



Methane fluxes from typical marine polyculture ponds of swimming crab with kuruma shrimp and short-necked clam in eastern China

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ABSTRACT: Methane (CH₄) fluxes at the water–air interface in 2 typical seawater polyculture systems were determined during the farming season. The mean CH₄ fluxes were 66.0 ± 44.1 μg m⁻² h⁻¹ in the bispecies polyculture system of swimming crab *Portunus trituberculatus* with kuruma shrimp *Marsupenaeus japonicus* (PM) and 68.7 ± 39.5 μg m⁻² h⁻¹ in the trispecies polyculture system of swimming crab with shrimp and short-necked clam *Ruditapes philippinarum* (PMR), and no significant differences were observed between them. CH₄ emissions showed temporal variability during the farming season, peaking in mid-August. The linear mixed-effect model demonstrated that air temperature was the main regulator of CH₄ fluxes rather than the internal physical and chemical properties of the systems. Air, water and sediment temperatures explained 58.9, 61.4 and 55.3% of the CH₄ flux variations in the PM and 64.6, 57.1 and 60.8% of the variations in the PMR, respectively. Reducing organic matter accumulation in the sediment by means of improving feeding efficiency and the application of integrated aquaculture are likely to be effective in reducing CH₄ emissions from aquaculture systems.

KEY WORDS: Methane fluxes · Temperature · Sediment characteristics · Seawater polyculture systems

1. INTRODUCTION

Methane (CH₄) is an important greenhouse gas (GHG) contributing to global warming. The atmospheric concentration of CH₄ in 2016 was 1.853 ppm (WMO 2017), which exceeded its preindustrial level by 150% and was held responsible for 17% of the radiative forcing (IPCC 2013). Although its atmospheric concentration is relatively low compared to the major GHG carbon dioxide (CO₂), the global warming potential of CH₄ is 84 times that of CO₂ over a 20 yr time horizon (IPCC 2014).

CH₄ fluxes at the water–air interface in aquatic ecosystems depend on CH₄ formation, which occurs

exclusively in anaerobic environments (Utsumi et al. 1998), and CH₄ oxidation during transmission from the sediment to the water–air interface (Duc et al. 2010). Any chemical, physical and biological factors that could affect CH₄ formation and oxidation might potentially enhance or decrease CH₄ fluxes across the water–air interface. Temperature is a strong regulator of CH₄ fluxes (Svensson 1984, Williams & Crawford 1984). Since methanogenesis is temperature dependent (Westermann et al. 1989), CH₄ fluxes usually show temporal variability when measured over a long time period (Xing et al. 2005, Tong et al. 2009, Xiao et al. 2013a). Macrofaunal activities profoundly impact biogeochemical processes and micro-

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bial diversity (Aller 1980, Laverock et al. 2010). However, an understanding of the impact of macrofauna on CH₄ release is still limited. According to the results of an incubation experiment (Bonaglia et al. 2017), polychaetes indirectly enhance CH₄ efflux by a factor of 8 through bioturbation, while bivalves have a direct positive effect on CH₄ release through the colonization of active methanogens in the bivalve body, i.e. the anoxic intestine. To date, no field investigation of the effect of bivalves on CH₄ emission in aquaculture systems has been reported.

Generally, lakes, reservoirs, tidal marshes, paddy fields, etc., because of the prevalence of waterlogged and anoxic conditions, are critical CH₄ sources, which has been documented by numerous studies (Naser et al. 2007, Tong et al. 2010, Schubert et al. 2012, Xiao et al. 2013b). Moreover, these systems are strong CH₄ emitters because sulfate reduction is generally absent or very low in nonsaline systems (Reeburgh & Heggie 1977). However, CH₄ fluxes across the water–air interface from mariculture ponds have been rarely studied until now (Chen et al. 2016, Yang et al. 2018). Mariculture ponds play important roles in coastal ecosystems. According to statistical data maintained by the Agriculture Ministry of China, the combined area of mariculture ponds in 2015 was approximately 4.6×10^3 km², accounting for nearly 20% of the total mariculture area in China, and production was approximately 2.35×10^6 tons (Fisheries Department of Agriculture Ministry of China 2016), accounting for approximately 8.5% of the world's mariculture production (FAO 2017).

In mariculture ponds, feeds are normally supplied daily. However, only a small portion of the feed is converted into production, and this could be as low as 4.0 to 27.4% (Chen et al. 2016). Most of the input feed is retained in the systems or is discharged to adjacent water bodies (Su et al. 2009). This residual feed as well as the feces generated during aquaculture production could be major sources of substrates for CH₄ formation. To date, some efforts have been made to characterize CH₄ fluxes from mariculture ponds, and results indicated that mariculture ponds might be important CH₄ emitters (Chen et al. 2016, Yang et al. 2018). However, the number of field studies on CH₄ fluxes from mariculture ponds remains limited in comparison to those from other aquatic systems (e.g. lakes and reservoirs). As a result, it is difficult to assess the role that mariculture ponds play in global CH₄ emissions. More importantly, the maintenance of mariculture ponds depends on the management of stocks, feeding, water exchange, etc. Different mariculture ponds often have different

aquaculture management requirements, which might lead to large variations in CH₄ emissions. Therefore, to better understand the potential contribution of mariculture ponds to the global CH₄ budget, it is of great significance and necessity to conduct extensive studies on CH₄ emissions from various mariculture ponds.

