



# Summer methane emissions from sewage water–fed tropical shallow aquaculture ponds characterized by different water depths

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## Abstract

Aquaculture practices are steadily increasing to meet the fish demand, especially in tropical countries like India. However, efforts to characterize the contribution of these aquaculture ponds towards greenhouse gas emission like CH<sub>4</sub> are still very few. CH<sub>4</sub> concentration in water [pCH<sub>4</sub>(water)] and air–water CH<sub>4</sub> fluxes were estimated (during the summer months) in two sewage-fed ponds having different depths situated in the East Kolkata Wetlands, India (a Ramsar site). pCH<sub>4</sub>(water) in both of these ponds showed significant positive correlation with water temperature ( $R^2 = 0.68$  and  $0.71$ ,  $p < 0.05$ ). Daily mean chlorophyll-*a*, turbidity, biochemical oxygen demand (BOD) and gross primary productivity (GPP) also showed positive correlation with pCH<sub>4</sub>(water). This indicated that higher primary production and presence of turbid materials acted as substrates for methanogenesis, which favoured air–water CH<sub>4</sub> effluxes towards atmosphere. Mean air–water CH<sub>4</sub> fluxes in the ponds having depth of 1.1 m and 0.6 m were observed to be  $24.79 \pm 12.02$  mg m<sup>-2</sup> h<sup>-1</sup> and  $6.05 \pm 3.14$  mg m<sup>-2</sup> h<sup>-1</sup> respectively. Higher depth facilitated net heterotrophic conditions, which led to lower dissolved oxygen levels, which, in turn, led to lower rate of CH<sub>4</sub> oxidation. Moreover, under reduced photosynthetically active radiation (in the pond having greater depth), the pH values were comparatively lower (~7.7), which further facilitated a favourable condition for the methanogens to grow. On the whole, it was inferred that apart from pre-established physicochemical factors, depth was also found to play a decisive role in regulating the air–water CH<sub>4</sub> fluxes from these aquaculture ponds. In future, continuous sampling should be carried out (by chamber method) to take into account the ebullition CH<sub>4</sub> fluxes, and more number of ponds should be sampled throughout a complete annual cycle to have a more holistic understanding about this cluster of sewage-fed aquaculture ponds.

**Keywords** Methane emission · Aquaculture ponds · Sewage · Wetlands · Ramsar site · Depth

## Introduction

Ecosystem services of wetlands to mankind by acting as biodiversity reserves, long-term sink for nitrogen and carbon, flood control, improvement of water quality through pollutant removal etc. have been long recognized; however, these

wetlands are also responsible for being one of the largest sources of methane (CH<sub>4</sub>), a greenhouse gas (GHG) towards the atmosphere (Bridgham et al. 2006, 2013). The global warming potential of CH<sub>4</sub> is 25 times more compared with that of carbon dioxide (CO<sub>2</sub>) and its emission accounts for ~18% of the total human-induced radiative forcing, rendering it the second most important GHG, CO<sub>2</sub> being the first (Forster et al. 2007). Owing to multifarious natural and anthropogenic factors, atmospheric CH<sub>4</sub> concentration has become more than double of its pre-industrial revolution concentration in the last 250 years (Flury et al. 2010). The variability in the global atmospheric CH<sub>4</sub> levels is found to be largely controlled by the CH<sub>4</sub> fluxes from the wetlands (Bridgham et al. 2013). According to the most recent estimate, wetlands occupy 29.83 million km<sup>2</sup> area (~5.84% of global land surface) (Hu et al. 2017) and emits CH<sub>4</sub> at a rate of 164 Tg year<sup>-1</sup> (Bridgham et al. 2013). Bridgham et al.

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(2013) also argued that CH<sub>4</sub> emission rates in the past were significantly controlled by the climatic conditions and in future CH<sub>4</sub> fluxes from wetlands would be responsive to the anthropogenically driven changing climate scenario.

Recent researches show that small lentic bodies acts as significant source of GHGs, especially CH<sub>4</sub> (Panneer Selvam et al. 2014). Inland waters encompass ~3.7% of Earth's land surface (Raymond et al. 2013) and emit CH<sub>4</sub> at the rate of 0.65 Pg C year<sup>-1</sup>, which can substantially offset the terrestrial carbon sink (Bastviken et al. 2011). CH<sub>4</sub> generation in static aquatic bodies mainly originates due to degradation of organic matter under anaerobic environment by means of methanogenesis (Panneer Selvam et al. 2014). A substantial part of this CH<sub>4</sub> produced within the water column is consumed by methanotrophs by means of oxidative processes; however, a significant portion of the remnant CH<sub>4</sub> finds its way to the atmosphere by ebullition, diffusion, storage flux, plant-mediated flux etc. (Bastviken 2009). Among the inland water bodies, most of the studies are carried out on lakes and reservoirs from the perspective of characterizing the CH<sub>4</sub> flux (Jacinthe et al. 2012; Xiao et al. 2013a, b; Xiao et al. 2014; Yang et al. 2013a); however, ponds that are smaller in area and mostly shallow in depth have not received that much attention (Yang et al. 2015). Repo et al. (2007) reported that small ponds and lakes that are often found in peatlands emit substantial amount of CH<sub>4</sub>; however, their role in regulating global carbon dynamics are largely underestimated (Downing et al. 2006; Bastviken et al. 2008). Burger et al. (2016) pointed out that ponds having an area less than 10,000 m<sup>2</sup> comprises almost one-third of the global ponds and CH<sub>4</sub> emission from such small ponds, which are often neglected, might substantially affect the global budget.

Among the shallow water bodies, researches on aquaculture ponds (which are mostly situated in wetland areas) have gained impetus as these have been recognized to be significant sources of atmospheric CH<sub>4</sub> mainly due to exhaustive use of organic materials and fertilizers (Liu et al. 2016). Wetlands, especially the ones situated close to the coastline act as an ecotone between terrestrial and marine ecosystems and are known to foster substantial biological production (Yang et al. 2017). The present trend of land use change from natural wetlands and marshes to aquaculture ponds aggravated the deterioration of more than 50% of the world's coastal wetlands (Barbier et al. 2011; Liu and Mo 2016). It is presumed that such change in land use would enhance the release of stored carbon in the form of GHG; however, the estimates of such emission are poorly constrained as of present date (Yang et al. 2017), which, in turn, makes the global as well as regional emission budgets poorly constrained. According to FAO (2014), in the recent past, large-scale conversion of agricultural plots to aquaculture ponds also enhanced the CH<sub>4</sub> emission towards atmosphere. Due to the increase in area and frequent loading of chemical nutrients and organic feeding

material (Adams et al. 2012; Yang et al. 2015), studying the CH<sub>4</sub> dynamics in inland aquaculture ponds has become an urgent need of the hour (IPCC 2014). The existing global budgets of GHG emission from aquaculture ponds are mostly based on extrapolated modelling approach taking into consideration the surface GHG concentrations and comparatively more attention is paid towards nitrous oxide (N<sub>2</sub>O) than CH<sub>4</sub> (Williams and Crutzen 2010; Hu et al. 2012; Paudel et al. 2015). This kind of approach would not suffice to draw a holistic scenario about the CH<sub>4</sub> emission from the inland aquaculture ponds, and hence more ground measurements should be carried out to fill the gap in data and strengthen the global estimates (Wu et al. 2018).

