



Valorization of nutrients and CO₂ from a recirculating aquaculture system into high-value astaxanthin: A circular bioeconomy approach with *Haematococcus pluvialis*

Hemanta Timilsina¹ · Jussi S. Vesamäki¹ · Marco L. Calderini² · Minna Hiltunen¹ · Juhani Pirhonen¹ · Katja Pulkkinen¹

Received: 10 April 2026 / Revised: 1 June 2026 / Accepted: 6 June 2026
© The Author(s) 2026

Abstract

Upcycling CO₂-rich exhaust air and nutrient-rich effluent water from recirculating aquaculture systems (RAS) into high-value bioproducts can enhance aquaculture sustainability and promote a circular bioeconomy. This study investigated the effects of RAS-derived CO₂ supplementation on the growth and astaxanthin production of the microalga *Haematococcus pluvialis* cultivated in RAS water. Cultures were grown in 1.5-L photobioreactors and aerated with either ambient room air (~400 ppm CO₂) or RAS exhaust air (RAS CO₂; 1900–2500 ppm) at ~18 °C, representative of Nordic RAS conditions. During the stationary phase, room-air cultures were subdivided and aerated with either room air or RAS CO₂, while RAS-CO₂ cultures continued under the same aeration. All cultures were then subjected to high light (200–400 μmol photons m⁻²s⁻¹) to induce astaxanthin synthesis. Compared with room air, RAS CO₂ increased *H. pluvialis* growth rate by 15%, cell density by 57%, and dry weight (DW) by 76%. The removal of total N, NO₃-N, and PO₄-P exceeded 95% in all treatments. Regardless of initial growth conditions, RAS CO₂ during the stress phase increased astaxanthin content by 17–19%, reaching up to 1.12% of dry weight, and increased astaxanthin concentration by 92–119%, reaching up to 23.26 mg L⁻¹, relative to room air. These findings indicate that CO₂ stripped directly from a RAS is sufficient to promote both growth and astaxanthin production in *H. pluvialis* relative to ambient room air. Overall, this study demonstrates RAS exhaust CO₂ as a viable carbon source for high-value astaxanthin production under realistic aquaculture operating conditions.

Keywords Chlorophyceae · Microalgae · Aquaculture · Bioremediation · Nitrogen · Phosphorus · RAS-CO₂

Introduction

Global seafood demand is rising rapidly, while capture fisheries cannot expand further because of ecological limits and regulatory restrictions (FAO 2024). Fed aquaculture has become the primary solution to meet future seafood demand. Aquaculture currently supplies about 50% of global seafood and is expected to exceed 60% by 2030 (World Bank 2013; FAO 2024). Recirculating aquaculture systems (RAS) have emerged as technically advanced, land-based systems that

support intensive fish farming under controlled conditions. Unlike traditional, single-pass flow-through or net-pen systems, RAS recycle more than 90% of water through mechanical, biological and/or chemical treatment units (Ahmed and Turchini 2021). These systems substantially reduce water demand, effluent discharge, and land footprint. They also improve biosecurity, waste management, and environmental control, thus supporting year-round fish production regardless of geographic or climatic conditions (Piedrahita 2003; Ahmed and Turchini 2021; Bregnballe 2022; Timmons and Vinci 2022; Ende et al. 2024). Although initial investment and operational costs are higher than in traditional aquaculture, the popularity of RAS in commercial aquaculture operations continues to expand (European Commission 2020a).

Despite their advantages, RAS unavoidably produce side streams (nutrient-rich effluent water, CO₂-rich exhaust air) that may pose environmental and economic challenges (Ende et al. 2024). RAS can have multiple inline treatment units, but compounds such as nitrate (NO₃-N), phosphate

✉ Hemanta Timilsina
hemanta.h.timilsina@jyu.fi

¹ Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

² Faculty of Information Technology, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

(PO₄-P) (van Rijn 2013), off-flavor metabolites (e.g., geosmin and methylisoborneol) (Lindholm-Lehto and Vielma 2019; Ben-Asher et al. 2024), dissolved organic carbon (DOC) and fine colloids (Chiam and Sarbatly 2011) accumulate in the water over time. To maintain water quality, 5–10% of system water is typically replaced daily with fresh water (Piedrahita 2003; Bregnballe 2022). The discharged water can contain nutrients at levels 10–100 times higher than those in flow-through systems (Blancheton 2007), with PO₄-P and NO₃-N reaching up to 45 and 200 mg L⁻¹, respectively (van Bussel et al. 2013; Böppe et al. 2025). A RAS producing ~1000 t of fish per year may release ~21 t of nitrogen through its effluent water (Bregnballe 2022; Böppe et al. 2025). If released untreated, such water can cause eutrophication in recipient water bodies (Arbour et al. 2024; Ende et al. 2024). In addition to nutrient-rich water, RAS also produce CO₂ through fish and microbial respiration. To maintain optimal water quality and ensure fish health, dissolved CO₂ must be stripped before the water is recycled back to fish tanks (Aslam et al. 2019; Mota et al. 2019; Timmons and Vinci 2022). Degassing units such as trickling filters or cascade columns are commonly used for this purpose (Moran 2010; Timmons and Vinci 2022), which emit exhaust air with elevated CO₂.

Although often considered waste, RAS side streams (effluent water and exhaust air) could be used as valuable resources for cultivating microalgae. Compared to municipal and industrial wastewaters or flue gases, RAS side streams are relatively clean due to their origin in food production (Ende et al. 2024). Moreover, the RAS water generally contains a balanced mix of essential nutrients (favorable N:P ratio) suitable for microalgal cultivation (Silkina et al. 2019; Ende et al. 2024). Since microalgal photosynthesis is CO₂-limited under ambient air (Chekanov et al. 2017), supplementing cultures with CO₂-rich RAS exhaust air can increase growth rate, biomass yield, and nutrient uptake. Thus, not only nutrient-rich effluent water but also CO₂-rich exhaust air from RAS can be harnessed for microalgal cultivation (Pirhonen et al. 2023; Hiltunen et al. 2026). This approach provides a synergistic bioremediation strategy that simultaneously recovers nutrients, captures CO₂, and produces valuable biomass. As a biological alternative to conventional physicochemical treatments, microalgal bioremediation of RAS effluents promotes resource efficiency, minimizes environmental hazard, and transforms waste into bioproducts. This approach supports EU goals for low-carbon food and feed production, sustainable aquaculture, and climate change mitigation, while aligning with circular bioeconomy principles and global sustainability goals (United Nations 2015; European Commission 2020b; 2021).

The microalga *Haematococcus pluvialis* (syn. *H. lacustris*) is of particular interest because it synthesizes astaxanthin, a ketocarotenoid with high antioxidant capacity.

This freshwater, unicellular, green microalga transitions from green, motile vegetative cells to red, non-motile cysts (aplanospores) under stress conditions such as high light and nutrient deprivation (Boussiba and Vonshak 1991; Shah et al. 2016). This green-to-red phase transition involves morphological and biochemical changes, including deflagellation, secondary cell wall thickening, and intracellular accumulation of astaxanthin (up to 5% per dry weight), as a protective response to oxidative stress (Boussiba and Vonshak 1991; Boussiba et al. 1999; Boussiba 2000; Shah et al. 2016; Hu et al. 2019, 2020). Astaxanthin is widely used in aquaculture as a pigmentation and health-promoting feed additive (Lu et al. 2021; Tuan Harith et al. 2024) and it also has applications in the nutraceutical and cosmetic industries (Bjørklund et al. 2022; Dutta et al. 2023). Natural astaxanthin has been reported to have higher antioxidant capacity and pigmentation efficiency than synthetic astaxanthin (Capelli et al., 2013; Villaró et al., 2021). However, due to the high cost of natural astaxanthin, salmonid feeds are currently supplemented with cheaper synthetic astaxanthin derived from petrochemical sources (Behnam et al. 2025). Developing sustainable and cost-effective production systems, including approaches that valorize aquaculture side streams, may support the growing global demand for natural astaxanthin.

Previous studies have investigated the integration of microalgal cultivation with RAS through the simultaneous utilization of nutrient-rich RAS effluent water and CO₂-rich RAS exhaust air. Supplementation with CO₂ (805–1166 ppm) stripped from the RAS trickling filter enhanced the growth of *H. pluvialis* and *Monoraphidium griffithii* compared with ambient room air (Pirhonen et al. 2023). Similarly, *Scenedesmus armatus* cultivated using nitrate-rich RAS effluent water and RAS-derived CO₂ (925–1619 ppm) showed improved growth under RAS CO₂ compared with ambient air, while nitrate availability influenced biomass production and CO₂ utilization efficiency (Sucunthowong et al. 2023). In addition, *Chlorella vulgaris* was cultivated using RAS effluent water and exhaust CO₂ (600–1100 ppm) (Hiltunen et al. 2026). However, the integrated valorization of RAS-derived CO₂ and effluent water as complementary carbon and nutrient sources for astaxanthin production by *H. pluvialis* has received limited attention. To address this, we investigated the effect of CO₂ supplementation derived from RAS on the growth and astaxanthin production of *H. pluvialis* cultivated in RAS effluent water. Cultures were aerated with either ambient room air or RAS exhaust CO₂ under low-temperature conditions (~18 °C), typical of Nordic RAS facilities, and high light was applied at the stationary phase to induce astaxanthin synthesis. Growth rate, cell density, dry biomass weight, nutrient (N, P) removal efficiencies, biochemical composition in both green- and red-phase biomass,

and astaxanthin production were assessed and compared between aeration treatments. We also assessed the effect of shifting cultures grown under room air to RAS CO₂ during the stress phase on astaxanthin biosynthesis and biochemical responses.

