



# Species-specific effects of light and temperature on photosynthesis and respiration among Symbiodiniaceae (*Dinophyceae*)

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**Abstract** Coral reefs are exposed to various environmental stressors that cause bleaching events, whereby endosymbiotic microalgae (Symbiodiniaceae) disassociate from coral hosts. Bleached corals are compromised and face mortality. The combination of high-light exposure and elevated seawater temperature often lead to coral bleaching. The physiological properties of the Symbiodiniaceae within the coral tissues contribute to the thermal tolerance of the holobiont (the host and all its symbionts). The present study aimed to investigate the effects of light and temperature stress on four Symbiodiniaceae species from three genera with respect to photosynthetic oxygen production and consumption. Under control conditions, the species displayed predominantly low-to-moderate light requirements for photosynthesis with increased photoinhibition at higher photon flux rates. After 30 days of heat acclimation at 32 °C, maximum

photosynthetic activity declined in *Effrenium voratum*, doubled in *Fugacium kawagutii*, and remained unchanged in *Breviolum psygmophilum*. In subsequent acute heating assays, species-specific effects on maximum photosynthetic activity were observed. Photosynthesis in all species declined across a temperature gradient between 25 and 39 °C in the acute heating assays; full inhibition occurred at 37 °C in *B. psygmophilum* and *E. voratum* and at 39 °C in *B. aenigmaticum* and *F. kawagutii*. In contrast, respiration remained largely constant in all species across temperatures. Our data point to species-specific photophysiological traits that lead to different thermal tolerances among Symbiodiniaceae.

**Keywords** Acclimation · Coral reef · Cnidarian-algal symbiosis · Heat stress · Irradiance · Photodamage · Zooxanthellae

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## Introduction

Reef-building corals are unable to efficiently form reef structure (i.e., precipitate calcium carbonate) at low temperatures and are thus restricted to oligotrophic tropical regions between 30° north and south latitude (Kampmann 2002). Tropical coral reefs are hotspots of biodiversity and contribute multiple ecological and economic goods and services such as high productivity, complex habitat formation, food provisioning for numerous marine organisms, and coastal protection (Moberg and Folke 1999). In addition, tropical coral reefs rely on the mutualistic symbiosis between unicellular dinoflagellates (Symbiodiniaceae) and coral hosts (Decelle et al. 2018; LaJeunesse et al. 2018). Coral reefs are threatened by numerous natural and anthropogenic stressors, such as ocean warming and acidification, freshwater runoff, and water pollution, all of which can lead to coral bleaching

(reviewed by van Oppen and Lough 2018). This phenomenon involves the dissociation of corals and their symbionts as a consequence of environmental stress. The longer this situation persists, the more likely it is that the corals will die. Coral bleaching has been observed multiple times on a global scale over the last decades (Hughes et al. 2017; Roth 2014). According to the latest Status Report of Coral Reefs of the World (Souter et al. 2021), there was a progressive loss of about 14% of the coral from the world's reefs between 2009 and 2018, and as the main stressor a rapid rise in sea surface temperature due to marine heatwaves. The tropical coral-algal symbiosis typically exists within a narrow temperature range close to its upper thermal limit, and thus even small temperature anomalies of just 1–2 °C above mean local summer maxima are sufficient to cause coral bleaching (Lesser 2011; Rådecker et al. 2023).

Absorbed sunlight is essential for photosynthesis as it drives photochemical reactions, but the strength of light determines whether Symbiodiniaceae species are stressed or not (Roth 2014). Due to the dynamic nature of solar radiation, the photosynthetic machinery is often confronted with higher light levels than can be processed through photochemistry, and hence excess absorbed excitation energy must be removed by other pathways to minimize photooxidative damage (Müller et al. 2001; Niyogi 1999). These include reemission as chlorophyll fluorescence, dissipation as heat (non-photochemical quenching) or decay via the chlorophyll triplet state in which reactive oxygen species (ROS) are produced (Asada 1999; Müller et al. 2001). ROS often cause photooxidative damage to proteins and other biomolecules of the photosynthetic system and are inevitably produced during photosynthesis (Niyogi 1999). Consequently, the photosynthetic apparatus is constantly repairing itself from photodamage (Niyogi 1999). If the rate of damage exceeds the rate of repair, there will be a decline in photosynthetic performance (Roth 2014). Nevertheless, the photosynthetic apparatus is known as a plastic and flexible molecular machine, and thus capable of acclimating to dynamic and changing light conditions (Falkowski and Raven 2007), such as those on a coral reef.

The algal endosymbionts that form mutualisms with corals belong to the family Symbiodiniaceae (Dinophyceae, Alveolata). Though originally classified as a single species due to morphological homogeneity (Freudenthal 1962), the application of various molecular markers has revealed a genetically diverse group of dinoflagellates, which were assigned to multiple phylogenetic clades (e.g., “Clade A,” “Clade B,” etc.) (Rowan and Powers 1991). Most of these “Clades” were recently elevated to genera within the family Symbiodiniaceae (LaJeunesse et al. 2018, 2022; Nitschke et al. 2020; Pochon and LaJeunesse 2021). Species within each genus exhibit different ecophysiological traits, such as levels of tolerance to solar radiation (Suggett et al. 2015) and

temperature fluctuations (Swain et al. 2017). The underlying mechanisms for this functional diversity are not fully understood, but can be, at least partially, explained by molecular and biochemical processes of the photosynthetic apparatus and other modes of resource acquisition (Takahashi et al. 2008; Suggett et al. 2017).

