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Decreased motility of flagellated microalgae long-term acclimated to CO₂-induced acidified waters

Yitao Wang^{1,2}, Xiao Fan¹, Guang Gao³, John Beardall^{3,4}, Kazuo Inaba⁵, Jason M. Hall-Spencer^{5,6}, Dong Xu¹, Xiaowen Zhang¹, Wentao Han¹, Andrew McMinn⁷ and Naihao Ye^{1,2} ✉

¹Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China. ²Function Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China. ³State Key Laboratory of Marine Environmental Science & College of Ocean and Earth Sciences, Xiamen University, Xiamen, China. ⁴School of Biological Sciences, Monash University, Clayton, Victoria, Australia. ⁵Shimoda Marine Research Center, University of Tsukuba, Shizuoka, Japan. ⁶School of Biological and Marine Sciences, University of Plymouth, Plymouth, UK. ⁷Institute of Antarctic and Southern Ocean Studies, University of Tasmania, Tasmania, Australia. ✉e-mail: yenh@ysfri.ac.cn

Supplementary Information

Decreased motility of flagellated microalgae grown in CO₂-induced acidified waters for over five years

Yitao Wang^{1,2}, Xiao Fan¹, Guang Gao³, John Beardall^{3,4}, Kazuo Inaba⁵, Jason M. Hall-Spencer^{5,6}, Dong Xu¹, Xiaowen Zhang¹, Wentao Han¹, Andrew McMinn⁷ and Naihao Ye^{1,2*}

¹Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China

²Function Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

³State Key Laboratory of Marine Environmental Science & College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China

⁴School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia

⁵Shimoda Marine Research Center, University of Tsukuba, Shizuoka, Japan

⁶School of Biological and Marine Sciences, University of Plymouth, PL4 8AA, UK.

⁷Institute of Antarctic and Southern Ocean Studies, University of Tasmania, P.O. Box 252-77, Tasmania 7001, Australia

*E-mail: yenh@ysfri.ac.cn

Supplementary Methods

Blue light induced *Microglena* sp. movement. Blue light density distribution with variations in incident irradiance was measured during the positive/negative phototaxis assays of *Microglena* sp., (Supplementary Fig. 8e). The average and instantaneous velocities of *Microglena* sp. under blue light were measured by referring to the method in main text.

Vertical cell spatial distribution. To visualize the cell distribution at the end of the light induction experiment, we calculated the cell numbers under each CO₂ gradient at different sampling sites after the vertical cell motion experiment (the section of **Vertical cell motion in the main text**). Algal cells cultured under different CO₂ levels were placed at one end of separate channels. The size and structure of these channels were exactly same and they were placed side by side. After the same light intensity induction, algal cells are redistributed in the channels. We considered cells numbers in the no. 1 sampling point for all (280-1, 400-1, 700-1, 1000-1, 1500-1, 2000-1) CO₂ gradients as a whole (100%), calculated the percentage of cells number for each CO₂ concentration, and marked the actual cell number (mean) on it (the bar). Sampling points no.2-10 were calculated in the same way. This data presentation shows the distribution of cells in the channels at the end of the experiment.

Horizontal cell spatial distribution. To visualize the cell distribution at the end of the light induction experiment, we calculated the cell numbers under each CO₂ gradient at different sampling sites after the horizontal cell motion experiment (the section of **Horizontal cell motion in the main text**). Algal cells cultured under different CO₂ levels were placed at one end of separate channels. The size and structure of these channels were exactly same and they were placed side by side. After the same light intensity induction, algal cells are redistributed in the channels. We considered cells numbers in the no. 1 sampling point for all (280-1, 400-1, 700-1, 1000-1, 1500-1, 2000-1) CO₂ gradients as a whole (100%), calculated the percentage of cells number for each CO₂ concentration, and marked the actual cell number (mean) on it (the bar). Sampling points no.2-10 were calculated in the same way. This data presentation shows the distribution of cells in the channels at the end of the experiment.

Distribution of *Microglena* sp. via spontaneous fluorescence. The procedure was based on Ueki et al (2016) method with improvements³⁰. The distribution of cells reflects the velocity of cells with biomass estimated by chlorophyll fluorescence. Cells were washed in experimental solutions of different pH and kept under red light for more than 50 min before the assays. For the positive and negative phototaxis assays, 2 mL cell suspensions ($\sim 10^7$ cells/mL) were placed into Petri dishes (35 mm in diameter, 10 mm thick), illuminated with a LED (positive phototaxis assays White, $\sim 2 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; negative phototaxis assays White, $\sim 200 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) from one side for 20 min, and photos taken with an Imaging-PAM (Walz, Effeltrich, Germany).

CO₂/pH manipulation. To distinguish the effects of carbonation vs. acidification, seawater medium was prepared using synthetic ocean water (SOW) to obtain the targeted CO₂ and pH levels, i.e., carbonated (CO₂ 400 ppm + pH 8.1, CO₂ 1000 ppm + pH 8.1 and CO₂ 2000 ppm + pH 8.1), acidified (CO₂ 400 ppm + pH 8.1, CO₂ 400 ppm + pH 7.8 and CO₂ 400 ppm + pH 7.5) conditions using benchtop fermentor (Minifors Bacteria, INFORS, Swiss). The carbonate chemistry of the *Microglena* sp. and *D. salina* medium was manipulated by adding different amounts of NaHCO₃ to the medium while maintaining a constant medium pH with 2 mM 4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS, Sigma Ultra), to achieve independent variation of CO₂ and pH³⁴. The carbonate chemistry of the *C. reinhardtii* medium was manipulated by adding different

amounts of NaHCO₃ to the medium while maintaining a constant medium pH with 2 mM 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethane sulfonic acid (HEPES, Sigma Ultra), to achieve independent variation of CO₂ and pH (Supplementary Tables 18-20). The cultures were grown semi-continuously and were pre-acclimated to experimental conditions for 30 days. The experimental methods refer to Vertical cell motion and Horizontal cell motion of are the same as the main text.

Detection of cytosolic Ca²⁺ concentrations. The Ca²⁺ level was indicated by a Ca²⁺-sensitive fluorescent dye, Fluo-3/AM (Molecular Probe). The method was based on Chen et al (2014) method with improvements³⁵. The log phase cells were cultured for 20 minutes under light intensity for positive and negative phototaxis, respectively. Then the algal pellet was obtained by centrifugation at the corresponding culture temperature at 4000 g for 3 minutes. The algal pellets were loaded with the fluorescent dyes of 5 mmol/L final concentration of acetoxymethyl ester form (AM) of fluo-3 and 50 mmol/L final concentration of sorbitol in the dark for 1 h. Loaded cells was subsequently rinsed 3 times in fresh iso-osmotic medium, and then harvested by centrifugation. Fluorescence images and mean fluorescence of cells were measured after 20 min of positive and negative phototaxis induction. The fluorescence settings were as follows: excitation = 488 nm, emission = 510–530 nm. Imaging of the cells were obtained with laser scanning confocal microscope (TCS SP5 II, LEICA, Germany).

Genetic adaptation to long term acidification. To query the potential genetic adaptation in genome, we performed the whole genome resequencing on the samples under 5 years CO₂ acidification in 400 ppm, 1000 ppm and 2000 ppm, and the algal samples of 5 years ago were also be sequenced as negative control. For each of the samples, three biological replicates were assayed independently. Genomic DNA was extracted using the Plant Genomic DNA kit (Tiangen, Beijing, China), and RNA was removed by incubating the DNA solution at 37°C with a DNase-free RNase A (Tiangen) for 30 min. DNA integrity was assessed using agarose gels and a NanoPhotometer[®] spectrophotometer (IMPLEN, Westlake Village, CA, USA). DNA was quantified with a Qubit[®] 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) using the Qubit[®] DNA Assay Kit. High quality DNA was used to perform whole-genome sequencing (WGS) employing an Illumina Hiseq 2500 platform. We used FastQC (Babraham Institute, <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) to examine the overall sequencing quality of the raw reads. The FASTQ data were cleaned and trimmed using Trimmomatic³⁶ (SLIDINGWINDOW: 4:15, LEADING: 3, TRAILING: 3, ILLUMINACLIP: adapter.fa: 2: 30: 10). All short reads (< 36 bps) and broken pairs were discarded. The resulting high-quality reads were aligned to the corresponding genomes assemblies using SAMTOOLS³⁷, and Single nucleotide polymorphisms (SNPs) were called using bam-readcount (<https://github.com/genome/bam-readcount>). SNP counts per 100k bp length bin of each chromosome were calculated using in-house python script. To construct back-translated nucleotide alignments, protein alignments between HGTs and the corresponding MMSHs were generated using MUSCLE version 3.8.31 under the default settings³⁸, and thereafter, protein-coding DNA alignments were guided by their protein alignments and limited to un-gapped regions using ParaAT (Parallel Alignment and back-Translation tool)³⁹. The Ka, Ks, ω (Ka/Ks) between HGTs and the corresponding MMSHs were calculated using KaKs Calculator 2.0 (<http://evolution.genomics.org.cn/software.htm>) under the model-selected (MS) setting.

Light distribution. Under laboratory conditions, a LED lamp was used as the light source, and the distribution of light in the experimental channel was measured by an underwater light meter (LI-

192, LI-COR, USA). In a uniform culture ($\sim 4 \times 10^5$ cells/mL), light is attenuated rapidly due to absorption by the algal cells. The light distribution in seawater was different to that in the cultures during the positive and negative phototaxis assays.

Growth curve. Culture growth rates were measured in 500 ml conical flasks containing 300 ml medium. The conical flasks were placed in the same phytotron chamber under a series of CO₂ concentrations (280, 400, 700, 1000, 1500, 2000 ppm). Cell density was measured every two days using a blood count plate. The generations were calculated as follows: $G = n \cdot d \cdot (\ln(N_{t_2}/N_{t_1})) / ((t_2 - t_1) \cdot \ln 2)$ (Equation 3) G: generations; n: year = 5; d: days = 365; N_{t1}: cell numbers of on day t₁; N_{t2}: cell numbers of on day t₂; t₁, t₂: sampling time; the factor ln2 reflects the that binary fission growth of cells.

Carbonate chemistry. Total alkalinity (TA) was measured using an 848 Titrino plus automatic titrator (Metrohm, Riverview, FL, USA) on 50 ml of GF/F filtered samples. pH was measured using a pH electrode (6010M, Jenco, USA). Temperature was measured using a mercury thermometer (YHZ-90271, Yuhuaze, China). Salinity was measured using a salinity meter (YK31SA, Luchang, China). Carbonate system parameters were calculated using the CO2SYS Package (Pierrot, Lewis, & Wallace, 2006) in Excel based on pH, temperature, salinity, and TA⁴⁰.

Supplementary Results and Discussion

Distribution of *Microglena* sp. via spontaneous fluorescence. In this experiment, the higher the fluorescence intensity, the higher the number of cells. The aggregation of cells is reflected by the color of fluorescence. The fluorescence intensity is shown in bar on the left of the picture, with red being the weakest and green the strongest (Supplementary Fig. 4). As shown in Supplementary Fig. 8, when the induced light intensity was $2 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the cells showed positive phototaxis response, and the fluorescence intensity on the near side was higher than that on the far side. Comparing the algae treated with different CO_2 , the 280 ppm treatment group showed the greatest difference in fluorescence intensity between the near-light side of the plate and the far-light side, with a black color on the far-light side indicating almost no cells. With the increase of carbon dioxide concentration, the fluorescence intensity on the far-light side began to increase, indicating that the movement distance of cells in a certain period of time decreased due to the decreased velocity. Similarly, in the negative phototaxis response, the difference fluorescence intensity on both sides of the plate decreased with decreased velocity.

