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Production and uptake of dissolved carbon, nitrogen and phosphorus in overlying water of aquaculture shrimp ponds in subtropical estuaries, China

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Abstract

Water quality deterioration can adversely affect the long-term sustainability of aquaculture industry. Understanding the processes of nutrient regeneration and uptake is important for improving water quality and the overall ecosystem health of aquaculture systems. In spite of the importance of dissolved nutrients (DOC, DIC, $\mathrm{N\text{-}NO_x^{-}}$, $\mathrm{N\text{-}NH_4^{+}}$, and $\mathrm{P\text{-}PO_4^{3-}}$) in governing water quality and ecosystem functioning, the spatiotemporal variations in the production and uptake of dissolved nutrients in aquaculture ponds is still poorly understood. In this study, the nutrient production and uptake rates in the overlying water were quantified among different shrimp growth stages in the aquaculture ponds in the Min River Estuary (MRE) and Jiulong River Estuary (JRE), southeast China. Significant differences in the nutrient production and uptake rates in the overlying water were observed among the three growth stages and two estuaries. The temporal variations of DOC and DIC production rates in both estuarine ponds closely followed the seasonal cycle of temperature, while the difference in DOC and DIC production rates between the two estuaries was likely caused by differences in water salinity. The changes in the production and uptake rates of dissolved inorganic nitrogen ($\mathrm{N\text{-}NO_x^{-}}$ and $\mathrm{N\text{-}NH_4^{+}}$) and $\mathrm{P\text{-}PO_4^{3-}}$ in the water column over time were partly related to the interactions between thermal conditions and phytoplankton biomass (e.g., chlorophyll *a* concentrations) in the ponds. Our results demonstrate the complex dynamics and environmental risk of dissolved nutrients in subtropical shrimp ponds, and call for a more effective management of nutrient-laden wastewater in safeguarding the long-term sustainability of aquaculture production.

Keywords Nutrients · Uptake · Production · Overlying water · Aquaculture pond · Subtropical estuary

Nomenclature table

Nomenclature	Abbreviations	Nomenclature	Abbreviations
Min River Estuary	MRE	DOC production rates in the overlying water	$\mathrm{DOC}_{\_\mathrm{prod}}$
Jiulong River Estuary	JRE	DIC production rates in the overlying water	$\mathrm{DIC}_{\_\mathrm{prod}}$
Litopenaeus vannamei	*L. vannamei*	Dissolved organic carbon	DOC
Metric tons	Mt	Dissolved inorganic carbon	DIC
Nitrite	$\mathrm{N\text{-}NO_2^{-}}$	Chlorophyll *a*	Chl-*a*
Nitrate	$\mathrm{N\text{-}NO_3^{-}}$	Dissolved oxygen	DO
Ammonium	$\mathrm{N\text{-}NH_4^{+}}$	Dissolved organic matter	DOM
Phosphate	$\mathrm{P\text{-}PO_4^{3-}}$		

Introduction

As a result of rising living standards, the demand for protein-rich seafood has been growing rapidly in recent years. The production of shrimps, as one of the most favoured seafood, has been on an increasing trend around the world. The global total production of shrimp was about 2.1 million tonnes in the year 2015 (FAO 2016), with prawn (*Litopenaeus vannamei*) being a highly popular aquaculture product in the Asia-Pacific region. Intensive shrimp aquaculture has been considered to be important in sustaining a steady aquaculture production (FAO 2016; Molnar et al. 2013; Silva et al. 2013). The successful operation of intensive shrimp aquaculture ponds depends on active human management activities, such as the stocking of fries, daily supply of feeds, and others (Chen et al. 2016; Yang et al. 2018). These pond systems receive a large quantity of nutrients from residual feeds and shrimp excreta (Chen et al. 2016), which can subsequently lead to water quality deterioration and harmful algae blooms once nutrient levels in the water column exceed certain thresholds (Huang et al. 2016; Yang et al. 2017). Hence, a thorough understanding of the nutrient dynamics in pond water is critical for assessing the trophic status, primary productivity, yield, and environmental risks of intensive aquaculture shrimp ponds.

The variations of nutrient concentrations in aquatic systems are closely related to the processes of nutrient production and uptake. The production of nutrients in aquatic systems can contribute to a negative feedback (e.g., water quality deterioration, toxics) on the environment through releasing nutrients (e.g., $N\text{-}NO_x^-$, $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$) into the water column (Holmer et al. 2015). In contrast, nutrient uptake can potentially reduce the ambient nutrient concentrations in water and thereby create a positive feedback on the ecosystem (Holmer et al. 2015). Nutrient regeneration and uptake in natural aquatic ecosystems (e.g. lake, river, and ocean) have received great attention in recent studies (e.g., Bronk et al. 2014; Fernández et al. 2009; Fields et al. 2014; Finkler et al. 2018; McCarthy et al. 2016). Researchers have found that the variations in nutrient regeneration and uptake are associated with phytoplankton biomass (e.g., Fellows et al. 2006; Hall et al. 2003; Hargreaves 1998), hydrology and hydrogeomorphology (e.g., Fields et al. 2014; Gücker and Pusch 2006), carbon availability (e.g., Ensign and Doyle 2006; Yang et al. 2008), and nutrient limitation and availability (e.g., Finkler et al. 2018). Nutrient regeneration and uptake have also been measured in long-line mussel farms and pearl oyster culture systems (e.g., Carlsson et al. 2012;Christensen et al. 2003; Holmer et al. 2015; Jansen et al. 2012; Lacoste and Gaertner-Mazouni 2016; Srisunont and Babel 2015; van Broekhoven et al. 2014). These studies were based on measurement of bivalve (and associated fauna) excretion providing regenerated nutrient to the system. In this kind of studies, empty chambers are used as control and fluxes are generally negligible (e.g. Richard et al. 2007). Studies measured nutrient process using tracers (e.g., N^{15} isotopes) or addition of nutrients such degradation occurs because sediment accumulate along aquaculture ropes and between bivalves so microbial process take place in the suspended sediment compartment of water. However, the data on nutrient production and uptake of aquaculture ponds is still scarce. The biogeochemical cycling of nutrients in aquaculture systems generally involve multiple processes, e.g. nutrient fluxes across the sediment-water interface (SWI), nutrient regeneration and uptake in the water column, nutrient release through animal excretion, etc. However, the potential contribution of nutrient regeneration and uptake in the water column to the biogeochemical cycling of nutrients from the aquaculture systems remains poorly documented.

As an indispensable part of the global aquaculture system, aquaculture ponds are widely distributed in the temperate and tropical regions, in particular in the developing countries. These ponds are generally semi-artificial ecosystems maintenance supported by daily feed supply to culture aquatic animals (Aiméa et al. 2018; Chen et al. 2016; Yang et al. 2018). Consequently, the pond water nutrient dynamics is crucial to the long-term sustainability of the aquaculture industry. While Yang et al. (2017) have estimated the N-NO_x^-, N-NH_4^+ and P-PO_4^{3-} fluxes across the sediment-water interface in the estuarine aquaculture ponds, the process of nutrient production in the water column of aquaculture ponds, particularly in China with the world's largest aquaculture area, is still largely unknown. Moreover, the spatiotemporal variability of the rates of production or uptake of dissolved nutrients in aquaculture ponds is poorly characterized, which could lead to considerable biases in estimating the nutrient fluxes in this ecosystem. Understanding the dynamics of nutrient regeneration and uptake in aquaculture ponds is thus essential for promoting the sustainable development of the aquaculture industry.