Materials and methods

Microalgal stock and preculture conditions

Haematococcus pluvialis (syn. *H. lacustris*) strain K-0084 was obtained from the Norwegian Culture Collection of Algae (NORCCA). The microalga was maintained as stock monocultures in modified Wright's cryptophyte (MWC) medium (Guillard and Lorenzen 1972; Table S1). For the experiment, *H. pluvialis* was precultured in 5-L Erlenmeyer flasks with a 4 L working volume of MWC medium at ~20 °C under an 18:6 h photoperiod, and a light intensity of 40–50 μmol photons m⁻²s⁻¹ (6500 K fluorescent lamps, 36 W/965, Osram, Germany). Cultures were continuously aerated with ambient room air (~400 ppm CO₂) using air pumps (Eheim air400, Germany). Green vegetative cells in the early exponential phase were used as inoculum for the experiment.

Recirculating aquaculture system water

Nutrient-rich effluent water (hereafter RAS water) was obtained from a pilot-scale RAS operated by the Natural Resources Institute Finland (Luke), in Laukaa, Finland. The RAS farm sources its influent water from the oligotrophic Lake Peurunka (62.44886, 25.85201). No antibiotics were used at the RAS farm. A detailed description of the RAS configuration and operation is provided in Pulkkinen et al. (2021). RAS water was collected in mid-November 2024 from the clear outflow of four RAS tanks rearing rainbow trout (*Oncorhynchus mykiss*), after drum filtration and fixed-bed bioreactor treatment. At the time of sampling, fish had an average weight of 0.43–0.46 kg and were maintained at a stocking density of 40–97 kg m⁻³. The system operated with a water circulation rate of 30 L s⁻¹ (7–8 L s⁻¹ per tank) and used ~700 L of replacement water per kg of feed supplied, typically varying 600–800 L kg⁻¹. After collection, the water was stored at 4 °C in the dark for less than a week. Prior to the experiment, the water was sieved through a 27 μm mesh, disinfected by ultraviolet irradiation for 3 h (VIQUA D4, Canada), and equilibrated to ~18 °C. Initial water parameters were assessed at the start of the experiment (Table 1).

Table 1 Initial physicochemical characteristics (mean ± SD, n=3–4) of the RAS water used in the experiment

Parameter	Value
pH	6.81 ± 0.02
Total nitrogen, TN (mg L ⁻¹)	40.17 ± 1.33
Nitrite, NO ₂ -N (mg L ⁻¹)	0.52 ± 0.02
Nitrate, NO ₃ -N (mg L ⁻¹)	32.11 ± 0.22
Ammonia, NH ₄ -N (mg L ⁻¹)	0.55 ± 0.03
Phosphate, PO ₄ -P (mg L ⁻¹)	2.52 ± 0.02
N:P ratio ^a (mass)	12.74 ± 0.16
N:P ratio ^b (molar)	28.16 ± 0.35
Dissolved organic carbon, DOC (mg L ⁻¹)	21.37 ± 0.15
Dissolved inorganic carbon, DIC (mg L ⁻¹)	9.72 ± 0.26

^{ab}N:P ratio was calculated as NO₃-N:PO₄-P

Experimental setup

Twelve photobioreactors (transparent plastic (PET) funnels, 9 cm inner diameter, 30 cm length; 1.5 L total volume, 1.2 L working volume) were arranged in a row and covered with transparent lids as described in Pirhonen et al. (2023). A detailed engineering schematic and a real photograph are provided in Figs. S1 and S2. Each reactor was inoculated with 16% (v/v) of precultured *H. pluvialis* cell suspension (density: ~18.50 × 10⁴ cells mL⁻¹) and 84% (v/v) of RAS water with starting densities of 2.90 × 10⁴ cells mL⁻¹. Cultures were continuously aerated with either ambient room air (n=6) or CO₂-rich exhaust air from the RAS trickling filter (hereafter RAS CO₂; n=6) at a flow rate of 1.2–1.4 L min⁻¹, consistent with our previous study (Hiltunen et al. 2026), and illuminated with 6500 K fluorescent lamps (Osram, Germany) at 18:6 h photoperiod with intensity of 55–67 μmol photons m⁻²s⁻¹ at ~18 °C (RAS facility temperature).

RAS CO₂ was collected from the headspace of the trickling filter of an experimental-size RAS (total volume ~4.5 m³, raising *O. mykiss* at a stocking density of 20 kg m⁻³) in the laboratory of the Department of Biological and Environmental Science at the University of Jyväskylä. In the trickling filter, aeration facilitates gas exchange by stripping CO₂ from the culture water into the exhaust air and increases the dissolved O₂ concentration in the water. The resulting CO₂-rich exhaust air from the headspace of the covered trickling filter was directed through a PVC hose into a plastic reservoir bag housing air pumps (Eheim air400, Germany) and then supplied to the photobioreactors. Room air was drawn directly from the RAS facility atmosphere using identical air pumps (Fig. S1). CO₂ concentrations in both room air and RAS exhaust air were continuously monitored (every 30 s) using CO₂ sensors (Air-Control COACH, Dostmann Electronic GmbH, Germany). The sensor used to monitor RAS-derived CO₂ concentration was placed inside the reservoir bag, whereas the sensor for

monitoring room air CO₂ was placed in the laboratory near the photobioreactors. RAS CO₂ varied between 1900–2500 ppm (except on day 3, exceeding 3000 ppm), while room air CO₂ remained at ~400 ppm (Fig. 1).

On day 14, room-air cultures (n=6) were pooled and subdivided into two conditions: continued aeration with room air (n=3) or switched to RAS CO₂ (hereafter room-RAS CO₂, n=3). RAS-CO₂ cultures (n=6) were continued with the same aeration. All cultures were then exposed to continuous high light (200–400 μmol photons m⁻²s⁻¹; 18 W AP67 T8 LED lamps, Valoya Oy, Finland) for 10 days to induce astaxanthin biosynthesis. Continuous aeration ensured homogeneous mixing and uniform high light exposure within each reactor, despite spatial variation in irradiance, with lower intensity at the top and higher intensity near the light source.

Analytical methods

H. pluvialis growth

Haematococcus pluvialis growth was assessed by analyzing cell density, specific growth rate, cell productivity, and dry cell weight. Cell density (cells mL⁻¹) was estimated daily using a hemocytometer (Bürker, Marienfeldt, Germany) under 100× magnification (light microscope). Cell productivity (CP, cells mL⁻¹ day⁻¹) and the specific growth rate (μ, day⁻¹) were determined using Eqs. (1) and (2), respectively:

$$CP = \frac{(C_2 - C_1)}{(T_2 - T_1)} \quad (1)$$

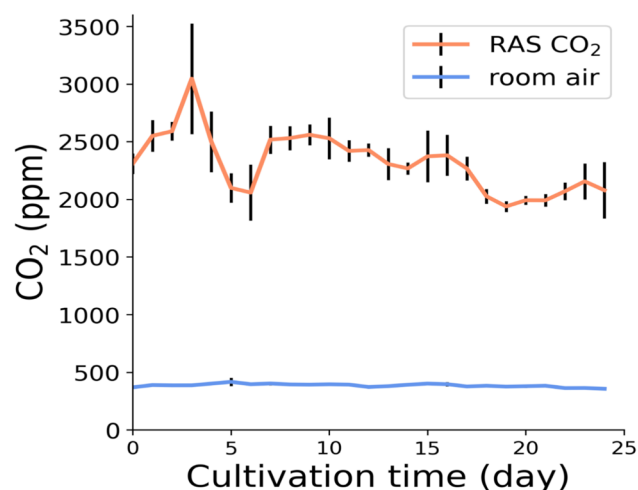


Fig. 1 CO₂ concentrations (mean ± SD) in room air and exhaust air from a recirculating aquaculture system (RAS) used for aeration of cultures

$$\mu = \frac{\ln(C_2/C_1)}{(T_2 - T_1)} \quad (2)$$

where C_1 and C_2 are cell densities at times T_1 and T_2 , respectively. For CP, T_1 and T_2 correspond to days 0 and 14 (green growth phase before high light induction). For μ, T_1 and T_2 correspond to days 0 and 7 (exponential-growth phase).

Dry weight (DW, g L⁻¹) was determined as described in Timilsina et al. (2025). Briefly, a known volume of culture aliquot (5–10 mL) was filtered through a pre-washed (with 50 mL DI water and dried at 105 °C) and pre-weighed glass fiber filter (Whatman GF/C) using a vacuum filtration system. The biomass retained on the filter was dried overnight at 105 °C and subsequently reweighed.

Water parameters

Nutrients in the RAS water were analyzed at: (i) day 0 (start of the experiment) and ii) day 14 (at the end of the green growth phase prior to the high-light exposure). NO₃-N, NO₂-N, NH₄-N, and PO₄-P were analyzed spectrophotometrically (LASA 100, Dr. Lange, Germany) with reagent kits LCK 339 (NO₃-N), LCK 341 (NO₂-N), LCK 304 (NH₄-N), and LCK 349 (PO₄-P) (Hach, USA), following the manufacturer's instructions.

