

Contrasting Diffusive Methane Emission from Two Closely Situated Aquaculture Ponds of Varying Salinity Situated in a Wetland of Eastern India



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Abstract Inland aquaculture practice is becoming popular throughout the world to suffice the increasing protein demand of the growing population. Aquaculture ponds in general emit methane (CH₄) towards the atmosphere. However, available data are scarce from India, where the number of aquaculture plots is growing at a fast pace. We measured the partial pressure of CH₄ in surface water [pCH₄(w)], the atmosphere-pond CH₄ fluxes, and several relevant biogeochemical parameters in sewage-fed freshwater (FWP) and oligohaline (OHP) aquaculture ponds situated in an eastern Indian wetland. We hypothesized that pCH₄(w) and the atmosphere-pond CH₄ effluxes would significantly vary between FWP and OHP as salinity plays a crucial role in regulating the methanogens in any water column. Measurements were carried out in both FWP and OHP throughout an annual cycle. FWP and OHP emitted CH₄ at the rate of $22.4 \pm 16.2 \text{ mg m}^{-2} \text{ h}^{-1}$ and $13.4 \pm 13.6 \text{ mg m}^{-2} \text{ h}^{-1}$, respectively. Apart from low salinity, turbidity was higher in FWP, which in turn led to reduced photosynthetic activities and lower dissolved oxygen levels compared to OHP. pH was also substantially lower in FWP compared to OHP. More anaerobic and low pH conditions in FWP compared to OHP favored methanogenic activities and methane oxidation was discouraged, which led to higher atmosphere-pond CH₄ fluxes from FWP compared to OHP. However, both FWP and OHP exhibited annual mean CH₄ effluxes much higher than the efflux rates observed in most of the Chinese aquaculture ponds.

Keywords Methane emission · GHG · Aquaculture · Sewage-fed · Freshwater · Brackish water · Wetland · East Kolkata Wetland · Sundarban Biosphere Reserve

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1 Introduction

Aquaculture ponds have become an essential land-use class and encompass a substantial part of the surface water ecosystems of the Earth (Yang et al., 2018a). Since the 1970s, aquaculture ponds have come up as an alternative to capture fisheries and it has been serving well to meet the ever-increasing demand for aquatic foods like fish, shrimp, crabs, etc. (Hu et al., 2012). Distributed over a wide range of tropical to temperate regions, the freshwater and brackish water aquaculture ponds comprise an area of about 1,10,832 km² throughout the world (Verdegem & Bosma, 2009). However, like many other inland lentic ecosystems (e.g. lakes, ponds, reservoirs), aquaculture ponds have been also found to emit a substantial quantity of carbon dioxide, methane, and nitrous oxides (Boyd et al., 2010; Chen et al., 2016; Yang et al., 2015a). Among the several greenhouse gases, methane (CH₄) emission has perhaps received the highest attention (Bastviken et al., 2011; Hu et al., 2014, 2016). The aquaculture ponds receive a substantial amount of organic load which when remains unutilized acts as a substrate for the microbes to act upon and under anaerobic conditions, the methanogens produce a substantial amount of CH₄ from it (Yang et al., 2019). According to the estimates made at the beginning of the present decade, inland aquatic bodies are capable of emitting 650 Tg C year⁻¹ in the form of methane (Bastviken et al., 2011). However, it is believed that these magnitudes include considerable uncertainty as the CH₄ emissions from shallow aquaculture ponds are mostly not considered while drawing these estimates (Long et al., 2016; Yang et al., 2018a). Moreover, field observations of CH₄ emission are still very few and mostly concentrated in Chinese pisciculture plots (Chen et al., 2016; Long et al., 2016; Yang et al., 2015a, 2018b, 2019).

CH₄ transport from the water column of any aquatic ecosystem towards the atmosphere mainly takes place through either diffusion (Chen et al., 2016) or ebullition (Dutta et al., 2013). A wide range of biotic and abiotic factors are known to regulate the production of CH₄ by the methanogens and its consumption by methanotrophs which in turn govern the partial pressure of CH₄ in surface water [pCH₄(w)] and hence the atmosphere-pond CH₄ flux (Yang et al., 2019). Earlier pieces of research have highlighted that water temperature plays a critical role in governing the CH₄ biogeochemistry (Knox et al., 2016; Olsson et al., 2015; Palma-Silva et al., 2013). Factors like pH (Hu et al., 2017), dissolved oxygen (DO) (Liu et al., 2015), primary productivity (Xiao et al., 2017), water table (Yang et al., 2013) and substrate availability (Venkiteswaran et al., 2013) also regulates the water column CH₄ production. In addition to these factors, one of the most important and decisive factors that substantially alters the pCH₄(w) is the salinity of the water (Hu et al., 2017; Vizza et al., 2017; Welti et al., 2017), based on which aquaculture ponds are differentiated into freshwater and brackish water categories. An increase in salinity is often found to reduce pCH₄(w) and hence lead to lower atmosphere-pond CH₄ fluxes (Yang et al., 2018b). Earlier studies exhibited that higher salinity leads to ion stress to methanogens (Chambers et al., 2013; Neubauer et al., 2013). It provides alternative electron acceptors like sulfate ion to the water medium, which in turn suppresses

the methanogens from producing CH_4 in the water column (Laanbroek, 2010; Sun et al., 2013). So far, very few attempts have been made to analyze the difference in $\text{pCH}_4(\text{w})$ dynamics in aquaculture ponds of varying salinity (Yang et al., 2018a, 2019) and most of these studies were carried out in highly saline mariculture ponds. Moreover, almost all of the studies where $\text{pCH}_4(\text{w})$ dynamics of aquaculture ponds are characterized are carried out in such aquaculture ponds where daily fish feeds are provided. Comparisons between sewage-fed freshwater aquaculture ponds and oligohaline aquaculture ponds are not at all available at the present date.

Keeping in view this background, the present research work was carried out to expand the knowledge by quantifying atmosphere-pond CH_4 fluxes from sewage fed freshwater as well as an oligohaline aquaculture pond in the eastern part of India, located in East Kolkata Wetlands (EKW), and Minakhan block, within the Sundarban Biosphere Reserve (SBR), respectively (West Bengal, India). It is worth mentioning that aquaculture practice is steadily increasing in India and they encompass a substantial area (7,900 km^2) of India's total areal extent (Adhikari et al., 2012). However, endeavors of quantifying the atmosphere-pond CH_4 fluxes from such water bodies are very few (Adhikari et al., 2012; Pathak et al., 2013). This is why; this data set generated from this study is expected to contribute to the global database of CH_4 fluxes from aquaculture ponds. Atmosphere-pond CH_4 fluxes have been found to exhibit potential variations in different seasons (Heyer & Berger, 2000). Thus we have carried out sampling all-round the year covering three seasons [monsoon season (June, July, August, and September), pre-monsoon season (February, March, April, and May), and post-monsoon season (October, November, December, and January)] for this piece of research. We hypothesized that $\text{pCH}_4(\text{w})$ dynamics and hence atmosphere-pond CH_4 flux would significantly vary between freshwater and an oligohaline aquaculture pond situated close and experience the same climate conditions due to different salinity. Following this hypothesis, the main aims of this research were to (i) characterize and compare the $\text{pCH}_4(\text{w})$ and atmosphere-pond CH_4 fluxes from freshwater as well as an oligohaline aquaculture pond throughout an annual cycle, (ii) examine seasonal variations of CH_4 fluxes from the two aquaculture ponds and (iii) characterize the relationship between $\text{pCH}_4(\text{w})$ and the associated biogeochemical factors, with special emphasis on salinity.

