



Methane distribution, sources, and sinks in an aquaculture bay (Sanggou Bay, China)

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ABSTRACT: From 2012 to 2015, we investigated methane (CH₄) distribution, air–sea fluxes, and sediment–water fluxes in an aquaculture bay (Sanggou Bay, China), and estimated the input of CH₄ from potential land sources including rivers and groundwater. Surface water CH₄ in the bay ranged from 3.0 to 302 nM, while bottom CH₄ was usually higher due to sediment release. Water column CH₄ in summer and autumn was 3 to 10 times that in spring and winter due to seasonal variation in water temperature and land source inputs. Surface CH₄ was higher in kelp and scallop polyculture zones than in other culture zones and outside the bay, suggesting the influence of aquaculture activities. CH₄ concentrations were 123 to 2190 nM in rivers around the bay, and 1.6 to 405 nM in groundwater along the shoreline; both showed great spatial and temporal variations. Sediment–water CH₄ fluxes ranged from 0.73 to 8.26 μmol m⁻² d⁻¹, with those in bivalve culture zones higher than in polyculture zones. Sea–air CH₄ fluxes ranged from 2.1 to 123.2 μmol m⁻² d⁻¹ (mean 48.2 μmol m⁻² d⁻¹) and showed seasonal variations. CH₄ budget in Sanggou Bay showed that groundwater input (4.2 × 10⁵ mol yr⁻¹) was the largest source of CH₄, followed by sediment release (2.6 × 10⁵ mol yr⁻¹) and riverine input (1.4 × 10⁵ mol yr⁻¹), while sea-to-air release (2.5 × 10⁶ mol yr⁻¹) and export from the bay to the Yellow Sea (8.8 × 10⁵ mol yr⁻¹) were the dominant CH₄ sinks. Net water column production-oxidation was estimated preliminarily to produce 1.7 × 10⁵ mol CH₄ yr⁻¹. However, there was a great imbalance of sources and sinks, with an apparent missing source of 2.4 × 10⁶ mol yr⁻¹ that was mostly due to an underestimate of *in situ* water column production and CH₄ release from the sediments.

KEY WORDS: CH₄ · Sanggou Bay · Production · Sediment–water exchanges · Air–sea fluxes · Aquaculture

INTRODUCTION

Methane (CH₄), the most abundant hydrocarbon in the atmosphere, plays an important role in regulating the Earth's radiation balance and atmospheric chemistry in the troposphere (Cicerone & Oremland 1988, Lashof & Ahuja 1990). Although the current atmospheric mixing ratio of CH₄ (~1.8 ppm) is much less than that of CO₂ (~390 ppm), it is actually responsible for about 20% of the greenhouse effect (IPCC 2013). The atmospheric CH₄ mixing ratio has increased by a factor of 2.5, from 722 ppb in 1750 to 1803 ppb in

2011, as a result of human activities since the Industrial Revolution (IPCC 2013).

Oceans are a natural source of atmospheric CH₄, and there are large spatial and temporal variations of oceanic CH₄ emissions. Typically, oligotrophic waters are only slightly supersaturated (by about 5%) in CH₄ with respect to atmospheric equilibrium (Bates et al. 1996, Bange et al. 1998, Karl et al. 2008), resulting in low sea-to-air CH₄ fluxes. High sea-to-air CH₄ emissions can occur in biologically productive regions such as estuaries and coastal and upwelling areas, which contribute to about 75% of the oceanic

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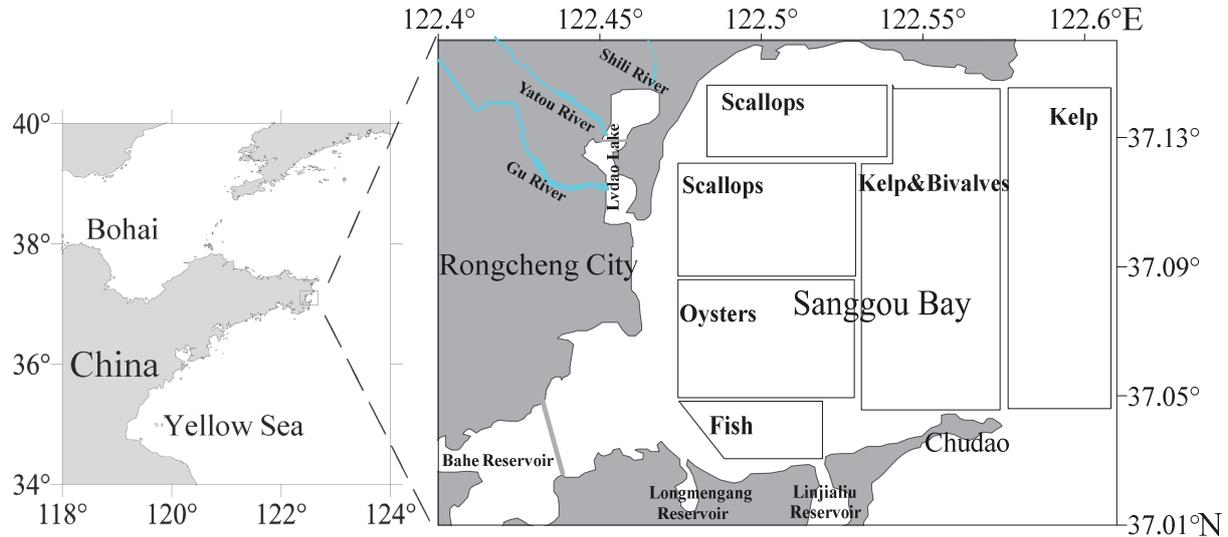


Fig. 1. Location of Sanggou Bay, China, and the main aquaculture practices

CH₄ emissions (Bange et al. 1994, EPA 2010). However, CH₄ emissions from coastal areas still have great uncertainties, due to poor coverage of CH₄ measurements and great spatial variations. Potential sources of CH₄ in coastal waters include riverine inputs, *in situ* water column production, and sediment release (Martens & Klump 1980, Kelley et al. 1990, Hornafius et al. 1999, Mau et al. 2007, Canet et al. 2010). It is generally assumed that CH₄ production and emission from coastal waters will be enhanced with the increase in human perturbations such as increased nutrient loading and intensive marine aquaculture. With the continuous decline in fishery harvests, aquaculture has become the world's fastest growing sector of food production, increasing nearly 60-fold during the last 5 decades, to meet the increasing demand for seafood (FAO 2007). However, the rapid increase in aquaculture production may also cause some environmental concerns, such as the release of greenhouse gases (Ferrón et al. 2007, Green et al. 2012). The discharge of effluent with high concentrations of organic matter and nutrients from coastal aquaculture systems to adjacent marine waters can lead to organic pollution and provide favorable conditions for the production of CH₄. However, most studies on coastal methane emissions have focused on estuaries and coastal waters, and few studies have been conducted on coastal aquaculture systems. The aims of this study were to determine the temporal and spatial distributions of CH₄ in an intensive coastal aquaculture bay (Sanggou Bay) in China, to identify various CH₄ sources and sinks, and to evaluate CH₄ emissions from this bay and the possible impact of aquaculture activities.

MATERIALS AND METHODS

Study area

Sanggou Bay (SGB) is a semi-circular bay on the north-eastern coast of China (Fig. 1). The bay is crescent-shaped, facing the Yellow Sea in the east, and has an average water depth of approximately 7.5 m and an area of approximately 144 km² (Zhang et al. 2009). Water renewal between the bay and the Yellow Sea is driven by a semi-diurnal tide with the largest tide range of 3.5 m. When the tide is rising, the tidal water enters the bay from the north, rotates counterclockwise, and flows out from the south through the west coast of the bay. The ebb tide has the reverse process, and the velocity of the residual flow is slow (Chinese Gulf Compilation Committee 1991). The main rivers emptying into the bay include the Sanggan, Ba, Shili, and Gu Rivers, with the total annual water discharge ranging between 1.7×10^8 and 2.3×10^8 m³ yr⁻¹ (Jiang et al. 2015). The largest river, i.e. the Gu, provides almost 70% of the total water discharge.