The swimming crab *Portunus trituberculatus* has been widely cultured in coastal China, with production reaching 117 772 tons in 2015 (Fisheries Department of Agriculture Ministry of China 2016). Multi-species polyculture in marine ponds is very popular for this species, and polycultures of swimming crabs with shrimp and clams are 2 representative systems (Dong et al. 2013). In this study, we chose 2 typical seawater polyculture systems, i.e. polyculture of swimming crabs with kuruma shrimp *Marsupenaeus japonicus* (PM) and polyculture of swimming crabs with kuruma shrimp and short-necked clams *Ruditapes philippinarum* (PMR), and monitored CH₄ fluxes at the water–air interface, determined during the farming season. The aims of this study were (1) to quantify CH₄ fluxes at the water–air interface in the 2 typical polyculture systems and their differences and (2) to determine the main environmental factors influencing CH₄ fluxes in mariculture systems.

2. MATERIALS AND METHODS

2.1. Experimental ponds

The study was carried out in the Modern Agriculture Industrial Park in Ganyu County, Jiangsu Province, China (34.97° N, 119.20° E), in 2014 from July to November, which represents a typical temperate monsoonal climate. Two typical polyculture systems in marine ponds were selected: the PM was stocked with swimming crab *Portunus trituberculatus* and kuruma shrimp *Marsupenaeus japonicus*, and the PMR was stocked with swimming crab, kuruma shrimp and short-necked clam *Ruditapes philippinarum*. Three ponds were employed for each system, and all the ponds were oriented north–south. The pond dimensions were as follows: 170 m length, 60 m width and 2.3 m depth. Information on stocking biomass and yield is shown in Table 1. During the farming season, the crab and shrimp were fed mainly with *Aloides laevis* and supplemented with frozen trash fish. The total feed input in the PM was the same as that in the PMR. The seawater in the ponds was routinely exchanged through water inlets and outlets during spring tides.

Table 1. Stocking density and yield in 2 mariculture systems (mean \pm SD). PM: polyculture system of swimming crab with kuruma shrimp; PMR: polyculture system of swimming crab with kuruma shrimp and shortnecked clam. (–) data not available

Farming system	Stocking density (10 ⁴ ind. ha ⁻¹)	Yield (kg ha ⁻¹)
PM		
Swimming crab	7.2	758.3 \pm 15.2
Kuruma shrimp	48	303.3 \pm 15.3
Short-necked clam	–	–
PMR		
Swimming crab	7.2	850.0 \pm 86.6
Kuruma shrimp	48	283.3 \pm 28.9
Short-necked clam	50	1866.7 \pm 189.3

2.2. CH₄ gas collection and determination

Our study was carried out during the farming season from July to November with a sampling interval of approximately 15 d. Three different sites in each pond were selected for the measurement of CH₄ flux at the water–air interface using a static chamber technique (Xing et al. 2005, 2006). The sampling chambers, made of transparent acrylic resin, were open bottomed and cylindrical (inner diameter 30 cm, height 50 cm) and covered with aluminum foil to avoid high temperatures inside the chamber resulting from direct sunlight. A small vertical vent stopped by a silicon septum on the top was used for sampling, and a 4.5 V fan driven by a dry battery was used inside the chamber to better mix the air without disturbing the water–air interface. The chambers were placed on a flotation device installed at the measurement sites during sampling, keeping the lower parts of the chambers approximately 5 cm below the water surface. All the gas samples were taken between 09:00 and 12:00 h, and 4 gas samples of 100 ml were transferred from the chambers into vacuum sampling bags via polypropylene syringes at 0, 10, 20 and 30 min after deployment. All the gas samples were placed in a cool box first and then taken to the laboratory.

The gas samples were analyzed as soon as possible with a GC-2010 Plus gas chromatograph (Shimadzu), which was connected to an MGS-4 gas sampler. After being driven into the MGS-4 gas sampler, CH₄ was separated on the column (2 m \times 2 mm stainless steel, 40°C, packed with TDX [60–80 mesh]) and then determined with a flame ionization detector at 100°C. The flow rate of the carrier gas (N₂) was set at 22 ml min⁻¹, and the flow rates of the flame gases

(H₂ and compressed air) were 20 and 30 ml min⁻¹, respectively. The detection limit for CH₄ was 0.3 ppm. To evaluate the precision of the measurements as well as determine the sample concentrations, standard gases (10.4 ppm for CH₄) were measured every 4 gas samples. CH₄ fluxes were calculated from the linear regression of the change in CH₄ concentrations over time. The equation is as follows:

$$F = \frac{M}{V_0} \cdot \frac{P}{P_0} \cdot \frac{T_0}{T} \cdot H \cdot \frac{dc}{dt}$$

where F is the flux ($\mu\text{g m}^{-2} \text{h}^{-1}$), M is the molar mass ($\mu\text{g mol}^{-1}$) of CH₄, P is the atmospheric pressure (kPa), T is the air absolute temperature (K) during sampling and H is the height (m) of the chamber. V_0 , P_0 and T_0 are the gas molar volume ($\text{m}^3 \text{mol}^{-1}$), atmospheric pressure (kPa) and air absolute temperature (K) under standard conditions, respectively, and dc/dt is the slope of the gas concentration (c , ppm) curve variation over time (t , h).