2 Methodology

2.1 Study Sites

The EKW (Fig. 1) lies on the east of the Kolkata metropolis. Being tagged as a 'wetland of international importance', EKW found its place in the list of Ramsar Sites in 2002. EKW is known to be the 'kidney of the city of Kolkata' because of its unique natural purification system (Kundu et al., 2008). This wetland complex stands tall as the largest conglomeration of human-built pisciculture ponds in the world.

EKW receives the wastewater load from the adjacent city of Kolkata and treats the water mass through activities like pisciculture, agriculture, and solid waste farms. In this way, the bulk sewage water load of Kolkata is naturally treated. The sewage canal flows into the Bidyadhari River that ends in the Bay of Bengal through the Sundarban mangrove ecosystem. According to the estimates of Aich et al. (2012), EKW has almost 250 functional aquaculture ponds that encompass 12 km². This system altogether produces substantial fish and vegetables that engage close to 0.5 million people and acts as the primary food source to the residents of the metropolis (Chaudhuri et al., 2012). EKW's performance has continued in this fashion since the late eighteenth century and it has been acting as an economic, ecosystem-resilient, and effective system of both aquatic and solid waste management (Kundu et al., 2008). These ponds are usually very shallow (1 to 1.5 m depth). The fishermen maintain a flat bottom in these ponds. These ponds vary in size from 10,000 m² to 100,000 m². The infrastructural characteristics of these ponds are portrayed in detail by Ghosh and Furedy (1984) and Ghosh (2005). The concept behind this natural engineering is discussed by Chaudhuri et al. (2007) and Chaudhuri et al. (2008). All the ponds within this system are mostly freshwater as a mixture of groundwater and sewage water is utilized for pisciculture in this setup.

The OHP which was sampled in this study is situated in the Minakhan community development Block situated almost 25 km to the east of EKW near the bank of Bidyadhari River. This block is a part of the SBR, known to shelter the world's largest continuous stretch of mangrove forest. Local people of this region mainly practiced agriculture since the 1970s (Naskar, 1985), however, after the construction of dikes and embankments by the Department of Irrigation, Govt. of West Bengal people started switching for aquaculture with the help of the oligohaline water flowing through the Bidyadhari River (Bunting et al., 2017). Coupled rice–shrimp farming is quite popular in Minakhan. In the present date, the number of pisciculture ponds has observed a drastic increase in the Minakahn Block (Mondal & Bandyopadhyay, 2015).

2.2 Sampling Strategy

Samples were collected once a month covering the entire annual cycle from March 2018 to February 2019. Sampling was conducted in two aquaculture ponds; one situated within the EKW (freshwater pond) and the other in Minakhan Block (oligohaline pond) [hereafter referred to as FWP (22.514744 N, 88.482124 E) (depth: 1.2 m; area: ~ 45,000 m²) and OHP (22.50693 N, 88.75452 E) (depth: 3 m; area: 54,000 m²) respectively]. Samples were collected at every 2 h intervals over a complete diel cycle. The ambient temperature, wind velocity, and atmospheric pressure were measured by deploying a portable weather station. The water surface physicochemical parameters were monitored in-situ using typical probes. For other parameters like CH₄ concentration in water and air, chlorophyll-*a* (chl-*a*), and biochemical

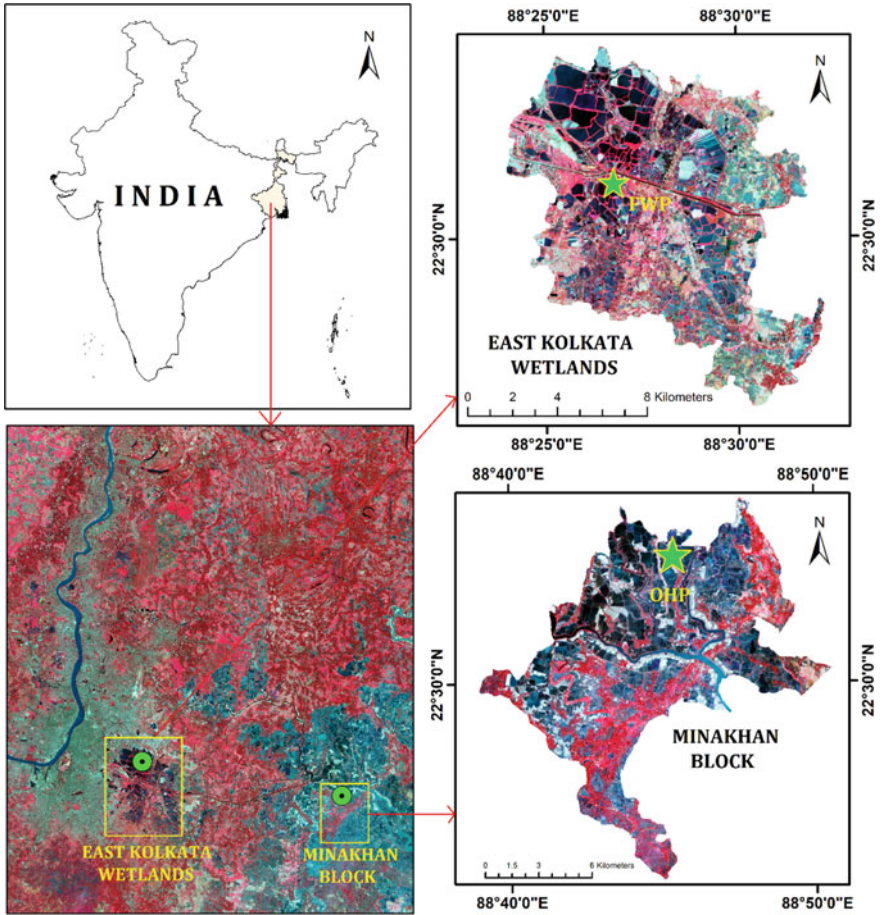


Fig. 1 The location map portraying the freshwater aquaculture pond (FWP) situated in the EKW and the oligohaline aquaculture pond (OHP) located in the Minakhan Block, West Bengal, India

oxygen demand (BOD), samples were retrieved and relocated to the laboratory after taking necessary measures of preservation.

2.3 Pisciculture in FWP and OHP

Oreochromis nilotica (Tilapia) and *Penaus monodon* (Tiger prawn) was cultured in FWP and OHP respectively. Unlike other aquaculture ponds, no external fish feed is used in these ponds. In the EKW ponds, the organic detritus of the sewage are utilized by the fish as their food throughout the year. However, during the monsoon season, the sewage load sometimes becomes overdiluted and can not provide sufficient food

to the fish. Bunting et al. (2010) mentioned that under such occasional circumstances, external fish feed is deployed by the fisher community. Quantifying the total quantity of feed or the feed conversion ratio is an almost impossible endeavor in EKW ponds, as the quality and quantity of sewage vary significantly over a short-term temporal scale (Chanda et al., 2019). Ponds of the Minakhan area utilize the oligohaline water Bidhyadhari River channelized through lock gates to maintain the salinity levels. The fisher community of this region practices variable stocking density and periodically harvest fish at new moon and full moon phases of the lunar cycle (Alagarswamy, 1995; De Roy, 2012).