Ca^{2+} concentrations effect on cell velocity. In terms of biological function of Ca^{2+} , it has been found that Ca^{2+} plays a major role in regulating the motion of cells^{2,11,13,31}. Schmidt showed that low concentrations of Ca^{2+} ($<10^{-6}$ M) induced forward swimming and high concentrations of Ca^{2+} (10^{-3} M) induced backwards swimming of *C. reinhardtii*⁴¹. Data presented here, with a Ca^{2+} concentration of $2\cdot 10^{-4}$ M (between 10^{-6} M and 10^{-3} M), *Microglena* sp. excreted Ca^{2+} under the positive phototaxis assay conditions and absorbed Ca^{2+} under the negative phototaxis assay treatment (Fig. 3g). In addition, elevated CO_2 /acidification reduced the Ca^{2+} flux under positive and negative phototaxis assays. This suggests that *Microglena* sp. can regulate Ca^{2+} concentrations and that this plays a role in positive and negative phototaxis (Supplementary Fig. 20) and that acidification may reduce the Ca^{2+} flux resulting in a decrease in the velocity of cell motion (Fig. 1)^{42,43}. Similar results were found in *C. reinhardtii* and *D. salina* assays (Extended data Fig. 1).

Growth curve. Although high CO_2 increases population growth in many chlorophytes and larger phytoplankton may better adapt to variable pH conditions^{8,44}, the individual velocity of *Microglena* sp. becomes slower, increasing predation and survival risks. In addition, the lengthened DVM at higher CO_2 levels may become unfavorable (Fig. 4b).

Supplementary References

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Supplementary Figures

Supplementary Figure 1: Spatial distribution of *Microglena* sp. in the channel after adding sample at one end.

Supplementary Figure 2: Spatial distribution of *C. reinhardtii* and *D. salina* in the channel after adding the sample at one end.

Supplementary Figure 3: Spatial distribution of *Microglena* sp. induced by sunlight in the channel after adding the sample at one end.

Supplementary Figure 4: Positive and negative phototaxis were shown by chlorophyll fluorescence of *Microglena* sp..

Supplementary Figure 5: Average velocity of *Microglena* sp. under pH and CO₂.

Supplementary Figure 6: Average velocity of *C. reinhardtii* under pH and CO₂.

Supplementary Figure 7: Average velocity of *D. salina* under pH and CO₂.

Supplementary Figure 8: Channels for experiment and intensity distribution with variation of incident irradiance in channel at laboratory micro-small scale.

Supplementary Figure 9: Channels for experiment and intensity distribution with variation of incident irradiance in channel on the sea at field meso-scale.

Supplementary Figure 10: Diagram of laboratory micro-small scale and field mesoscale for cell motion and spatial distribution assay.

Supplementary Figure 11: Average velocity of *Microglena* sp. induced by blue light.

Supplementary Figure 12: Instantaneous velocity of *Microglena* sp. under blue light.

Supplementary Figure 13: Effect of CO₂ concentration on growth of *Microglena* sp..

Supplementary Figure 14: Effect of CO₂ concentration on growth of *C. reinhardtii* and *D. salina*.

Supplementary Figure 15: Difference of gene expression pattern between 400 and 1000ppm CO₂ treatment.

Supplementary Figure 16: Ca²⁺ concentration in *Microglena* sp. under CO₂ (280, 400, 700, 1000, 1500, 2000 ppm).

Supplementary Figure 17: Ca²⁺ concentration in *C. reinhardtii* and *D. salina* under CO₂ (400, 1000, 2000 ppm). Mean value represents the fluorescence intensity of the cells. Bar, 10μm.

Supplementary Figure 18: Comparison between overall SNPs count of *Microglena* sp. grown under normal (control, samples five years ago, marked in grey), 400ppm (red), 1000ppm (green) and 2000ppm (blue) CO₂ conditions.

Supplementary Figure 19: Profile of genetic mutations across reference genomes of *Microglena* sp. grown under normal (control, samples five years ago, marked in grey), 400ppm (blue), 1000ppm (yellow) and 2000 ppm (red) CO₂ conditions.

Supplementary Figure 20: The relative SNP level of genes of *Microglena* sp. related to algal motility under acidification gradients.

Supplementary Figure 21: The ratio of synonymous and non-synonymous mutations of *Microglena* sp. under acidification gradients.

Supplementary Tables

Supplementary Table 1. Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *Microglena* sp. grown under various pCO₂ in the laboratory microscale experiment.

Supplementary Table 2. Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *Microglena* sp. grown under various pCO₂ in the field mesoscale experiment.

Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *C. reinhardtii* grown under various pCO₂ in the laboratory microscale experiment.

Supplementary Table 4. Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *D. salina* grown under various pCO₂ in the laboratory microscale experiment.

Supplementary Table 5. Repeated analysis of variance for the effects of laboratory microscale and field mesoscale on average velocity in *Microglena* sp. grown under various pCO₂.

Supplementary Table 6. Repeated analysis of variance for the effects of positive and negative phototaxis on instantaneous velocity in *Microglena* sp., grown under various pCO₂, in the laboratory microscale experiment.

Supplementary Table 7. Repeated analysis of variance for the effects of positive and negative phototaxis on instantaneous velocity in *C. reinhardtii* grown under various pCO₂, in the laboratory microscale experiment.

Supplementary Table 8. Repeated analysis of variance for the effects of positive and negative phototaxis on instantaneous velocity in *D. salina* grown under various pCO₂ in the laboratory microscale experiment.

Supplementary Table 9. Repeated analysis of variance for the effects of white and blue light on average velocity in *Microglena* sp., grown under various pCO₂, in the vertical direction in the laboratory microscale experiment.

Supplementary Table 10. Repeated analysis of variance for the effects of white and blue light on average velocity in *Microglena* sp., grown under various pCO₂, in the horizontal direction in the laboratory microscale experiment.

Supplementary Table 11. Repeated analysis of variance for the effects of white and blue light on instantaneous velocity in *Microglena* sp., grown under various pCO₂, in the vertical direction in the laboratory microscale experiment.

Supplementary Table 12. Repeated analysis of variance for the effects of white and blue light on instantaneous velocity in *Microglena* sp., grown under various pCO₂, in the horizontal direction in the laboratory microscale experiment.

Supplementary Table 13. Annotations of flagella bending genes for RT-PCR.

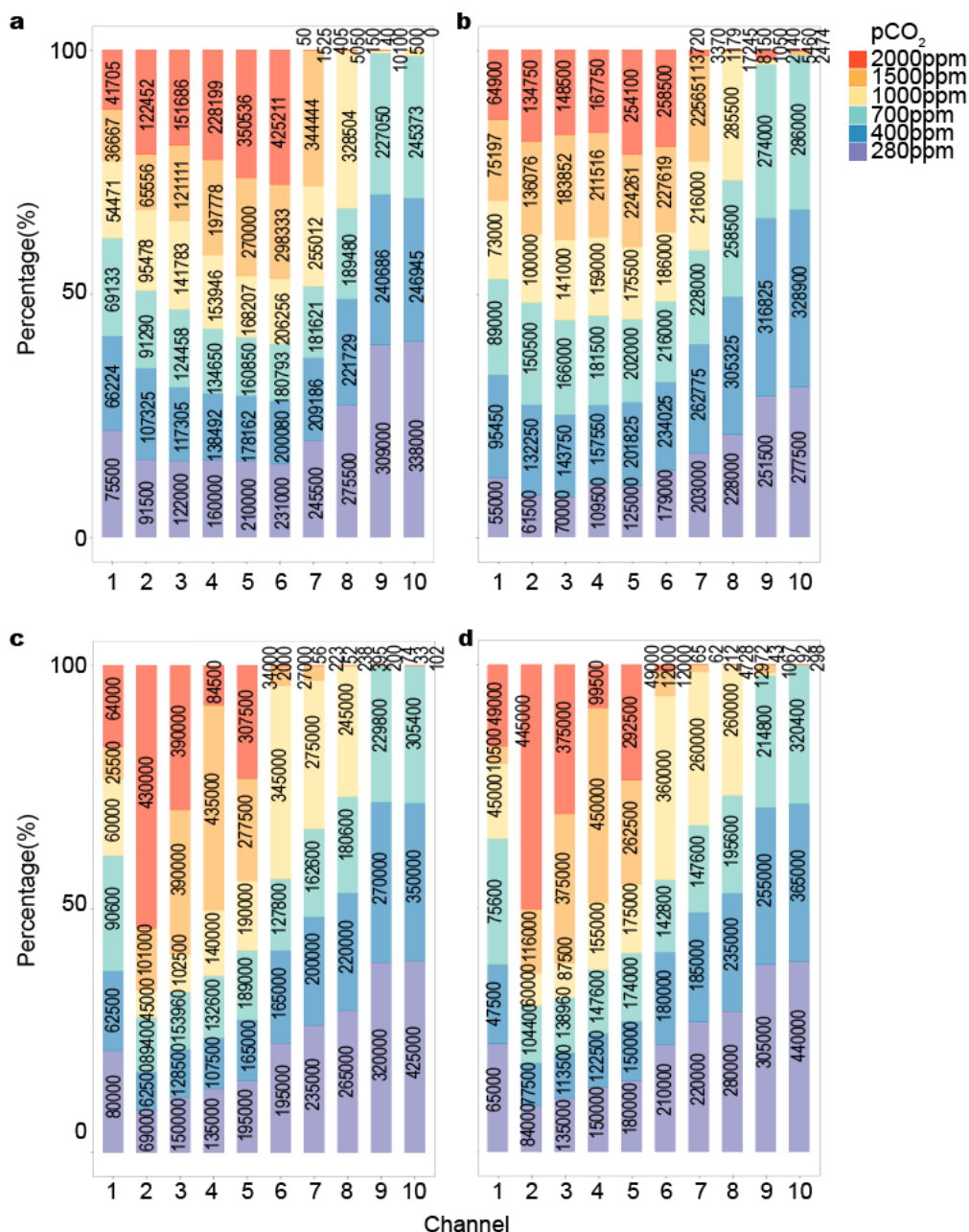
Supplementary Table 14. Medium for *Microglena* sp..

Supplementary Table 15. Medium for *C. reinhardtii*.