China, as the world's leading aquaculture producer, had a total cultivation area of 58,579 km^2 (Verdegem and Bosma 2009) and annual production of 29.4 million metric tons (Mt) in 2015 (Fisheries Department of Agriculture Ministry of China 2015). Shrimp pond is one of the major types of aquaculture ponds in China, with a wide distribution along the coastal regions (Yang et al. 2017). This study aims to investigate the nutrient dynamics of shrimp ponds located in two different estuaries in Fujian Province, southeast China. The specific objectives of this study are to: (1) quantify the production and uptake rates of dissolved nutrients among three different growth stages of shrimps; (2) compare the production and uptake rates of dissolved nutrients between two different estuaries; (3) identify the factors influencing the production and uptake of dissolved nutrients in pond water.

Materials and methods

Study sites

The study was conducted in two subtropical estuaries, namely Min River Estuary (MRE) and Jiulong River Estuary (JRE), situated in southeast China (Fig. 1). The Min River Estuary has a typical subtropical monsoonal climate with relatively warm and wet weather. The multi-year average temperature and precipitation are 19.6°C and 1,350 mm, respectively (Tong et al. 2010). The Jiulong River Estuary has a subtropical oceanic climate with multi-year mean air temperature of 21.0°C and annual rainfall of 1,371 mm (Wang et al. 2016). Both estuaries receive significant amount of southeast monsoonal rain between May and September. The two estuaries have semi-diurnal tides with an average range of 4.5 m and 4.0 m in the MRE and JRE, respectively. The wetland soil surface is submerged for 7 h over a 24 h cycle. The catchment areas of the Min River and Jiulong River are 60,092 and 14,741 km^2, respectively, with annual discharge of 58.6×10^9 and 12.4×10^9 m^3 y^{-1}, respectively (Zhou et al. 2016). The mean salinity of tidal water at MRE is 5.1±2.6‰, which is significantly lower than that in JRE (23.6±3.2‰). The shrimp ponds, being one of the dominant landscape features in these estuarine zone, were mostly generated by the complete removal of marsh vegetation.

Shrimp pond system and management

Aquaculture production in the majority of the shrimp ponds occurs between June and November (Yang et al. 2017a), with only one crop of shrimps being produced per year. Prior to shrimp production, these ponds are filled with estuarine water from an adjacent estuary using a submerged pump. The water is first filtered through a 2 mm mesh bag in order to prevent the entry of predators and competitors (Guerrero-Galván et al. 1999; Yang et al. 2017a). Additional input of freshwater into the ponds takes place occasionally during rainfall events. There is no water exchange with the spillways of the ponds during the farming period. The water depths in the shrimp ponds at the MRE and JRE range between 1.1 -1.5 m, and 1.3-1.8 m, respectively, with the average values of 1.3 m and 1.5 m, respectively.

To assess the production and uptake processes of dissolved nutrients in the overlying water during the culture period, water samples were collected from three replicate commercial shrimp ponds in the Shanyutan Wetland of the MRE and Humao Island of the JRE (Fig. 1). The basic details about the selected shrimp ponds can be found in Table S1 in the Supporting Information. The shrimp production cycle began on May 15, 2015, and lasted for about 163 days. *Litopenaeus vannamei* were fed with artificial feeds containing 42% of crude protein (Yuehai^{TM}, Guangzhou, China) twice per day at 07:00 and 16:00 (local standard time), respectively, by direct application from a small boat. The shrimp grow-out cycle was divided into three stages (initial, middle, and final stages) according to the management practices (e.g. feeding rate, water depth, etc.), water salinity, and shrimp weight (Table S2), which was similar to the classification scheme adopted by Páez-Osuna et al. (1997) The feeding rates were maintained at approximately 10–16, 50–55, and 40–45 kg ha^{-1} d^{-1} during the initial, middle, and final stages, respectively. The amount of feed added was determined according to the response of shrimps to the previous feeding. At each pond, three to five 1,500-W paddlewheel aerators were activated four times per day at 07:00–09:00, 12:00–14:00, 18:00–20:00, and 00:00–03:00 (local standard time). Pond water was completely drained and surface sediment (0–10 cm) was removed after shrimp harvest.

Collection and analysis of water samples

Taking into account the shrimp grow-out cycle, the field sampling campaigns were carried out in the middle of June, August, and October of 2015 to represent the three stages. Three replicate sites were chosen in each pond for the sampling of overlying water (approximately 5-10 cm above the sediment surface). Overlying water was collected during each sampling campaign using a 5 L Niskin bottle, and then immediately transported to the laboratory for the incubation experiment and the measurement of chlorophyll *a* (chl-*a*) concentrations (Zhang et al. 2013). Chl-*a* concentrations in the water samples were determined using a UV-visible spectrophotometer (Shimadzu UV-2450, Japan) following the methods of Jeffrey and Humphrey (1975) and Yang et al. (2017a). Physico-chemical properties of the overlying water, including temperature, pH, salinity, and dissolved oxygen (DO), were measured *in situ* simultaneously. The temperature and pH were determined using a portable pH/mV/Temp system (IQ150, IQ Scientific Instruments, USA). Salinity and DO were determined using a salinity meter (Eutech Instruments-Salt6, USA) and a multi-parameter water quality meter (HORIBA, Japan), respectively. All the instruments were calibrated in the laboratory before each field campaign according to the instruction manuals.

Laboratory incubation and analysis of nutrient fluxes

Triplicate overlying water samples were retrieved from each pond for use in an incubation experiment to examine the production and uptake of nutrients including dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), N-NO_x^-(N-NO_2^-+N-NO_3^-), N-NH_4^+ and P-PO_4^{3-}. The rates of nutrient production and uptake in the overlying water were determined using an *ex situ* incubation. The incubation device (Fig. S1, Supporting Information) was designed following Chen et al. (2014), Cowan and Boynton (1996), and Xiong et al. (2017). The incubation chambers were constructed using transparent Plexiglas with an internal diameter of 6.0 cm and a height of 30 cm. In the laboratory, overlying water samples were filtered through a 0.45 μm cellulose acetate filter and transferred into the incubation chambers, while the cores were sealed using a Teflon plunger with inlet and outlet tubes. Before the incubation, the DO concentration in the overlying water was adjusted to achieve the *in situ* level. We monitored the DO level and introduced air into the water column occasionally to replenish the oxygen consumed, as well as providing mixing of the water column. The chambers were incubated in light in a temperature-regulated incubator (QHZ-98A, China) for 9 h to simulate the typical conditions experienced during the daytime period. The incubation temperature was selected according to the *in situ* temperature (MRE: 22.5 (June), 28.5 (August), and 22.5 (October); JRE: 25.5 (June), 29.0 (August), and 26.5 (October)). We recognize that this *ex situ* incubation would only take into account the biogeochemical processes that take place within the water column, while under actual field conditions, nutrient dynamics in pond water might also be affected by sediment-water interactions as well as faunal activities.