2.4 Biogeochemical Analysis

2.4.1 Ancillary Environmental Measurements

Atmospheric temperature and wind velocity were recorded by a field-operable weather station (WS-2350, La Crosse Technology, USA). Electrical conductivity (EC) (precision: 1 $\mu\text{S}/\text{cm}$) and water surface temperature (precision: 0.1 $^{\circ}\text{C}$) were recorded by a digital EC meter (Thermo Scientific, Eutech, Germany). Dissolved oxygen (DO) (accuracy: $\pm 1\%$; precision: 0.01 mg l^{-1}) was recorded using a FiveGo portable F4 Dissolved Oxygen meter, Mettler Toledo. The DO readings were cross-checked by performing Winkler's titration. pH was monitored by an Orion PerpHecT ROSS Combination pH Micro Electrode fitted to a micro-pH data reader (Thermo Scientific, USA) (analytical resolution – 0.001; precision – 0.009). NBS scale technical buffer solutions were used to calibrate the glass-calomel electrodes. Nephelometric turbidity was measured with Eutech TN-100 turbidity meter. Determination of underwater photosynthetically active radiation (UWPAR) was carried out using LI-192SA, LiCor, USA (precision 0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and a data reader (Li-250A, LiCor, USA). Quantification of chl-*a* was carried out using a spectrophotometer (precision 0.01 mg m^{-3}). The community respiration (CR) and gross primary productivity (GPP) in both the ponds were monitored by the standard light-bottle-dark-bottle incubation method. A 24-h incubation was followed to monitor the alterations in DO concentrations. BOD was estimated by incubating water samples from each pond at 27 $^{\circ}\text{C}$ for 3 days. Chl-*a*, BOD, CR, and GPP were quantified according to the protocols of APHA (2005).

2.4.2 Measuring CH₄ Concentrations in Water and Air

Surface water from FWP and OHP was directly filled in 40 ml glass ampoules equipped with a latex septum. No headspace was left during the sampling. Supersaturated HgCl_2 solution (100 μl) was pushed through the septum to cease all microbial activities till further analysis. Before analysis, half of the sample was injected out of the vial and 99.99% pure nitrogen gas was used to purge the remaining half to

equilibrate the sample. The samples were equilibrated for 2 h. A Hamilton syringe was used to collect the headspace gas (5 ml). The collected gas was flown through a gas chromatograph (GC) (Systronics GC-8205) to estimate CH₄ concentrations. The uncertainty in estimation was $\pm 2.9\%$. The carrier gas was pure nitrogen and the retention time for CH₄ was 37s. Moisture removal was done from the system by enhancing the injector temperature to 105 °C. The GC was regularly calibrated by reference standard CH₄ gas of known concentrations. A battery-operated pump was attached to a glass sampling bulb to draw in air samples from above the pond water interface. The glass bulbs were carefully evacuated and washed with distilled water before sampling. While bringing the air samples to the laboratory, parafilm coverings were used to seal the knobs and outlets. These samples were analyzed in GC using the same method discussed above.

2.4.3 Air–water CH₄ Flux Estimation

pCH₄(w) and pCH₄(a) were transformed to concentrations of CH₄ in surface water (CH₄wc) and air (CH₄ac) as per the Eqs. (1) and (2) (Morel, 1983) and (3) (Lide, 2007).

$$\text{CH}_4\text{wc} = K_H \times \text{pCH}_4(\text{w}) \quad (1)$$

$$\text{CH}_4\text{ac} = K_H \times \text{pCH}_4(\text{a}) \quad (2)$$

$$\begin{aligned} \ln K_H = & -115.6477 + 155.5756/(T_K/100) + 65.2553 \\ & \times \ln(T_K/100) - 6.1698 \times (T_K/100) \end{aligned} \quad (3)$$

where K_H stands for the gas partition coefficient of CH₄ in water at sampling temperature, expressed in mole l⁻¹ atm⁻¹, and T_K refers to the temperature (Kelvin).

The CH₄ flux is estimated as per Eq. (4) (MacIntyre et al., 1995).

$$\text{CH}_4\text{Flux} \text{ (mg m}^{-2}\text{h}^{-1}\text{)} = k_x(\text{CH}_4\text{wc} - \text{CH}_4\text{ac}) \quad (4)$$

where k_x denotes the mass transfer coefficient (cm h⁻¹) and it is computed according to Eq. (5) (Wanninkhof, 1992)

$$k_x = k_{600} \times (S_c/600)^{-x} \quad (5)$$

where S_c is the Schmidt number for CH₄. It depends on water temperature as per Eq. (6). k_{600} is computed from the wind velocity (U_{10}), as per Cole and Caraco (1998) (Eq. 7) and 'x' = 0.66 for wind speed $\leq 3 \text{ m s}^{-1}$ and 'x' = 0.5 for wind speed $> 3 \text{ m s}^{-1}$.

$$S_c = 1897.8 - 114.28 \times T + 3.290 \times T^2 - 0.039061 \times T^3 \quad (6)$$

$$k_{600} = 2.07 + (0.215 \times U_{10}^{1.7}) \quad (7)$$

2.4.4 Statistical Computations

We carried out a one-way analysis of variance (ANOVA) to test whether the seasonality exhibited by all the parameters in each of the ponds is statistically significant or not. Independent samples Student's t-test was applied to examine the difference in the average of all the parameters between FWP and OHP. Pearson correlation coefficients were computed to study the relationship between pCH₄(w) and the measured physicochemical parameters. We used the SPSS version 16.0 (SPSS, Inc., USA) to carry out these analyses. 95% confidence level ($p < 0.05$) was set as the threshold in this study to determine the statistical significance.

3 Results

3.1 Variability of Physicochemical Parameters

Seasonal mean pH values were higher in OHP compared to FWP in all the seasons [pre-monsoon: 8.189 ± 0.096 (FWP) and 8.291 ± 0.108 (OHP); monsoon: 8.030 ± 0.066 (FWP) and 8.071 ± 0.068 (OHP); post-monsoon: 8.187 ± 0.089 (FWP) and 8.273 ± 0.122 (OHP)] (Table 1). The difference in seasonal pH between OHP and FWP were significant in all the seasons (pre-monsoon: $t = -4.9$, $p < 0.001$; monsoon: $t = -3.0$, $p = 0.003$; post-monsoon: $t = -3.9$, $p < 0.001$). The seasonal variability in pH within FWP ($F = 55.7$, $p < 0.001$) and OHP ($F = 69.3$, $p < 0.001$) were also statistically significant (Fig. 2a).

The EC values were also significantly different in OHP and FWP in all the seasons (see Table 1) with almost 5 to 7 times higher values in OHP compared to FWP. EC varied in FWP from $434 \mu\text{S cm}^{-1}$ to $1403 \mu\text{S cm}^{-1}$, whereas, in OHP it varied from $3018 \mu\text{S cm}^{-1}$ to $6267 \mu\text{S cm}^{-1}$. The seasonal variability in EC was statistically significant in both FWP ($F = 46.1$, $p < 0.001$) and OHP ($F = 30.8$, $p < 0.001$) with considerably lower values during monsoon season compared to the other two seasons (Fig. 2b).

