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Photosynthesis and circadian rhythms regulate the buoyancy of marimo lake balls

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Marimo are unusual, attractive and endangered spherical aggregations of the filamentous green macroalga *Aegagropila linnaei* (Figure 1A–E) [1]. Globally rare, marimo populations persist in cold freshwater lakes in Japan, Iceland and Ukraine. Marimo occupy both the lake bed and rise to the lake surface [2,3]. Here, we show that marimo buoyancy is conferred by bubbles arising from photosynthesis. We find that light-induced acquisition of buoyancy by marimo is circadian-regulated. We identify that there are circadian rhythms of photosynthesis in marimo, which might explain the circadian rhythm of buoyancy in response to light. This identifies a circadian-regulated buoyancy response in an intriguing and little-studied plant.

Marimo balls occupy the lake bed and also rise to the surface [2,3]. These movements occur under laboratory conditions, with marimo rising rapidly after acquiring buoyancy (Figure 1A,B). Bubbles form on the surface of buoyant marimo (Figure 1C) [2]. We reasoned that the bubbles might be products of photosynthesis and confer buoyancy, because marimo sink in darkness (Figure 1B) [2]. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which inhibits photosynthetic electron transport from photosystem II (PSII) to plastoquinone, prevented surface bubble formation and marimo buoyancy (Figure 1C–E). DCMU-incubated marimo did not float during 48 h of continuous illumination (Figure 1F). Therefore, the dynamic day–night buoyancy of marimo is due to photosynthesis.

Given that photosynthesis in plants and algae is controlled by the circadian clock [4], we asked whether light-induced acquisition of buoyancy

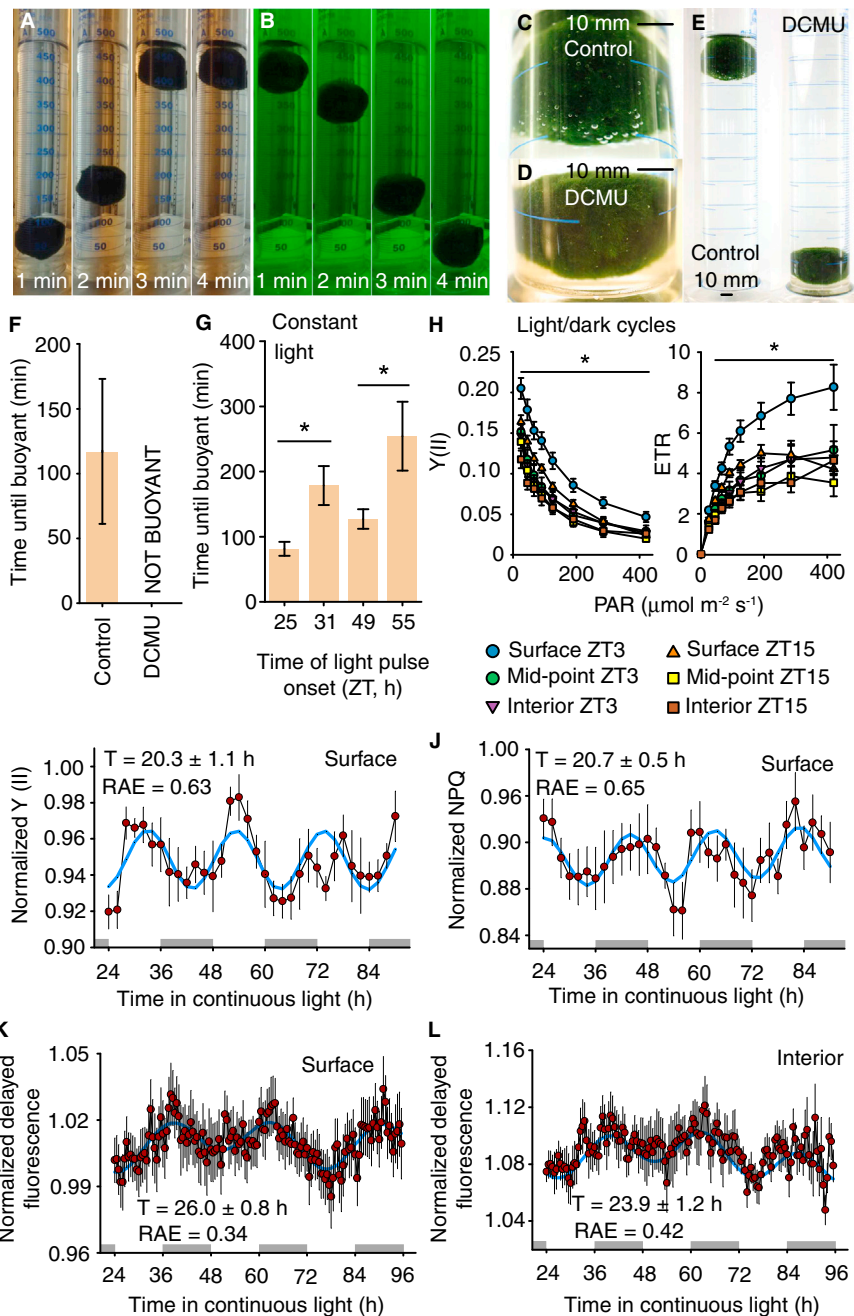


Figure 1. Photosynthesis, circadian rhythms and marimo buoyancy.