2.3. Environmental factor determination

Meteorological factors were collected *in situ* when collecting the gas. Wind velocity (3 m above the water surface) and air temperature were measured by a portable anemometer (AVM-03, TES Electrical Electronic). Meanwhile, 9 water samples (surface, middle and bottom water layers at 3 different sites) were taken from each pond with a horizontal sampler. Water temperature and dissolved oxygen (DO) were measured with a YSI DO meter (Model 5000, 230 V), water pH and redox potential were determined with a pH/mV meter (IQ150, Spectrum), and water salinity was determined by a portable refractometer *in situ* when collecting the water samples. Sediment pH and redox potential (at 3 cm depth) were also measured with a pH/mV meter (IQ150, Spectrum) after sediment cores were taken with a sediment corer.

Water samples were stored in 1 l polyethylene bottles separately and then immediately taken to the laboratory. The total nitrogen (TN) and total phosphorus (TP) concentrations of the water samples were analyzed by the method of simultaneous digestion introduced by Valderrama (1981), and the detection limits for TN and TP were 0.05 and 0.003 mg l⁻¹, respectively. After water samples were filtered through GF/F glass microfiber filters, the filters were extracted with acetone (90%) in darkness for 24 h, and then chl *a* was analyzed according to the method of the National Standardization Management Coun-

cil (2007). NO_3^- -N, NO_2^- -N, NH_4^+ -N and PO_4^{3-} -P concentrations were determined using the filtrate. NO_3^- -N was analyzed with the cadmium–copper column reduction method according to Hansen & Koroleff (1999), and the detection limit was $0.6 \times 10^{-3} \text{ mg l}^{-1}$. NO_2^- -N concentration was determined by the method of Bendschneider & Robinson (1952), with a detection limit of $0.3 \times 10^{-3} \text{ mg l}^{-1}$. NH_4^+ -N was measured with the indophenol blue method according to Sagi (1966), and the detection limit was $0.7 \times 10^{-3} \text{ mg l}^{-1}$. PO_4^{3-} -P concentration was analyzed by the method introduced by Murphy & Riley (1962), with a detection limit of $0.6 \times 10^{-3} \text{ mg l}^{-1}$. The sediment cores (0–10 cm) were sampled using a cylindrical metal corer (diameter 8 cm) and then dried at 60°C to a constant weight, ground and sieved with a sample sifter. The organic matter content of the sediment cores was measured by the combustion method according to Piron et al. (1990).

2.4. Data analysis

The data were analyzed with the statistical software SPSS 17.0. Differences in the environmental variables between the different culture systems were analyzed using a Student's *t*-test. Correlations between CH_4 flux and the measured variables were tested using curve estimation or Pearson correlation analysis. To examine the associations between CH_4 flux and the environmental factors measured, a linear mixed-effect model was applied to the PM and PMR datasets. In the model, the intercept, sampling time, aquaculture system, interaction between sampling time and system, wind velocity, air temperature, water pH, DO, redox potential in water, chl *a*, NO_3^- -N, NO_2^- -N, NH_4^+ -N, PO_4^{3-} -P, TN, TP, sediment redox potential, pH and organic matter content were fitted as fixed effects, and the intercept and the system were specified as random effects. The first-order autoregressive was selected as the covariance structure.

3. RESULTS

3.1. Variations in environmental factors

Wind velocity during the farming season averaged 2.0 m s^{-1} (0.8 – 3.9 m s^{-1}). Air and water temperatures varied similarly during the farming season, with the maximum (28.1 and 27.2°C , respectively) occurring in mid-August and the minimum (12.9 and 12.0°C ,

respectively) occurring at the end of the farming season (Fig. 1b). Water salinity in the PM and the PMR varied similarly during the farming season, with the minimum occurring on 3 August and 18 September (Fig. 1a), respectively, which was mostly related to a heavy rainstorm before the sampling days.

As shown in Fig. 1c, water pH in the PM averaged 8.32 (7.96–8.79) during the farming season, which was significantly higher than that in the PMR ($p < 0.01$) (mean 7.90, 7.78–7.97). During the farming season, no significant differences in DO concentrations were found between the PM and the PMR ($p > 0.05$) (mean 8.42 and 7.36 mg l^{-1} , respectively) (Fig. 1d), and there was also no significant difference in the water redox potential between the PM and the PMR (mean 86.8 and 102.0 mV , respectively) ($p > 0.05$) (Fig. 1e). Chl *a* concentrations in the water are shown in Fig. 1f. Chl *a* concentrations in the PM (mean 44.5 mg m^{-3} , 21.2 – 103.0 mg m^{-3}) were significantly higher than those in the PMR (mean 15.8 mg m^{-3} , 7.8 – 30.3 mg m^{-3}) ($p < 0.05$).