Based on studies of cultured algae, the effects of elevated temperature on members of the Symbiodiniaceae are not uniform (Lesser 1996; Karim et al. 2015; Klueter et al. 2017). For example, Karim et al. (2015) revealed that growth and photochemical efficiencies ( $F_v/F_m$ ) of photosystem II (PSII) remained unchanged between 25 and 30 °C in various Symbiodiniaceae lineages. Both physiological traits strongly declined at 33 °C in thermally sensitive taxa, but not in thermally tolerant ones. In contrast, at temperatures > 33 °C, the thermal tolerance of all investigated species was exceeded. Other studies confirmed a similar pattern, showing that different Symbiodiniaceae cultures exhibit specific tolerances with respect to light and temperature gradients (Robison and Warner 2006; Diaz-Almeyda et al. 2017). Such variation is not restricted to the free-living condition; light and temperature tolerance are species-specific traits among Symbiodiniaceae residing within coral hosts (Berkelmans and van Oppen 2006; Sampayo et al. 2008; van Oppen et al. 2009). Ultimately, the interaction between host and symbiont genotype dictates various aspects of holobiont physiology (Parkinson and Baums 2014).

The main goal of the present study was to evaluate variation in the light requirements and thermal tolerances of several different Symbiodiniaceae species from multiple genera. *Breviolum psygmophilum* is a cold-tolerant symbiotic species from the Western Atlantic Ocean, whereas *B. aenigmaticum* is a closely-related but likely free-living Symbiodiniaceae with unknown thermal tolerance (Bayliss et al. 2019; Parkinson et al. 2015; Thornhill et al. 2008). *Effrenium voratum* is a free-living, highly motile species distributed globally in temperate waters (Jeong et al. 2014; Lee et al. 2016). *Fugacium kawagutii* is also a free-living species mostly found in the Pacific Ocean (LaJeunesse et al. 2018). These four Symbiodiniaceae were comparatively investigated under controlled experimental conditions (i.e., standard maintenance at 26 °C followed by 30 days incubation at 32 °C). Photosynthetic and respiratory responses were evaluated to address light requirements, thermal tolerance, and acclimation capacity.

## Material and methods

### Culture and cultivation

In this study, we evaluated the photophysiological properties and thermal tolerance of four isoclinal cultures representing

four Symbiodiniaceae species: *Breviolum aenigmaticum* (strain 04–180), *B. psygmophilum* (strain Pur.P.flex), *Effrenium voratum* (strain CCMP 421), and *Fugacium kawagutii* (strain MV) (Tab.1). The cultures were maintained in marine sterilized North Sea water (Helgoland, 30.7 S<sub>A</sub> (absolute salinity)) enriched with 30 mL L<sup>-1</sup> Guillard's f/2 medium (Guillard and Ryther 1962) which provides trace metals and vitamins. For each species, three replicate 50 mL Erlenmeyer flasks were established which were then covered with foam stoppers and aluminum foil. These glass vessels were kept in a water bath to ensure a stable temperature for cultivation at 26 °C (± 0.8 °C, Jumo, TDA-300, Fulda, Germany) by establishing a constant water flow (Profi Cool, UKT 300-1, National Lab GmbH, Mölln, Germany). Using a 36W neon tube (Osram Lumilux® De Luxe L36W/840 Cool Light, Osram, Munich, Germany), an average photon flux density of 74 μmol photons m<sup>-2</sup> s<sup>-1</sup> was applied in combination with a light–dark rhythm of 12 h:12 h. All cultures were acclimated at 26 °C for 2–3 months and the media regularly changed to grow sufficient biomass (5–10 mg chlorophyll *a* L<sup>-1</sup>) for the experiments. Media composition was identical to that mentioned before (Table 1).

### Temperature treatments

For each culture, after sufficient biomass was produced as indicated by the algal cultures reaching log phase (see above), each replicate flask was split into two equal sub-volumes. One sub-volume was maintained at 26 °C for an additional 30 days (control treatment) while in parallel, the other sub-volume was maintained in a separate water bath at 32 °C for 30 days (elevated temperature treatment) under the same light conditions.

### Fixed temperature, variable light experiments

For each species and treatment, photosynthesis-irradiance curves (PE curves) were recorded employing a custom-built PE box (Prelle et al. 2022, Supplemental Fig. 1) which

determines oxygen production per chlorophyll *a* as a function of increasing photon flux densities (PFDs). PE curves were generated in three separate water-surrounded oxygen electrode chambers (Hansatech Instruments, King's Lynn, United Kingdom), each filled with 3 mL of culture media on top of a magnetic stirrer (Hansatech Instruments, King's Lynn, United Kingdom). These chambers were temperature-controlled by connecting to a water supply (K10, Thermo Haake, Karlsruhe, Germany) and a thermostat (DC10, Thermo Haake, Karlsruhe, Germany) to ensure constant conditions during the measurements at 26 or 32 °C (± 0.1 °C). As external light sources, LEDs were applied (LUXEON Rebel1 LXML-PWN1-0100, neutral white, Phillips, Amsterdam), which were implemented in the PE box. To reduce the PFDs, neutral density filters were placed between each LED and the respective cuvette to generate a gradient of PFDs between 0 and 1,600 μmol photons m<sup>-2</sup> s<sup>-1</sup>. The irradiance was measured directly inside the cuvettes using a small spherical light sensor (Light meter LI-250, LI-COR, Lincoln, USA). Each cuvette was equipped with an oxygen dipping probe DP sensor (optode) (PreSens Precision Sensing GmbH, Regensburg, Germany) and connected via a fiber optic to an oxygen transmitter (Oxy 4-Mini, PreSens Precision Sensing GmbH, Regensburg, Germany) in combination with the PreSens software OXY4v2\_30 for measuring and calibration (two-point calibration, 0 and 100% oxygen saturation). To calibrate the oxygen dip probes, each cuvette was treated at 0 and 100% oxygen saturation at 20 °C, respectively. Oxygen saturation at 100% was performed by aeration of the culture media for 15 min. Sodium dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) was used to achieve oxygen free media. The chambers were tempered to the desired temperature (26 and 32 °C, respectively, for recording PE curves), and always 30 μL of sodium bicarbonate (NaHCO<sub>3</sub>, final concentration 2 mM) was added to each sample to avoid carbon deficiency (for details, see Prelle et al. 2022). After each PE curve, Symbiodiniaceae cell suspensions from each cuvette were filtered onto an individual Whatman GF/6 glass fiber filter (Ø 25 mm) for chlorophyll *a* determination