SGB has been used for aquaculture since the mid-1980s and is among the largest aquaculture sites in China (Guo et al. 1999, Zhang et al. 2009, Jiang et al. 2015). About 2/3 of the bay area is used for farming of bivalve shellfish, seaweed, and fish, with 4 major types of culture model, i.e. the monoculture of kelps, the monoculture of scallops, the monoculture of oysters, and the polyculture of kelps and bivalves (Fig. 1) (Shi et al. 2013). The main cultivation method is long-line culture, and cultivated species include kelp *Saccharina japonica*, scallops *Chlamys farreri*, and oys-

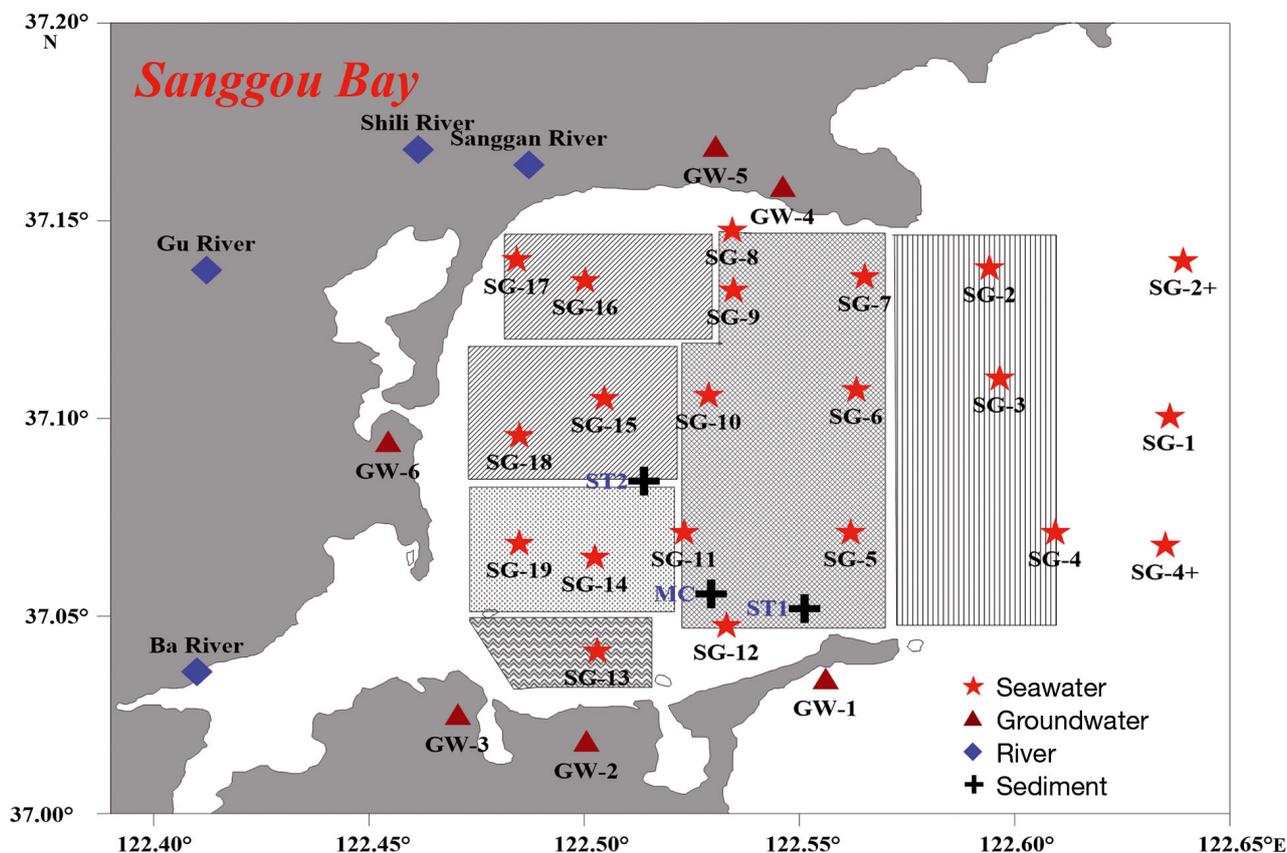


Fig. 2. Sampling locations in Sanggou Bay. Polygons delineate aquaculture types, see Fig. 1

ters *Crassostrea gigas* (Zhang et al. 2009). Kelp is tied to ropes and scallops are contained either in lantern nets or by ear-hanging (Zeng et al. 2015). Macroalgae (i.e. kelp) are grown outside the bay and only between November and May (Fang et al. 1996). During the seeding and harvesting period, kelp competes with phytoplankton for the assimilation of dissolved inorganic nitrogen. The aquaculture of bivalve shellfishes occurs from early spring to November. Shellfishes filtrate and ingest particulate matter and digest phytoplankton and particulate organic matter (POM), especially oysters when they have spawned in August, demanding more energy and stored substances (Mao et al. 2006). Most fish culture is clustered together in the southern part of the bay where water is calm and cages are within easy access from the shore (Fig. 1).

Water sampling and analysis

Eight cruises were carried out in SGB during June and September 2012, April, July, and October 2013, January and May 2014, and May 2015. The sampling

locations are shown in Fig. 2. Duplicate samples of surface and bottom seawater were collected using 10 l Niskin bottles, and then filled into 116 ml glass bottles. After overflow of approximately 1.5- to 2-fold of bottle volume, 1 ml of saturated solution of HgCl_2 was added to inhibit microbial activity. The sample bottle was then immediately sealed with a butyl rubber stopper and an aluminum cap and stored upside down in a dark box (Zhang et al. 2008). All water samples were analyzed after return to the shore laboratory within 60 d of collection (Zhang et al. 2004). Salinity and seawater temperature were measured with a multi-parameter probe (WTW 350i), and wind speeds were measured with an anemometer at about 10 m above the sea surface.

To evaluate the CH_4 input from potential terrestrial sources, water samples were collected from 4 rivers (Sanggan, Ba, Shili, and Gu) and 6 groundwater wells (GW1–GW6, Fig. 2) along the shoreline of SGB in June and September 2012, April, July, and October 2013, and January 2014. River water and groundwater were collected using a 5 l plastic sampler, and the samples were processed as for seawater described above.

Dissolved CH₄ in seawater was measured using a gas-stripping method described by Zhang et al. (2004). After purging with high-purity N₂, samples were passed through a drying tube with calcium chloride to remove water vapor. CH₄ was then separated on a 3 m × 3 mm i.d. stainless steel column packed with 80/100 mesh Porapak Q and measured with a gas chromatograph (Shimadzu, model GC-14B) equipped with a flame ionization detector (FID) (Zhang et al. 2004). FID responses were calibrated using known volumes of CH₄ standards (2.05, 4.22, and 50.4 ppmV; Research Institute of China National Standard Materials). The FID response signal and CH₄ concentration had a linear relationship, so a multi-point calibration method was used to determine CH₄ concentration based on chromatographic peak areas. The precision of this method was about 3% (Zhang et al. 2004).

Sediment sampling and incubation experiments

CH₄ emission from the sediments was measured by the closed chamber incubation method previously described by Sun et al. (2015), which was modified from Barnes & Owens (1999). Sediment samples were collected by a box corer at different sampling stations (Fig. 1), and only samples with undisturbed sediment surfaces were used. At each station, 15 sediment cores were collected using plexiglass tubes (i.d. = 5 cm, height = 30 cm) and sealed using air-tight rubber bungs. After ambient bottom water was added carefully with no gas headspace, the core was capped with a plexiglass top with 2 sampling ports. All cores were placed in a water-filled tank held at ambient room temperature, and the overlying water was stirred by magnetic stirrers rotated at 60 rpm. Ten glass bottles filled with ambient bottom water were placed in the same tank as water column controls. Cores and bottled waters were incubated in the dark for ~24 to 48 h. Overlying water samples (56.5 ml) from 3 cores were collected each time at intervals of 4 to 8 h to measure CH₄ concentration. At the same time, 2 bottled water samples were also treated with 0.5 ml HgCl₂ as a water column control. The CH₄ concentrations of all samples were measured by the gas-stripping method described above. Sediment–water CH₄ flux was estimated from the slope of the CH₄ increase in the overlying water versus time. The discrepancy in the CH₄ emission rate that resulted from differences between incubation and *in situ* temperatures was calibrated by the Arrhenius empirical equation as described by Aller et al. (1985) and Song et al. (2016).