(A,B) Marimo position within a water column changes rapidly and is bimodal, with balls (A) buoyant in light and (B) sunken in physiological darkness (time in minutes). (C–E) 20 μM DCMU prevents buoyancy and surface bubble formation. (F) DCMU-treated marimo do not rise within the water column ($n=3$). (G) Circadian regulation of the acquisition of buoyancy in response to illumination ($n=4$). (H) Quantum yield of PSII (Y(II)) and electron transport rate (ETR) within rapid light curves (RLCs) from three positions within the marimo ball at ZT3 (daytime) and ZT15 (night), under 12 h light/12 h dark cycles ($n=10$). The marimo exterior during the day differs significantly from the other measures. (I,J) Circadian oscillations in two photosynthetic parameters on the marimo surface. Data are from a timecourse of RLCs, with traces shown for the signal under $33 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n=5$). (K,L) Circadian rhythms of delayed chlorophyll fluorescence at the marimo surface and interior ($n=3$). (I–L) Red circles represent data and blue lines represent FFT-NLLS waveform fit to data, and grey boxes on x-axes indicate subjective dark period. Asterisks indicate statistically significant differences ($p<0.05$) using (G) Student's t-test and (H) one-way ANOVA with Student-Neuman-Keuls post-hoc analysis. Data are mean \pm s.e.m.



in marimo is also under circadian control. We tested whether the light-induced acquisition of buoyancy by marimo (Figure 1A,B) is circadian regulated. Marimo were entrained to 12 h light/12 h dark cycles and then transferred to continuous dim red light to maintain circadian rhythmicity. When these marimo were exposed to buoyancy-inducing light intensities ($20 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density) the marimo became buoyant more rapidly at the start of the subjective day than halfway through the subjective day, during both the second and third days of constant dim light (Figure 1G). This indicates that there is circadian regulation of light-induced buoyancy in marimo. Under light/dark cycles, marimo position within the water column oscillated (Figure S1F in Supplemental Information, published with this article online) whereas marimo remained buoyant under constant light conditions (Figure S1G). Persistent marimo buoyancy under constant light is expected because photosynthesis always occurs under constant light, even when circadian oscillations are present [4].

We identified day–night fluctuations in photosynthetic rates at the marimo surface. Under light/dark cycles, we measured photosynthesis at the marimo surface and interior using a chlorophyll fluorescence instrument with a narrow fibre optic probe. The quantum yield of photosystem II (Y(II)) and electron transport rate (ETR) was significantly greater at the marimo surface during the light period than dark period, under light-limited and light-saturated conditions (Figure 1H). This day–night fluctuation was absent from the marimo interior (Figure 1H). Interior day-time and night-time Y(II) and ETR was comparable to the marimo exterior during the dark period (Figure 1H). Therefore, there are anatomically dependent day–night fluctuations in the photosynthetic rate of marimo.

Next, we identified low amplitude circadian rhythms of photosynthesis in marimo. Marimo were entrained to 12 h light/12 h dark cycles, and intact marimo and dissected marimo interiors then transferred to constant light conditions. Under constant light, rapid photosynthetic light response curves (RLCs) were obtained every 2 h using chlorophyll fluorescence imaging

(Figure S1H). To our knowledge, this is the first time the RLCs have been used to study circadian rhythms in plants. Circadian oscillations in Y(II) (essentially the proportion of light entering photochemistry) and non-photochemical quenching (NPQ, thermal dissipation of energy) occurred within a subset of areas of interest analyzed, at many light intensities within the RLCs (Table S1A; Figure 1I,J). More areas of interest were rhythmic at the marimo surface under lower light intensities, whereas this trend was absent from the marimo interior (Table S1). For example, under $33 \mu\text{mol m}^{-2} \text{s}^{-1}$ light the marimo surface was clearly rhythmic whereas the interior was arrhythmic (Figures 1I,J and S1I,J). Circadian period estimates obtained using fast Fourier transform–non linear least squares method (FFT–NLLS) ranged from 20–28 h, depending on light intensity and marimo anatomy (Figures 1I,J and S1I,J). The relative amplitude error (RAE) quantifies the rhythmic robustness of the oscillation, with low RAE indicating a better FFT–NLLS fit to data. Across the light intensities within the RLC timecourses, RAE was in the range of 0.6–0.97 for marimo exterior and 0.45–0.98 for marimo interior (Table S1). This exceeds the RAE of timecourses of promoter–luciferase reporters and chlorophyll fluorescence analysis of *Arabidopsis* (RAE=0.3–0.35 [5]), and is somewhat greater than RAE from chlorophyll fluorescence analysis of barley (RAE=0.2–0.6; some barley varieties 0.4–0.9 [6]). The RAE from marimo might indicate lower amplitude circadian oscillations, and potentially explains the variable phase estimates (Table S1). The mathematically distinct maximum entropy spectral analysis (MESA) also identified circadian rhythms in these photosynthetic parameters (Table S1). The slope of the straight-line portion of the RLC did not oscillate significantly under constant light (Figure S1K). Circadian rhythms were also detected using the delayed chlorophyll fluorescence (DF) method [7] (Figure 1K,L). DF oscillations likely derive from rhythms of charge recombination during photosynthetic electron transport [7]. Together, this identifies low amplitude circadian rhythms of PSII activity in marimo.

Marimo buoyancy might maximise photosynthetic light capture because

light penetration decreases with depth. Buoyancy alterations might also rearrange balls within marimo accumulations and underlie seasonal changes in marimo position [3]. There are daily changes in the water column position of jellyfish harbouring endosymbiotic algae [8], and daily valve opening by giant clams exposes endosymbiotic algae to sunlight [9]. Similarly, higher plant organs undergo diel and circadian changes in position [10]. Therefore, circadian and diel movements might optimize photosynthesis in several kingdoms of aquatic and terrestrial organisms.

SUPPLEMENTAL INFORMATION

Supplemental Information contains one figure, one table, and experimental procedures, and can be found with this article online at <https://doi.org/10.1016/j.cub.2018.07.027>.

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