrient concentration are shown in Table 1. The significant positive correlation between

NO₃-N, DON and EI ($R^2=0.978$ and 0.522 , $P<0.01$, respectively) suggests that NO₃-N and DON were the most significant factors for EI. Because DIP is limiting in the MWS, the bioavailability of DOP can become an important DIP source for primary productivity (Bentzen *et al.*, 1992; Correll *et al.*, 1998). On the other hand, large amounts of lower molecular weight DON can be directly taken up and utilized by phytoplankton, which may result in algal bloom (Bronk *et al.*, 2007). Thus, the relatively high amount of DON in this study may result from oyster mariculture and surrounded natural mangrove reserve in the MWS, as well as large input of anthropogenic N load from the nearby farmlands and wastewater discharge. There was no relationship between NO₂-N, NH₄-N and EI, which may be related to the relatively low concentrations of NO₂-N and NH₄-N, and their little or no contribution to eutrophication status in the MWS, during this study. Hydrodynamic can also be another important factor influencing eutrophication (Schanz *et al.*, 2002). The tidal phase in the MWS is mainly diurnal, and the average velocity (95.68 cm s^{-1}) during the ebb and flood tides is the largest in the coast of Guangxi (He *et al.*, 2004). Hydrodynamic in the MWS is also one of the drivers of the freshwater discharge from the QJR and the MLJR (Yang *et al.*, 2014). The mouth of the MWS is characterized by strong currents, which enhances pollutant transport. The relationship between DIN, P, DSi and salinity underlines that the quality of water in the MWS is not only influenced by nutrient load because of anthropogenic activities, but also by hydrodynamic conditions.

5 Conclusions

In the present study, the compositions and spatiotemporal distributions of different species of nitrogen and phosphorus were investigated in the MWS. An improved eutrophication index method was also developed to assess the eutrophication status of the MWS. The following results were obtained. 1) Decreasing trends in the concentrations of almost all the studied nutrients species from the inner bay to the bay mouth indicates that the MWS is largely influenced by nutrients from land-based sources. 2) Dissolved organic nitrogen (DON) and inorganic phosphorus (DIP) were the main component of total nitrogen (TN) and phosphorus (TP), and averagely accounted for 65.32% and 54.58% of TN and TP, respectively. 3) The improved eutrophication index applied in this study corrects the perceived deficiencies in eutrophication assessment, and may be adopted for effectively evaluation of the eutrophication status of other coastal water bodies. 4) Based on the improved eutrophication index (EI), the water quality of the MWS is within the moderate to poor eutrophication status. The eutrophication level decreases from the inner bay to the bay mouth. Therefore, management efforts to reduce nutrient (especially N) load into the MWS is encouraged to maintain its water quality and safeguard its ecosystem services and functions.

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