Water incubation experiments

Time series incubation experiments were conducted to determine net CH₄ production-oxidation rates and understand the potential production mechanism in April, July, and October 2013 and January, May, and September 2014. To test for the effects of methylated compounds on CH₄ production, surface water samples were incubated with or without the addition of dimethylsulfoniopropionate (DMSP; final concentration 50 μM), or trimethylamine (final concentration 1 μM), or with added 2-bromoethane sulfonic acid (BES; final concentration 10 mM) to inhibit methanogenesis, and the CH₄ concentrations were monitored for more than 9 d in October 2013. Seawater for incubation experiments was transferred from the 10 l polyvinylchloride sampling bottles into clean polycarbonate carboys before the start of each experiment. DMSP, trimethylamine, or BES were added to the final concentration, and subsamples were transferred into 56.5 ml glass serum bottles that were capped with gas-tight Teflon-lined silicone stoppers and crimp-sealed with aluminum caps. Subsamples with no added reagents were used as controls. The incubations were conducted at approximately *in situ* water temperatures (3°C for January, 10°C for April, 15°C for May, 26°C for July, 23°C for September, 18°C for October) under an approximately 12:12 h light:dark cycle. Duplicate water samples were poisoned by addition of 0.5 ml of saturated HgCl₂ solution at 1 to 2 d intervals and analyzed for CH₄ as described above. Two additional samples for dissolved oxygen (DO) were collected and measured using the Winkler titration method (Bryan et al. 1976). Net CH₄ production-oxidation rates were estimated from the initial slope of the increase of CH₄ over time.

Saturation and flux calculation

The saturation (R , %) and sea-to-air fluxes of CH₄ (F , μmol m⁻² d⁻¹) were calculated using the following equations:

$$R = C_{\text{obs}}/C_{\text{eq}} \times 100\% \quad (1)$$

$$F = k_w(C_{\text{obs}} - C_{\text{eq}}) \quad (2)$$

where C_{obs} is the observed concentration of dissolved CH₄, and C_{eq} is the air-equilibrated seawater CH₄ concentration calculated from the *in situ* temperature and salinity using the equation of Wiesenburg & Guinasso (1979). Atmospheric CH₄ was not measured during these cruises. Therefore, mean atmospheric

CH₄ mixing ratios of 1.896, 1.901, and 1.929 ppm by volume (ppmv) at 3 observation stations near the coastal seas of China (NOAA Stns LLN, TAP, and SDZ) for 2012, 2013, and 2014, from the NOAA/ESRL Global Monitoring Division *in situ* program (www.esrl.noaa.gov/gmd), were used for calculations. We found that the variation of assumed atmospheric CH₄ concentrations in the range of 1.85 to 1.95 ppmv make differences less than $\pm 2\%$ in the computed air–sea CH₄ fluxes. Hence use of the annual mean atmospheric CH₄ concentration from monitoring networks for the sea–air flux calculation will not introduce significant errors. k_w is the gas transfer coefficient in cm h⁻¹, which is a function of wind speed and Schmidt number (Sc). Various empirical equations were employed to estimate k , among which the equations from Liss & Merlivat (1986) and Wanninkhof (1992) were used most frequently and represent the estimation in a lower and higher level, respectively. Nightingale et al. (2000) proposed a gas exchange relationship that shows a dependence on wind speed, and the corresponding value lies near the median of extensive methods and models. Wanninkhof (2014) recently updated the most frequently used method of Wanninkhof (1992), and this update reflects advances that have occurred over the last 2 decades in quantifying the gas transfer coefficient. Hence the methods from Nightingale et al. (2000) and Wanninkhof (2014) (hereafter N2000 and W2014) were chosen to calculate air–sea fluxes in this study.

RESULTS

Water column CH₄ and other parameters

Table 1 shows the temperature, salinity, and CH₄ concentration in surface and bottom waters of SGB during the 8 cruises. Water temperature ranged from

3.1 to 23.2°C, with the extremes in January and September. Salinity varied slightly, from 29.3 to 31.6 psu, with the lowest in September. DO in the water column ranged from 4.6 to 12.3 mg l⁻¹ with an average of 10.2 ± 2.6 for June 2012, 6.3 ± 0.8 for September 2012, 9.8 ± 0.8 for April 2013, 9.1 ± 1.3 for July 2013, 10.5 ± 1.9 for January 2014, and 6.2 ± 0.4 mg l⁻¹ for May 2015. DO in the surface water is usually comparable or slightly higher than at the bottom. Suspended particulate matter (SPM) in surface and bottom waters, respectively, was 18.6 ± 2.8 (mean \pm SD) and 26.9 ± 13.9 mg l⁻¹ for September 2012, 13.6 ± 9.1 and 14.9 ± 11.6 for April 2013, 11.3 ± 6.9 and 37.6 ± 26.8 for July 2013, 14.1 ± 5.7 and 27.6 ± 15.0 for October 2013, and 15.9 ± 14.1 and 13.5 ± 12.6 mg l⁻¹ for January 2014. Obvious high bottom SPM was observed during the period from July to October. CH₄ concentrations in the water column ranged between 3.0 and 356 nM, and showed clear seasonal variation, with higher levels occurring in summer and autumn and lower levels in winter and early spring. CH₄ concentrations in autumn were comparable to those in summer and about 7- to 8-fold higher than those in winter and spring. Bottom CH₄ concentrations were higher than those at the surface during all cruises except in September 2012, during which surface CH₄ was 50% higher than in bottom water together with the lowest salinity among all cruises.

Geographical distributions of CH₄ in SGB

Fig. 3 shows the geographical distributions of temperature, salinity, and CH₄ in surface waters of SGB measured during this study. Two cruises were carried out in spring (April 2013 and May 2015), during which the kelp thrived and the long kelp enhanced frictional effects in the upper layers and influenced the water exchange between the bay and the Yellow

Table 1. Water temperature (°C), salinity (psu), and CH₄ concentrations (nM) in surface and bottom waters of Sanggou Bay, China, during 8 cruises. Mean \pm SD

Date (yyyy-mm)	Station	Temperature		Salinity		Surface CH ₄		Bottom CH ₄	
		Surface	Bottom	Surface	Bottom	Range	Average	Range	Average
2014-01	21	3.5 \pm 1.1	3.1 \pm 1.1	31.3 \pm 0.1	31.3 \pm 0.1	4.3–10.1	6.2 \pm 1.4	4.3–19.4	8.7 \pm 4.95
2013-04	20	7.7 \pm 1.2	7.8 \pm 1.2	31.6 \pm 0.2	31.6 \pm 0.2	5.2–10.1	7.2 \pm 1.3	5.6–10.6	8.0 \pm 1.5
2014-05	10	13.8 \pm 2.2	14.1 \pm 1.1	31.3 \pm 0.4	31.3 \pm 0.3	3.0–24.9	9.3 \pm 7.9	3.0–29.5	12.5 \pm 8.4
2015-05	21	14.2 \pm 2.4	12.6 \pm 1.8	31.5 \pm 0.2	31.4 \pm 0.1	5.4–43.4	22.6 \pm 10.1	5.3–46.7	25.0 \pm 11.0
2012-06	20	18.6 \pm 3.2	17.3 \pm 2.1	30.6 \pm 0.3	30.4 \pm 0.2	12.4–91.3	38.3 \pm 21.9	5.8–98.4	43.9 \pm 27.9
2013-07	27	21.3 \pm 1.9	18.4 \pm 0.8	29.8 \pm 1.2	30.4 \pm 0.1	10.8–82.1	53.0 \pm 17.3	7.5–114	75.9 \pm 23.4
2012-09	13	23.2 \pm 0.9	22.3 \pm 1.0	29.3 \pm 1.0	29.8 \pm 0.7	23.5–128	53.8 \pm 33.5	21.7–136	35.5 \pm 32.8
2013-10	21	18.6 \pm 1.4	18.6 \pm 0.9	30.0 \pm 0.2	29.9 \pm 0.1	21.5–302	63.8 \pm 59.5	34.3–356	99.6 \pm 81.4