Dissolved organic carbon (DOC) and total nitrogen (TN) in RAS water was analyzed using a TOC/TN analyzer (TOC-L, Shimadzu, Japan). Dissolved inorganic carbon (DIC) was determined by filtering 5 mL of water or culture aliquot (0.45 μm pore size, Sartorius) through a syringe filter into vacuum-cleaned exetainer vials (12 mL; Labco, UK) preloaded with 200 μL of 85% H₃PO₄. The resulting vial headspace CO₂ was analyzed using gas chromatography (7890B, Agilent, USA), and concentrations were expressed as mg L⁻¹ (Vesamäki et al. 2024). The pH was assessed with a pH meter (SevenEasy, Mettler Toledo, Switzerland).

Biochemical composition in green and red phases

Biomass samples were collected at the end of the green growth phase (day 14, prior to high-light exposure) and during the red phase (days 15, 17, and 24). At each sampling point, 50 mL of culture aliquot from each reactor was centrifuged, and the resulting pellets were stored at -80 °C until analysis. Carbon (C) and nitrogen (N) contents (% of DW) were determined from freeze-dried biomass (~0.9 mg) using an elemental analyzer (Flash EA1112, Thermo Fisher Scientific Inc.), with birch (*Betula alba*) leaf powder as the reference material.

Protein, lipid, and carbohydrate contents (relative % of DW) were analyzed from freeze-dried biomass using a Fourier-transform infrared (FTIR) spectrometer (Nicolet iS50, Thermo Fisher Scientific Inc.) as described in Vesamäki

et al. (2026). The FTIR-based method provides a rapid, non-destructive estimation of biochemical composition and has been previously validated for microalgal biomass (Wagner et al. 2010). Briefly, spectra were recorded in attenuated total reflectance (ATR) mode over 2000–600 cm^{-1} (32 scans, 4 cm^{-1} resolution). All spectra were background- and baseline-corrected, as well as min–max scaled to standardize spectral intensity among samples. Biochemical components were identified based on characteristic absorbance peaks: lipids at 1750–1730 cm^{-1} , carbohydrates at 1160–1140 cm^{-1} (Wagner et al. 2010), and proteins as the average of the amide I (1680–1640 cm^{-1}) and amide II (1550–1540 cm^{-1}) bands (Stehfest et al. 2005; Wagner et al. 2010). Relative proportions of lipids, proteins, and carbohydrates were obtained by expressing the normalized peak intensity of each biomolecule as a fraction of the combined intensities of the lipid, protein, and carbohydrate peaks.

Astaxanthin extraction, de-esterification, and analysis

Biomass was sampled after 1, 3, and 10 days of high-light exposure (culture days 15, 17, and 24). For each sampling day, 40 mL of *H. pluvialis* suspension from each replicate was centrifuged (5000 $\times g$, 10 min, 18 °C), and the resulting pellets were stored at –80 °C until analysis. The procedures for astaxanthin extraction, de-esterification, and quantification are described in detail in Calderini et al. (2025). Briefly, 1 ± 0.1 mg of freeze-dried biomass was placed in ZR Bashing Bead tubes (0.1 and 0.5 mm; Zymo Research, USA), and 3.11 μg of internal standard (trans- β -apo-8'-carotenal) was added. Total pigments were extracted with 100% acetone. Cell disruption was performed with a Bead Ruptor Elite instrument (OMNI, USA) at 4 m s^{-1} for four cycles of 20 s. De-esterification of astaxanthin was carried out through enzymatic hydrolysis using cholesterol esterase enzyme (final concentration: 0.36 U mL^{-1} ; MP Biomedicals, Germany). De-esterified astaxanthin extracts were then separated and analyzed by high-performance liquid chromatography (HPLC; Nexera, Shimadzu, Japan) equipped with a diode array detector (SPD-M20A, Shimadzu) and an Acclaim C30 column (150 $\text{mm} \times 3$ mm, 3 μm ; Thermo Fisher Scientific Inc.). Astaxanthin isomers (all-*E*, 13-*Z*, and 9-*Z*) were identified based on retention times and absorption spectra and were quantified against a calibration curve of synthetic (3*S*, 3'*S*)-astaxanthin (DHI). Astaxanthin content was normalized to dry weight and expressed as mg g^{-1} . Extraction losses were corrected based on the recovery % of the internal standard. The astaxanthin data presented in this study was previously utilized for the development and validation of a hyperspectral spectral imaging (HSI) model for non-invasive quantification of astaxanthin (Calderini et al. 2025).

Statistical analyses

Statistical analyses were performed in IBM SPSS (version 30.0.0). Data from the green vegetative phase (days 0–14) and the red stress phase (days 15–24) were analyzed separately. In the green phase, two treatments were compared (RAS CO_2 , $n = 6$; room air, $n = 6$). One-way ANOVA was used to test the treatment effects on growth rate (μ), cell productivity (CP), dry weight (DW, day 14), biomass C:N ratio (day 14), and nutrient removal on day 14. A repeated-measures ANOVA (rmANOVA) was used to assess whether changes in cell density over time differed between treatments. Time (day) was included as a within-subject factor and treatment as between-subject factor. In the red phase, three treatments were compared (RAS CO_2 , $n = 6$; room-RAS CO_2 , $n = 3$; room air, $n = 3$). rmANOVAs were used to assess the effects of treatment on dry weight, astaxanthin content and concentration, and biomass C:N ratio. Biochemical composition data (protein, carbohydrate, lipid; normalized to 100%) from the green phase (day 14) and red phase (day 24) was first transformed using Centered Log-Ratio (CLR) transformation because the data are compositional. Then, treatment effect on overall biochemical composition was first analyzed by multivariate analysis of variance (MANOVA, Pillai's trace), with CLR-transformed lipid, protein, and carbohydrate as dependent variables and treatment as the fixed factor. Significant multivariate effect was further analyzed using univariate ANOVAs. Data normality was tested with the Shapiro–Wilk test, homogeneity of variances with Levene's test, and sphericity with Mauchly's test (Greenhouse–Geisser correction used when sphericity violated). When variances were unequal, Welch's *t*-test (two treatment groups) or Welch's ANOVA with Games–Howell post hoc tests (three treatment groups) were used. When statistically significant effect of treatment (in three treatment groups), pairwise comparisons were performed with Bonferroni adjusted post hoc tests. Statistical significance was set at $p < 0.05$.

Results

Effect of RAS CO_2 on *H. pluvialis* growth

Supplementation with RAS-derived CO_2 significantly enhanced the growth of *H. pluvialis* compared to room air. During the green growth phase (days 0 to 14), cultures supplied with RAS CO_2 had consistently higher cell densities than those aerated with room air (rmANOVA, between-subject effects, $p < 0.001$; Fig. 2a; Table S2). By day 14, RAS- CO_2 cultures reached a mean density of 71×10^4 cells mL^{-1} , whereas room-air cultures attained only 45×10^4 cells mL^{-1} (Fig. 2a). RAS CO_2 increased the growth rate (μ) by 15% and