**Table 1** Symbiodiniaceae cultures used in this study. Information includes species name, strain ID, ITS2-Type, lifestyle, and oceanic region of strain

Species	Authority	Strain ID	ITS2-Type	Lifestyle	Oceanic region of culture
<i>Breviolum aenigmaticum</i>	(J.E.Parkinson, Coffroth & LaJeunesse) J.E.Parkinson & LaJeunesse	04–180	B19	Free living	Atlantic
<i>Breviolum psygmophilum</i>	(LaJeunesse, J.E.Parkinson & J.D.Reimer) J.E.Parkinson & LaJeunesse	Pur.P.flex	B2	Symbiotic	Atlantic
<i>Effrenium voratum</i>	(H.J.Jeong, S.Y.Lee, N.S.Kang & LaJeunesse) LaJeunesse & H.J.Jeong	CCMP421	E1	Free living	Pacific
<i>Fugacium kawagutii</i>	LaJeunesse	MV	F1	Free living	Pacific

as a reference parameter according to spectrophotometric assay of HELCOM (2015) which uses 96% ethanol (v/v) for pigment extraction. The photosynthetic model of Walsby (1997) was used for fitting and calculating different PE curve parameters:

$$\text{NPP} = \text{NPP}_{\max} \left( 1 - \exp(-\alpha I / \text{NPP}_{\max}) \right) + R + \beta I,$$

where NPP is the net photosynthetic rate,  $\text{NPP}_{\max}$  is the maximum photosynthetic rate at light-saturating irradiances ( $I$ ),  $R$  is the respiration rate,  $\alpha$  is the light utilization coefficient, and  $\beta$  is the photoinhibition coefficient. The light saturation point ( $E_k$ ) is calculated as  $\text{NPP}_{\max} / \alpha$  and the light compensation point ( $E_c$ ) is calculated as  $-R/\alpha$ .

### Fixed light, variable temperature experiments

For each species and treatment, changes in photosynthesis and respiration rates were recorded as the Symbiodiniaceae responded to rising temperatures in the PE box following the approach of Russnak et al. (2021). These authors used the same batch of culture during the variable temperature experiment. Starting at 25 °C, a 20-min dark incubation phase was followed by a 10 min dark respiration phase and afterward by an additional 10 min photosynthesis phase in the light (340  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , which typically stimulates maximal photosynthesis in Symbiodiniaceae; Falkowski and Raven 2007). After determining photosynthetic oxygen production, the temperature was increased by 2 °C within 3–4 min and a new incubation cycle (20 min incubation, 10 min respiration, and 10 min photosynthesis) was started after reaching the new temperature in the thermostat chamber. Finally, oxygen consumption in the dark and oxygen production in the light per unit time was related to the chlorophyll  $a$  concentration per sample as described above. From the data, the optimum temperatures for photosynthesis and respiration were calculated by applying the nonlinear model of Blanchard et al. (1996):

$$P_{\max}(T) = P_{\max} \left( \frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}} \right)^{\beta} \exp \left[ -\beta \left( \frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}} - 1 \right) \right]$$

where  $P_{\max}(T)$  is the net photosynthetic rate,  $P_{\max}$  is the maximum value of  $P_{\max}$ ,  $T$  is the temperature (°C),  $T_{\max}$  is the maximum temperature,  $T_{\text{opt}}$  is the optimum temperature, and  $\beta$  is a dimensionless parameter for sensitivity.

### Statistical analysis

Data calculations were performed using Microsoft Office 365 Excel. To calculate the PE curves according to the Walsby model (Walsby 1997), the solver function was used to minimize the normalized deviation squares. The statistical

