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Using algal biomass to evaluate numeric nutrient criteria in an estuary: A case study of Daliaohe Estuary in China

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ABSTRACT

This paper presents an initial inquiry into model-based numeric nitrogen and phosphorus criteria for estuaries based on stressor-response models between nutrients and algal biomass (Chl-*a* concentration) and further studies the effect of salinity (*S*) on nutrient criteria. Field data collection and associated indoor modeling were conducted in Daliaohe Estuary. Dissolved inorganic nitrogen (DIN), total nitrogen (TN), phosphate (PO_4^{3-}) and total phosphorus (TP) eco-criteria of Daliaohe Estuary in China were calculated at salinities of 15, 25 and 31. The results suggested that the variation in *S* had significant influence on the DIN and TN eco-criteria and no obvious effect on the PO_4^{3-} and TP eco-criteria. The values of DIN and TN eco-criteria increased with either higher or lower salinity concentrations, and were strongly linked to the effects of salinity on algal nutrient absorption and utilization, growth, metabolism, and osmotic pressure.

1. Introduction

Estuaries located in the confluence of rivers and oceans present a dynamic environment for phytoplankton, with large changes in salinity. It is universally acknowledged that salinity is an important ecological factor affecting the growth and reproduction of phytoplankton. Anthropogenic eutrophication of estuaries and adjacent coastal waters, largely a result of nitrogen and phosphorus pollution, is becoming increasingly serious, leading to the frequent occurrence of nuisance or undesirable harmful algae blooms and destroying the eco-environment and the integrity of the ecosystem (Bricker et al., 1999; Elliott and De Jonge, 2002; Paerl, 2006; Wang, 2006; Whitall et al., 2007; Anderson et al., 2008; Bricker et al., 2008; Howarth et al., 2011). Even more concerning, a majority of the estuaries and adjacent seas in China are under threat of serious eutrophication (Huang et al., 2003; Liu et al., 2013; Wang et al., 2013; Li et al., 2014; Strokal et al., 2014) without available and appropriate standards that are consistent across a wide variety of estuarine environments. Therefore, it is necessary and urgent to develop numeric nutrient criteria, which not only are the scientific basis of nutrient monitoring, evaluation and management but also can provide theories and methods for the establishment of corresponding standards (Wu et al., 2010).

Nutrient criteria in estuarine waters are defined as the acceptable maximum dose or concentration of the nutritional parameters that will not produce toxic or harmful effects on the estuarine environment (US EPA, 1998; Meng et al., 2006). The development and application of nutrient criteria is needed to effectively protect estuaries from eutrophication. The United States has systematically studied the theoretical approaches to nutrient criteria for rivers, lakes, estuaries, coastal waters and wetlands (US EPA, 2000a,b,c, 2001, 2008, 2010). Three types of scientifically defensible empirical approaches for setting numeric criteria were recommended: reference condition approaches, mechanistic modeling and stressor-response analysis (Dodds and Oakes, 2004; US EPA, 2000a,b,c, 2010; Heatherly, 2014). Similarly, the European Union has developed strategies to control cultural eutrophication of shared waters (European Communities, 2003; Pardo et al., 2012). On the basis of these reliable and advanced approaches, some researchers in China have attempted to develop estuarine numeric nutrient criteria, conforming to water quality characteristics and eco-environment parameters. For example, a population distribution approach was used to determine reference states and establish numeric nutrient criteria in estuaries and adjacent waters (Hu et al., 2011; Zheng et al., 2013a,b). Compared with the methods for developing nutrient criteria for estuaries, few studies have focused on

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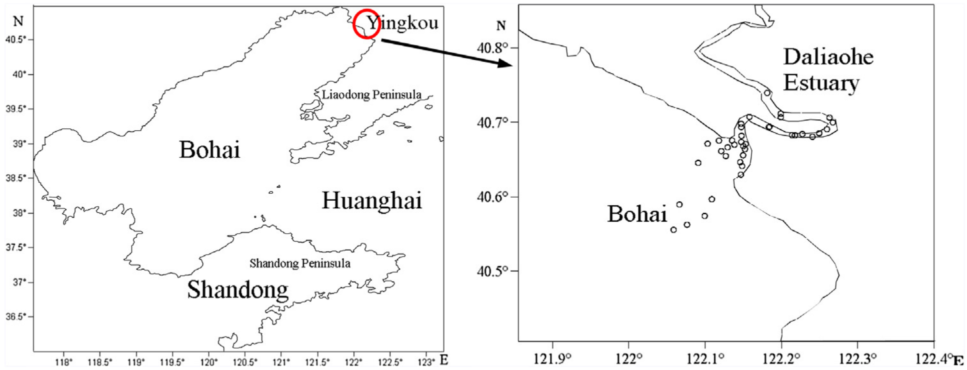

Fig. 1. Study area and location of sampling stations in the Daliaohe Estuary and adjacent Bohai for field cruises in the period 2009–2013.

the factors influencing nutrient criteria, which play a crucial role in developing more accurate nutrient criteria.