According to the records of Food and Agriculture Organization (FAO) of the United Nations, freshwater and brackish aquaculture ponds together cover an area of ~1,10,832 km<sup>2</sup> throughout the globe (Verdegem and Bosma 2009; Yang et al. 2018a), most of these being situated in the tropics and subtropics. Till date most of the efforts in quantifying the GHG emissions from aquaculture ponds have been largely concentrated in China (Hu et al. 2014; Yang et al. 2015; Chen et al. 2015, 2016; Hu et al. 2016; Wu et al. 2018) perhaps because they have the world's largest mariculture industry (FAO 2016) comprising a total aquaculture pond area of about 25,700 km<sup>2</sup> (Chen et al. 2016). Indian aquaculture ponds also cover a substantial area of the country's landmass (7900 km<sup>2</sup>) (Adhikari et al. 2012); however, studies based on characterizing the GHG and/or carbon dynamics of these ponds are scarce (Adhikari et al. 2012; Pathak et al. 2013). With respect to the above-mentioned background, the present study was focused to quantify and characterize the air–water CH<sub>4</sub> fluxes from a tropical aquaculture pond of India, situated within a Ramsar wetland site, namely East Kolkata Wetlands (EKW), which also happens to be the largest man-made aquaculture system of the world. Only the summer fluxes have been monitored in this study in order to get an idea about the higher range of fluxes as several studies pointed out that CH<sub>4</sub> emission from lentic bodies has a direct relationship with temperature (Pelletier et al. 2014; Sachs et al. 2010; Wik et al. 2013). Methanogenesis as well as methane fluxes has been recently found to exhibit positive exponential relation with temperature (Yvon-Durocher et al. 2014; Natchimuthu et al. 2016). Highest magnitudes of CH<sub>4</sub> fluxes across air–water interface from aquaculture ponds are usually observed in the peak of summer (Yang et al. 2017). Thus we have chosen the summer months for the present study. The main objectives of the study were to (i) quantify the air–water CH<sub>4</sub> fluxes from the aquaculture ponds during the summer months and to (ii) characterize the environmental controlling factors regulating these fluxes giving special emphasis to the depths of the ponds.

## Materials and methods

### Study area

Kolkata metropolis (one of the biggest, populous and polluted cities of India) is situated in the eastern bank of Hugli River, a 260-km-long distributary of River Ganges in the state of West Bengal, India. EKW formerly known as East Calcutta Wetlands (Fig. 1) is situated on the eastern suburbia of the city, and it was recognized as a ‘wetland of international importance’ as well as enlisted as a ‘Ramsar site’ in November 2002. EKW is renowned because of its unique natural purification system, and as a result it is popularly known as the ‘kidney of the city of Kolkata’ (Kundu et al. 2008). EKW comprises the world’s largest cluster of aquaculture ponds (man-made) situated within a wetland. EKW stands as a classic instance of a natural resource recovery system, wherein the untreated wastewater discharge from the Kolkata metropolis is efficiently exploited in activities like agriculture, pisciculture, solid waste farms and aquaculture ponds by means of which the city’s drainage water is purified before it meets the Bidyadhari River, which eventually drains the water in Bay

of Bengal (Kundu et al. 2008). The initiation of the conversion of marsh lands to aquaculture ponds in EKW dates back to the year 1876 (Bunting et al. 2010 and the references therein). EKW at present consists more than 250 aquaculture ponds covering an area of  $\sim 12 \text{ km}^2$  (Aich et al. 2012) and at the same time furnishes  $\sim 150$  tons of fresh vegetables per day and  $\sim 10,500$  tons of table fish per year, which, in turn, provides livelihood for about  $\sim 50,000$  people and a food security to Kolkata (Chaudhuri et al. 2012). EKW has been mechanized in such a way since the late nineteenth century such that it acts as a cheap, eco-friendly and efficient system of sewerage and solid waste treatment (Kundu et al. 2008). The aquaculture ponds of EKW vary in size from few hectares to hundreds of hectares and are very shallow (depths varying from 100 to 150 cm) in nature with flat bottom. The architectural characteristics of these aquaculture ponds are detailed in Furedy and Ghosh (1984) and Ghosh (2005). Chaudhuri et al. (2007, 2008) detailed the reasons behind opting for such architecture of these ponds. The seasons in this part of the world is mainly demarcated as pre-monsoon (February–May), monsoon (June–September) and post-monsoon (October–January).

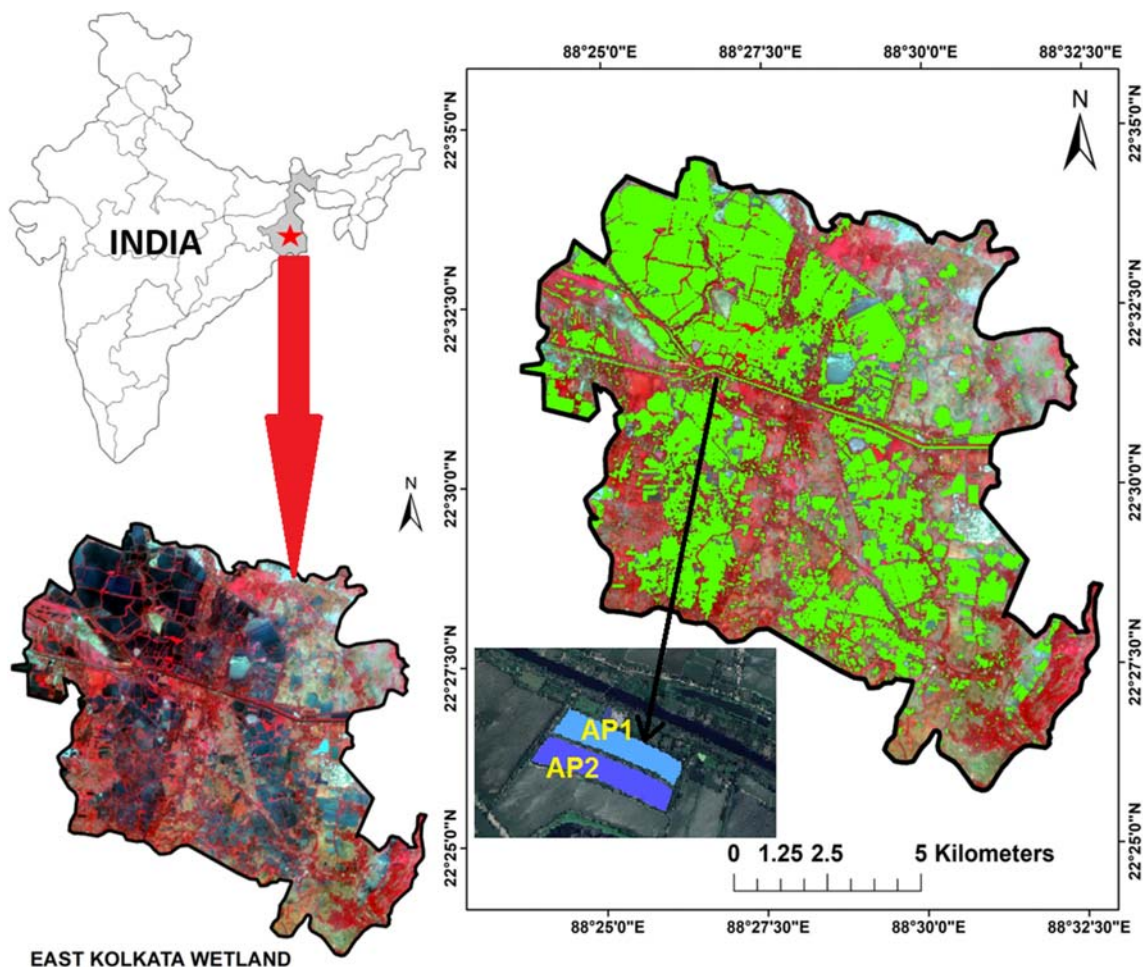


Fig. 1 The study area map showing the location of the ponds studied in the East Kolkata Wetlands, India

## Plan of sampling

Sampling was conducted in summer months of April and May 2018, in two aquaculture ponds [hereafter referred to as AP1 (22° 33' 16.26" N 88° 24' 47.34" E) (having a depth of 1.1 m and area of 44,019 m<sup>2</sup>) and AP2 (22° 31' 49.95" N 88° 25' 58.30" E) (having a depth of 0.6 m and area of 44,284 m<sup>2</sup>) respectively]. Samples were taken on 5 days covering the entire extent of summer season [1st April, 2018 (onset of summer), 15th April (early summer), 30th April (peak summer), 15th May, 2018 (peak summer) and 30th May, 2018 (summer end)]. High ambient temperatures are observed in the month of June, too; however, the southwest monsoon hits this part of the world in early June bringing with it heavy rains, and eventually the biogeochemical characteristics of the APs change significantly (mostly regulated by phenomenon-like surface runoff and dilution). Sampling in all the above-mentioned dates was done at 2-h intervals for 24 h duration. The atmospheric parameters were monitored with the help of a weather station and most of the physico-chemical parameters were measured in-situ by standard probes. For other parameters like air and water CH<sub>4</sub> concentration and chlorophyll-*a*, samples were collected and transferred to laboratory with appropriate measures of preservation being taken.