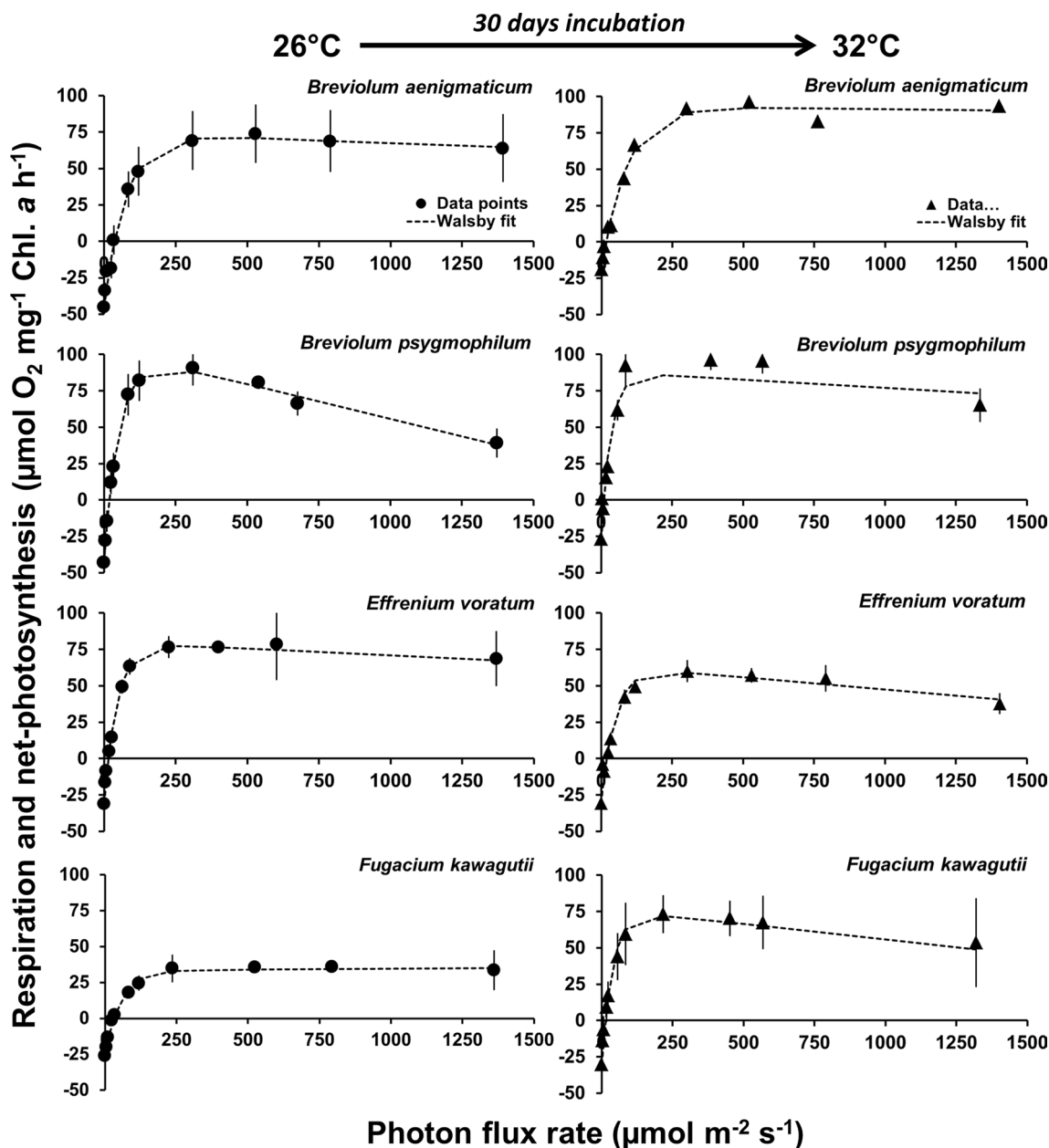
analysis was performed using SPSS Statistics (version 27) and the program  $R$  (version 4.2.2). To calculate significance levels among all means, one-way ANOVA was used followed by a post hoc Tukey test. If the data did not fulfill the assumptions of variance homogeneity or normal distribution for one-way ANOVA, the Kruskal–Wallis test was applied. The significance level was set to  $\alpha < 0.05$  for all analyses.

## Results

### Light requirements for photosynthesis under ambient and elevated temperatures

Photosynthetic oxygen production in all four Symbiodiniaceae species at 26 °C (control treatment) revealed slightly different PI curves (Fig. 1). While *Fugacium kawagutii* showed no indication of photoinhibition up to 1,361  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , *Breviolum aenigmaticum* and *Effrenium voratum* exhibited very small degrees of photoinhibition ( $\beta = -0.01 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$ ) (Fig. 1, Table 2). In contrast, photosynthesis of *Breviolum psygmophilum* was moderately photoinhibited ( $\beta = -0.05 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$ ) at the highest PFD (Fig. 1). In addition, the maximal oxygen production rate was similar in both *Breviolum* species and *E. voratum* ( $\text{NPP}_{\max}$  range: 76.0–90.8  $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ), and 2.5-fold lower in *F. kawagutii* (32.6  $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ) (Fig. 1, Table 2). Among all species, the  $\alpha$ -values ranged from 1.3 to 2.6  $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$ , the  $E_k$  values from 40.4 to 78.5  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , and that of  $E_c$  were between 18.4 and 32.5  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Table 2). All these data indicate generally low-to-moderate light requirements for photosynthesis at 26 °C, although some species-specific response patterns can be observed.

Incubations of 30 days at 32 °C led to species-specific effects on the PE curve parameters of all investigated Symbiodiniaceae (Fig. 1, Table 2). While in *B. psygmophilum*  $\text{NPP}_{\max}$  was unaffected, in *E. voratum* a significant reduction in  $\text{NPP}_{\max}$  could be observed, dropping from 76.0 to 59.4  $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$  with higher temperature ( $p < 0.05$ ), and the opposite was the case in *F. kawagutii*, where  $\text{NPP}_{\max}$  more than doubled under the higher temperature (from 32.6 to 70.4  $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ;  $p < 0.05$ ) (Table 2). In *B. aenigmaticum*, there also appeared to be a slight enhancement of  $\text{NPP}_{\max}$  after treatment with 32 °C, but only one replicate could be measured due to a technical failure. While most PE curve parameters of the four species exhibited minor changes under increased temperatures, photoinhibition in *B. psygmophilum* was much less pronounced at 32 °C compared to 26 °C as reflected in a fivefold reduction in the  $\beta$ -value at elevated temperature ( $-0.05$  to



**Fig. 1** Photosynthesis and respiration rates ( $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ) as a function of increasing photon flux densities ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) of four Symbiodiniaceae species kept at 26 °C and at 32 °C for 30 days. The endosymbionts were kept in f/2 Baltic Sea water

medium,  $^{33}\text{S}_A$ , and oxygen changes were measured using optodes. Data represent mean values  $\pm$  SD ( $n=3$ ), except for *Breviolum aenigmaticum* ( $n=1$ , due to technical problems). Data points were fitted using the model of Walsby (1997)

$-0.01 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1}$ ;  $p < 0.05$ , Table 2).

**Temperature effects on photosynthesis and respiration**

Temperature had a strong effect on photosynthetic oxygen production and a much weaker influence on respiratory oxygen consumption in all four Symbiodiniaceae species (Fig. 2). Using cultures from the control treatment, the

optimum temperature for photosynthesis was calculated using the Blanchard et al. (1996) model, resulting in optimum values ranging from 26.8 to 29.5 °C depending on the species (data not shown). With the same approach, the optimum temperature for respiration was calculated, and here a broader range was observed. While both *Breviolum* species showed a respiration optimum at 25 °C, *E. voratum* and *F. kawagutii* showed optima between 37 and 39 °C.