Approximately 60 mL of overlying water was withdrawn from each incubation chamber using a 100 mL plastic syringe at the beginning and the end of the 9-hour incubation period. The water samples were subsequently transferred to 60 mL polyethylene bottles, and injected with about 0.2 mL of saturated $HgCl_2$ solution to inhibit microbial activities (Taipale and Sonninen 2009; Zhang et al. 2013). Approximately 30 mL of water samples were then filtered through a 0.45 μm cellulose acetate filter (Biotrans™ nylon membranes), and the filtrates were stored in 30 mL polyethylene bottles before the analysis of $N\text{-}NO_x^-$ ($N\text{-}NO_2^-$+$N\text{-}NO_3^-$), $N\text{-}NH_4^+$, and $P\text{-}PO_4^{3-}$ concentrations by a flow injection analyser (Skalar Analytical SAN^{++}, Netherlands). The detection limits for $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, and $P\text{-}PO_4^{3-}$ were 0.02, 0.1, 0.1, and 0.05 μmol L^{-1}, respectively. The relative standard deviations of all analyses were in the range of 0.1-4.0%. The remaining water samples were also filtered immediately through a 0.45 μm cellulose acetate filter (Biotrans™ nylon membranes) that was pre-combusted at 450 °C for 4 h, and the filtrates were then stored in 30 mL pre-combusted glass bottles before the analysis of DOC and DIC concentrations by a total organic carbon analyzer (TOC-VCPH/CPN, Shimadzu, Japan). The detection limits for DOC and DIC were both 0.4 μg L^{-1}. The relative standard deviations of all analyses were in the range of 0.5-3.0%. All samples were stored at 4°C in the dark until further analysis.

The production and uptake rates of dissolved nutrients in the overlying water were determined as follows (Cheng et al. 2015; Yang et al. 2017a):

$$F = (C_{W\text{-}E} - C_{W\text{-}B}) \times V / S / T / H$$

where F (mg m^{-3} h^{-1}) is the rate of nutrient production and uptake in the overlying water (positive and negative values indicate a net production and a net uptake, respectively, by the overlying water), $C_{W\text{-}E}$ and $C_{W\text{-}B}$ are the nutrient concentrations (mg L^{-1}) in the overlying water at the end and the beginning of incubation, respectively, V is the volume of overlying water (L), S is the cross-sectional area of the incubation tube (m^2), T is the incubation time (h) and H is the height of the incubation tube (m).

Statistical analyses

Two-way analysis of variance (ANOVA) was conducted to explore whether aquaculture stages, sampling estuaries or their interaction have fixed effects on environmental variables, the production and uptake of dissolved carbon, nitrogen and phosphorus in overlying water of aquaculture shrimp ponds. Pearson correlation analyses were conducted to examine the relationships between various environmental variables and the production/uptake rates of dissolved nutrients. A stepwise regression analysis was used to identify the major environmental factors governing the temporal variations of dissolved carbon, nitrogen and phosphorus production rates in the overlying water of different estuarine ponds. All statistical analyses were performed using the SPSS statistical software package (SPSS v 17.0, Inc., USA), and were considered significant at the 0.05 significance level. All statistical plots were generated using OriginPro 7.5 (OriginLab Corp. USA).

Results and discussions

Overlying water characteristics

Table 1 shows the physio-chemical and biological properties of overlying water in the shrimp ponds during the farming period. The temporal patterns of overlying water properties were similar between the two estuaries. Both temperature and Chl-*a* concentrations in the overlying water decreased in the order of middle stage > final stage > initial stage (Table 1). The overlying water pH varied significantly among the three growth stages (p<0.05), with considerably lower values observed during the middle stage (Table 1). DO concentrations in the water column showed an increasing trend over time (Table 1), whereas salinity exhibited an opposite trend (Table 1). There were significant differences in DO concentration and salinity among the three stages (p<0.05).

Large variations in overlying water characteristics were also found between the two estuaries. Water temperature and salinity at the JRE ponds were significantly higher than those at the MRE ponds over the study period (p<0.01; Table 1). Mean DO concentration at the JRE ponds was significantly lower than that at the MRE ponds during the middle and final stages (p<0.001; Table 1), while no significant differences were detected during the initial stage (p>0.05; Table 1). Overlying water Chl-*a* concentrations at the JRE ponds were significantly lower than those at the MRE ponds across all three growth stages (p<0.001; Table 1). Significant difference in overlying water pH between the two estuaries was recorded during the initial and final stages (p>0.05; Table 1).

Production of DOC and DIC in the overlying water

During the production cycle, aquaculture ponds receive daily feed supply and subsequently retain a large amount of organic matter from residual feeds and feces (Chen et al. 2016). Therefore, dissolved / particulate organic matter decomposition in the aerobic water column might be an important contributor to the internal DOC and DIC production ($DOC_{\_prod}$ and $DIC_{\_prod}$) in the shrimp ponds. The results of our laboratory incubation experiment on the DOC and DIC production rates in the overlying water are presented in Fig. 2. The mean $DOC_{\_prod}$ rates in the MRE and JRE ponds across the three shrimp growth stages were 277.79±67.51 mg m^{-3} h^{-1} (range: 162.93-396.64) and 248.08±34.15 mg m^{-3} h^{-1} (range: 182.11-296.35) (Fig. 2a). The mean $DIC_{\_prod}$ rates in the MRE and JRE ponds were 552.95±202.08 mg m^{-3} h^{-1} (range: 186.99-884.50) and 316.89±73.18 mg m^{-3} h^{-1} (range: 186.52-439.66), respectively. The mean $DIC_{\_prod}$ rate was significantly higher than that of $DOC_{\_prod}$ (p<0.05), showing that inorganic carbon species was the major products of dissolved organic matter (DOM) decomposition in the water column of shrimp ponds. A clear temporal pattern of $DOC_{\_prod}$ and $DIC_{\_prod}$ rates was observed across the three shrimp growth stages in all ponds in the descending order of middle stage > final stage > initial stage (Fig. 2 and Table 2). The temporal variations in $DOC_{\_prod}$ and $DIC_{\_prod}$ rates were, to a large extent, dependent on the interactions between thermal conditions and other environmental factors (such as organic matter, salinity, pH and DO) (Table 3 and Table 4). Firstly, the significant and positive relationships observed between water temperature, and the rates of $DOC_{\_prod}$ and $DIC_{\_prod}$ ($p<0.01$; Table 3) suggested that temperature was likely an important driver of the temporal changes in the rates of $DOC_{\_prod}$ and $DIC_{\_prod}$. An increase in temperature could stimulate the microbial activity in the water column, and subsequently accelerate the decomposition of organic matter (Pinho et al. 2016; Porcal et al. 2015) and the production of DOC and DIC during the middle stage. Secondly, changes in DO concentrations might alter the activity of microorganisms in the water column, which in turn affect the temporal dynamics of $DOC_{\_prod}$ and $DIC_{\_prod}$ rates in the MRE ponds, as supported by the significant correlations observed between DO concentrations and the rates of $DOC_{\_prod}$, $DIC_{\_prod}$ (Table 3). Previous studies have also suggested that microbial mineralization of organic matter under aerobic conditions in the water column plays a key role in governing aquatic carbon cycling (Cory et al. 2014; Wang et al. 2017; Weyhenmeyer et al. 2015; Yang et al. 2015),

Salinity is also an important driver of ecosystem processes in coastal wetlands (Neubauer et al. 2013; Tong et al. 2017). The negative relationships observed between water salinity and both $DOC_{\_prod}$ and $DIC_{\_prod}$ rates in the current work (Table 3) were consistent with the results reported in several previous studies in tidal wetlands (Craft 2007; Morrissey et al. 2014; Neubauer 2012; Roache et al. 2006). Under increasing salinity, the activity of soil microorganisms and enzymes will be suppressed, resulting in an overall decrease in the rate of microbial decomposition of organic carbon (Chambers et al. 2013; Morrissey et al. 2014; Neubauer 2012). Thus, the significantly lower $DOC_{\_prod}$ and $DIC_{\_prod}$ rates observed during the initial stage of shrimp growth in this study might be partly related to the significantly higher water salinity, which exerted a negative effect on microbial decomposition of organic matter in the water column. We also found significant differences in $DOC_{\_prod}$ and $DIC_{\_prod}$ rates between the two estuaries (Table 2), except for $DOC_{\_prod}$ in the final stage and $DIC_{\_prod}$ in the initial stage (Fig. 2). Over the whole study period, the mean values of $DOC_{\_prod}$ and $DIC_{\_prod}$ were higher in MRE than JRE ponds ($p<0.001$), which might be related to the significantly lower average salinity, and higher DO and Chl-*a* concentrations (Table 1) in the former that promoted organic matter decomposition .