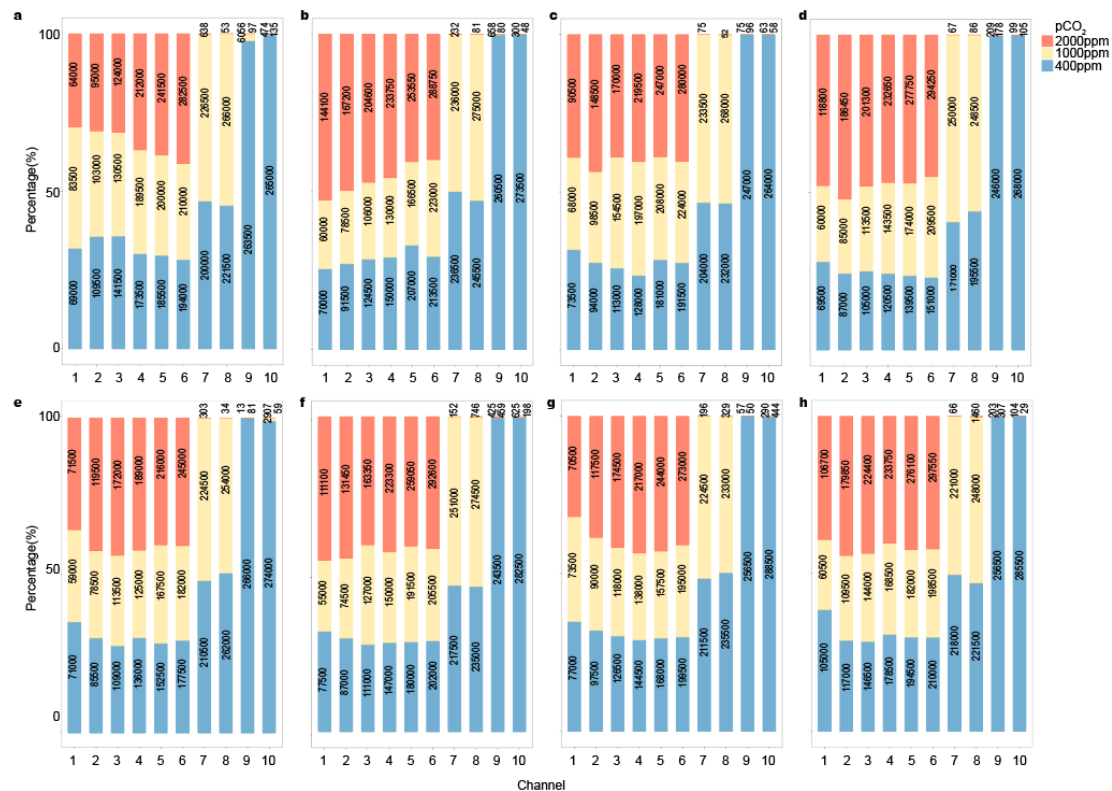
Supplementary Table 16. Medium for *D. salina*.

Supplementary Table 17. Original carbonate system data (mean ± standard errors) determined from total alkalinity (TA) and pH at 6 °C, 32‰ salinity in the laboratory experiment using the CO2SYS Package.

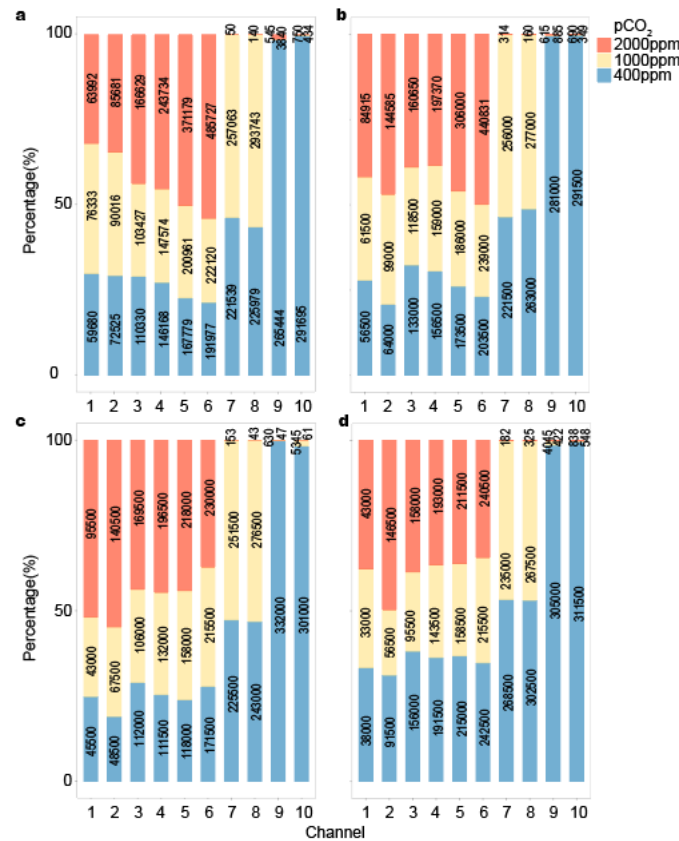
266 Supplementary Table 18. Original carbonate system data (mean $\pm$ standard errors)
267 determined from total alkalinity (TA) and pH at 6 °C, 32‰ salinity in the laboratory
268 experiment using the CO2SYS Package in CO₂/pH manipulation experiment.
269 Supplementary Table 19. *C. reinhardtii* and *D. salina* culture conditions in natural
270 seawater.
271 Supplementary Table 20. *C. reinhardtii* and *D. salina* culture conditions in artificial
272 seawater.
273 Supplementary Table 21. Photosynthesis vs light curve.



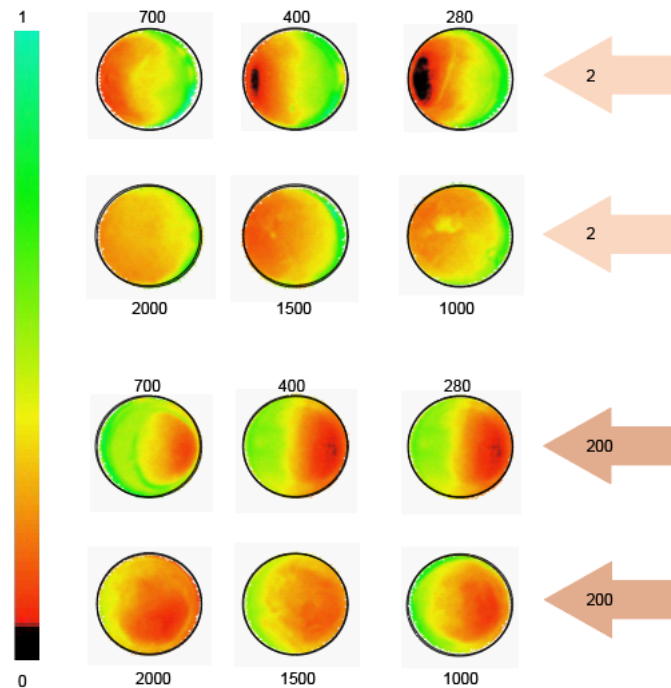
Supplementary Figure 1 | Spatial distribution of *Microglena* sp. in the channel after adding sample at one end. **a**, Positive phototaxis induced by white light in the vertical direction. **b**, Negative phototaxis induced by white light in the vertical direction. **c**, Positive phototaxis induced by white light in the horizontal direction. **d**, Negative phototaxis induced by white light in the horizontal direction. The ordinate (percentage) represents the proportion of cell number under different CO₂ concentration (280, 400, 700, 1000, 1500, 2000 ppm) to sum of all cells at the same sampling point. Bars of different colours represent different CO₂ concentrations. Numbers in the bars represent the actual number of cells at each sampling point. Mean values per experimental assay are given (n = 3).



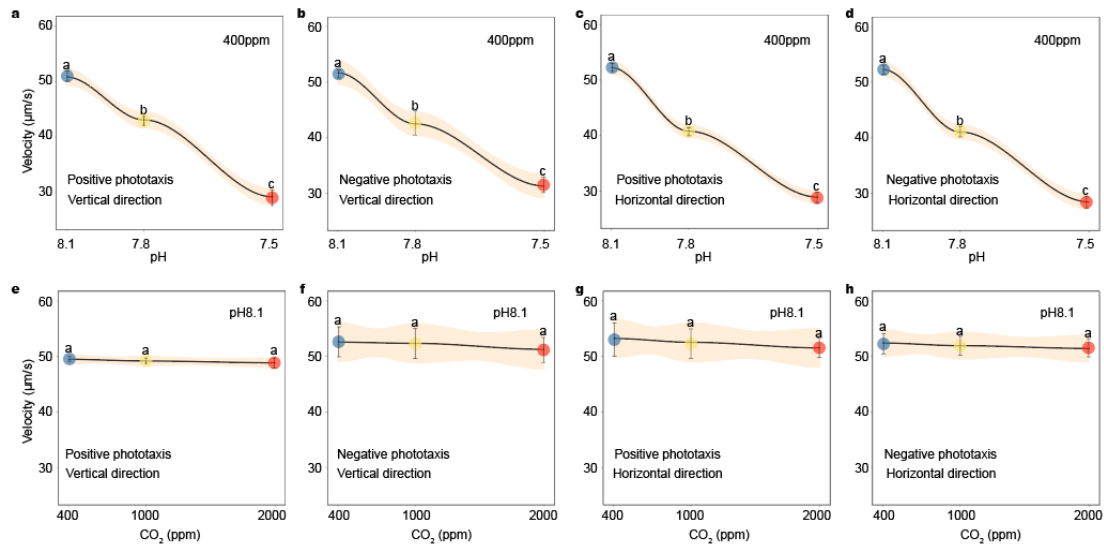
Supplementary Figure 2 | Spatial distribution of *C. reinhardtii* and *Dunaliella salina* in the channel after adding the sample at one end. **a**, *C. reinhardtii* positive phototaxis induced by white light in the vertical direction. **b**, *C. reinhardtii* negative phototaxis induced by white light in the vertical direction. **c**, *D. salina* positive phototaxis induced by white light in the vertical direction. **d**, *D. salina* negative phototaxis induced by white light in the vertical direction. **e**, *C. reinhardtii* positive phototaxis induced by white light in the horizontal direction. **f**, *C. reinhardtii* negative phototaxis induced by white light in the horizontal direction. **g**, *D. salina* positive phototaxis induced by white light in the horizontal direction. **h**, *D. salina* negative phototaxis induced by white light in the horizontal direction. The ordinate (percentage) represents the proportion of cell number under different CO₂ concentration (280, 400, 700, 1000, 1500, 2000 ppm) to sum of all cells at the same sampling point. Bars of different colours represent different CO₂ concentrations. Numbers in the bars represent the actual number of cells at each sampling point. Mean values per experimental assay are given (n = 3).



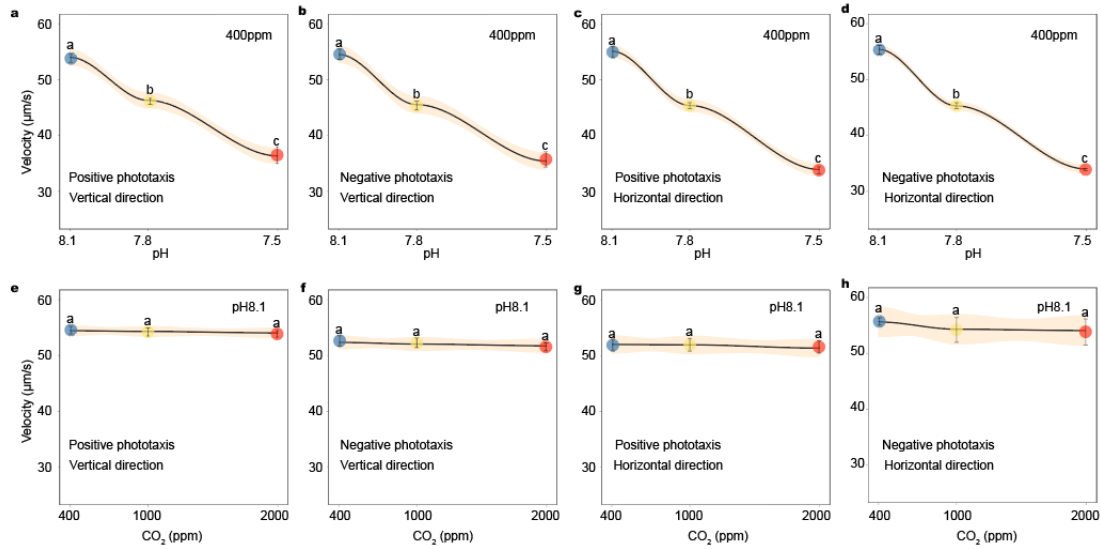
Supplementary Figure 3 | Spatial distribution of *Microglena* sp. induced by sunlight in the channel after adding the sample at one end. **a**, Positive phototaxis in the vertical direction. **b**, Negative phototaxis in the vertical direction. **c**, Positive phototaxis in the horizontal direction. **d**, Negative phototaxis in the horizontal direction. The ordinate (percentage) represents the proportion of cell number under different CO₂ concentration (280, 400, 700, 1000, 1500, 2000 ppm) to sum of all cells at the same sampling point. Bars of different colours represent different CO₂ concentrations. Numbers in the bars represent the actual number of cells at each sampling point. Mean values per experimental assay are given (n = 3).