**Table 2** Parameters of respective PE curves (Fig. 2) of four Symbiodiniaceae species ( $n=3$ ) kept at 26 °C and after 30 days treatment at 32 °C

Species	NPP <sub>max</sub> (μmol O <sub>2</sub> mg <sup>-1</sup> Chl <i>a</i> h <sup>-1</sup> )	Respiration (μmol O <sub>2</sub> mg <sup>-1</sup> Chl <i>a</i> h <sup>-1</sup> )	$\alpha$ (μmol O <sub>2</sub> mg <sup>-1</sup> Chl <i>a</i> h <sup>-1</sup> ) (μmol photons m <sup>-2</sup> s <sup>-1</sup> ) <sup>-1</sup>	$\beta$ (μmol O <sub>2</sub> mg <sup>-1</sup> Chl <i>a</i> h <sup>-1</sup> ) (μmol photons m <sup>-2</sup> s <sup>-1</sup> ) <sup>-1</sup>	$E_k$ (μmol photons m <sup>-2</sup> s <sup>-1</sup> )	$E_c$ (μmol photons m <sup>-2</sup> s <sup>-1</sup> )
<i>Breviolum aenigmaticum</i> 26 °C	78.3 ± 9.1	-45.1 ± 13.1	1.9 ± 0.9	-0.01 ± 0.01	78.5 ± 11.6	32.5 ± 7.6
<i>Breviolum aenigmaticum</i> 32 °C	92.2	-19.1	1.3	-0.00	86.6	16.3
<i>Breviolum psygmophilum</i> 26 °C	90.8 ± 12.2a	-43.1 ± 6.9a	2.9 ± 0.4a	-0.05 ± 0.01a	46.8 ± 4.9a	18.4 ± 4.0a
<i>Breviolum psygmophilum</i> 32 °C	85.1 ± 20.0a	-26.7 ± 3.7b	2.6 ± 0.2a	-0.01 ± 0.02b	43.5 ± 6.9a	11.9 ± 0.8b
<i>Effrenium voratum</i> 26 °C	76.0 ± 7.6c	-31.0 ± 1.9c	1.8 ± 0.1c	-0.01 ± 0.02c	58.5 ± 1.7c	20.0 ± 1.4c
<i>Effrenium voratum</i> 32 °C	59.4 ± 5.9d	-30.7 ± 9.9c	1.9 ± 0.3c	-0.02 ± 0.01c	46.8 ± 4.1d	19.4 ± 1.8ce
<i>Fugacium kawagutii</i> 26 °C	32.6 ± 9.1e	-26.0 ± 4.7e	1.3 ± 0.4e	-0.00 ± 0.01e	40.4 ± 17.8e	27.1 ± 3.0e
<i>Fugacium kawagutii</i> 32 °C	70.4 ± 11.2f	-30.2 ± 9.9e	2.1 ± 0.5e	-0.02 ± 0.03e	49.6 ± 6.9e	18.4 ± 8.9e

Different lowercase letters represent significant levels among the means of each species at 26 and 32 °C as calculated by a one-way ANOVA (Tukey's test,  $p < 0.05$ ). NPP<sub>max</sub> represents the maximal oxygen production rate, alpha the initial slope of production in the light limited range, beta the terminal slope of production in extensive light range (photoinhibition),  $E_k$  the light saturation point, and  $E_c$  the light compensation point. For *Breviolum aenigmaticum* at 32 °C, only one replicate was available due to technical problems

In *B. psygmophilum* and *E. voratum* photosynthesis was fully inhibited at 37 °C (Fig. 2). Photosynthesis in *B. aenigmaticum* and *F. kawagutii* was still measurable at 37 °C (c. 10–40% of the maximum), and fully inhibited at 39 °C (Fig. 2). In contrast, respiration essentially remained constant regardless of temperature (Fig. 2).