## Fishing practice of the two ponds

*Oreochromis nilotica* was cultured in both the ponds (AP1 and AP2). Around 25 kg of fingerlings were stocked per 1000 m<sup>3</sup> of water volume in both of the ponds during the end of March. No harvest was performed during the study period. Usually this species is allowed to grow for 3 months and harvested altogether around June end or July. Unlike other aquaculture ponds, no external fish feed was added to the ponds during the sampling. In EKW, the sewage water acts as the fish feed throughout the year. In monsoon season, sometimes external fish feed are added by the fishermen, as sewage water gets diluted due to heavy rain (Bunting et al. 2010). However, since sewage acts as the main source of food for the fishes, it is very difficult to quantify the total feed mass or to estimate the feed conversion ratio as the organic load in the sewage is principally dependent on the quality of the swage, which, in turn, varies spatially and temporally within EKW (Chanda et al. 2019). Since the two ponds are situated adjacent to each other, it was assumed that the same quality of sewage (having same organic load) entered both of the ponds during the time of sampling. This assumption was very essential behind carrying out a comparative analysis of CH<sub>4</sub> dynamics between the two ponds. Almost 1.1 million m<sup>3</sup> day<sup>-1</sup> sewage gets drained through the EKW having a TIC and TOC concentration of 95.6 ± 22.0 mg l<sup>-1</sup> and 348.05 ± 154.98 mg l<sup>-1</sup> respectively (Pal et al. 2018). On an average, a sewage water retention time of 33.8 ± 2.9 days is maintained in the aquaculture ponds of EKW (Roy Goswami et al. 2017). The inflow rate of

sewage water to the aquaculture ponds of EKW varies between 0.06 and 0.33 m s<sup>-1</sup> (Chattopadhyay et al. 2004).

## Analytical protocol

### Environmental variables

Air temperature and wind speed were measured by a portable weather station (WS-2350, La Crosse Technology). Conductivity and water temperature in the pond surface were monitored by a digital salinity meter (Thermo Scientific, Eutech, Germany) having a precision of 1 μS/cm and 0.1 °C respectively. Dissolved oxygen (DO) was monitored using a standard probe (FiveGo portable F4 Dissolved Oxygen meter, Mettler Toledo) having an accuracy of ± 1% and a precision of 0.01 mg l<sup>-1</sup>. Winkler's titrimetric method was also adopted to measure DO once during each sampling day and re-check the DO sensor's reading. pH was measured with the help of a Orion PerpHecT ROSS Combination pH Micro Electrode fitted to a micro-pH data logger (Thermo Scientific, U.S.A.) (precision—0.001). The glass electrodes were calibrated on the NBS scale with standard buffers at constant temperature (25 °C). Eutech TN-100 turbidity meter was implemented to monitor the turbidity of the surface water. Underwater photosynthetically active radiation (UWPAR) was monitored using a standard sensor (LI-192SA, Li-Cor, USA, precision 0.1 μ mol m<sup>-2</sup> s<sup>-1</sup>) and a data logger (Li-250A, Li-Cor, USA). Chlorophyll-*a* was measured according to standard spectrophotometric procedures (precision 0.01 mg m<sup>-3</sup>). The gross primary productivity (GPP) and community respiration (CR) in the APs were measured by standard light-dark bottle method (three replicates for each pond in each sampling day were sampled). Incubation was done for 12 h from 6 a.m. to 6 p.m., and the changes in DO level were measured with DO probes. Biochemical oxygen demand (BOD) was also measured by taking three replicate samples from each pond during each sampling day. Chlorophyll-*a*, GPP, CR and BOD were measured following standards methods as outlined in APHA (2005).

### Sampling of CH<sub>4</sub> concentration in air and surface water

Surface water samples were collected in 40-ml glass vials fitted with rubber septum leaving no headspace. Collected water samples were preserved with 8% HgCl<sub>2</sub> solution in order to arrest microbial activity. Before analysis, 20 ml of water from the glass vial was removed with the help of a disposable syringe and the remaining 20 ml of water sample in the glass vial was purged with nitrogen gas (99.99% pure) in the laboratory. After equilibration of the water sample for 2 h, 5 ml of the headspace gas was collected by a Hamilton syringe and analysed using a gas chromatograph (Systronics GC-8205) with a mean relative uncertainty of ± 2.9%. The detection limit and precision for CH<sub>4</sub> measurements were

0.4 ppm and 0.1 ppm respectively. Pure nitrogen gas used as the carrier gas and the retention time for CH<sub>4</sub> was 0.623 min. Injector temperature was raised to ~105 °C in order to remove the available moisture. Certified standard methane (10 ppm) was used for calibration of the GC. Ambient air samples above the respective pond surface were collected with the help of a battery operated pump in glass sampling bulbs, which were evacuated before collection; carefully made airtight. Extra precaution was taken by covering the stopcock by parafilm. Air samples were brought to the laboratory and analysed in GC by using the above-mentioned injection method.

### Air–water CH<sub>4</sub> flux estimation

pCH<sub>4</sub>(water) and pCH<sub>4</sub>(air) were converted to concentration of methane in water (CH<sub>4</sub>wc) and air (CH<sub>4</sub>ac) according to the Eqs. (1) and (2) (Morel 1983) and (3) (Lide 2007).

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

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

$$\ln K_H = -115.6477 + 155.5756/(T_K/100) \quad (3)$$

$$+ 65.2553 \times \ln(T_K/100) - 6.1698 \times (T_K/100)$$

where  $K_H$  stands for the gas partition constant of CH<sub>4</sub> in water at sampling temperature, expressed in mole l<sup>-1</sup> atm<sup>-1</sup>, and  $T_K$  denotes the temperature in Kelvin.

The CH<sub>4</sub> flux is calculated according to Eq. (4) (MacIntyre et al. 1995).

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

where  $k_x$  denotes the mass transfer coefficient (cm h<sup>-1</sup>) 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 CH<sub>4</sub> and it is dependent on water temperature according to the Eq. (6).  $k_{600}$  is computed from the wind speed ( $U_{10}$ ), according to Cole and Caraco (1998) (Eq. 7), and 'x' is equal to 0.66 for wind speed ≤ 3 m s<sup>-1</sup> and is equal to 0.5 for wind speed > 3 m s<sup>-1</sup>.

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

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

### Statistical analyses

Descriptive statistics of all the analysed parameters were computed by using Microsoft Excel 2007. The significance of the intra-seasonal variation of all the physico-chemical parameters along with air and water CH<sub>4</sub> concentration and air–water

CH<sub>4</sub> fluxes in each pond was tested by conducting one-way analysis of variance (ANOVA). Repeated measures ANOVA was applied to test the difference in mean of pH, conductivity, water temperature, DO and UWPAR, CH<sub>4</sub> concentration in water and air and air–water CH<sub>4</sub> fluxes between the two ponds (AP1 and AP2). Independent samples Student's *t* test was performed to test the difference in mean of turbidity, chlorophyll-*a*, BOD, GPP and CR between AP1 and AP2. Pearson correlation coefficients were computed to examine the relationship between pCH<sub>4</sub>(water) and the measured physico-chemical parameters. All these statistical analyses were carried out using SPSS version 16.0 (SPSS, Inc., USA), and the outcomes were considered significant at the 95% confidence level ( $p < 0.05$ ). All data were reported as mean ± standard deviation.