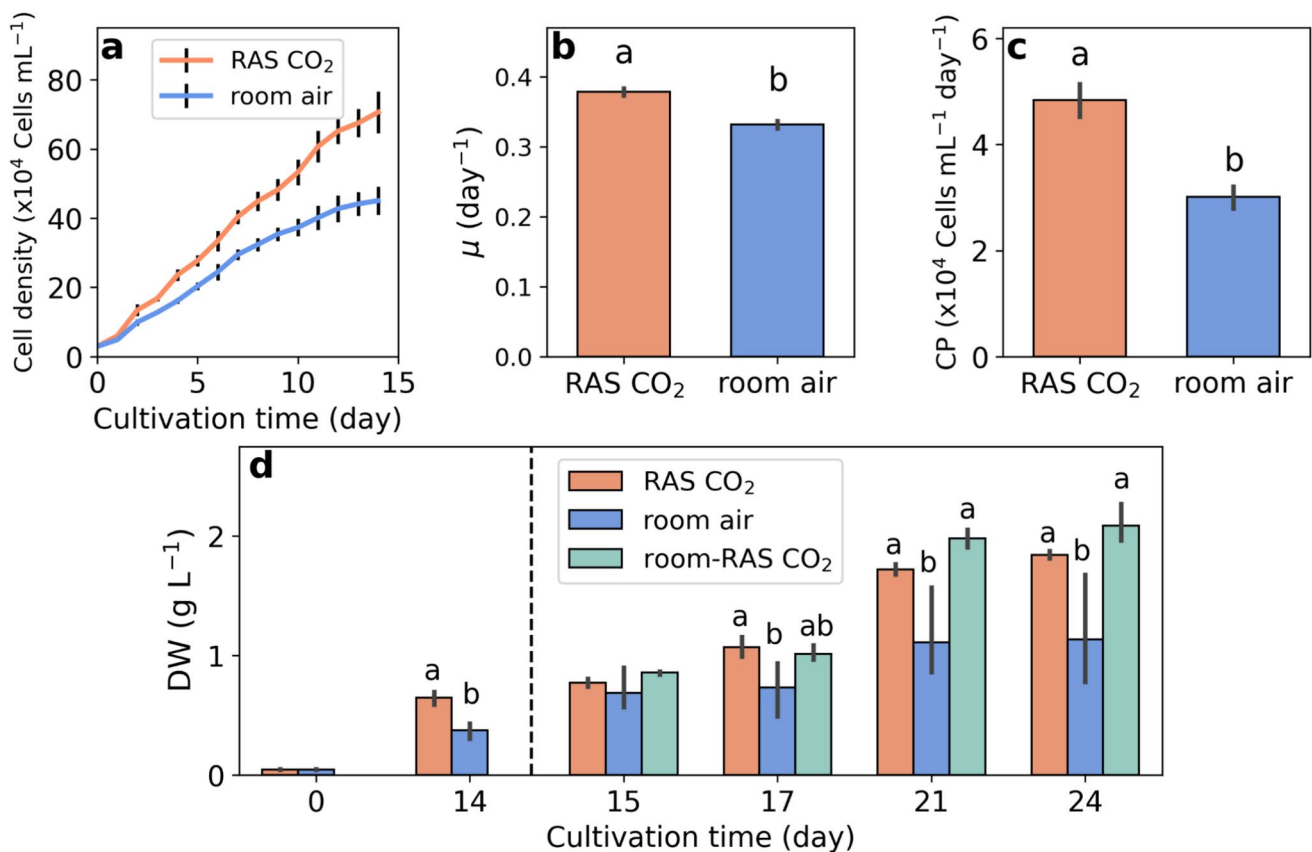


Fig. 2 Mean ($\pm$ SD) of (a) cell density, (b) specific growth rate (μ), (c) cell productivity (CP), (d) dry weight (DW) of *H. pluvialis* cultivated in recirculating aquaculture system (RAS) effluent water under exhaust air from the RAS trickling filter (RAS CO₂: 1900–2500 ppm) or room air ($\sim$ 400 ppm CO₂). High light (200–400 μ mol photons

m⁻²s⁻¹) was initiated on day 14 for 10 days to induce astaxanthin biosynthesis. The dashed vertical line in Fig. 2d separates the green growth and red stress phases. Different letters indicate statistically significant differences ($p < 0.05$) among treatments at each sampling point

cell productivity (CP) by 60% (both $p < 0.001$; Fig. 2b, c; Table S3). At the end of growth phase (day 14), dry biomass weight (DW) was 76% higher in RAS-CO₂ cultures than in room-air cultures, yielding up to 0.65 g L⁻¹ ($p < 0.001$; Fig. 2d, day 14; Table S3).

RAS CO₂ also increased DW during the red phase, regardless of the aeration used during the growth phase. Under high-light exposure which started on day 14, DW increased in all treatments (rmANOVA within-subjects, main effect of time, $p < 0.001$; Fig. 2d, red phase; Table S4), and the rate of increase differed among treatments, as indicated by time-treatment interaction ($p < 0.001$, Table S4). There was also a main effect of treatment (rmANOVA between-subjects, $p = 0.003$; Table S4). Pairwise comparisons showed that cultures receiving RAS CO₂, either continuously or only during the red phase (i.e., room-RAS CO₂), had higher DW than cultures aerated continuously with room air ($p = 0.009$ and $p = 0.004$, respectively). The continuous RAS CO₂ and room-to-RAS CO₂ treatments did not differ significantly. By the end of red phase (day 24), RAS CO₂

had higher DW by 62–84% compared with continuous room air (Fig. 2d).

Effect of RAS CO₂ on water parameters

Nitrogen removal from RAS water by *H. pluvialis* differed between the two aeration sources, whereas PO₄-P removal remained similar. Supplementing the cultures with RAS-derived CO₂ supported higher removal of TN and NO₃-N from RAS water than aeration with room air (TN: $p = 0.02$; NO₃-N: $p = 0.03$; Table 2; Table S5). By the end of growth phase, almost 99% of both TN and NO₃-N had been removed in the RAS CO₂ treatment. PO₄-P removal reached up to 91% by the end of the growth phase, with no statistically significant difference between RAS CO₂ and room air treatments ($p = 0.33$; Table 2; Table S6). DOC was higher in the RAS CO₂ treatment than in room air treatment ($p < 0.001$; Table 2; Table S6). However, DIC was slightly higher under room air (Table 2; Table S6). The RAS CO₂ treatment had lower pH than the room air treatment (Table 2).

Table 2 Mean ($\pm$ SD) of pH, nutrient concentrations, and removal efficiencies (%R.E.) of *H. pluvialis* cultivated in recirculating aquaculture system (RAS) effluent water aerated with either exhaust air from the RAS trickling filter (RAS CO₂: 1900–2500 ppm) or room air (~400 ppm CO₂), measured on day 14 (end of the growth phase). Dissolved inorganic and organic carbon (DIC and DOC) are presented as concentrations only

Parameter	RAS CO ₂	Room air
pH	7.85 $\pm$ 0.05	8.56 $\pm$ 0.07
TN (mg L ⁻¹)	0.51 $\pm$ 0.02	1.37 $\pm$ 0.60
TN (%R.E.)	98.72 $\pm$ 0.06	96.59 $\pm$ 1.50
NO ₃ -N (mg L ⁻¹)	0.41 $\pm$ 0.09	1.08 $\pm$ 0.56
NO ₃ -N (%R.E.)	98.71 $\pm$ 0.28	96.64 $\pm$ 1.75
PO ₄ -P (mg L ⁻¹)	0.22 $\pm$ 0.12	0.28 $\pm$ 0.08
PO ₄ -P (%R.E.)	91.29 $\pm$ 4.93	88.83 $\pm$ 2.36
DIC (mg L ⁻¹)	18.41 $\pm$ 1.27	20.27 $\pm$ 1.08
DOC (mg L ⁻¹)	70.89 $\pm$ 2.42	50.73 $\pm$ 3.14

Effect of RAS CO₂ on biochemical composition

The molar carbon-to-nitrogen (C:N) ratio of *H. pluvialis* biomass increased significantly from the green to the red phase, rising from 8–11 on day 14 to 55–60 on day 24 (Fig. 3a). During the green phase, the C:N ratio was higher under RAS CO₂ than under room air ($p < 0.001$; Fig. 3a; Table S7). During the red phase, the C:N ratio increased significantly overtime in all treatments (rmANOVA within-subjects, main effect of time, $p < 0.001$; Fig. 3a; Table S8). The C:N ratio also differed among treatments (rmANOVA between-subjects, $p = 0.02$; Table S8). Pairwise comparisons showed that RAS CO₂ had a higher C:N ratio than room air ($p = 0.03$). No difference was found between the RAS CO₂ and room-RAS CO₂ treatments ($p = 1.00$).

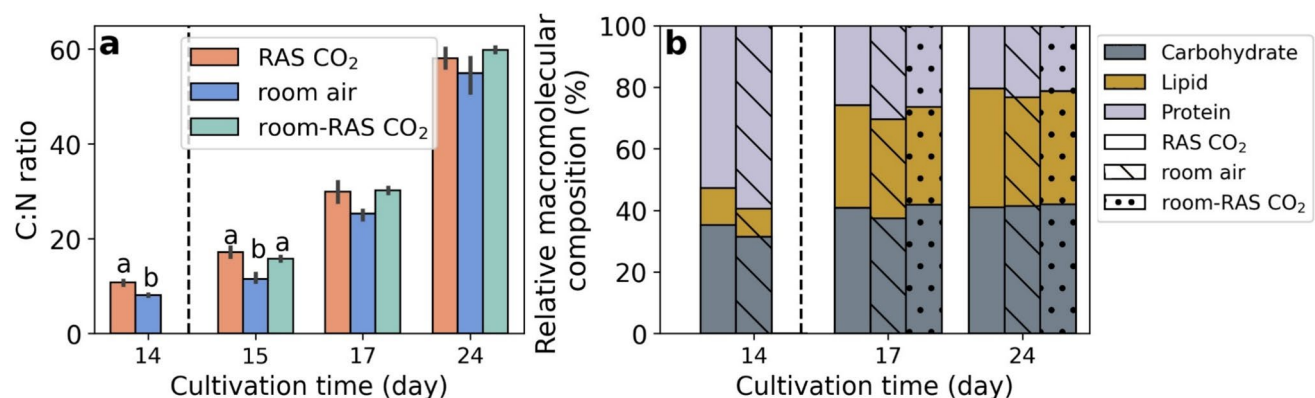


Fig. 3 Mean ($\pm$ SD) of (a) C:N molar ratio of dry biomass (b) Mean of biochemical composition (relative abundance, % DW; normalized to 100%) of *H. pluvialis* biomass cultivated in recirculating aquaculture system (RAS) effluent water under exhaust air from the RAS trickling filter (RAS CO₂: 1900–2500 ppm) or room air (~400 ppm

CO₂). High light (200–400 μ mol photons m⁻²s⁻¹) was initiated on day 14 for 10 days to induce astaxanthin biosynthesis. The dashed vertical line separates the green growth and red stress phases. Different letters indicate statistically significant differences ($p < 0.05$) among treatments at each sampling point

The biochemical composition of *H. pluvialis* biomass (protein, carbohydrate, and lipid) shifted between the green phase and the red phase (Fig. 3b). Green phase biomass was mainly protein-rich (53–60%), with comparatively lower proportions of carbohydrate (31–35%) and lipid (9–12%). Red phase biomass showed a substantial redistribution of macromolecules, with carbohydrate (37–42%) and lipid (32–39%) becoming the major fractions, while protein decreased to 20–30% of dry weight (Fig. 3b). During the green phase, biochemical composition differed between the RAS CO₂ and room air treatments ($p = 0.005$; Table S9). Lipid proportion was higher under RAS CO₂ ($p = 0.002$; Table S9) and protein was higher under room air ($p = 0.001$; Table S9), while carbohydrate did not differ between treatments ($p = 0.56$; Table S9). In the red phase, biochemical composition also differed among the treatments (RAS CO₂, room-RAS CO₂, and room air; $p = 0.002$; Table S9). Lipid proportion was highest in RAS CO₂ and lowest in room air (pairwise comparisons: RAS CO₂ vs. room-RAS CO₂, $p = 0.002$; RAS-CO₂ vs. room air, $p < 0.001$; room-RAS CO₂ vs. room-air, $p = 0.005$). Protein showed the reverse trend, being lower in RAS CO₂ and room-RAS CO₂ compared to room air ($p < 0.001$ and $p = 0.003$, respectively). Carbohydrate proportion again showed no treatment effects ($p = 0.21$).