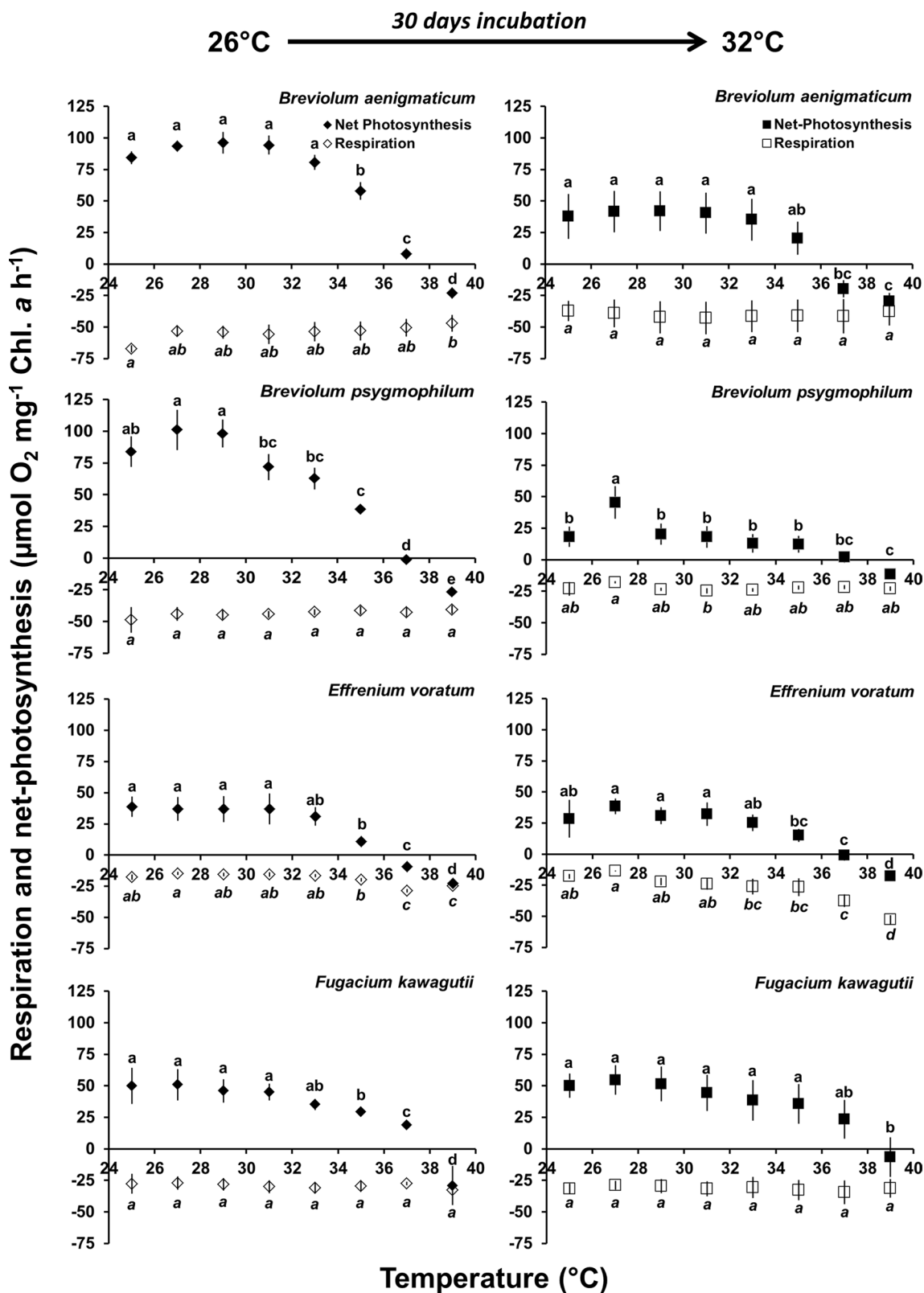
After 30 days of incubation at 32 °C, photosynthesis and respiration responses varied relative to counterparts in the control treatment for some species but not others (Fig. 2). In both *Breviolum* species NPP<sub>max</sub> decreased > twofold and the maximum respiration rate declined 1.3–1.9 fold compared to the control treatment (Fig. 2).

Applying the model of Blanchard et al. (1996), we first calculated the optimum temperature for photosynthesis and respiration, followed by determination of the percentiles of < 20%, 20–80%, and > 80% (Fig. 3). This approach led to a visualization of the thermal limits of both physiological processes when reared at either 26 °C or 32 °C. Both *Breviolum* species lost photosynthetic capacity when reared at higher temperatures, while *E. voratum* and *F. kawagutii* were less affected. No species could photosynthesize when exposed to temperatures in excess of 37 °C, and all species maintained comparable respiration rates across a broad temperature range regardless of initial rearing temperature (Fig. 3).

## Discussion

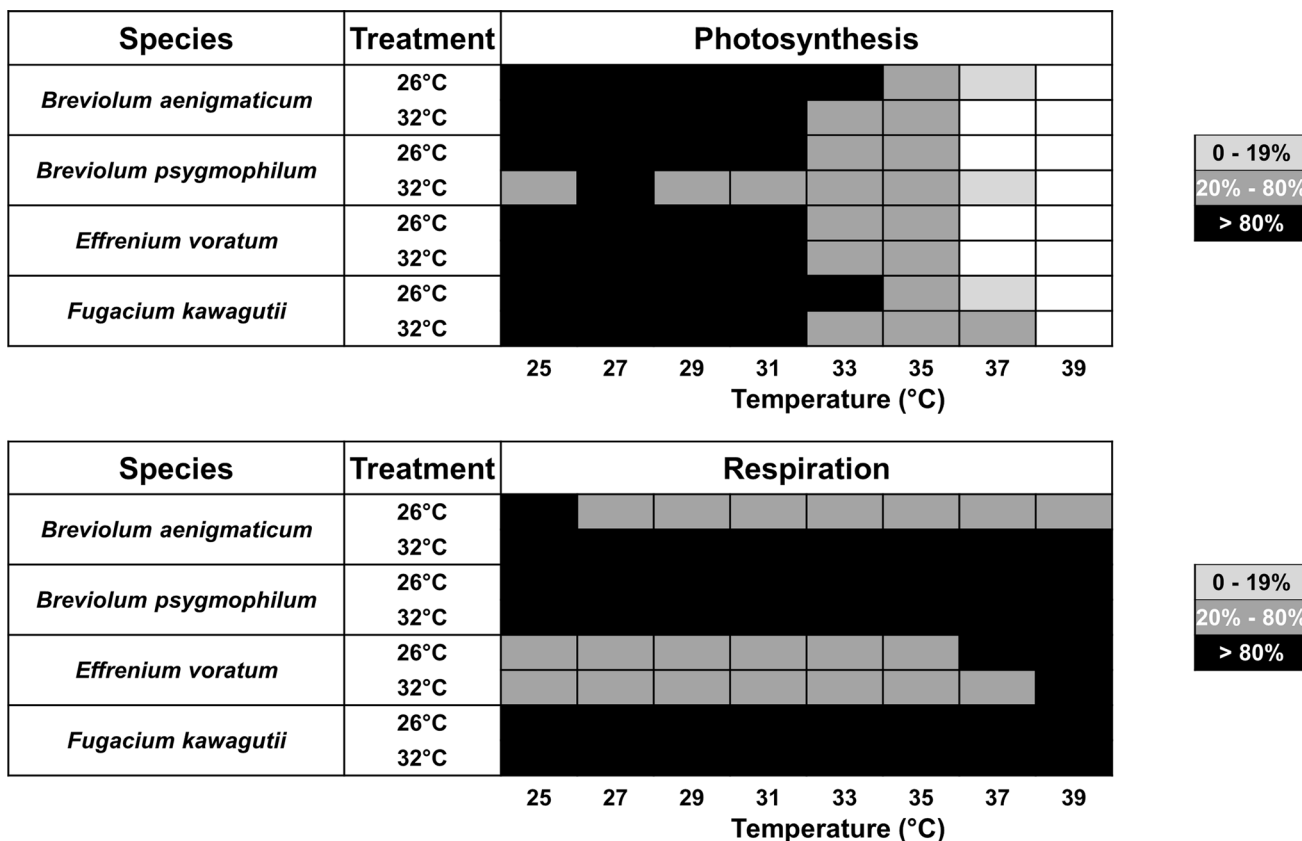
Corals are susceptible to abiotic stressors such as increased solar radiation and heat waves, and the physiological plasticity and genetic diversity of the endosymbiotic microalgae are a key element explaining the different response patterns of their hosts (Berkelmans and van Oppen 2006; Sampayo et al. 2008; Baird et al. 2009; DeSalvo et al. 2010). Therefore, we grew four Symbiodiniaceae species under fixed light conditions and different temperatures, and evaluated photosynthesis and respiration activity to better understand physiological tolerance and plasticity.