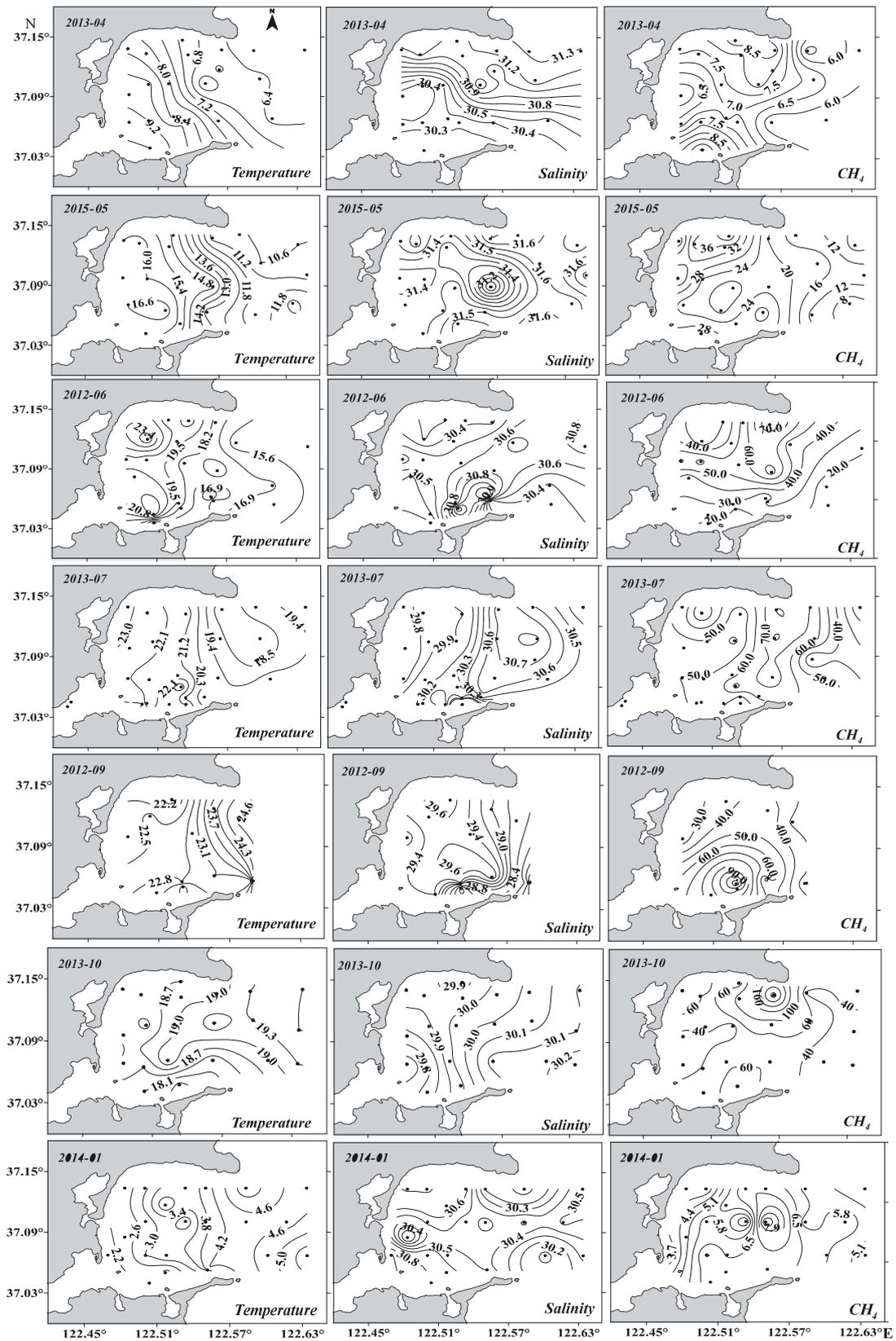


Fig. 3. Geographical distributions of water temperature (°C), salinity, and CH₄ concentration (nM) in surface waters of Sanggou Bay (SGB) during 2012 to 2015 (dates are yyyy-mm)

Sea (Zeng et al. 2015). Surface water temperature was higher in May than in April and presented a similar trend, which decreased gradually from nearshore to offshore, and showed an obvious gradient. In contrast, surface salinity increased from nearshore to offshore in May, while it decreased gradually from the northeast to the southwest in April. Dissolved CH₄ in April decreased gradually from the southwest and the northeast to the central bay, with concentrations ranging between 6 and 8 nM. In May, CH₄ concentrations in the inner bay were higher than those in outer bay, with highest dissolved CH₄ occurring in the northern part of the bay.

During summer cruises (June 2012 and July 2013), kelps had already been harvested, and water exchange with the Yellow Sea was not influenced by suspended kelp. Water temperature had a similar trend as in spring, i.e. decreasing from nearshore to offshore. Surface salinity varied in a narrow range (30.4–31.0) in June and increased slightly from the coast to the center of the bay, while in July, salinity increased gradually from the inner to the outer bay with low salinity (<30) in nearly half of the bay. CH₄ concentrations were much lower in June (mean \pm SD: 38.3 \pm 21.9 nM) than in July (53.0 \pm 17.3 nM), and decreased gradually from the inner to the outer bay. In July, dissolved CH₄ concentrations increased from about 50 nM in the inner bay to >70 nM at the mouth of the bay, then decreased to <40 nM in the outer bay.

During autumn (September 2012 and October 2013), water temperature increased gradually from the inner to the outer bay, but the gradient was less pronounced. Salinity was relatively low (<30) compared to other seasons due to heavy rainfall and freshwater input. In September, low salinity (<29) together with high CH₄ (>90 nM) was observed in the southern part of the bay. However, CH₄ concentrations presented an opposite trend in October, with the highest value (>140 nM) measured in the northeastern part of the bay, while salinity increased gradually from inner to outer bay.

During winter (January 2014), the Bohai South Coast Current enters the bay from the north and flows out from the south through the west coast of the bay (Sun et al. 2007). Surface seawater temperature decreased gradually from the outer to the inner bay, while surface salinity (30.2–30.6) showed little variation over the whole bay. Dissolved CH₄ concentrations in January were the lowest during the whole year, with highest CH₄ (8 nM) occurring near the center of the bay. It then decreased rapidly seaward to <4 nM at the bay mouth.

Riverine and groundwater input

Rivers and groundwater are potential sources for dissolved CH₄ in SGB. CH₄ concentrations in the main rivers around the bay are shown in Fig. 4, which ranged from 123 to 2190 nM and were 1 to 2 orders of magnitude higher than those (3–356 nM) observed in the water column of the bay. Riverine CH₄ also presented obvious spatial and seasonal variations. For example, lowest CH₄ values usually occurred in winter, and the highest values occurred in summer and early fall for the smaller rivers (i.e. Sanggan, Shili, and Ba Rivers), while higher CH₄ values occurred in winter and summer, and lower CH₄ occurred in spring and late fall in the Gu River.

Considering that the runoff from the Sanggan, Shili, and Ba Rivers is limited and we lack discrete flow rate data for each river, we attributed 70% of the total runoff to the Gu River and 30% to the other rivers (Jiang et al. 2015). Riverine CH₄ flux to the SGB was estimated to be 1.4×10^5 mol yr⁻¹, using the average CH₄ concentrations in the Gu River (665 nM) and other small rivers (857 nM), and mean annual runoff (2.0×10^8 m³).