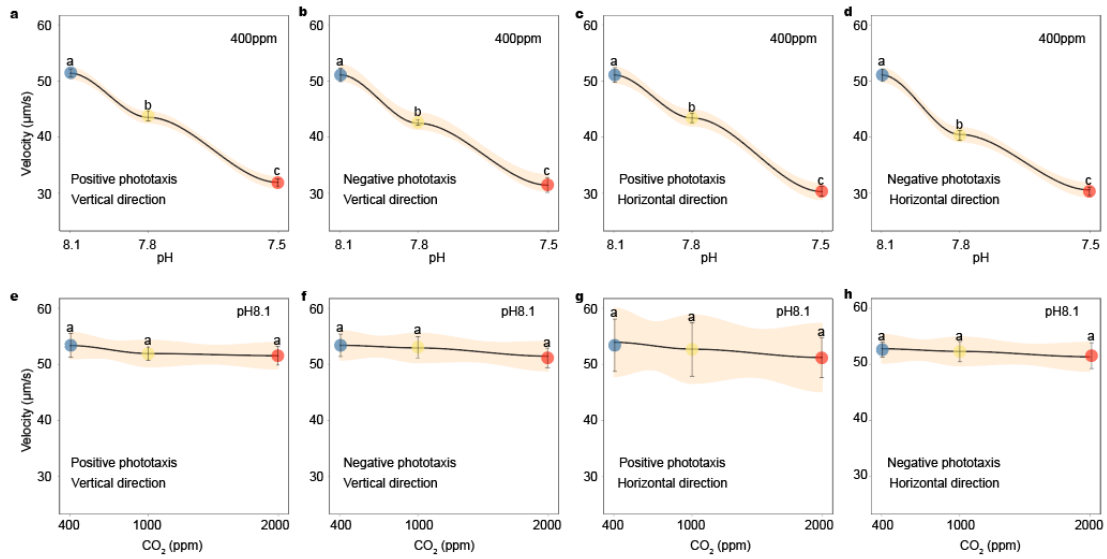
Supplementary Figure 4 | Positive and negative phototaxis was shown by chlorophyll fluorescence of *Microglena* sp.. Positive phototaxis induced by 2 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ white light (light brown arrow). Negative phototaxis induced by 200 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ white light (pale brown arrow). The fluorescence intensity reflects the number of cells. The scale of fluorescence as a proxy for cell number is shown on the left, the greener the colour, the more cells are present.



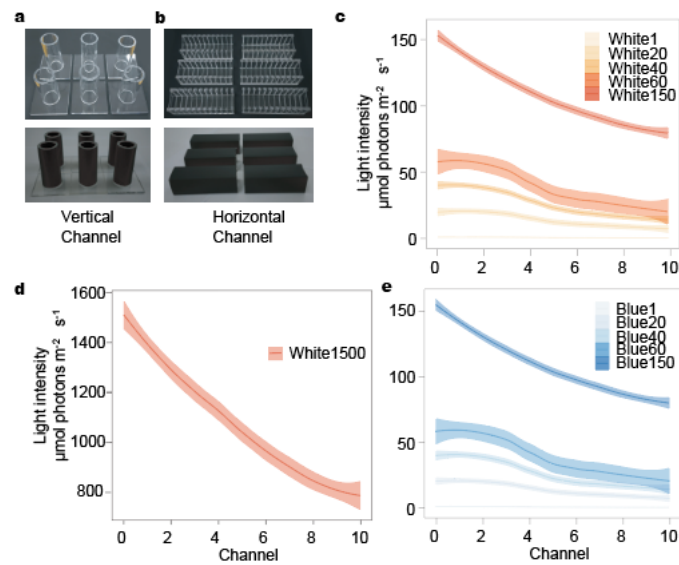
Supplementary Figure 5 |. Average velocity of *Microglena* sp. under pH and CO₂. **a**, Positive phototaxis response in the vertical direction under different pH. **b**, Negative phototaxis response in the vertical direction under different pH. **c**, Positive phototaxis response in the horizontal direction under different pH. **d**, Negative phototaxis response in the horizontal direction under different pH. **e**, Positive phototaxis response in the vertical direction under different CO₂. **f**, Negative phototaxis response in the vertical direction under different CO₂. **g**, Positive phototaxis response in the horizontal direction under different CO₂. **h**, Negative phototaxis response in the horizontal direction under different CO₂. Mean ± SD values per experimental assay are given (n = 3). Different letters in superscript indicate significant differences (p < 0.05) among treatments.



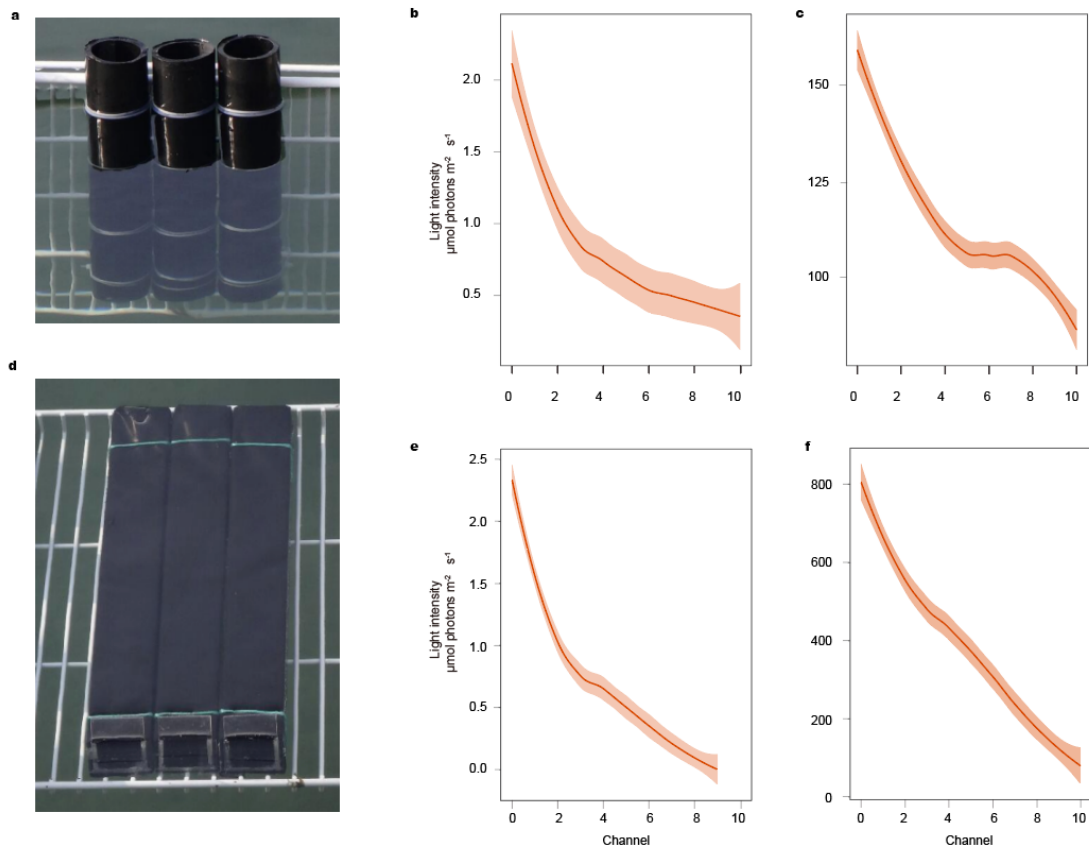
Supplementary Figure 6 | Average velocity of *C. reinhardtii* under pH and CO₂. **a**, Positive phototaxis response in the vertical direction under different pH. **b**, Negative phototaxis response in the vertical direction under different pH. **c**, Positive phototaxis response in the horizontal direction under different pH. **d**, Negative phototaxis response in the horizontal direction under different pH. **e**, Positive phototaxis response in the vertical direction under different CO₂. **f**, Negative phototaxis response in the vertical direction under different CO₂. **g**, Positive phototaxis response in the horizontal direction under different CO₂. **h**, Negative phototaxis response in the horizontal direction under different CO₂. Mean ± SD values per experimental assay are given (n = 3). Different letters in superscript indicate significant differences (p < 0.05) among treatments.



Supplementary Figure 7 |. Average velocity of *D. salina* under pH and CO₂ treatments. **a**, Positive phototaxis response in the vertical direction under different pH. **b**, Negative phototaxis response in the vertical direction under different pH. **c**, Positive phototaxis response in the horizontal direction under different pH. **d**, Negative phototaxis response in the horizontal direction under different pH. **e**, Positive phototaxis response in the vertical direction under different CO₂. **f**, Negative phototaxis response in the vertical direction under different CO₂. **g**, Positive phototaxis response in the horizontal direction under different CO₂. **h**, Negative phototaxis response in the horizontal direction under different CO₂. Mean ± SD values per experimental assay are given (n = 3). Different letters in superscript indicate significant differences (p < 0.05) among treatments.

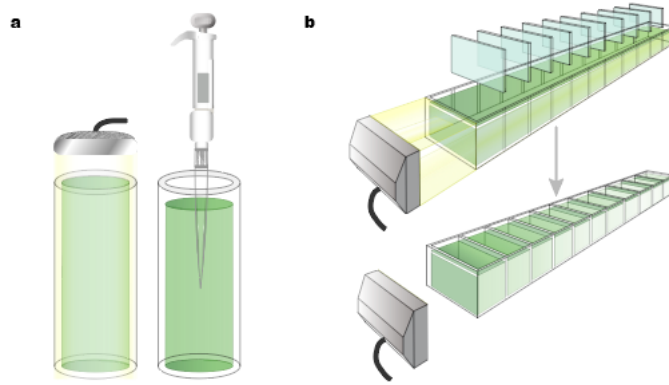


Supplementary Figure 8 | Channels for experiment and light intensity distribution with different incident irradiances in a channel at the laboratory micro- scale. **a**, Vertical experimental channels. For cell motion experiments, channel was cylindrical, with an inner diameter of 4.4 cm and a height of 11 cm. For cell spatial distribution experiments, channel was cylindrical, with an inner diameter of 4.4 cm and a height of 10 cm. **b**, Horizontal experimental channels. For cell motion experiments, channel was oblong with a section of a square with a diameter of 4 cm, and channels were evenly divided into 11 parts with a width of 1cm each. For cell spatial distribution experiments, channel was oblong with a section of a square with a diameter of 4cm, and channels were evenly divided into 10 parts with a width of 1cm each. **c**, Distribution of white light in pure seawater. The x axis represent position in the channel, the width of each position is 1cm (same in below figures). **d**, Distribution of white light as photophobic light of *C. reinhardtii* and *D. salina* in pure seawater. **e**, Distribution of blue light in pure seawater. Mean $\pm$ SD values per experimental assay are given (n = 3).