Within a given temperature treatment, all Symbiodiniaceae species seem to exhibit similar patterns of photoacclimation when exposed to a range of light levels (Fig. 1). Symbiodiniaceae are capable of photoacclimation under changing photon flux rates (Iglesias-Prieto and Trench 1994; Roth 2014; Russnak et al. 2021), and our results align with this notion. In addition, Symbiodiniaceae increase chlorophyll and carotenoid concentrations in response to low-light conditions, thereby maximizing light absorption and utilization as well as improving photosynthetic efficiency (Anthony and Hoegh-Guldberg 2003). The conditions under which the algae were cultured are reflective of typical low-light levels within coral host tissue (Anthony et al. 2005), and were equivalent for all species, so it was assumed that



**Fig. 2** Photosynthetic oxygen production and respiratory consumption rates ( $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ) as function of increasing temperature (25–39  $^{\circ}\text{C}$ ) at a saturating photon fluence density (PFD) of approx.  $350 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in four Symbiodiniaceae species kept at 26  $^{\circ}\text{C}$  and after 30 days treatment at 32  $^{\circ}\text{C}$ . The endosym-

bionts were kept in f/2 Baltic Sea water medium, 33S<sub>A</sub>, and oxygen changes were measured using optodes. Data represent mean values  $\pm$ SD ( $n=3$ ). Lowercase letters at photosynthesis and respiration indicate significantly means ( $p<0.05$ ; one-way ANOVA with post hoc Tukey-HSD test)



**Fig. 3** Influence of temperature between 25 °C and 39 °C on photosynthetic oxygen production and respiratory oxygen consumption of four tested Symbiodiniaceae species using the fit of Blanchard et al. (1996). The black box represents area of highest photosynthesis more

than >80% percentile, dark gray symbols within 20 and 80% percentile, light gray symbols <20% percentile, and white spaces no photosynthesis or respiratory signal. The data represent mean values (n = 3)

chlorophyll levels remained constant within each species. However, because *P:E* data were normalized to chlorophyll content rather than cell counts, it is possible that certain differences in acclimation were masked (e.g., if pigment density changed with temperature). Future work should incorporate cell counts and verification of chlorophyll content per cell to increase confidence in species-specific patterns.

Our light experiment data are in agreement with those of Russnak et al. (2021) who investigated two genetically distinct genotypes of *Breviolum psygmophilum* (strains MAC HIAP and 1046) and reported  $E_k$ ,  $E_c$  and respiration values similar to those measured in this study for *B. psygmophilum* (strain Pur.P.flex) under control conditions (70–80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Furthermore, Russnak et al. (2021) examined the same isolate (genotype) of *Effrenium voratum* (strain CCMP 421), which yielded PE curve parameters comparable to those in the present investigation.

However, it is important to keep in mind that the physiology of cultured algae (*in vitro*) may not reflect their physiology when engaged in symbiosis (*in hospite*) (Maruyama and Weis 2021). Bhagooli and Hidaka (2003) studied the consequences of light stress on the maximum quantum

yield ( $F_v/F_m$ ) of PSII of different Symbiodiniaceae species *in vitro* and *in hospite*. They found that within host cells, symbiont  $F_v/F_m$  decreased significantly at high-light conditions (1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), while in the respective isolated cultures,  $F_v/F_m$  declined precipitously at 70  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and higher, supporting the photoprotective role of host tissue. The coral host has a set of mechanisms to protect their endosymbionts against high-light conditions, while also optimizing their intracellular light environment depending on incident solar radiation (Lichtenberg et al. 2016). These mechanisms include, for example, the production of chromoproteins or other fluorescent pigments to quench harmful UV radiation, the formation of antioxidative enzymes, contraction and expansion of the tissue (gastrodermis) leading to modulation of intra-tissue light scattering, or the modification of the calcium carbonate skeleton to enhance light absorption (Lichtenberg et al. 2016; Roth 2014). Consequently, the coral host controls and regulates antioxidants, pigments, gene expression, behavior, and architectural levels over multiple timescales in response to changing light conditions to optimize the photobiological performance of the coral-algal symbiosis (Roth 2014).

Further study of the performance of symbiotic *Breviolum psygmophilum* in hospite is warranted to better understand these protective mechanisms.

