

1 **Seasonal variations in source-sink balance of CO₂ in**
2 **subtropical earthen aquaculture ponds: Implications**
3 **for carbon emission management**

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22 **ABSTRACT**

23 Aquaculture ponds serve as focal points for carbon cycling and act as anthropogenic
24 contributors to the emission of carbon dioxide (CO₂). To understand the seasonal CO₂
25 dynamics within the ponds, we measured the CO₂ concentrations in sediment porewater
26 and the water column in aquaculture ponds in the Shanyutan Wetland in China.
27 Subsequently, the sediment-to-water and water-to-air CO₂ fluxes were calculated based
28 on the gas transfer coefficient model. Our results showed that that CO₂ flux ranged 0.01–
29 4.58 mmol m⁻² h⁻¹ across the sediment-to-water interface and -0.08–0.45 mmol m⁻² h⁻¹
30 across the water-to-air interface throughout the farming period. Photosynthetic activity
31 was the key driver of the temporal variations in water column CO₂ concentration and
32 water-to-air CO₂ flux, while the change in porewater CO₂ concentration and sediment-to-
33 water CO₂ flux were governed by sediment temperature which drive the microbial
34 decomposition of organic matter. Based on a simple mass balance approach, the apparent
35 CO₂ consumption (ACC) in the water column across all seasons ranged from 0.24 to 2.32
36 mmol m⁻² h⁻¹, indicating that the pond water body had a high capacity to “consume” the
37 excess CO₂. Our results highlight that the contrasting roles between the sediment
38 compartment and water column compartment in CO₂ dynamics, and the possibility to
39 manipulate ACC to reduce the aquaculture carbon footprint.

40 **Keywords:** Carbon dioxide; Aquaculture ponds; Carbon footprint; Photosynthesis;
41 Climate impact

42 **1. Introduction**

43 Carbon dioxide (CO₂) is a major greenhouse gas, accounting for approximately 60%
44 of the overall atmospheric radiative forcing (Le Quéré et al., 2018; IPCC, 2014). Since
45 1750, there has been a 150% increase in the global atmospheric CO₂ concentration,
46 reaching 417.2 ppm in 2022 (Friedlingstein et al., 2022), with many aquatic systems (e.g.
47 lakes, reservoirs, rivers) acting as important CO₂ sources to the atmosphere (Borges et al.,
48 2015; Li et al., 2018; Raymond et al., 2013; Tangen et al., 2016; Tranvik et al., 2009).

49 Small and shallow ponds, being integral components of global aquatic ecosystems,
50 are hotspots for carbon cycling (Holgerson, 2015; Hou et al., 2023) and CO₂ emission
51 (Holgerson and Raymond, 2016; Jensen et al., 2023; Prėskienis et al., 2021; Zhang et al.,
52 2023). Small-scale aquaculture ponds are extensively found across various locations (FAO,
53 2018; Kosten et al., 2020; Luo et al., 2022), covering about 5.4×10^5 km² of surface area
54 by 2016 (FAO, 2016). CO₂ emission from aquaculture has gained much attention
55 (MacLeod et al., 2020) thanks to the fast-growing aquaculture sector world-wide (Naylor
56 et al., 2021). In developing countries, unfortunately, proper monitoring of small-scale
57 aquaculture ponds is often lacking (Soares and Henry-Silva, 2019; Xu et al., 2023; Zhang
58 et al., 2022). Generally, these aquaculture ponds receive substantial quantities of organic
59 matter as feeds, autochthonous carbon input and animal wastes (Chen et al., 2015; Tong
60 et al., 2020). Organic carbon deposited into the sediment could be remineralized into CO₂
61 (Yang et al., 2022a); processes such as diffusion and bioturbation at the sediment-water
62 interface would then determine the CO₂ flux to the overlying water (Gruca-Rokosz and

63 [Tomaszek, 2015](#); [Xiong et al., 2017](#)). Meanwhile, processes within the water column
64 would further modulate the gains and losses of CO₂ ([Morales-Williams et al., 2017, 2021](#);
65 [Yang et al., 2022b](#)), which would then determine the magnitude and direction of CO₂ flux
66 across the water-air interface.

67 In China, small-scale earthen shrimp ponds are among the fastest growing aquaculture
68 systems ([Duan et al., 2020](#); [Ren et al., 2019](#)), many of which were created by clearing
69 areas of coastal wetlands. The Shanyutan Wetland is one the largest wetlands in Fujian,
70 China. Large swaths of the Shanyutan Wetland were converted to shrimp ponds in the past
71 decades, but environmental monitoring of the aquaculture operation here has been lacking
72 ([Tong et al., 2020](#); [Yang et al., 2022b](#)), but which will be critical to assess its carbon
73 footprint and climate impact. We have recently begun to investigate the greenhouse gas
74 dynamics in these aquaculture ponds, and observed that the water-to-air CO₂ flux varied
75 seasonally, and was negative during certain time of the year (i.e., CO₂ going from air into
76 water) ([Tan et al., 2023](#)). This suggests capacity for the aquaculture ponds to switch
77 between being a CO₂ source and a CO₂ sink. To fully understand this source-sink dynamics,
78 it is necessary to include sediment CO₂ flux data and study how it varies seasonally.

79 In this study, the concentration of CO₂ was measured in both the water column and
80 the sediment porewater, and the sediment-to-water and water-to-air CO₂ fluxes were
81 calculated. Using a simple mass balance approach, we examined the CO₂ source-sink
82 balance within the aquaculture ponds, how it was influenced by environmental factors and
83 varied between seasons. This research aimed to determine the seasonal variations in CO₂

84 fluxes across the sediment-water-air interfaces and its main driving factors.

85 **2. Materials and methods**

86 *2.1. Study area*

87 The Shanyutan Wetland (22°00'36"26°03'42"N, 119°34'12"–119°41'40"E, [Figure 1](#)) in
88 the Fujian Province, China, served as the location for this study. Situated at the southern
89 tip of the Min River Estuary, the region experiences a subtropical marine monsoon climate
90 characterized by an average air temperature of 19.6 °C and 139 cm of precipitation per
91 year ([Yang et al., 2020a, 2023](#)). The dominant vegetation in the area consists of native
92 species such as *Cyperus malaccensis* and *Phragmites australis*, along with the invasive
93 *Spartina alterniflora* ([Tan et al., 2023; Tong et al., 2018](#)). Extensive areas of the tidal
94 saltmarshes were cleared and converted to aquaculture shrimp ponds over the past decades
95 ([Yang et al., 2020b](#)). Shrimp farming typically take place from May to November, yielding
96 a single crop per year.

97 *2.2. Collection of sediment and water samples*

98 Three shrimp (*Litopenaeus vannamei*) ponds were selected for the study ([Figure 1](#)).
99 Each pond was ~1.5 m deep and had an area of 1.5–2.0 ha. Field sampling was carried out
100 monthly from April 2019 to January 2020. Samples of sediment and water were collected
101 from three different locations with each pond: one near the bank, one in the feeding zone,
102 and one at the center of the pond ([Tian et al., 2023](#)). The upper 15 cm sediments were
103 collected using a steel cylinder with a diameter of 5 cm then transferred into sterile plastic
104 bags. For the water column samples, a 1.5-L organic glass hydrophore was utilized to

105 collect samples from the surface layer (approximately 10 cm below the surface), mid-
106 depth, and the bottom layer (around 5 cm above the sediment). The collected water
107 samples were immediately divided into two portions. One portion of the water sample was
108 transferred into a pre-weighed serum glass bottle (55 mL) without bubbles and preserved
109 with 0.5 mL HgCl₂ for the analyses of dissolved CO₂ concentrations (Zhang et al., 2021).
110 The other portion was transferred into a 150 mL polyethylene bottle for analyzing physico-
111 chemical characteristics and chlorophyll *a* (Chl-*a*) concentrations. Prior to analysis in the
112 laboratory, the samples were stored in the dark at 4 °C for 4–6 hours.