Chlorophyll *a* (Chl-*a*) concentrations, which are strongly correlated with algal biomass, serve as a commonly used response variable for nutrient criteria because algae sensitively and directly respond to nutrient variations (Porter et al., 2008; Royer et al., 2008; Black et al., 2011; Smucker et al., 2013). Thus, the Chl-*a* concentration is regarded as a response variable. Based on the stressor-response relationship model of algal biomass and nitrogen and phosphorus (US EPA, 2010; Huo et al., 2013; Smith et al., 2013; Zhang et al., 2014), the objectives of this study were: (1) to develop an indoor simulation method of calculating nutrient criteria and (2) to apply this method to study the effects of salinity (*S*) on the numeric nutrient criteria.

2. Materials and methods

2.1. Study area, samples and methods

Daliaohe Estuary is located in the north of Liaodong Bay and has a length of 94 km. Its average runoff is approximately 7.715×10^9 m^3 per year, accounting for approximately 55.32% of Liaodong Bay runoff into the Bohai Sea. Daliaohe Estuary is a middle tidal estuary with an average tidal range of 2.71 m and a maximum of 4.43 m. The Daliaohe River flows through many industrial towns, such as Shenyang, Liaoyang and Yingkou, receiving a large number of pollutants that mainly stem from the modernizations of industry, agriculture and urbanization; these pollutants contribute to serious pollution of the Daliaohe Estuary. As a result of elevated nutrient levels, Daliaohe Estuary is now very eutrophic, resulting in severe damage to the marine resources and environment (Wang et al., 2013).

Five field surveys (Fig. 1) were conducted in Daliaohe Estuary: in July 2009, in April, July and November 2010, and in April 2013. Water samples were collected and immediately filtered through a pre-cleaned 0.45-μm pore-sized cellulose acetate membrane, placed in acid-cleaned polyethylene bottles and immediately frozen. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+), nitrite (NO_2^-) and phosphate (PO_4^{3-}) were determined by a Quaatro Continuous-Flow Analyzer with detection limits of 0.02, 0.03, 0.02 and 0.01 μmol l^{-1}, respectively. The dissolved inorganic nitrogen (DIN) concentration was calculated as the sum of the concentrations of NO_3^-, NH_4^+ and NO_2^-. Total nitrogen (TN), total phosphorus (TP), dissolved total nitrogen (DTN) and dissolved total phosphorus (DTP) were measured by wet oxidation in acid persulfate with an analytical precision of 0.68 μmol l^{-1} for TN, 0.02 μmol l^{-1} for TP, 0.68 μmol l^{-1} for DTN and 0.02 μmol l^{-1} for DTP (Grasshoff et al., 1999).

Table 1
Concentrations of nitrogen and phosphorus in the experiment (μmol l^{-1}).

DIN	P groups								
180	0	0.2	0.5	1	1.5	2	2.5	3	4	5
PO_4^{3-}	N groups									
2.3	0	10	20	30	40	50	60	80	100	200

2.2. Diatoms source and culture

Skeletonema costatum, *Chaetoceros curvisetus* and *Ditylum brightwellii* were used as experimental subjects and are the dominant diatom species in Daliaohe Estuary. These algae species were obtained from the Ecotoxicity Lab at the Ocean University of China.

Firstly, the diatoms were cultured with natural seawater from the Bohai Sea that was filtered through a 0.45-μm cellulose acetate membrane. Secondly, the conical flasks were soaked in dish detergent, scrubbed with a bristled brush, rinsed in tap water, soaked with concentrated sulfuric acid, rinsed in tap water at least ten times, and finally sealed with tin foil and autoclaved at 120 °C for 20 min. Then, the diatom strains that were cultured in an illumination incubator were inoculated into 500 ml flasks containing 250 ml of f/2 medium (Guillard and Ryther, 1962) without nitrogen and phosphorus and grown at 23 ± 1 °C with a light intensity of 60 μmol m^{-2} s^{-1} and a 12:12 light/dark cycle. Ten different nutrient concentrations were tested in the experiment (Table 1), with triplicate parallel samples in each group. Background values of DIN and PO_4^{3-} in the seawater were 23.5 μmol l^{-1} and 0.23 μmol l^{-1}, respectively.

The values of 180 μmol l^{-1} for DIN and 2.3 μmol l^{-1} for PO_4^{3-} were derived using a frequency distribution curve obtained from field investigation data from Daliaohe Estuary.

2.3. S, Chl-a and data analyses

S, measured with portable multi-parameter meter, was adjusted with the addition of distilled water or NaCl. A 20 ml sample of the diatom suspension was collected and immediately filtered through a 47-mm GF/F membrane. The membranes were wrapped in tinfoil, placed into storage capsules, and then stored at −20 °C until further analysis. Chl-*a* concentrations were extracted from the membranes using 90% acetone, and samples were then stored in the dark at 4 °C for 24 h. Finally, the samples were centrifuged for 10 min at 4000 r/min, and Chl-*a* measurements were completed using a UV–vis spectrophotometer at wavelengths 630 nm, 647 nm, 664 nm and 750 nm. Absorbance at 750 nm was used to correct for