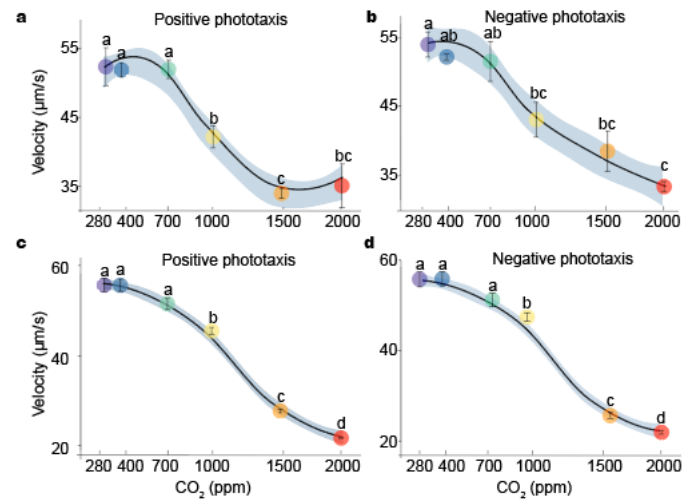


365

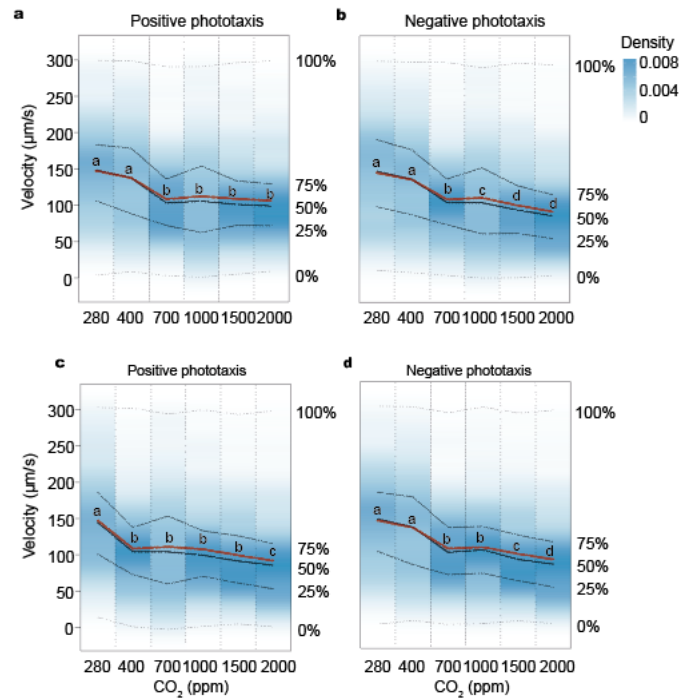
366 **Supplementary Figure 9** | Channels for experiment and light intensity distribution
 367 with variation of incident irradiance in a channel at sea at the field meso-scale. **a**,
 368 Vertical experimental channels. **b**, Distribution of natural light in vertical channels
 369 under positive phototaxis. **c**, Distribution of natural light in vertical channels under
 370 negative phototaxis. **d**, Horizontal experimental channels. **e**, Distribution of natural
 371 light in horizontal channels under positive phototaxis. **f**, Distribution of natural light in
 372 horizontal channels under negative phototaxis. Mean $\pm$ SD values per experimental
 373 assay are given ($n = 3$).



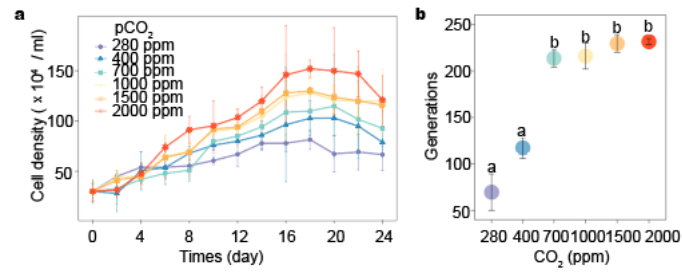
Supplementary Figure 10 | Diagram of laboratory micro-small scale and field mesoscale for cell motion and spatial distribution assay. **a**, Positive and negative phototaxis motion assay at the vertical in an 11cm channel. **b**, Positive and negative phototaxis motion assay at the horizontal in an 11cm channel, which is divided into 11 equal parts.



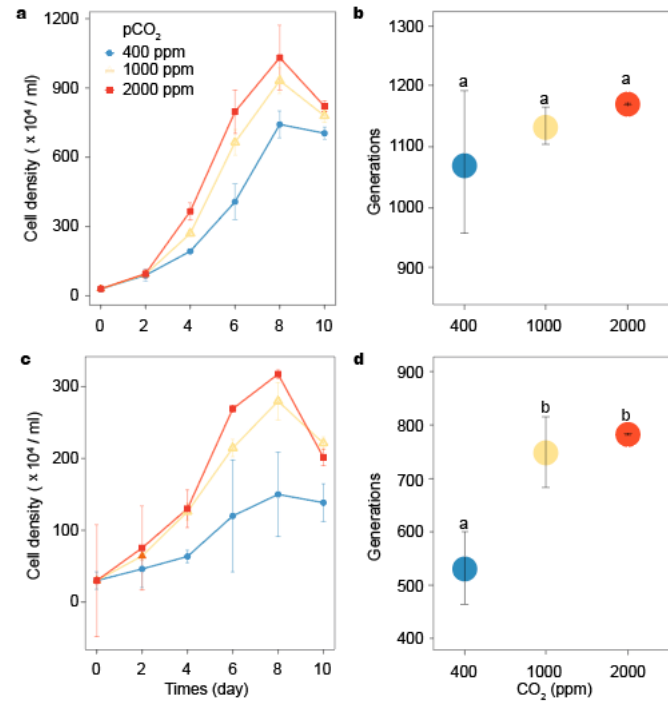
Supplementary Figure 11 | Average velocity of *Microglena* sp. induced by blue light. **a**, Positive phototaxis induced by blue light in the vertical direction. **b**, Negative phototaxis induced by blue light in the vertical direction. **c**, Positive phototaxis induced by blue light in the horizontal direction. **d**, Negative phototaxis induced by white light in the horizontal direction.



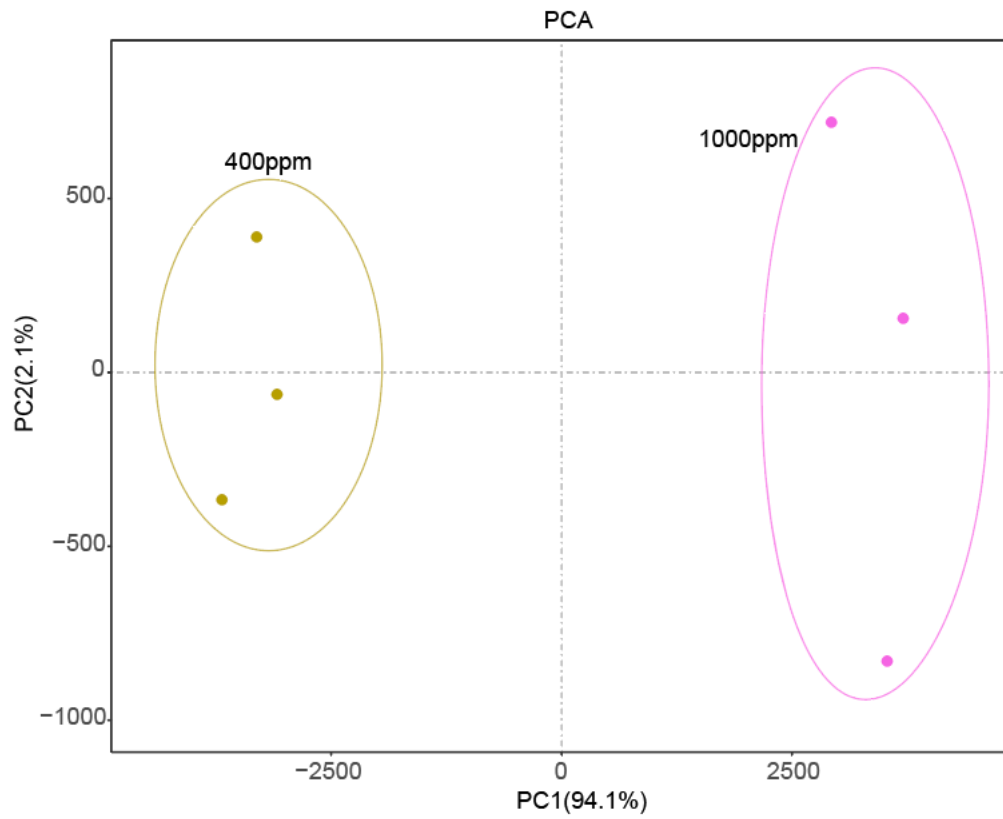
Supplementary Figure 12 | Instantaneous velocity of *Microglena* sp. under blue light. **a**, Positive phototaxis induced by blue light in the vertical direction. **b**, Negative phototaxis induced by blue light in the vertical direction. **c**, Positive phototaxis induced by blue light in the horizontal direction. **d**, Negative phototaxis induced by blue light in the horizontal direction. The colour scale indicates the density of the cell distribution under various velocities. A total of 300 data points was collected for each experimental treatment. The five dotted lines correspond to 0%, 25%, 50%, 75%, 100%, respectively. Each dotted line represents the cumulative ratio, the number of cells below the dotted line as a percentage of the total number of cells. The solid line represents the average velocity and letters indicate significant differences between CO₂ treatments ($p < 0.05$). Different letters in superscript indicate significant differences ($p < 0.05$) among treatments (Kruskal Wallis test followed by Dunn's post-hoc test).



Supplementary Figure 13 | CO₂ concentration effects on growth of *Microglena* sp.. **a**, Growth curve. **b**, Generations in five years. Mean $\pm$ SD values per experimental assay are given (n = 3). Different letters in superscript indicate significant differences (p < 0.05) among treatments.