Concentrations of water nutrients in the PM and the PMR during the farming season are shown in Table 2. No significant differences in the concentrations of NO_2^- -N, NH_4^+ -N and PO_4^{3-} -P were found between the PM and the PMR. NO_3^- -N concentrations in the PM were significantly lower than those in the PMR ($p < 0.05$), while TN and TP concentrations in the PM were significantly higher than those in the PMR ($p < 0.05$).

As shown in Table 3, pH and redox potential in the sediment were lower than those in the water, while the average sediment temperature was higher than the water temperature (Fig. 1). Among the measured physical and chemical factors of the sediment, a significant difference was only found in the redox potential ($p < 0.05$), while the pH, temperature and organic matter content of the sediment showed no significant differences between the PM and the PMR ($p > 0.05$).

3.2. CH_4 fluxes in the PM and the PMR during the farming season

Variations in CH_4 fluxes in the PM and the PMR during the farming season are shown in Fig. 2. The PM and PMR culture systems both acted as stable CH_4 sources to the atmosphere, with average fluxes of 66.0 and $68.7 \mu\text{g m}^{-2} \text{ h}^{-1}$, respectively, and no difference was observed between them ($p > 0.05$). During the farming season, CH_4 fluxes in the PM and

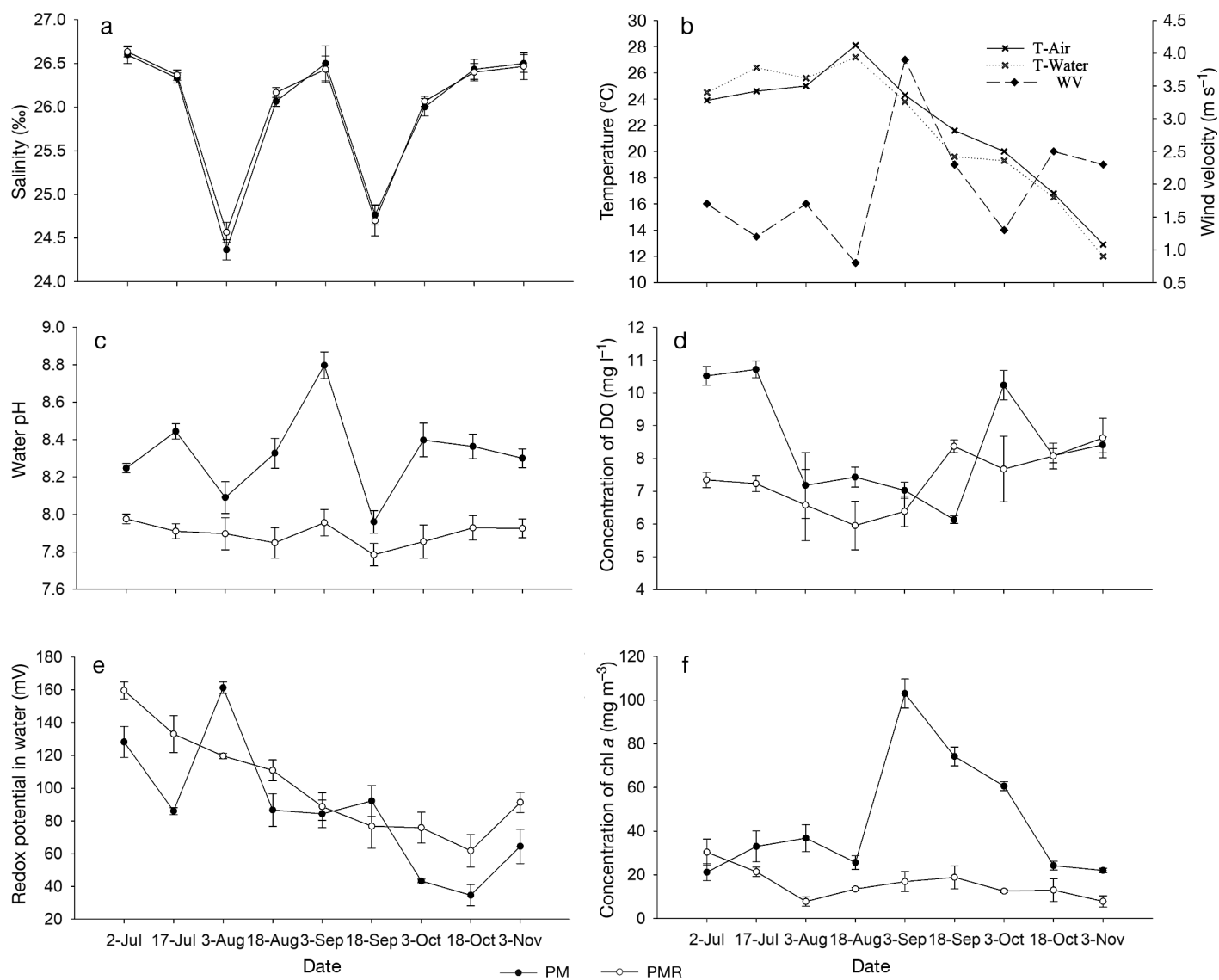


Fig. 1. Variations in salinity, wind velocity (WV), temperature (T), water pH, dissolved oxygen (DO), water redox potential and chl a in the PM and the PMR (defined in Table 1) during the farming seasons