113 *2.3. Analyses of sediment and water samples*

114 Sediment water content (SWC), bulk density (BD) and sediment porosity (POR) were
115 measured in the laboratory using a subsample from each sediment sample. The
116 measurement was based on weight loss before and after drying the subsample (Yin et al.,
117 2019; Zhang et al., 2013). The freeze-dried subsample was subjected to homogenization
118 and grinding until it formed a fine powder. Afterward, some of the powder was mixed with
119 deionized water in a 1:2.5 ratio (v/v) for measuring pH (Orion 868 pH meter, USA) and
120 in 1:5 ratio for measuring salinity (Eutech Instruments-Salt6 salinity meter, USA).
121 Sediment total carbon (TC) was measured via an elemental analyzer (Elementar Vario
122 MAX CN, Germany).

123 Porewater in raw sediment samples was extracted by centrifugation (Cence® L550,
124 Bodmer et al., 2020; De Vittor et al., 2012; Matos et al., 2016). Acetate fiber membranes
125 (0.45 µm-Biotrans™ nylon membranes) were employed to filter approximately 20 mL of

126 porewater and 100 mL of water column sample. The filtrates were analysed for the
127 concentrations of Cl^- and SO_4^{2-} using an ion chromatograph (Dionex 2100, USA), and
128 $\text{PO}_4^{3-}\text{-P}$, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ using a flow injection analyzer (Skalar Analytical SAN⁺⁺,
129 Netherlands). Concentrations of DOC in sediment porewater were analyzed with a TOC
130 Analyzer (TOC-*V*CPH/CPN, Shimadzu, Japan). Furthermore, Chl-*a* in water column samples
131 was extracted in 90% ethanol solution for 24 h and measured on a UV-visible
132 spectrophotometer (Shimadzu UV-2450, Japan) (Jeffrey and Humphrey, 1975; Kang et al.,
133 2023; Xu et al., 2017). In each sampling campaign, ancillary data including water
134 temperature (T_w), sediment temperature (T_s), and dissolved oxygen (DO) were measured
135 with a portable temperature meter (IQ150, USA) and a multiparameter probe (550A YSI,
136 USA). Air temperature (T_A) and wind speed (W_s) were collected from the automatic
137 meteorological station at the Min River Estuary (MRE) weather station.

138 *2.4. Measurement of CO₂ concentration in water column and sediment porewater*

139 The measurement of CO₂ concentration in water column and sediment porewater was
140 conducted using the headspace equilibrium technique and a gas chromatograph (Bellido
141 et al., 2009; Li et al., 2023; Zhang et al., 2021). A 6 mL subsample of sediment (or water)
142 was transferred into a serum glass bottle with 24 mL of CO₂-free water (Dutta et al., 2015;
143 Yang et al., 2019a). After the bottle was sealed, nitrogen gas (N₂) with a purity exceeding
144 99.999 %) was injected to replace a 25-mL headspace. The sealed bottle was then placed
145 in an oscillator (IS-RDD3, China) and shaken for 10 min to reach an equilibrium in CO₂
146 concentration. After settling for 30 minutes, 5 ml of the headspace was extracted and

147 introduced into a gas chromatograph (GC-2010, Shimadzu, Japan). The gas
 148 chromatograph was equipped with a flame ionization detection (FID) for CO₂. The
 149 dissolved CO₂ concentrations (μmol CO₂ L⁻¹) in sediment porewater and water column
 150 were calculated according to [Ding et al. \(2010\)](#) and [Wanninkhof \(1992\)](#).

151 2.5. Diffusive CO₂ fluxes across the sediment-water interface and water-air interface

152 Calculation of diffusive CO₂ fluxes across the sediment-water interface (F_{S-W} , mmol
 153 m⁻² h⁻¹) followed the methods proposed by [Tan \(2014\)](#) and [Gruca-Rokosz and Tomaszek
 154 \(2015\)](#):

$$155 \quad F_{S-W} = D_S \times \Delta C / \Delta Z = (D_w \times \varphi^2) \times (C_S - C_W) / \Delta Z \quad (\text{Eq. 1})$$

156 where D_S (cm² s⁻¹) and D_w (cm² s⁻¹) are the diffusion coefficients of CO₂ in sediment and
 157 water, respectively; $\Delta C / \Delta Z$ is the depth gradient of dissolved CO₂ concentration (μmol L⁻¹
 158 cm⁻¹); C_S and C_W are the dissolved CO₂ concentrations (μmol L⁻¹) in sediment porewater
 159 and overlying water, respectively; φ is the sediment porosity. D_w was calculated as:

$$160 \quad D_w = 3.7 \times 10^{-11} T^3 + 2.6 \times 10^{-9} T^2 + 3.1 \times 10^{-7} T + 9.2 \times 10^{-6} \quad (\text{Eq. 2})$$

161 where T was the sediment temperature (°C). F_{S-W} was expressed in mmol m⁻² h⁻¹ with the
 162 proper unit conversion, and positive values represented CO₂ flux from sediment into the
 163 water column.

164 Diffusive CO₂ fluxes across the water-air interface (F_{W-A} , mmol m⁻² h⁻¹) was calcula
 165 ted according to [Musenze et al. \(2014\)](#):

$$166 \quad F_{W-A} = [2.07 + (0.215 \times U_{10}^{1.7})] \times (Sc/660)^{-n} \times (C_W - C_{eq}) \quad (\text{Eq. 3})$$

167 where U_{10} was the frictionless wind speed (m s⁻¹) at a height of 10 m above the water

168 surface (Crusius and Wanninkhof, 2003); Sc was the Schmidt number for CO_2 , which was
169 dependent on *in situ* water temperature and salinity, and was calculated according to
170 Wanninkhof (1992); n was the proportionality coefficient that ranges from 0.50 (wind
171 speed $> 3 \text{ m s}^{-1}$) to 0.66 (for wind speed $\leq 3 \text{ m s}^{-1}$) (Cole and Caraco, 1998); C_w was the
172 dissolved CO_2 concentration ($\mu\text{mol L}^{-1}$) in the surface water at a depth of 20 cm; C_{eq} was
173 the dissolved CO_2 concentration ($\mu\text{mol L}^{-1}$) at equilibrium with the atmosphere under the
174 prevailing *in situ* conditions. F_{S-W} was expressed in $\text{mmol m}^{-2} \text{ h}^{-1}$ with positive values
175 representing CO_2 flux from surface water to air.

176 2.6. Data analysis

177 Water-column CO_2 concentrations within the aquaculture ponds would be determined
178 by the balance between addition (e.g., through internal respiration and diffusive input) and
179 subtraction (e.g., through photosynthesis and emission). We examined how this balance
180 changed through time in two ways: First, because the sediment was a stronger diffusive
181 source of CO_2 than the atmosphere (based on flux data), in the absence of thermal
182 stratification (based on water temperature data), we assumed the bottom-layer CO_2
183 concentration to be the end-member value and the lower CO_2 concentrations in shallower
184 depths would indicate photosynthetic consumption of CO_2 . We therefore fitted a linear
185 regression function to the depth-specific CO_2 concentrations for each month, with the
186 slope ($\Delta CO_2 / \Delta z$) indicating the rate of change in CO_2 with depth. For the second approach,
187 we reasoned that if the pond water-column CO_2 pool was in a steady-state, CO_2 flux from
188 sediment into water column (F_{S-W}) should equal CO_2 flux from surface water to air (F_W).

189 A). Therefore, we calculated the seasonal average of F_{S-W} and F_{W-A} ; the difference between
190 the two values would then represent the apparent CO₂ consumption (ACC) within the
191 water column due to autotrophic CO₂ fixation and/or CO₂ accumulation. Note that we used
192 the seasonal average values for this calculation to eliminate any short-term temporal and
193 spatial mismatch between F_{S-W} and F_{W-A} , and to illustrate the influence of seasonal
194 environmental conditions.

