BRIEF COMMUNICATION

Photophysiological response of Symbiodiniaceae single cells to temperature stress

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Ocean warming can disrupt the symbiosis between corals and photosynthetic dinoflagellates in the family Symbiodiniaceae, a process referred to as bleaching [1]. Corals can recover from bleaching by repopulating more stress-tolerant symbiont cells [2] and/or recruiting new cells from the environment [3, 4]. The bleaching susceptibility of corals is thus partly linked to the thermal sensitivity of Symbiodiniaceans and many studies have experimentally tested lineage-specific thermal tolerances of coral symbionts [5–8]. However, the considerable heterogeneity between Symbiodiniaceans remains underexplored [9], despite strong indications that the phenotypic richness among symbiont lineages is useful for mitigating bleaching effects [4, 10–13].

Here we studied the effect of temperature stress on Symbiodiniaceae single cells via microwebs, microscale temperature control and pulse amplitude-modulated chlorophyll fluorometry (PAM) imaging. Specifically, we asked: (i) how heterogeneous are single-cells of Symbiodiniaceae under temperature stress and, (ii) does the initial photophysiology of a cell predict its thermal tolerance?

To address these questions, we selected five representative species from three Symbiodiniaceae lineages: *Effrenium* sp. (‘S1’, clade E, non-symbiotic), *Symbiodinium* sp. (‘S2’, ‘S3’ and ‘S4’, clade A, all symbiotic), and *Fugacium* sp. (‘S5’, clade F, symbiotic). The identity of all cell cultures was determined by phylogenetic analysis (Table S1 and Fig. S1) and their growth was inspected via absorbance measurements (Fig. S2). Following this characterization, single cells were loaded into individual microwebs within a microfluidic device that was attached to a miniaturized temperature regulation system [14] and a PAM microscope (Fig. 1a–c). Together, this setup enabled us to measure single-cell maximum quantum yields of PSII, i.e., $F_{v}/F_{m}$, under user-defined temperatures. As certain representative species (i.e., *Effrenium* sp. ‘S1’, *Fugacium* sp. ‘S5’) exhibited significantly reduced $F_{v}/F_{m}$ during stationary phase (Fig. S3), all experiments were conducted using exponentially growing cells.

To understand the photophysiological heterogeneity among Symbiodiniaceae under temperature stress, we repeatedly measured single-cell $F_{v}/F_{m}$ under stepwise increasing temperatures (22–39 °C, +1 °C every 15 min). This temperature range does not represent an environmentally realistic scenario but was chosen according to known growth conditions for coral symbionts [15] and in order to probe their thermal tolerance in a rapid assay (akin to [8], but using single cells). At cultivation temperature (22 °C), all five species demonstrated average $F_{v}/F_{m}$ ranging between 0.38–0.53; however, under increasing temperatures, these values gradually declined in a lineage-specific manner (Fig. 1d, e). A significant ($p < 0.05$, one-way ANOVA with post hoc Tukey test) decline in average $F_{v}/F_{m}$ started to occur at 28 °C (‘S2’, ‘S3’, and ‘S4’), 31 °C (‘S5’), and 34°C (‘S1’) compared to their respective $F_{v}/F_{m}$ obtained at 22 °C (Table S2).

As stepwise increasing temperature exposes cells to aggregate stress, we calculated the cumulative thermal dose [14] (D) with a...
unit of °Ch via:

\[ D = \int_{t_0}^{t_s} (T(t) - T_0) \, dt \]

where \( t_0 \) represents the starting time in hours of the temperature profile, \( t_s \) represents the investigated time point in hours, \( T(t) \) and \( T_0 \) (°C) represent the temperature at \( t_s \) and \( t_0 \), respectively. By plotting the percentage of cells with inactive PSII as a function of \( D \) and fitting a dose-response curve (Fig. 1f), we determined the half-maximal effective distress dose (\( D_{50\%} \)) and the corresponding temperature where \( D_{50\%} \) occurred. This revealed \( D_{50\%} \) values of 140 °Ch for Effrenium sp. ‘S1’ (occurring at 37 °C), 126 °Ch, 121 °C, and 116 °Ch for Symbiodinium sp. ‘S2’, ‘S3’, and ‘S4’ (at 36 °C, 35 °C, and 35 °C, respectively), and 103 °C for Fugacium sp. ‘S5’ (at 33 ° C). Single cells of Symbiodiniaceae thus respond differently to cumulative short-term temperature stress and certain species (e.g., Effrenium sp.) appear more temperature resilient than others (e.g., Fugacium sp.). These findings are broadly consistent with earlier studies on coral symbionts, where \( F_v/F_m \) typically decreased above 31 °C [16–18].