Dissolved CH₄ concentrations in groundwater ranged from 1.6 to 405 nM and showed large spatial and temporal variations (Fig. 5). Groundwater near the mouth of the bay (GW4) had the highest CH₄ concentrations (26.2–255 nM, mean 98.6 nM), and Stn GW1 had medium values (6.7–109 nM, mean 40.7 nM), while those in the other areas usually had low CH₄ (<10 nM). For each region, lowest groundwater CH₄ usually occurred in winter (January) and early spring (April), while the highest values all occurred in late summer (September). In general, CH₄ concentrations in groundwater were much lower than those in rivers, but the submarine groundwater discharge to the SGB was ~50 times larger than river

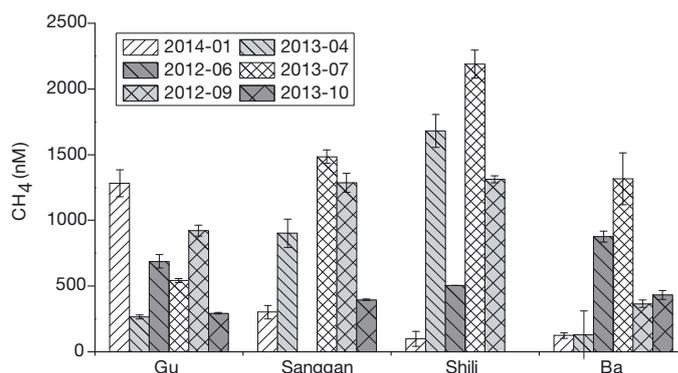


Fig. 4. CH₄ concentrations (mean \pm SD) in different rivers flowing into Sanggou Bay in different seasons and years (yyyy-mm)

runoff, and estimated to be $(2.59\text{--}3.07) \times 10^7 \text{ m}^3 \text{ d}^{-1}$ based on the non-conservative inventory of ^{226}Ra and ^{228}Ra in the water column (Wang et al. 2014). Hence, we estimated the CH_4 flux to the bay via the submarine groundwater by multiplying mean CH_4 concentration (40.2 nM) in the end-member well samples by the radium-derived mean submarine groundwater discharge ($2.83 \times 10^7 \text{ m}^3 \text{ d}^{-1}$). It yielded a flux of $4.2 \times 10^5 \text{ mol yr}^{-1}$, which was 3 times that of riverine CH_4 flux.

Sediment–water CH_4 fluxes

CH_4 fluxes across the sediment–water interface were measured at Stns MC, ST1, and ST2 (Fig. 6), among which Stns MC and ST1 were located in the kelp and bivalve polyculture zone, and Stn ST2 was located in the oyster monoculture zone. Sediment–water CH_4 fluxes from Stn ST1 ranged from 0.73 to $1.65 \mu\text{mol m}^{-2} \text{ d}^{-1}$ with a mean ($\pm\text{SD}$) of $1.19 \pm$

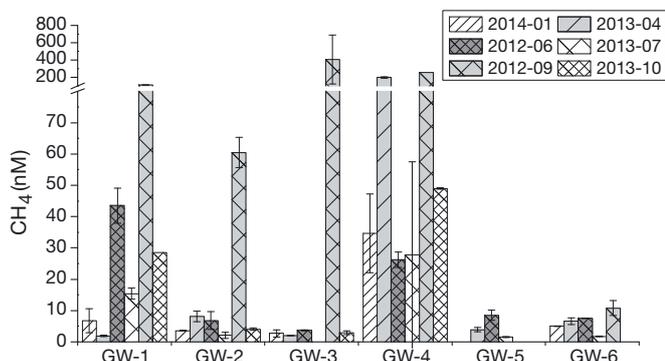


Fig. 5. CH_4 concentrations in groundwater measured at 6 stations (GW1–GW6) along the shoreline of Sanggou Bay in different seasons and years (yyyy-mm)

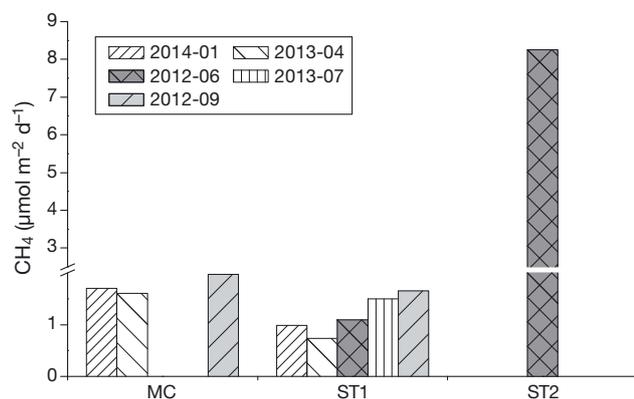


Fig. 6. Sediment–water CH_4 fluxes ($\mu\text{mol m}^{-2} \text{ d}^{-1}$) at Stns MC, ST1, and ST2 in Sanggou Bay (see Fig. 2) in different seasons and years (yyyy-mm)

$0.38 \mu\text{mol m}^{-2} \text{ d}^{-1}$, which showed obvious seasonal variation and correlated well with bottom water temperature ($F = 0.039T + 0.66$, $n = 5$, $r^2 = 0.67$). Sediment–water CH_4 fluxes from Stn MC ranged from 1.60 to $1.97 \mu\text{mol m}^{-2} \text{ d}^{-1}$ with a mean of $1.76 \pm 0.19 \mu\text{mol m}^{-2} \text{ d}^{-1}$. Sediment–water CH_4 fluxes from Stn ST1 ($1.19 \mu\text{mol m}^{-2} \text{ d}^{-1}$) was lower than that from Stn ST2 ($8.26 \mu\text{mol m}^{-2} \text{ d}^{-1}$) in June 2012. Although the sediment–water CH_4 flux from the oyster culture zone was measured only during 1 cruise, considering the seasonal variation trend in the polyculture zone, it is reasonable to deduct this flux as an annual average value. Based on the total surface area (about 144 km^2) (Zhang et al. 2009), average sediment–water CH_4 fluxes of $8.26 \mu\text{mol m}^{-2} \text{ d}^{-1}$ (bivalve culture zone) and $1.48 \mu\text{mol m}^{-2} \text{ d}^{-1}$ (polyculture zone), and assuming that the area ratio of the 2 culture zones is 1:1, annual CH_4 emission from sediments of SGB was estimated to be about $2.6 \times 10^5 \text{ mol}$.

Water column methane production-oxidation

Net water column CH_4 production-oxidation rates (CH_4 formation- CH_4 oxidation) were estimated to be 0.25, 0.41, 0.19, 0.39, and 0.31 nM d^{-1} for Stn MC in April and July 2013 and January, May, and September 2014, which showed significant seasonal variation and correlated well with temperature ($R = 0.01T + 0.19$, $n = 5$, $r^2 = 0.70$). Net water column CH_4 production-oxidation rates were 0.28 and 0.67 nM d^{-1} for Stns ST2 and SG-3 in September 2014. If we take the mean value of 0.42 nM d^{-1} at these stations as the net CH_4 production rate in the water column, together with the area of 144 km^2 and a mean water depth of 7.5 m , total net CH_4 production-oxidation in the water column was estimated to be $1.7 \times 10^5 \text{ mol yr}^{-1}$.

Surface CH_4 saturation and sea-to-air fluxes

CH_4 saturation in the surface waters of SGB ranged from 202 to 2734% (Fig. 7), with great spatial and temporal variation. Average saturation was higher in autumn and summer than in spring and winter. In general, the surface waters of SGB were all over-saturated with CH_4 , except for a few stations during winter. Thus, SGB is a net source of atmospheric CH_4 .

Sea-to-air CH_4 fluxes calculated with the W2014 equation ranged from 2.1 to $123 \mu\text{mol m}^{-2} \text{ d}^{-1}$ with a mean of $48.2 \mu\text{mol m}^{-2} \text{ d}^{-1}$, which was comparable to the results from the N2000 equation (2.3– $126 \mu\text{mol}$

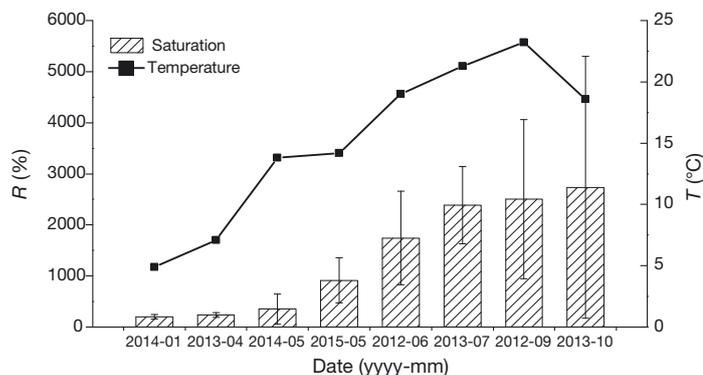


Fig. 7. CH₄ saturation (R , %; mean \pm SD) and water temperature (T , °C) in surface waters of Sanggou Bay