195 Taking the average depth-specific values for each month, we used two-way ANOVA
196 to examine the differences in Chl-*a* and CO₂ concentrations between months and depths.
197 One-way ANOVA was employed to assess temporal change in diffusive CO₂ fluxes (F_{S-w}
198 and F_{W-A}). Pearson's correlation analysis was employed to analyze the correlation between
199 environmental parameters and dissolved CO₂ concentration (or diffusive CO₂ fluxes)
200 using the vegan package in R (v. 4.1.0). Partial least square structural equation modeling
201 (PLS-SEM) was employed in software R (v. 3.5.3) with the 'semPLS' package to evaluate
202 the direct or indirect relationships between environmental variables and dissolved CO₂
203 concentration (or diffusive CO₂ fluxes). Details about the PLS-SEM analysis can be found
204 in [Tan et al. \(2022, 2023\)](#). A significance level of $p < 0.05$ was applied in all statistical
205 tests.

206 **3. Results**

207 *3.1. Temperature, PO₄³⁻ and porewater CO₂*

208 Across the study period, the T_A , T_W and T_S ranged from 15.8–33.4 °C, 14.7–32.1 °C
209 and 14.8–25.4 °C ([Figure S1](#)), respectively. T_W was nearly identical between depths for

210 each month (Figure S2). Temperatures were noticeably higher in the summer months
211 (June-August) and early autumn (September-October). Water-column PO_4^{3-}
212 concentrations remained low at around $60 \mu\text{g L}^{-1}$ for most of the months, except in autumn
213 and early winter when it reached up to $138.9 \mu\text{g L}^{-1}$ (Figure 2a). Sediment porewater CO_2
214 concentration was rather low in spring ($\sim 53.2 \mu\text{mol L}^{-1}$), but it increased steadily to 436.2
215 $\mu\text{mol L}^{-1}$ in September, before it decreased sharply to $21.9 \mu\text{mol L}^{-1}$ by January (Figure
216 2b). The detailed results of other physico-chemical properties of sediment and water
217 samples can be found in Supporting Information (Figures S3 and S4).

218 3.2. Water-column chlorophyll-*a* and CO_2 concentrations

219 The average Chl-*a* and CO_2 concentrations in the water column varied significantly
220 between months and between depths (Table 1). Chl-*a* concentrations were higher in the
221 surface layers, and it decreased slightly from spring to summer, then increased noticeably
222 in autumn before dropping in winter (Figure 3a). The seasonal average Chl-*a*
223 concentration varied between 86.9 and $175.8 \mu\text{g L}^{-1}$ throughout the study.

224 CO_2 concentrations increased sharply in the summer months, reaching a average of
225 $27.4 \mu\text{mol L}^{-1}$; it remained high in early autumn before it decreased sharply into late
226 autumn and winter (Figure 3b). The vertical concentration gradient was also steeper in the
227 summer. By fitting linear regression functions to the depth-specific CO_2 concentrations,
228 the seasonal average rate of change was 2.26 , 4.21 , 1.25 and $0.20 \mu\text{mol L}^{-1} \text{m}^{-1}$ for spring,
229 summer, autumn and winter, respectively (Figure 3b; Table S1).

230 3.3. Diffusive CO_2 fluxes and apparent CO_2 consumption

231 CO₂ flux across the sediment-water interface (F_{S-W}) varied significantly with time (p
232 < 0.001) and was always positive (i.e., CO₂ going from sediment to water) (Figure 4a). F_{S-}
233 w was low in spring, between 0.25 and 0.39 mmol m⁻² h⁻¹; it then increased steadily to
234 4.58 mmol m⁻² h⁻¹ in September, before it decreased to 0.01 mmol m⁻² h⁻¹ by January.

235 Diffusive CO₂ flux across the water-air interface (F_{W-A}) varied significant with time
236 ($p < 0.001$) and was overall much lower than F_{S-W} (Figure 4b). The F_{W-A} values were
237 highest in the summer and early autumn, and close to zero or slightly negative (i.e., CO₂
238 going from air to water) in late autumn and winter.

239 We defined apparent CO₂ consumption (ACC) in the water column as the difference
240 between seasonal F_{S-W} and F_{W-A} . The calculated ACC average value was positive for all
241 four seasons, meaning a net sink of CO₂ in the pond water body (Figure 4c). ACC was
242 highest in autumn (2.32 ± 1.01 mmol m⁻² h⁻¹), followed by summer (1.02 ± 0.69) and
243 winter (0.41 ± 0.42), and it was lowest in spring (0.24 ± 0.14) ($p < 0.01$).

244 3.4. Environmental drivers of CO₂ concentrations and fluxes

245 Pearson's correlation analysis showed that the water-column dissolved CO₂
246 concentration and F_{W-A} was positively correlated with T_W and NO₃⁻-N ($p < 0.01$), but
247 negatively correlated with DO, pH, salinity, PO₄³⁻, Cl⁻, SO₄²⁻ and Chl-*a* ($p < 0.01$) (Figure
248 5a). The sediment porewater dissolved CO₂ concentration was positively correlated with
249 T_S , NO₃⁻-N, NH₄⁺-N and DOC ($p < 0.05$ or < 0.01), but negatively with salinity, Cl⁻ and
250 SO₄²⁻ ($p < 0.05$ or < 0.01) (Figure 5b). F_{S-W} was positively correlated with T_S , SWC, POR,
251 NO₃⁻-N and DOC ($p < 0.01$), but negatively with salinity ($p < 0.01$), SO₄²⁻ ($p < 0.001$), BD

252 ($p < 0.01$) and Cl^- ($p < 0.05$) (Figure 5b).

253 As indicated in Figure 6a of the partial least square structural equation model (PLS-
254 SEM), PO_4^{3-} had a positive effect on dissolved CO_2 in water column by way of Chl-*a*.
255 Changes in F_{W-A} were influenced by T_W and further mediated through changes in Chl-*a*
256 and DO (Figure 6a). T_S and DOC positively affected F_{S-W} , directly and indirectly via
257 porewater dissolved CO_2 (Figure 6b). F_{S-W} was also negatively affected by SO_4^{2-} indirectly
258 via P_{CO_2} (Figure 6b).

259 4. Discussion

260 During the shrimp farming period between May and November, farmers added animal
261 feeds twice per day to the ponds. The amount of added feeds increase as shrimp grow.
262 Unconsumed feeds and animal wastes might settle to the bottom and add to sediment
263 porewater CO_2 through decomposition (Hou et al., 2023; Lin and Lin, 2022; Xiong et al.,
264 2017; Yang et al., 2019a), as reflected by the positive correlations between P_{CO_2} and DOC
265 and TC (Figure 5b). The higher sediment temperature in August and September (Figure
266 S1) would have accelerated decomposition and increased porewater CO_2 concentration
267 during that time (Figure 2b, 5b); this would also introduce remineralized nutrients such as
268 PO_4^{3-} to the water column (Yang et al., 2019b), which was noticeable after a slight delay
269 (Figure 2a). The increasing PO_4^{3-} concentration may explain the higher autotroph biomass
270 (as Chl-*a*) (Lapointe et al., 2015; Morales-Williams et al., 2021) in the autumn (Figure
271 3a), as confirmed by correlation analysis (Figure 5a).

272 Water column CO_2 concentrations were higher in the summer, likely reflecting the

273 results of increasing respiration stimulated by the higher water temperatures (Figure 5a
274 and Figure S1; Yang et al., 2019a). Overall, the CO₂ concentrations in water column were
275 lower, sometimes by an order of magnitude, than sediment porewater CO₂ concentrations;
276 as a result, CO₂ would diffuse from the sediment to the overlying water, as confirmed by
277 the positive F_{S-W} values (Figure 4a). The lower CO₂ concentrations in the upper water
278 column could be attributed to a higher photosynthetic CO₂ consumption in the shallower
279 water (Liu et al., 2010), driven by the higher Chl-*a* (Figure 3a) and light availability (Ni
280 et al., 2021; Ni et al., 2022), as supported by the strong negative correlation between W_{CO_2}
281 and Chl-*a* (Figure 5a). Although the water-column Chl-*a* was highest in the autumn
282 (Figure 3b), the rate of change in CO₂ was largest in the summer (4.21 μmol L⁻¹ m⁻¹),
283 likely because the higher seasonal light intensity and temperature (Figure S1) increased
284 the overall photosynthetic drawdown of CO₂.