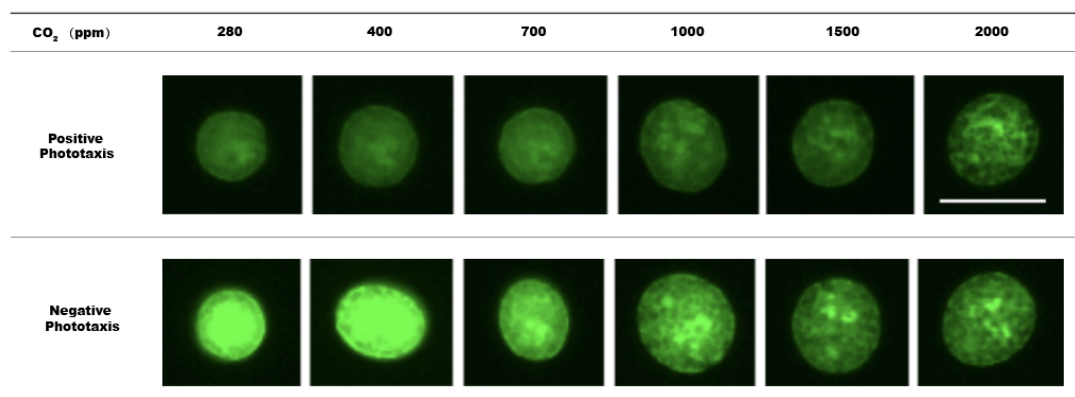
Supplementary Figure 14 | CO₂ concentration effects on growth of *C. reinhardtii* and *D. salina*. **a**, Growth curve of *C. reinhardtii*. **b**, Generations of *C. reinhardtii* in five years. **c**, Growth curve of *D. salina*. **d**, Generations of *D. salina*. Mean $\pm$ SD values per experimental assay are given (n = 3). Different letters in superscript indicate significant differences (p < 0.05) among treatments.



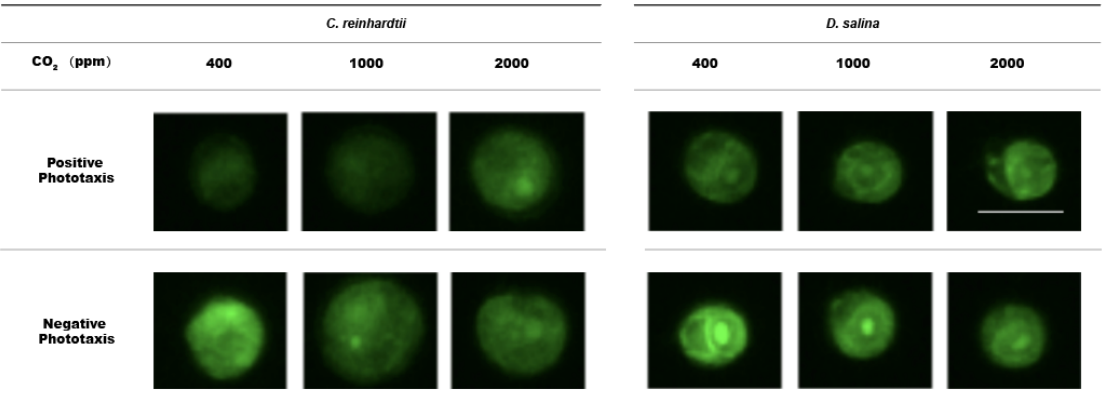
411

412 **Supplementary Figure 15** | Difference of gene expression pattern between 400 and

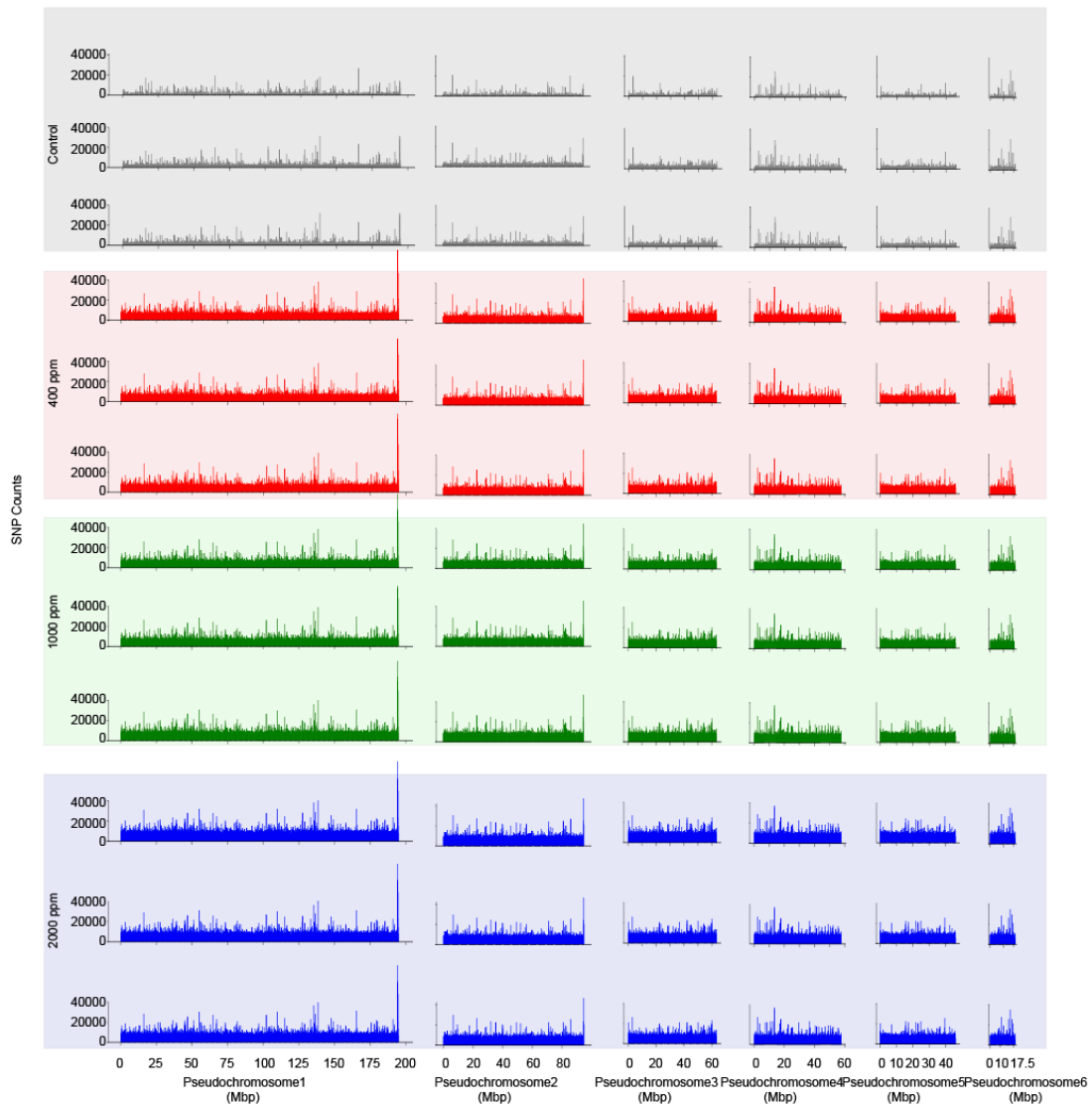
413 1000ppm CO₂ treatment.



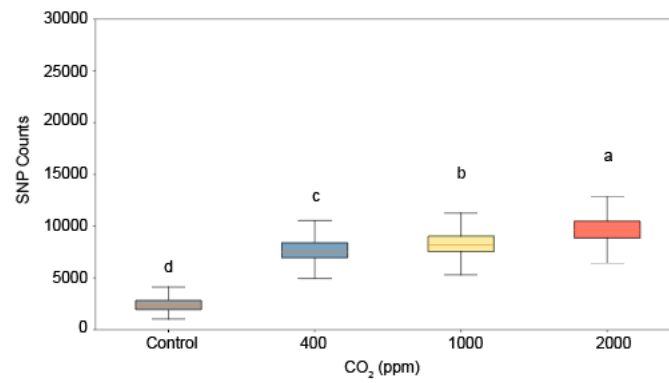
Supplementary Figure 16 | Ca²⁺ concentration in *Microglena* sp. under CO₂ concentration (280, 400, 700, 1000, 1500, 2000 ppm). The white bar represents the length of 20 µm.



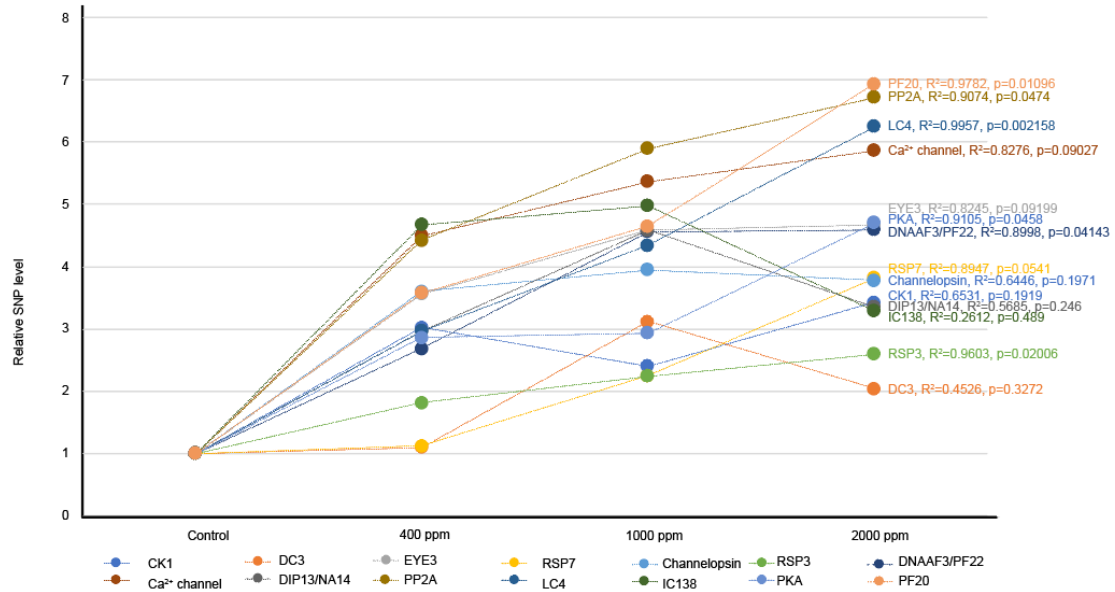
Supplementary Figure 17 | Ca²⁺ concentration in *C. reinhardtii* and *D. salina* under CO₂ concentration (400, 1000, 2000 ppm). Bar, 10μm.



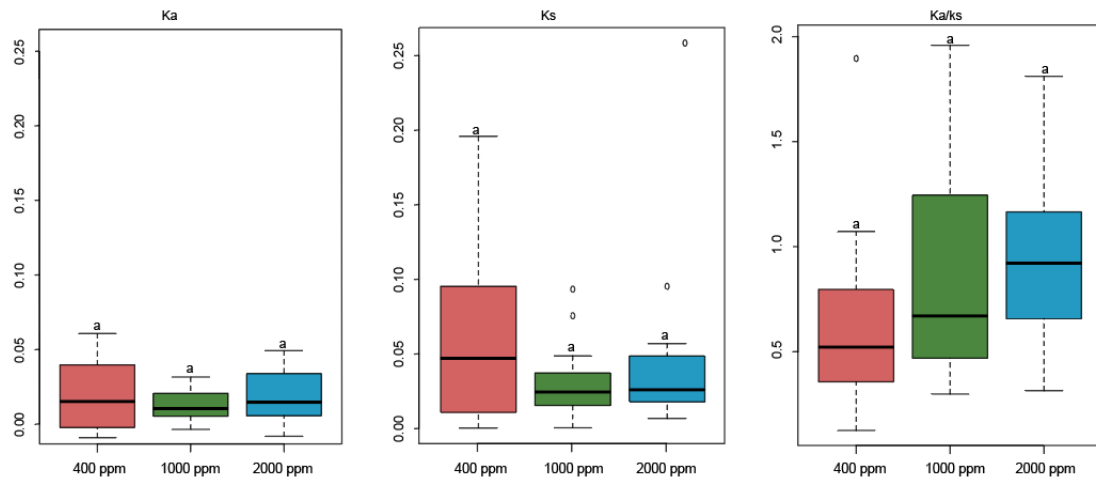
Supplementary Figure 18 | Comparison between overall SNPs count of *Microglena* sp.. grown under normal (control, samples five years ago, marked in grey), 400 ppm (red), 1000 ppm (green) and 2000 ppm (blue) CO₂ conditions.