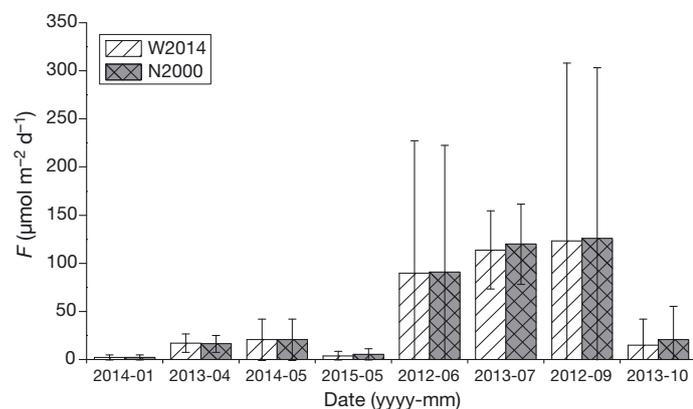


Fig. 8. Seasonal variation of air-sea CH₄ fluxes (F , $\mu\text{mol m}^{-2} \text{d}^{-1}$; mean \pm SD) in Sanggou Bay, estimated using 2 different equations (W2014 and N2000); see 'Materials and methods' for details

$\text{m}^{-2} \text{d}^{-1}$ with a mean of $50.3 \mu\text{mol m}^{-2} \text{d}^{-1}$; Fig. 8). CH₄ fluxes showed clear seasonal variation, with those in early autumn (September) comparable to those in summer (July and June), and more than 8-fold higher than those in spring (April) and autumn (October); the lowest values occurred in winter. In addition, we estimated the CH₄ emission from SGB to be $2.5 \times 10^6 \text{ mol yr}^{-1}$ based on the annual mean atmospheric CH₄ flux ($48.2 \mu\text{mol m}^{-2} \text{d}^{-1}$) from this study and the area of SGB (144 km^2).

CH₄ exchange with the Yellow Sea

Considering that dissolved CH₄ in SGB was much higher than in the adjacent Yellow Sea, water exchange with the Yellow Sea should cause net loss of CH₄ from the bay. Aquaculture activities may influence water exchange between SGB and the Yellow Sea; however, suspended kelp culture mainly changed

the spatial pattern of the tidal flux but not the tidal prism (Zeng et al. 2015). Jiang et al. (2015) estimated the annual water exchange volume to be about $8.3 \times 10^{10} \text{ m}^3$. CH₄ concentrations gradients between the inner and outer bay were 3.6 nM for September 2012, 1.5, 11.9, and 40.7 nM for April, July, and October 2013, and 0.9 nM for January 2014, respectively. During the kelp seeding to harvesting period (November to May), the CH₄ concentration gradient (mean: 1.2 nM) was lower than during the non-aquaculture period (mean: 18.7 nM). Hence, based on the annual mean observed CH₄ concentration gradient between the inner and outer bay (10.0 nM), and the annual water exchange volume of the bay ($8.3 \times 10^{10} \text{ m}^3$), we estimated the CH₄ flux exported out of the bay to be $8.8 \times 10^5 \text{ mol yr}^{-1}$.

DISCUSSION

Comparison with previous research

Previous studies have reported CH₄ concentrations for some bays, estuaries, and coastal and shelf areas of northern China (Table 2). Average CH₄ concentrations in SGB during the corresponding seasons were lower than those reported for the adjacent Rushan Bay (mean \pm SD: $59.9 \pm 7.8 \text{ nM}$, May 2007; Wang et al. 2008), Dalian Bay ($59.4 \pm 82.0 \text{ nM}$, January 2010; $56.0 \pm 69.4 \text{ nM}$, November 2009; Wang et al. 2011), and Jiaozhou Bay ($137 \pm 224 \text{ nM}$ for August 2003 and 34.8 ± 75.5 for December 2003; Zhang et al. 2007), but obviously higher than the Yellow River estuary ($3.9\text{--}14.3 \text{ nM}$; Gu et al. 2011) and Changjiang estuary ($7.95 \pm 5.24 \text{ nM}$; Zhang et al. 2008). CH₄ concentrations of SGB were 2- to 10-fold of those in the adjacent marginal shelf sea, i.e. the Yellow Sea ($3.4\text{--}12.0 \text{ nM}$; Zhang et al. 2004, Yang et al. 2010, Ye et al. 2016).

The estimated atmospheric CH₄ fluxes from the SGB in this study were close to those from Jiaozhou Bay (Zhang et al. 2007) and Dalian Bay (Wang et al. 2011), and slightly higher than those from some estuaries, e.g. the Changjiang estuary (Zhang et al. 2008). However, CH₄ fluxes from SGB were far higher than those from shelf areas, e.g. 2-fold higher than those from the adjacent North Yellow Sea during Spring (April), and 5 times higher than during winter (January) and summer (July for SGB, August for North Yellow Sea), respectively, but comparable to those from the adjacent North Yellow Sea during October (Yang et al. 2010). Hence SGB is a hot spot of CH₄ emissions to the atmosphere.

Table 2. Compilation of surface concentrations and sea-to-air fluxes of CH₄ in different coastal areas of China (see Fig. 2 for station locations). Data are mean ± SD

Sea area	Date	Sampling station	CH ₄ (nM)	Flux (μmol m ⁻² d ⁻¹)	Reference
Jiaozhou Bay	Aug 2003	16	137 ± 224	132 ± 220	Zhang et al. (2007)
	Dec 2003	14	34.8 ± 75.5	36.9 ± 87.3	Zhang et al. (2007)
Rushan Bay	May 2007	8	59.90 ± 7.75	–	Wang et al. (2008)
Dalian Bay	Jan 2010	17	59.41 ± 81.97	133.07 ± 193.37 ^a , 286.96 ± 416.98 ^b	Wang et al. (2011)
	Nov 2009	17	56.01 ± 69.39	52.88 ± 68.42 ^a , 113.46 ± 146.82 ^b	Wang et al. (2011)
Yellow River estuary	Jun 2009	28	3.9–14.3	7.2 ^a , 14.2 ^b	Gu et al. (2011)
Changjiang estuary	Dec 2004	10	7.95 ± 5.24	21.1 ± 9.6 ^a , 41.1 ± 18.7 ^b	Zhang et al. (2008)
East China Sea	Apr 2001	29	3.24 ± 0.59	1.63 ± 1.67 ^a , 2.77 ± 2.71 ^b	Zhang et al. (2004)
	Aug 2013	65	6.26 ± 4.96	6.5 ± 7.4 ^a , 11.5 ± 111.9 ^b	Ye et al. (2016)
Bohai Sea	Aug 2008	28	5.87 ± 2.02	3.1 ± 1.6 ^a , 8.1 ± 4.2 ^b	Li et al. (2010)
North Yellow Sea	Jan 2007	78	3.40 ± 0.58	0.2 ± 1.0 ^a , 0.4 ± 1.7 ^b	Yang et al. (2010)
	Aug 2007	76	6.43 ± 2.52	4.2 ± 4.7 ^a , 6.9 ± 7.3 ^b	Yang et al. (2010)
	Apr 2007	59	5.70 ± 3.50	11.8 ± 10.2 ^a , 21.1 ± 16.4 ^b	Yang et al. (2010)
	Oct 2006	80	12.02 ± 7.51	8.5 ± 12.7 ^a , 14.6 ± 22.3 ^b	Yang et al. (2010)
Yellow Sea	Mar-Apr 2001	14	3.43 ± 0.23	0.81 ± 0.50 ^a , 1.33 ± 0.76 ^b	Zhang et al. (2004)
Yellow and East China Seas	Aug 2011	38	8.21 ± 6.02	16.98 ± 21.40 ^c , 17.65 ± 21.72 ^d	Sun et al. (2015)
	Oct 2011	55	5.03 ± 1.68	9.88 ± 9.97 ^c , 9.87 ± 9.61 ^d	Sun et al. (2015)
	Dec 2011	59	4.07 ± 0.63	6.82 ± 6.86 ^c , 6.64 ± 6.44 ^d	Sun et al. (2015)

^ak_w was estimated by the LM86 equation (Liss & Herliav 1986)
^bk_w was estimated by the W92 equation (Wanninkhof 1992)
^ck_w was estimated by the W2014 equation (see 'Materials and methods' for details)
^dk_w was estimated by the N2000 equation (see 'Materials and methods' for details)