285 Based on the concentration data, we calculated the CO₂ diffusive fluxes across the
286 sediment-water and water-air interfaces. While *in situ* CO₂ production within the sediment
287 did not necessarily translate directly to CO₂ diffusive flux, the seasonal variation in F_{S-W}
288 in this study was consistent with the seasonal variation in sediment CO₂ production
289 measured by slurry incubation (Tan et al., 2023), with higher values in the summer and
290 early autumn. F_{S-W} value was positive throughout the study (Figure 4a), meaning that the
291 sediment was a consistent source of CO₂ for the water column.

292 The magnitude and seasonal pattern of F_{W-A} were comparable to those measured
293 empirically with a floating chamber (Tan et al., 2023), with highest values in the summer

294 (Figure 4b). F_{W-A} values were considerably lower than F_{S-W} , and in later autumn F_{W-A} was
295 close to or below zero, indicating that the surface water acted as a CO₂ sink, which is also
296 consistent with earlier direct measurements (Tan et al., 2023).

297 By considering the overall CO₂ fluxes in and out of the aquaculture ponds across the
298 sediment-water and water-air interfaces, we can deduce the apparent CO₂ consumption
299 (ACC) within the water column, which would represent a combination of biological and
300 chemical fixation of CO₂, as well as accumulation of dissolved CO₂ in the water column.
301 We considered this to be “apparent” consumption because it did not include conversion of
302 CO₂ to other carbon gases (e.g. CH₄) and volatiles (Costa and Leigh, 2014; Elizabeth
303 Holmes et al., 2015; Li et al., 2023). ACC increased from spring to summer by >4 fold
304 (Figure 3a); however, the water-column CO₂ concentration increased only by 1.7-fold
305 between spring (seasonal average 15.98 μmol L⁻¹) and summer (27.42 μmol L⁻¹) (Figure
306 3b). Therefore, we suggest that much of the ACC was due to photosynthetic uptake, as
307 supported by the high rate-of-change in CO₂ through depth (Figure 3b). ACC was highest
308 in autumn, which coincided with an increase in Chl-*a* (Figure 3a) but not dissolved CO₂
309 (Figure 3b) in the water column, suggesting that the excess CO₂ had been incorporated
310 into autotroph and heterotroph biomass. This was consistent with the observations by Yang
311 et al. (2022b), who showed that the majority of the ‘new’ carbon within the aquaculture
312 ponds was introduced through photosynthesis. Some of the ACC in autumn could be
313 attributed to biomass harvested from the ponds, especially toward the end of the farming
314 period, but this is usually a small fraction (ca. 9-11%) of the carbon loss from the system

315 (Yang et al., 2022b).

316 Because CO₂ concentrations and fluxes could be influenced to different degrees and
317 in different directions by a multitude of variables (Figure 5), we used PLS-SEM to tease
318 out the key environmental factors and their effects (positive or negative) on CO₂
319 concentrations and fluxes. PLS-SEM results showed that temperature (T_w and T_s) had a
320 strong positive effect on CO₂ flux (F_{W-A} and F_{S-W} , respectively) directly, or indirectly by
321 influencing other parameters (Figure 7). Higher temperature would increase the system
322 respiration and the mineralization of DOC thereby increasing CO₂ concentrations and
323 fluxes (Huttunen et al., 2003; Gudasz et al., 2010; Xiao et al., 2020; Yang et al., 2019). In
324 the water column, Chl-*a* and DO were the two key factors with a strong negative effect on
325 F_{W-A} (Figure 7a), showing the importance of photosynthetic uptake of CO₂ (which releases
326 oxygen as byproduct) in determining the water-column CO₂ dynamics (Almeida et al.,
327 2016; Jensen et al., 2023; Morales-Williams et al., 2021; Yan et al., 2020). On the other
328 hand, DOC had the second strongest positive effect on F_{S-W} (Figure 7b), showing that
329 decomposition of organic detritus within the sediment was the primary process in
330 controlling sediment-to-water CO₂ flux (Gruca-Rokosz and Tomaszek, 2015; Kristensen
331 et al., 2008; Xiong et al., 2017; Yang et al., 2019).

332 **5. Implications and conclusions**

333 The fast-expanding aquaculture sector worldwide has raised concern about its
334 environmental impacts through effluent pollutions (Naylor et al., 2021) and greenhouse
335 gas emissions (MacLeod et al., 2020; Zhang et al., 2022). Decomposition of organic

336 carbon in the aquaculture pond sediment could produce both CO₂ and CH₄ (Yang et al.,
337 2022a). Although the production rate of CH₄ in the sediment is orders of magnitude lower
338 than that of CO₂ (Yang et al., 2022a), CH₄ is less soluble and could by-pass water-column
339 consumption via ebullition (Tong et al., 2020), making aquaculture ponds hotspots for CH₄
340 emission (Zhang et al., 2022). In contrast, CO₂ dynamics within the aquaculture ponds is
341 influenced by a more intricate network of gains and losses. In this study, significant
342 temporal variations in dissolved CO₂ concentrations and fluxes were observed in the
343 coastal aquaculture ponds. In addition to temperature, photosynthetic activity was the key
344 driver of change in water column CO₂ concentration and water-to-air CO₂ flux. Porewater
345 CO₂ concentration and sediment-to-water CO₂ flux were strongly influenced by sediment
346 temperature, which would drive the microbial decomposition of organic matter.

347 Using a relatively simple system mass balance approach, we showed that, despite the
348 strong and persistent source of CO₂ in the sediment, the water column had a high capacity
349 to “consume” the excess CO₂, leading to a much lower than expected CO₂ emission to air;
350 in some cases even a net sink of atmospheric CO₂. Understanding the underlying process
351 and the potential to manipulate or enhance such a capacity, along with carbon burial into
352 the sediment (Boyd et al., 2010; Yang et al., 2022b), may allow farmers to better assess
353 and lower their carbon footprints.