To describe the phenotypic heterogeneity among Symbiodiniaceae single cells under temperature stress, we calculated a...
measure of photophysiological heterogeneity, $H$ by [19]:

$$H = \frac{\text{std}(F_v/F_m)}{F_v/F_m}$$

where $\bar{F_v/F_m}$ represents the average $F_v/F_m$ at a specific temperature and std$(F_v/F_m)$ represents the corresponding standard deviation. All species demonstrated increasing $H$ values under elevated temperatures, but significantly higher $H$ after...
exceeding the temperature where \( D_{50\%} \) occurred (Fig. 2a, b). Maximal \( H \) values were 2.46 (‘S1’), 16.3 (‘S2’), 8.3 (‘S3’), 12.4 (‘S4’), and 12.8 (‘S5’) (Table S3 for all \( H \) values). Applying a thermal dose above \( D_{50\%} \) thus increases the photophysiological heterogeneity among single cells. Notably, mid-exponentially growing cells exhibited lower \( H \) values compared to cells grown at stationary phase (Table S3), which suggests that the single cell heterogeneity also increases with culture age or, alternatively, due to other factors that change with time (e.g., microenvironments). In other microorganisms, single cell heterogeneity occurs due to stochastic gene expression or molecular-level ‘noise’ which increases the probability of specific phenotypes to persist in challenging environments [20]. Similar mechanisms could be in play for coral symbionts and our platform, in combination with single-cell selection [9] and sequencing methods, is uniquely suited to explore the molecular underpinnings of this heterogeneity and to accelerate targeted phenotyping efforts.

We explored whether the innate photophysiology of a cell could reflect its ability to resist future temperature stress by plotting the initial \( F_v/F_m \) at 22°C of a cell against its \( F_v/F_m \) at increasing temperatures and linear regression fitting (Fig. 2c and Fig. S4–S8). This revealed a linear relationship \( (R^2 > 0.5) \) at temperatures below 31°C for all species and a collapse of this linearity \((R^2 < 0.5)\) for three out of five species above 31°C (Fig. 2d). This suggests that the initial photophysiology of a cell at 22°C is useful in predicting its PSII activity at temperatures up to 31°C. Our inability to predict PSII activity above 31°C is likely related to the simultaneous increase in phenotypic heterogeneity beyond this temperature. Despite these observed trends we cannot rule out that Symbiodiniaceae cells with low initial \( F_v/F_m \) could still exhibit high thermal tolerance. For example, photoacclimation can result in lower \( F_v/F_m \) while cells maintain thermal tolerance [17]. Conceivably, non-ideal cultivation temperatures could also have led to the reduction of initial \( F_v/F_m \) values among our cultures and so could have microenvironmental gradients within cultivation vessels (e.g., small differences in irradiance or gas transfers due to stratified growth of cells). Despite these potential shortcomings, earlier work corroborated that the application of acute heat stress to corals could resolve fine differences in host thermal tolerance [8]. We therefore speculate that our minimally-invasive method holds potential to provide bottom-up information on the thermal sensitivity of corals but also emphasize that further experimental verification is needed.

In summary, our study (i) uncovered increasing levels of single cell heterogeneity under elevated temperatures and (ii) reliably predicted photophysiological responses of single cells to temperatures below 31°C. Finally, besides temperature, our approach can also be used to reproduce other environmental features experienced by symbionts in hospite (e.g., pH and nutrients) and thus help elucidate the interplay between temperature stress, chemical microenvironment and acidification on coral symbionts.

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AUTHOR CONTRIBUTIONS
LB and LX designed the study. LX performed experiments and analyzed the data, with support from SJ and MT who fabricated the heat-stage. SR performed DNA extraction and amplification, FB and MS performed phylogenetic analysis. The manuscript was written by LX and LB with contributions from all coauthors.

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COMPETING INTERESTS
The authors declare no competing interests.