The seasonal mean water temperature was found highest during the monsoonal months, followed by the pre-monsoonal months and the lowest was observed during the post-monsoonal months. The seasonal variability in water temperature was statistically significant in both FWP ($F = 86.8$, $p < 0.001$) and OHP ($F = 82.5$, $p < 0.001$). However, water temperature did not exhibit any statistical difference between FWP

Table 1 Mean \pm standard deviation of all the parameters observed in the FWP and the OHP during the three seasons

Parameters	Pre-monsoon		Monsoon		Post-monsoon	
	FWP	OHP	FWP	OHP	FWP	OHP
pH	8.189 \pm 0.096 (7.998 – 8.375)	8.291 \pm 0.108 (8.088 – 8.504)	8.030 \pm 0.066 (7.904 – 8.181)	8.071 \pm 0.068 (7.977 – 8.214)	8.187 \pm 0.089 (8.023 – 8.370)	8.273 \pm 0.122 (8.063 – 8.482)
Conductivity ($\mu\text{S cm}^{-1}$)	1043 \pm 212 (706 – 1403)	5393 \pm 536 (4706 – 6267)	814 \pm 253 (434 – 1205)	4351 \pm 1173 (3018 – 6101)	667 \pm 60 (555 – 782)	4219 \pm 519 (3393 – 4873)
Water temperature ($^{\circ}\text{C}$)	30.7 \pm 4.1 (23.1 – 37.9)	30.7 \pm 4.2 (22.8 – 37.5)	34.8 \pm 1.5 (32.1 – 38.0)	34.9 \pm 1.6 (32.3 – 41.3)	25.6 \pm 4.0 (19.0 – 32.2)	25.6 \pm 4.1 (17.9 – 32.4)
DO (mg l^{-1})	8.3 \pm 1.4 (6.1 – 11.2)	9.4 \pm 1.5 (6.7 – 12.8)	8.0 \pm 1.3 (5.9 – 10.5)	8.0 \pm 1.3 (5.6 – 10.5)	9.1 \pm 1.5 (6.3 – 12.1)	9.8 \pm 1.4 (6.8 – 12.4)
UWPAR ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	6.5 \pm 2.9 (0.9 – 12.1)	7.4 \pm 3.5 (1.3 – 15.5)	7.0 \pm 2.9 (2.1 – 11.5)	9.0 \pm 4.0 (2.3 – 16.4)	2.8 \pm 1.7 (0.4 – 6.6)	3.8 \pm 1.6 (1.2 – 7.1)
BOD (mg l^{-1})	12.8 \pm 0.9 (11.4 – 14.3)	8.3 \pm 0.6 (7.1 – 9.2)	12.4 \pm 1.5 (10.3 – 14.7)	7.9 \pm 0.7 (6.9 – 9.1)	10.4 \pm 0.5 (9.4 – 11.2)	7.4 \pm 0.5 (6.5 – 8.1)
Chl- <i>a</i> (mg m^{-3})	40 \pm 4 (32 – 46)	29 \pm 3 (24 – 35)	34 \pm 6 (24 – 42)	30 \pm 5 (24 – 38)	35 \pm 4 (28 – 41)	27 \pm 4 (20 – 34)
Turbidity (NTU)	42.8 \pm 3.0 (37.9 – 47.5)	31.4 \pm 2.9 (27.6 – 37.2)	38.2 \pm 5.2 (30.5 – 46.9)	28.9 \pm 3.5 (23.5 – 34.7)	37.9 \pm 3.4 (32.6 – 43.8)	31.2 \pm 2.8 (26.5 – 35.4)
GPP ($\text{gO}_2 \text{ m}^{-2} \text{ d}^{-1}$)	5.6 \pm 0.5 (4.9 – 6.5)	5.0 \pm 0.3 (4.3 – 5.4)	5.5 \pm 0.5 (4.5 – 6.2)	5.1 \pm 0.2 (4.6 – 5.5)	5.6 \pm 0.3 (5.2 – 6.4)	5.7 \pm 0.2 (5.4 – 5.9)
CR ($\text{gO}_2 \text{ m}^{-2} \text{ d}^{-1}$)	22.2 \pm 3.4 (17.9 – 27.4)	16.1 \pm 1.1 (14.2 – 17.5)	28.3 \pm 1.5 (25.9 – 30.5)	17.3 \pm 0.7 (16.3 – 18.5)	23.6 \pm 2.2 (20.5 – 27.5)	14.7 \pm 1.3 (12.9 – 16.4)
pCH ₄ (w) (μatm)	8.53 \pm 2.36 (4.75 – 14.38)	5.57 \pm 1.33 (3.46 – 9.15)	8.83 \pm 2.98 (5.18 – 14.50)	6.04 \pm 2.99 (2.02 – 13.59)	6.97 \pm 1.84 (3.80 – 12.46)	4.59 \pm 2.30 (1.32 – 10.69)

(continued)

Table 1 (continued)

Parameters	Pre-monsoon		Monsoon		Post-monsoon	
	FWP	OHP	FWP	OHP	FWP	OHP
pCH ₄ (a) (μ atm)	1.866 \pm 0.007 (1.854 – 1.881)	1.861 \pm 0.009 (1.843 – 1.880)	1.872 \pm 0.007 (1.858 – 1.887)	1.867 \pm 0.007 (1.855 – 1.888)	1.863 \pm 0.006 (1.850 – 1.875)	1.860 \pm 0.006 (1.849 – 1.871)
K ₆₀₀	4.29 \pm 0.35 (3.79 – 4.60)		4.76 \pm 0.36 (4.24 – 5.06)		3.86 \pm 0.41 (3.44 – 4.34)	
CH ₄ Flux (mg m ⁻² h ⁻¹)	23.1 \pm 14.7 (5.3 – 72.2)	12.9 \pm 8.0 (2.9 – 40.0)	32.4 \pm 17.7 (12.3 – 78.9)	20.7 \pm 18.8 (0.7 – 97.6)	11.6 \pm 6.8 (2.8 – 35.1)	6.4 \pm 6.7 (-0.9 – 29.7)