## Results

### Atmospheric conditions and comparative analysis of physico-chemical parameters between AP1 and AP2

The ambient air temperature varied between 32.7 °C and 35.3 °C during the summer and the daily mean temperature gradually increased from 33.1 ± 0.3 °C (on 1st April, 2018; onset of summer) to 34.2 ± 0.6 °C (on 31st May, 2018; peak of summer). Wind speed fluctuated between 3.7 m s<sup>-1</sup> and 5.3 m s<sup>-1</sup>, having a seasonal mean of 4.5 ± 0.4 m s<sup>-1</sup>. Based on the wind speed, the daily mean  $k_x$  ranged between 4.64 cm h<sup>-1</sup> and 5.06 cm h<sup>-1</sup>.

Seasonal mean pH of AP1 (7.777 ± 0.042) was significantly lower compared with the mean pH of AP2 (8.035 ± 0.041) (RMANOVA;  $F_{1, 59} = 4876.0$ ;  $p = 0.000$ ). Significant intra-seasonal variation of pH was observed in both AP1 and AP2 (see Table 1 for detailed statistical results). Though significant difference in mean conductivity was observed between the two ponds (RMANOVA;  $F_{1, 59} = 44.13$ ;  $p = 0.000$ ), the mean magnitudes differed over a small margin (1430 ± 46 μS cm<sup>-1</sup> in AP1; 1438 ± 50 μS cm<sup>-1</sup> in AP2). Mean water temperature also exhibited marginal difference between AP1 (33.0 ± 0.8 °C) and AP2 (33.1 ± 0.8 °C); however, the differences were found significant (RMANOVA;  $F_{1, 59} = 23.27$ ;  $p = 0.000$ ). Both water temperature and conductivity portrayed significant intra-seasonal variability. Like pH, DO and UWPAR were significantly lower in AP1 (5.8 ± 0.9 mg l<sup>-1</sup> and 7.71 ± 2.92 μmol m<sup>-2</sup> s<sup>-1</sup> respectively) compared with AP2 (7.8 ± 1.3 mg l<sup>-1</sup> and 10.55 ± 4.72 μmol m<sup>-2</sup> s<sup>-1</sup> respectively), and both of these parameters did not exhibit significant intra-seasonal variability in neither AP1 nor AP2. BOD, on the contrary, exhibited higher mean magnitudes in AP1 (13.4 ± 0.4 mg l<sup>-1</sup>) compared with AP2 (9.2 ± 0.5 mg l<sup>-1</sup>), and the difference was statistically significant ( $t = 25.49$ ;  $p = 0.000$ ).

**Table 1** Descriptive statistics of all the physico-chemical parameters as well as methane concentration and fluxes [mean ± standard deviation (minimum–maximum)] observed in AP1 and AP2 along with the results for statistical significance of the difference in the mean within each pond during the 5 days of sampling throughout the summer and between the two ponds

Parameters	Sampling no. per pond	AP1	AP2	Intra-seasonal variability within AP1 <sup>§</sup>	Intra-seasonal variability within AP2 <sup>§</sup>	Variability between AP1 and AP2
pH	60	7.777 ± 0.042 (7.702–7.873)	8.035 ± 0.041 (7.969–8.131)	F = 12.69; <i>p</i> = 0.000	F = 8.55; <i>p</i> = 0.000	F <sub>1, 59</sub> = 4876.0; <i>p</i> = 0.000 <sup>#</sup>
Conductivity (µS cm <sup>-1</sup> )	60	1430 ± 46 (1369–1567)	1438 ± 50 (1375–1580)	F = 24.07; <i>p</i> = 0.000	F = 28.84; <i>p</i> = 0.000	F <sub>1, 59</sub> = 44.13; <i>p</i> = 0.000 <sup>#</sup>
Water temperature (°C)	60	33.0 ± 0.8 (31.4–34.8)	33.1 ± 0.8 (31.5–34.8)	F = 18.46; <i>p</i> = 0.000	F = 16.30; <i>p</i> = 0.000	F <sub>1, 59</sub> = 23.27; <i>p</i> = 0.000 <sup>#</sup>
D.O. (mg l <sup>-1</sup> )	60	5.8 ± 0.9 (4.0–8.1)	7.8 ± 1.3 (6.1–10.9)	F = 1.57; <i>p</i> = 0.194	F = 1.42; <i>p</i> = 0.240	F <sub>1, 59</sub> = 437.80; <i>p</i> = 0.000 <sup>#</sup>
UWPAR (µmol m <sup>-2</sup> s <sup>-1</sup> )	35	7.71 ± 2.92 (3.15–14.25)	10.55 ± 4.72 (3.69–18.58)	F = 0.51; <i>p</i> = 0.728	F = 0.25; <i>p</i> = 0.907	F <sub>1, 34</sub> = 49.89; <i>p</i> = 0.000 <sup>#</sup>
Turbidity (NTU)	15	85.2 ± 5.8 (75.8–95.2)	61.4 ± 5.7 (49.8–69.4)	F = 14.83; <i>p</i> = 0.000	F = 19.26; <i>p</i> = 0.000	<i>t</i> = 11.31; <i>p</i> = 0.000 <sup>*</sup>
Chlorophyll- <i>a</i> (mg m <sup>-3</sup> )	15	51 ± 5 (42–59)	51 ± 4 (44–58)	F = 3.89; <i>p</i> = 0.037	F = 6.05; <i>p</i> = 0.010	<i>t</i> = -0.11; <i>p</i> = 0.910 <sup>*</sup>
BOD (mg l <sup>-1</sup> )	15	13.4 ± 0.4 (12.7–14.1)	9.2 ± 0.5 (8.2–9.9)	F = 7.83; <i>p</i> = 0.004	F = 4.81; <i>p</i> = 0.020	<i>t</i> = 25.49; <i>p</i> = 0.000 <sup>*</sup>
GPP (gO <sub>2</sub> m <sup>-2</sup> day <sup>-1</sup> )	15	5.8 ± 0.4 (5.1–6.4)	5.8 ± 0.4 (5.1–6.4)	F = 3.21; <i>p</i> = 0.061	F = 5.84; <i>p</i> = 0.011	<i>t</i> = 0.15; <i>p</i> = 0.882 <sup>*</sup>
CR (gO <sub>2</sub> m <sup>-2</sup> day <sup>-1</sup> )	15	34.8 ± 2.4 (30.8–39.1)	24.5 ± 1.7 (21.6–27.9)	F = 5.61; <i>p</i> = 0.012	F = 6.26; <i>p</i> = 0.009	<i>t</i> = 13.73; <i>p</i> = 0.000 <sup>*</sup>
pCH <sub>4</sub> (water) (ppm)	60	8.076 ± 2.528 (5.250–15.130)	3.373 ± 0.691 (2.230–5.130)	F = 3.19; <i>p</i> = 0.020	F = 4.33; <i>p</i> = 0.004	F <sub>1, 59</sub> = 344.01; <i>p</i> = 0.000 <sup>#</sup>
pCH <sub>4</sub> (air) (ppm)	60	1.876 ± 0.008 (1.859–1.890)	1.876 ± 0.008 (1.857–1.891)	F = 10.28; <i>p</i> = 0.000	F = 11.67; <i>p</i> = 0.000	F <sub>1, 59</sub> = 0.22; <i>p</i> = 0.641 <sup>#</sup>
CH <sub>4</sub> flux (mg m <sup>-2</sup> h <sup>-1</sup> )	60	24.79 ± 12.02 (11.40–59.36)	6.05 ± 3.14 (1.21–14.79)	F = 4.61; <i>p</i> = 0.003	F = 5.623; <i>p</i> = 0.001	F <sub>1, 59</sub> = 243.11; <i>p</i> = 0.000 <sup>#</sup>