Supplementary Figure 19 | Profile of genetic mutations across reference genomes of *Microglena* sp.. grown under normal (control, samples five years ago, marked in grey), 400 ppm (blue), 1000 ppm (yellow) and 2000 ppm (red) CO₂ conditions. Different letters in superscript indicate significant differences ($p < 0.05$) among treatments.



Supplementary Figure 20 | The relative SNP level of genes of *Microglena* sp.. related to algal motility under acidification gradients. SNP level=SNP counts/gene length. The SNP level under each acidification gradient is divided by that of the control, and is termed the relative SNP level.



Supplementary Figure 21 | The ratio of synonymous and non-synonymous mutations of *Microglena* sp.. under acidification gradients.

439 Supplementary Table 1. Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *Microglena* sp.
 440 grown under various pCO₂ used in the laboratory microscale experiment. R_V means ratio of velocity of positive phototaxis to velocity of negative
 441 phototaxis and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _V	Sig.
Vertical direction	280	<1	0.075
Vertical direction	400	<1	0.69
Vertical direction	700	>1	0.149
Vertical direction	1000	>1	0.471
Vertical direction	1500	>1	0.01
Vertical direction	2000	>1	0.02
Horizontal direction	280	<1	0.768
Horizontal direction	400	<1	0.709
Horizontal direction	700	<1	0.679
Horizontal direction	1000	<1	0.514
Horizontal direction	1500	<1	0.808
Horizontal direction	2000	<1	0.29

443 Supplementary Table 2. Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *Microglena* sp.
444 grown under various pCO₂ in the field mesoscale experiment. R_v means ratio of velocity of positive phototaxis to velocity of negative phototaxis
445 and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Vertical direction	400	<1	0.834
Vertical direction	1000	>1	0.916
Vertical direction	2000	>1	0.401
Horizontal direction	400	>1	0.331
Horizontal direction	1000	<1	0.764
Horizontal direction	2000	<1	0.283

447 Supplementary Table 3. Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *C. reinhardtii*
448 grown under various pCO₂ in the laboratory microscale experiment. R_v means ratio of velocity of positive phototaxis to velocity of negative
449 phototaxis and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Vertical direction	400	<1	0.247
Vertical direction	1000	<1	0.13
Vertical direction	2000	>1	0.087
Horizontal direction	400	>1	0.603
Horizontal direction	1000	<1	0.738
Horizontal direction	2000	<1	0.791

451 Supplementary Table 4. Repeated analysis of variance for the effects of positive and negative phototaxis on average velocity in *D. salina* grown
452 under various pCO₂ in the laboratory microscale experiment. R_v means ratio of velocity of positive phototaxis to velocity of negative phototaxis
453 and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Vertical direction	400	<1	0.74
Vertical direction	1000	<1	0.514
Vertical direction	2000	>1	0.6
Horizontal direction	400	>1	0.233
Horizontal direction	1000	>1	0.798
Horizontal direction	2000	>1	0.288

455 Supplementary Table 5. Repeated analysis of variance for the effects of laboratory microscale and field mesoscale on average velocity in
 456 *Microglena* sp. grown under various pCO₂. R_v means ratio of velocity of positive phototaxis to velocity of negative phototaxis and Sig. means p
 457 value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	Reaction to light	CO ₂ treatment (ppm)	Velocity	
			R _v	Sig.
Vertical direction	Phototaxis	400	<1	0.214
Vertical direction	Phototaxis	1000	>1	0.91
Vertical direction	Phototaxis	2000	<1	0.611
Vertical direction	Photophobism	400	<1	0.393
Vertical direction	Photophobism	1000	<1	0.275
Vertical direction	Photophobism	2000	<1	0.182
Horizontal direction	Phototaxis	400	<1	0.627
Horizontal direction	Phototaxis	1000	>1	0.665
Horizontal direction	Phototaxis	2000	<1	0.001
Horizontal direction	Photophobism	400	>1	0.122
Horizontal direction	Photophobism	1000	>1	0.87
Horizontal direction	Photophobism	2000	<1	0.00

Supplementary Table 6. Repeated analysis of variance for the effects of positive and negative phototaxis on instantaneous velocity in *Microglena* sp. grown under various pCO₂ in the laboratory microscale experiment. R_v means ratio of velocity of positive phototaxis to velocity of negative phototaxis and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Vertical direction	280	>1	0.000
Vertical direction	400	>1	0.000
Vertical direction	700	>1	0.000
Vertical direction	1000	>1	0.000
Vertical direction	1500	>1	0.139
Vertical direction	2000	>1	0.000
Horizontal direction	280	>1	0.000
Horizontal direction	400	<1	0.000
Horizontal direction	700	>1	0.000
Horizontal direction	1000	>1	0.003
Horizontal direction	1500	>1	0.000
Horizontal direction	2000	>1	0.000

463 Supplementary Table 7. Repeated analysis of variance for the effects of positive and negative phototaxis on instantaneous velocity in *C. reinhardtii*
464 grown under various pCO₂ in the laboratory microscale experiment. R_v means ratio of velocity of positive phototaxis to velocity of negative
465 phototaxis and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Vertical direction	400	<1	0.000
Vertical direction	1000	<1	0.000
Vertical direction	2000	>1	0.003
Horizontal direction	400	>1	0.000
Horizontal direction	1000	>1	0.000
Horizontal direction	2000	>1	0.001

467 Supplementary Table 8. Repeated analysis of variance for the effects of positive and negative phototaxis on instantaneous velocity in *D. salina*
468 grown under various pCO₂ in the laboratory microscale experiment. R_v means ratio of velocity of positive phototaxis to velocity of negative
469 phototaxis and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Vertical direction	400	<1	0.004
Vertical direction	1000	<1	0.000
Vertical direction	2000	>1	0.140
Horizontal direction	400	>1	0.010
Horizontal direction	1000	>1	0.000
Horizontal direction	2000	>1	0.000

471 Supplementary Table 9. Repeated analysis of variance for the effects of white and blue light on average velocity in *Microglena* sp. grown under
 472 various pCO₂ in vertical direction in the laboratory microscale experiment. R_v means ratio of velocity of white light to velocity of blue light and
 473 Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Positive phototaxis	280	>1	0.940
Positive phototaxis	400	<1	0.084
Positive phototaxis	700	<1	0.086
Positive phototaxis	1000	<1	0.801
Positive phototaxis	1500	>1	0.000
Positive phototaxis	2000	<1	0.786
Negative phototaxis	280	>1	0.419
Negative phototaxis	400	<1	0.788
Negative phototaxis	700	<1	0.359
Negative phototaxis	1000	<1	0.528
Negative phototaxis	1500	<1	0.186
Negative phototaxis	2000	<1	0.346

475 Supplementary Table 10. Repeated analysis of variance for the effects of white and blue light on average velocity in *Microglena* sp. grown under
 476 various pCO₂ in horizontal direction in the laboratory microscale experiment. R_v means ratio of velocity of white light to velocity of blue light
 477 and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Positive phototaxis	280	<1	0.708
Positive phototaxis	400	<1	0.693
Positive phototaxis	700	<1	0.664
Positive phototaxis	1000	>1	0.684
Positive phototaxis	1500	>1	0.530
Positive phototaxis	2000	<1	0.932
Negative phototaxis	280	>1	0.609
Negative phototaxis	400	<1	0.562
Negative phototaxis	700	<1	0.538
Negative phototaxis	1000	<1	0.756
Negative phototaxis	1500	>1	0.143
Negative phototaxis	2000	>1	0.905

479 Supplementary Table 11. Repeated analysis of variance for the effects of white and blue light on instantaneous velocity in *Microglena* sp. grown
 480 under various pCO₂ in vertical direction in the laboratory microscale experiment. R_V means ratio of velocity of white light to velocity of blue light
 481 and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _V	Sig.
Positive phototaxis	280	>1	0.000
Positive phototaxis	400	>1	0.000
Positive phototaxis	700	<1	0.000
Positive phototaxis	1000	<1	0.000
Positive phototaxis	1500	<1	0.000
Positive phototaxis	2000	<1	0.000
Negative phototaxis	280	>1	0.000
Negative phototaxis	400	>1	0.000
Negative phototaxis	700	<1	0.000
Negative phototaxis	1000	<1	0.000
Negative phototaxis	1500	<1	0.000
Negative phototaxis	2000	<1	0.000

483 Supplementary Table 12. Repeated analysis of variance for the effects of white and blue light on instantaneous velocity in *Microglena* sp. grown
484 under various pCO₂ in horizontal direction in the laboratory microscale experiment. R_v means ratio of velocity of white light to velocity of blue
485 light and Sig. means p value. Mean ± SD values per experimental assay are given (n = 3).

Motion direction	CO ₂ treatment (ppm)	Velocity	
		R _v	Sig.
Positive phototaxis	280	>1	0.000
Positive phototaxis	400	>1	0.000
Positive phototaxis	700	>1	0.000
Positive phototaxis	1000	<1	0.000
Positive phototaxis	1500	<1	0.000
Positive phototaxis	2000	>1	0.000
Negative phototaxis	280	>1	0.000
Negative phototaxis	400	>1	0.000
Negative phototaxis	700	>1	0.000
Negative phototaxis	1000	>1	0.000
Negative phototaxis	1500	<1	0.009
Negative phototaxis	2000	<1	0.992

Supplementary Table 13. Annotations of flagella bending genes for RT-PCR.