the PMR generally increased from the beginning of the farming season, peaked in mid-August ($143.8 \mu\text{g m}^{-2} \text{h}^{-1}$ in the PM and $139.0 \mu\text{g m}^{-2} \text{h}^{-1}$ in the PMR), and were followed by a reduction before reaching the lowest points at the end of the farming season ($21.9 \mu\text{g m}^{-2} \text{h}^{-1}$ in the PM and $11.2 \mu\text{g m}^{-2} \text{h}^{-1}$ in the PMR).

3.3. Correlations between CH_4 fluxes and environmental factors measured

As shown in Fig. 3, the exponential model could be used to describe the relationship between CH_4 fluxes from the PM and the PMR and the temperatures of air, water and sediment, and all the fitting equations

Table 2. Concentrations of water nutrients in different polyculture systems (PM and PMR, defined in Table 1) during the farming season (mean \pm SD). Data in the same column with different superscripts indicate significant differences ($p < 0.05$)

System	$\text{NO}_3^- \text{-N}$ (mg l^{-1})	$\text{NO}_2^- \text{-N}$ (mg l^{-1})	$\text{NH}_4^+ \text{-N}$ (mg l^{-1})	$\text{PO}_4^{3-} \text{-P}$ (mg l^{-1})	TN (mg l^{-1})	TP (mg l^{-1})
PM	0.21 ± 0.16^a	0.057 ± 0.039	0.063 ± 0.054	0.014 ± 0.005	3.66 ± 0.52^b	0.22 ± 0.065^b
PMR	0.47 ± 0.25^b	0.089 ± 0.030	0.052 ± 0.027	0.022 ± 0.015	3.13 ± 0.40^a	0.14 ± 0.032^a

Table 3. Physical and chemical factors of the sediment in different polyculture systems (PM and PMR, defined in Table 1) during the farming season (mean \pm SD). Data in the same column with different superscripts indicate significant differences ($p < 0.05$)

Systems	pH	Redox potential (mV)	Temperature ($^{\circ}$ C)	Organic matter (%)
PM	7.72 \pm 0.27	-152.2 \pm 25.9 ^a	23.0 \pm 4.8	3.96 \pm 0.51
PMR	7.58 \pm 0.15	-121.5 \pm 26.0 ^b	22.9 \pm 4.9	4.16 \pm 0.51

were significant ($p = 0.000$). Air, water and sediment temperatures explained 58.9, 61.4 and 55.3% of the CH₄ flux variations in the PM and 64.6, 57.1 and 60.8% of the CH₄ flux variations in the PMR, respectively.

According to the Pearson correlation analysis, CH₄ flux in the PM was correlated significantly with NO₃⁻-N ($r = -0.553$, $p < 0.01$) and NO₂⁻-N ($r = -0.465$, $p < 0.05$) concentrations in the water. In the PMR, a significant correlation was found only between CH₄ emission and wind velocity ($r = -0.515$, $p < 0.01$).

The results of estimates of the fixed effects demonstrated that sampling time was not significant in predicting CH₄ flux and neither was aquaculture system ($p > 0.05$, see Table A1 in the Appendix). The interaction between sampling time and aquaculture system was also found to be insignificant ($p > 0.05$). Among all the environmental variables measured, air temperature was the only factor significantly correlated with CH₄ flux ($p < 0.01$). According to estimates of the covariance parameters (see Table A2), the variance of the random errors for repeated measures was estimated to be 528.899, which was significant ($p = 0.000$). The variance of the random errors for the random variables in the model was estimated to be 3.140 ($p = 0.874$).

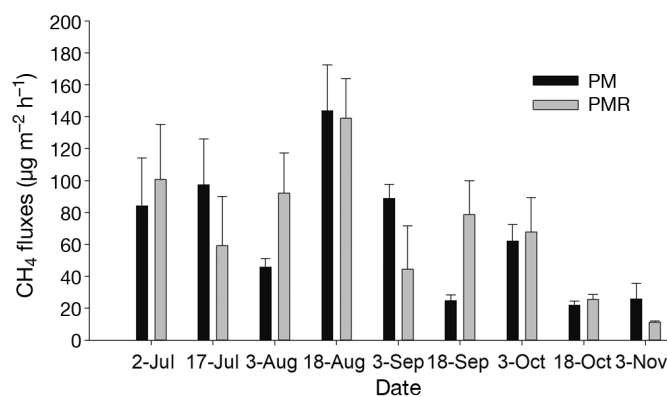


Fig. 2. Variations in methane (CH₄) fluxes in the PM and the PMR (defined in Table 1) during the farming season