Non-significant *p* values are given in italic fonts

<sup>§</sup> Results of one-way analysis of variance (ANOVA) is presented for the difference in mean for the five sampling dates

<sup>#</sup> Results of repeated measures analysis of variance (RMANOVA) is presented for difference in mean between AP1 and AP2

<sup>\*</sup> Results of independent samples Student's *t* test is presented for difference in mean between AP1 and AP2

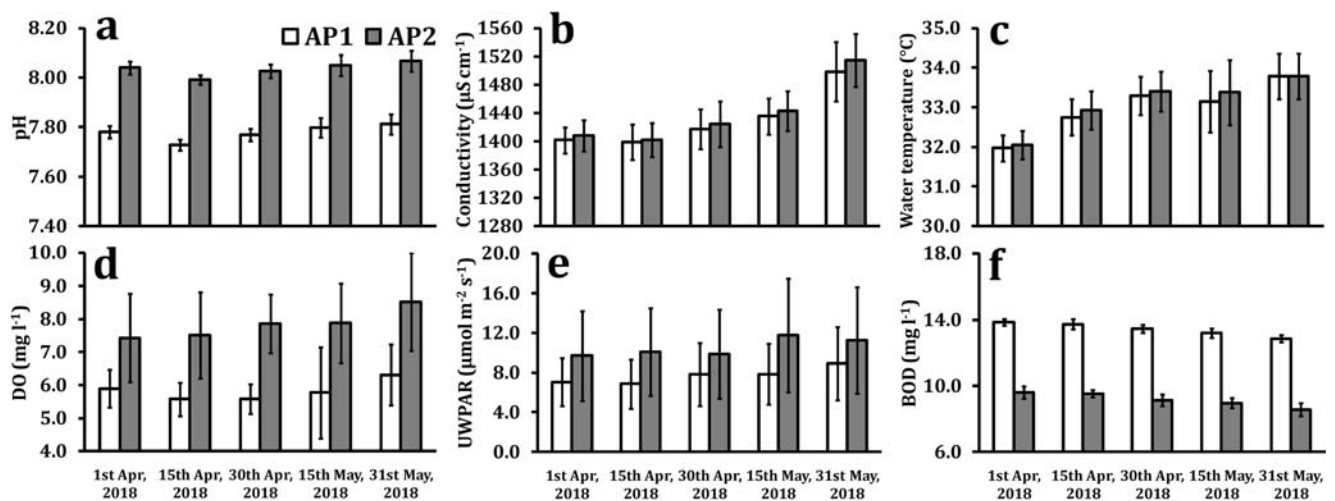
The intra-seasonal variation along with the difference between AP1 and AP2 of all the parameters discussed in this paragraph are illustrated in Fig. 2.

Mean chlorophyll-*a* concentrations were almost identical in AP1 (51 ± 5 mg m<sup>-3</sup>) and AP2 (51 ± 4 mg m<sup>-3</sup>), and there was no statistically significant difference (*t* = -0.11; *p* = 0.910); however, intra-seasonal variation was significant in both of the ponds (Fig. 3). Turbidity in the water column was substantially higher in AP1 (85.2 ± 5.8 NTU) than AP2 (61.4 ± 5.7 NTU). Not only was the mean but the range of turbidity magnitudes altogether were completely different in AP1 (75.8–95.2 NTU) and AP2 (49.8–69.4 NTU). Like chlorophyll-*a* concentration, GPP also exhibited similar mean magnitudes in AP1 (5.8 ± 0.4 gO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>) and AP2 (5.8 ± 0.4 gO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>) with no significant difference (*t* = 0.15; *p* = 0.882). However, CR was significantly higher in AP1 (34.8 ± 2.4 gO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>) than AP2 (24.5 ± 1.7 gO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>).

Apart from GPP in AP1, all the parameters mentioned in this paragraph exhibited significant intra-seasonal variability.

### Diurnal variation and difference in pCH<sub>4</sub>(water) and air–water CH<sub>4</sub> flux between AP1 and AP2

Seasonal mean pCH<sub>4</sub>(water) was double in AP1 (8.076 ± 2.528 ppmv) compared with AP2 (3.373 ± 0.691 ppmv). Moreover, pCH<sub>4</sub>(water) varied over a wider range in AP1 (5.250 ppmv to 15.130 ppmv) with respect to AP2 (2.230 ppmv to 5.130 ppmv) exhibiting statistically significant differences between the two ponds (RMANOVA; F<sub>1, 59</sub> = 344.01; *p* = 0.000). Seasonal mean pCH<sub>4</sub>(air) did not vary was absolutely identical in the ambience of both of the ponds with no significant variation (RMANOVA; F<sub>1, 59</sub> = 0.22; *p* = 0.641). However, both pCH<sub>4</sub>(water) and pCH<sub>4</sub>(air) showed

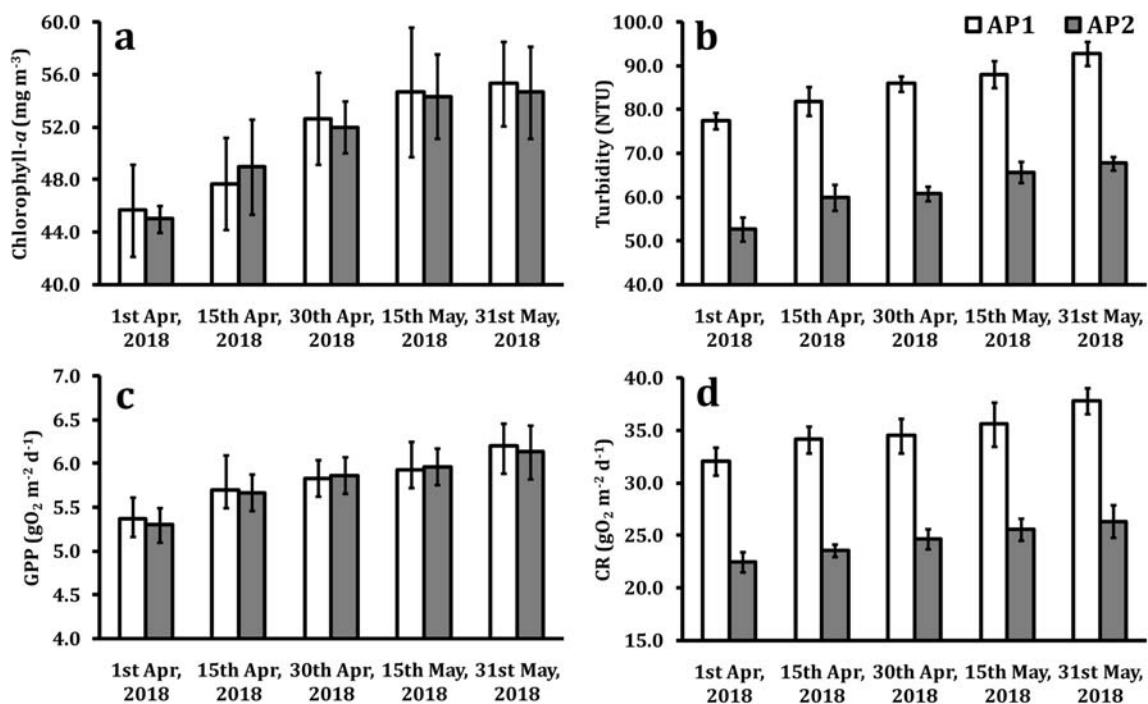


**Fig. 2** The intra-seasonal variation of mean **a** pH, **b** conductivity, **c** water temperature, **d** dissolved oxygen, **e** underwater photosynthetically active radiation (UWPAR) and **f** biochemical oxygen demand (BOD) in AP1 and AP2. The error bars showing the standard deviation from the diel

mean observed in the respective dates of sampling. The origin of the y-axis in most of the plots is shifted suitably to clearly portray the difference between AP1 and AP2