Gene ID	Predicted function	Nr Annotation
MigICE16000	Voltage gated calcium channel CAV7	XP_001690493.1 voltage-gated Ca ²⁺ channel, alpha subunit [<i>Chlamydomonas reinhardtii</i>]
MigICE11569	RSP7	AOM63275.1 calmodulin [<i>Chlamydomonas</i> sp. ICE-L]
MigICE12547	Channelopsin1	AER58219.1 channelopsin 1 [<i>Haematococcus lacustris</i>]
MigICE10361	Eyespot assembly protein EYE3	XP_001699977.1 eyespot assembly protein, ABC1 kinase family [<i>Chlamydomonas reinhardtii</i>]
MigICE08486	CK1	XP_001693999.1 ser/thr kinase [<i>Chlamydomonas reinhardtii</i>]
MigICE16283	PP2A	XP_001700181.1 PP2A-twin subunit [<i>Chlamydomonas reinhardtii</i>]
MigICE04581	PKA	XP_001693385.1 predicted protein, partial [<i>Chlamydomonas reinhardtii</i>]
MigICE14893	RSP3	KXZ49444.1 RSP3 protein [<i>Gonium pectorale</i>]
MigICE05195	PF20	AAB41727.1 PF20 [<i>Chlamydomonas reinhardtii</i>]
MigICE09498	DC3	GAQ81162.1 calmodulin [<i>Klebsormidium nitens</i>]
MigICE16837	LC4	XP_001690249.1 flagellar outer dynein arm 18 kDa light chain LC4 [<i>Chlamydomonas reinhardtii</i>]
MigICE04131	IC138	XP_001696921.1 flagellar inner dynein arm I1 intermediate chain IC138 [<i>Chlamydomonas reinhardtii</i>]
MigICE16200	Flagella Deflagellation inducible protein DIP13/NA14	XP_001697145.1 deflagellation inducible protein [<i>Chlamydomonas reinhardtii</i>]
MigICE14891	Dynein assembly protein DNAAF3/PF22	AEC04845.1 axonemal dynein assembly protein PF22 [<i>Chlamydomonas reinhardtii</i>]

489 Supplementary Table 14. Medium for *Microglena* sp..

Provasoli Stock Solution			
Component	Element type	Initial concentration	End concentration
II	Fe(NH ₄) ₂ (SO ₄) ₂ •6H ₂ O	2.738 mg L ⁻¹	/
	Na ₂ EDTA	2.578 mg L ⁻¹	/
	Na ₂ EDTA	3.906 mg L ⁻¹	/
	H ₃ BO ₃	4.453 mg L ⁻¹	/
III	FeCl ₃ •6H ₂ O	0.191mg L ⁻¹	/
	MnCl ₂ •4H ₂ O	0.57mg L ⁻¹	/
	ZnSO ₄ •7H ₂ O	0.008 mg L ⁻¹	/
	CoCl ₂ •6H ₂ O	0.0156 mg L ⁻¹	/
N	NH ₄ NO ₃	51.418 mg L ⁻¹	15-25 mg L ⁻¹
P	NaH ₂ PO ₄ •2H ₂ O	8.512 mg L ⁻¹	0.01-0.02 mg L ⁻¹

490

491 Supplementary Table 15. Medium for *C. reinhardtii*.

Provasoli Stock Solution			
Component	Element type	Initial concentration	End concentration
I	H ₃ BO ₃	1 mg L ⁻¹	/
	ZnSO ₄ •7H ₂ O	1 mg L ⁻¹	/
	MnSO ₄ •H ₂ O	0.303 mg L ⁻¹	/
	CoCl ₂ •6H ₂ O	0.2 mg L ⁻¹	/
	Na ₂ MoO ₄ •2H ₂ O	0.2 mg L ⁻¹	/
	CuSO ₄ •5H ₂ O	0.0625 mg L ⁻¹	/
II	Na ₃ C ₆ H ₅ O ₇ •2H ₂ O	500 mg L ⁻¹	/
III	FeCl ₃ •6H ₂ O	10 mg L ⁻¹	/
IV	CaCl ₂ •2H ₂ O	53 mg L ⁻¹	/
V	MgSO ₄ •7H ₂ O	300 mg L ⁻¹	/
VI	NH ₄ NO ₃	300 mg L ⁻¹	1-5 mg L ⁻¹
VII	KH ₂ PO ₄	100 mg L ⁻¹	0.01-0.02 mg L ⁻¹
VIII	K ₂ HPO ₄ •3H ₂ O	131 mg L ⁻¹	

492 The final pH should be 6.7-6.9.

493 Supplementary Table 16. Medium for *D. salina*.

Provasoli Stock Solution			
Component	Element type	Initial concentration	End concentration
I	CaCl ₂ •2H ₂ O	10 mg L ⁻¹	/
II	MgSO ₄ •7H ₂ O	1230 mg L ⁻¹	/
III	NaNO ₃	420 mg L ⁻¹	10-15 mg L ⁻¹
	NaH ₂ PO ₄ •2H ₂ O	15.6 mg L ⁻¹	0.01-0.02 mg L ⁻¹
	NaHCO ₃	840 mg L ⁻¹	/
	KCl	74 mg L ⁻¹	/
IV	C ₆ H ₈ FeNO ₇	5 mg L ⁻¹	/
V	NaCl	30 g L ⁻¹	/
VI	H ₃ BO ₃	2.86 mg L ⁻¹	/
	MnCl ₂ •4H ₂ O	1.86 mg L ⁻¹	/
	ZnSO ₄ •7H ₂ O	0.22 mg L ⁻¹	/
	Na ₂ MoO ₄ •2H ₂ O	0.39 mg L ⁻¹	/
	CuSO ₄ •5H ₂ O	0.08 mg L ⁻¹	/
	Co(NO ₃) ₂ •6H ₂ O	0.05 mg L ⁻¹	/

494 The final pH should be 7.5.

495 Supplementary Table 17. Original carbonate system data (mean ± standard errors) determined from total alkalinity (TA) and pH at 6 °C, 32‰
 496 salinity in the laboratory experiment using the CO2SYS Package.

Treatment	pH	CO ₂ (ppm)	pCO ₂ (μatm)	TA (μmol L ⁻¹)	DIC (μmol L ⁻¹)	CO ₂ (μmol L ⁻¹)	CO ₃ ²⁻ (μmol L ⁻¹)	HCO ₃ ⁻ (μatm)
280	8.21 ± 0.07	291 ± 8	241 ± 54	2147 ± 91	1940 ± 108	12 ± 3	145 ± 16	1783 ± 116
400	8.13 ± 0.06	408 ± 8	272 ± 4	1937 ± 153	1779 ± 166	14 ± 3	110 ± 5	1655 ± 166
700	7.91 ± 0.06	703 ± 15	560 ± 102	2298 ± 93	2201 ± 108	29 ± 5	85 ± 7	2088 ± 110
1000	7.82 ± 0.02	1037 ± 35	789 ± 42	2622 ± 40	2548 ± 42	40 ± 2	80 ± 3	2428 ± 41
1500	7.67 ± 0.02	1520 ± 34	1153 ± 47	2644 ± 39	2621 ± 36	59 ± 2	58 ± 3	2504 ± 34
2000	7.51 ± 0.01	1960 ± 21	1733 ± 61	2694 ± 42	2723 ± 44	88 ± 3	42 ± 1	2593 ± 42

Supplementary Table 18. Original carbonate system data (mean ± standard errors) determined from total alkalinity (TA) and pH at 6 °C, 32‰ salinity in the laboratory experiment using the CO2SYS Package in CO₂/pH manipulation experiment.

Treatment	pH	CO ₂ (ppm)	pCO ₂ (μatm)	TA (μmol L ⁻¹)	DIC (μmol L ⁻¹)	CO ₂ (μmol L ⁻¹)	CO ₃ ²⁻ (μmol L ⁻¹)	HCO ₃ ⁻ (μatm)
400+8.1	8.10 ± 0.01	404 ± 9	481 ± 26	3238 ± 134	3025 ± 130	25 ± 1	177 ± 6	2824 ± 123
400+7.8	7.81 ± 0.01	404 ± 9	486 ± 8	1578 ± 42	1524 ± 40	25 ± 0.4	46 ± 2	1452 ± 38
400+7.5	7.52 ± 0.02	404 ± 9	542 ± 17	874 ± 8	871 ± 6	28 ± 1	14 ± 1	830 ± 6
1000+8.1	8.11 ± 0.01	1025 ± 33	920 ± 9	6031 ± 23	5690 ± 19	47 ± 1	329 ± 4	5315 ± 17
2000+8.1	8.09±0.02	1981 ± 19	1683 ± 114	11467 ± 767	10842 ± 725	86 ± 6	650 ± 49	10106 ± 674

502 Supplementary Table 19. *C. reinhardtii* and *D. salina* culture conditions in natural seawater.

	Treatment	CO ₂ (ppm)	pH
<i>C. reinhardtii</i>	400 ppm	404 ± 10	7.01 ± 0.01
	1000 ppm	1022 ± 30	6.92 ± 0.01
	2000 ppm	1978 ± 36	6.85 ± 0.01
<i>D. salina</i>	400 ppm	404 ± 10	8.22 ± 0.03
	1000 ppm	1022 ± 30	7.85 ± 0.02
	2000 ppm	1978 ± 36	7.51 ± 0.02

503

504 Supplementary Table 20. *C. reinhardtii* and *D. salina* culture conditions in artificial seawater.

	Treatment	CO ₂ (ppm)	pH
<i>C. reinhardtii</i>	400ppm+7.0	408 ± 8	7.00 ± 0.01
	400ppm+6.9	408 ± 8	6.91 ± 0.01
	400ppm+6.8	408 ± 8	6.86 ± 0.01
	1000ppm+7.0	1020 ± 39	7.00 ± 0.01
	2000ppm+7.0	1971 ± 17	7.01 ± 0.01
<i>D. salina</i>	400ppm+8.2	408 ± 8	8.24 ± 0.03
	400ppm+7.8	408 ± 8	7.82 ± 0.03
	400ppm+7.5	408 ± 8	7.51 ± 0.02
	1000ppm+8.2	1020 ± 39	8.21 ± 0.07
	2000ppm+8.2	1971 ± 17	8.21 ± 0.09

506 Supplementary Table 21. Photosynthesis vs light curve. P means photosynthesis, I means light.

CO ₂ (ppm)	Equation	R ²
280	$P = -(6.04 \times 10^{-8} \cdot I + 7.14 \times 10^{-7} - \text{SQRT}((6.04 \times 10^{-8} \cdot I + 7.14 \times 10^{-7}) \cdot (6.04 \times 10^{-8} \cdot I + 7.14 \times 10^{-7}) + 2.5 \times 10^{-17}))/157446.3 - 1.84 \times 10^{-9}$	0.983
400	$P = (3.04 \times 10^{-10} \cdot I + 7.62 \times 10^{-9} - \text{SQRT}((3.04 \times 10^{-10} \cdot I + 7.62 \times 10^{-9}) \cdot (3.04 \times 10^{-10} \cdot I + 7.62 \times 10^{-9}) - 1.11 \times 10^{-26}))/1.652 - 1.46 \times 10^{-9}$	0.996
700	$P = (8.11 \times 10^{-11} \cdot I - \text{SQRT}((8.11 \times 10^{-11} \cdot I) \cdot (8.11 \times 10^{-11} \cdot I))) + 0.001 \times 10^{-9}$	0.781
1000	$P = (3.53 \times 10^{-10} \cdot I + 1.01 \times 10^{-8} - \text{SQRT}((3.53 \times 10^{-10} \cdot I + 1.01 \times 10^{-8}) \cdot (3.53 \times 10^{-10} \cdot I + 1.01 \times 10^{-8}) - 1.59 \times 10^{-26}))/1.822 - 1.22 \times 10^{-9}$	0.998
1500	$P = (3.10 \times 10^{-10} \cdot I + 7.88 \times 10^{-9} - \text{SQRT}((3.10 \times 10^{-10} \cdot I + 7.88 \times 10^{-9}) \cdot (3.10 \times 10^{-10} \cdot I + 7.88 \times 10^{-9}) - 7.23 \times 10^{-27}))/1.558 - 9.5 \times 10^{-10}$	0.998
2000	$P = (2.12 \times 10^{-10} \cdot I - 5.31 \times 10^{-9} - \text{SQRT}((2.12 \times 10^{-10} \cdot I - 5.31 \times 10^{-9}) \cdot (2.12 \times 10^{-10} \cdot I - 5.31 \times 10^{-9}) - 2.27 \times 10^{-26}))/0.794 + 1.27 \times 10^{-8}$	0.983

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