Factors influencing spatial and temporal distribution of CH₄ in SGB

The concentration, saturation, water column production, and sediment–water fluxes of CH₄ in SGB all had obvious seasonal variation and were closely related to water temperature. Mean water column CH₄ concentrations and saturations during different cruises correlated positively with mean water temperature (CH₄ conc. = 2.33T – 4.70, r² = 0.93, n = 11, p < 0.0001; R(CH₄) = 124.8T – 380.4, r² = 0.8 n = 7, p < 0.008). This is consistent with the positive correlations observed for net water column CH₄ production-oxidation rates at Stn MC (R = 0.01T + 0.19, n = 5, r² = 0.82, p < 0.09) and sediment–water CH₄ fluxes at Stn ST1 (F = 0.039T + 0.66, n = 5, r² = 0.82, p < 0.09), which showed that CH₄ production rates in both water column and sediments increase with rising temperature. Temperature mainly controls the organic matter decomposition and the activity of methanogenesis, and the rising temperature may increase the relative abundance and diversity of methanogenic communities (Metje & Frenzel 2005, Høj et al. 2008). Yvon-Durocher et al. (2014) also reported seasonal variation in CH₄ emissions from diverse ecosystems using meta-analysis, and showed that CH₄ emissions

increased significantly with seasonal increases in temperature. Hence our results suggest that water temperature plays a significant role in regulating the seasonal variation of CH₄ in SGB.

Although the salinity of SGB only showed a slight fluctuation throughout the year, mean water column CH₄ concentrations during different cruises correlated negatively with mean salinity (S) (CH₄ conc. = –0.33S + 5.43, r² = 0.94, n = 11, p < 0.001), suggesting that terrestrial input (i.e. rivers and groundwater) also play a role in the seasonal variation of CH₄ in the bay. In general, rivers and groundwater are primary routes for delivery of dissolved and particulate carbon and nutrients from land to coastal areas, and they are usually supersaturated with CH₄ (Taniguchi et al. 2002, Striegl et al. 2012). Observed CH₄ (123.3 to 2189.7 nM) in rivers around SGB are within the CH₄ range (5–5000 nM) reported for rivers worldwide (de Angelis & Lilley 1987, Upstill-Goddard et al. 2000) and much higher than those in the water column of the bay. Especially during the wet seasons (summer and early autumn), high discharges of river water and groundwater with rich CH₄ enter the bay, affecting its spatial distribution. For example, observed surface CH₄ in the bay was 50 % higher than that in bottom water in September 2012 together

with the lowest salinity (Table 1), while CH₄ concentrations were usually higher at the bottom than at the surface during other cruises.

CH₄ distribution in SGB may also be influenced by aquaculture activities. The bay is extensively used for culture of macroalgae and shellfish. Previous studies showed that CH₄ can be produced in anaerobic microenvironments of SPM and digestive tracts of zooplankton and fish in oxygenic surface water (Marty 1993, Karl & Tilbrook 1994). Obvious high bottom SPM was observed during the shellfish culture period from July to October. Dense populations of bivalve shellfish (i.e. scallop and oyster) in shallow water can produce a large amount of feces and pseudo-feces, hence the digestive tract of shellfish and their waste provide favorable environments for potential water column methanogenesis. Suspended shellfish culture also accelerates biodeposition and results in sediments with rich organic matter and high microbial activity (Green et al. 2012), which in turn enhances rates of anaerobic decomposition of organic matter and lead to high CH₄ production in the sediment (Nizzoli et al. 2006, Jiang et al. 2015). Due to the sediment release, bottom CH₄ concentrations in SGB were usually higher than those at the surface, especially in the culture zones with bivalve shellfish in summer (Fig. 9). For example, we observed CH₄ concentrations at Stn ST2 in the oyster culture zone to be 4.0 nM at the surface (0 m), 3.8 nM at 2 m, 5.9 nM at 4 m, and 12.0 nM at the bottom (6 m) in May 2014. We also observed significant high sediment–water CH₄ fluxes in the oyster culture zone (8.26 $\mu\text{mol m}^{-2} \text{d}^{-1}$) compared to the polyculture zone (1.09 $\mu\text{mol m}^{-2} \text{d}^{-1}$) in June 2012. Green et al. (2012) observed greater CH₄ emissions from the sediment in areas that had the highest cover of oysters compared to areas with medium cover. They attributed this to more CH₄ produced by the reduction of CO₂ and the stimulation by the ‘priming effect,’ whereby the addition of fresh labile organic matter (such as from oyster biodeposits) temporarily stimulates microbial decomposition, including that of older, buried, recalcitrant organic matter (Green et al. 2012).

Recent research has shown that under certain nutrient-limited conditions, a variety of methyl-rich organic phosphorus or sulfur compounds are likely to be utilized by microorganism and serve as precursors of CH₄ production in aerobic surface waters (Damm et al. 2008, Karl et al. 2008, Zindler et al. 2013). Integrated multi-trophic aquaculture in SGB can enhance the recycling of organic matter and nutrients and provide favorable conditions for aerobic CH₄ production. Bivalve shellfish ingest algae, POM, bac-

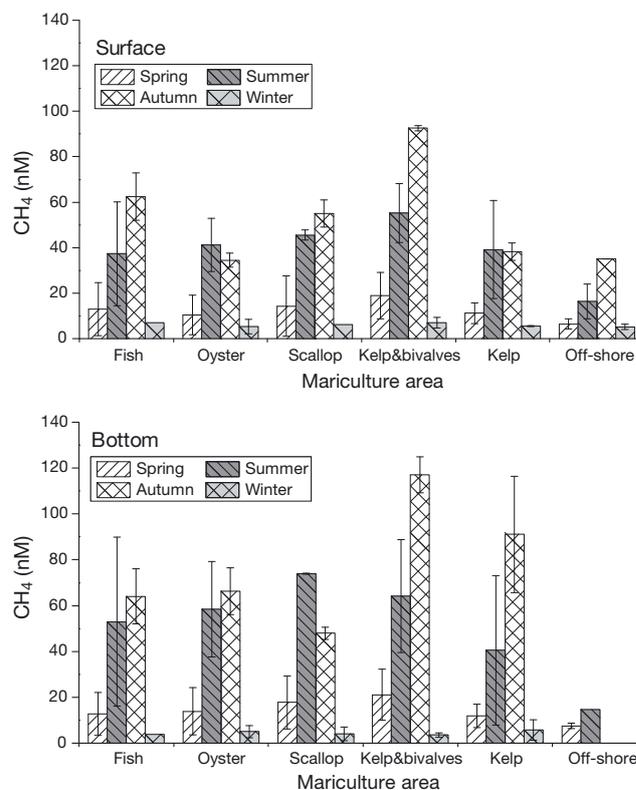


Fig. 9. Comparison of seasonal mean (\pm SD) CH₄ concentrations in surface and bottom waters in different culture areas of Sanggou Bay

teria, and other micro-organisms, while kelp absorbs organic and inorganic wastes from the shellfish (Fang et al. 1996). Commonly cultivated seaweeds (*Gracilaria lemaneiformis* and *Laminaria japonica*) in SGB have a high nutrient uptake efficiency (Mao et al. 2009, Xu et al. 2011) and may lead to nitrogen and phosphorus limitation in spring as well as phosphorus limitation in summer (Zhang et al. 2010), which in turn implies a potential formation of CH₄ from methyl-rich organic phosphorus or sulfur compounds (Damm et al. 2008, Karl et al. 2008, Zindler et al. 2013). DMSP can be produced by macroalgae, and grazing by bivalves appears to facilitate the release of DMSP from kelp (Smit et al. 2007). Hence the water column in polyculture zones may contain higher levels of DMSP and enhance the production of CH₄. Seasonal variation in mean CH₄ in different mariculture areas of SGB support this hypothesis and show that higher surface CH₄ concentrations usually occur in the polyculture areas of kelp and bivalve shellfish (Fig. 9). We also observed a highly significant and rapid increase in CH₄ during incubations with DMSP spikes in October 2013 (Fig. 10). During the incubations, CH₄ concentration increased sharply by more than 60-fold and reached 460 nM on Day 3.5, then decreased