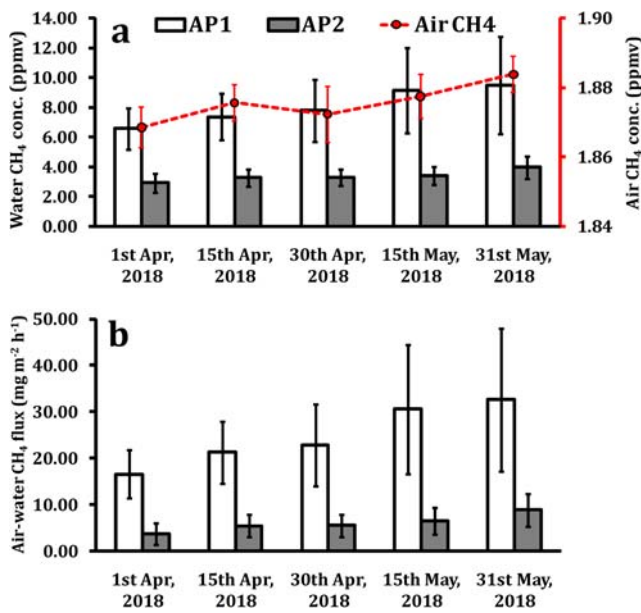
significant intra-seasonal variability in both AP1 and AP2. Especially  $\text{pCH}_4(\text{water})$  magnitudes were found to increase steadily starting from the onset of summer and climbed to the highest values in the end of summer (Fig. 4). The air–water  $\text{CH}_4$  fluxes mirrored the variability of  $\text{pCH}_4(\text{water})$  in both AP1 ( $24.79 \pm 12.02 \text{ mg m}^{-2} \text{ h}^{-1}$ ) and AP2 ( $6.05 \pm$

$3.14 \text{ mg m}^{-2} \text{ h}^{-1}$ ) and exhibited significant intra-seasonal variability as well. The diurnal variation of  $\text{pCH}_4(\text{water})$  and air–water  $\text{CH}_4$  fluxes is illustrated in Fig. 5. Highest  $\text{pCH}_4(\text{water})$  and air–water  $\text{CH}_4$  flux magnitudes were observed in the late noon hours, and it reached a steady value during the night time, much lower than the day time magnitudes.



**Fig. 3** The intra-seasonal variation of mean **a** chlorophyll-*a*, **b** turbidity, **c** gross primary productivity (GPP) and **d** community respiration (CR) in AP1 and AP2. The error bars showing the standard deviation from the

diel mean observed in the respective dates of sampling. The origin of the y-axis in all the plots is shifted suitably to clearly portray the difference between AP1 and AP2



**Fig. 4** The intra-seasonal variation of mean **a** CH<sub>4</sub> concentration in surface water and ambient air along with **b** the air–water CH<sub>4</sub> fluxes observed in AP1 and AP2. The error bars showing the standard deviation from the diel mean observed in the respective dates of sampling

**Relationship between pCH<sub>4</sub>(water) and other physico-chemical variables**

The scatter plots of the hourly pH, conductivity and water temperature exhibited statistically significant positive correlation with pCH<sub>4</sub>(water) in both AP1 ( $R^2 = 0.61, p < 0.05$ ;  $R^2 = 0.69, p < 0.05$  and  $R^2 = 0.68, p < 0.05$  respectively) and AP2 ( $R^2 = 0.61, p < 0.05$ ;  $R^2 = 0.59, p < 0.05$  and  $R^2 = 0.71, p < 0.05$  respectively) (Fig. 6). The daily mean chlorophyll-*a*, turbidity, BOD, GPP and CR also depicted statistically significant positive

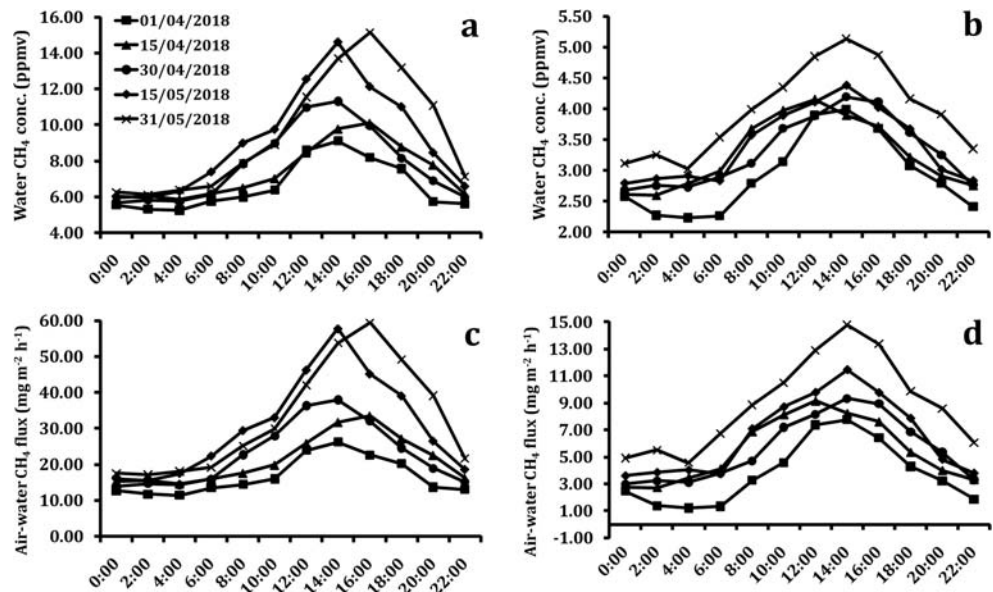
correlation with daily mean pCH<sub>4</sub>(water) ( $p < 0.05$ ) in both AP1 and AP2. Comparatively, the goodness of fit ( $R^2$  values) was much better in case of AP1 than AP2 (Fig. 6).