Effect of RAS CO₂ on astaxanthin accumulation

Astaxanthin increased over time during the red phase and differed among aeration treatments (RAS CO₂ vs. room air). Astaxanthin content increased steadily from 1.7–2.1 mg g⁻¹ on day 15 to 9.4–11.2 mg g⁻¹ on day 24 (rmANOVA within-subjects, main effect of time, $p < 0.001$; Fig. 4a; Table S10). Although the temporal pattern of increase was similar across

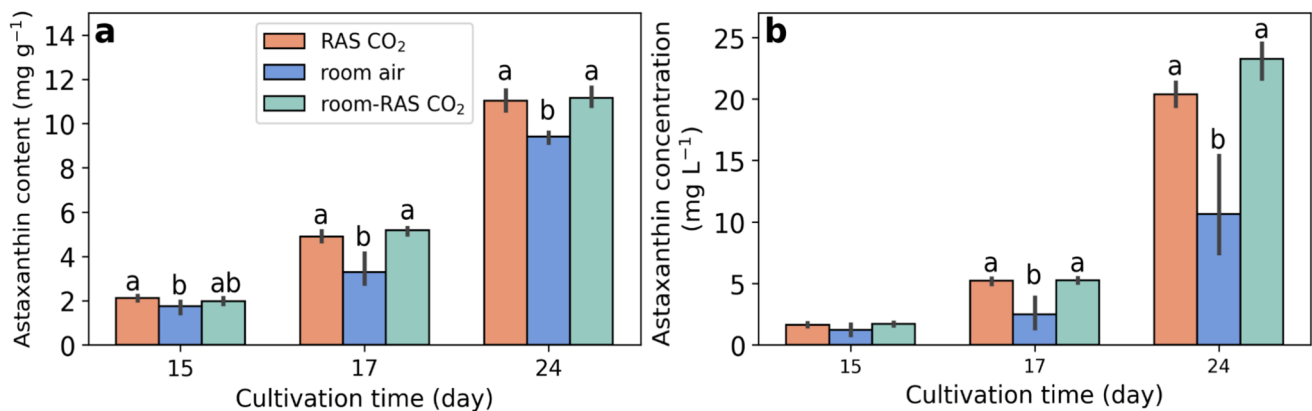


Fig. 4 Mean ($\pm$ SD) of (a) astaxanthin content (mg g^{-1}) and (b) astaxanthin concentration (mg L^{-1}) yielded by *H. pluvialis* cultivated in recirculating aquaculture system (RAS) effluent water under either exhaust air from the RAS trickling filter (RAS CO₂: 1900–2500 ppm)

treatments (i.e., no time-treatment interaction, $p = 0.05$; Table S10), overall astaxanthin content differed among treatments (rmANOVA between-subjects, $p < 0.001$; Fig. 4a; Table S10). Cultures supplied continuously with RAS CO₂ accumulated more astaxanthin than cultures remained under continuous room air (pairwise tests, $p < 0.001$). Cultures grown under room air during the green phase and switched to RAS CO₂ during the red phase (i.e., room-RAS CO₂) also accumulated higher astaxanthin than room-air cultures (pairwise tests, $p < 0.001$). No difference was found between the RAS CO₂ and room-RAS CO₂ treatments (Fig. 4a).

Astaxanthin volumetric concentration also showed a similar pattern, rising from 1.2–1.7 mg L^{-1} on day 15 to 10.6–23.3 mg L^{-1} on day 24 (rmANOVA within-subjects, main effect of time, $p < 0.001$; Fig. 4b; Table S10). In contrast to astaxanthin content, the rate of increase in volumetric concentration differed among treatments (i.e., significant time-treatment interaction, $p < 0.001$; Table S10). Concentration also differed among treatments (rmANOVA between-subjects, $p < 0.001$; Table S10). Both continuous RAS CO₂ and room-RAS CO₂ treatments had higher astaxanthin concentrations than continuous room air (pairwise tests, $p < 0.001$ for both). No difference in astaxanthin concentration was observed between RAS CO₂ and room-RAS CO₂ treatments (Fig. 4b).

Discussion

In this study, we investigated the potential of valorizing RAS side streams (both CO₂-rich exhaust air and nutrient-rich effluent water) through the cultivation of *H. pluvialis* for natural astaxanthin production. Conducting a 24-day experiment, we found that supplementing *H. pluvialis* cultures

or room air (~ 400 ppm CO₂). High light ($200\text{--}400 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) was initiated on day 14 for 10 days to induce astaxanthin biosynthesis. Different letters indicate statistically significant differences ($p < 0.05$) among treatments at each sampling point

with stripped CO₂ from RAS enhanced growth performance and astaxanthin yield compared with cultures aerated with ambient room air.

During the green growth phase, both room air (~ 400 ppm CO₂) and RAS exhaust CO₂ (1900–2500 ppm) supported active growth of *H. pluvialis* in RAS water. However, cultures aerated with RAS CO₂ consistently outperformed those supplied with room air, attaining a 15% higher growth rate, 61% greater cell productivity, and 76% higher dry weight. This response indicates that CO₂ enrichment with RAS exhaust air, despite being lower than the 1–5% CO₂ commonly used to enhance microalgal growth, can still substantially improve *H. pluvialis* growth rate and biomass accumulation (i.e., DW) relative to room air. The higher growth and biomass accumulation observed in the RAS CO₂ supplemented cultures were likely due to the alleviation of C limitation commonly associated with ambient room air aeration. This may have enhanced C fixation, reduced photorespiration, lowered the energy required for inorganic C acquisition, and promoted greater allocation of metabolic resources toward cell growth and biomass accumulation. In addition, RAS CO₂ supplementation helped to stabilize pH against photosynthetic alkalization, thereby maintaining more favorable conditions for vegetative growth. Chekanov et al. (2017) and Haque et al. (2017) investigated the effects of different CO₂ levels (0.04–20%) on *H. pluvialis* growth and found that growth and photosynthetic performance were highest at 5% CO₂, whereas higher concentrations ($\geq 10\%$) impaired growth due to medium acidification and physiological stress. In this study, RAS exhaust air had 0.19–0.25% CO₂, which is substantially lower than the concentration tested in those studies, yet still resulted in significant improvements in growth and biomass production. Importantly, the CO₂ levels used here are representative of

exhaust streams produced by degassing units in RAS and thus reflect realistic, scalable operating conditions. Unlike direct flue-gas injection or high-percentage pure CO₂ sparging, RAS exhaust air provides a continuous, low-cost CO₂ supply (up to 0.25%) substantially higher than that of room air for *H. pluvialis* cultivation.

The cell density and DW observed here in the growth phase under room air were comparable to our earlier results (Timilsina et al. 2025). The growth rate of *H. pluvialis* under RAS CO₂ (0.38 day⁻¹) was comparable to previously reported maximum values of up to 0.40 day⁻¹ (Aflalo et al. 2007), whereas the growth rate under room air (0.33 day⁻¹) was slightly lower. The increase in DW during the stress phase reflects stress-induced cell enlargement, cell wall thickening, and accumulation of carbon-rich structural and storage components during encystment, as cell size generally increases from 8–20 µm in the green growth phase to 20–50 µm in the red cyst phase (Triki et al. 1997; Shah et al. 2016). In the present study cultures supplied with RAS CO₂ either continuously or only during the red stress phase had similar final DW, and both exceeded that of cultures remained under room air throughout. This indicates that elevated CO₂ availability during stress likely promotes greater C incorporation into biomass, even when cell growth is limited or ceased (Guo et al. 2025). Comparable increases in DW (more than twofold) during the red phase have also been reported by Huang et al. (2019). This suggests that continued per-cell biomass accumulation during the encystment is a characteristic physiological response of *H. pluvialis* under stress conditions.

During the green growth phase, *H. pluvialis* efficiently removed nutrients (N and P) from RAS water. By the end of this phase, prior to high-light exposure, N was nearly depleted, with TN and NO₃-N removal reaching 99% under RAS CO₂ and 97% under room air. N removal in this study was higher than that reported previously for *H. pluvialis* cultivated in RAS water (Calderini et al. 2021; Timilsina et al. 2025), despite differences in initial N concentrations and photobioreactor configurations among studies. PO₄-P removal was 91% by the end of growth phase and increased to 98% by the end of the experiment, with no significant difference between RAS CO₂ and room air treatments (Tables 2, S11, S12). In microalgal systems, P removal can occur through multiple mechanisms, such as cellular uptake, surface adsorption, and chemical precipitation (Molitor et al. 2024). Despite its relatively slow growth rate, *H. pluvialis* achieved nutrient removal comparable to that of fast-growing microalgae such as *C. vulgaris* (Hiltunen et al. 2026), highlighting its potential for RAS water bioremediation.