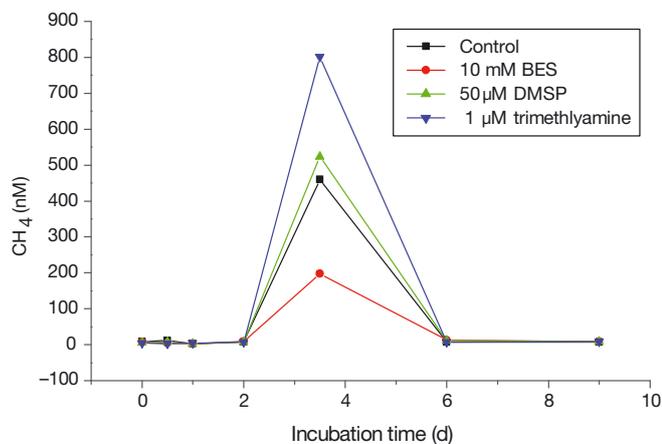


Fig. 10. CH₄ time series incubation experiments at Station MC (see Fig. 2) during October 2013. BES: 2-bromoethane sulfonic acid, DMSP: dimethylsulfoniopropionate

sharply to the initial concentration around 7 nM. The addition of 10 μM BES reduced the CH₄ production by more than half, while the addition of 50 μM DMSP and 1 μM trimethylamine significantly enhanced CH₄ production. During all of these incubations, DO in the bottles ranged from 5 to 8 mg l⁻¹, suggesting that CH₄ might be produced under aerobic conditions with the degradation of methylated compounds such as DMSP and trimethylamine in the water column in SGB.

Preliminary CH₄ budget and its implication

In order to understand the contributions of different sources and sinks to dissolved CH₄ in SGB, a preliminary CH₄ budget was constructed, although there are still great uncertainties in the estimate of each term. Considering that the sea–air flux values from W2014 and N2000 were quite similar, we took the results estimated by W2014 for the budget estimation. From the budget (Fig. 11), we can see that the groundwater input (4.2×10^5 mol yr⁻¹) was the largest quantified CH₄ source, followed by sediment

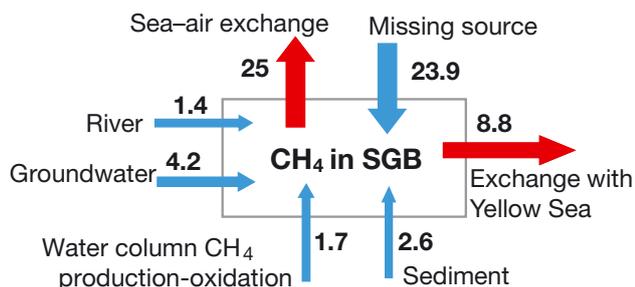


Fig. 11. Preliminary CH₄ budget (10^5 mol yr⁻¹) estimation for Sanggou Bay (SGB)

release (2.6×10^5 mol yr⁻¹) and riverine input (1.4×10^5 mol yr⁻¹), while sea-to-air release (2.5×10^6 mol yr⁻¹) and export from the bay to the Yellow Sea (8.8×10^5 mol yr⁻¹) were the dominant sinks for CH₄ in SGB. Net water column production-oxidation was estimated preliminarily to produce 1.7×10^5 mol CH₄ yr⁻¹. However, there is still a large imbalance between the sources and sinks of methane in the water column of SGB, with an apparent missing source of 2.4×10^6 mol yr⁻¹ needed to balance the budget, although this value might be overestimated due to the propagation of the errors in the other terms in the budget.

Because of the large spatial and temporal variations in CH₄ concentrations in the groundwater samples, our ability to provide an accurate estimate for the CH₄ flux via submarine groundwater discharge is rather limited due to the small number of groundwater end-member samples used in the calculation ($n = 6$) and lack of seasonal variation in groundwater fluxes. Hence the source item of groundwater may have large uncertainties.

Previous studies have demonstrated that sediments are a significant CH₄ source for bays and coastal waters (Sansone et al. 1998, Ferrón et al. 2010). Ferrón et al. (2010) found that benthic CH₄ fluxes from the shelf of the Gulf of Cádiz ranged from 0.5 to 24.1 μmol m⁻² d⁻¹ with an average of 5 ± 6 μmol m⁻² d⁻¹ using benthic chambers. Sansone et al. (1998) reported that CH₄ benthic fluxes from Tomales Bay ranged from 0.4 to 16 μmol m⁻² d⁻¹ with an average of 5.5 and 2.5 μmol m⁻² d⁻¹ for summer and winter, respectively. These results are comparable to or slightly higher than our results (0.7–8.3 μmol m⁻² d⁻¹) for SGB. However, given the high rates of labile organic matter loading in SGB, this strongly indicates that the fluxes assessed during the sediment incubations in this study might be underestimated. The most influential factors for this underestimation are likely to be an insufficient number of sampling stations and the *ex situ* sediment incubation method we used. Sediment–water CH₄ fluxes from SGB showed large spatial variation, although we only measured benthic fluxes at 3 stations and did not measure benthic fluxes from eelgrass beds in the southern region of SGB near Chudao. Previous studies showed that CH₄ benthic fluxes from eelgrass beds may be about 10-fold higher than those from unvegetated areas in Tomales Bay (Sansone et al. 1998). Due to lack of a benthic chamber, the emission of CH₄ from sediments was measured by a modified closed chamber incubation method (Barnes & Owens 1999). This method changes the environment (i.e. pressure and

temperature) and cannot simulate real *in situ* conditions such as resuspension, deposit feeding, burrowing, and irrigation, which often significantly change the geochemical characteristics of sediments and overlying water and increase the benthic fluxes (Sansone et al. 1998, Upstill-Goddard et al. 2000). Hence, an underestimation of the CH₄ released from the sediments may account for part of the missing source.

Underestimating *in situ* water column production is also likely to contribute to the missing source. As discussed above, CH₄ might be produced in aerobic water columns of the bay with the degradation of methylated compounds such as DMSP produced from the macroalgae, and grazing by bivalves appeared to facilitate the release of DMSP from kelp (Smit et al. 2007). However, the time series incubation method employed in this study only focuses on the microbial activity in the water column itself and neglects the interaction of algae, shellfish, and microbes, which may result in great underestimation of potential contributions from *in situ* production in the water column. If the *in situ* production in the water column is indeed the only missing source for CH₄ budget in the bay, the net water column production-oxidation rate is estimated to be about 7 nM d⁻¹. This is reasonable based on our incubation results in October 2013, which showed a net water column CH₄ increase of ~40 nM d⁻¹ with a DMSP spike. However, our incubation results also showed that CH₄ produced might be oxidized rapidly. Hence it is difficult to evaluate how much CH₄ is accumulated in the water column over time. Considering the complicated interactions between macroalgae, shellfish, and microbes, mesocosm experiments should be carried out in the future to further understand the *in situ* water column CH₄ production and consumption and to understand the CH₄ budget in multi-trophic aquaculture systems like that in SGB.

CONCLUSIONS

CH₄ concentrations in SGB showed obvious seasonal and spatial variation. CH₄ concentrations were 3 to 10 times higher in summer and autumn than in spring and winter. Bottom CH₄ concentrations were obviously higher than those in the surface water due to sediment release. Higher surface CH₄ concentrations occurred in the polyculture areas of kelp and bivalves. Seasonal variation in water temperature, terrestrial freshwater input, and aquaculture activities play significant roles in regulating the spatial and temporal variation of CH₄ in the bay. Ground-

water input (4.2×10^5 mol yr⁻¹) was the largest quantified source of CH₄, followed by sediment release (2.6×10^5 mol yr⁻¹), and riverine input (1.4×10^5 mol yr⁻¹), while sea-to-air release (2.5×10^6 mol yr⁻¹) and export from the bay to the Yellow Sea (8.8×10^5 mol yr⁻¹) were the dominant CH₄ sinks. Net water column production-oxidation was estimated preliminarily to produce 1.7×10^5 mol CH₄ yr⁻¹; however, this value may have been underestimated due to the neglect of interactions between algae, shellfish, and microbes. There was a great imbalance of sources and sinks, with an apparent missing source of 2.4×10^6 mol yr⁻¹, most of which might be attributed to underestimates of *in situ* water column production and CH₄ released from the sediments. Benthic chamber measurements and mesocosm experiments should be carried out in the future to further understand the CH₄ budget in multi-trophic aquaculture systems like that in SGB.

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