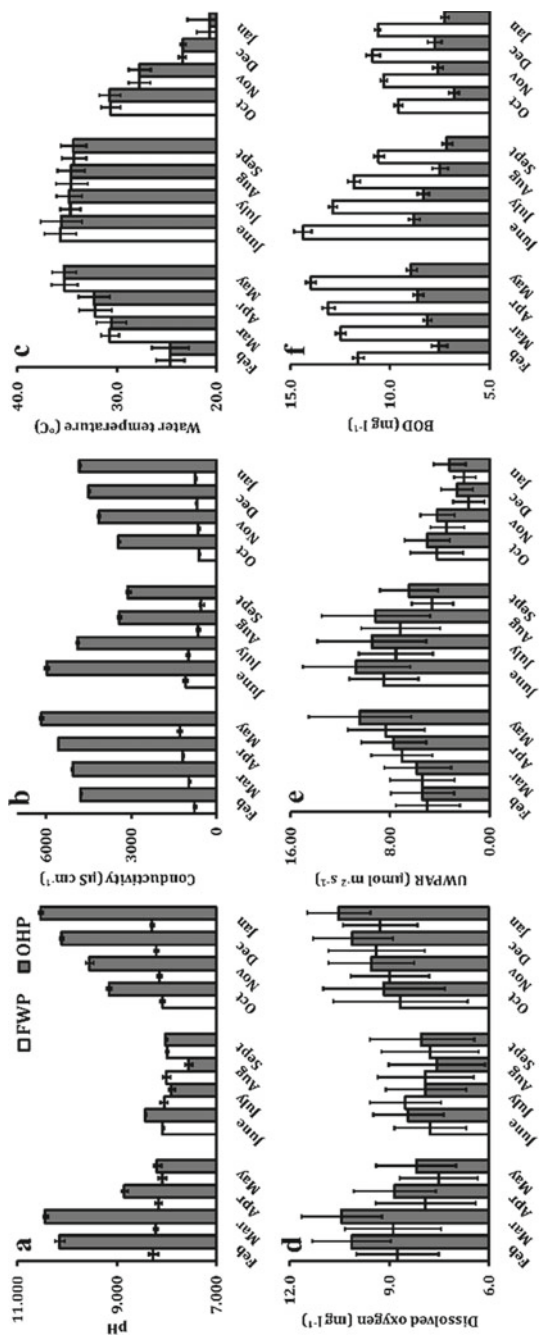


Fig. 2 The monthly observations of daily mean **a** pH, **b** electrical conductivity, **c** water temperature, **d** DO, **e** UWPAR, and **f**BOD observed in FWP and OHP throughout the year. The error bars indicate the standard deviation from the average value

and OHP in any of the seasons (pre-monsoon: $t = -0.02$, $p = 0.981$; monsoon: $t = -0.14$, $p = 0.883$; post-monsoon: $t = -0.02$, $p = 0.986$) (Fig. 2c).

The seasonal mean DO concentration was significantly higher in OHP (pre-monsoon: $8.3 \pm 1.4 \text{ mg l}^{-1}$; monsoon: $8.0 \pm 1.3 \text{ mg l}^{-1}$; post-monsoon: $9.1 \pm 1.5 \text{ mg l}^{-1}$) compared to FWP (pre-monsoon: $9.4 \pm 1.5 \text{ mg l}^{-1}$; monsoon: $8.0 \pm 1.3 \text{ mg l}^{-1}$; post-monsoon: $9.8 \pm 1.4 \text{ mg l}^{-1}$) during pre-monsoonal months ($t = -3.8$, $p < 0.001$) and post-monsoonal months ($t = -2.57$, $p = 0.011$), however, no significant difference ($t = -0.05$, $p = 0.957$) was observed during the monsoonal months. The seasonal difference in DO was statistically significant for both FWP ($F = 7.8$, $p = 0.001$) and OHP ($F = 22.6$, $p < 0.001$) (Fig. 2d).

Like pH and EC, UWPARG exhibited higher magnitudes in OHP compared to FWP in all the three seasons, however, the difference was not statistically significant in any of the three seasons (pre-monsoon: $t = -0.99$, $p = 0.326$; monsoon: $t = -2.0$, $p = 0.052$; post-monsoon: $t = -1.9$, $p = 0.062$). In monsoon and post-monsoon seasons the p -value was marginally higher than 0.05. However, the seasonal difference in UWPARG within FWP ($F = 18.2$, $p < 0.001$) and OHP ($F = 16.4$, $p < 0.001$) was statistically significant (Fig. 2e).

BOD in FWP was significantly higher than OHP in all the three seasons (pre-monsoon: $t = 13.8$, $p < 0.001$; monsoon: $t = 9.3$, $p < 0.001$; post-monsoon: $t = 14.8$, $p < 0.001$). Over the annual cycle, BOD ranged between 6.5 mg l^{-1} and 9.2 mg l^{-1} in OHP, whereas, it varied between 9.4 mg l^{-1} and 14.7 mg l^{-1} in FWP. The seasonal difference in BOD was also statistically significant within FWP ($F = 18.2$, $p < 0.001$) and OHP ($F = 7.3$, $p = 0.002$) (Fig. 2f).

3.2 Variability of Primary Productivity-Related Parameters

The chl-*a* concentration was higher in FWP compared to OHP in all the seasons, however, the difference was significant during pre-monsoon season ($t = 7.4$, $p < 0.001$) and post-monsoon season ($t = 4.5$, $p < 0.001$). During monsoon season the p -value was marginally not significant ($t = 1.9$, $p = 0.059$). The inter-seasonal variation in chl-*a* was statistically significant in case of FWP ($F = 5.1$, $p = 0.012$), however, in OHP there was no significant variation over the annual cycle ($F = 1.8$, $p = 0.172$) (Fig. 3a).

There was significant difference in turbidity between FWP and OHP in all the three seasons (pre-monsoon: $t = 9.5$, $p < 0.001$; monsoon: $t = 5.1$, $p < 0.001$; post-monsoon: $t = 5.2$, $p < 0.001$), with higher values in FWP compared to OHP. The seasonal mean difference in turbidity varied from ~ 6 NTU to ~ 11 NTU. The seasonal variability of turbidity was significant in FWP ($F = 5.8$, $p = 0.007$), however, like chl-*a*, it was not significant in OHP ($F = 2.4$, $p = 0.110$) (Fig. 3b).

The difference in GPP between FWP and OHP was statistically significant in pre-monsoon season ($t = 3.6$, $p = 0.001$) and monsoon season ($t = 2.4$, $p = 0.024$), however, during post-monsoon season the difference was not significant ($t = -0.32$, $p = 0.747$). Though the difference in GPP was significant in two seasons, in terms of

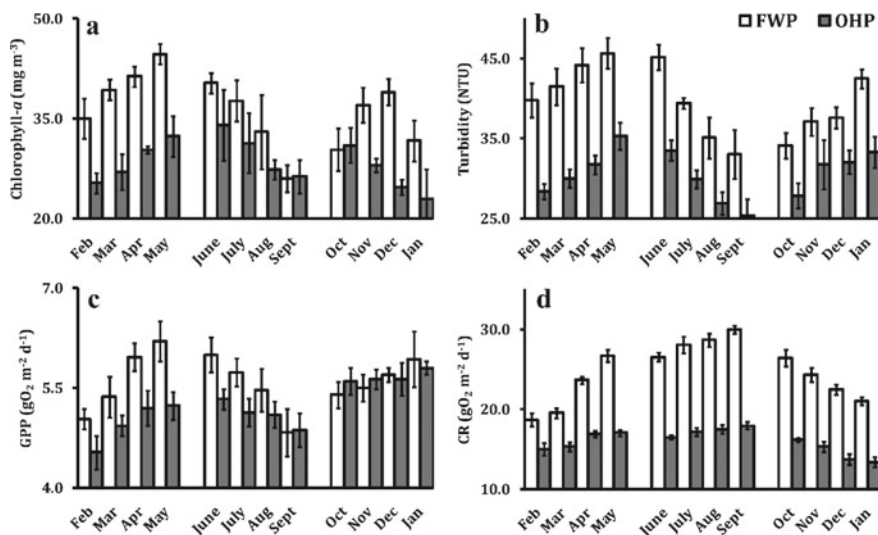


Fig. 3 The monthly variability of the daily mean **a** chl-*a*, **b** turbidity, **c** GPP, and **d** CR observed in FWP and OHP throughout the year. The error bars indicate the standard deviation from the average value

magnitude, the mean difference ranged from 0.4 gO₂ m⁻² d⁻¹ to 0.6 gO₂ m⁻² d⁻¹. In contrast to chl-*a*, the inter-seasonal variation in GPP was not significant in FWP ($F = 0.31, p = 0.733$), however, it was statistically significant in case of OHP ($F = 22.6, p < 0.001$) (Fig. 3c).