Production and uptake of dissolved inorganic nitrogen in the overlying water

The results of our laboratory incubation experiment on the production and uptake rates of nitrogen ($N\text{-}NO_x^-$ and $N\text{-}NH_4^+$) in the overlying water of shrimp ponds are shown in Fig. 3. The average rates of $N\text{-}NO_x^-$ production and $N\text{-}NH_4^+$ uptake in MRE ponds were 2.16±0.44 mg m^{-3} h^{-1} (range: 1.40-2.92) and 26.65±10.69 mg m^{-3} h^{-1} (range:

10.83-47.03). In contrast, the JRE ponds showed a net uptake of N-NO_x^- but a net production of N-NH_4^+, with mean rates of 1.07±0.31 mg m^{-3} h^{-1} (range: 0.54-1.63) and 27.31±12.05 mg m^{-3} h^{-1} (range: 14.02-51.37). The rates of production and uptake of dissolved inorganic nitrogen varied greatly between the two estuaries (Table 5), and exhibited a clear seasonal trend with generally higher values in the middle stage (Fig. 3a and Fig. 3b).

Dissolved organic matter (DOM) and particulate organic matter (POM) is widely existed in almost every species of water bodies, which are rich in biogenic element (e.g., carbon, nitrogen and phosphorus) (e.g., Simon et al. 2002; Yao et al. 2014; Zhu et al. 2019). DOM and POM in the aquatic systems (e.g., lake) is usually derived from the allochthonous input and autochthonous biological degradation (e.g., phytoplankton degradation) (e.g., Simon et al. 2002; Yao et al. 2011, 2014; Zhang et al. 2009; Zhu et al. 2019). The degradation of (DOM) and POM is an important link in the biogeochemical process of nutrients regeneration (Yao et al. 2014). In general, a large supply of DOM and POM provides a reliable source of substrates for the microbial mineralization of nitrogen in water. The middle stage is considered to be the main period for boosting shrimp growth (Yang et al. 2017), during which a large amount of organic matter is introduced through feed inputs and shrimp metabolism, and subsequently dissolved in water (Zhu et al. 2019). The combination of high water temperature and large supply of DOM during the middle stage likely contributed to the higher rates of N-NO_x^- and N-NH_4^+ production observed in the MRE and JRE ponds, respectively, as compared to other stages, as supported by the significant, positive relationships observed between water temperature and N-NO_x^- (MRE ponds) or N-NH_4^+ (JRE ponds) ($p<0.05$, Tables 4 and 6). More detailed investigations of the DOM and POM in the two estuaries during the culture period are further needed to confirm the above hypothesis.

We observed a net uptake of N-NH_4^+ and N-NO_x^- in the MRE (Fig. 3a) and JRE ponds (Fig. 3b), respectively, which were probably related to the phytoplankton dynamics (e.g., community composition and biomass). Different phytoplankton species have different physiological abilities to use a given nutrient source (Cunha et al. 2017; Glibert et al. 2006). Blomqvist et al. (1994) found that cyanobacteria and diatoms prefer N-NH_4^+ and N-NO_x^-, respectively, as a nitrogen source. In general, cyanobacteria are widely distributed in freshwater or low salinity aquatic ecosystems (Paerl et al. 2001), while diatoms live mostly in oceans and other saltwater environments (Armbrust 2009). Due to a lower amount of freshwater input, the annual mean salinity in Jiulong River Estuary was ~5 times higher than that in Min River Estuary, which in turn led to a significantly higher salinity in the JRE than MRE ponds (6.1–15.1‰ vs. 1.9–3.4‰) throughout the study period (Yang et al. 2018). Therefore, the net uptake of N-NO_x^- observed in the JRE ponds might be related to the preferred use of N-NO_3^- by diatoms, while the net uptake of N-NH_4^+ observed in the MRE ponds could be linked to the preferred utilization of N-NH_4^+ as a nitrogen source by cyanobacteria. More detailed investigations of the phytoplankton community in the two estuaries are further needed to confirm the above hypothesis. On the other hand, the strong temporal variability observed in the net uptake rates of N-NH_4^+ and N-NO_x^- in the estuarine ponds might be related to phytoplankton activities. The significant and positive relationships found between Chl-*a* concentrations and N-NH_4^+/N-NO_x^- uptake rates in the estuarine ponds (p<0.01; Tables 4 and 6) suggested that an increase in phytoplankton biomass could enhance the rate of N-NH_4^+ and N-NO_x^- uptake from the water column, which was consistent with the findings in some tropical reservoirs (e.g., Cunha et al. 2017).

Uptake of dissolved P-PO_4^{3-} in the overlying water

The mean uptake rate of P-PO_4^{3-} in the MRE and JRE ponds over the study period were 3.27±0.72 mg m^{-3} h^{-1} (range: 2.13-4.60) and 2.18±0.55 mg m^{-3} h^{-1} (range: 1.38-3.24) (Fig. 3c). The P-PO_4^{3-} uptake rates in the shrimp ponds of both estuaries varied greatly among the three growth stages, which decreased in the order of middle stage > final stage > initial stage (p<0.001; Fig. 3c). Phosphorus uptake in the aquatic systems is driven mostly by the interactions between biotic and abiotic process. Previous studies have reported that phosphorus uptake is governed by primary production (e.g., Fellows et al. 2006; Gücker and Pusch 2006), carbon availability (e.g., Ensign and Doyle 2006; Yang et al. 2008), and nutrient supply (Tromboni et al. 2018). In the present study, the P-PO_4^{3-} uptake rate was significantly and positively correlated with water temperature (P<0.001; Table 6), indicating that thermal energy was an important factor driving the temporal patterns of P-PO_4^{3-} uptake in the estuarine ponds. The positive effect of temperature on P-PO_4^{3-} uptake could be related to biological mechanisms (Angelo et al. 1991). A higher temperature can stimulate microbial activity and algal bloom, which in turn would increase the microbial demand for phosphorus and biotic uptake of P-PO_4^{3-} (Brown and Shilton 2014; Finkler et al. 2018). This hypothesis was supported by the significant and positive relationship found between P-PO_4^{3-} uptake rate, water temperature, and Chl-*a* concentrations (p<0.001; Table 6). Moreover, we found that P-PO_4^{3-} uptake rate was negatively correlated with water pH in JRE ponds (Table 6). Baldy et al. (2015) have similarly observed a stronger phosphorus uptake in softwater (pH = 7.2, conductivity = 72 μS cm^{-1}) than in hardwater (pH = 8.4, conductivity = 669 μS cm^{-1}), especially under nutrient-enriched conditions. This is because under more alkaline conditions, orthophosphates can combine more easily with calcium to form apatite ($Ca_5(PO_4)_3OH$), which is a form of phosphorus unavailable to aquatic organisms (e.g., planktonic algae and aquatic plant) (Baldy et al. 2015; Chambers et al. 2001).