**Discussion**

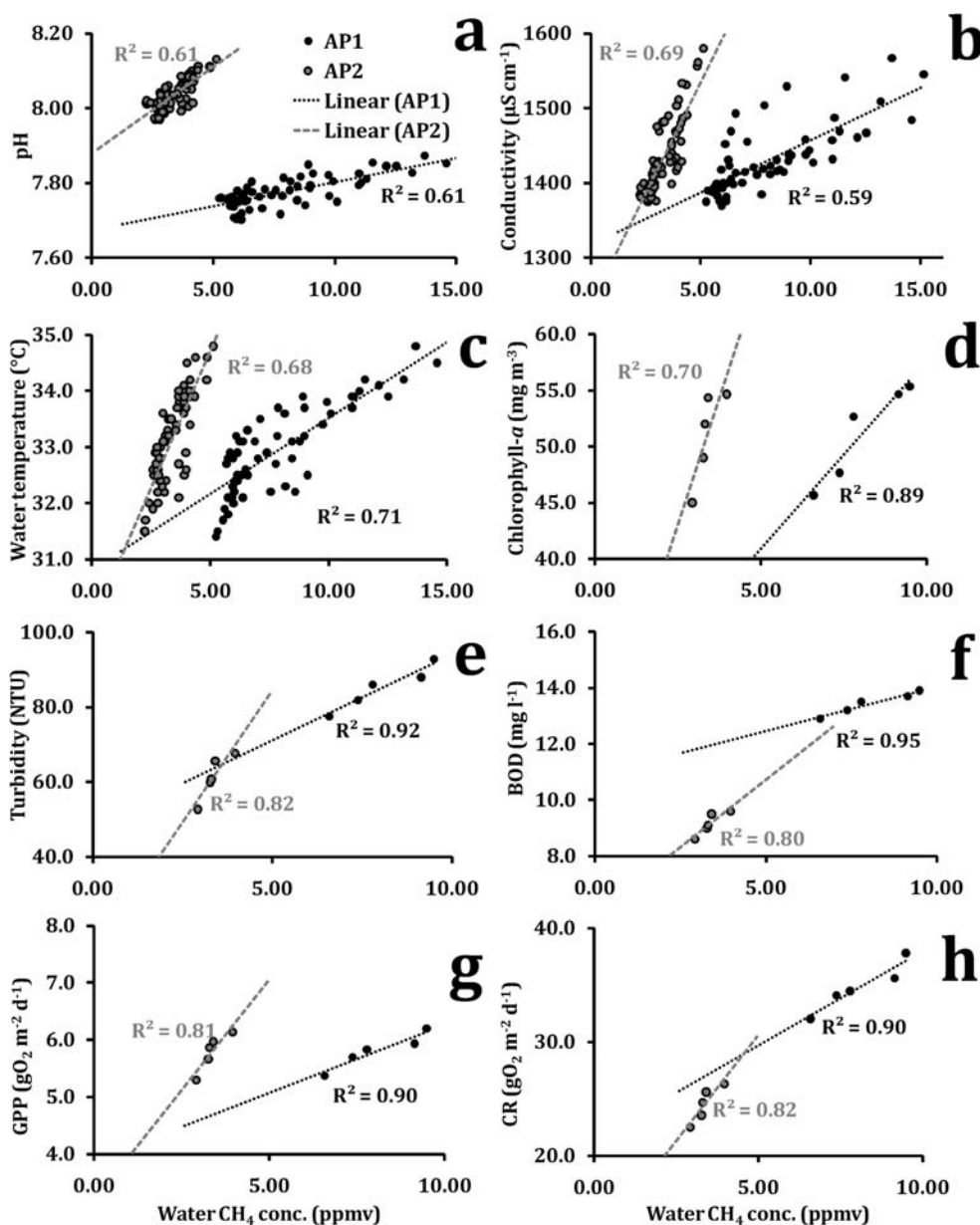
**pCH<sub>4</sub>(water) variability and environmental factors**

The pCH<sub>4</sub>(water) magnitudes in both of the ponds exhibited significant positive correlation with water temperature. The ambient air temperature and the water temperature increased simultaneously from the onset of summer to its peak. This implied that water temperature played a crucial role in governing the pCH<sub>4</sub>(water) and hence the air–water CH<sub>4</sub> fluxes. Similar effect of temperature on CH<sub>4</sub> fluxes were observed by Olsson et al. (2015) while working in the coastal wetlands of Liaohe Delta situated in Northeast China and Natchimuthu et al. (2016) while studying in Skogaryd Research Catchment situated in the southwest of Sweden. Increase in temperature is known to favour the methanogenic bacteria in the sediment and aquatic columns of shallow ponds and also facilitates organic matter degradation at a much higher rate (Wang et al. 2016; Vizza et al. 2017). Man-made reservoirs and natural lakes have exhibited similar findings in the past (Yang et al. 2015). Moreover, highest CH<sub>4</sub> emission was observed during the summer months compared with the other months in many studies (Xing et al. 2005). The significant role of temperature was further justified by the fact that highest pCH<sub>4</sub>(water) and air–water CH<sub>4</sub> flux magnitudes were observed in the late noon hours, which coincided with daily water temperature maxima. Like water temperature, a positive correlation was also observed between pCH<sub>4</sub>(water) magnitudes and pH. Yang et al. (2017) also observed similar results. They argued that

**Fig. 5** The diel variation of CH<sub>4</sub> concentration in surface water in **a** AP1 and **b** AP2 along with the diel variation of air–water CH<sub>4</sub> fluxes in **c** AP1 and **d** AP2







**Fig. 6** The scatter plots showing the goodness of fit (linear) between the hourly  $\text{CH}_4$  concentration in surface water with **a** hourly pH, **b** hourly conductivity, **c** water temperature and scatter plots with the goodness of fit (linear) between the daily mean  $\text{CH}_4$  concentration in surface water with

daily mean **d** chlorophyll-*a*, **e** turbidity, **f** BOD, **g** GPP and **h** CR. The origin of the y-axis in all the plots is shifted suitably to clearly portray the difference between AP1 and AP2

increased hydrogenotrophic methanogenesis leads to consumption of  $\text{CO}_2$  from the water column, which leads to enhancement in pH. This, in turn, led Yang et al. (2017) to infer that positive correlation between air–water  $\text{CH}_4$  flux and pH was observed as an outcome of the processes that is taking place during methanogenesis rather than the elevated pH being a direct driver for methane production.

Chlorophyll-*a* magnitude is known to serve as a proxy of trophic status and algal production rate in a eutrophic and/or hypereutrophic shallow aquatic system (Yang et al. 2015; Liu et al. 2017). The warmer phase of the year (i.e.

the summer months) often experience higher algal production rates (exhibited by higher chlorophyll-*a* magnitudes), and it leads to production of new autochthonous organic matter as observed by Palma-Silva et al. (2013). In the present study, a significant positive correlation was exhibited by the daily mean chlorophyll-*a* and GPP with the  $\text{pCH}_4(\text{water})$  which implied that the autochthonous production of new organic carbon acted as substrates for methanogenesis (Flury et al. 2010; Furlanetto et al. 2012). BOD was measured in this study in order to have an idea about the biodegradable organic matter load in

these aquatic systems.  $p\text{CH}_4(\text{water})$  exhibited a positive correlation with BOD in both the ponds, which further supports the fact that higher organic matter loading led to higher methane concentration in the water and hence higher air–water  $\text{CH}_4$  fluxes. A positive correlation was also observed between the  $p\text{CH}_4(\text{water})$  and turbidity in these waters. Usually the BOD and turbidity in this kind of sewage-fed aquaculture ponds are measured to assess the quality of sewage entering the aquaculture systems (Sarkar et al. 2017). It is clear from the above-mentioned findings that both allochthonous input of sewage and autochthonous regeneration of organic matter within the aquaculture system are facilitating methanogenesis in the aquaculture ponds of EKW.

### Comparative analysis of $p\text{CH}_4(\text{water})$ and air–water $\text{CH}_4$ flux in ponds of different depths

Though the environmental factors, which are known to regulate the  $p\text{CH}_4(\text{water})$  and hence air–water  $\text{CH}_4$  flux, exhibited similar relationships in both AP1 and AP2, there was a statistically significant difference in magnitudes of both  $p\text{CH}_4(\text{water})$  and air–water  $\text{CH}_4$  flux between the two ponds. Both of the aquaculture ponds can be categorized into the class of conventional shallow aquaculture ponds; however, AP1 had a depth almost double of that of AP2. It was clearly observed that AP1 exhibited ~2 times  $p\text{CH}_4(\text{water})$  and ~4 times air–water  $\text{CH}_4$  exchange compared with AP2. Based on the results obtained from this study, we argue that higher depth led to higher rate of methanogenesis and vice-versa. Chanda et al. (2019) in a recent study observed that the photosynthetically active radiation penetrating these waters was very low and that these ponds have an extremely shallow euphotic zone. In the present study, the UWPAR was found to be lower in AP1 compared with AP2. Due to this reason,