Unlike GPP, CR was significantly higher in FWP compared to OHP in all the three seasons (pre-monsoon: $t = 5.8, p < 0.001$; monsoon: $t = 23.6, p < 0.001$; post-monsoon: $t = 12.1, p < 0.001$). The magnitude of difference varied from 6gO₂ m⁻² d⁻¹ to 11gO₂ m⁻² d⁻¹, which was much higher than the difference in GPP between FWP and OHP. At the same time, the seasonal variation within FWP ($F = 20.1, p < 0.001$) and OHP ($F = 19.2, p < 0.001$) was also statistically significant (Fig. 3d).

3.3 Variability in pCH₄(a), pCH₄(w) and Air-Water CH₄ Flux

pCH₄(a) varied over a very short range of 1.843 ppmv to 1.888 ppmv, however, it was marginally higher near FWP compared to OHP in all the three seasons (pre-monsoon: $t = 3.3, p = 0.002$; monsoon: $t = 3.7, p < 0.001$; post-monsoon: $t = 3.1, p < 0.001$). pCH₄(a) also exhibited significant seasonal variation near FWP ($F = 21.5, p < 0.001$) as well as OHP ($F = 15.2, p < 0.001$) (Fig. 4a).

Seasonal mean pCH₄(w) followed the trend monsoon > pre-monsoon > post-monsoon in both FWP and OHP, with significantly higher values in FWP compared to

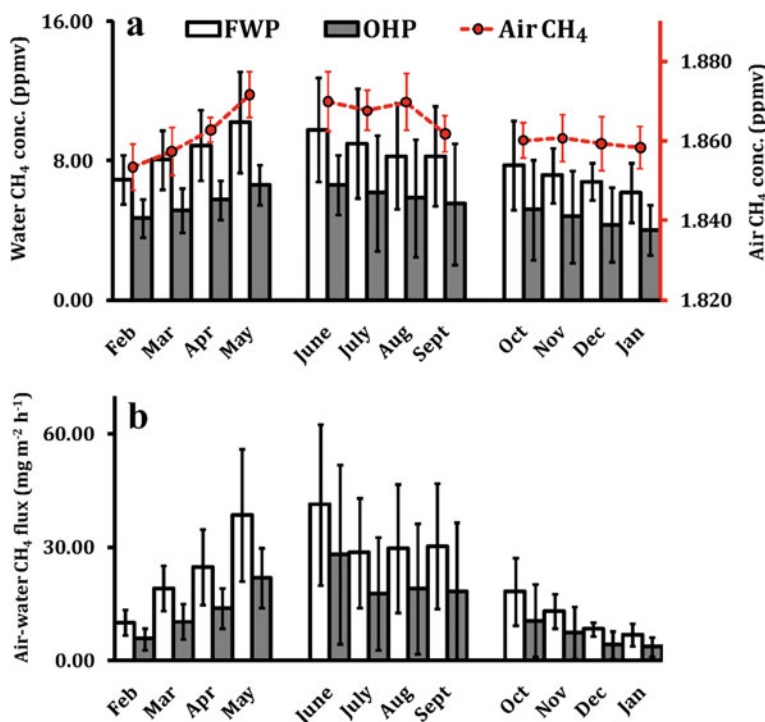


Fig. 4 The monthly variation of the daily mean (a) air CH₄ concentration [pCH₄(a)] and water CH₄ concentration [pCH₄(w)] along with (b) air–water CH₄ fluxes observed in FWP and OHP throughout the year. The error bars indicate the standard deviation from the average value

OHP (pre–monsoon: $t = 7.5$, $p < 0.001$; monsoon: $t = 4.6$, $p < 0.001$; post–monsoon: $t = 5.6$, $p < 0.001$). The seasonal difference in mean pCH₄(w) was statistically significant in both FWP ($F = 8.1$, $p < 0.001$) and OHP ($F = 4.9$, $p = 0.009$) (Fig. 4a).

Both FWP ($22.4 \pm 16.2 \text{ mg m}^{-2} \text{ h}^{-1}$) and OHP ($13.4 \pm 13.6 \text{ mg m}^{-2} \text{ h}^{-1}$) acted as source of CH₄ towards atmosphere throughout the year, with significantly higher values of CH₄ efflux from FWP compared to OHP in all the seasons (pre–monsoon: $t = 4.2$, $p < 0.001$; monsoon: $t = 3.1$, $p = 0.002$; post–monsoon: $t = 3.7$, $p < 0.001$). Mirroring the trend of seasonal mean pCH₄(w), air–water CH₄ flux also followed the same trend monsoon > pre–monsoon > post–monsoon in both FWP and OHP. The inter–seasonal difference in air–water CH₄ flux was statistically significant in both FWP ($F = 27.3$, $p < 0.001$) and OHP ($F = 15.9$, $p < 0.001$) (Fig. 4b).

3.4 Relationship Between $pCH_4(w)$ and Biogeochemical Variables

pH exhibited significant negative relationship with $pCH_4(w)$ in both FWP ($r = -0.62$, $p = 0.031$) and OHP ($r = -0.68$, $p = 0.015$) (Fig. 5a). EC showed significant positive relationship with $pCH_4(w)$ in FWP ($r = 0.72$, $p = 0.008$), however, in OHP the relationship was not significant ($r = 0.38$, $p = 0.220$) (Fig. 5b). Water temperature exhibited very strong positive relationship with $pCH_4(w)$ in both FWP ($r = 0.91$, $p < 0.001$) and OHP ($r = 0.94$, $p < 0.001$) (Fig. 5c). The relationship between DO and $pCH_4(w)$ was significantly negative in both FWP ($r = -0.86$, $p < 0.001$) and OHP ($r = -0.83$, $p = 0.001$) (Fig. 5d). Like water temperature, BOD also showed significant positive relationship with $pCH_4(w)$ in both FWP ($r = 0.81$, $p = 0.001$) and OHP ($r = 0.65$, $p = 0.021$) (Fig. 5e). Chl-*a* exhibited significant positive relationship with $pCH_4(w)$ in OHP ($r = 0.86$, $p < 0.001$), however, the relationship was not significant in case of FWP ($r = 0.50$, $p = 0.098$) (Fig. 5f). Turbidity and GPP did not exhibit any significant relationship with $pCH_4(w)$ (Fig. 5g,h). CR showed significant positive relationship with $pCH_4(w)$ in OHP ($r = 0.83$, $p = 0.001$), but in FWP it was marginally beyond the significance limit ($r = 0.56$, $p = 0.057$) (Fig. 5i).