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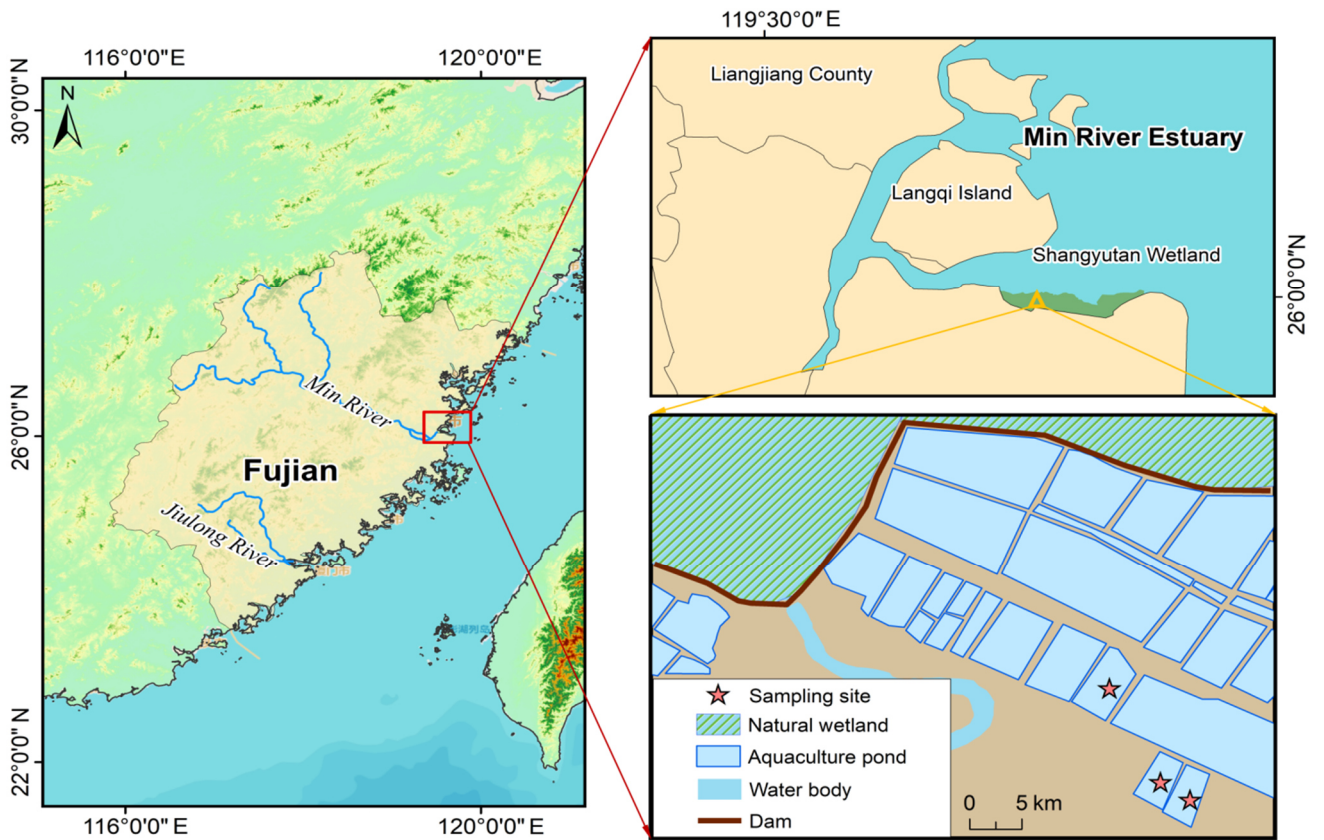
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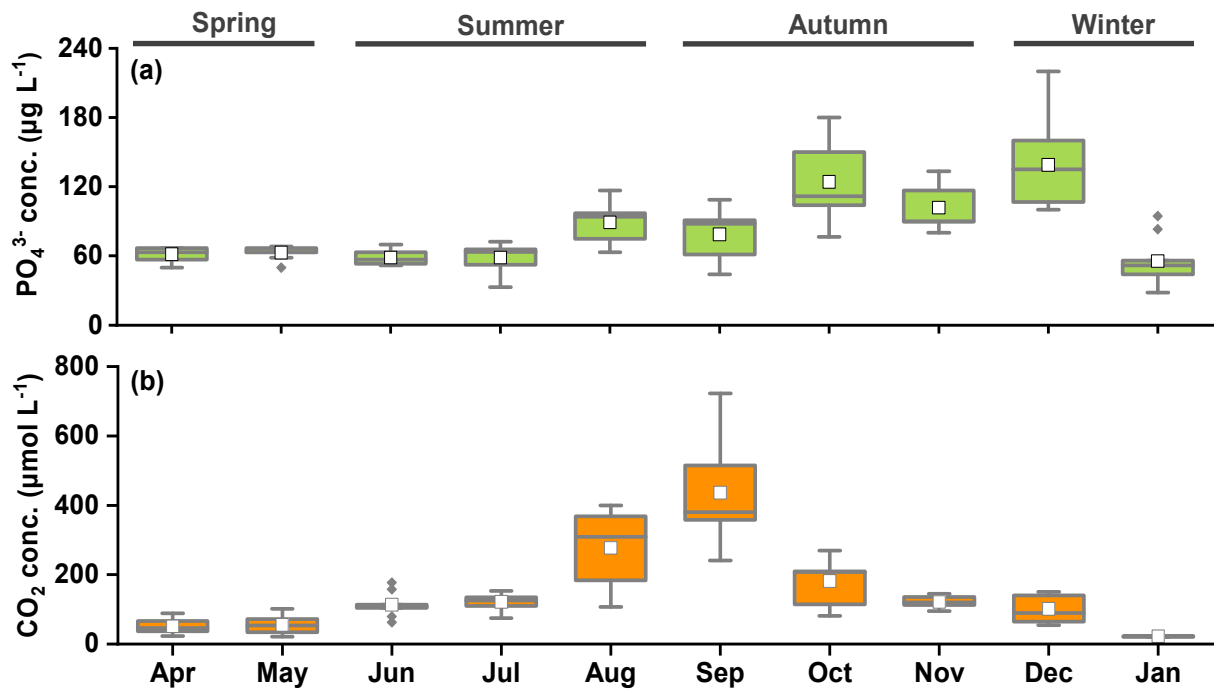
- 1 **Table 1** Summary of two-way ANOVAs examining the effects of water depth, month and their interactions on average Chl-*a* and dissolved
- 2 CO₂ concentrations in the water column of the aquaculture ponds.

	<i>df</i>	Chl- <i>a</i> concentration			CO ₂ concentration				
		<i>SS</i>	<i>MS</i>	<i>F value</i>	<i>P value</i>	<i>SS</i>	<i>MS</i>	<i>F value</i>	<i>P value</i>
Depth	2	930.36	465.18	9.91	0.001	41.51	20.75	12.37	4E-4
Month	9	47359.1	5262.12	112.06	6E-14	969.17	107.69	64.20	7.7E-12
Error	18	845.22	46.96			30.19	1.68		
Total	29	49134.7				1040.87			



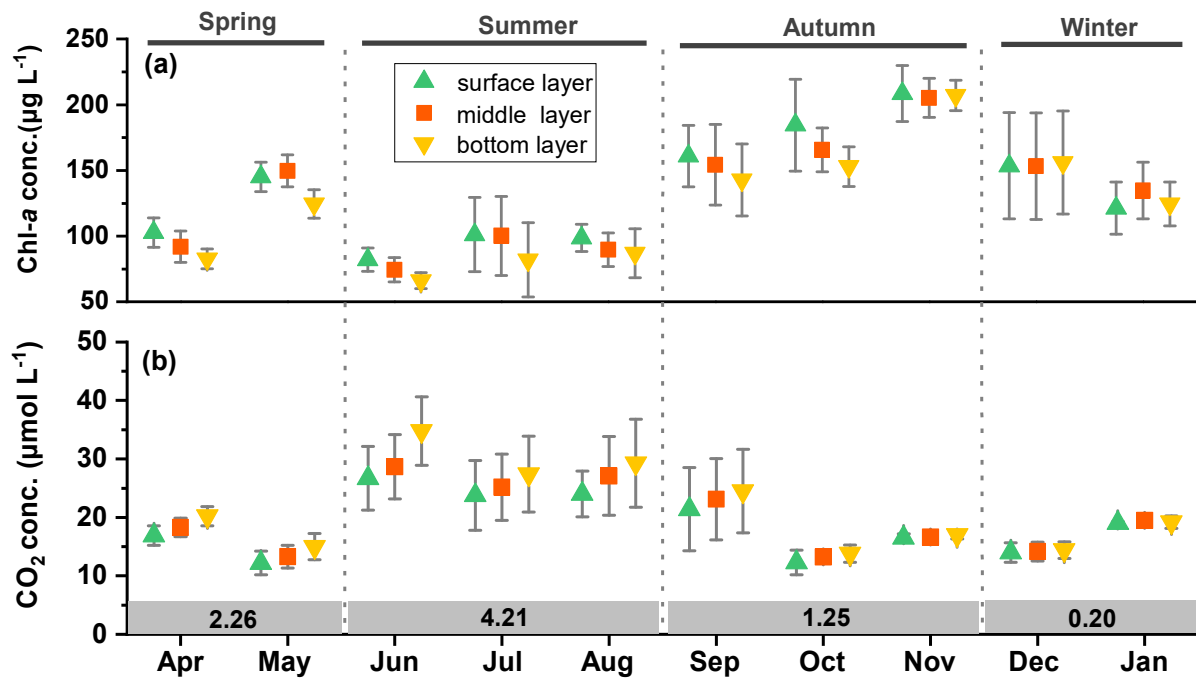
1

2 **Figure 1.** Map of the Shanyutan wetland within the Min River estuary showing the aquaculture
 3 ponds used in this study.