Water quality parameters (pH, DIC, and DOC) also differed between aeration treatments at the end of the growth phase. Cultures supplemented with RAS CO₂ had lower pH and DIC, but higher DOC than those grown under room air

(Table 2). The lower pH under RAS CO₂ reflects enhanced carbonic acid (H₂CO₃) formation due to higher CO₂ dissolution in the culture medium (RAS water). Although CO₂ enrichment generally increases DIC availability, the slightly lower residual DIC under RAS CO₂ at the end of growth phase likely indicates higher inorganic carbon uptake by *H. pluvialis* compared with cultures supplied with room air. The higher DOC observed under RAS CO₂ likely reflects increased cellular exudation of organic compounds, which is commonly associated with elevated photosynthetic activity and biomass production under CO₂-enriched microalgal systems (Hulatt and Thomas 2010).

As expected, the transition of *H. pluvialis* from green growth phase to the red cyst phase had a strong shift in biomass composition, which was further modulated by aeration conditions. Biomass C:N ratios increased from 8–11 at the end of the green phase to 56–60 by the end of the red phase. This transition was accompanied by a shift from protein-rich biomass in the green phase to biomass enriched in lipids and carbohydrates in the red phase. These compositional changes represent the well-known survival strategy of *H. pluvialis* to N limitation combined with high light-induced oxidative stress, which promotes the degradation of N-rich cellular components and redirects metabolic flux toward C-based storage and protective compounds such as carbohydrates (starch), lipids (triacylglycerols), and carotenoids (mainly astaxanthin) (Boussiba 2000; Grünewald et al. 2001; Shah et al. 2016). Relative to room air, RAS CO₂ had higher C:N ratios and higher lipid contents in both phases, together with lower protein contents. Carbohydrate content increased during stress in all treatments, but differences among treatments were smaller than those observed for lipids, suggesting that elevated CO₂ primarily improved lipid synthesis rather than uniformly increasing all C pools. In *H. pluvialis*, the photosynthetic machinery continues even during the stress phase, but in a reduced and reorganized form (Scibilia et al. 2015; Mascia et al. 2017). Under N limitation, cell division and protein synthesis are arrested, yet residual photosynthetic activity allows continued C fixation. Because this fixed C cannot be incorporated into N-containing biomass, it is preferentially redirected toward C-rich storage pools. Accordingly, the higher lipid content and elevated C:N ratios observed under RAS CO₂ are consistent with increased inorganic C availability improving C allocation into neutral lipids and related storage compounds. Lipid accumulation is closely linked to astaxanthin synthesis because lipids provide the main storage matrix for esterified astaxanthin during encystment (Hu et al. 2021).

Astaxanthin content increased steadily during the 10-day high-light induction period, reaching 9.4–11.2 mg g⁻¹ dry weight. Cultures supplied with RAS CO₂ accumulated more astaxanthin than cultures under room air, both in terms of cellular content and volumetric concentration, although

carotenogenesis was consistent across treatments, with most cells in the aplanospores stage (Fig. S3). Particularly, *H. pluvialis* cultures grown under room air shifted to RAS CO₂ during the stress phase accumulated astaxanthin at levels comparable to cultures maintained under RAS CO₂ throughout both growth and stress phases. This result indicates that elevated CO₂ during the stress phase is sufficient to enhance astaxanthin accumulation, regardless of CO₂ conditions during the growth phase. Since N was almost depleted at the start of high-light exposure, N limitation combined with high-light stress was the main trigger for astaxanthin synthesis (Scibilia et al. 2015). Nevertheless, RAS CO₂ increased the extent of astaxanthin accumulation compared with room air, likely by alleviating inorganic C limitation and supporting continued C fixation during high-light induction. The relatively strong response of the room air-to-RAS CO₂ transfer condition may also be explained by its lower initial biomass density, which likely reduced self-shading and increased effective light exposure per cell during high-light induction (Li et al. 2020; Samhat et al. 2023).

Astaxanthin content of *H. pluvialis* grown under room air in this study was similar to previously reported values (Christian et al. 2018; Timilsina et al. 2025). However, the maximum astaxanthin content obtained (1.12% DW) was lower than the 2–5% DW reported in studies using optimized algal culture media under varying light intensities and CO₂ concentrations (Imamoglu et al. 2007; Christian et al. 2018; Cui et al. 2020; Chen et al. 2022). The astaxanthin yields observed in this study can be attributed to several factors related to cultivation conditions. First, RAS effluent water was used as the growth medium, which may be deficient in essential macro- and micronutrients. Elements such as magnesium (Mg²⁺) and iron (Fe³⁺) are known to influence *H. pluvialis* growth and astaxanthin synthesis (Fábregas et al. 1998; Kim et al. 2020; Zhu et al. 2021). Second, our experimental-size RAS emitted relatively low CO₂ concentrations (0.19–0.25%) compared with the 5–15% CO₂ levels used in other studies to enhance astaxanthin accumulation (Imamoglu et al. 2007; Christian et al. 2018). In addition, astaxanthin accumulation in *H. pluvialis* is known to be strain dependent. Allewaert et al. (2017) compared astaxanthin content among different *H. pluvialis* strains, including K-0084, and reported that this strain can accumulate up to ~3% DW when grown in synthetic culture media. Despite these differences, our results show that utilizing stripped CO₂ from RAS substantially increased astaxanthin production compared with room air, and that the use of RAS water can eliminate the need for synthetic culture media.

This study confirmed the potential of *H. pluvialis* for high-value astaxanthin production through RAS water bioremediation and CO₂ biofixation, providing a circular bioeconomy approach that transforms RAS liabilities into value-added bioproducts. The resulting astaxanthin-rich red-phase

biomass can be utilized as a sustainable resource for aqua-feed applications. In addition to astaxanthin, biomass may also contain other carotenoids, including canthaxanthin, β -carotene, adinorubin, adonixanthin, and echinenone (Shah et al. 2016), as well as lipid-rich compounds such as α -linolenic acid (omega-3) and linoleic acid (omega-6) (Azizi et al. 2024). These compounds may further enhance the nutritional and functional value of *H. pluvialis* biomass for aquaculture applications. Commercial RAS facilities typically operate at high rainbow trout stocking densities (50–100 kg m⁻³) (Roque d'Orbcastel et al. 2009), may produce much higher concentration of low-cost onsite CO₂ and discharge large volume of effluent water daily, providing substantial resources of nutrients and CO₂ that could be valorized for sustainable and low-cost astaxanthin production. Nonetheless, some limitations need to be considered. The experiment was carried out in 1.5 L photobioreactors, and the translation of these findings to industrial applications remains to be examined. Larger pilot- or commercial-scale cultivation trials will be needed to test the full feasibility of this approach. The batch cultivation technique used in this study does not fully reflect the continuous nature of RAS operation, and cultivation approaches such as continuous or semi-continuous systems may be more appropriate. Moreover, in this study, effluent water was collected from the pilot-scale RAS because the lab-scale RAS used for stripped CO₂ had insufficient NO₃-N (~16 mg L⁻¹) and PO₄-P (~0.8 mg L⁻¹) to support adequate *H. pluvialis* growth. Future studies could use both CO₂ and effluent water from the same pilot- or commercial-scale RAS to further develop integrated RAS–*H. pluvialis* systems for sustainable astaxanthin production.

The composition and stability of RAS exhaust air are important considerations for future integration of *H. pluvialis* cultivation with RAS. In this study, RAS CO₂ concentrations were relatively stable (1900–2500 ppm); however, under commercial operations, they are expected to fluctuate with changes in fish biomass, respiration rate, feed input, stocking density, and degassing efficiency. Such variability could affect the consistency of *H. pluvialis* growth and potentially compromise biomass production and astaxanthin biosynthesis if carbon supply becomes limiting. To maintain stable cultivation conditions, CO₂ monitoring and regulation strategies (e.g., blending RAS CO₂ with ambient air at adjustable ratios or using automated sensor-based flow controllers) may be required. Along with elevated CO₂, RAS exhaust air may also contain trace volatile compounds such as NH₃, H₂S, and N₂O originating from fish metabolism and microbial processes, and their potential effects on *H. pluvialis* physiology warrant further investigation.

Future RAS–microalgae integrated systems can consider strategies to improve CO₂ utilization, as microalgae are unlikely to utilize all supplied CO₂ because CO₂ capture

efficiency is significantly influenced by photobioreactor design, gas–liquid mass transfer, bubble retention time, aeration strategy, light distribution, and nutrient availability. Potential strategies to improve CO₂ utilization include increasing gas–liquid contact time (longer tubular or airlift photobioreactors), reducing bubble size (fine-pore spargers), and optimizing aeration and mixing conditions. In addition, unutilized CO₂ from the microalgal photobioreactor outlet could be directed to a secondary microalgal cultivation unit or other photosynthetic production systems, such as greenhouses or plant-factory systems (Sucunthowong et al. 2023). The phased CO₂ (room-RAS CO₂) strategy may be advantageous because continuous elevated CO₂ supplementation throughout cultivation may not be necessary to enhance astaxanthin biosynthesis. When RAS exhaust CO₂ availability is limited, *H. pluvialis* could be grown under ambient room air during the green growth phase and shifted to RAS exhaust CO₂ during the red astaxanthin induction phase.