4. DISCUSSION

4.1. Factors influencing CH₄ emissions

Temperature is a strong regulator of CH₄ fluxes (Svensson 1984, Williams & Crawford 1984). The temporal variability of CH₄ emissions is normally found to be positively related to temperature when measuring over longer time periods, and the connections between temperature

and CH₄ fluxes range from linear to exponential (e.g. Xing et al. 2005, Frei et al. 2007, Tong et al. 2009, Xiao et al. 2013a). In the present study, CH₄ fluxes in both the PM and the PMR showed an obvious seasonal trend, peaking in mid-August and being relatively low at the beginning and later parts of the farming season, and the CH₄ fluxes tended to be consistent with the temperature. Results of the correlation analysis showed that the relationships between CH₄ emissions and temperature could be best described as an exponential model, and the temperatures of air, water and sediment explained 58.9, 61.4 and 55.3% of the CH₄ flux variations in the PM and 64.6, 57.1 and 60.8% of the CH₄ flux variations in the PMR, respectively. Additionally, results of the linear mixed-effect model also demonstrated that air temperature, among all the variables measured, was the only factor positively correlated with CH₄ flux ($p < 0.05$). Furthermore, sampling time and the different aquaculture systems were found to have no significant effect on CH₄ flux, which means that under the conditions of the present study, CH₄ emissions were barely regulated by the stocked animals in the systems or the different developmental stages of the aquaculture systems. These results suggest that the external meteorological factors (mainly temperature) rather than the internal physical and chemical properties of the system dominate the CH₄ emission pattern in this study. In contrast, our study on CO₂ flux from the present aquaculture systems indicated that CO₂ flux at the water–air surface was strongly driven by their internal biological and physicochemical characteristics, and any factor that influenced their internal biogeochemical process (e.g. management methods, biological characteristics of stocked species) could have impacts on CO₂ emission patterns in aquaculture systems (D. Zhang et al. unpubl. data). The positive effect of temperature on CH₄ emissions may be related to the following aspects. First, methanogenesis is temperature dependent, similar to most other metabolic activities (Westermann et al. 1989). Temperature can affect the population of methano-

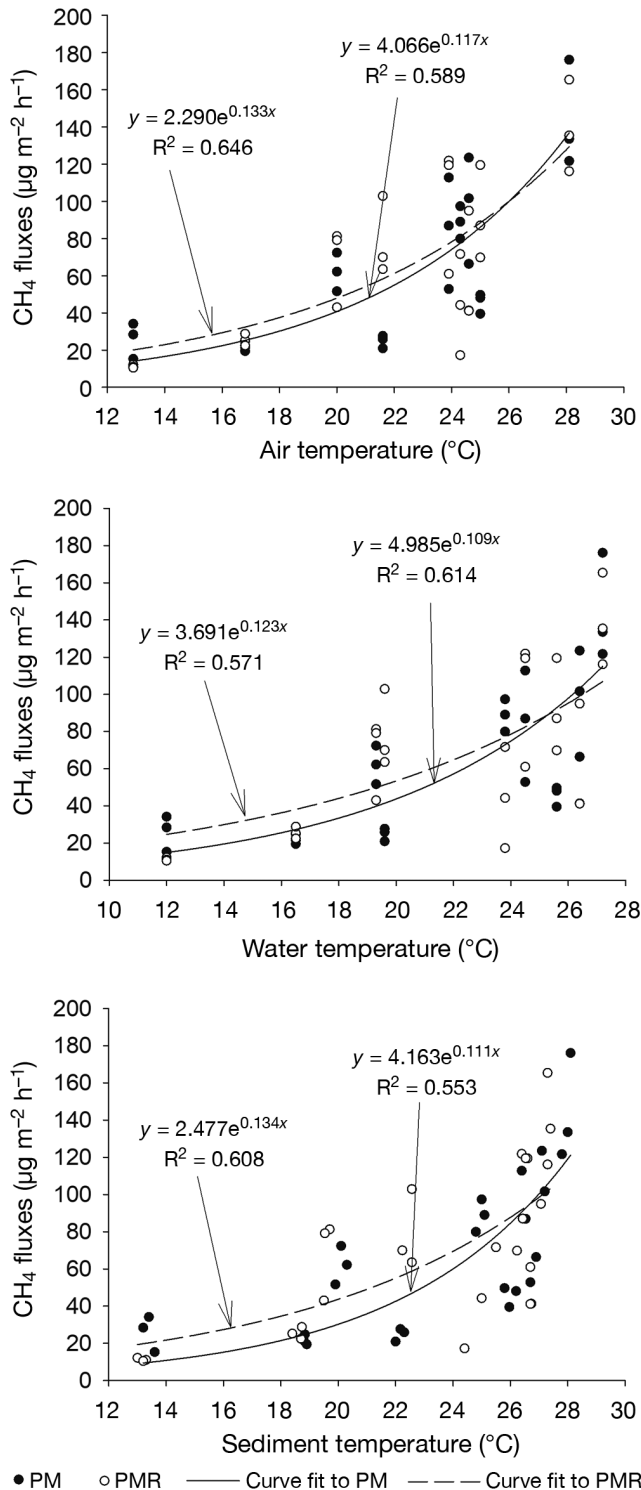


Fig. 3. Relationships between methane (CH_4) fluxes and air, water and sediment temperature in the PM and the PMR (defined in Table 1)

gens, and higher temperatures favor the domination of species that are more powerful in utilizing substrates and producing CH_4 (Sekiguchi et al. 1998, Ding