Abiotic P uptake can occur through sorption processes, which include both adsorption on the surfaces of cationic minerals and precipitation with electrolytes (Finkler et al. 2018; House and Warwick 1999; Reddy et al. 1999). Iron (Fe) and aluminum (Al) can form complexes with organic matter to support P sorption (Finkler et al. 2018; Reddy et al. 1999). Future studies should quantify the metal dynamics and examine their relationship with P-PO_4^{3-} production/ uptake more explicitly.

The P-PO_4^{3-} uptake rates in the overlying water of shrimp ponds differed significantly between the two estuaries, with the mean values being 1.5 times higher in the MRE than JRE ponds (3.27±0.72 vs. 2.18±0.55 mg m^{-3} h^{-1}), which might be related to the significantly lower salinity in the former (p<0.01; Table 1). Salinity can induce stress in the coastal ecosystems primarily through its effects on ionic strength and the microbial pathway of organic matter mineralization (Chambers et al. 2013; Wang et al. 2015). With an increase in salinity, the competition between various species of anions (e.g. Cl^-, SO_4^{2-}, HCO_3^-, OH^- and Br^-) for the surface adsorption sites would intensify, while at the same time displacing the adsorbed phosphate from the particle surface (Meng et al. 2015; Millero et al. 2001; Zhang and Huang 2011). In addition, we found a significantly higher mean Chl-*a* concentration in the MRE than JRE ponds ($p<0.01$; Table 1), which could support a higher uptake rate of P-PO_4^{3-} by phytoplankton as discussed previously.

Role of sediments in the biogeochemical cycling of nutrients in shrimp ponds

The biogeochemical cycling of nutrients in aquaculture ponds generally involve multiple processes, e.g. nutrient fluxes across the sediment-water interface (SWI), nutrient regeneration and uptake in the water column, nutrient inputs through animal excretion, etc. (Hargreaves 1998). Lacoste and Gaertner-Mazouni (2016) reported that nutrient regeneration by pearl oyster culture in the water column often had the largest contribution to the nutrient concentrations in the water column of aquaculture systems. In our previous study, the average N-NO_x^-, N-NH_4^+ and P-PO_4^{3-} fluxes across the SWI in the Min River estuarine ponds were found to be 0.25±0.16, 41.42±6.69, and 2.74±0.71 mg m^{-2} h^{-1}, respectively (Yang et al. 2017). Jiang et al. (2000) and Li et al (2006) reported that the rates of N-NO_x^-, N-NH_4^+ and P-PO_4^{3-} input from shrimp excreta ranged between 1.08-4.32, 31.86-126.72, and 0.69-7.94 mg m^{-2} h^{-1}, respectively. Assuming that these reported rates of shrimp excretion of nutrients were representative of those in the Min River estuarine ponds, the rate of N-NO_x^- flux across the SWI was substantially lower than that produced by either shrimp excretion rates or regeneration in the overlying water. Our results showed that the sediment was likely not the most dominant contributor of N-NO_x^- levels in the water column of MRE ponds. Meanwhile, the sediment might play a more significant role in governing the dissolved N-NH_4^+ and P-PO_4^{3-} concentrations in the subtropical estuarine aquaculture ponds, as shown by the significantly higher fluxes of both N-NH_4^+ and P-PO_4^{3-} across the SWI as compared to the rates of shrimp excretion and regeneration of these nutrients in the water column.

Implications of dissolved nutrient production in the shrimp pond water

Shrimp culture has expanded remarkably worldwide, accompanied by increasing concerns about shrimp diseases (Castillo-Soriano et al. 2013; da Silva et al. 2013; Yang et al. 2017). In this study, we observed the emergence of shrimp diseases in the ponds of both estuaries, with a higher rate of shrimp mortality during the middle and late stages. The mean shrimp survival rates in the MRE and JRE ponds were 65% and 70%, respectively (unpublished data), which were lower than the average level of 80% reported nationally (Lai 2014). At the meantime, the nutrient production rates (e.g., DOC, DIC and N-NO_x^-) in Min River estuary were higher than those in Jiulong River estuary (Fig. 2 and Fig. 3). Given the similar temporal patterns between the emergence of shrimp diseases and the production rates of dissolved nutrients in both estuaries (Fig.

2 and Fig. 3), the presence of excessive nutrients in the water column might exert a negative impact on shrimp survival. An increased nutrient level or imbalanced N:P ratios could lead to the attainment of toxic levels of nitrogen (e.g., ammonia and nitrite) and undesirable growth of harmful algae (da Silva et al. 2013; Mook et al. 2012). Sediment release of dissolved nutrients is also a potentially important source of nutrients in supporting harmful algal blooms in the water column (Yang et al. 2017; Zhang et al. 2006). In the present study, the observed high rates of nutrient production in the water column could help satisfy the majority of nutrient requirements for the primary productivity in shrimp ponds, which in turn lead to the formation of harmful algal blooms. We found a net production of $N\text{-}NO_x^-$ and $N\text{-}NH_4^+$ in the MRE (Fig. 5a) and JRE ponds (Fig. 5b), respectively, which might have contributed to the emergence of shrimp diseases during the middle and late stages. Moreover, the relatively low rates of shrimp survival in the ponds of both estuaries were probably related to the high production rates of DOC and DIC, as the decomposition of organic matter into dissolved carbon in aquaculture systems would consume a large amount of DO and thus pose a threat to shrimp lives (Mook et al. 2012; Zhang et al. 2016). Given the high nutrient production rate in the water column, this internal biogeochemical process could contribute significantly to dissolved nutrient levels in coastal aquaculture ponds, leading to the degradation of water quality and hampering the long-term sustainability of shrimp production systems. Therefore, it is important to take effective measures to mitigate environmental pollution from aquaculture (Yang 2014; Yang et al. 2015).

Limitations and future research

This research has a number of limitations, as is the case of many other studies. We used *ex situ* incubation under controlled conditions to determine the production and uptake rates of nutrients in the overlying water of shrimp ponds. Conducting an *in situ* experiment in future might be useful to produce results that can better represent the natural variability of environmental parameters experienced under field conditions. Also, researchers have found that the dynamics of DIC and DOC are associated with the activities of microorganisms and enzymes in the water column (Chambers et al. 2013; Morrissey et al. 2014; Neubauer, 2012), which were excluded in the current study and deserve further investigations. Moreover, given the potential importance of Fe and Al complexes in the dynamics of $P\text{-}PO_4^{-3}$ (Finkler et al. 2018; Reddy et al. 1999), future studies should quantify the metal dynamics and examine their relationship with $P\text{-}PO_4^{3-}$ production more explicitly. Furthermore, the specific role of shrimp excretion in the nutrient dynamics in pond water should be studied in greater detail, since previous studies have shown that the shrimp excretion could be an important contributor to dissolved nutrients (e.g., $N\text{-}NO_x^-$, $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$) in water (Jiang et al. 2000; Li et al. 2006; Zhong et al. 2015). Lastly, our results were based on field sampling in a limited number of shrimp ponds, and future studies should increase the spatial representativeness by covering a greater number of sites over a larger region in characterizing the nutrient dynamics in shrimp ponds.