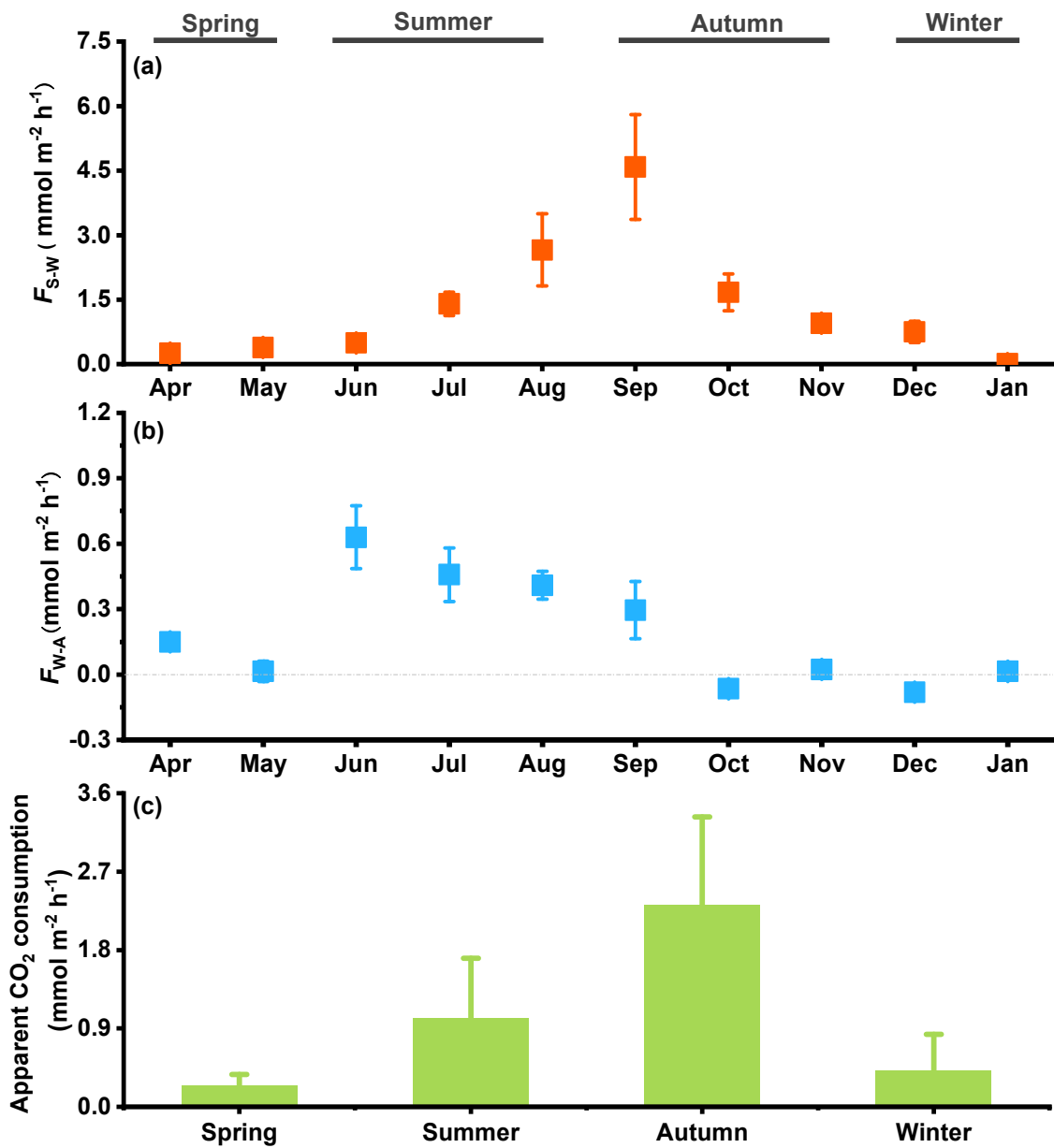
4

5 **Figure 2.** Box plots of monthly values of (a) water-column PO_4^{3-} concentration; and (b) pore-
 6 water CO_2 concentrations.



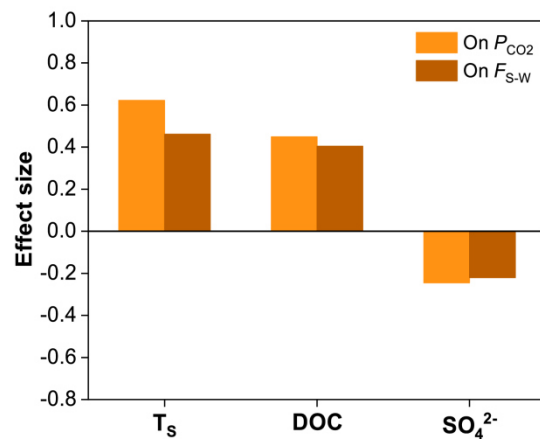
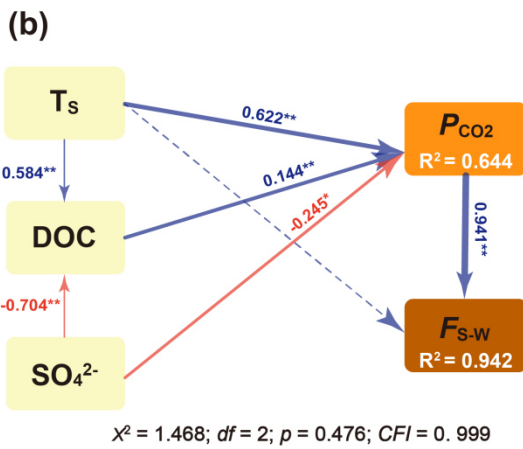
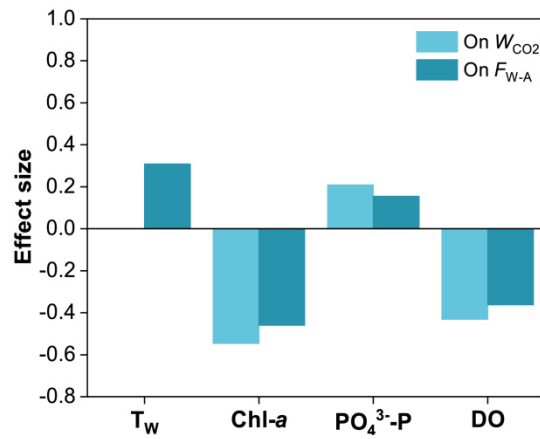
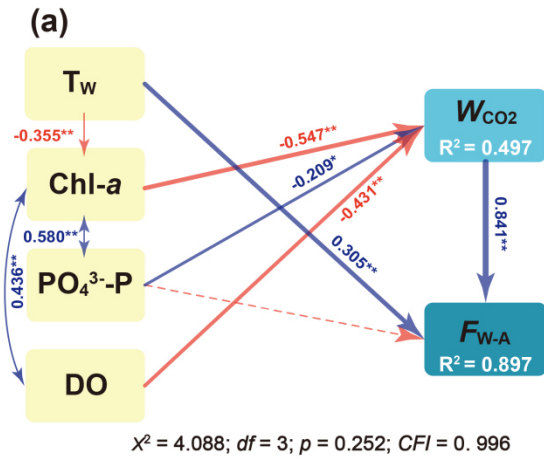
7

8 **Figure 3.** Monthly water column concentrations of (a) chlorophyll-*a* and (b) dissolved CO_2 at
 9 different water depths (mean \pm SE; $n = 9$). Numbers within panel b are seasonal average of
 10 rate of change in $[\text{CO}_2]$ with depth, based on linear regressions. See text and Table S1 for
 11 explanation.