& Cai 2003). Second, a substrate is an essential requirement for methanogenesis. Since methanogenic substrates mostly originate from the metabolic activities of other microorganisms, substrate availability is always involved in temperature responses. For instance, temperature could limit hydrogen turnover and hence the availability of a substrate to a greater extent than methanogenic activity (Conrad et al. 1987). Third, higher temperatures reduce the solubility of CH_4 in the water column, promoting CH_4 evasion from water during transport.

In the present study, we found that NO_3^- -N and NO_2^- -N concentrations were both negatively correlated with CH_4 emissions in the PM ($p < 0.01$ and $p < 0.05$, respectively), which might be interpreted as being due to the competition for substrates by denitrifiers. CH_4 is mainly generated from precursor substrates, such as acetic acid and H_2/CO_2 , by methanogens. However, denitrifying bacteria could compete for common substrates with methanogens (Klüber & Conrad 1998), resulting in a decrease in CH_4 production. Meanwhile, NO_2^- -N, an important denitrification intermediate, is toxic to methanogens and hence inhibits methanogenesis and suppresses CH_4 production (Balderston & Payne 1976, Roy & Conrad 1999).

In this study, CH_4 emissions from the PM and the PMR were 66.0 and $68.7 \mu\text{g m}^{-2} \text{h}^{-1}$, respectively, during the farming season, and on the whole, no significant difference was found between the 2 systems ($p > 0.05$). The correlation analysis showed that the differences in sediment variables between the PM and the PMR were observed to be insignificant ($p > 0.05$), though some water environmental variables, such as pH, TN, TP and chl *a*, were remarkably different ($p < 0.05$), which suggests that CH_4 emissions from the present systems may mostly be correlated with the characteristics of sediment rather than the water environmental factors, especially given the shallow water table in the present study. Bivalve activities such as biodeposition and bioturbation could alter the physical and chemical characteristics of sediment (Vaughn & Hakenkamp 2001, Newell 2004) and thus affect CH_4 emissions (Bonaglia et al. 2017). However, the results of the present study do not seem to fit well with previous understandings. This is probably because even though biodeposition could increase the organic matter content, the bioturbation of sediments through bivalve movements could increase the oxygen content in the sediments and consequently inhibit methanogenesis. Moreover, it was reported that a higher stocking density of bivalves could promote

CH₄ production (Wang 2008), and the stocking density of the clam in this study may have been too low to lead to a significant difference between the PM and the PMR. However, it is worth noting that CH₄ fluxes on 3 August and 18 September in the PMR were significantly higher than those in the PM ($p < 0.05$). This may be related to a heavy rainstorm before the sampling days. The sudden drop in water salinity could cause metabolic changes in the clam, such as increasing their oxygen consumption (Nie et al. 2017), which can result in oxygen depletion in the interstitial water and thereby promote CH₄ production and emission.

4.2. Comparisons of CH₄ flux with other aquatic ecosystems

CH₄ fluxes across the water–air interface from mariculture ponds have rarely been studied until now. Compared with other aquatic systems, CH₄ fluxes from the PM and the PMR are 2 or 3 orders of magnitude lower than those reported for natural ecosystems such as lakes and reservoirs (Xing et al. 2005, Schrier-Uijl et al. 2011). Chen et al. (2016) reported that the CH₄ flux in a shrimp mariculture pond was $65.1 \mu\text{g m}^{-2} \text{h}^{-1}$, which is quite close to the results of the present study. We speculated that the lower organic matter content might be the main reason explaining the lower CH₄ fluxes. Average organic matter contents in the PM and the PMR were 4.16 and 4.06%, respectively, 1 order of magnitude less than those in lakes and reservoirs (e.g. Huttunen et al. 2006, Hahn-Schöfl et al. 2011, Schrier-Uijl et al. 2011). The low availability of organic matter could have greatly limited CH₄ production. In aquaculture ponds, residual feeds are an important source of organic matter in the sediment. However, in this study, the use of live feed together with a reasonable feeding strategy may likely have mitigated the accumulation of organic matter in the sediment and hence avoided deterioration of the sediment environment. Moreover, crab and shrimp in the present study normally inhabit the bottom. Bioturbation of the sediment by stocked animals could cause sediment resuspension and increase organic matter degradation (Xiong et al. 2017). In addition, unlike lakes and reservoirs that have been inundated for decades or even centuries, aquaculture ponds are dried and dredged annually after harvesting. As a result, a large amount of organic matter in the sediments could have been oxidized after being exposed to air and sunlight.