4. Conclusions

Owing to rapid expansion of aquaculture production to meet the increasing demand of protein-rich seafood, there is an increasing worldwide concern on the environmental impacts arising from such activities. In the study, the rates of production and uptake of dissolved nutrients in the shrimp ponds of two subtropical estuaries were measured at three different growth stages over the grow-out cycle. The following key findings on the biogeochemical cycling of dissolved nutrients in the aquaculture ponds in the southeastern coast of China were obtained:

1) Large DOC and DIC production rates were observed in the overlying water of shrimp ponds, indicating that the overlying water of shrimp ponds during the culture period of shrimps were "hotspots" of DOC and DIC production.

2) A net uptake of N-NH_4^+ but a net production of N-NO_3^- were found in the overlying water of shrimp ponds in the Min River Estuary, while an opposite pattern was observed for N-NH_4^+ and N-NO_3^- in the Jiulong Min River Estuary, which might be related to the preferential use of different nitrogen forms by phytoplankton species with different physiological abilities between the two estuaries.

3) High rate of P-PO_4^{3-} uptake was observed in the overlying water of shrimp ponds in both estuaries, implying that phosphorus could potentially become a limiting nutrient for phytoplankton growth in the shrimp ponds.

4) Marked variations in the nutrient production and uptake rates were observed between the two estuaries and among the three shrimp growth stages, indicating a significant spatiotemporal variability of dissolved nutrient dynamics in shrimp ponds.

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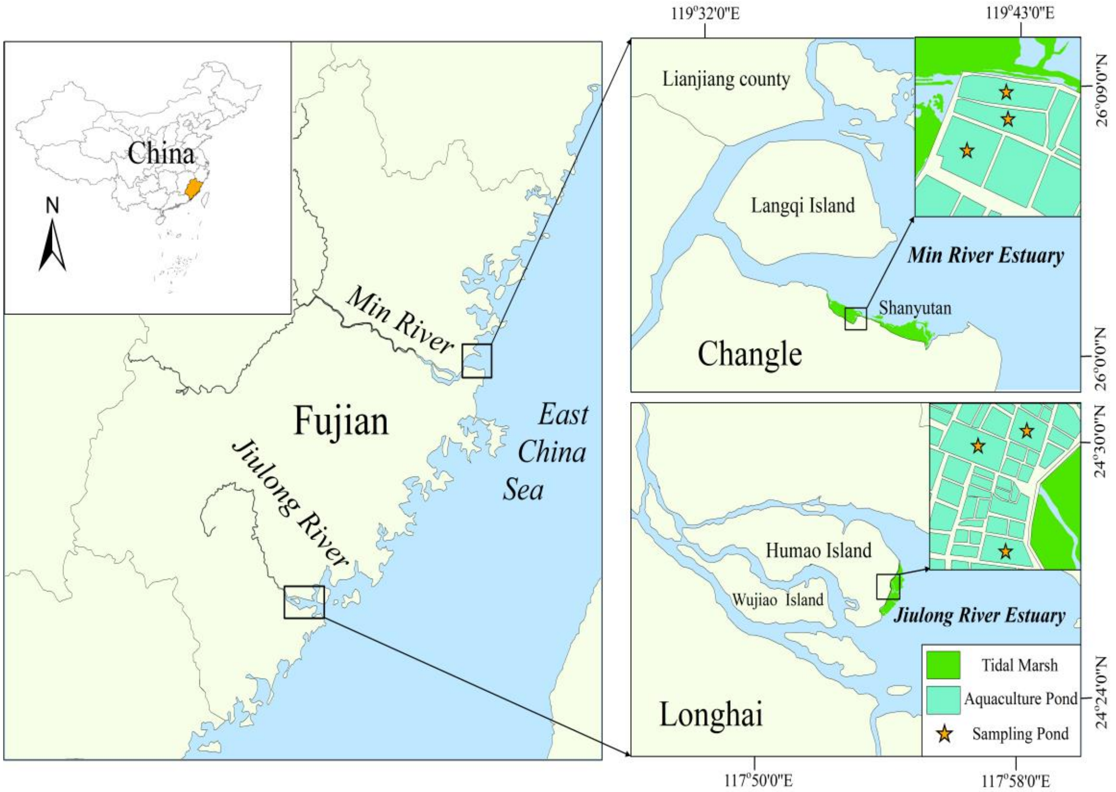

Fig. 1 Location of the study area and sampling sites in the Min River and Jiulong River estuaries in southeast China.