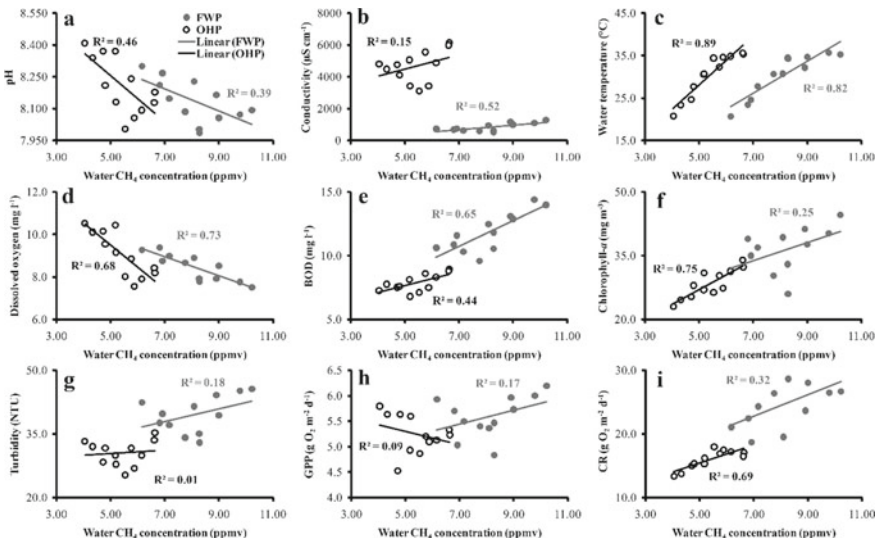


Fig. 5 The scatter plots displaying the relationship between monthly mean water CH_4 concentration [$pCH_4(w)$] and monthly mean **a** pH, **b** electrical conductivity, **c** water temperature, **d** DO, **e** BOD, **f** chl-*a*, **g** turbidity, **h** GPP, and **i** CR. Linear trend lines along with the goodness of fit (R^2) are shown separately for FWP and OHP

3.5 Diurnal Variation in $p\text{CH}_4(w)$ and Atmosphere-Pond CH_4 Flux

During all the seasons the $p\text{CH}_4(w)$ and hence the atmosphere-pond CH_4 efflux exhibited a steady increase from dawn till noon and the peak was observed during 1400 h to 1600 h (Fig. 6). During night time both the $p\text{CH}_4(w)$ and the atmosphere-pond CH_4 efflux was much lower compared to the day time.

4 Discussion

Analyzing the results it can be observed that OHP had significantly lower $p\text{CH}_4(w)$ and atmosphere-pond CH_4 fluxes compared to FWP all-around the year. In terms of physicochemical variables, the difference in salinity was the major reason behind choosing these two ponds and comparing their dissolved CH_4 dynamics. EC values in OHP were substantially higher than FWP throughout the year and this could be the most crucial factor which led to lower $p\text{CH}_4(w)$ in OHP due to reduced methanogenesis; as also observed in earlier studies like Poffenbarger et al. (2011) and Welti et al. (2017). Thus we could accept our hypothesis that a difference in salinity (or EC) leads to a difference in $p\text{CH}_4(w)$ and atmosphere-pond CH_4 fluxes between OHP and FWP. However, unlike previous studies like Cotovicz et al. (2016)

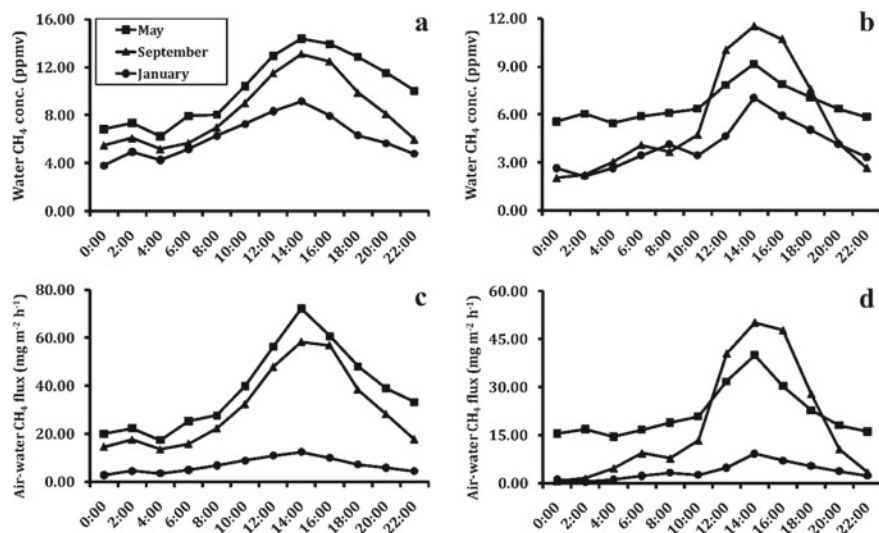


Fig. 6 The diurnal variability of water CH_4 concentration [$p\text{CH}_4(w)$] in **a** FWP and **b** OHP and the diurnal variability of atmosphere-pond CH_4 flux in **c** FWP and **d** OHP in May (pre-monsoon season), September (monsoon season) and January (post-monsoon season)

and Yang et al., (2018a, 2019), no significant negative relationship was observed between EC and $p\text{CH}_4(\text{w})$ in neither FWP nor OHP. This shows that there are some other factors as well which are regulating the $p\text{CH}_4(\text{w})$ in these two ponds apart from EC.

Water temperature has been regarded as one of the crucial factors which govern both methanogenesis (Inglett et al., 2012; Yang et al., 2015b) and methane oxidation (Lofton et al., 2014; Osudar et al., 2015). In the present study, a strong positive association between water temperature and $p\text{CH}_4(\text{w})$ was portrayed in both the ponds, and no significant difference was observed in water temperature as well between the two ponds. Hence it can be affirmed in both the ponds increase in water temperature aided methanogenesis by enhancing organic matter degradation and hence providing suitable labile substrates from which CH_4 is produced by microbial activity. Similar observations were made by Xiang et al. (2015) in coastal marshes and Yang et al. (2019) in aquaculture ponds. The difference in $p\text{CH}_4(\text{w})$ magnitudes between FWP and OHP despite having the same water temperature further shows that methane oxidation is not prompted by the effect of temperature fluctuation, rather the intrinsic difference in water column substrates led to the difference in $p\text{CH}_4(\text{w})$ between FWP and OHP as also observed by Roland et al. (2017).

Earlier studies emphasized that the growth and thriving of the methanogens are quite dependent on the pH of the aquatic column, and they prefer to grow at lower pH close to ~ 7.7 (Chang & Yang, 2003). The pH all over the annual cycle was significantly lower in FWP compared to OHP, which could have facilitated better growth of methanogens in FWP compared to OHP. This reason could be further ascertained as both the ponds exhibited significant negative relation between pH and $p\text{CH}_4(\text{w})$, which showed that with decreasing pH, methanogens flourished and led to enhancement of $p\text{CH}_4(\text{w})$. Datta et al. (2009) also recorded a similar negative association between pH and $p\text{CH}_4(\text{w})$ while working in a rain-fed fish farming pond situated in Eastern India. Yang et al. (2018b) also observed a similar relationship in aquaculture ponds of China and attributed the reduced activity of methanogens behind such observations.