Conclusions

This study successfully demonstrated the feasibility of valorizing RAS side streams (both nutrient-rich effluent water and CO₂-rich exhaust air) through the cultivation of *H. pluvialis* for high-value astaxanthin production. More than 95% of total N, NO₃-N and PO₄-P removal from RAS effluent water, as well as astaxanthin yields of 1.12% of dry weight were achieved. Importantly, the use of RAS exhaust CO₂ significantly improved growth, biomass production, and astaxanthin yield compared with ambient room air. As aquaculture sustainability is a global concern, integrating *H. pluvialis* with RAS may provide a pathway toward a circular bioeconomy. Nevertheless, the scalability and industrial applicability of this approach remain to be thoroughly investigated.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-026-03940-7>.

Acknowledgements The authors gratefully acknowledge financial support from the European Union – NextGenerationEU instrument through the Research Council of Finland under (grant 352765 to Katja Pulkkinen and grant 352764 to Pauliina Salmi). Thanks to lab technicians Ahti Karusalmi, Juha Ahonen, Nina Honkanen, and Emma Pajunen for their technical support during the experiments.

Author contributions Hemanta Timilsina: Writing – original draft, Writing – review & editing, Visualization, Investigation, Methodology, Formal analysis, Conceptualization. Jussi S. Vesamäki: Writing – review & editing, Investigation, Methodology, Conceptualization. Marco L. Calderini: Writing – review & editing, Visualization, Investigation, Methodology, Conceptualization. Minna Hiltunen: Writing – review & editing, Supervision, Investigation, Conceptualization. Juhani Pirhonen: Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. Katja Pulkkinen: Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Funding Open Access funding provided by University of Jyväskylä (JYU). The European Union – NextGenerationEU instrument through the Research Council of Finland under (grant 352765 to Katja Pulkkinen and grant 352764 to Pauliina Salmi).

Data availability The datasets supporting the findings of this study are publicly available in Zenodo at: <https://doi.org/10.5281/zenodo.20411928>.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Aflalo C, Meshulam Y, Zarka A, Boussiba S (2007) On the relative efficiency of two- vs. one-stage production of astaxanthin by the green alga *Haematococcus pluvialis*. *Biotechnol Bioeng* 98:300–305
- Ahmed N, Turchini GM (2021) Recirculating aquaculture systems (RAS): environmental solution and climate change adaptation. *J Clean Prod* 297:126604
- Allewaert CC, Vanormelingen P, Daveloose I, Verstraete T, Vyverman W (2017) Intraspecific trait variation affecting astaxanthin productivity in two *Haematococcus* (Chlorophyceae) species. *Algal Res* 21:191–202
- Arbour AJ, Bhatt P, Simsek H, Brown PB, Huang J-Y (2024) Life cycle assessment on environmental feasibility of microalgae-based wastewater treatment for shrimp recirculating aquaculture systems. *Bioresour Technol* 399:130578
- Aslam SN, Navada S, Bye GR, Mota VC, Terjesen BF (2019) Effect of CO₂ on elemental concentrations in recirculating aquaculture system tanks. *Aquac* 511:734254
- Azizi M, Motesafi H, Hashemi M (2024) Novel paradigm of light and CO₂ feeding to improve the co-production of astaxanthin and polyunsaturated fatty acids in *Haematococcus pluvialis*. *J CO2 Util* 84:102855
- Behnam BM, Mahmoud H, Shafigh S, Sadegh S, Esmaeil PK (2025) The red gold of aquaculture: The dual role of astaxanthin in aquatic nutrition and human health. *Aquac Res* 2025:7000490
- Ben-Asher R, Zohar Y, Stromberg M, Lahav O (2024) A new approach for eliminating off-flavors from RAS fish, as part of the normal grow-out period. *Water Res* 249:121015. <https://doi.org/10.1016/j.watres.2023.121015>
- Bjørklund G, Gasmi A, Lenchyk L, Shanida M, Zafar S, Mujawdiya PK, Lysiuk R, Antonyak H, Noor S, Akram M, Smetanina K, Piscopo S, Upry T, Peana M (2022) The role of astaxanthin as

- a nutraceutical in health and age-related conditions. *Molecules* 27:7167
- Blancheton J (2007) Intensification of landbased aquaculture production in single pass and reuse systems. In: Bergheim A (ed) *Aquacultural engineering and environment*. Research Signpost, Poniac, USA, pp 21–47
- Böpple H, Slegers PM, Breuhaus P, Kleinegris DMM (2025) Comparing continuous and perfusion cultivation of microalgae on recirculating aquaculture system effluent water. *Bioresour Technol* 418:131881
- Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. *Physiol Plant* 108:111–117
- Boussiba S, Bing W, Yuan J-P, Zarka A, Chen F (1999) Changes in pigments profile in the green alga *Haematococcus pluvialis* exposed to environmental stresses. *Biotechnol Lett* 21:601–604
- Boussiba S, Vonshak A (1991) Astaxanthin accumulation in the green alga *Haematococcus pluvialis*. *Plant Cell Physiol* 32:1077–1082
- Bregnballe J (2022) A guide to recirculation aquaculture. An introduction to the new environmentally friendly and highly productive closed fish farming systems. Food and Agriculture organization of the United Nations, Rome
- Calderini ML, Pääkkönen S, Yli-Tuomola A, Timilsina H, Pulkkinen K, Pölonen I, Salmi P (2025) Accurate non-invasive quantification of astaxanthin content using hyperspectral images and machine learning. *Algal Res* 103979
- Calderini ML, Stevčić Č, Taipale S, Pulkkinen K (2021) Filtration of Nordic recirculating aquaculture system wastewater: effects on microalgal growth, nutrient removal, and nutritional value. *Algal Res* 60:102486
- Capelli B, Bagchi D, Cysewski GR (2013) Synthetic astaxanthin is significantly inferior to algal-based astaxanthin as an antioxidant and may not be suitable as a human nutraceutical supplement. *Nutraceuticals* 12:145–152. <https://doi.org/10.1007/s13749-013-0051-5>
- Chekanov K, Schastnaya E, Solovchenko A, Lobakova E (2017) Effects of CO₂ enrichment on primary photochemistry, growth and astaxanthin accumulation in the chlorophyte *Haematococcus pluvialis*. *J Photochem Photobiol B* 171:58–66
- Chen F, Xu N, Liu K, Lv R, Shi J, Liu J, Sun X, Hu C (2022) Increasing production and bio-accessibility of natural astaxanthin in *Haematococcus pluvialis* by screening and culturing red motile cells under high light condition. *Bioresour Technol* 364:128067
- Chiam C, Sarbatly R (2011) Purification of aquacultural water: conventional and new membrane-based techniques. *Sep Purif Rev* 40:126–160
- Christian D, Zhang J, Sawdon AJ, Peng C-A (2018) Enhanced astaxanthin accumulation in *Haematococcus pluvialis* using high carbon dioxide concentration and light illumination. *Bioresour Technol* 256:548–551
- Cui J, Yu C, Zhong D, Zhao Y, Yu X (2020) Melatonin and calcium act synergistically to enhance the coproduction of astaxanthin and lipids in *Haematococcus pluvialis* under nitrogen deficiency and high light conditions. *Bioresour Technol* 305:123069
- Dutta S, Kumar SPJ, Banerjee R (2023) A comprehensive review on astaxanthin sources, structure, biochemistry and applications in the cosmetic industry. *Algal Res* 74:103168
- Ende S, Henjes J, Spiller M, Elshobary M, Hanelt D, Abomohra A (2024) Recent advances in recirculating aquaculture systems and role of microalgae to close system loop. *Bioresour Technol* 407:131107
- European Commission (2020a) Recirculation aquaculture systems. European Market Observatory for Fisheries and Aquaculture Products (EUMOFA)
- European Commission (2020b) Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions: A farm to fork strategy for a fair, healthy and environmentally-friendly food system COM (2020) 381 final. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:52020DC0381>
- European Commission (2021) Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions: Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030 COM(2021) 236 final. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:52021DC0236>
- Fábregas J, Domínguez A, Álvarez DG, Lamela T, Otero A (1998) Induction of astaxanthin accumulation by nitrogen and magnesium deficiencies in *Haematococcus pluvialis*. *Biotechnol Lett* 20:623–626
- FAO (2024) The state of world fisheries and aquaculture 2024. Blue transformation in action. FAO, Rome
- Grünwald K, Hirschberg J, Hagen C (2001) Ketocarotenoid biosynthesis outside of plastids in the unicellular green alga *Haematococcus pluvialis*. *J Biol Chem* 276:6023–6029
- Guillard RRL, Lorenzen CJ (1972) Yellow-green algae with chlorophyllide c. *J Phycol* 8:10–14
- Guo S, Li F, Wang J, Zhou H, Yuan Z, Yang R, Ke H, Chen H, Wang C, Cai M (2025) Two-stage carbon sequestration by *Haematococcus pluvialis*: integrated research from small-scale to pilot-scale cultivation and data quality monitoring. *Bioresour Technol* 416:131828
- Haque F, Dutta A, Thimmanagari M, Chiang YW (2017) Integrated *Haematococcus pluvialis* biomass production and nutrient removal using bioethanol plant waste effluent. *Process Saf Environ Prot* 111:128–137
- Hiltunen M, Kannangara R, Nakandalage B, Söderena O, Timilsina H, Pirhonen J, Pulkkinen K (2026) Growth and biomass composition of *Chlorella vulgaris* using nutrient-rich water and CO₂ from a recirculating aquaculture system. *Aquaculture* 610:742956
- Hu C, Cui D, Sun X, Shi J, Song L, Li Y, Xu N (2019) Transcriptomic analysis unveils survival strategies of autotrophic *Haematococcus pluvialis* against high light stress. *Aquaculture* 513:734430
- Hu C, Cui D, Sun X, Shi J, Xu N (2020) Primary metabolism is associated with the astaxanthin biosynthesis in the green algae *Haematococcus pluvialis* under light stress. *Algal Res* 46:101768
- Hu Q, Song M, Huang D, Hu Z, Wu Y, Wang C (2021) *Haematococcus pluvialis* accumulated lipid and astaxanthin in a moderate and sustainable way by the self-protection mechanism of salicylic acid under sodium acetate stress. *Front Plant Sci*
- Huang L, Gao B, Wu M, Wang F, Zhang C (2019) Comparative transcriptome analysis of a long-time span two-step culture process reveals a potential mechanism for astaxanthin and biomass hyperaccumulation in *Haematococcus pluvialis* JNU35. *Biotechnol Biofuels* 12:18
- Hulatt CJ, Thomas DN (2010) Dissolved organic matter (DOM) in microalgal photobioreactors: a potential loss in solar energy conversion? *Bioresour Technol* 101:8690–8697
- Imamoglu E, Vardar Sukan F, Conk Dalay M (2007) Effect of different culture media and light intensities on growth of *Haematococcus pluvialis*. *Int J Nat Eng Sci* 1:5–9
- Kim Y-E, Matter IA, Lee N, Jung M, Lee Y-C, Choi S-A, Lee SY, Kim JR, Oh Y-K (2020) Enhancement of astaxanthin production by *Haematococcus pluvialis* using magnesium aminoclay nanoparticles. *Bioresour Technol* 307:123270
- Li F, Cai M, Lin M, Huang X, Wang J, Ke H, Wang C, Zheng X, Chen D, Yang S, Li F, Cai M, Lin M, Huang X, Wang J, Ke H, Wang C, Zheng X, Chen D, Yang S (2020) Enhanced biomass and astaxanthin production of *Haematococcus pluvialis* by a cell transformation strategy with optimized initial biomass density. *Mar Drugs* 18:341
- Lindholm-Lehto PC, Vielma J (2019) Controlling of geosmin and 2-methylisoborneol induced off-flavours in recirculating