12

13 **Figure 4.** Monthly diffusive CO₂ flux across (a) sediment-water interface and (b) water-
 14 air interface (mean \pm SE); and (c) seasonal apparent CO₂ consumption within the water
 15 column (mean \pm SE). See text for explanation.



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Figure 6. Partial least square structural equation modeling (PLS-SEM) to evaluate the direct and indirect effects of environmental factors on (a) water CO_2 concentration (W_{CO_2}) and CO_2 diffusive flux across the water-air interface (F_{W-A}), and (b) sediment porewater dissolved CO_2 concentration (P_{CO_2}) and CO_2 diffusive flux across the sediment-water interface (F_{S-W}). Solid blue and red arrows indicate significant positive and negative effects, respectively, and dotted arrow indicates insignificant effect on the dependent variable. Numbers adjacent to arrows are standardized path coefficients, indicating the effect size of the relationship. R^2 represents the variance explained for target variables. * $p < 0.05$; ** $p < 0.01$.

1 **Supporting Information**

2 **Seasonal variations in source-sink balance of CO₂ in**
3 **subtropical earthen aquaculture ponds: Implications**
4 **for carbon emission management**

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23 **Supporting Information Summary**

24 **No. of pages: 5 No. of Table: 1 No. of Figures: 4**

25 **Page S3:** Table S1. Linear regression parameters for Fig. 3b.

26 **Page S4:** Figure S1 Monthly temperatures in the aquaculture ponds during the
27 farming period. The bars represent the means + 1 standard error ($n = 3$). T_S , T_W and T_A
28 represent sediment temperature, water temperature and air temperature, respectively.

29 **Page S5:** Figure S2 Monthly watertemperatures at different water depths in the
30 aquaculture ponds during the farming period (mean \pm SE; $n = 3$ ponds).

31 **Page S6:** Figure S3 Seasonal values of sediment pH, salinity, total carbon (TC),
32 porewater DOC concentration, NO_3^- -N concentration, NH_4^+ -N concentration, Cl^-
33 concentration and SO_4^{2-} concentration in the aquaculture ponds during the study
34 period. Data are after Tian et al. (2023) and Yang et al. (2022) for reference and
35 review only.

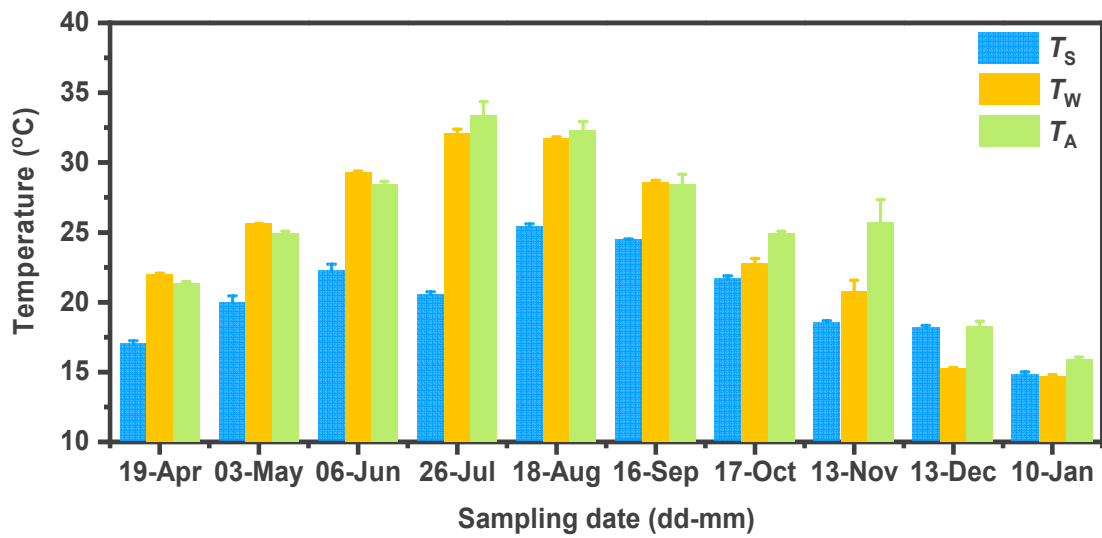
36 **Page S7:** Figure S4 Seasonal values of pH, salinity, NO_3^- -N, NH_4^+ -N, Cl^- and SO_4^{2-}
37 concentration in water column of the aquaculture ponds during the study period. Data
38 are after Tian et al. (2023) and Yang et al. (2022) for reference and review only.

39 **Page S8:** Reference

40 **Table S1** Linear regression parameters for Fig. 3b.

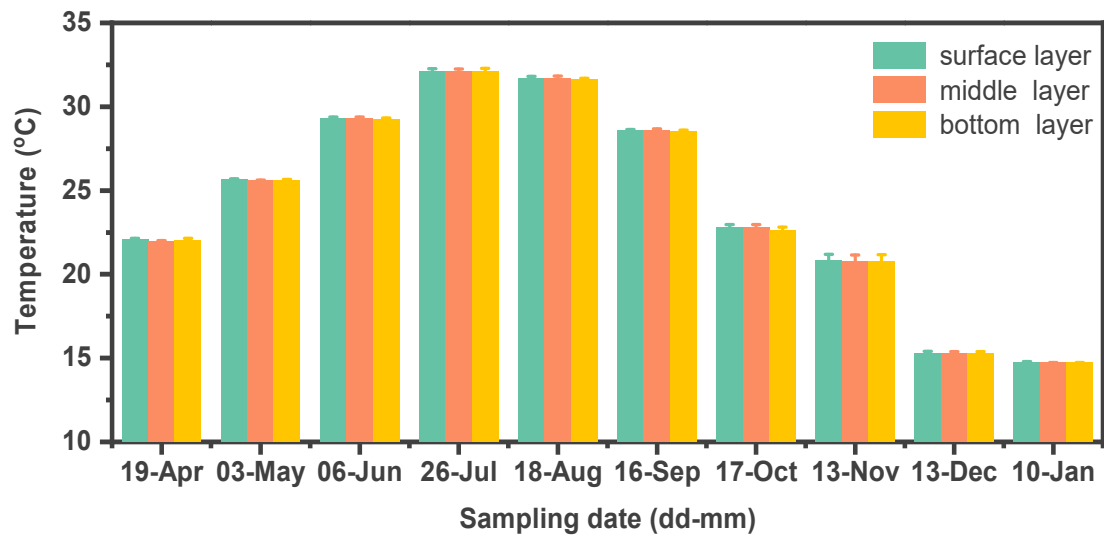
Month	[CO₂] vs depth linear regression			season avg slope
	slope	intercept	r²	
April	2.457	16.62	0.999	2.263
May	2.069	11.94	0.996	
June	6.056	25.51	0.952	4.205
July	2.712	23.41	0.994	
August	3.848	23.9	0.974	
September	2.269	21.29	0.984	
October	1.118	12.27	0.952	1.252
November	0.369	16.45	0.972	
December	0.316	13.93	1	0.2
January	0.084	19.17	0.06	