Based on the above understanding, to reduce CH₄ emissions from aquaculture systems, reducing the accumulation of organic matter in the sediment is likely an effective method. Accordingly, the amount of uneaten feed as well as the feces from stocked animals, which constitute the major portion of the organic matter in the sediment, should be decreased as much as possible. On the one hand, given that the feed utilization efficiency is normally low in many aquaculture systems, reasonable feeding strategies that correspond to specific aquaculture patterns should be established. A proper feeding method and feeding frequency could improve feed utilization (Biswas et al. 2006, Casillas-Hernández et al. 2006, Silva et al. 2007) and thus minimize the production of organic solids. On the other hand, reductions in solid waste can also be achieved through feed formulation optimization. Highly digestible diets have been introduced as a solution to reduce solid waste excretion (Amirkolaie 2011). In addition, applications of integrated aquaculture could also be practical. A proper combination of different trophic levels of animals in aquaculture systems could make the most efficient use of the nutrients and thus reduce organic matter accumulation in the sediment or even improve the sediment environment. For example, as a type of deposit-feeding animal, sea cucumber could efficiently ingest the organic solids in polyculture systems (Yokoyama 2013, Yu et al. 2014). Therefore, it is usually considered an ideal candidate species to improve the properties of the sediment environment. A recent study indicated that a polyculture system of shrimp with sea cucumber was a significantly weaker CH₄ emitter than the shrimp culture system (Chen et al. 2016). In future studies, the role of deposit-feeding animals on CH₄ dynamics should be given more attention.

5. CONCLUSIONS

CH₄ emissions from the PM and the PMR were 66.0 and $68.7 \mu\text{g m}^{-2} \text{h}^{-1}$, respectively, during the farming season. Clam farming seems to have little impact on CH₄ emissions in the present study, although in situations such as a sudden salinity drop, clams could promote CH₄ emissions. Temperature factors were positively correlated with CH₄ fluxes, and CH₄ emissions showed temporal variability during the farming season. Mariculture ponds in this study served as weak CH₄ sources to the atmosphere, and the low availability of organic matter in the sediment might be the main reason why CH₄ fluxes from the aqua-

culture ponds were much lower than those reported for lakes and reservoirs. Feeding efficiency improvement by establishing corresponding feeding strategies and optimizing feed formulation for specific aquaculture patterns as well as the application of integrated aquaculture are likely to be practicable and effective ways to reduce CH₄ emissions and mitigate global warming.

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Appendix. Estimates of fixed effects and covariance parameters

Table A1. Estimates of the fixed effects. T: sampling time; PM: polyculture system of swimming crab with kuruma shrimp; PMR: polyculture system of swimming crab with kuruma shrimp and short-necked clam; WV: wind velocity; AT: air temperature; S: salinity; pH_{water} : water pH; DO: dissolved oxygen; RP_{water} : redox potential in water; TN: total nitrogen; TP: total phosphorus; $\text{pH}_{\text{sediment}}$: pH in sediment; $\text{RP}_{\text{sediment}}$: redox potential in sediment; $\text{OM}_{\text{sediment}}$: organic matter content in sediment; (-) no data available

Parameter	Estimate	SE	df	t	p
Intercept	-694.178	419.726	25.408	-1.654	0.110
T	0.035	6.977	23.515	0.005	0.996
PM	-31.112	42.350	33.508	-0.735	0.468
PMR (reference)	0	0	-	-	-
PM × T	10.494	5.446	29.555	1.927	0.064
PMR × T (reference)	0	0	-	-	-
WV	-10.436	6.458	32.882	-1.616	0.116
AT	13.535	3.306	28.896	4.094	0.000
S	-15.079	8.509	32.601	-1.772	0.086
pH_{water}	101.496	42.311	24.043	2.399	0.025
DO	9.424	4.599	32.881	2.049	0.048
RP_{water}	0.261	0.267	31.349	0.979	0.335
Chl <i>a</i>	-0.689	0.368	28.750	-1.872	0.071
NO_3^- -N	74.319	51.618	32.654	1.440	0.159
NO_2^- -N	-132.472	152.682	33.701	-0.868	0.392
NH_4^+ -N	-171.887	196.769	27.989	-0.874	0.390
PO_4^{3-} -P	461.918	650.985	30.665	0.710	0.483
TN	-13.226	14.951	21.715	-0.885	0.386
TP	-161.099	100.709	30.424	-1.600	0.120
$\text{pH}_{\text{sediment}}$	12.000	24.776	30.501	0.484	0.632
$\text{RP}_{\text{sediment}}$	0.656	0.285	31.262	2.305	0.028
$\text{OM}_{\text{sediment}}$	-2.878	11.988	30.941	-0.240	0.812

Table A2. Estimates of covariance parameters. AR1: first-order autoregressive

Parameter		Estimate	SE	Wald Z	Sig.
Repeated measures	AR1 diagonal	488.439	125.223	3.901	0.000
	AR1 rho	-0.101	0.260	-0.389	0.697
Intercept + system	AR1 diagonal	7.131	25.726	0.277	0.782
	AR1 rho	0.414	2.398	0.173	0.863