All four Symbiodiniaceae species exhibited optimum photosynthesis between 26.8 and 29.5 °C, and declining photosynthetic rates became evident with rising temperatures up to 35 °C (Fig. 2). While at 37 °C photosynthesis in *B. aenigmaticum* and *F. kawagutii* was still detectable, it was not in *B. psygmophilum* and *E. voratum*, and at 39 °C it was completely inhibited in all species. Gregoire et al. (2017) reported that their culture of *B. psygmophilum* (strain Mf11.05.01) was completely inhibited at just 33 °C, as was a culture of *Symbiodinium tridacnidorum*, whereas cultures of *Breviolum minutum*, *Cladocopium infistulum*, *Durusdinium trenchii*, and *Fugacium kawagutii* could still photosynthesize to varying degrees at this temperature. Russnak et al. (2021) comparatively investigated nine Symbiodiniaceae cultures (representing five species) and found that in four isolates (including *E. voratum* strain CCMP 421) photosynthesis was completely inhibited at 35 °C. The remaining five strains exhibited photosynthetic efficiencies between 20 and 80% of the maximum at 35 °C (Russnak et al. 2021), while at 40 °C photosynthesis was inhibited in all cultures.

The results from the present study point to clear species-specific and even strain-specific upper temperature thresholds for Symbiodiniaceae photosynthesis, typically in the range of 30 to 37 °C, and an absolute limit at 39 °C (Fig. 3). These thresholds are consistent with the 32–34 °C range typically reported for other cultured Symbiodiniaceae (Iglesias-Prieto et al. 1992; Warner et al. 1996; Iglesias-Prieto and Trench 1994; Jones et al. 1998; Brown et al. 1999). Given the thermal sensitivity of holobionts, shuffling or switching of Symbiodiniaceae communities in some corals after severe bleaching events may provide one of several mechanisms to acclimate (Huang et al. 2020). Although shifts in the dominant Symbiodiniaceae toward more thermotolerant species are commonly observed on the reef following bleaching, most novel associations do not persist long-term (Hume et al. 2020). Even if we consider that novel associations may persist under altered environmental regimes (i.e., if conditions don't return to their previous state), our data argue that there is an upper thermal limit of algal symbionts that is close to the summer conditions in the warmest sea where corals exist: the Persian/Arabian Gulf (Hume et al. 2016). These limitations must be considered in the current debate over active interventions, in particular regarding nature-based solutions (Buerger et al. 2020; Quigley et al. 2021; Voolstra et al. 2021). Under natural conditions tropical surface water temperatures typically range from 27 to 32 °C, but anomalies of up to 2.5 °C above the summer thermal maximum have been observed during strong El-Niño events (NOAA). Factoring in climate change, extremes are likely

to increase to +3.0 to 5.0 °C in the coming decades (IPCC 2023). Such temperature rise would reach the photosynthesis threshold of many symbiont species, with negative consequences for reef-building corals.

The conspicuous variation in Symbiodiniaceae thermal tolerance across cultures might be explained by an array of mechanisms. Enhanced temperatures can inhibit the repair mechanism of PSII, particularly the D1 proteins in the reaction centers, resulting in photoinhibition. This occurs when the damage to PSII exceeds its repair capacity, and symbiont strains capable of photosynthesis at temperatures above 32 °C likely have efficient thermoprotective mechanisms (Amario et al. 2023). These mechanisms may include the upregulation of various ROS scavenging proteins and molecular chaperones (Howells et al. 2012). For example, members of the Symbiodiniaceae synthesize heat shock protein 70 under thermal stress in order to repair and refold proteins (Ellison et al. 2017).

While photosynthesis exhibited a clear upper thermal limit for complete inhibition, respiration in all four species was mostly unaffected by temperature between 25 °C and 39 °C (Fig. 3). These data indicate that even under the highest temperature the endosymbionts were not dead because respiration was detectable. Such conspicuously distinct temperature requirements and tolerances for photosynthesis and respiration have previously been reported in closely-related Symbiodiniaceae (Gregoire et al. 2017; Russnak et al. 2021), as well as in other dinoflagellates (Lindström 1984), benthic diatoms (Prelle et al. 2022), and green algae (Karsten et al. 2014). It seems that in many microalgal taxa, respiration under low temperature is just detectable compared to photosynthesis, while the opposite is true for higher temperatures. This is not surprising, as key differences in both physiological processes exist. For example, the upstream mechanisms of photosynthesis are controlled mostly by light-related processes such as light absorption and energy transfer, whereas respiration is controlled almost entirely by temperature-dependent enzymatic reactions (Atkin and Tjoelker 2003). Nevertheless, the downstream process of carbon fixation involves various enzymes and hence might also be affected by temperature. In addition, photosynthesis is restricted to chloroplasts and respiration to mitochondria, and both organelles differ in their internal membrane systems and stability. The thylakoid structure of chloroplasts is much more vulnerable to light and temperature stress compared to mitochondrial cristae (Kirchoff 2014; Gounaris et al. 1984; Rurek 2014), which likely explains the greater functionality of respiration compared to photosynthesis under elevated temperatures. Under high temperature conditions photosynthesis is inhibited, while respiration becomes more and more essential to maintain the cellular energy demand (Slot et al. 2014).

## Conclusion

In conclusion, we show species-specific physiological traits for Symbiodiniaceae cultures under controlled and simulated light and temperature conditions, which helped to identify light requirements and thermal tolerances of photosynthesis and respiration in four species. Our data confirm that photosynthesis and respiration responses are mostly uncoupled in these organisms. Further, respiration is typically less sensitive to temperature than photosynthesis. While results from culture-based studies cannot be directly transferred to the natural conditions of corals on the reef, where symbionts are buffered from the external environment by their hosts and colonies are exposed to more variable temperature fluctuations on different time scales, they nevertheless provide valuable insights into Symbiodiniaceae physiology.

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**Data availability** All data are presented in this study. The raw data are available on request by the corresponding author.

## Declarations

**Conflict of interest** Authors declare that they have no conflict of interest.

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