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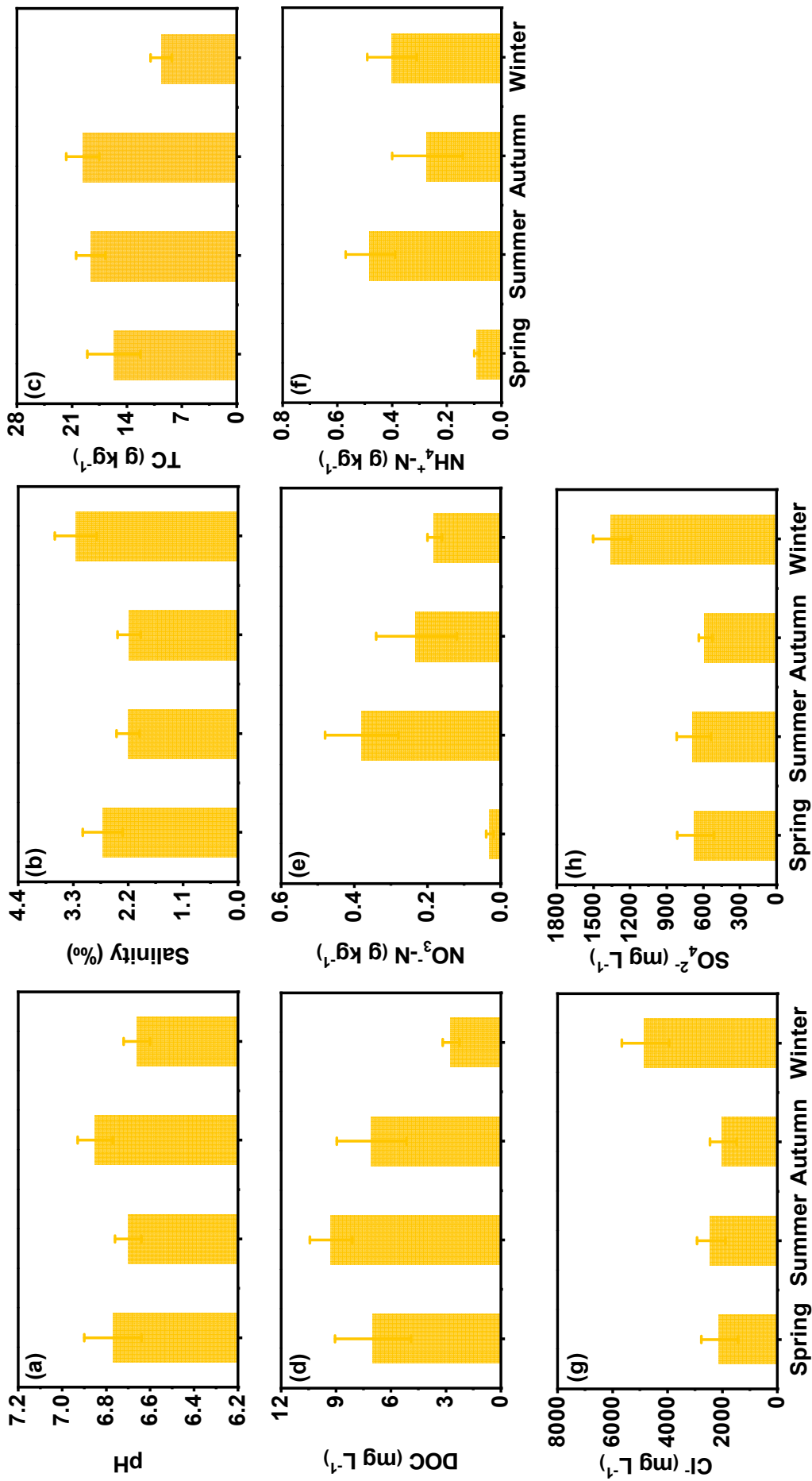
43 **Figure S1** Monthly temperatures in the aquaculture ponds during the farming period
 44 (Tian et al., 2023). The bars represent the means + 1 standard error ($n = 3$ ponds). T_S ,
 45 T_W and T_A represent sediment temperature, water temperature and air temperature,
 46 respectively.



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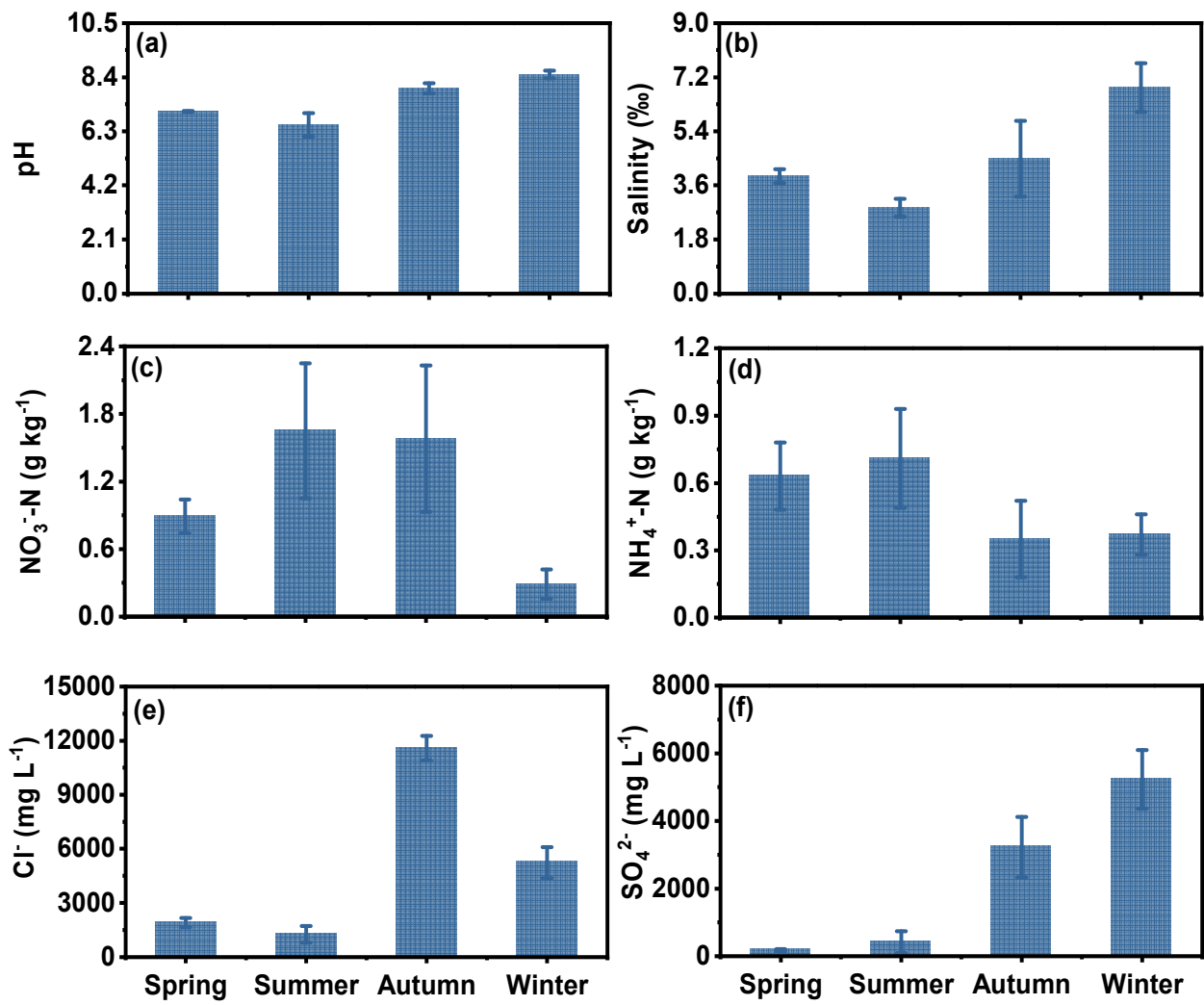
48 **Figure S2.** Monthly water temperatures at different depths in the aquaculture ponds

49 during the farming period (mean \pm SE; $n = 3$ ponds).



50

51 **Figure S3** Seasonal values of sediment pH, salinity, total carbon (TC), porewater DOC concentration, $\text{NO}_3\text{-N}$ concentration, $\text{NH}_4\text{-N}$
 52 concentration, Cl^- concentration and SO_4^{2-} concentration in the aquaculture ponds during the study period. Data are after Tian et al. (2023) and
 53 Yang et al. (2022) for reference and review only.



54
 55 **Figure S4** Seasonal values of pH, salinity, NO₃⁻-N, NH₄⁺-N, Cl⁻ and SO₄²⁻ concentration in
 56 water column of the aquaculture ponds during the study period. Data are after Tian et al. (2023)
 57 and Yang et al. (2022) for reference and review only.

58 **References**

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Data Curation: Ping Yang, Kam W. Tang, Chuan Tong

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Writing - Review & Editing: Ping Yang, Kam W. Tang

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