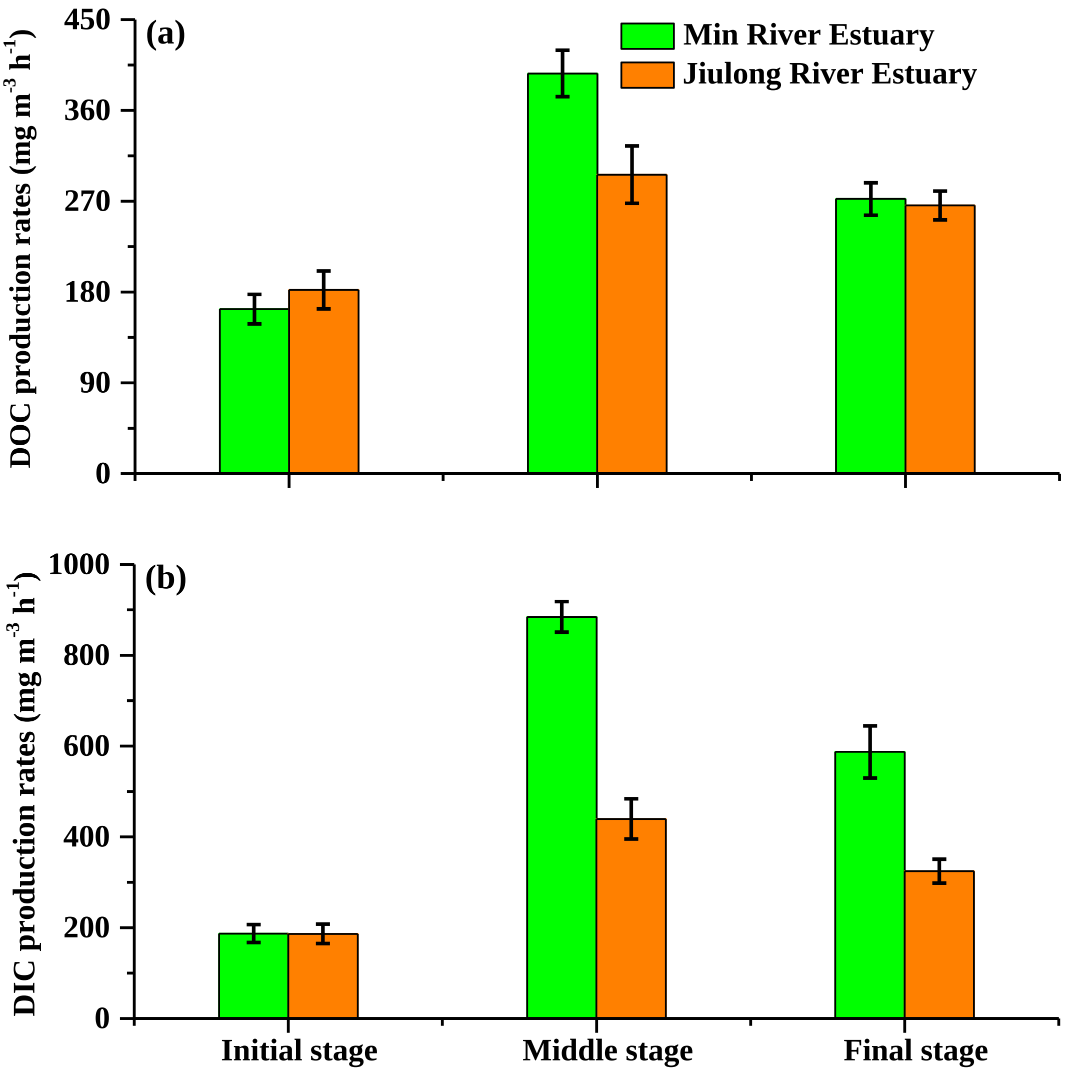

Fig. 2 Mean production rates of (a) dissolved organic carbon (DOC), and (b) dissolved inorganic carbon (DIC) in the overlying water (approximately 5-10 cm above the sediment) of shrimp ponds in the Min River Estuary (MRE) and Jiulong River Estuary (JRE) among the three aquaculture stages. The bars represent the mean ± SE (n = 9).

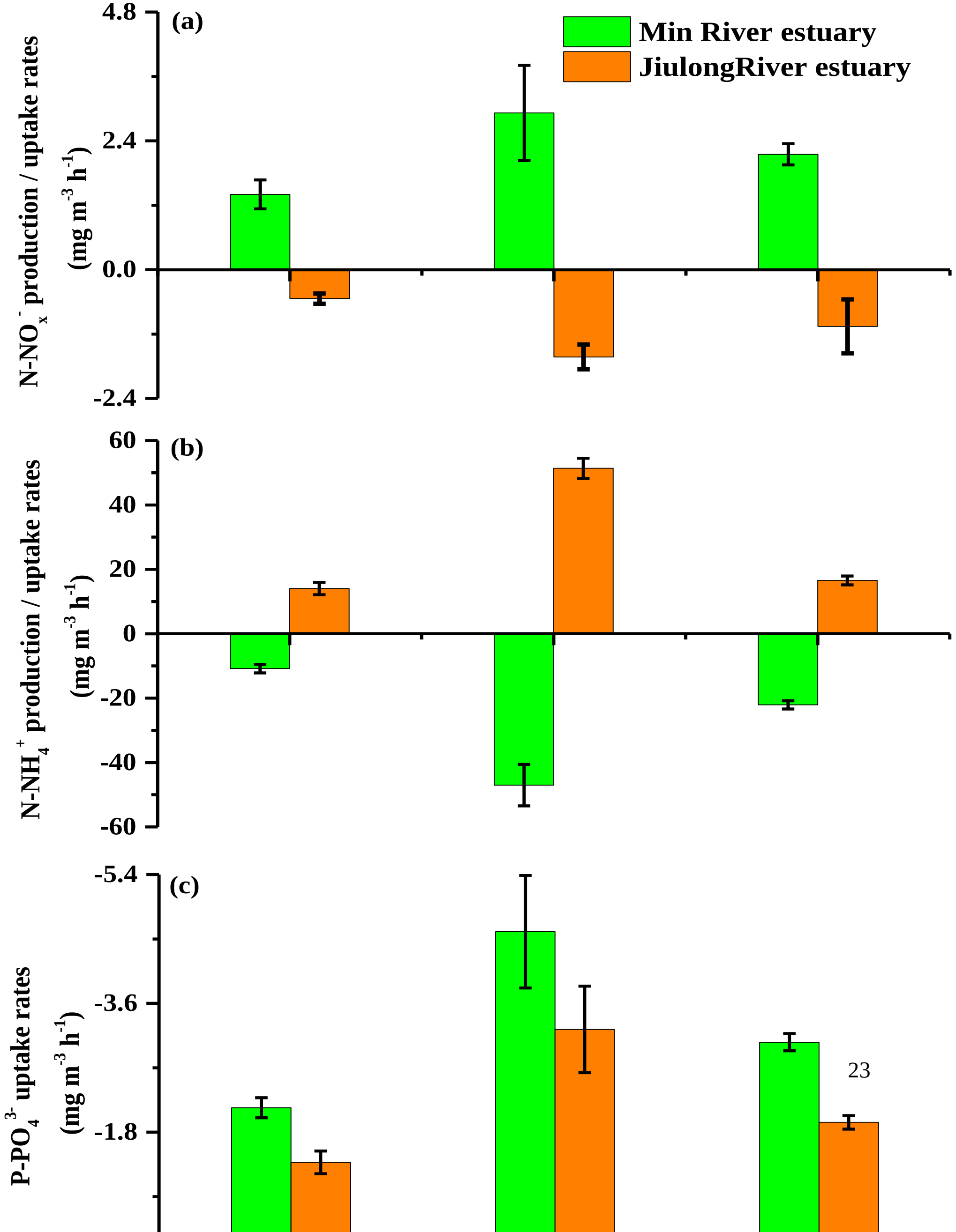
(a)
🟩 Min River estuary
🟧 JiulongRiver estuary
N-NO$_x^-$ production / uptake rates
(mg m^{-3} h^{-1})
4.8
2.4
0.0
-2.4
(b)
N-NH$_4^+$ production / uptake rates
(mg m^{-3} h^{-1})
60
40
20
0
-20
-40
-60
(c)
P-PO$_4^{3-}$ uptake rates
(mg m^{-3} h^{-1})
-5.4
-3.6
-1.8

Fig. 3 Mean production rates of (a) N-NO_x^-, and (b) N-NH_4^+, and (c) mean uptake rate of P-PO_4^{3-} in the overlying water of shrimp ponds in the Min River Estuary (MRE) and Jiulong River Estuary (JRE) among the three aquaculture stages. The bars represent the mean ± SE (*n* = 9).

Table 1 Physicochemical and biological properties in the overlying water of shrimp ponds in the Min River Estuary (MRE) and Jiulong River Estuary (JRE).

Environmental Variables	**Min River Estuary**			**Jiulong River Estuary**		
	Initial stage	**Middle stage**	**Final stage**	**Initial stage**	**Middle stage**	**Final stage**
Water temperature (°C)	23.99±0.03	30.66±0.12	24.05±0.24	26.34±0.12	31.69±0.04	27.88±0.06
pH*	8.96±0.02	8.63±0.07	9.99±0.04	9.21±0.07	8.67±0.13	9.48±0.08
Dissolved oxygen (mg L^{-1})*	7.76±0.12	11.01±0.95	11.69±0.52	8.19±0.19	8.35±0.16	9.94±0.37
Salinity (‰)	3.27±0.01	2.51±0.00	1.91±0.00	13.61±0.30	7.39±0.20	6.39±0.13
Chlorophyll *a* (μg L^{-1})	125.09±1.98	248.21±2.72	242.70±3.69	29.31±1.09	94.37±12.33	72.09±5.88

* Data are after Yang et al. (2019) for reference and review only.

Table 2 Summary of two-way ANOVAs that examining the effect of aquaculture stages, sampling estuaries and their interactions on the DOC and DIC production rates in the overlying water of shrimp ponds.