The present study also showed that OHP had significantly higher DO levels compared to FWP. This could be attributed to the lower turbidity and higher UWP/AR in OHP which in turn facilitated a higher degree of autotrophic activities and hence higher production of DO compared to that in FWP. Several studies have observed that *Oreochromis nilotica* which is cultured in FWP is a fast-moving bottom feeder (Adeyemi et al., 2009; Jihulya, 2014; Njiru et al., 2004) and the strong fish movement in the sediment–water interface often causes enhanced turbidity (Chapman & Fernando, 1994; Frei & Becker, 2005). *Penaeus monodon*, on the other hand, being cultured in OHP is also known to be a bottom feeder but shrimps and prawns are usually more sluggish than fish species, hence the bottom churning due to their movement is expected to be quite less. This differential photosynthesis-induced difference in DO content between the two ponds could play a crucial role in having different $p\text{CH}_4(\text{w})$ magnitudes between FWP and OHP. Kettunen et al. (1999) and Yang et al. (2013) reported that reduced DO levels promote methane production by enhancing

anaerobic decomposition rates and at the same time lower DO levels reduce methane oxidation.

BOD serves as a proxy of biodegradable organic matter in any aquatic column and the present study observed significantly higher BOD in FWP compared to OHP. FWP utilizes sewage water to carry out fishing practice and since BOD concentration in city sewage remains very high, FWP reflected the higher levels of BOD (Sarkar et al., 2017). On the contrary, OHP utilizes the brackish water of the adjacent Bidhyadhari River, which though receives the sewage water of the Kolkata metropolis but the BOD levels are quite reduced when the sewage effluent reaches Bidhyadhari River after passing through EKW (Ghosh, 2018). Higher BOD in any aquatic ecosystem indicates the presence of the labile biodegradable substance and under reduced DO and lower pH levels and leads to a higher rate of methane emission [as also observed by Yang (1998) while working in the rivers and lakes of Taiwan].

The effect of lower DO and higher BOD was also reflected on the CR of the two selected ponds. Though both FWP and OHP showed CR magnitudes greater than the magnitudes of GPP, CR in FWP was significantly higher than OHP, which further indicated that the degree of net heterotrophy was substantially higher in FWP. Previous studies have clearly shown that there is a strong relationship between net heterotrophic conditions and supersaturation of CH₄, especially in small ponds like that of the chosen aquaculture ponds (Holgerson, 2015). It should be also mentioned in this regard, that chl-*a* concentrations were also substantially higher in FWP compared to OHP. Chl-*a* magnitude in any lentic ecosystem acts as a proxy of trophic status and provides an idea about the primary productivity in shallow lentic ecosystems (Liu et al., 2017; Yang et al., 2015a). Higher chl-*a* magnitudes indicate higher algal production rates which in turn consequences the creation of autochthonous organic substrates (Palma-Silva et al., 2013). Chl-*a* and GPP portrayed a positive (significant) relation with the pCH₄(w) clearly emphasizing that the autochthonous production of organic substrates facilitated methanogenesis (Flury et al., 2010; Furlanetto et al., 2012) and hence facilitated higher methane emission.

In terms of the annual mean magnitude of atmosphere-pond CH₄ efflux, the methane emission observed in this piece of research (FWP: $22.4 \pm 16.2 \text{ mg m}^{-2} \text{ h}^{-1}$ and OHP: $13.4 \pm 13.6 \text{ mg m}^{-2} \text{ h}^{-1}$) was found higher than many of the recent measurements. Datta et al. (2009) observed a mean CH₄ emission of $2.5 \text{ mg m}^{-2} \text{ h}^{-1}$ from the refuge rain-fed ponds of Cuttack, India. In Chinese aquaculture ponds, most of the recent estimates exhibited lower magnitudes than those observed in the present study. Wu et al. (2018) observed a mean CH₄ emission of only $0.5 \text{ mg m}^{-2} \text{ h}^{-1}$ in the experimental farm of Nanjing Agricultural University, China. Yang et al (2018b) observed a mean CH₄ emission of $1.1 \pm 0.9 \text{ mg m}^{-2} \text{ h}^{-1}$ and $10.5 \pm 4.9 \text{ mg m}^{-2} \text{ h}^{-1}$ from the undrained and drained ponds of Shanyutan Wetlands, China. Yang et al. (2015a) recorded mean CH₄ efflux of $1.6 \pm 0.5 \text{ mg m}^{-2} \text{ h}^{-1}$ from mixed polyculture ponds of China, whereas, from shrimp they observed a mean CH₄ emission rate of $19.9 \pm 4.3 \text{ mg m}^{-2} \text{ h}^{-1}$ (which was higher than that observed in the OHP of the present study). However, very few studies like Yang et al. (2017) observed substantially higher effluxes ($123 \pm 48 \text{ mg m}^{-2} \text{ h}^{-1}$) than the present estimates while working in the shrimp ponds near Min River Estuary, China. Thus in

totality, it can be inferred that atmosphere-pond CH_4 fluxes from the aquaculture ponds of EKW and Minakhan Block were found higher than the recent observations being made throughout the world, especially in China.

4.1 Uncertainties and Scope for Future Studies

The present study implemented the bulk formula method and thus the estimation of atmosphere-pond CH_4 flux was carried out from the difference of CH_4 concentration between pond and atmosphere. This is why we could measure only the diffusive flux in this study. In the future, the chamber method should be deployed to characterize the CH_4 ebullition rates from this region. More aquaculture ponds of this region should be sampled based on different species being cultured, different depths, and so forth to draw a holistic CH_4 budget of this crucial region. In the present study, sampling was conducted in all the months of a calendar year. However, different stages of aquaculture should be distinctly studied as they usually exhibit different signatures of fluxes in several annual studies (Liu et al., 2016; Wu et al., 2018). In addition to these, the dissolved organic carbon should be measured in the future and the methane dynamics in the sediment–water interface should be also studied.

5 Conclusion

Analyzing all the results and outcomes of the present study, it can be concluded that partial pressure of CH_4 [$\text{pCH}_4(\text{w})$] and hence atmosphere-pond CH_4 fluxes were much higher in the freshwater aquaculture ponds (FWP) of East Kolkata Wetlands compared to the oligohaline aquaculture ponds (OHP) of Minakhan Block, both being situated under the same climatic regime in eastern India. Higher salinity was found to inhibit methanogens which resulted in lower $\text{pCH}_4(\text{w})$ in the OHP compared to FWP. The difference in fishing practice in the two ponds was also found to regulate the turbidity and hence the presence of photosynthetically active radiation in the two ponds. The higher turbidity in FWP led to lower dissolved oxygen levels and enhanced community respiration which in turn facilitated anaerobic conditions and thus the methanogen activity was much more in FWP compared to OHP. Aquaculture practice is becoming popular day by day in India, however, efforts of characterizing the methane fluxes from these ecosystems stand very few in the present date. The annual study revealed that the mean CH_4 emission observed in these sites of India was much higher than most of the recent estimates being carried out in the Chinese aquaculture ponds. Thus this study is expected to provide an impetus to carry out similar measurements in other aquaculture ponds of India to meet the present need of the hour and fill the data gap.

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Conflict of Interest: All the authors state that they do not have any competing conflict of financial or any other form of interest.

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