- aquaculture system farmed fish—A review. *Aquac Res* 50:9–28. <https://doi.org/10.1111/are.13881>
- Lu Q, Li H, Zou Y, Liu H, Yang L (2021) Astaxanthin as a microalgal metabolite for aquaculture: a review on the synthetic mechanisms, production techniques, and practical application. *Algal Res* 54:102178
- Mascia F, Girolomoni L, Alcocer MJP, Bargigia I, Perozeni F, Cazaniga S, Cerullo G, D'Andrea C, Ballottari M (2017) Functional analysis of photosynthetic pigment binding complexes in the green alga *Haematococcus pluvialis* reveals distribution of astaxanthin in photosystems. *Sci Rep* 7:16319
- Molitor HR, Kim G-Y, Hartnett E, Gincley B, Alam MM, Feng J, Avila NM, Fisher A, Hodaie M, Li Y, McGraw K, Cusick RD, Bradley IM, Pinto AJ, Guest JS (2024) Intensive microalgal cultivation and tertiary phosphorus recovery from wastewaters via the EcoRecover process. *Environ Sci Technol* 58:8803–8814
- Moran D (2010) Carbon dioxide degassing in fresh and saline water. I: Degassing performance of a cascade column. *Aquac Eng* 43:29–36
- Mota VC, Nilsen TO, Gerwins J, Gallo M, Ytteborg E, Baeverfjord G, Kolarevic J, Summerfelt ST, Terjesen BF (2019) The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (*Salmo salar*) in recirculating aquaculture systems. *Aquaculture* 498:578–586
- Piedrahita RH (2003) Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture* 218:35–44
- Pirhonen J, Koukka S, Pulkkinen K (2023) Supplementing air with CO₂ stripped from recirculating aquaculture improves growth of two green microalgae in aquaculture wastewater. *Aquac Eng* 257:739242
- Pulkkinen JT, Ronkanen A-K, Pasanen A, Kiani S, Kiuru T, Koskela J, Lindholm-Lehto P, Lindroos A-J, Muniruzzaman M, Solismaa L, Klöve B, Vielma J (2021) Start-up of a “zero-discharge” recirculating aquaculture system using woodchip denitrification, constructed wetland, and sand infiltration. *Aquac Eng* 93:102161
- Roque d'Orbcastel E, Person-Le Ruyet J, Le Bayon N, Blancheton J-P (2009) Comparative growth and welfare in rainbow trout reared in recirculating and flow through rearing systems. *Aquac Eng* 40:79–86
- Samhat K, Kazbar A, Takache H, Ismail A, Pruvost J (2023) Influence of light absorption rate on the astaxanthin production by the microalga *Haematococcus pluvialis* during nitrogen starvation. *Bioresour Bioprocess* 10:78
- Scibilia L, Girolomoni L, Berteotti S, Alboresi A, Ballottari M (2015) Photosynthetic response to nitrogen starvation and high light in *Haematococcus pluvialis*. *Algal Res* 12:170–181
- Shah MMR, Liang Y, Cheng JJ, Daroch M (2016) Astaxanthin-producing green microalga *Haematococcus pluvialis*: from single cell to high value commercial products. *Front Plant Sci*
- Silkina A, Ginnever NE, Fernandes F, Fuentes-Grünwald C (2019) Large-scale waste bio-remediation using microalgae cultivation as a platform. *Energies* 12:2772
- Stehfest K, Toepel J, Wilhelm C (2005) The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. *Plant Physiol Biochem* 43:717–726
- Sucunthowong K, Lee JH, Powtongsook S, Nootong K (2023) Simultaneous utilization of CO₂ and nitrate wastes from compact recirculating aquaculture system for improving algal biomass (*Scenedesmus armatus*) production. *Algal Res* 74:103224, 103224
- Timilsina H, Hiltunen M, Calderini ML, Salmi P, Pirhonen J, Pulkkinen K (2025) Flow-through and cyclic fed-batch cultivation of the green microalga *Haematococcus pluvialis* in recirculating aquaculture effluent: strategies for sustained biomass production and astaxanthin biosynthesis. *Aquac Eng* 111:102573
- Timmons MB, Vinci BJ (2022) Recirculating aquaculture, 5th ed. Cayuga Aqua Ventures, Ithaca, New York
- Triki A, Maillard P, Gudin C (1997) Gametogenesis in *Haematococcus pluvialis* Flotow (Volvocales, Chlorophyta). *Phycologia* 36:190–194
- Tuan Harith Z, Mohd Sukri S, Remlee NFS, Mohd Sabir FN, Zakaria NNA (2024) Effects of dietary astaxanthin enrichment on enhancing the colour and growth of red tilapia, *Oreochromis* sp. *Aquac Fish Sci* 9:52–56
- United Nations (2015) Transforming our world: The 2030 agenda for sustainable development. UN, New York
- van Bussel CGJ, Mahlmann L, Kroeckel S, Schroeder JP, Schulz C (2013) The effect of high ortho-phosphate water levels on growth, feed intake, nutrient utilization and health status of juvenile turbot (*Psetta maxima*) reared in intensive recirculating aquaculture systems (RAS). *Aquac Eng* 57:63–70
- van Rijn J (2013) Waste treatment in recirculating aquaculture systems. *Aquac Eng* 53:49–56
- Vesamäki JS, Laine MB, Nissinen R, Taipale SJ (2024) Plastic and terrestrial organic matter degradation by the humic lake microbiome continues throughout the seasons. *Environ Microbiol Rep* 16:e13302
- Vesamäki JS, Timilsina H, Hiltunen M, Pirhonen J, Pulkkinen K (2026) Co-cultivation of mixotrophic non-green algae with the green alga *Haematococcus pluvialis* in aquaculture effluent. *J Appl Phycol* 38:161–174
- Villarro S, Ciardi M, Morillas-España A, Sánchez-Zurano A, Acien-Fernández G, Lafarga T (2021) Microalgae derived astaxanthin: Research and consumer trends and industrial use as food. *Foods* 10:2303. <https://doi.org/10.3390/foods10102303>
- Wagner H, Liu Z, Langner U, Stehfest K, Wilhelm C (2010) The use of FTIR spectroscopy to assess quantitative changes in the biochemical composition of microalgae. *J Biophotonics* 3:557–566
- World Bank (2013) Fish to 2030: Prospects for fisheries and aquaculture (English). World Bank Group, Washington DC, USA
- Zhu Y, Zhang Z, Xu X, Cheng J, Chen S, Tian J, Yang W, Crocker M (2021) Simultaneous promotion of photosynthesis and astaxanthin accumulation during two stages of *Haematococcus pluvialis* with ammonium ferric citrate. *Sci Total Environ* 750:141689

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.