	DOC_$_{\text{prod}}$ rate					**DIC_$_{\text{prod}}$ rate**				
	df	*Sum of squares*	*Mean square*	*F values*	*P values*	*df*	*Sum of squares*	*Mean square*	*F values*	*P values*
Stages	2	24614.107	12307.054	38.545	<0.001	2	184081.104	92040.552	86.141	<0.001
Estuaries	1	1036.150	1036.150	3.245	=0.078	1	67703.708	67703.708	63.364	<0.001
Stages × Estuaries	2	3202.959	1601.480	5.016	0.011	2	40422.450	20211.225	18.916	<0.001
Residuals	48	15325.889	319.289			48				

Table 3 Pearson correlation coefficients for DOC, DIC production rates, and environmental variables of shrimp ponds in subtropical estuaries during the aquaculture period[a].

Environmental variables	**$DOC_{\_prod}$ rate**			**DIC_{prod} rate**		
	MRE	**JRE**	**Both estuaries**	**MRE**	**JRE**	**Both estuaries**
Water temperature	**0.881****	**0.834****	**0.698****	**0.821****	**0.931****	NS
pH	NS	NS	NS	NS	NS	NS
Dissolved oxygen	**0.708****	NS	**0.636****	**0.805****	NS	**0.769****
Salinity	NS	**-0.868****	NS	**-0.621***	**-0.808****	**-0.582***
Chlorophyll *a*	**0.858****	**0.936****	**0.617****	**0.915****	**0.965****	**0.837****

[a] *MRE* and *JRE* represent Min River Estuary and Jiulong River Estuary, respectively. * and ** indicate significance at the 0.05 and 0.01 levels, respectively. $n = 27$ for environmental variables, DOC and DIC production rates of each estuary from the shrimp ponds. NS denotes not significant.

Table 4 Multiple regression equations between environmental variables (e.g., water temperature, salinity, pH, dissolved oxygen, and chlorophyll *a*) and the production/uptake rates of dissolved carbon, nitrogen and phosphorus in shrimp ponds of subtropical estuaries during the aquaculture period[a] .

		Regression equations	***F***	$\boldsymbol{R^2}$	***p***
DOC production rates	MRE	$Y = 335.776 - 5.112x_{\text{WT}} - 171.191x_{\text{WS}} + 1.560x_{\text{Chl-}a}$	42.468	0.847	<0.001
	JRE	$Y = 39.930 + 0.529x_{\text{Chl-}a}$	44.164	0.639	<0.001
	Both estuaries	$Y = -66.577 + 5.967x_{\text{WT}} - 3.131x_{\text{WS}}$	26.893	0.513	<0.001
DIC production rates	MRE	$Y = -421.585 + 29.568x_{\text{WT}} - 0.916x_{\text{Chl-}a}$	118.250	0.908	<0.001
	JRE	$Y = -1043.875 + 20.473x_{\text{WT}} + 60.610x_{\text{pH}}$	35.927	0.750	<0.001
	Both estuaries	$Y = -315.470 + 14.420x_{\text{DO}} + 13.169x_{\text{WT}} - 8.924x_{\text{WS}}$	34.018	0.671	<0.001
N-NO_x^- production/uptake rates	MRE	**NV**	/	/	/
	JRE	$Y = 1.248 - 0.055x_{\text{WT}}$	4.940	0.165	=0.036
	Both estuaries	$Y = 1.387 - 0.055x_{\text{WT}}$	24.038	0.591	<0.001
N-NH_4^+ production/uptake rates	MRE	$Y = 27.843 + 0.006x_{\text{Chl-}a} - 0.085x_{\text{DO}} - 0.047x_{\text{WT}}$	36.757	0.595	<0.001
	JRE	$Y = 0.351 + 1.684x_{\text{WT}} - 4.429x_{\text{PH}}$	144.297	0.923	<0.001
	Both estuaries	$Y = -12.919 - 0.082x_{\text{Chl-}a} + 0.880x_{\text{WT}}$	36.436	0.588	<0.001
P-PO_4^{3-} uptake rates	MRE	$Y = 1.723 - 0.074x_{\text{WT}} - 0.075x_{\text{DO}}$	10.080	0.457	<0.001
	JRE	$Y = 2.285 - 0.103x_{\text{WT}}$	13.974	0.359	=0.001
	Both estuaries	$Y = 1.225 - 0.003x_{\text{Chl-}a} - 0.061x_{\text{WT}}$	15.746	0.382	<0.001

[a] MRE and JRE represent Min River Estuary and Jiulong River Estuary, respectively. WT, WS, Chl-*a*, and DO represent water temperature, salinity, chlorophyll *a*, and dissolved oxygen, respectively. *n* = 27 for environmental variables, and dissolved carbon, nitrogen and phosphorus production/uptake rates in the shrimp ponds of each estuary. NV denotes no input variables in the equation.

Table 5 Summary of two-way ANOVAs that examining the effect of sampling stages, sampling estuaries and their interactions on the production and uptake of dissolved nitrogen and phosphorus in the overlying water of shrimp ponds.

	df	**N-NO$_x^-$ production/uptake rates**[a]				**N-NH$_4^+$ production/uptake rates**[a]				**P-PO$_4^{3-}$ uptake rates**			
		Sum of squares	*Mean square*	*F values*	*P values*	*Sum of squares*	*Mean square*	*F values*	*P values*	*Sum of squares*	*Mean square*	*F values*	*P values*
Stages	2	0.037	0.019	0.114	=0.893	23.602	11.801	1.457	=0.243	3.907	1.953	13.739	<0.001
Estuaries	1	12.686	12.686	77.377	<0.001	3538.142	3538.142	436.818	<0.001	1.425	1.425	10.024	=0.003
Stages × Estuaries	2	1.374	0.687	4.190	=0.021	1237.676	618.838	76.402	<0.001	0.075	0.037	0.262	=0.771
Residuals	48	7.869	0.164			388.791	8.100			6.824	0.142		

[a] N-NO$_x^-$ production and N-NH$_4^+$ uptake phenomenon occurred in MRE ponds, and N-NO$_x^-$ uptake and N-NH$_4^+$ production phenomenon occurred in JRE ponds

Table 6 Pearson correlation coefficients between nitrogen (N-NO_x^- and N-NH_4^+) production (or uptake) rates, phosphorus (P-PO_4^{3-}) uptake rates, and environmental variables of shrimp ponds in subtropical estuaries during the aquaculture period[a].

Environmental variables	**N-NO_x^- production/uptake rates[b]**			**N-NH_4^+ production/uptake rates[b]**		
	MRE	**JRE**	**Both estuaries**	**MRE**	**JRE**	**Both estuaries**
Water temperature	**0.781***	**0.711***	NS	**-0.947****	**0.972****	**0.864****
pH	NS	NS	NS	NS	**-0.921****	**-0.591****
Dissolved oxygen	NS	NS	**0.615****	NS	NS	NS
Salinity	NS	NS	**-0.653****	NS	NS	NS
Chlorophyll *a*	**0.709***	**0.696***	**0.811****	**-0.753***	**0.795***	NS

[a] *MRE* and *JRE* represent Min River Estuary and Jiulong River Estuary. * and ** indicate significance at the 0.05 and 0.01 levels. *n* = 27 for environmental variables, nitrogen and phosphorus rates of each estuary from the shrimp ponds. NS denotes not significant. [b]N-NO_x^- production and N-NH_4^+ uptake phenomenon occurred in MRE ponds, and N-NO_x^- uptake and N-NH_4^+ production phenomenon occurred in JRE ponds