autotrophic activities were facilitated at a higher rate in the AP2 since its depth itself was very small and a substantial part of the water column could engage in photosynthetic activities, whereas in AP1, due to higher depth, net heterotrophy was more prevalent. This hypothesis can be justified from the observations that AP1 and AP2 despite having similar chlorophyll-*a* concentration and GPP in the surface waters, exhibited significant difference in DO and CR magnitudes. DO levels in AP1 were significantly lower than that observed in AP2 and in case of CR, AP1 exhibited much higher values than that of AP2. Yang et al. (2013b) examined that under reduced environment, i.e. lower DO levels, both in water column and bottom sediment,  $\text{CH}_4$  oxidation decreased substantially and eventually it gave rise to higher  $p\text{CH}_4(\text{water})$  and hence higher  $\text{CH}_4$  emission. We can thus infer that higher depth is making the pond sediment and the water column much more prone to exhibit net heterotrophy and the reduction in DO level as a consequence is facilitating  $\text{CH}_4$  emission. One of the main reasons why UWPAR was low in AP1 compared with AP2 could be attributed to the higher turbidity in AP1. Boyd et al. (2016) specified that turbidity in this kind of aquaculture ponds are mostly imparted by particulate organic carbon (POC) and soil re-suspended sediments. If we assume that the soil sediment churning is taking place at a similar rate in both the ponds, the amount of POC would be much higher in AP1 as its water column is almost double that of AP2, thus were capable of providing more organic materials for the methanogens to feed upon. Moreover, apart from DO level, the rate of autotrophic activities can, in turn, regulate the pH of the water column. Higher autotrophic production consumes the  $\text{CO}_2$  from the water column, and it leads to rise in pH (Yang et al. 2018b). The mean pH observed in AP1 was > 8.0, whereas in AP2, pH varied between 7.7 and 7.8. Chang and Yang (2003) observed that the methanogens are quite sensitive to pH and that these methanogens thrive best at an

**Table 2** Comparison of air–water  $\text{CH}_4$  fluxes ( $\text{mg m}^{-2} \text{h}^{-1}$ ) observed in the air–water interface of aquaculture ponds

Sampling site	Type of aquaculture	Sampling period	$\text{CH}_4$ fluxes	Reference
East Kolkata Wetlands, West Bengal, India	Sewage-fed fish pond (AP1—depth: 1.1 m)	April and May, 2018	11.40 to 59.36 (24.79)	Present study
East Kolkata Wetlands, West Bengal, India	Sewage-fed fish pond (AP2—depth: 0.6 m)	April and May, 2018	1.21 to 14.79 (6.05)	Present study
Shanyutan Nature Reserve, Min River Estuary, China	Undrained polyculture pond	December, 2011 to January, 2012	0.05 to 4.96 (1.12)	Yang et al. (2018a)
Shanyutan Nature Reserve, Min River Estuary, China	Drained polyculture pond	December, 2011 to January, 2012	0.06 to 28.32 (10.56)	Yang et al. (2018a)
Min River Estuary, China	Shrimp	June–October, 2015	5.53 to 189.38 (86.01)	Yang et al. (2018b)
Jiulong River Estuary, China	Shrimp	June–October, 2015	2.54 to 23.54 (9.64)	Yang et al. (2018b)
Nanjing Agricultural University (Experimental farm), Xinghua, Jiangsu province, China	Rice paddy converted to fish aquaculture pond	June, 2014 to June, 2015	(0.51)	Wu et al. (2018)
Shanyutan wetland, Min River Estuary, China	Marsh ecosystem converted shrimp pond	June, August and October, 2015	6 to 200 (123)	Yang et al. (2017)
Nanjing Agricultural University (Experimental farm), Xinghua, Jiangsu province, China	Rice paddy converted to mixed crab–fish aquaculture pond	November, 2013–November, 2014	(0.37)	Liu et al. (2016)
Xinghua, China	Mixed crab–fish	June–October, 2013	0.04 to 0.10 (0.07)	Hu et al. (2016)
Shanyutan wetland, Min River Estuary, China	Shrimp	June–November, 2012	1.44 to 94.60 (19.95)	Yang et al. (2015)
Shanyutan wetland, Min River Estuary, China	Mixed shrimp–fish	September–November, 2011	0.12 to 7.18 (1.65)	Yang et al. (2015)

optimum pH of 7.7. This further indicates that the heterotrophic conditions observed in AP1 led to lower pH values, which, in turn, gave rise to favourable conditions for the methanogens.

### Comparison with global scenario

Efforts towards characterizing of the CH<sub>4</sub> emission potential (along with other greenhouse gases like CO<sub>2</sub> and N<sub>2</sub>O) from various types of aquaculture ponds were largely made by China. Though aquaculture practice is becoming very popular in India day by day, reports of GHG emission studies from this ecosystem are scarce till date. Datta et al. (2009) quantified the CH<sub>4</sub> emission in a sub-humid tropical rice field where integrated rice–fish farming was practiced under rain-fed lowland conditions. However, direct measurements in aquaculture ponds are not yet reported. The range of CH<sub>4</sub> fluxes along with the mean observed from various studies conducted in China is given in Table 2. The mean CH<sub>4</sub> emission rate from AP1 was found substantially high compared with all the observations made so far, except the reports of Yang et al. (2017, 2018b) from the shrimp ponds situated in Min River Estuary, China. However, it should be considered that the temporal span of most of the studies tabulated in Table 2 are much more than the present study and the present study was conducted exclusively in the summer months, when the CH<sub>4</sub> emission rates usually remain very high. On the contrary, the mean CH<sub>4</sub> emission rate from AP2 was less than many of the observations and almost comparable with few. Thus it can be inferred that the two aquaculture ponds of EKW taken up for this study having different depths exhibited considerable variation in CH<sub>4</sub> flux magnitudes when compared with the global results, despite being situated under same climatic regime and experiencing same fishing practice using exactly the same sewage water. This further enabled us to testify the importance of the depth of these aquaculture ponds in regulating the air–water CH<sub>4</sub> fluxes.

### Uncertainties and scope for future studies

The sampling conducted for the present study was limited only to the summer months and that too for only two aquaculture ponds having different depths. In order to draw more holistic inference about the CH<sub>4</sub> emission scenario from the aquaculture ponds of EKW, annual cycle should be covered and sampling should be conducted in more number of ponds to examine the spatial variation of pCH<sub>4</sub>(water) within EKW. Since the bulk formula method from the gradient of CH<sub>4</sub> concentration between water and air was adopted for estimation of the CH<sub>4</sub> flux, only the diffusive flux could be taken into consideration. In future the CH<sub>4</sub> ebullition measurements should be also taken up preferably by chamber method. A year-round data covering more sampling sites would enable us to derive an annual estimate, which was not possible from this short term study. Moreover, special emphasis should be given on the different stages of aquaculture while

estimating the CH<sub>4</sub> fluxes like stocking, pre-sewage inflow, post-sewage inflow, liming, pre-harvesting and post-harvesting as earlier studies reported that different stages of aquaculture exhibited varying magnitudes of CH<sub>4</sub> fluxes (Liu et al. 2016; Wu et al. 2018).

### Conclusion

Analysing the outcomes obtained from the present study, it can be inferred that the aquaculture ponds of EKW alike all other aquaculture ponds studied so far acted as strong sources of CH<sub>4</sub> in the summer months, when both the ambient and water temperature remain substantially high compared with other months of a year. Apart from water temperature, primary productivity and turbidity notably exhibited a significant positive relationship with CH<sub>4</sub> concentration in water and hence air–water CH<sub>4</sub> fluxes. Higher productivity and turbid conditions were found to furnish more substrates for methanogenesis, which, in turn, led to higher air–water CH<sub>4</sub> fluxes. Out of the two ponds sampled, the pond having higher depth was found to emit more CH<sub>4</sub> than the one comparatively shallower. Lower level of dissolved oxygen due to net heterotrophic character of the pond having greater depth was principally found responsible for triggering more methanogenesis. DO is known to oxidize the methane, which are comparatively more prevalent in the shallower pond. Moreover, in the shallower pond, due to higher photosynthetic rate, the pH values were higher, which was not favourable for the methanogens to thrive properly. All these factors together indicated that depth could be a principal governing factor of diffusive air–water CH<sub>4</sub> fluxes from an aquaculture pond. In future, more thrust needs to be given on the spatial and temporal resolution of the sampling and characterization of the CH<sub>4</sub> ebullition from this ecosystem to draw a more holistic scenario and end up with a comprehensive annual estimate.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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